2012-11

Visual Performance, and it's Response to Intervention, in Subjects with Age-Related Macular Degeneration.

Sarah Sabour-Pickett
Technological University Dublin, sarah.sabour-pickett@tudublin.ie

Visual performance, and its response to intervention, in subjects with age-related macular degeneration

Sarah Sabour-Pickett, BSc

Department of Optometry,

Dublin Institute of Technology,

Kevin St., Dublin 8

Supervisors:

Dr. James Loughman, PhD,

Department of Optometry,

Dublin Institute of Technology,

Kevin St., Dublin 8.

Dr. John M. Nolan, PhD,

and

Prof. Stephen Beatty, MD,

Macular Pigment Research Group,

Vision Research Centre,

Waterford Institute of Technology,

Waterford.

Submitted to Dublin Institute of Technology, for the award of PhD.

November 2012

Dedication

To the Bahá'i youth of Iran, and all those throughout the world, who are being actively denied their human right to an education.

"O Lord, help Thou Thy loved ones to acquire knowledge and the sciences and arts, and to unravel the secrets that are treasured up in the inmost reality of all created beings. Make them to hear the hidden truths that are written and embedded in the heart of all that is." ***- Bahá'i Writings***

Abstract

Objectives:

1. To explore visual performance status through a range of psychophysical methods beyond corrected distance visual acuity (CDVA), in subjects with age-related macular degeneration (AMD).

2. To investigate the effects on these visual performance parameters in subjects with neovascular age-related macular degeneration (nv-AMD) and in subjects with early AMD undergoing anti-VEGF (vascular endothelial growth factor) therapy and macular carotenoid supplementation, respectively.

3. To understand the role of a supplement containing *meso*-zeaxanthin (MZ; the third, and currently least explored, macular carotenoid) on the augmentation of macular pigment (MP), on visual performance and on disease progression (graded according to the AREDS [Age-Related Eye Disease Study] criteria), in subjects with early AMD.

4. To explore the impact of macular carotenoid supplementation on vision in subjects presenting with atypical macular pigment optical density (MPOD) spatial profiles at baseline.

Outcomes: This study has shown that CDVA is not the most appropriate measure of visual function and does not reflect retinal morphology in cases of early AMD or in cases of nv-AMD. Retinotopic ocular sensitivity (ROS), however, appears to be a more reflective measure of disease severity, where it correlates well with AMD-severity grade (in cases of early AMD) and also with mean foveal thickness (MFT; in cases of nv-AMD).

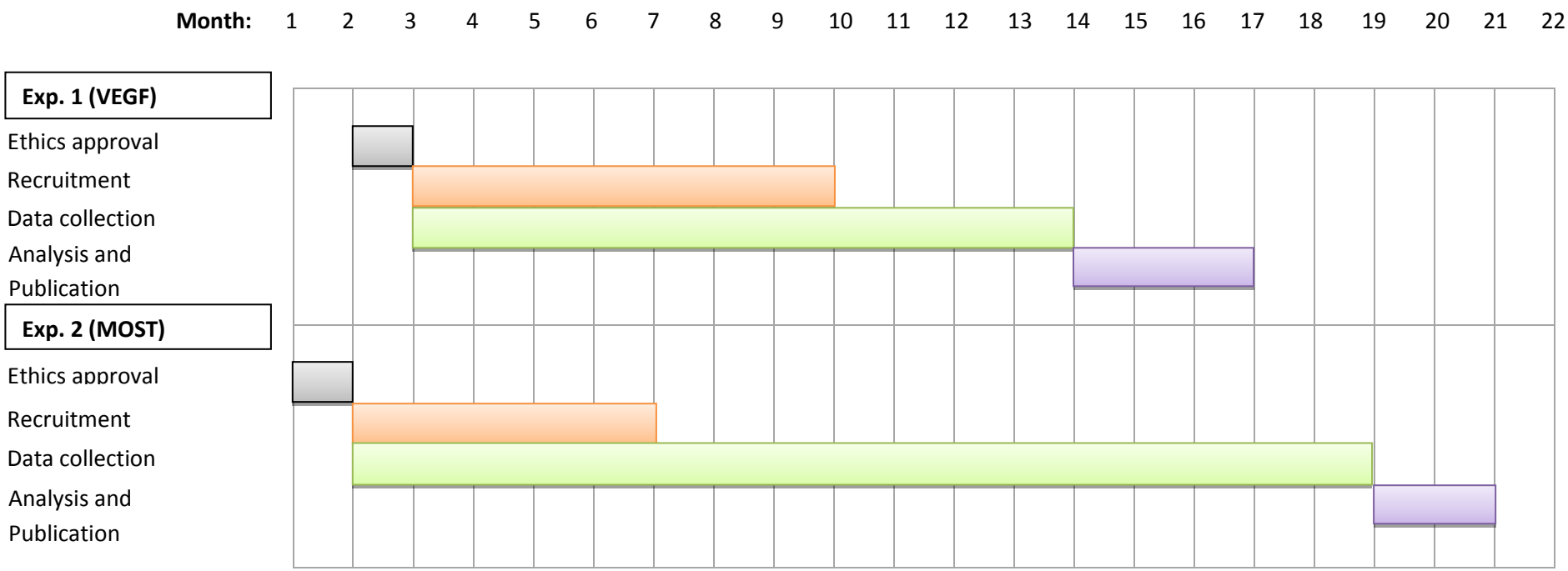
In eyes with nv-AMD undergoing monthly intravitreal ranibizumab injections, there have been demonstrable improvements in a range of parameters of visual function, namely, contrast sensitivity (CS), glare disability (GD), and ROS but no significant change in CDVA, despite a reduction in MFT.

MP can be augmented, and CS enhanced, in subjects with early AMD who receive supplemental macular carotenoids. Subjects with low baseline central MPOD had the greatest increases in MPOD and the greatest improvements in CS, when compared with subjects with medium or high baseline MPOD, suggesting that the

optimisation of CS (and putatively visual performance in general) is somewhat dependent on central MP levels.

The literature review has concluded that supplementation with the macular carotenoids offers the best means of fortifying the antioxidant defenses of the macula, thus putatively reducing the risk of AMD and/or its progression, and of optimising visual performance.

Conclusions: The findings of this work suggest the incorporation of tests, complimentary to CDVA, such as CS, GD, and particularly ROS, when attempting to understand disease severity in cases of AMD. In terms of monitoring change over time, the results of this study do seem to indicate that measures of ROS may be particularly useful in monitoring subjects with nv-AMD, while measures of CS and GD may be more apt in monitoring change in subjects with early AMD. Macular carotenoid supplementation can enhance visual performance in subjects with early AMD.


Month: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Exp. 1 (VEGF)
Ethics approval
Recruitment
Data collection
Analysis and Publication
Exp. 2 (MOST)
Ethics approval
Recruitment
Data collection
Analysis and Publication

Key findings

1. CDVA poorly reflects retinal morphology in cases of early AMD and in cases of nv-AMD. ROS, however, appears to be a measure which is more reflective of disease severity in these conditions, where it correlates well with AMD-severity grade (in cases of early AMD) and also with MFT (in cases of nv-AMD).
2. In eyes with nv-AMD undergoing monthly intravitreal ranibizumab injections, there have been demonstrable improvements in a range of parameters of visual function, including CS, GD and ROS, but no significant change in CDVA, despite a reduction in mean MFT.
3. Early AMD is visually consequential: while CDVA may not be greatly affected by early stages of the condition, it is clear that measures such as CS and GD are depressed compared to normal subjects and, therefore, should be considered in the diagnosis and monitoring of patients with AMD.
4. MP can be augmented, and CS enhanced, in subjects with early AMD who receive supplemental macular carotenoids. A formulation containing MZ appears to offer advantages over a formulation that does not contain MZ, in terms of improvements in psychophysical function and in terms of MP augmentation.
5. Optimisation of CS (and putatively visual performance in general) is influenced by central MP levels. Subjects with low baseline central MPOD had the greatest increases in MPOD and the greatest improvements in CS, when compared with subjects with medium or high baseline MPOD.

Declaration

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

This thesis was prepared according to the regulations for postgraduate study by research of the Dublin Institute of Technology and has not been submitted in whole or in part for another award in any other third level institution.

The work reported on in this thesis conforms to the principles and requirements of the DIT's guidelines for ethics in research.

DIT has permission to keep, lend or copy this thesis in whole or in part, on condition that any such use of the material of the thesis be duly acknowledged.

Signature _____ Date _____

Acknowledgements

"O compassionate God! Thanks be to Thee for Thou hast awakened and made me conscious. Thou hast given me a seeing eye and favoured me with a hearing ear…"

I would like to thank my parents, Maurice and Ala, for their invaluable support, love and encouragement, not only over the course of my study, but through all my life's experiences. To my brother, Ali, for always encouraging me to see the end in the beginning (and for taking me on a much needed skiing trip!). To my sister, Florence, who always manages to put a smile on my face. I would also like to thank my cousin, Saba, for her friendship and loving support over these years.

Not only did my move to Waterford provide me with the opportunity to do research, through it I have also been so lucky to have made such great friends. Thank you all for helping me settle so seamlessly into life here in Waterford.

To my supervisors – your advice and support have greatly contributed to what I have achieved. John, thank you for keeping me focussed, for having confidence in me, for your generosity, and above all for giving me the opportunity to work with the MPRG. It is a time in my life that I will look back on with great pleasure. Stephen, I have enjoyed, and have learned a great deal, working with you on our publications. Thank you also for giving me the opportunity to work at the Institute of Eye Surgery, which has furnished me with invaluable clinical experience as an Optometrist. James, thank you for your support from day one, for encouraging me to consider this path in my career, and for believing in me when I wasn't so sure myself. Thank you particularly for walking so patiently with me in the last few months as I wrote my thesis.

To the MPRG – thank you for your collaboration. It has been an absolute pleasure working alongside you all. I eagerly await what will emerge from the group in the coming years. I would like to particularly thank Eithne Connolly for her important and much appreciated collaboration on the MOST trial. I would like to also thank Dr. Jim Stack who assisted me greatly in understanding, and instilling in me an unexpected love for statistics.

I would like to thank Novartis for funding my period of research and also for facilitating my attendance of ARVO 2012, which was one of the highlights of my research experience. I would like to thank the Howard Foundation, not only for their

support of the MOST trial, but also for their scientific collaboration, and not least for giving me the opportunity to attend the research meetings in Cambridge over the past couple of years.

I would like to also thank Nutrasight Consultancy Ltd., the staff at the Institute of Eye Surgery, Prof. Ron Klein and the team at the University of Wisconsin, Dr. Konrad Pesudovs, MacuVision Europe and MacuHealth, USA. I would also like to particularly thank all the volunteers who kindly gave of their time to participate in the trials; it would not have been possible without you!

List of Abbreviations (in alphabetical order)

AD – artificial distortion

ALP – alkaline phosphatase

ALT – alanine aminotransferase

AST – aspartate aminotransferase

AMD – age related macular degeneration

AREDS – Age-Related Eye Disease Study

CAn – comparative analysis

CA – chromatic aberration

CARMA – Carotenoids in Age-Related Maculopathy

CDVA – corrected distance visual acuity

CFH – complement factor H

cHFP – customized heterochromatic flicker photometry

CNV – choroidal neovascularisation

COMPASS – The Collaborative Optical Macular Pigment ASsessment Study

cpd – cycles per degree

CRP – C-reactive protein

CS – contrast sensitivity

CSF – contrast sensitivity function

ETDRS – Early Treatment Diabetic Retinopathy Study

FFA – fundus fluorescein angiography

FVA – Functional Vision Analsyer

GA – geographic atrophy

GGT – gamma-glutamyl transferase

GD – glare disability

hsCRP- high sensitivity CRP

HDL – high density lipoprotein

L – lutein

LAST – Lutein Antioxidant Supplementation Trial

LDL – low-density lipo-protein

LED – light emitting diode

LFVFS – long-form visual function score

mfERG – multifocal elecroretinogram

MFT – mean foveal thickness

MFV – mean foveal volume

MOST – The Meso-zeaxanthin Ocular Supplementation Trial

MOST Vision – The Meso-zeaxanthin Ocular Supplementation Vision Trial

MP – macular pigment

MPRG – macular pigment research group

MPOD – macular pigment optical density

MZ – *meso*-zeaxanthin

NEI – National Eye Institute

NEI-VFQ – National Eye Institute Visual-Function Questionnaire

NHANES – National Health and Nutrition Examination Survey

Nv-AMD – neovascular age-related macular degeneration

NVS – near vision score

OCT – optical coherence tomography

PD – pathological distortion

PDT – photodynamic therapy

PGF – placenta growth factor

PHP – preferential hyperacuity perimetry

PRN – *pro re nata*

PUFA – polyunsaturated fatty acid

QoL – Quality of Life

RCT – randomised control trial

ROI – reactive oxygen intermediate

ROS – retinotopic ocular sensitivity

RPE – retinal pigment epithelium

SA – situational analysis

SSS – subjective satisfaction score

UV – ultraviolet

VA – visual acuity

VAR – visual acuity rating

VEGF – vascular endothelial growth factor

VPF – vascular permeability factor

wpm – words per minute

Z – zeaxanthin

Table of Contents

List of Tables

List of Figures

Chapter 1. Introduction

Age-related macular degeneration (AMD), a degenerative condition of the macula typically encountered amongst individuals fifty years or older, is the leading cause of blind registration in the developed world[1] (Figure 1.1). AMD affects an individual's central vision, whilst peripheral vision is preserved. The advanced stages of the condition, however, can have a considerable impact on an individual's quality of life and independence, as daily tasks, such as reading, driving, and recognising faces, are hampered.

The measurement of visual performance is a long established practice in the assessment and monitoring of ocular disease. It assists clinicians to understand disease severity and its corresponding impact on quality of life. It has also been used, in certain cases, to determine when to commence, continue or cease treatment, as well as to judge the efficacy of intervention, for clinical and research purposes, particularly following the introduction of new treatments or new treatment strategies.

Historically, the quantification of visual performance (in subjects with and without AMD) has been, and remains dominated by measures of corrected distance visual acuity (CDVA), a measure of the angular resolution limits of the eye at high contrast.[2] However, there is a general consensus that CDVA is not a true reflection of daily visual experience, and research studies have, in more recent times, started to incorporate alternative methods of assessing visual performance in clinical trials. Such trials have demonstrated the capacity of these additional measures to provide a more comprehensive overall assessment of visual performance compared to CDVA alone.[3-13] The universality of the measure of CDVA, however, along with other factors such as the cost and inconvenience associated with introducing additional methods of visual

assessment, has hindered the translation of these important research findings into clinical practice which, for the most part, has not materialised.[14] This has influenced a clinician's appreciation of disease severity and/or of the efficacy of intervention and also potentially influences decisions clinicians make with respect to commencing, continuing, or ceasing a given intervention.

AMD is generally classified as either 'early' or 'late'. The early form involves both hypotrophic and hypertrophic changes of the retinal pigment epithelium (RPE) underlying the central macula, accompanied by drusen formation beneath the RPE, and such changes are, typically at least, of little or moderate visual importance. Late AMD is sub-divided into atrophic and neovascular AMD (nv-AMD). The atrophic form causes degeneration and thinning of the RPE and choriocapillaris, weakening the RPE's capacity to nourish, and remove waste products from, the retina. Nv-AMD is characterised by the growth of abnormal blood vessels from the choroid, which penetrate Bruch's membrane and sometimes the RPE.[15] If left untreated, the leakage results in subretinal and/or retinal scarring, and associated photoreceptor damage with consequential and irreversible loss of central vision.[16] Nv- AMD can develop very rapidly relative to the atrophic form, which typically develops over months or years. Approximately 7% of people 75 years and older have progressed to the late stage of this disease.[17]

A global estimate has reported that 8.7% (approximately 14 million cases) of visual impairment is attributable to AMD.[18] It is estimated that the late form of the condition affects approximately 1.4 million individuals in the United States, 417,000 people in the United Kingdom and 70,000 people in the Republic of Ireland,[19] numbers which are likely to increase due to increasing longevity (Figure 1.2). Further, and as a result of a continued demographic shift towards an elderly population, the socio-economic implications of visually consequential AMD is becoming more important.[20]

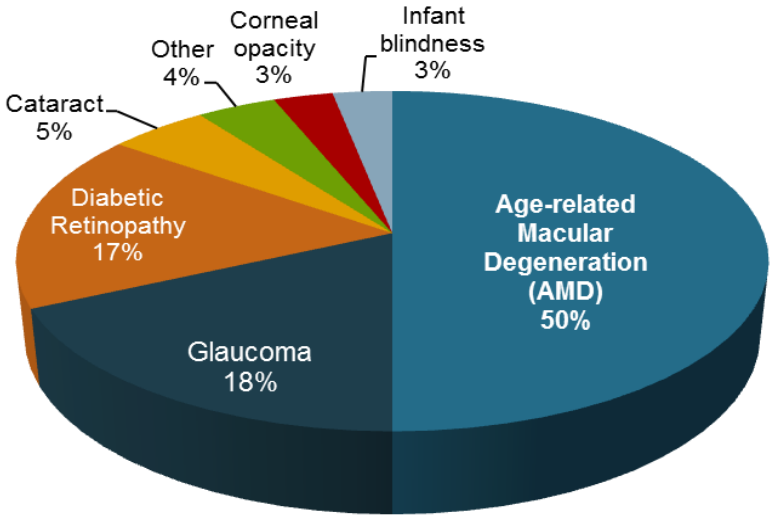


Figure 1.1 Distribution of the causes of blindness in the developed world. *(Data from the World Health Organisation: Magnitude and causes of visual impairment. Factsheet 282. November 2004; image courtesy of the Macular Pigment Research Group [MPRG], Waterford).*

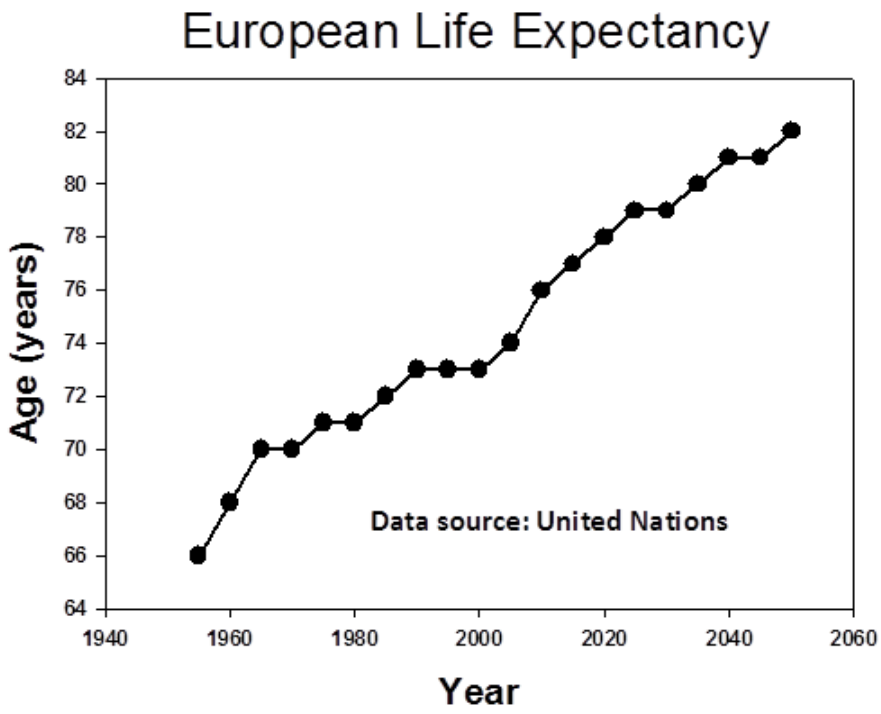


Figure 1.2 European life expectancy (1950 – 2050). *(Data from the United Nations, World Population prospects [2006] http://www.un.org/esa/population/publications/wpp2006/WPP2006_Highlights_rev.pdf ; figure adapted by the MPRG, Waterford*)

Intravitreal anti-VEGF therapy is now the most commonly used treatment for nv-AMD. Studies have conclusively demonstrated the morphological and visual benefits of treatment, yet anti-VEGF therapy has cost and logistical implications for the healthcare system and for the patient. In addition, there is, as yet, no effective treatment for atrophic AMD, which has similarly detrimental effects on a patient's quality of life.[21] One of the well-established risk factors for developing the late, more debilitating, forms of the condition is having early AMD.[22, 23] There is a clear and urgent need, therefore, to understand how the onset of this condition can be prevented, delayed or, at least, its progression retarded.

Macular pigment (MP), a yellow-coloured pigment located in the inner retinal layers of the macula, has generated interest in recent years because of its possible protective role for AMD, putatively (assumably) attributable to its antioxidant properties and/or its pre-receptoral filtration of damaging (short-wavelength) blue light, given that (photo)-oxidative retinal injury is known to be important in the pathogenesis of this condition.[24, 25] MP is composed of the two dietary carotenoids, lutein (L) and zeaxanthin (Z), and a third carotenoid, *meso*-zeaxanthin (MZ), which is not found in a typical diet.[26, 27] The anatomical (central retinal), biochemical (anti-oxidant) and optical (short-wavelength filtering) properties of MP have generated interest in the biologically plausible rationale that MP may confer protection against AMD.

A study design to conclusively prove MP's role in disease prevention would need to be at least fifteen years in duration, and would involve recruiting subjects who are not afflicted with the condition, and evaluating the incidence of AMD with respect to dietary intake of the carotenoids and with respect to MP optical density (MPOD). Such a study has yet to be undertaken, most likely due to the prohibitive cost and methodological difficulties inherent to the required study design. In the interim, the role of MP in reducing the risk of progression of the disease can be more readily

investigated. Evidence from a large scale clinical trial has shown that supplementation with dietary antioxidants can reduce the risk of progression from intermediate to late AMD by 25%.[28] Furthermore, there is now sufficient evidence to suggest that supplementation with the macular carotenoids can reduce the risk of disease progression, although conclusive evidence from a large scale randomised and placebo-controlled trial (RCT) is not yet available.

The influence of MP (and its augmentation) on visual performance has also been the focus of scientific investigation. The bulk of the experimental evidence supports the concept that MP has a functional influence on visual performance.[29-33] Supplementation with the macular carotenoids has been shown to be related to improvements in visual performance amongst subjects with and without AMD,[34-36] putatively through its ability to filter short-wavelength blue light, reducing the effects of chromatic aberration (CA) and light scatter, thereby enhancing contrast and reducing glare. A measure as crude as CDVA is unlikely to detect the changes in vision that might be attributable to MP carotenoid supplementation. Any improvement (or even stabilisation) in vision, however, has important implications for patients with or without macular disease. Considering the degenerative nature of AMD, it is important to assess visual performance as accurately and as comprehensively as possible, for the benefit of the patient, clinician and for the betterment of research.

In addition, the nature of the macular carotenoid formulation that maximises the visual benefit, if any, of MP at the macula has yet to be determined, i.e. the constituent carotenoids, individual carotenoid dosage.

This study was designed to look beyond CDVA in the assessment of visual performance, through a range of psychophysical methods, in subjects with AMD, in general. Also, this study investigates the effects on these visual performance parameters in subjects with nv-AMD and in subjects with early AMD undergoing anti-VEGF

therapy and macular carotenoid supplementation, respectively. This study has also sought to investigate the role of a supplement containing MZ (the third, and currently least explored, macular carotenoid) on the augmentation of MP, on visual performance and on disease progression, in subjects with early AMD.

Chapter 2. Age-related macular degeneration

Age-related macular degeneration (AMD) is a degenerative condition of the macula, a specialised area of the retina, responsible for central and colour vision.[37] Whilst peripheral (navigational) vision is maintained in cases of AMD (regardless of stage), it is central vision that is needed for seeing fine detail and for common daily tasks such as reading, driving and face recognition.[38-40] Therefore, the loss of central vision has a significant impact on an individual's independence and his/her quality of life.

The retina is a light-sensitive tissue at the back of the eye, which converts light images into electrical impulses, which are sent to the brain. The macula, the centre of which is the foveola, has a diameter of approximately 5mm at the posterior retina and, unlike the rest of the retina, has a particularly high density of cones. Typically, the macula has a characteristic yellow colour, which is usually detectable on fundoscopy. Its yellow colour attracted the attention of anatomists in the late 18th century, who later coined the term "*macula lutea*" or "yellow spot". It is now known that the yellow colouration is a characteristic of the highly concentrated presence of the macular carotenoids, collectively referred to as MP.

2.1 Classification of AMD

The International age-related maculopathy Epidemiological Study Group delineated the parameters of a core grading system to create a universal method with which to classify and define AMD for future clinical and epidemiological purposes.[41] "Age-related maculopathy" was the term used to define early stages of the condition and "AMD" was used to describe late, more advanced, stages of the disease, namely choroidal neovascularisation (CNV; a manifestation of nv-AMD), and geographic atrophy (GA).

For the purposes of this thesis, AMD will be used to define the condition in general, "early AMD" will be used to describe early manifestations of the disease and "late AMD" will be used to denote CNV and/or GA.

2.1.1 Early AMD

Early AMD is defined as a disorder of the macular area and is apparent clinically in subjects typically after the age of fifty. It is characterised by any of the following findings, when not associated with another disorder:

1. Soft drusen $\geq 63\mu m$ in diameter. Drusen are focal collections of extracellular material that lie external to the neurosensory retina and the RPE and appear as whitish-yellow spots on fundoscopy (Figure 2.1). Drusen may be soft and confluent, soft distinct, or soft indistinct. Hard drusen appear as small, round, discrete, yellow-white spots, while soft drusen are larger and have indistinct edges. Soft drusen may enlarge and coalesce (confluent drusen).[42] Hard drusen, alone, does not characterise AMD.
2. Hyperpigmentation (increased pigment) in the outer retina or choroid, associated with drusen (hard or soft).
3. Hypopigmentation (depigmentation) of the RPE, typically more sharply demarcated than drusen, without any visible choroidal vessels, associated with drusen (hard or soft). (See Figure 2.2 for hypo- and hyperpigmentation, in association with drusen).

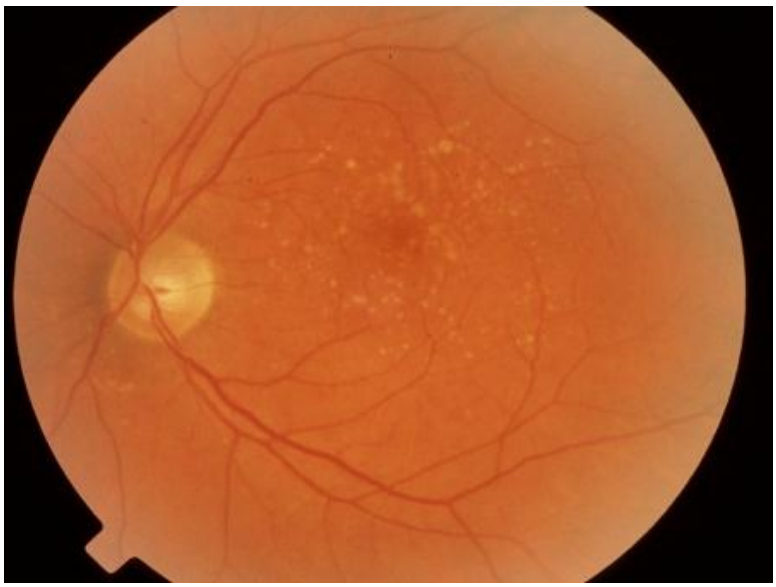


Figure 2.1 Macular drusen (hard and soft present). *(Image obtained from the Institute of Eye Surgery, Waterford).*

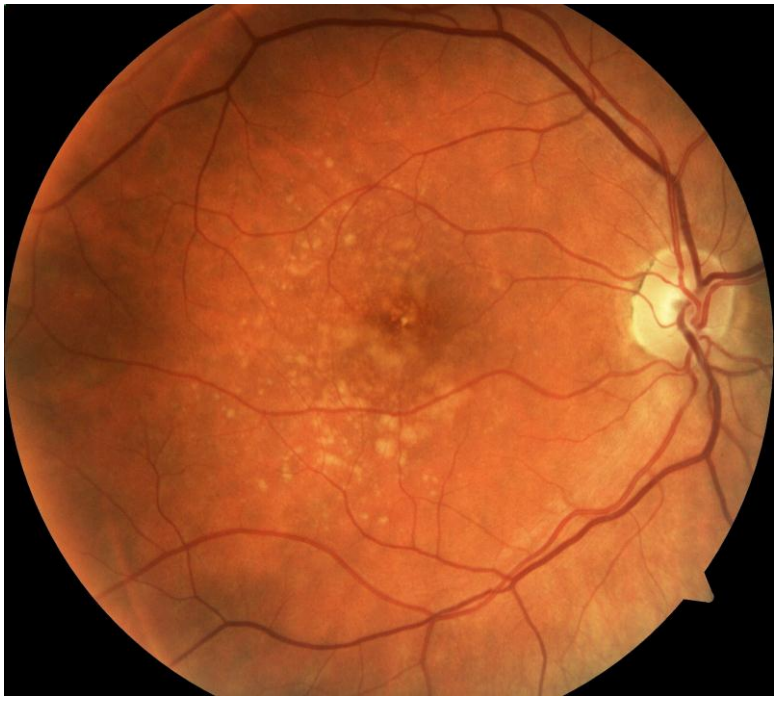


Figure 2.2 Macular drusen and pigmentary (hypo and hyper) changes *(Image obtained from the Institute of Eye Surgery, Waterford).*

2.1.2 Late AMD

The presence of early AMD predisposes the individual to late AMD, which is subdivided and defined as follows:

1. GA is described as any sharply delineated area of hypopigmentation or apparent absence of the RPE, in which the choroidal vasculature is more visible that the surrounding area. The area of atrophy must be ≥ 175μm in diameter (see example Figure 2.3).

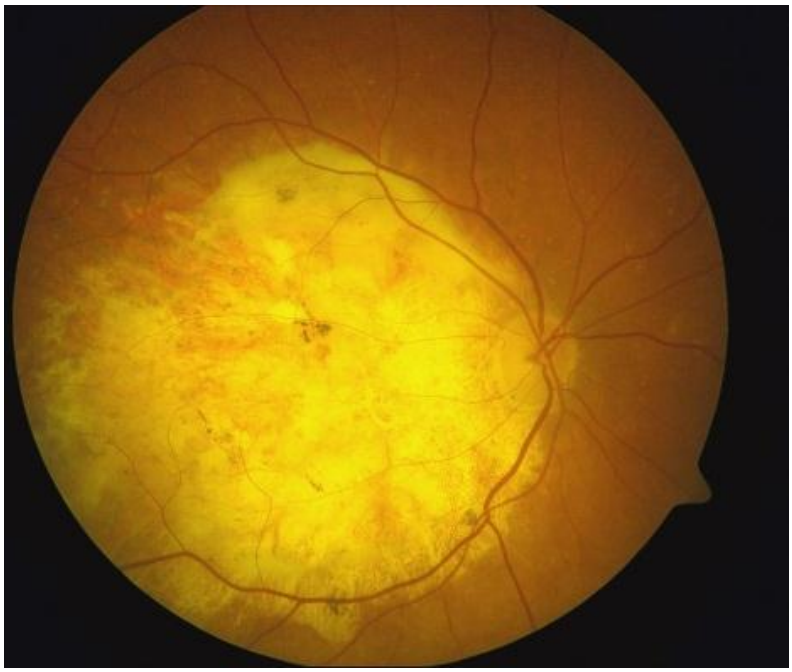


Figure 2.3 Geographic atrophy. *(Image obtained from the Institute of Eye Surgery, Waterford).*

2. Nv-AMD, also commonly referred to as "wet AMD" or "exudative AMD", is characterised by any of the following:
    a. RPE detachment(s), which may/may not be associated with neurosensory retinal detachment, associated with other signs of early AMD.
    b. Sub-retinal or sub-RPE neovascularisation.
    c. Epiretinal, intraretinal, subretinal, or sub-RPE glial tissue of fibrin-like deposits.
    d. Subretinal haemorrhage, unrelated to other retinal vascular disease (Figure 2.4).

e. Hard exudates (lipids) within the macular area, related to any of the above, in the absence of other retinal vascular disease.

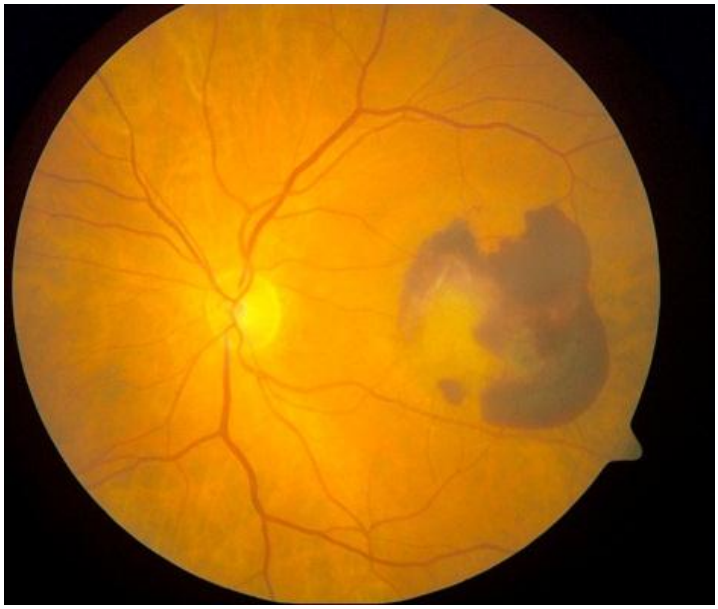


Figure 2.4 Neovascular AMD, showing subretinal haemorrhage. *(Image obtained from the Institute of Eye Surgery, Waterford).*

2.2 Aetiopathogenesis of AMD

2.2.1 Oxidative stress

As AMD is an age-related condition, the free radical theory of ageing is believed to be relevant to its aetiopathogenesis. This theory proposes that age-related disorders are the result of cumulative tissue damage following interaction with reactive oxygen intermediates (ROIs).[43, 44] ROIs, which include free radicals, hydrogen peroxide and singlet oxygen, are unstable by-products of oxygen metabolism. Free radicals, for example, lack an electron in their outer orbit (see Figure 2.5), and are therefore inherently unstable, causing them to scavenge an electron from another readily available source. The membranes of the photoreceptor outer segments have the highest concentration of polyunsaturated fatty acids (PUFAs) in the mammalian world.[45] These PUFAs are one such available source of electrons and are readily oxidised by ROIs, thus generating a cytotoxic chain reaction of events, thereby producing yet more ROIs

and further and consequential oxidative injury[46, 47] (in this case, impaired photoreceptor function and cell death).[48] The body's natural defence against ROIs includes their neutralisation by enzymes and/or antioxidants.[49] However, generation of ROIs increases in response to environmental stresses, such as atmospheric pollution, asbestos exposure, tobacco use, irradiation and alcohol consumption.[47, 50] Oxidative injury occurs, therefore, when the level of oxidants (ROIs) in a system exceeds the detoxifying capacity of its antioxidant defence system.[51]

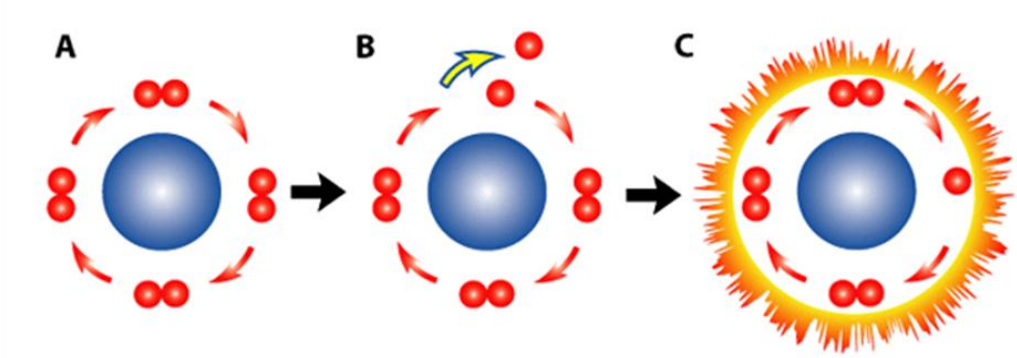


Figure 2.5 Schematic representation of the generation of a reactive oxygen intermediate (ROI). 'A' represents an atom in stable state (full array of electrons in its outer orbit); B shows the loss of an electron from the atom's outer orbit, thus generating a ROI (C), which is, as a result, unstable. *(Image obtained from http://www.health2know.com/2007-03/)*

The retina is made up of ten definable layers, nine of which are collectively termed the neurosensory retina (containing the photoreceptors and neuron axons), the remaining outermost layer being the dark, melanin-rich, RPE. The RPE plays an important role in facilitating visual function; not only does it nurture, and remove waste products from, the neurosensory retina,[52, 53] it also protects against photic injury through the absorbance of light-induced heat. The melanin pigment of the RPE also allows for the absorbance of scattered and excess light, thus providing optical benefits.[54]

AMD is characterised by loss of photoreceptors and by RPE cell dysfunction,[55] the latter being largely attributable to an age-related accumulation of lipofuscin (yellow-brown pigment granules representing lipid-containing residues of lysosomal digestion).[56] Of note, the accumulation of lipofuscin within the RPE cells increases as a result of incomplete digestion of oxidatively damaged photoreceptor outer segment membranes.[57] In turn, this yellow age pigment then acts as a chromophore (a compound which, when irradiated with light of an appropriate wavelength, emits an electron, thereby generating an ROI),[46, 58] thus provoking further oxidative injury.[57, 59]

The retina is an ideal tissue for the production of ROIs, because of its high oxygen demand and consumption, exposure to visible light, metabolic activities (such as RPE phagocytosis) and the presence of photosensitisers (chromophores).[60] In addition, the photoreceptor outer segments contain a high concentration of PUFAs, which are readily oxidised by ROIs, thus generating a cytotoxic chain reaction of events, thereby producing yet more ROIs and further and consequential oxidative injury.[46, 47]

Light of shorter wavelengths (blue, ultraviolet [UV]) has greater energy than that of longer wavelengths (e.g. red, yellow) and is, therefore, more injurious to retinal tissue.[61] In the human eye, the cornea and crystalline lens efficiently filter most of the UV light.[62] However, substantial amounts of damaging, high energy, short-wavelength (visible) light is incident upon the retina, even in an ambient setting.[63]

Damage to the RPE and to the photoreceptors by visible light was first demonstrated in 1966.[64] Later, it was shown that the short-wavelength component of the visible spectrum is most injurious.[65] Of note, it has also been demonstrated that such short-wavelength light induced photo-oxidative retinal damage is greater in the presence of high oxygen tension.[66] Lipofuscin also appears to play a decisive role in photo-oxidative stress in the retina, inducing the production of ROIs when irradiated

with short-wavelength light, as this pigment acts as a chromophore.[67] Indeed, and consistent with this, it has been shown that lipofuscin in RPE cells stimulates cell apoptosis when exposed to short wavelength visible light.[68, 69]

There is a growing consensus that cumulative lifetime exposure to visible light increases the risk of AMD,[70, 71] consistent with the aforementioned findings. AMD-like lesions have been demonstrated in laboratory rats reared in ambient levels of light, when compared with rats reared in the dark.[72] Subsequent investigators have demonstrated that the generation of AMD-like lesions in monkey retinas, following exposure to light of varying wavelengths, requires 70-1000 times less power when using short-wavelength light compared to infrared wavelengths.[73] Furthermore the administration of antioxidants to laboratory rats exposed to continuous illumination has been shown to confer protection against photoreceptor loss.[74] A recent analysis by the European Eye Study (n=4753) found a significant correlation between cumulative exposure to visible light and nv-AMD in those patients with low intake of dietary antioxidants, including L and Z.[75] There is, therefore, a compelling body of evidence to suggest that cumulative exposure to visible (short-wavelength) light is an important contributor to the development of AMD and that the mechanism of its contribution rests on the (photo)-oxidative injury that such short wavelengths of visible light inflict upon the retina.

Of interest, ROI production (and, therefore, oxidative injury) peaks at the macula,[76, 77] where, coincidently, MP peaks, and which is also the site where AMD manifests.

2.2.2 Inflammation

There is a consensus that inflammation also plays a role in the pathogenesis of AMD.[78, 79] Inflammation is part of the complex, biological, non-specific, immune response

of vascular tissue to harmful stimuli, such as pathogens, damaged cells, or irritants.[80] It is believed that inflammation within the retina is a precursor to the formation of drusen and the alteration of the extracellular matrix.[81, 82] These changes alter the RPE-choriocapillaris relationship, ultimately causing CNV and other manifestations of advanced AMD.[79, 83] Of note, drusen have been shown to contain proteins associated with immune-mediated response and inflammation.[83, 84] Indeed, histological studies have consistently demonstrated the presence of chronic inflammatory cells in retinas afflicted with AMD.[85, 86] It is believed that these inflammatory cells damage tissue by releasing proteolytic enzymes and oxidants, thus compounding the effects of oxidative stress.

The inflammatory hypothesis of AMD has generated a lot of interest, especially given the discovery that subjects with a certain gene variant, one which is closely connected to the mediation of inflammatory processes, are significantly more at risk of developing AMD.[87, 88]

It has been shown that oxidative damage-induced inflammation is the initiator of AMD.[89] The investigators demonstrated AMD-like lesions in mice immunized with carboxyethylpyrrole, a unique oxidation product of docosahexaenoic acid found in drusen from AMD donor eyes. As a result, immunized mice develop antibodies to this hapten, fix complement component-3, in Bruch's membrane (the site of drusen formation), accumulate drusen below the RPE during ageing, and develop atrophic changes within the RPE. It appears, therefore, that oxidative damage represents the trigger for the development of AMD, the pathogenesis of which is mediated by the inflammatory response to that insult, which in turn is determined by genetic background. It follows, therefore, that prevention or attenuation of the initial oxidative injury will reduce the risk of developing AMD, regardless of genetic background.[90]

2.3 AMD Risk Factors

The three undisputed risk factors for AMD are: ageing, genetic pre-disposition (positive family history of AMD) and tobacco use.

The free radical theory of ageing (discussed above) suggests that the observation that advancing age is a strong risk factor in the development of AMD, is attributable primarily to increasing levels of oxidative stress. Population-based studies have consistently shown that the prevalence, incidence and progression of AMD increase exponentially with advancing age.[91-93]

The prevalence of AMD among first-degree relatives of subjects with AMD, particularly those with nv-AMD, is greater than that of first-degree relatives of subjects without disease, suggesting a genetic component may contribute to the development of AMD.[94] A study compared patients with AMD (n = 457) with age- and sex-matched controls (n = 1071). Patients who carried the susceptibility alleles for either CFH (complement factor H) or LOC387715 were found to have a 3- to 8-fold increased risk for developing AMD.[95] A 50-fold increase in risk was reported in patients who had two copies of the susceptibility alleles in both genes. Tobacco use and obesity multiplied the risks associated with these variants.[96] This study (amongst others[97-99]) suggests that genetic predisposition to AMD is subject to environmental provocation.

Tobacco use is the third, and the only environmental (modifiable), established risk factor for AMD. Almost all epidemiological studies have shown that tobacco use is associated with increased incidence and prevalence of the condition, [100, 101] and has been confirmed by a number of meta-analyses.[102, 103]

Other possible risk factors, for which findings have been inconclusive thus far, include: obesity, female gender, previous cataract surgery, cardiovascular disease, Caucasian race, and lack of physical activity. There is a growing body of evidence that cumulative exposure to visible light, in association with a lack of dietary intake of key

antioxidants,[104] also represents an increased risk of AMD.[105] Of note, an inverse association has been shown between risk of AMD and the amounts of L and Z in the retina.[106]

Interestingly, the three established risk factors for AMD (age, genetic background and tobacco use) are associated with a relative lack of MP prior to disease onset.[107] Furthermore, obesity (a putative risk factor for AMD), which is known to be related to poor diet (and, therefore, consequential low serum and retinal carotenoid levels), is associated with increased oxidative stress and inflammation, and has also been shown to be inversely and significantly related to MP levels. Moreover, a recent study has identified that age and tobacco use are also associated with an atypical, and most likely undesirable, central dip in the spatial profile of MP,[108] which may be attributable to a relative lack of the macular carotenoid, MZ (this will be further discussed later).

2.4 AMD Treatment

2.4.1 Previous interventions for nv-AMD

Nv-AMD is the only form of AMD for which a proven treatment is available. Until recently, the available therapeutic interventions for nv-AMD were largely aimed at the preservation of the presenting visual acuity (VA). These included: laser photocoagulation, photodynamic therapy and submacular surgery, and are summarised below.

Laser photocoagulation: Laser was the first treatment introduced to retard the progression of nv-AMD and is still used in some cases of well-defined extrafoveal CNV. With the advent of newer therapies, however, and the concern for the impact of

iatrogenic scotoma in subfoveal CNV, laser photocoagulation of peri- and subfoveal CNV is no longer recommended.[109]

Photodynamic therapy (PDT): Choroidal neovascular tissue contains a high concentration of low-density lipo-protein (LDL) receptors. Verteporfin, (Visudyne®, Novartis, Basel, Switzerland) when infused intravenously complexes with LDLs and accumulates in CNV membranes. Non-thermal laser activation (689nm) of verteporfin induces endothelial damage with thrombus formation via ROI formation, without damage to the overlying retina.[110, 111] PDT has been effective in reducing the rate of vision loss in patients with subfoveal predominantly classic CNV due to AMD.[112] However, the VIO (Visudyne In Occult CNV) study failed to show any benefit in cases of subfoveal occult CNV.[113]

Surgery: Subretinal removal of CNV by pars plana vitrectomy has been a proposed treatment for nv-AMD. The Submacular Surgery Trial[114] found that submacular surgery was not a superior method to (less invasive) laser photocoagulation of subfoveal CNV and was abandoned with the advent of PDT and, later, anti-angiogenic therapies. The poor results of this particular study are attributed to the collateral damage to the RPE, responsible for the nutritional supply of the overlying macula.[115] Another surgical method, macular translocation surgery, has also been used in cases of nv-AMD. The procedure involves relocating the fovea to an area where the RPE is healthier than it is centrally, the aim allowing for the recovery of some useful vision.[116]

2.4.2 Anti-VEGF therapy

Current treatment interventions, specifically anti-VEGF agents, have resulted in better visual outcomes for patients with nv-AMD, providing, for the first time, a relatively strong probability of visual gain amongst patients with the condition.

VEGF, a signal protein (cytokine), is an important regulator of vascular permeability and angiogenesis.[117, 118] The functional role of VEGF is to stimulate new blood vessel growth, for example, during embryonic development, after injury, or to by-pass blocked blood vessels. The production of VEGF significantly increases in hypoxic conditions, and VEGF also has a role in tumour-angiogenesis and in other ischaemic and inflammatory conditions.[119]

VEGF encompasses a family of proteins, which include: PGF (Placenta Growth Factor), VEGF-A, VEGF-B, VEGF-C, VEGF-D and the viral and snake venom homologues, VEGF-E and F, respectively. VEGF-A is most relevant for angiogenesis and vascular permeability. Nine human VEGF-A isoforms have been identified to date, with varying numbers of amino acids: $VEGF_{121}$, $VEGF_{145}$, $VEGF_{148}$, $VEGF_{162}$, $VEGF_{165b5}$, $VEGF_{165}$, $VEGF_{183}$, $VEGF_{189}$ and $VEGF_{206}$. $VEGF_{165}$ is the most abundant isoform.[120, 121]

VEGF functions by binding to one of three VEGF receptors (VEGFR-1, -2 and -3) on the cell surface. VEGFR-2 appears to be the receptor that mediates the major signalling effects of VEGF-A (angiogenesis and vascular permeability).[122] The function of VEGFR-1 is less clear, although evidence suggests that it functions as a "decoy" receptor, thus regulating the activity of VEGF-A by making it less available for binding on VEGFR-2. VEGFR-3, on the other hand, does not bind VEGF-A, and is instead, a receptor for VEGF-C and -D.

The two most important forms of ocular angiogenesis are preretinal angiogenesis (originating from the retinal vasculature), and subretinal (choroidal) angiogenesis. Preretinal angiogensis is associated with capillary non-perfusion and neuroretinal ischaemia, which stimulate the growth of new blood vessel along the retina-vitreous interface, which can potentially haemorrhage, obscure vision and, in addition, increase the risk of retinal detachment. Severe retinal ischaemia can cause new

blood vessel formation on the iris, which can block the trabecular meshwork in the anterior chamber angle, resulting in neovascular glaucoma. Preretinal angiogenesis is an uncommon manifestation of diabetic retinopathy and retinal vein occlusions.[123, 124] Subretinal angiogenesis (CNV) is characterised by the growth of abnormal choroidal blood vessels, which penetrate Bruch's membrane and sometimes the RPE,[15] and is classified according to its appearance on fluorescein angiography. Classic CNV is located between the RPE and the neural retina, whereas occult CNV occurs between the RPE and Bruch's membrane. Mixed CNV also exists. If left untreated, the leakage results in subretinal and/or retinal scarring, with consequential and irreversible loss of central vision.[16] CNV is a manifestation of nv-AMD as well as other, less common, degenerative retinal conditions such as myopic maculopathy and angioid streaks.

It has been shown that VEGF-A is secreted basally by the RPE towards the choriocapillaris, influencing the permeability of its fenestrated capillaries. The secretion of VEGF-A increases 10-fold in hypoxic conditions. In addition, VEGF-2 receptors were preferentially located at the choriocapillaris endothelium facing the RPE, which is suggestive of a paracrine relationship between the RPE and the choriocapillaris endothelium. An imbalance in this relationship, which potentially produces an increase in VEGF production, or an increase in VEGF secretion simply due to hypoxic conditions, are possible contributing factors in the pathogenesis of CNV.[125]

2.4.2.1 The history of anti-VEGF therapy for nv-AMD

In 1948, Michaelson suggested that the avascular foetal retina induces vascular ingrowth by the release of a diffusible "metabolic" factor, one which also may play a role in vascular-related retinal disease, such as diabetic retinopathy.[126] In 1954, Ashton was the first to hypothesise that this factor "X" is stimulated by hypoxia.[127] It was not until 1983 that VEGF-A was discovered as a protein,[128] although its angiogenic properties were not perceived at this time. It was given the name, Vascular Permeability

Factor (VPF), in light of its discovered ability to influence vascular permeability. Ferrara et al, in 1992, described VEGF's major angiogenic role.[129] Also, in 1992, and thirty-eight years on, Ashton's original hypothesis, that VEGF is, indeed, induced by hypoxia, was proven.[130]

Further research in animal models demonstrated that the inhibition of VEGF-A prevents the development of CNV, causes regression of existing CNV, reduces pathological vascular permeability and prevents the development of iris neovascularisation due to retinal ischemia.[131-133] Important studies were also able to show that VEGF-A levels are elevated in the vitreous of subjects with nv-AMD, as well as in excised CNV membranes.[134, 135]

These provocative findings led to the design and the execution of clinical trials for the purpose of investigating the effects of anti-VEGF agents in subjects with vascular-angiogenic ocular disease.

2.4.2.2 Ocular anti-VEGF agents

Pegaptanib (Macugen®; OSI/Eyetech Pharmaceuticals, New York)

Pegaptanib is an oligonucleotide that binds and inactivates some, but not all, VEGF-A isoforms. The VISION trial, a randomised, multicentre study, investigated the effect of intravitreal pegaptanib (once every six weeks) in 1186 subjects with nv-AMD over a 54-week period. The study demonstrated that pegaptanib could reduce the rate of visual loss in patients with subfoveal nv-AMD. Seventy percent of subjects receiving 0.3mg pegaptanib lost fewer than 15 letters of VA over the study period, compared with 55% of subjects receiving sham treatment.[136] However, there was no statistically significant improvement in vision between the treated and sham groups and 1% of patients developed endophthalmitis. Other significant side-effects included retinal detachment and traumatic cataract.[136]

Bevacizumab (Avastin®; Genentech, South San Francisco, California)

Bevacizumab is a humanised full-size antibody that inactivates all isoforms of VEGF-A, and has been approved for the treatment of metastatic colorectal cancer.[137] Off-label systemically administered bevacizumab was studied in an uncontrolled open label trial in 18 patients with CNV attributable to AMD. VA improved in the study eyes within the first two weeks of treatment, and by 24 weeks, mean VA increased by 14 letters (and was accompanied by a decrease in mean central retinal thickness, determined by optical coherence tomography [OCT]). Complications included significant elevations in blood pressure in several patients.[138] Subsequently, intravitreal bevacizumab was administered with similar visual outcomes and a very low rate of ocular and systemic side-effects.[139-147] There is a paucity of RCTs investigating the role of intravitreal bevacizumab for AMD. One study showed that 1.25 mg of intravitreal bevacizumab, given as part of a six-weekly variable retreatment regimen, was superior to standard care (intravitreal pegaptanib or verteporfin therapy, depending on lesion type), with low rates of serious ocular adverse events.[148] Mean VA increased by seven letters (from baseline) in the bevacizumab group compared with a decrease of 9.4 letters in the standard care group ($p<0.001$) over the 54 week study period.

Ranibizumab (Lucentis®; Genentech, South San Francisco, California)

Ranibizumab is a humanised antigen-binding fragment of bevacizumab that has a strong affinity for all VEGF-A isoforms. The testing of intravitreal ranibizumab in a number of large scale clinical trials provided an important breakthrough in the treatment of nv-AMD.

2.4.2.3 Ranibizumab trials

The MARINA study (Minimally Classic/Occult Trial of the Anti-VEGF Antibody Ranibizumab in the Treatment of Neovascular AMD)[149]

The MARINA trial was a randomised, double-blind, controlled, multicentre phase III clinical trial, which investigated the response of patients with nv-AMD (either minimally classic or occult CNV) to ranibizumab. Seven-hundred and sixteen patients were randomly assigned to receive either 0.3mg ranibizumab, 0.5mg ranibizumab or sham injections every month for a period of two years. Results at 24 months were as follows:

- 90% of the 0.5mg ranibizumab-treated patients lost fewer than 15 letters compared with 53% in control-group ($p<0.001$).
- 33.3% and 26.1% of patients being treated with 0.5mg and 0.3mg ranibizumab, respectively, gained at least 15 letters of VA, whereas 3.8% had such gains in the control-group ($p<0.001$).
- Mean VA at 24 months increased by 6.6 lines amongst those receiving 0.5mg ranibizumab, compared to a decrease of 14.9 lines in the sham-injection group ($p<0.001$) (Figure 2.6).
- With similar baseline measurements, approximately 40% of patients treated with ranibizumab achieved VA of 20/40 (6/12), compared to 11% in the sham group ($p<0.001$).
- Approximately 12% of patients in the ranibizumab groups had vision of 20/200 (6/60; equivalent to legal blindness) or less at 24 months, compared to 43% in the control group ($p<0.001$); both groups were comparable in this respect at baseline.

- The area of the CNV lesion in the sham group increased by an average of 2.5 disc diameters in the control group and showed no change in the ranibizumab groups, over the course of 24 months ($p<0.001$).
- 1% of subjects (n=5) being treated with ranibizumab developed presumed endopthalmitis over the course of the study and 1.3% developed uveitis. The overall incidence of any serious or non-serious systemic adverse event was similar among the groups.

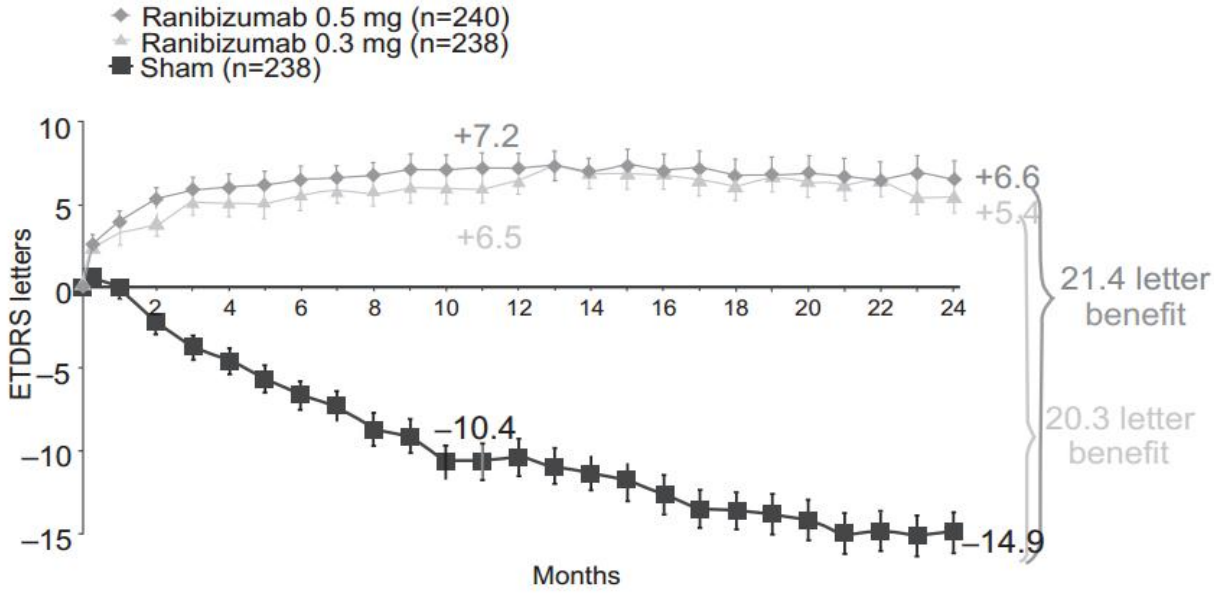


Figure 2.6 MARINA: mean change in visual acuity from baseline over time; from Rosenfeld et al.[149]

The ANCHOR Study (Anti-VEGF antibody for the treatment of predominantly classic choroidal neovascularisation in AMD)[150]

The ANCHOR trial, another randomised, double-blind, controlled, multicentre phase III clincial trial, assessed the effect of ranibizumab in patients with predominantly classic CNV. Four-hundred and thirty-two patients were randomly selected to receive PDT with verteporfin every three months plus a monthly sham injection, or sham PDT

combined with either 0.3 mg or 0.5 mg ranibizumab. The results after 24 months were as follows:

- 90% of both ranibizumab groups lost less than 15 letters compared with 65% in PDT group ($p<0.001$).
- 41% of those treated with 0.5 mg ranibizumab and 34% treated with 0.3 mg ranibizumab gained at least 15 letters, compared with 6% in PDT group ($p<0.001$) (Figure 2.7)
- Mean VA increased by 11.3 letters in the 0.5 mg ranibizumab-treated/sham PDT group whereas it decreased by 10.4 letters in the PDT/sham injection group ($p<0.001$).
- Changes in lesion anatomic characteristics on fundus fluorescein angiography (FFA) favoured ranibizumab (all comparisons $p<0.0001$ vs. PDT).
- Similar to the MARINA trial, the risk of presumed endopthalmitis was 1% amongst the ranibizumab-treated subjects and there was no imbalance among the groups in terms of rates of serious ocular and non-ocular adverse events.

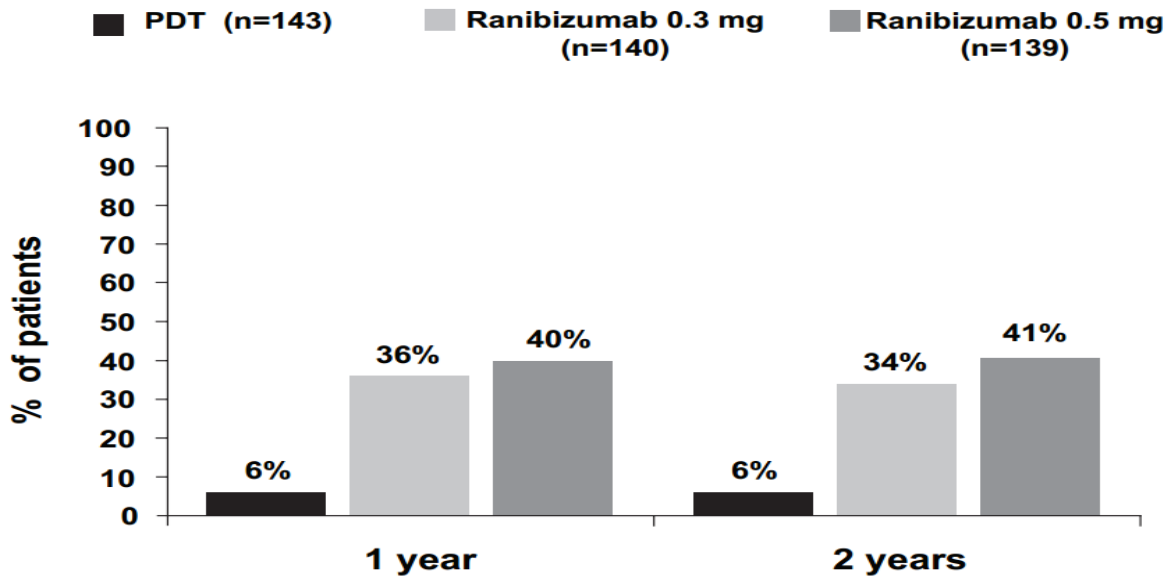


Figure 2.7 ANCHOR: Gain ≥15 letters after 1 and 2 years; from Brown et al.[150]

There was no significant evidence of toxicity with either dose of ranibizumab in either of the trials. It was suggested that the 0.5 mg dose was superior and has now been approved for intravitreal use in patients with nv-AMD.

The PIER study[151]

The PIER study was designed to evaluate the efficacy and safety of ranibizumab using a less frequent injection schedule than that used in MARINA and ANCHOR. One hundred and eighty-four patients were randomised to receive 0.3 mg ranibizumab, 0.5 ranibizumab or a sham injection every month for three months, followed by injections every three months. After one year, there was an observed difference, in terms of VA, between the ranibizumab-treated patients and those in the sham group:

- Mean change in VA between baseline and 12 months were -16.3, -1.6, and -0.2 letters for the sham, 0.3 mg, and 0.5 mg groups, respectively ($p \leq 0.0001$, each ranibizumab dose vs. sham) (Figure 2.8).
- Ranibizumab arrested CNV growth and reduced leakage from CNV compared to sham treatement ($p < 0.001$)

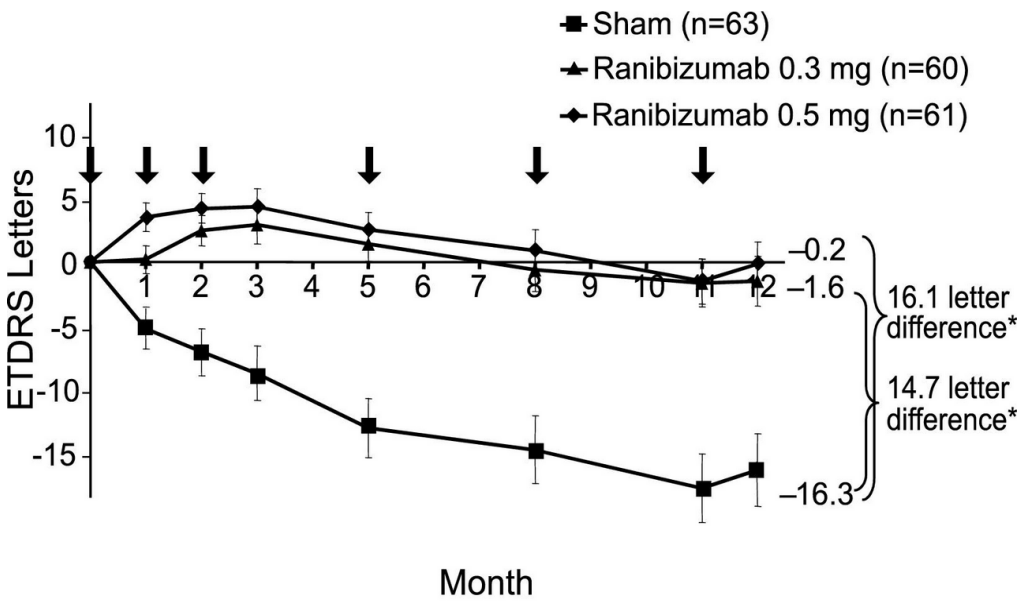


Figure 2.8 PIER: Mean change from baseline visual acuity at monthly intervals; from Regillo et al.[152]

However, in the ranibizumab groups, the treatment effect declined during quarterly dosing and the results 12 months showed poorer outcomes compared to those observed in the MARINA and ANCHOR trials for the same time period, which used monthly dosing. Between month 12 and month 24, changes in protocol meant that sham-injection patients crossed over to receive 0.5 mg ranibizumab quarterly after completing the month-12 visit. Subsequently, and in light of the 12-month PIER data, the protocol was further amended and all patients remaining in the study rolled over to receive 0.5 mg ranibizumab monthly for the remainder of the 2-year study. At month 24:

- VA decreased an average of 21.4, 2.2, and 2.3 letters from baseline in the sham, 0.3mg, and 0.5mg groups ($p<0.0001$ for each ranibizumab group vs. sham).
- VA of sham patients, who crossed over (and subsequently rolled over) to ranibizumab, decreased over time, with an average loss of 3.5 letters 10 months after crossover. This reduction in VA, in spite of the initiation of

treatment, suggests that ranibizumab has limited benefit following 12 months without treatment, further emphasising the importance of timely intervention.

- VA of 0.3 mg and 0.5 mg group patients who rolled over to monthly ranibizumab increased for an average gain of 2.2 and 4.1 letters, respectively, 4 months after rollover.
- The ocular safety profile of ranibizumab was favorable and consistent with previous reports, with no events of endophthalmitis or serious intraocular inflammation.

After 12 months, subjects in the treatment groups who rolled over to receive monthly ranibizumab, had further increases in VA, suggesting that more effective outcomes are obtained with a more frequent treatment regimen.

The PrONTO study (Prospective Optical Coherence Tomography Imaging of Patients with Neovascular Age-Related Macular Degeneration (AMD) Treated with intraOcular Ranibizumab)[153]

PrONTO investigated the effect of a variable-dosing regimen based on OCT findings and other clinical outcomes. Forty patients received three monthly injections of 0.5 mg ranibizumab and further injections thereafter, depending on the presence of defined criteria, as follows. During the first year, retreatment with ranibizumab was performed at each monthly visit if any of the following criteria were met: an increase central retinal thickness, as observed by OCT, of at least 100µm, or a loss of five letters or more on the acuity chart. The retreatment criteria were amended in the second year of the study to include any qualitative increase in the fluid detected using OCT.

- At month 24, mean VA improved from baseline by 11.1 letters ($p<0.001$).
- Central retinal thickness decreased by 212µ ($p<0.001$).
- VA improved by 15 letters or more in 43% of patients.

In brief, visual outcomes were similar to those acheived in MARINA and ANCHOR and were achieved with an average of 9.9 injections over 24 months. In other words, fewer intravitreal injections were required.

Other ranibizumab studies

The HORIZON trial was an open-label extension trial (of MARINA and ANCHOR) in which re-injections of ranibizumab were given at the clinician's discretion.[154] Half of the patients required re-injections within the first six months and the authors report that multiple ranibizumab injections were well tolerated for ≥ 4 years.

The EXCITE trial (Efficacy and safety of monthly versus quarterly ranibizumab treatment in neovascular age-related macular degeneration) compared 0.3mg quarterly, 0.5mg quarterly, and 0.3mg monthly ranibizumab.[155] Treatment consisted of a 3-month loading phase, followed by a 9-month maintenence phase (injection frequency depending on group). VA increased from baseline to month 12 by 4.9, 3.8, and 8.3 letters in the 0.3 mg quarterly (n=104), 0.5 mg quarterly (n=88), and 0.3 mg monthly (n=101) dosing groups, respectively, confirming the superiority of a monthly dosing regimen. The safety profile was similar to that reported in prior ranibizumab studies.

The SUSTAIN trial (Safety and efficacy of a flexible dosing regimen of ranibizumab in neovascular age-related macular degeneration), undertaken on 513 ranibizumab-naive subjects, investigated the efficacy of three initial monthly injections of ranibizumab (0.3 mg) followed by pro re nata (PRN) retreatment for the remaining nine months of the study.[156] Retreatment was based on the following pre-specified criteria: a) more than

five-letter loss in VA from the previous highest VA score during the first three months; or b) 100µm increase in central retinal thickness from the previous lowest measurement during the first three months. The average number of re-treatments from months three to 11 was 2.7. Mean VA increased steadily from baseline to month three (reaching +5.8 letters), decreased slightly from month three to six, and remained stable from month six to 12, reaching +3.6 at month 12. Central retinal thickness showed a rapid and significant decline in the first three months, which was maintained over the 12-month study period. The safety results were comparable to those observed in the previous clinical studies.

2.4.2.4 Bevacizumab versus Ranibizumab

The recently published 24-month results from CATT (Comparisons of Age-Related Macular Degeneration Treatments Trial) compared the effiacy and safety of bevacizumab and ranibizumab for nv-AMD.[157] Subjects were randomised to one of four groups: ranibizumab or bevacizumab, given either every month or as needed (*pro re nata*; PRN), with monthly review. In brief, the study showed that ranibizumab and bevacizumab had similar effects on VA over a 2-year period (p=0.21). Mean gain in VA was greater for monthly rather than for as-needed treatment (difference, -2.4 letters; p=0.046). There were no differences between drugs in terms of rates of death or arteriothrombotic events (p>0.6). However, the proportion of patients with one or more systemic serious adverse events was higher with bevacizumab than ranibizumab (39.9% vs. 31.7%; p=0.009).

The IVAN trial (A randomised controlled trial of alternative treatments to Inhibit VEGF in Age-related choroidal Neovascularisation) also compared the efficacy and safety of ranibizumab and bevacizumab in cases of nv-AMD, with the same randomisation protocol as CATT (above). Differences in VA at 12 months

between bevacizumab and ranibizumab were found to be inconclusive. In contrast to CATT, VA outcomes did not differ between monthly and as-needed treatment protocols. Drugs and treatment regimens were deemed to have similar efficacy and safety ($p=0.25$). Bevacizumab was less costly for both treatment regimens ($p<0.0001$). A recent safety review and meta-analyses of bevacizumab and ranibizumab has raised concern regarding the potentially increased risk of ocular and multiple systemic adverse effects with bevacizumab.[158] The authors also emphasised the need for studies that are sufficiently powered for safety outcomes, not just for efficacy.

2.5 Conclusion

Anti-VEGF therapy may even be termed "revolutionary" in terms of its capability to preserve and, indeed, improve vision for subjects with nv-AMD, who, if left untreated, would ultimately develop a disciform scar, leading to the irreversible loss of central vision. Yet, the current licensed form of anti-VEGF therapy is costly and cumbersome to the healthcare provider and to the patient. For example, in Ireland, the cost to the healthcare system of a year's treatment for one eye with (monthly) intravitreal injections of Lucentis® (ranibizumab) is in the range of €24,000. In addition, patients and their carers have to travel at least once a month (for injections and post-operative assessments), which is taxing on their time and finances e.g. travel costs, time off work. There is also no effective treatment for atrophic AMD, which has a similarly detrimental effect on a patient's quality of life. The increasing prevalence of AMD, and its associated consequences for the patient and the healthcare system, highlight the clear need for attention to be directed towards the prevention of AMD and its progression.

Chapter 3. Psychophysical assessment of visual performance in subjects with AMD

3.1 Introduction

Psychophysics quantitatively investigates the relationship between physical stimuli and the sensations and perceptions they affect in the observer. Psychophysics, described by Gescheider as *"the scientific study of the relation between stimulus and sensation"*,[159] provides valuable information about the functional status of the visual system, which includes the status of the retina, the visual pathways and the visual cortex. Psychophysical assessment can, therefore, reflect, compliment, and even inform physiological assessment.

AMD is the advanced stage of a degenerative process that occurs in all eyes. The presence of excessive lipofuscin in RPE cells (typically increases with age) is associated with RPE dysfunction and also with drusen formation (between Bruch's membrane and the RPE), causing further RPE damage. Loss of vision occurs due to the degeneration of RPE cells, which no longer facilitate the absorbance of excess light necessary for optimal visual function, nor provide nourishment to the overlying photoreceptors, leading to photoreceptor cell death. Visual loss can also be caused by leakage from neovascular membranes that invade the retina, disrupting its normal architecture, and which, if left untreated, results in scar formation and irreversible visual loss. Considering the delicate and precise nature of visual perception (in particular, that of central vision), and the fact that the optimum perception of an image relies on the intactness and health of a complete array of photoreceptors at the macula, a reduction in psychophysical function would, therefore, be expected in the presence of AMD.

An excellent review summarises psychophysical function in AMD;[160] (a) Spatial vision refers to our ability to resolve or discriminate spatially defined features and includes: (high contrast) VA, hyperacuity, reading speed and CS. (b) Visual field testing (perimetry): the systematic measurement of differential light sensitivity at topographically defined loci in the visual field. (c) Temporal vision represents how we perceive changes in luminance over time, e.g. the response of an eye to a flickering stimulus. (d) Visual adaptation describes the processes by which the visual system alters its functioning in response to changes in the environment. The human eye can function over a remarkably wide range of luminances (a range of greater than eight log units). The integrity of these visual processes can be assessed using tests, such as dark adaptation of both rods and cones and, also, the photostress recovery test. (e) Chromatic function (colour vision) represents the ability to discriminate between stimuli that differ with respect to their spectral composition, regardless of other parameters, such as intensity.

The assessment of psychophysical function is largely based on the concept of threshold testing, the threshold being defined as the point of intensity at which the presence of a stimulus, or the difference between two stimuli, is either just detectable or just undetectable. There are two types of thresholds, absolute thresholds and difference thresholds. An absolute threshold is the level of intensity of a stimulus at which it can be detected. A difference threshold is the magnitude of the smallest detectable difference between two stimuli of differing intensities. Humans, however, are not perfect observers, rendering the acquisition of precise thresholds challenging. The most common means of assessing thresholds are: the method of adjustment, the method of limits, the modified (staircase) methods of limits, and the method of constant stimuli.

For the purpose of this publication, I will review those measures of psychophysical function relevant to studies carried out as part of this PhD, and

comment on some of the studies germane to the impact of AMD on these particular measures of visual performance, where available.

3.2 Corrected distance visual acuity

Currently, CDVA a measure of the angular resolution limits of the eye at high contrast i.e. the smallest discernible black letter on a white chart,[2] represents the standard vision-related outcome measure for the management of AMD (and for vision in general, in subjects with and without ocular disease).[14] The inherent weaknesses of the 150 year-old, and still widely used, Snellen chart have been largely overcome with the introduction of the Bailey-Lovie and ETDRS (Early Treatment Diabetic Retinopathy Study) logMAR charts, which allows for a more standardised measure of high contrast acuity. These alterations, which are the novel features of the logMAR chart, include: equal numbers of letters per line, equal (logarithmic) graduations from line to line, the use of letters of equal legibility and the uniformity between-letter and between-row spacing.[161] However, and in spite of these important advances, there is a general consensus that CDVA is not a true reflection of daily visual experience in a world with few visual stimuli at such high levels of contrast, suggesting that perhaps other measures of visual function may be more appropriate in assessing visual performance and experience in patients with AMD.[162]

CDVA and AMD: Lesions associated with early AMD are associated with a decrease in CDVA of up to two letters or fewer when compared with eyes without such lesions,[163] which is neither clinically meaningful nor reliably detectable, considering that the test-retest variability can be up to two lines of letters on a logMAR chart.[164] Also, a report has shown no statistical difference in acuity between subjects with nv-AMD and subjects with GA.[163] CDVA is, therefore, unlikely to be a sensitive

psychophysical measure with the capacity to (a) detect the presence of early AMD, (b) detect change in visual function e.g. following intervention, or (c) provide a useful prognostic indicator of disease progression. Late AMD is associated with a more significant decrease in CDVA (approximately seven lines of letters), but only when signs of advanced AMD involved the central subfields of the macula.[163] CDVA is a poor indicator of disease severity. Acuity levels can vary dramatically despite similar areas of atrophy, although foveal involvement is a key predictor of VA.[165] Similarly, lesion size in subfoveal nv-AMD cannot explain the wide variations in VA.[166] Another study has shown that for the same level of VA, eyes with GA have worse function, particularly for dark-adapted vision tests and reading speed, than eyes with drusen (≥ 6/15).[167]

CDVA in response to anti-VEGF therapy: The large scale clinical trials (discussed in section 2.4.2.3) have demonstrated that CDVA improved significantly following ranibizumab therapy. Optimum visual outcomes are observed with monthly or criteria-based dosing regimens, compared to less-frequent dosing schedules.

CDVA in response to macular carotenoid supplementation: Various studies have also reported improvements in CDVA following macular carotenoid supplementation, in both normal and AMD-afflicted subjects.[168-171] However, there have been studies that have not reported such changes[35, 172] whilst reporting improvements in other parameters of visual function. It is generally accepted that CDVA is not a sensitive enough measure to detect subtle (but yet important) changes in visual performance. In this respect, other measures of psychophysical visual function, that might better reflect the functional status of the macula, should be considered in assessment and management of subjects with AMD.

3.3 Contrast sensitivity

Contrast threshold is the least amount of contrast required for an observer to discern a target, and is typically expressed as CS, its reciprocal. A graph, plotting CS as a function of spatial frequency, is known as the CS function (CSF) curve, which represents the minimum contrast required to detect a grating at varying spatial frequencies. In fact, losses in CS are associated with much smaller losses of VA (spatial resolution).[173] In normal subjects, under normal (photopic) viewing conditions, CS function peaks at 3-6 cycles per degree (cpd), with a steep reduction in CS approaching higher spatial frequencies and a more gradual decline in CS towards lower spatial frequencies.[174] A review has concluded that CS is an important measure of visual function in patients with AMD,[175] based on studies that have shown that, when compared with VA, CS better relates to the ability to perform tasks accurately and efficiently (including computer task accuracy),[176] to discriminate between objects[177] and to judge distances.[178]

CS and AMD: The aforementioned review (on psychophysical function), concluded that the available data indicates an observed loss in CS across all spatial frequencies in subjects with AMD. In normal subjects, reduction in CS is typically related to a reduction in CDVA. Importantly, however, patients with AMD may present with good CS (at low spatial frequencies) and poor CDVA (or vice versa),[179] suggesting that CDVA alone cannot account for the visual experience in subjects with AMD. In fact, a significant association has been observed between the loss of CS at high spatial frequencies and a number of AMD lesions including drusen confluence, focal

hyperpigmentation of the RPE, and RPE atrophy, whereas lesion size/type (in cases of late AMD) was not associated with CDVA (previously discussed).[180]

CS in response to anti-VEGF therapy: In light of the usefulness of CS as a measure of visual function in AMD, it is interesting to note that there is a paucity of studies that have investigated the impact of anti-VEGF therapy on CS, in cases of nv-AMD. An unpublished report reviewing the three large scale, Phase III clinical trials, namely, MARINA, ANCHOR and PIER, showed that, from the total of 1,323 enrolled participants in these studies, there was a significant improvement in CS at 12 months for all CNV lesion types, following intravitreal ranibizumab (0.3mg or 0.5mg).[181] Another much smaller-scaled study of three subjects with AMD, reported improvement in CS in one and stabilisation in two (subjects), following three consecutive ranibizumab injections.[182] The IVAN trial reported improvements in CS in subjects being treated with either intravitreal ranibizumab or intravitreal bevacizumab (with no significant difference reported between the two drugs).[183] Another study, investigating the impact of one injection of intravitreal ranibizumab, in combination with one PDT treatment, observed an improvement in CS in approximately 82% of subjects (n =17).[184] Also, improvements in CS scores were observed following one year of either intravitreal ranibizumab or intravitreal bevacizumab in eyes with CNV due to myopic maculopathy.[185]

CS in response to macular carotenoid supplementation: A statistically significant improvement in contrast acuity thresholds (the contrast threshold needed to detect and correctly identify the orientation of the gap in a Landolt ring) has been reported in normal subjects supplemented with L under mesopic conditions.[186] The MOST Vision trial (normal subjects; discussed in detail in section 4.4.3.2) also reported improvements

in CS following supplementation with a formulation containing all three macular carotenoids, an improvement which was not observed either amongst subjects taking placebo, or amongst subjects taking a supplement containing L primarily.[170] In 2004, the LAST study (Lutein Antioxidant Supplementation Trial) was carried out in an attempt to evaluate the effect of L, either alone or in combination with co-antioxidants, vitamins and minerals, on the progression of atrophic AMD.[187] In brief, results showed that the subjects taking MP carotenoid supplements (whether alone or in combination) demonstrated an improvement in VA, CS, glare recovery and visual distortion.

3.4 Glare disability

Glare can be categorized as (a) discomfort glare: the discomfort caused when the overall illumination is greater than the luminance to which the eyes are adapted, and can be caused by both direct and indirect light sources e.g. car headlights at night, reflections from water surfaces, snow, and (b) glare disability (GD): causes a reduction in target visibility against its background. Discomfort glare typically produces visual fatigue or annoyance, without necessarily reducing visual performance or visibility. It is thought to be related to neuronal interactions similar to that of the pupillary response to light.[188] GD, on the other hand, is caused by straylight (forward light scatter) either exterior to and/or within the eye, impairing visual performance and visibility. GD typically increases with age and/or ocular disease and can be used synonymously with straylight according to the definition by the CIE (*Commission International d'Eclairage*, or the International Committee on Illumination).[189]

Light travels in straight lines unless it is either absorbed, reflected or scattered by obstructing particles. The physics of light scatter has been eloquently described as follows: *"Scattering is the process by which a particle – any bit of matter – in the path*

of an electromagnetic wave continuously 1) abstracts energy from the incident wave, and 2) reradiates that energy into the total solid angle centered at the particle. Scattering only occurs when the particle's refractive index differs from the surrounding medium."[190]

The impact of light scatter on CS and visibility has been eloquently described in a review[33] where the effects of scatter by air particles are explored, in particular scatter caused by haze aerosols (a dispersed system of small particles suspended in a gas,[190] the most common component of the atmosphere), on visibility i.e. "how far one can see and how well details can be resolved." Light scatter is wavelength-dependent for small particles (e.g. 0.2μm), such as those found in haze aerosols. As light passes through the atmosphere, shorter wavelengths are more prone to scatter than longer wavelengths. The scattering of short-wavelength light creates a bluish veiling luminance, often termed "blue haze", which, when superimposed on the retinal image, reduces the contrast of targets being observed.

GD and AMD: The effect of straylight is exacerbated in the presence of retinal disease, such as AMD. AMD is associated with RPE dysfunction (discussed above), which in turn increases the effects of straylight. In addition, the orientation of photoreceptors is an important anatomical consideration with respect to glare. In a normal, healthy eye, photoreceptors are orientated in such a way that light entering the eye (though the pupil centre) is incident on the "top" of the photoreceptors. This limits the response to light scatter in the healthy eye (the Stiles-Crawford effect), particularly in the case of cones.[191] Photoreceptors that are irregularly oriented are less likely to respond to light than normally oriented receptors. In AMD, for example, the presence of drusen, retinal cysts, fluid, pigment epithelial detachments, alter the normal architecture (orientation)

of the photoreceptors, causing them to be less responsive to incoming light and more responsive to scattered light within the eye, exacerbated by an ageing RPE.

GD in response to anti-VEGF therapy: To my knowledge, no study has investigated the impact of anti-VEGF therapy in cases of nv-AMD on a subject's vision measured in the presence of glare.

GD in response to macular carotenoid supplementation: MP's short-wavelength filtering properties render it capable, in theory at least, of attenuating straylight incident upon the retina, reducing GD. Wooten and Hammond have proposed that having MPOD of e.g. 0.5 OD units (compared with having little or no MPOD) can attenuate the veiling luminance of a short-wavelength dominant background by 17%, thereby increasing the visibility and discriminability of objects in natural viewing conditions.[33] Studies have shown the inverse relationship between levels of MP and GD.[32] A study has shown that supplementation with 10mg of L and 2mg of Z for a period of 4-6 months significantly increases MPOD and improves visual performance in the presence of glare in normal healthy subjects.[36] Similarly, demonstrable improvements in mesopic and photopic GD for a range of spatial frequencies amongst normal healthy subjects supplementing with 10mg MZ, 10mg L and 2mg Z over a six-month period, have been observed. Of note, there were no statistically significant improvements in these parameters of visual performance amongst subjects supplementing with either placebo or L and Z alone.[170]

Improvements in GD have been reported in AMD subjects following supplementation with 15mg dietary L, improvements which were not observed amongst those supplementing with placebo, albeit in a small sample (n=5). To the best of my knowledge, no other study has looked at the impact of macular carotenoid

supplementation on GD in subjects with AMD. Of note, GD has also been shown to improve amongst subjects with cataract taking dietary supplementation of 15mg L three times a week, compared with subjects on placebo.[192] However, the presence of AMD was not an exclusion criterion in this study. Therefore, ascertaining whether or not the observed visual benefits of supplementation were related to regression in macular disease is not possible.

3.5 Reading speed

Word reading is a complex task comprising of a combination of visual, neural, motor and cognitive processes, and is influenced by stimulus conditions such as size of print, contrast, colour, and optical defocus.[193] Reading speed is also strongly associated with vision-related quality of life.[194] Reading speed is measured in words per minute (wpm) and is reported to have a mean (range) of 215 (169-273) wpm in subjects with normal vision.[38]

Reading speed and AMD: The intactness of the central field is one of the most important factors for accurate reading,[195] and is likely, therefore, to be of primary concern to subjects with late stages of AMD. Differences in maximum reading speed have been observed between subjects with nv-AMD and subjects with GA, with significantly higher reading speeds achieved by subjects with macular scotomas due to nv-AMD compared to those due to GA.[196] The authors postulate that this difference might be as a result of the different time-courses of the two conditions, which involve different types of visuo-motor and adaptation processes. The loss of central field elicits the use of eccentric viewing, thus impacting the size of the visual span (the number of letters recognised with each glance, which shrinks in peripheral compared to central

vision[197]) and, consequently, reading speed. Central field loss also impacts fixation ability, which is an important contributing factor to comfortable reading.[198] Bullimore et al reported that subjects with AMD (not defined by the authors as early or late) show similar fixation rates to normals, but they average fewer letters per forward saccade and make more frequent regressions.[199]

Reading speed and anti-VEGF therapy: There are very few studies that have investigated the impact of anti-VEGF therapy on reading speed. Two are of particular interest. A statistically significant improvement was reported in mean[±sd] reading speed (59[±40] to 85[±50] wpm; $p < 0.0001$) over a three month period in a group of thirty subjects being treated with ranibizumab for nv-AMD.[200] This study also showed that there was no significant relationship between change in CDVA and change in reading speed following intervention, indicating that change in CDVA alone cannot predict a change in reading speed, which was shown to relate more strongly to patient quality of life than CDVA. Another study has shown a shift in the critical print size towards smaller print sizes after three intravitreal injections of ranibizumab, i.e. subjects reached their maximum reading speed for smaller print sizes than those achieved at baseline, requiring less magnification for effortless reading following treatment.[201]

Reading speed and macular carotenoid supplementation: There is little information known about the relationship between MP and reading speed, or on the influence of macular carotenoid supplementation on this measure of visual function over time. Further research in this area is required. It has been reported, however, that the use of yellow filters (similar to MP) improve magnocellular function, which has been shown to enhance reading performance in children with reading difficulties.[202]

Another study investigated the effects of four different light filters (the yellow Corning Photochromic Filter [CPF] 450 [absorbing wavelengths below 450 nm], a grey neural density filter, an individualised filter obtained using the Intuitive Colorimeter®, and a clear filter) on reading speed in normal subjects and in subjects with (non-neovascular) AMD associated with central field loss. There was no statistically significant light filter effect on reading speed for normal subjects. However, the AMD group demonstrated a statistically significant (mean = 5%) improvement in reading speed with the CPF450 compared with the other filters, and some subjects had improvements of 10-15%. This suggests that the filtration of short-wavelength light may be of greater visual benefit for subjects with AMD than for normal subjects.[203]

3.6 Retinotopic ocular sensitivity

Retinotopic ocular sensitivity (ROS), as determined using Microperimetry (devices such as the Microperimeter (MP 1)®, Nidek Technologies, Padova, Italy), provides information regarding ocular functional performance by examining the differential light threshold at specific points on the retina, under direct visualisation of the fundus. It allows a point-to-point correlation between fundus lesions and functional defects and simultaneously corrects for eye movements. Other important features of the technique include real-time automated fundus tracking, the automatic, accurate mapping of the location and quality of fixation, and the facility to analyse eyes over time, using point to point comparisons from visit to visit. ROS uses a more sophisticated method of psychophysical assessment (compared to CDVA) and is, therefore, inherently more sensitive to subtle changes in retinal physiology.

ROS and AMD: ROS overlying areas of drusen and pigment abnormalities, using the MP 1, was examined in 13 patients with early AMD and good VA (6/6).[204] The results showed that, in subjects with early AMD, ROS diminishes in areas overlying drusen and/or pigment abnormalities, despite good VA. The reduction in sensitivity was greater when both types of lesion were present. These findings suggest that microperimetry provides additional and possibly more useful information than CDVA with respect to visual function in cases of AMD.

ROS and anti-VEGF therapy: Microperimetry has been shown to provide additional and valuable information in cases of nv-AMD undergoing anti-VEGF therapy. A study has demonstrated a progressive improvement of ROS, in response to ranibizumab therapy, as far as 24 months following the initiation of treatment, despite stabilisation of VA after six months.[7] Changes in macular morphology following anti-VEGF therapy have been shown to correlate with changes in central ROS, as measured by microperimetry.[205] Changes in microperimetry have also been shown to reflect changes in macular thickness for other eye conditions, such as diabetic macular oedema.[206, 207] A study has shown that, compared with microperimetry, CDVA seems to significantly underestimate the change in visual function experienced by patients following treatment (three consecutive monthly intravitreal ranibizumab injections) for nv-AMD. In that particular study, one patient exhibited a significant improvement in CDVA compared with eight patients who exhibited a significant improvement in mean ROS (but not CDVA).[208] This difference in the proportion of patients who had improved visual function as assessed by microperimetry compared with CDVA was statistically significant.

ROS and macular carotenoid supplementation: The first study that investigated the impact of macular carotenoid supplementation on macular function as examined by microperimetry in subjects with early AMD reported a tendency towards improvement following supplementation with L for six months (20mg for first three months and 10mg for the remaining three months), although the observed improvement did not reach statistical significance.[35] However, the authors report a significant correlation between the increase in MPOD and the increase in ROS over the study period. No other studies to date have investigated the impact of macular carotenoid supplementation on ROS.

3.7. Preferential Hyperacuity Perimetry

Preferential Hyperacuity Perimetry (PHP) is based on the phenomenon of vernier acuity, which reflects the ability to discern a subtle misalignment of an object. Vernier acuity, a form of hyperacuity, has a resolution threshold of three to six seconds of arc at the fovea, which is approximately 10-fold lower than that required for optimal resolution of an object on, for example, a letter chart (30 to 60 seconds of arc).[209] It is unaffected by patient age or physical condition,[210] as well as being quite resistant to image degradation e.g. as a result of lens opacities, when compared to resolution acuity.[211]

PHP and AMD: It has been suggested that measures of hyperacuity (such as vernier acuity) may better detect early loss of visual function in patients with age-related retinal disease, such as AMD.[212] The Foresee PHP® has demonstrated high sensitivity and specificity in differentiating between patients with intermediate AMD and recent-onset CNV,[213] and also greater sensitivity in detecting macular changes and AMD when

compared to the Amsler grid.[214] The PHP has, however, demonstrated a higher number of false-positive results amongst healthy individuals, compared with the Amsler grid.[215]

PHP and anti-VEGF therapy: A study has shown that improvements in the PHP metamorphopsia test score correlated closely with improvements in several OCT parameters, following a single intravitreal ranibizumab injection, amongst 14 subjects with nv-AMD.[216] A longer prospective study (by the same authors) in a similar group of subjects, over a period of six months has shown that improvements in OCT parameters correlated with functional improvements as evaluated by PHP ($r = 0.9$; $p < 0.05$), following intravitreal ranibizumab therapy. In addition, the PHP could predict the need for further injections with an accuracy of 75% (sensitivity, 83±12%; specificity, 67±15%), whereas a combination of all the measurements (PHP, CDVA, and OCT) yielded a higher accuracy of 87% (sensitivity, 83±12%; specificity, 90±10%),[217] rendering it a potentially useful tool for monitoring patients undergoing treatment for nv-AMD.

PHP and macular carotenoid supplementation: To date, PHP has not been assessed in conjunction with macular carotenoid supplementation.

3.8 Subjective Experience and Quality of Life.

Quantification of disease severity or any observed improvement or deterioration in a given patient's condition, judged by a clinician according to defined morphological or even psychophysical criteria, in many ways falls below the importance of subjective experience i.e. how the patient experiences the world as a result of the condition and/or its treatment. Whether or not the patient notices appreciable change in vision or in their

quality of life (QoL) is central to understanding the impact of any disease or resulting treatment strategy. This is interesting considering that ophthalmologists often underestimate the impact of AMD on a patient's QoL.[218] In addition, patient-reported outcomes are now part of US FDA guidelines for the design of clinical trials.[219]

There is considerable evidence to highlight the significantly negative impact of AMD on QoL.[220-223] Compared to age-matched normals, subjects with AMD are eight times more likely to report difficulty shopping, 13 times more likely to have difficulty managing finances, four times more likely to experience difficulty with meal preparation, 12 times more likely to have problems using a telephone, and nine times more likely to have difficulty carrying out housework.[223]

A number of studies have looked at the impact of AMD on psychological well-being. Patients with AMD and VA of 6/60 or worse in at least one eye are more likely to experience emotional distress than age-matched normals (from the Profile of Mood States).[224] However, QoL scores are dependent on stage, where late stages of the condition have a more profound impact.[39] Longer duration of the condition was associated with reduced levels of distress, most probably due to adaptation. However, poor adaptation was shown to be associated with depression.[225] A US-based cross-sectional study found that rates of depression amongst patients with advanced visual loss attributable to AMD were twice those found among a general sample of community-dwelling elderly subjects.[226]

There are obvious limitations to measuring QoL using standard questionnaires. As is implied, QoL is a subjective perception and will have a different meaning to different people. Many QoL measures, although obtaining a score or quantifying the degree of difficulty with respect to a given task, do not necessarily take into consideration the relevance of that particular task or aspect of daily life for the patient in question. For example, two patients with similar deterioration in reading speed may

exhibit vastly different QoL scores, depending on whether or not reading is an important part of their daily lives. The availability of support and rehabilitation may also contribute to variations in QoL scores. Patient to patient variability in QoL scores are further confounded when one considers the influence of the presence or absence of disease in the fellow eye.

Vision-related QoL questionnaires that have been validated for use amongst subjects with AMD include: the National Eye Institute Visual-Function Questionnaire (NEI-VFQ), the Visual Function-14 (VF-14) questionnaire, Daily Living Tasks dependent on Vision (DLTV) questionnaire and the Activities of Daily Vision (ADV) scale. Of these, NEI-VFQ[227] is the only questionnaire that investigates psychological aspects of visual impairment (social functioning, mental health, dependency), in addition to items specifically related to vision-related tasks.

A study investigating the responsiveness of the NEI-VFQ to changes in VA, using data from the MARINA and ANCHOR trials, has shown that the NEI-VFQ was a responsive and sensitive measure of vision-related function amongst patients with nv-AMD receiving anti-VEGF therapy.[228] However, it must be borne in mind that the criterion for change in VA in these studies was defined as >15 letters (three lines of a logMAR chart), which is a change of relatively large magnitude. The sensitivity of the NEI-VFQ to smaller changes, e.g. one or two lines, has yet to be ascertained.

NEI-VFQ has been critiqued with respect to its unidimensionality, a characteristic essential for a valid questionnaire. The overall composite score (a score between 0 and 100), which combines the scores of 11 subscales encompassing items related to socio-emotional state, and items related to visual functioning, should be interpreted with caution, considering the differing nature of these two concepts. Although there is overlap between the two, simply combining them in one common score will not accurately reflect the contribution of each to the overall output measure.

This has largely been remedied through recently derived scales (socioemotional and visual functioning scales) in combination with Rasch analysis.[229] Rasch analysis transforms raw nominal numeric questionnaire values into a continuous scale, reducing noise and allowing for parametric statistical analyses of the data.[230] Rasch analysis is now widely recognised as a valuable measure in the revalidation of questionnaires, including within the area of ophthalmology.[231-233]

All things considered, and in spite of limitations, a measurement designed to quantify a patient's QoL, either as a result of a condition such as AMD or following therapeutic intervention for the condition, should be given due consideration in studies investigating visual performance.

3.9 Conclusion

Considering the wide range and scope of psychophysical visual function and the importance of the information yielded with respect to assessment of subjective visual function, it would seem unwise to rely solely on one measure of visual performance when attempting to quantify disease severity, or when assessing the need for intervention, or when evaluating functional outcomes of intervention, both clinically and in research studies.

Chapter 4. The evidence germane to the role of macular pigment for the enhancement of vision, and its putative protective function against age-related macular degeneration

4.1 Study rationale, aims and objectives

There is a consensus that AMD is the result of (photo)-oxidative-induced retinal injury and its inflammatory sequelae, the latter being influenced by genetic background.

MP is a yellow-coloured pigment which accumulates primarily within the inner retinal layers at the macula,[234] and is optically undetectable beyond 7° eccentricity.[235] MP is composed of two dietary carotenoids, L and Z, and a third carotenoid, MZ, which is not found in a typical diet[26, 27] (chemical structure given in Figure 4.1) MP has generated interest in recent years because of its (now generally accepted) role in the enhancement of visual performance and its possible protective role for AMD, putatively attributable to its antioxidant properties and/or its pre-receptoral filtration of damaging short-wavelength visible light, given that photo-oxidative retinal injury is known to be important in the pathogenesis of this condition.[24, 25]

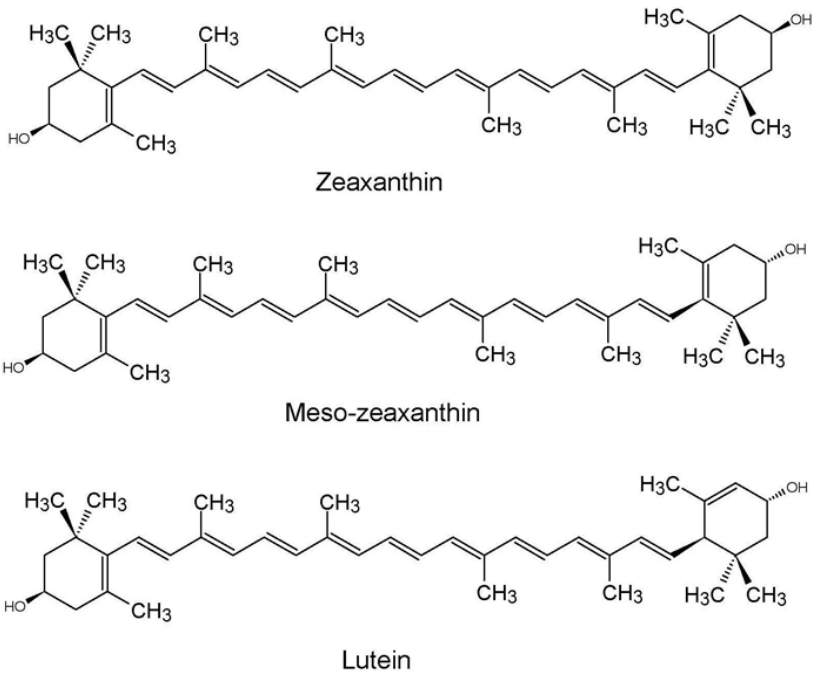


Figure 4.1 Chemical strutures of zeaxanthin ([3R,3'R]-β,β-Carotene-3,3'-diol), *meso*-zeaxanthin ([3R,3'S]-β,β-Carotene-3,3'-diol), and lutein ([3R,3'R,6R]-β,ε-Carotene-3,3'-diol). *Image obtained from the MPRG, Waterford.*

Evidence quality is typically graded on the basis of study design, where systematic reviews or meta-analyses of RCTs are widely accepted as providing the best evidence (Level 1) on the effects of preventative, as well as other, interventions in medicine.[236] (see Table 4.1)

RCTs are regarded as the "gold standard" in clinical research, yet they have certain limitations[237] such as inappropriate outcome measures and/or biased sample recruitment. Given that studies involving humans are laden with ethical issues and, in many cases, may not be feasible, practical or indeed appropriate,[237, 238] many important epidemiologic findings have been the result of observational studies. The weight accorded to RCTs can, in some instances, result in the exclusion of evidence arising from other valid study designs. In other words, studies with alternative designs should be seen as complementary, rather than an alternative, to RCTs.

Table 4.1 Levels of evidence for therapy or prevention

Level	Type of study
1a	Systematic review (homogeneous) of RCTs
1b	Individual RCT (with narrow confidence interval)
2a	Systematic review of (homogeneous) cohort studies
2b	Individual cohort study / Low quality RCT
3a	Systematic review of (homogeneous) case-control studies
3b	Individual case-control studies
4	Case series, low-quality cohort or case-control studies
5	Expert opinions without explicit critical appraisal, or based on physiology, bench research or "first principles"

Abbreviations: RCT=randomised control trial

Material adapted from the recommendations for evidence-based medicine in Oxford.[239]

Level 1 evidence has shown that dietary supplementation with broad-spectrum antioxidants results in risk reduction for AMD progression. Studies have demonstrated that MP rises in response to supplementation with the macular carotenoids, although Level 1 evidence that such supplementation results in risk reduction of AMD and/or its progression is still lacking. Although appropriately weighted attention should be accorded to higher levels of evidence, the totality of available data should be appraised in an attempt to inform clinical practice. In this context, I have reviewed the literature with respect to macular carotenoid supplementation and its putative protective role in the onset/progression of AMD and also its impact on visual performance, in subjects with and without the condition.

4.2 The Origins of Macular Pigment

The *macula lutea* ("yellow spot") was first identified more than two centuries ago (1792) by a Milanese ophthalmologist, Francesco Buzzi (1751-1805). Whilst dissecting and analysing eyes, he noticed a constant finding in the retina: the existence of a small area of yellowish colour lateral to the optic disc. He reported this finding in his famous work "*Nuovo sperienze fatte sull' occhio umano*" – new experiments on the human eye.[240]

Buzzi's finding was independently confirmed in 1795 by the German physician, Samuel Thomas von Soemmering (1775-1830), who observed yellow pigment at the macula during dissection of the eyes of a young man who had drowned. He described it as a "yellow round spot, and a small hole in the middle." Soemmering did, in fact, believe it to be a hole at the centre of the retina and named it (in Italian), *foramine centrali limbo luteo* - the central yellow-edged hole. He published his finding in a communication in 1799.[241]

Sir Everard Home, a British physician, took great interest in the discovery and carried out further research to investigate the presence of the pigment in human eyes, as well as those of other species, such as monkeys, cows and sheep. He concluded that only human and monkey eyes had the pigment. In 1798, he published the first review on the "macular yellow",[242] beginning an era of investigation into the composition, and function, of what has become known as macular pigment,[243] a term first coined in 1933 by Walls et al.[244]

The visual performance and protective hypotheses of MP was first discussed by Schultze in 1866 where, in his paper, "The retina's yellow spot – it's influence on vision and on colour-blindness", he concluded, "*Therefore, under an otherwise equal organisation, a retina without a yellow spot would see more blue light than one with such a spot.*" He believed that absorption of the "*most refractable violet*" reduced CA, but also hypothesised that macular yellow might provide some protection against the hazards of short-wavelength visible light.[245] MP's function was further discussed in a series of studies in the early 20th century.[246-249]

In 1945, Wald demonstrated the spectral sensitivity of MP (using a spectral adaptometer), indicating that it had a characteristic carotenoid absorption spectrum and belonged to a family of xanthophylls found in green leaves. Extraction of pigment yielded a hydroxy-carotenoid that Wald believed to be L.[250]

However, it was not until 1985 that Bone and Landrum first reported that the pigment was composed of the carotenoids L and Z,[251] and this was later confirmed in 1993, at which point the authors also identified MZ as being the third carotenoid present in the central retina, where it is the dominant carotenoid at the epicentre of the macula.[252] Bone et al proposed that MZ was primarily formed at the macula following conversion from retinal L,[253] and this has subsequently been confirmed.[254-256]

4.3 The Functions of Macular Pigment

The putative protective role of MP for AMD derives from its anatomical position in the retina (central and pre-receptoral), and from two functional properties of this pigment: its absorbance spectrum (peak absorption of this pigment is 460nm), and its ability to quench ROIs, referred to as antioxidant capacity (see Figure 4.2).

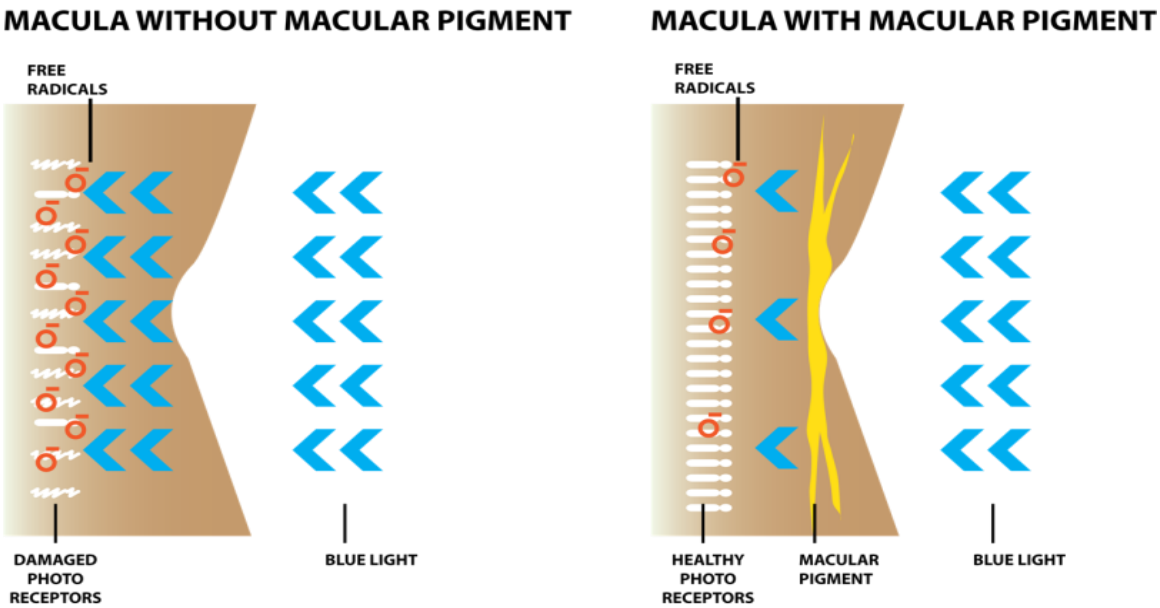


Figure 4.2 The antioxidant and blue-light filtering properties of macular pigment. *Image obtained from the Macular Pigment Research Group, Waterford, Ireland.*

4.3.1 Short wavelength light filtration

Although almost all UV-B (320-290nm) and UV-A (320-400nm) light is absorbed by the cornea and lens, light of slightly longer wavelength (400-520nm) passes through the anterior media, and irradiates the retina.[257] Given that the peak absorption of MP is at 460nm,[250] it has the ideal light filtration properties to screen short-wavelength light pre-receptorally. This allows MP to attenuate the amount of short-wavelength light incident upon the central retina.

L is reported to be a superior filter of short-wavelength light when compared to Z, due to its orientation with respect to the plane of the phospholipid bilayer of the cell membrane (see Figure 4.3),[258] which is both parallel and perpendicular. In contrast, Z and MZ only exhibit perpendicular orientation to this layer. However, it is important to note that the different absorption spectra of these pigments (L, Z and MZ) result in a collective optimal filtration of short-wave light at the macula, which would not be achieved by any of these carotenoids in isolation.[258-260]

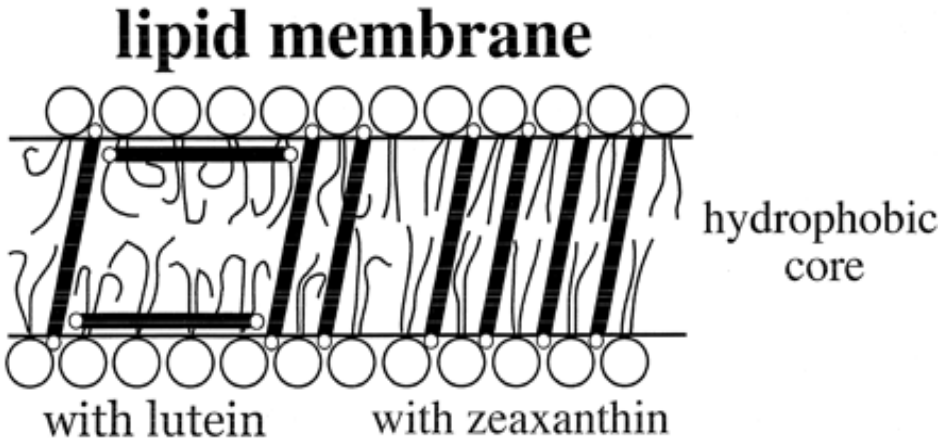


Figure 4.3 Lutein and zeaxanthin within the cell membrane. *Image obtained from Krinsky et al.*[261]

A recent analysis by the European Eye Study (n=4753) found a significant correlation between cumulative exposure to visible light and nv-AMD in those patients with low intake of dietary antioxidants, including L and Z.[75] A further study has

recently reported the effect of low-power laser light (476nm [blue]) on the retinae of eight rhesus monkeys who had lifelong deprivation of the dietary xanthophylls, and therefore no detectable MP. A further four monkeys (controls) had a typical dietary intake of L and Z from birth. The retinae of primates deprived of dietary xanthophylls until exposed to the low-power laser light, but then supplemented with either L or Z, were then exposed once again to the same laser light six months later. The relationship between lesion size and exposure energy was then analysed. The controls (primates with typical dietary intake of L and Z from birth) exhibited less severe short-wavelength light induced lesions in the foveal region of the retina when compared to the parafoveal region (where there is no MP), whereas those with lifetime deprivation of xanthophylls, and no measurable MP, exhibited no difference between the fovea and parafovea in terms of blue light induced retinal damage prior to supplementation, thus supporting the hypothesis that foveal photo-protection is indeed attributable to MP. This was further confirmed by the observation that, following either L or Z supplementation, relative foveal protection was restored, and those animals with prior lifelong deprivation of dietary xanthophylls no longer exhibited greater relative vulnerability of the fovea when compared with the parafovea, and were, therefore, similar to the control group in this respect following supplementation.[262]

4.3.2 Antioxidant Properties

L, Z and MZ are structural isomers of one another and are characterised, biochemically, by their high number of double-bonds.[253] Their supply of readily available electrons enables these carotenoids to quench ROIs, thus limiting membrane phospholipid peroxidation and attenuating oxidative injury.[258, 263, 264] Kirschfeld was the first to propose the idea that carotenoids protect the macula against oxidative stress.[265] However, it was not until 1997 that the presence of direct oxidation products of L and

Z in human retinal tissue was confirmed, supporting the hypothesis that MP does indeed protect against oxidative damage in this tissue.[266]

The antioxidant capacity of Z (and other carotenoids), however, has been shown to decrease with increasing oxygen tensions in tissue.[267] Of note, MP is at its highest concentration in the receptor axon layer of the foveola and in the inner plexiform layer.[268, 269] Also, the concentration of the carotenoids within each retinal layer peaks at the foveola. Importantly, it is at this central retinal location where ROI production is greatest.[270]

In vitro studies of human RPE cells, subjected to oxidative stress, have shown enhanced survival of these cells in the presence of Z and other antioxidants, when compared with controls.[271] Furthermore, L and Z are also more resistant to degradation than other carotenoids when subjected to oxidative stress.[272] Z appears to be a more potent antioxidant than L[273] and MZ is yet more efficacious, but only in conjunction with its binding protein[274] (binding proteins are likely to mediate the uptake of the carotenoids at the macula[275]). Another study has demonstrated that light-induced photoreceptor apoptosis is limited in response to supplemental Z in quail (the retinae of which, like those of primates, selectively accumulate L and Z).[276] Chucair et al provided the first evidence of direct neuroprotection of photoreceptors by the macular carotenoids,[277] by demonstrating that the retinal neurons of rats in culture were protected from oxidative stress when pre-treated with L and Z, compared to those not pre-treated with these carotenoids. Recently, it has been demonstrated that a mixture of L, Z and MZ (in a ratio of 1:1:1) quenches more singlet oxygen than any of these carotenoids individually at the same total concentration.[278]

4.3.3 MP for vision

The optical properties of MP, and its selective accumulation at the macula, prompted the original hypothesis that MP is important for visual performance and comfort. Indeed, the evidence-based consensus is that the principal function of MP at the central retina relates to its contribution to visual performance and experience. MP contributes in this respect through its short-wavelength light-filtering properties at a pre-receptoral level, thereby attenuating CA and light scatter (which are the result of defocus and scatter, primarily of short-wavelength visible light), with consequentially enhanced CS and reduced GD, respectively. The dichroic properties of MP may further contribute to glare reduction due to the preferential absorption of plane-polarised light.[279] Furthermore, MP's antioxidant properties may also contribute to the enhancement of visual function by neutralising damaging ROIs, which would otherwise, over time, impair the physiological functionality of the photoreceptors, and this putative contribution of MP to visual performance has been termed "neural efficiency".[170]

Many cross-sectional studies have shown a positive association between MP and measures of visual performance, including VA, CS, photostress recovery and GD (amongst others).[29-33] Early AMD is associated with the loss of psychophysical function,[160] and it has been shown that supplementation with the macular carotenoids improves parameters of visual function in patients afflicted with the early form of this condition.[34, 169, 171, 280] However, no study has yet investigated the impact of a formulation containing MZ on visual function in subjects with early AMD, or on the natural course of this condition.

4.4 The Source of the Macular Carotenoids

An average western diet contains 1.3-3 mg/day of L and Z combined, with significantly more L than Z (represented by an estimated ratio of circa 7:1).[281] It has been reported that approximately 78% of dietary L and Z is sourced from vegetables, with L found in highest concentrations in dark green leafy vegetables (including spinach, broccoli, kale, and collard greens).[282] However, as most current dietary databases report intakes of L and Z combined, it has been difficult to assess the respective and relative intakes of the individual macular carotenoids. However, a recent study reported concentrations of L and Z separately within the major food sources, as determined by the National Health and Nutrition Examination Survey (NHANES).[283] The authors confirmed that green leafy vegetables were the richest source of L (e.g. cooked spinach and kale), whereas corn and corn products were confirmed as being a major source of Z. Eggs are also a good source of L and Z, especially given the enhanced bioavailability of these carotenoids in this form because of co-ingestion of fat.[284]

It appears that humans ingest relatively low concentrations of MZ (if any). Eggs from hens fed MZ are known to be a rich human dietary source of this carotenoid.[285] Also, in 1986 a study reported that MZ and Z are present in twenty-one species of edible fish, shrimp, and sea turtles.[286] However, it should be noted that there is a paucity of studies conducted to test foods for the presence of MZ, and further study is this area is needed. The presence of MZ in the serum of unsupplemented individuals has never been unambiguously demonstrated, although efforts to extract and quantify MZ in human blood have demonstrated that, if it is present, the concentrations of this carotenoid are low.[287] Interestingly, and in spite of its absence or low concentration in a normal diet, MZ accounts for about one third of total MP at the macula, consistent with the finding that retinal MZ is produced primarily by isomerisation of retinal L at the macula.[253, 256] L differs from MZ (structurally) with respect to the location of the

double bond in one of the end rings (see Figure 4.1). The conversion of L into MZ requires a shift in this carbon-carbon double bond. The exact mechanism of the conversion, however, remains unknown.

4.5 The Evidence

4.5.1 Types of Evidence

There is the notable challenge of fitting carotenoid research into the, sometimes rigid, paradigm of evidence-based medicine.

A systematic review is a thorough, comprehensive, and explicit means by which to identify, critically appraise and evaluate medical literature related to a specific research question. A meta-analysis is a statistical approach to combine and analyse the data derived from a systematic-review. RCTs are studies in which participants are allocated at random, rather than by conscious decision of clinician or patient (which is the case in non-randomised trials), to receive one of several clinical interventions, one of which typically acts as a control (placebo). The greater the sample size, the reduced likelihood of bias. In contrast, an observational study is one in which conclusions are drawn by observation alone, examples of which may include case-control and cohort studies.

AMD is a slow, complex disorder, and the carotenoids under review, particularly L and Z, are already commonly found in the daily diet and are easily available in supplement form on the open market. This makes the conduct of gold standard RCTs particularly difficult. What is important to acknowledge is that all study designs contribute to an ever-growing body of knowledge in a given area. This point has been eloquently made by Hennekens: *"Every research strategy within a discipline, contributes importantly relevant and complimentary information to a totality of evidence upon which rational clinical decision making and public policy can be reliably based. In this context, observational evidence has provided and will continue to make unique and important contributions to this totality of evidence upon which to support a judgment of proof beyond a reasonable doubt in the evaluation of interventions."*[288]

While recognising the importance of study design in public health research, we are encouraged to give adequate attention to the completeness and transferability of evidence when interpreting the results of such studies. This has been eloquently articulated, as follows: *"Care is needed that the use of evidence hierarchies to compare the potential for bias between study designs does not translate into unrealistic or overly expensive demands for level 1 or 2 evidence, particularly if there is a good or adequate level 3 evidence to inform a decision."*[289]

The reader should also be aware that the capacity and resources of competing stakeholders (e.g. pharmaceutical companies, academic institutions) to generate and disseminate evidence has a profound influence on the prestige and volume of available and published literature on a given subject.[289]

4.5.2 Clinical trials investigating the macular carotenoids in subjects with AMD

4.5.2.1 Proof of Principle

In 2001, the Age-Related Eye Disease Study (AREDS) was published, having been conducted by the National Eye Institute (NEI). This was a double-masked, randomised, placebo-controlled trial of 4757 subjects over a period of 5 years. In brief, it was shown that supplementation with vitamins C and E, beta carotene, and zinc, in combination, resulted in a 25% risk reduction of progression from intermediate to advanced AMD.[28] Of note, the AREDS formulation did not contain any of the macular carotenoids, primarily because these compounds were not available in supplement form at the inception of that study. This landmark work did, however, provide Level 1 evidence that supplemental dietary antioxidants were of benefit to patients with AMD.

4.5.2.2 Interventional studies

Following AREDS, and in consideration of the possible protective role that MP plays in AMD, given its anatomical, antioxidant and optical properties, investigators began to direct their attention towards studies designed to explore the possible benefits of supplementation with MP's constiuent carotenoids. There now exists a plethora of published interventional studies reporting on supplementation with macular carotenoids and its impact on AMD (Table 4.2), ranging from case series to RCTs.[35, 168, 169, 171, 187, 280, 290-293]

In 2004, the LAST study was carried out in an attempt to evaluate the effect of L, either alone or in combination with co-antioxidants, vitamins and minerals, on the progression of atrophic AMD.[187] This study was a prospective, 12-month, randomised, double-masked, placebo-controlled trial, involving 90 subjects with atrophic (dry) AMD. The subjects were assigned to one of three groups: group 1 received L (10mg) only; group 2 received a broad-spectrum supplementation formula containing L (10mg) as well as co-antioxidants, vitamins and minerals; group 3 received a placebo. Results showed that the subjects in groups 1 and 2 demonstrated an increase in mean MP optical density as well as an improvement in VA, CS, glare recovery and visual distortion. This study, therefore, demonstrated that visual function is improved in patients with atrophic AMD following supplementation with either L alone or L in combination with co-antioxidants, vitamins and minerals. However, the LAST study is open to legitimate criticism on the basis of the small number of patients recruited into each arm of the investigation, and the short follow-up i.e. only 12 months (compared to e.g. AREDS).

The Carotenoids in Age-Related Maculopathy (CARMA) study was a randomised, double blind, placebo controlled clinical trial of L (12mg) and Z (0.6mg) supplementation with co-antioxidants versus placebo in patients with AMD.[294] This study included 433 subjects, who were recruited and randomly assigned to the treatment

or the placebo arms of the study. Although the primary outcome measure (CDVA at one year) did not differ between the placebo and the intervention arms of the study, it was noted that CDVA was significantly better in the intervention arm of the study at 36 months follow-up. In addition, an increase in serum L was associated with significantly improved CDVA and slowing of progression along the AMD severity scale.[171] It is important, however, to note there are several limitations in the CARMA study design, despite it being an RCT. These limitations include a relatively small sample size, particularly at 36 months (n = 41, 20 in the intervention group and 21 in the placebo group), and the questionable appropriateness of its primary outcome measure (CDVA at 12 months), given the chronic nature of AMD.

Table 4.2 Interventional studies investigating the effect of supplementation with the macular carortenoids in subjects with AMD

Principal Author	Study	Year	n	Study Design	Age	Carotenoids	Finding
Richer et al	-	1999	14	Case Series	61-79	L (14mg)	Improved VP
Olmedilla et al	-	2001	5	Case Series	69-75	L (15mg)	Improved VP
Richer et al	LAST	2004	90	RCT	68-82	L (10mg)	Improved VP[1]
Bartlett et al	-	2007	25	RCT	55-82	L (6mg)	No benefit
Beatty et al	CARMA	2007	433	RCT	50+	L (12mg) & Z (0.6mg)	Improved VP[1]
Weigart et al	LISA	2011	126	RCT	50-90	L (20mg/10mg)*	Improved VP[1]
Richer et al	ZVF	2011	60	RCT	75(±10)†	Z (8mg) & L (9mg)	Improved VP[1]
Sasamoto et al	-	2011	33	Case Series	65(±9)†	L (6mg)	Improved VP[1]
Piermarocchi et al	CARMIS	2011	145	PRS	-	L (10mg) & Z (1mg)	Improved VP
Jentsch et al	Lutega	2011	172	RCT	50+	L (10mg/20mg) & Z (1mg/2mg)	Improved VP[1]

Carotenoids = Macular carotenoids assessed in the study; L = Lutein; Z = Zeaxanthin; VP = Visual Performance; n = number of subjects participating in study; Age = Age range (years) of subjects in study; RCT = Randomised control trial; LAST = Lutein Antioxidant Supplementation Trial; CARMA = Carotenoids in Age-Related Maculopathy; LISA = Lutein Intervention Study Austria; ZVF = Zeaxanthin Visual Function; CARMIS = Carotenoids in Age-related Maculopathy Italian Study; PRS = prospective randomised study; - = data unavailable.

*20mg taken for first 3 months and 10mg taken for remaining 3 months

†mean(±sd)

[1]macular pigment measurements were obtained in the study

The optical, anatomical and antioxidant properties of MP have generated a consensus that MP plays an important role in vision. Many studies have already demonstrated the positive cross-sectional association between measures of MPOD and measures of visual performance, including: CDVA, CS, GD, photostress recovery,

critical flicker fusion frequency, colour vision (amongst others).[29-33, 295, 296] One might hypothesise, therefore, that an increase in MPOD will be paralleled by an improvement in vision. Indeed, increases in MPOD correlated significantly with decreases in mean differential light threshold (assessed by microperimetry), suggesting that augmentation of MPOD enhances ROS.[35] It is important to note that psychophysical function is adversely affected in AMD,[160] and this is confounded by age-related decline in many aspects of visual function in the absence of macular pathology.[297-299] Therefore, a demonstrable improvement (or even stabilisation) in visual function in response to supplemental macular carotenoids in an older population with a known degenerative disease should be deemed beneficial. In this context, it is interesting to note that nine of the ten studies investigating changes in visual performance following supplementation with macular carotenoids in AMD subjects have demonstrated an improvement in visual function, and the remaining study consisted of only 25 subjects supplemented with only 6mg L (alone), and even here vision did not deteriorate.

Trials awaiting completion

There are a number of trials underway investigating the putative protective role of L and Z in individuals with AMD. The AREDS 2 is an on-going multi-centre RCT (n=circa. 4000) evaluating the impact of supplemental L and Z (and/or omega-3) on the progression of intermediate to advanced AMD and the influence of these supplements on VA. Additionally, it seeks to assess whether modified forms of the original AREDS supplement, with reduced zinc and no beta-carotene, work as effectively as the original supplement in reducing the risk of progression to advanced AMD.

AREDS 2 is expected to be completed in December 2012 (http://clinicaltrials.gov/ct2/show/NCT00345176?term=AREDS2&rank=1). The results of AREDS 2 will provide valuable and timely data on the potential role of antioxidants,

including L and Z, in delaying AMD progression, and will inform current professional practice with respect to the role of dietary modification and/or supplementation in patients with AMD. A limitation of the trial, however, rests on the fact that MP is not being measured. Therefore, a finding that supplemental L and Z in AREDS 2 are not beneficial cannot be interpreted to mean that MP augmentation is not beneficial, as the latter will not have been demonstrated. Further, it is likely that a very high proportion of participants in the US-based AREDS 2 will have been supplementing with dietary antioxidants for many years, thereby contaminating the baseline findings for all study groups (further hindered by a short wash-out period of only thirty days in subjects who may have been supplementing for many years), and, therefore, compromising the trial's capacity to demonstrate a beneficial effect of supplementation. In fact, a recent baseline analysis on AREDS 2 subjects from one AREDS 2 centre that is assessing MP levels has reported unusually high baseline MPOD levels relative to an age-matched control group which did not regularly consume carotenoid supplements.[300] Also, since AREDS 2 is only investigating rates of progression among high risk patients (for advanced AMD), it therefore, cannot answer one of the most crucial questions with respect to carotenoid supplementation – does it prevent/delay AMD onset, or does it reduce progression in earlier stages of the condition?

4.5.2.3 Observational studies

A large number of studies have investigated the relationship between dietary intake of the macular carotenoids and AMD.[104, 301-307] Of these ten published observational studies, six reported that a high dietary intake of the carotenoids was associated with a reduced risk of AMD. The relationship between AMD and serum concentration of the macular carotenoids has also been investigated,[75, 303, 308-315] and of the ten published studies in this respect, seven have shown that low serum concentrations of the macular carotenoids are associated with increased risk of this condition. (Table 4.3)

Table 4.3. Observational studies investigating the relationship between the macular carotenoids and age-related macular degeneration.

Observational Dietary Studies							
Principal Author	**Study**	**Year**	**n**	**Study Design**	**Age**	**Carotenoids**	**Nutrient/AMD relationship**
Seddon et al	EDCCS	1994	356/520*	Case Control	55-80	L&Z	Inverse
VandenLangenberg et al	BDES	1996	1968	Cohort	45-86	L&Z	None
Mares-Perlman et al	NHANES III	2001	8222	Cross-sectional	40+	L&Z	Inverse
Flood et al	BMES	2002	2335	Cohort	49+	L&Z	None
Snellen et al	-	2002	72/66*	Case Control	60+	L	Inverse
Moeller et al	CAREDS	2006	1787	Cross-sectional	50-79	L&Z	None
San Giovanni et al	AREDS	2007	4519	Case Control	60-80	L&Z	Inverse
Tan et al	BMES	2008	2454	Cohort	49+	L&Z	Inverse
Cho et al		2008	66,993	Cohort	50+	L&Z	None
Olea et al	-	2012	52	Cross-sectional	mean=79	L&Z	Inverse‡
Observational Serum Studies							
-	EDCCS	1993	421/615*	Case Control	-	L&Z	Inverse
Mares-Perlman et al	BDES	1995	167/167*	Case Control	43-86	L&Z	None
Mares-Perlman et al	NHANES III	2001	8222	Cross-sectional	40+	L&Z	Inverse
Simonelli et al	-	2002	48/46*	Case Control	mean=67	L&Z	None
Gale et al	-	2003	380	Cross-sectional	66-75	L&Z; L; Z	Inverse (Z only)
Cardinault et al	-	2005	34/21*	Case Control	72-74	L; Z	None
Delcourt et al	POLA	2006	899	Cohort	60+	L&Z	Inverse (esp. Z)
Fletcher et al	EES	2008	2283/2117*	Cross-sectional	65+	L; Z	Inverse (esp. Z)
Michikawa et al	-	2009	722	Cross-sectional	65+	L&Z	Inverse‡
Zhao et al	-	2011	263	Cross-sectional	50-88	L&Z	Inverse‡

Abbreviations: carotenoids = macular carotenoids assessed in the study; L = lutein; Z = zeaxanthin; n = number of subjects; Age = age range (years) of subjects in study; EDCCS = Eye Disease Case Control Study; BDES = Beaver Dam Eye Study; NHANES = National Health and Nutrition Examination Survey; BMES = Blue Mountains Eye Study; CAREDS = Carotenoids in Age-Related Eye Disease Study; AREDS = Age-Related Eye Disease Study; POLA = Pathologies Oculaires Liées à l'Age; EES = European Eye Study; - = data unavailable.

*cases/controls

‡for late stages of AMD

4.5.3 Clinical trials investigating the macular carotenoids in normal subjects

Many studies have reported on the cross-sectional relationship between MP and a plethora of visual performance parameters, and a number of trials have investigated the impact of supplementation with the macular carotenoids on visual performance in subjects without disease (see Table 4.4)

Table 4.4 Interventional studies investigating the impact of the macular carotenoids on visual performance in normal subjects.

Principal author(s)	Year	n	Placebo-control	Carotenoids	Visual performance tests	Study duration (months)	Observed visual benefit following supplementation
Monje[a]	1948	14	No	L dipalmitate	Dark adaptation & scotopic VA	2-6	Yes‡
Wustenberg[a]	1951	7	No	L dipalmitate	Dark adaptation	-	No
Klaes & Riegel	1951	-	No	L dipalmitate	Dark adaptation	-	Yes
Andreani & Volpi[a]	1956	10	No	L dipalmitate	Dark adaptation	-	Yes
Mosci[a]	1956	-	No	L dipalmitate	Light sensitivity	-	Yes
Hayano[a]	1959	-	No	L dipalmitate	Dark adaptation	-	Yes†
Wenzel	2006	10	Yes	30mg L + 2.7mg Z	Photophobia	3	Yes
Rodriguez-Carmona	2006	24	Yes*	10mg/20mg of L/Z/L+Z	B/Y colour discrimination	12	No
Kvansakul	2006	34	Yes	10mg L/10mg Z/combination	Mesopic CS	6	Yes
Barlett & Eperjesi	2008	29	Yes	6mg L	VA (dist.&near), CS, photostress recovery	18	No
Stringham & Hammond	2008	40	No	10mg L + 2mg Z	Photostress recovery & grating visibility	6	Yes; both
Nolan	2010	121	Yes	12mg L + 1mg Z	VA, CS, GD, photostress recovery	12	Yes; CS, GD
Loughman	2012	36	Yes	10mg L+2mg Z+10mg MZ/20mg L+2mg Z	VA, CS, GD, photostress recovery	6	Yes; VA, CS, GD

Abbreviations: carotenoids=macular carotenoids investigated; L=lutein; VA=visual acuity; Z=zeaxanthin; B/Y=blue/yellow; CS=contrast sensitivity; GD=glare disability; MZ=meso-zeaxanthin; - =data not available.

[a] data obtained from Nussbaum[316]

*for second 6 months of the study

†proportional to serum L

‡described as having a "transient" benefit

4.5.3.1 COMPASS

The Collaborative Optical Macular Pigment ASsessment Study (COMPASS) was a RCT designed to investigate the impact of supplementation with macular carotenoids versus placebo, on MPOD and visual performance. One hundred and twenty-one normal subjects were recruited (age range: 18 - 41 years) to COMPASS. The active group consumed 12mg of L and 1mg of Z (but no MZ) every day for 12 months (n=61), while the remaining subjects were assigned to placebo. A range of psychophysical tests were used to assess visual performance, including: CDVA, CS, GD and photostress recovery. Subjective visual function was determined by questionnaire and MPOD was measured using customized heterochromatic flicker photometry (cHFP). The results of this study showed that central MPOD increased significantly in the active group (but only at the 12 month time point), whereas no such augmentation was demonstrable in the placebo group. Although this observed increase in MPOD did not result, generally, in a demonstrable improvement in visual performance, statistically significant differences in mesopic CS (with and without glare) were observed between those who had high MPOD and those who had low MPOD at 12 months, whereas this was not the case at baseline.[172]

4.5.3.2 MOST Vision

The widest range of short-wavelength light filtration is achieved in the presence of all three macular carotenoids (L, Z and MZ).[317, 318] Emerging data further indicates that supplementation with all three macular carotenoids increases MPOD faster and to a greater extent when compared to a formulation that does not contain MZ. *In vitro* studies have also shown that maximum anti-oxidant capacity of the pigment is dependent upon the presence of all three macular carotenoids.[319] Investigators,

therefore, have begun to study the impact of supplementation with a formulation containing L, Z and MZ on MPOD and on visual performance.

The *Meso*-zeaxanthin Ocular Supplementation Vision Trial (MOST Vision) investigated the effect of supplementation of different carotenoid dose combinations, on visual performance in normal subjects.[170] The 36 recruited subjects were assigned to one of three groups, as follows: the first was given a high dose (20mg) of L and 2mg Z (Group 1); the second group was given 10mg L, 10mg MZ and 2mg Z (Group 2); and the third group was given placebo (Group 3), every day for six months. A statistically significant rise in MP was observed (notably, three months following commencement of supplementation) only among subjects supplemented with a formulation containing all three macular carotenoids, including MZ (Group 2). Statistically significant improvements in CDVA were observed at six months, but only for subjects in Group 2. Statistically significant improvements in CS were noted across a range of spatial frequencies, under photopic (3, 12 and 18cpd) and mesopic conditions (1.5, 3, 12 and 18cpd), again only among subjects in Group 2 (with a single exception of improved CS at a single spatial frequency [6cpd] in the high L group [Group 1]). There were no statistically significant improvements in mesopic or photopic GD between baseline and six months in Groups 1 and 3. However, there was a demonstrable improvement in mesopic and photopic GD for subjects in Group 2 for all spatial frequencies tested (with the exception of 18cpd).

4.5.3.3 Supplementation with the macular carotenoids in subjects with an atypical MPOD spatial profile

A study investigated the relationship between MP and known risk factors for developing AMD amongst 828 normal subjects between the ages of 18 and 55. The study demonstrated a relative lack of MP in association with tobacco use and with a

family history of AMD. Also, the authors report an age-related decline in MP, suggesting that the risk that such variables represent for AMD may be attributable, at least in part, to a parallel lack of MP. It appears, therefore, that, prior to disease onset, known risk factors for AMD are independently associated with a relative lack of MP.[320]

Of the 828 subjects, a proportion (12%) exhibited a "central dip" (i.e. they did not exhibit the typical central peak that declines in from the foveal centre) in their MPOD spatial profile. Interestingly, this central dip was associated with tobacco use and increasing age,[321] suggesting that such atypical spatial profiles of MP may (independently) represent risk for AMD. Given that MZ is the dominant carotenoid in the foveal centre, it has been hypothesised that the observed central dip in the MP spatial profile (found in 12% of the study population) is attributable to a relative lack of this carotenoid. Further, and since retinal MZ is formed from retinal L (but not retinal Z), the observed central dip in the MP spatial profile may be the result of an inability among these subjects to convert retinal L to MZ, and therefore such subjects may require this carotenoid in supplement form if they are to achieve a typical and desirable spatial profile characterised by a central peak and an associated decline from the foveal centre.

The effect of supplementation on a group of subjects that exhibited a central dip in their MP spatial profile has also been investigated.[322] Thirty-one subjects were assigned to one of three intervention groups, as follows: one given a 20mg of L and 2mg of Z (Group 1); the second group was given 10mg L, 10mg MZ and 2mg Z (Group 2); and the third group was supplemented 17mg MZ and 3mg L (Group 3). Subjects took one capsule a day for eight weeks. A significant increase in MPOD was not demonstrable among subjects supplemented with high doses of L (Group 1), at any eccentricity. Subjects supplemented with high doses of MZ (Group 3) exhibited significant increases in MPOD at the centre of the MP spatial profile, but at no other

eccentricity. Subjects in the combined carotenoid group (Group 2, containing L, Z and MZ) exhibited a significant augmentation of MPOD at 0.25° and at 0.5° eccentricity, and a trend towards a rise in MP approaching statistical significance at all other eccentricities. The authors concluded that these atypical spatial profiles of MP, characterised by central dips, which have been shown to be associated with risk for AMD,[321] can be normalised following supplementation with a formulation containing MZ, but not with a formulation that is lacking this carotenoid, at least not over an eight-week study period. Augmentation of MPOD across its spatial profile was best achieved with a formulation containing all three macular carotenoids during the study period. Further trials, of longer duration and that explore different supplement doses/combinations, are required to support this finding.

4.5.4 Serum and retinal response to supplementation with the macular carotenoids

There have been many published studies on serum (Table 4.5) and retinal response (i.e. MPOD; Table 4.6) to supplementation with the macular carotenoids, in normal and in AMD subjects, and it is clear that serum carotenoid levels and MPOD rise in response to supplementation with MP's constituent carotenoids. However, it is important to point out that the magnitude of response is influenced by many factors, including the type of carotenoid used (i.e. L, Z, MZ, independently or in combination), the concentration of carotenoid present in the supplement (dose), the duration of supplementation (time), individual characteristics (e.g. adiposity), and baseline MP levels.[323]

Table 4.5 Serum carotenoid response per milligram of supplemental carotenoid, following supplementation with the macular carotenoids.

Principal Author	Journal	Year	*n*	Age	L(mg)	Z(mg)	MZ(mg)	L Response (µmol/L/mg)	Z Response (µmol/L/mg)	MZ Response (µmol/L/mg)	Duration
Normal Subjects											
Bone et al.	JN	2003	21	19-59	2.4	-	-	0.100 (**p <0.05**)	-	-	24
Bone et al.	ABB	2010	17	18-30	5	-	-	0.035 (**p<0.0001**)	-	-	20
			22	18-30	10	-	-	0.071 (**p<0.0001**)	-	-	20
			24	18-30	20	-	-	0.053 (**p<0.0001**)	-	-	20
			14	51-64	20	-	-	0.071 (**p<0.0001**)	-	-	20
Koh et al.	EER	2004	6	58-72	10	-	-	0.168 (p n/a)	-	-	19
Berendschot et al.	IOVS	2000	8	18-50	10	-	-	0.072 (**p<0.001**)	-	-	12
Zhao et al.	AJCN	2006	8	50-70	12	-	-	0.116 (**p<0.01**)	-	-	8
Hughes et al.	JID	2000	21	26–56	15	-	-	0.092 (**p<0.01**)	-	-	4
Hartmann et al.	AJCN	2004	10	28-38	-	1	-	-	0.152 (p n/a)	-	42
			10	28-43	-	10	-	-	0.087 (p n/a)	-	42
Schalch et al.	ABB	2007*	16	18-45	-	12.6	-	-	0.064 (p n/a)	-	24
Bone et al.	JN	2003	2	21-53	-	30	-	-	0.014 (p n/a)	-	12
Thürmann et al.	AJCN	2005	8	21-37	4.1	0.58		0.093 (p n/a)	-	-	42
	AJCN	2005	8	24-34	20.5	2.9		0.064 (p n/a)	-	-	42
Schalch et al.	ABB	2007*	18	18-45	10.7	0.8	-	0.078 (p n/a)	0.063 (p n/a)	-	24
			19	18-45	10.2	11.9	-	0.037 (p n/a)	0.046 (p n/a)	-	24
Huang et al.	IOVS	2008	40	64-86	10	2	-	0.041 (p n/a)	0.046 (p n/a)	-	24
Johnson et al.	AJCN	2008	11	60-80	12	0.5	-	0.022 (**p<0.001**)	0.030 (p n/a)	-	16
Nolan et al.	VR	2011	61	18-41	12	1	-	0.053 (**p<0.001**)	-0.003 (p>0.05)	-	48
Johnson et al.	AJCN	2000	7	33–54	19.7	1	-	0.018 (**p<0.05**)	0.016 (**p<0.003**)	-	15
Bone et al.	JN	2003	2	42-53	30	1.5	-	0.063 (p n/a)	0 (p n/a)	-	20
Connolly et al.	CER	2010	5	18-60	3.7	0.8	7.3	0.019 (**p<0.05**)	-0.028 (p>0.05)	0.006 (**p<0.05**)	8
Thurnham et al.	BJN	2008	19	21-46	10.8	1.2	8	0.056 (**p<0.01**)	0.088 (**p<0.001**)	0.026 (**p=0.004**)	3
Bone et al.	NM	2007*	10	21-58	5.5	1.4	14.9	0.014 (p n/a)	0.121^ (p n/a)	-	17
Loughman et al.‡		2012	12	56±8	20	1	-	0.014 (p=0.139)	0.010 (**p=0.045**)	-	24
			12	51±13	10	2	10	0.066 (**p=0.001**)	0.015 (**p=0.023**)	0.009 (**p=0.001**)	24

AMD Subjects											
Connolly et al.	CER	2010	5	18-60	3.7	0.8	7.3	0.012 (**p<0.05**)	0.035 (p>0.05)	0.004 (**p<0.05**)	8
Koh et al.	EER	2004	7	60-81	10	-	-	0.157 (p n/a)	-	-	19
Khachik et al.	IOVS	2006	15	60+	10	0.5	-	0.079 (**p<0.0001**)	0.076 (**p<0.0001**)	-	24
Trieschmann et al.	EER	2007	97	51-87	12	1	-	0.036 (**p<0.001**)	0.004 (**p=0.007**)	-	36

Abbreviations: L=lutein; Z=zeaxanthin; MZ=*meso*-zeaxanthin; *n*=number of subjects participating in study; Age=age range (years) of subjects in study; duration=duration of supplementation (weeks); ABB=Archives of Biochemistry and Biophysics; BJN=British Journal of Nutrition; IOVS=Investigative Ophthalmology and Visual Science; AJCN=American Journal of Clinical Nutrition; JN=Journal of Nutrition; JID=Journal of Infectious Diseases; VR=Vision Research; EER=Experimental Eye Research; CER=Current Eye Research; NM=Nutrition and Metabolism; OPO=Ophthalmic and Physiological Optics; (-)=data unavailable; n/a=not available.

*free (un-esterified) carotenoid supplement

†includes MZ supplementation

^refers to total Z+MZ

‡ARVO abstract

Table 4.6 Studies reporting on macular pigment optical density response to supplementation with the macular carotenoids.

Principal Author	Year	n	Age	L mg/d	Z mg/d	MZ mg/d	Duration (weeks)	Tec	Retinal ecc	PF	MP rise	Sig.
NORMAL subjects - dietary modification												
Hammond et al.[324]	1997	10	30-65	11.2	0.6	0	15	HFP	0.5°	5.5°	~ 0.05	**p < 0.05**
		2	30-65	0.4	0.3	0	15	HFP	0.5°	5.5°	~ 0.05	-
		1	30-65	10.8	0.3	0	15	HFP	0.5°	5.5°	~ 0.05	**p < 0.05**
Johnson et al.[325]	2000	7	33-54	11.2	0.57	0	15	HFP	0.5°	5.5°	~ 0.07	**p < 0.05**
NORMAL subjects - supplement modification												
Landrum et al.[326]	1997	2	42-51	30	0	0	20	HFP	0.75°	8°	~ 0.20	-
Berendschot et al.[327]	2000	8	18-50	10	0	0	12	SLO	0.75°	14°	~ 0.05	**p = 0.022**
		8	18-50	10	0	0	12	SA	0.75°	-	~ 0.04	**p < 0.001**
Aleman et al.[328]	2001	8	11-59	20	0	0	24	HFP	0.17°	5-7°	0.07	**p = 0.04**
		8	11-59	20	0	0	24	HFP	0.5°	5-7°	0.07	-
		8	11-59	20	0	0	24	HFP	1°	5-7°	0.08	-
		8	11-59	20	0	0	24	HFP	2°	5-7°	0.04	-
Bone et al.[329]	2003	2	19-59	30	1.5	0	20	HFP	0.75°	8°	~ 0.20	-
		1	53	0	30	0	17	HFP	0.75°	8°	~ 0.07	-
		21	19-59	2.4	0	0	17	HFP	0.75°	8°	~ 0.04	-
		12	19-60	20	0	0	17	HFP	0.75°	8°	~ 0.06	**p < 0.05**
		2	26-27	5	0	0	17	HFP	0.75°	8°	~ 0.03	-
Koh et al.[330]	2004	6	64-81	20	0	0	20	HFP	0.5°	6°	0.07	p > 0.05
Bernstein et al.[331]	2004	8	<61	20	0	0	16	HFP	0.75°	8°	0.04	-
		8	<61	20	0	0	16	RRS	-	-	76RC	-
Bone et al.[332]	2007	10	21-58	5.5	1.4	15	17	HFP	0.75°	8°	~ 0.07	**p < 0.05**
Wenzel et al.[333]	2007	3	24-52	30	2.7	0	17	HFP	0.33°	7°	0.07	**p < 0.001**
		3	24-52	30	2.7	0	17	HFP	0.5°	7°	0.07	**p < 0.002**
		3	24-52	30	2.7	0	17	HFP	1°	7°	0.046	**p< 0.002**
		3	24-52	30	2.7	0	17	HFP	2°	7°	0	-
Schalch et al.[334]	2007	23	18-45	10.7	0.8	0	17	HFP	0.5°	5.5°	0.06	**p = 0.04**
		23	18-45	0	12.6	0	17	HFP	0.5°	5.5°	0.01	p > 0.1
		23	18-45	10.2	11.9	0	17	HFP	0.5°	5.5°	0.06	**p = 0.04**
Johnson et al.[335]	2008	11	60-80	12	0.5	0	16	HFP	1.5°	7°	-	p < 0.05
		11	60-80	12	0.5	0	16	HFP	3°	7°	-	**p < 0.01**
Stringham et al.[336]	2008	40	17-41	10	2	0	24	HFP	0.25°	10°	0.19	-

		40	17-41	10	2	0	24	HFP	0.5°	10°	0.16	-
		40	17-41	10	2	0	24	HFP	1°	10°	0.1	-
		40	17-41	10	2	0	24	HFP	3°	10°	0.07	-
		40	17-41	10	2	0	24	HFP	7°	10°	0.03	-
Connolly et al.[287]	2010	5	30-85	3.7	0.8	7.3	8	HFP	0.25°	7°	0.16	**p < 0.05**
		5	30-85	3.7	0.8	7.3	8	HFP	0.5°	7°	0.16	**p < 0.05**
Nolan et al.[337]	2011	61	18-41	12	1	0	52	HFP	0.25°	7°	0.12	**p = 0.001**
		62	18-42	12	1	0	52	HFP	0.5°	7°	0.11	**p = 0.001**
Loughman et al.[170]	2012	12	56±8	20	2	0	24	HFP	0.25°	7°	0.09	p = 0.092
	2012	12	51±13	10	2	10	24	HFP	0.25°	7°	0.13	**p = 0.002**

AMD subjects

Principal Author	**Year**	**n**	**Age**	**L** mg/d	**Z** mg/d	**MZ** mg/d	**Duration (weeks)**	**Tech**	**Retinal ecc.**	**PF**	**MP rise**	**Sig.**
Koh et al.[330]	2004	7	64-81	20	0	0	20	HFP	1°	6°	0.07	p > 0.05
Trieschmann et al.[338]	2007	108	51-87	12	1	0	24	AF	1°	6°	0.1	**p < 0.001**
Richer et al.[339]	2007	76	-	10	0	0	52	HFP	1°	7°	0.25	**p < 0.05**
Connolly et al.[287]	2010	5	30-85	3.7	0.8	7.3	8	HFP	0.25°	7°	1.6	**p < 0.05**
		5	30-85	3.7	0.8	7.3	8	HFP	0.5°	7°	1.6	**p < 0.05**
Weigert[35]	2011	84	72±9	15	0	0	24	HFP	0.25°	7°	0.08	**p <0.001**
Richer[280]	2011	25	76±9	0	8	0	52	HFP*	1°	7°	0.13†	**p = 0.03**
		25	74±11	9	8	0	52	HFP	1°	7°	0.20†	p = 0.06
		10	74±9	9	0	0	52	HFP	1°	7°	0.18†	**p = 0.03**
Beatty et al.	2012	246/63‡	55+	12	0.6	0	104	RRS	central 3°	-	61 (RC)	**p < 0.001**

Abbreviations: L = Lutein (mg/day); Z = Zeaxanthin (mg/day); MZ = *Meso*-zeaxanthin (mg/day); Tec = technique used to measure MPOD (macular pigment optical density); n = Number of subjects participating in study; Age = Age range (years) of subjects in study; Retinal ecc.= retinal eccentricity; PF = Parafovea stimulus; AJCN = American Journal of Clinical Nutrition; IOVS = Investigative Ophthalmology and Visual Science; ABB = Archives of Biochemistry and Biophysics; OPO = Ophthalmic and Physiological Optics; EER = Experimental Eye Research; NM = Nutrition and Metabolism; OPT = Optometry; JN = Journal of Nutrition; OVS = Optometry and Vision Science; RC = Raman counts; ODU = Optical density units; HFP = Heterochromatic flicker photometry; AF = Autofluorescence; SLO = Scanning Laser ophthalmoscope; SA = Spectral Analysis; AMD = Age related Macular Degeneration; RRS = Resonance Raman Spectroscopy; RC = raman count- = data unavailable.

*modified HFP technique (QuantifEYE®)

†measurements from right eyes in the study

‡246 at baseline, 63 at year 2

The data suggest that supplementation with all three macular carotenoids results in the greatest and broadest response in terms of MP augmentation and changes in its spatial profile. Therefore, and given that the antioxidant capacity of MP is maximised in the presence of all three macular carotenoids,[319] and where the objective is to augment MP and to thereby putatively confer protection against AMD, current evidence suggests that supplementation with all three macular carotenoids is most likely to (1) limit (photo)-oxidative retinal injury with a consequential reduction in risk of AMD development or progression and (2) maximally enhance visual performance and ameliorate GD.

Interestingly, a study by Bone and Landrum has shown that serum levels of L and Z rise and fall rapidly following commencement and discontinuation of supplementation with the macular carotenoids, respectively. In contrast, MP optical density increases more slowly from baseline following commencement of supplementation with the macular carotenoids, and returns to baseline levels more slowly following discontinuation of supplementation, reflecting a slow biological turnover or these carotenoids at the macula.[326] A recent study investigated the impact of dietary deprivation of all L- and Z-containing foodstuffs on serum carotenoid levels and on MPOD, over a period of six weeks. In brief, a rapid decline in serum levels of L and Z, and also in MPOD, was observed in response to this dramatic dietary change by week three. The resumption of a normal diet resulted in a 40% recovery in MPOD levels within two weeks.[340]

4.5.5 Conclusion

In summary, the properties of MP, namely its central retinal location, its pre-receptoral filtration of damaging short-wavelength light and its ability to quench free radicals, suggest that it plays a key role in the aetiopathogenesis of AMD and its progression, in

addition to contributing to the optimisation of visual performance (in subjects with and without disease). Level 1 evidence has demonstrated that supplemental dietary antioxidants reduce the risk of vision loss in AMD, although evidence of this quality for supplementation with the macular carotenoids is still lacking. The visual performance hypothesis of MP, on the other hand, is now accepted, with many clinical trials reporting that macular carotenoid supplementation demonstrably enhances visual performance in subjects with and without disease. Clinical trials have repeatedly shown that dietary supplementation with the macular carotenoids (L, Z and/or MZ) results in augmentation of MP, and the best response in terms of augmentation, changes in spatial profile of the pigment, global fortification of the antioxidant defenses of the tissue to be protected and in terms of visual performance, appears to be a supplement containing all three macular carotenoids. These trials (involving all three macular carotenoids), however, have been limited by several factors, including small numbers of subjects and inadequate masking, such that definitive conclusions cannot yet be drawn.

To effectively investigate the putative protective role of carotenoid supplements in AMD, including a possible role in prevention of this condition, an RCT of considerable length (at least a decade) would be required. As a consequence, it is important that we appraise the totality of currently available evidence in order to assist eyecare professionals to make well-informed decisions with respect to the prevention and/or delay of AMD onset and/or its progression. In this context, it would appear that supplementation with the macular carotenoids offers the best means of fortifying the antioxidant defenses of the macula, thus putatively reducing the risk of AMD and/or its progression, and of optimising visual performance.

This work is divided into four principal areas; the rationale, methods, results and discussion of each is contained within its own chapter (Chapters 5-8).

Chapter 5. Visual performance in patients undergoing intravitreal ranibizumab for neovascular AMD.

5.1 Study rationale, aims and objectives.

Considering the emergence of new therapies and treatment regimens for subjects with nv-AMD, and considering the complex nature of the visual experience, it is essential that visual performance is not judged solely on the outcomes of one visual task (most often, CDVA), either in clinical or research settings. This study was designed to assess the effect of anti-VEGF therapy in cases of nv-AMD on a subject's visual performance and experience, through a range of psychophysical tests, which take into greater consideration the complexity of the visual environment.

There is a strong rationale to support the administration of anti-VEGF therapy in spite of good presenting CDVA,[341] as early treatment is essential in terms of preventing visual loss. However, it is well documented that the extent of visual improvement following anti-VEGF therapy is inversely dependent upon presenting CDVA, i.e. presenting CDVA is a prognostic indicator for improvement in CDVA following treatment, with greater acuity benefits accruing in those with poorest baseline CDVA.[342, 343] Therefore, a patient who presents for anti-VEGF therapy with relatively good CDVA e.g. 6/7.5, will not exhibit the same level of improvement as a patient who presents with CDVA of 6/30, for example, purely due to ceiling effects. This finding may lead one to believe that the treatment is not having a functional benefit in the case of the high acuity patient, whereas there may be important parameters of visual function that are improving/changing but that are not being detected by a measure as crude as CDVA. In addition, if other measures of vision are depleted (and CDVA preserved),

then CDVA is also not detecting a certain amount of functional loss, which is important with respect to (re)treatment strategies.

The vast majority of studies investigating visual function in subjects with nv-AMD (i.e. assessing disease severity, determining when to commence, cease or recommence treatment), have depended, for the most part, on the measurement of CDVA. Considering the complexity of visual experience, and the known range of methods available to ascertain a more realistic and thorough appreciation of visual function, it would seem reasonable to conclude that the full extent of the effect of anti-VEGF therapy on visual function has yet to be elucidated. The current study has sought to more deeply probe and investigate visual performance beyond CDVA, both in terms of understanding how to evaluate disease severity, and also in terms of assessing functional outcomes of visual performance following intravitreal anti-VEGF therapy in subjects with nv-AMD.

5.2 Methods

5.2.1 Study design

Suitability for inclusion in this prospective study was confirmed by an ophthalmologist, in compliance with the following inclusion criteria: the study eye must be suffering from active nv-AMD, and be scheduled to commence, recommence or continue a course of intravitreal ranibizumab and have a baseline CDVA of logMAR 0.7 or better. Exclusion criteria included a history of diabetes mellitus, the presence of physical or mental impairment, or any visually important ocular comorbidity. All patients were recruited from the Institute of Eye Surgery, Whitfield Clinic, Waterford. In cases where both eyes were being treated, the eye with the better CDVA was selected for the study. Ethics approval was granted by the Dublin Institute of Techonology Ethics Committee

(Appendix 1), and informed consent was secured from each subject (Appendix 2). The research was conducted in accordance with the principles of the Declaration of Helsinki.

A diagnosis of nv-AMD was made by a retinal specialist on the basis of clinical examination, OCT and FFA. The standard regime of treatment (following initial diagnosis) included three consecutive monthly injections, followed by monthly evaluation for further treatment. Subsequent injections were administered based on signs of lesion activity on OCT and FFA, as per previously described protocol,[344] and typically upon resolution of fluid and/or cysts (determined by OCT), one more intravitreal injection of ranibizumab was administered. Two weeks following that intraocular injection, FFA was repeated. Where lesion inactivity was angiographically confirmed, treatment was discontinued.

Data were collected at baseline, and at monthly intervals (midway between monthly ranibizumab injections) within the 11 month study period. An exit visit was defined as the patient's final study visit (two weeks after the preceding and final intravitreal injection in the study). Subjects exited the study either when the study period came to an end (n=20; after a maximum follow-up of 11 months; some of these patients may have continued with further intravitreal injections of ranibizumab following closure of the study), when treatment was discontinued on clinical grounds (n=23; in these cases it was deemed, clinically, that maximum realisable benefits of treatment had been achieved i.e. fluid/cysts resolution and absence of leakage on FFA), when the patient was unable to continue in the study for unrelated health reasons (n=2) or when the patient elected to discontinue his/her participation in the study (n=2).

Patients were naïve to all the tests involved, with the exception of CDVA.

5.2.2 The technique for the administration of an intravitreal injection of a pharmacological agent (ranibizumab 0.5mg)

Valid and informed consent was obtained prior to the procedure, which included informing the patient of all risks inherent in the procedure. Patients were instructed to instill prophylactic antibiotics drops (chloramphenicol or exocin [ofloxacin]) into the conjunctival sac four times daily for three days prior to the date of each injection.

On the day of injection, the following steps were taken: the pupil was pharmacologically dilated (tropicamide); povodine iodine was instilled into the conjunctival sac ten minutes prior to the injection; anesthetic (proxymethacaine) was instilled into the conjunctival sac five minutes prior to the injection; povodine iodine was once again instilled into the conjunctival sac approximately two minutes prior to the injection.

The technique of intravitreal injection. Patient was supine. A sterile drape was used to cover the eye, and a speculum was used keep the eye open during the procedure. The intravitreal pharmocological agent (ranibizumab 0.5mg) was drawn up in a sterile syringe. Using a callipers, the intravitreal pharmocological agent was then injected (at 90 degrees to the sclera), through the pars plana into the vitreous cavity (3.5mm and 4mm from the limbus in pseudophakic and phakic eyes, respectively; Figure 5.1). Using the indirect ophthalmoscope, the central retinal artery was examined (the central retinal artery should be either pulsatile and/or pink in colour, and if pale in colour and non-pulsatile, paracentesis should be considered). A topical antibiotic was then instilled, the lid speculum removed, and the eye and lid margins rinsed with sterile saline. Following the procedure, the patient was told to continue the antibiotic drops for five or six days, and to contact the eye clinic should any problems arise (a detailed information leaflet with contact numbers, was furnished to the patient with respect to such a need).

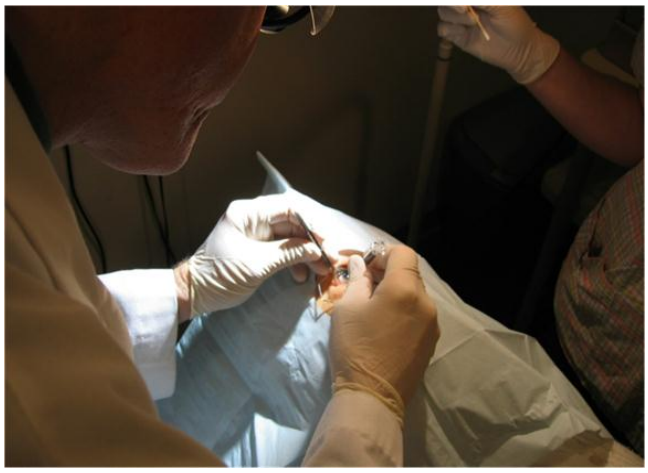


Figure 5.1 Left: The administration of intravitreal ranibizumab. *Image courtesy of the Institute of Eye Surgery, Waterford, Ireland.* Right: Schematic representation of an intravitreal injection through the pars plana. *Image obtained from My Vision Test - www.myvisiontest.com/newsarchive.php?id=1321*

5.2.3 Visual acuity

CDVA was measured for the study eye monocularly using the logMAR chart provided by Test Chart 2000 PRO® (Thomson Software Solutions, Herts, UK) at a test distance of 4m. The logMAR form of the ETDRS letterset were selected due to the benefits of regular logarithmic progression and equal legibility of letters.[345, 346] CDVA was determined with the patient's best subjective (distance) refraction. All tests were performed at a constant room illuminance of 870 lux. The patients were told that the charts have letters only, that they are allowed to guess, and that they should read slowly to achieve the best identification of each letter. The letters were presented in one isolated row at a time. The testing did not proceed until the patient had given a definite response. A visual acuity rating (VAR) was calculated for each patient (see below).[29] Points were awarded for all fully read lines. At the first incompletely read line, the letters of the line were changed using the software's randomisation function and the patient was encouraged to attempt to read the new line. This process was repeated resulting in the patient being shown three different lines of letters of equal size. Each time, the score was recorded (each correctly identified letter was awarded one point, and

a full line of five correctly identified letters was awarded five points) and an average of the three scores was taken as the score of the line with the smallest legible letters read. After that, the next (smaller) line was presented and the patient was encouraged to read it. If any of the letters were identified correctly, one point per letter was added to the previous score and that was the VAR for the eye of that patient. For example, a patient achieving CDVA of logMAR 0.0 would receive a VAR of 100, and all additional letters identified would be added, so that logMAR -0.1, for example, would be recorded as 105, while logMAR 0.1 would be recorded as 95.

5.2.4 Contrast sensitivity and glare disability (Functional Vision Analyzer™)

CS was measured using the Functional Vision Analyser™ (FVA; Stereo Optical Co., Inc – Chicago, USA), a sine wave grating contrast test system. CS was measured for the study eye, monocularly, at a constant room illuminance of 1.5 lux, with distance correction. Each patient was asked to look at five linear sine-wave grating charts of 1.5, 3, 6, 12 and 18 cpd, respectively. Each chart consisted of nine circular patches containing gratings of decreasing contrast. The background of each patch tapered into a grey field (i.e. Gabor patch) to maintain the retinal illumination and avoid ghost images. The nine patches were arranged in two rows (five patches above, four patches below). The contrast step between each patch was 0.15 log units i.e. there was a 50% loss of contrast between consecutive patches. The patch subtended an angle of approximately 1.7 degrees. Patients were instructed to identify the orientation of the gratings by choosing one of three options: gratings tilted left (+15°), gratings upright (0°) or gratings tilted right (-15°). Patients were instructed not to guess the orientation of the gratings (in order to optimize the accuracy of the measurement, as guessing would yield a 33% chance of a correct response). In cases of uncertainty, patients were advised to report that they were unable to determine the orientation of the gratings. The three-

alternative forced-choice method was stopped after the first incorrect or "don't know" response, and the last correct answer was recorded. The CS corresponding to that grating was taken as the CS score for that spatial frequency. The test was then repeated for the other spatial frequencies (increasing while testing), with the grating charts mounted as slides on a rotatable drum.

Testing was performed under: mesopic (3 cd/m^2) and photopic (85 cd/m^2) background illumination conditions, in that order. Each patient was tested at a maximum of nine contrast levels at five spatial frequencies. If a subject could not determine the orientation of the highest contrast stimulus at any particular spatial frequency, they were given a nominal baseline value, which was chosen as half of the lowest CS value achievable for the particular spatial frequency.

The procedure was repeated in a similar manner for GD, but with an additional glare light; 1 Lux for mesopic conditions, and 10 Lux for photopic conditions, inducing an estimated luminance increase of 30% and 12%, respectively. Glare light was achieved by 12 inbuilt white light emitting diodes (LEDs) arranged circumferentially in an oval pattern surrounding the gratings (ranging from 4.5° to 6° from central fixation).[347] The LED glare source rendered a daylight simulating colour temperature of 6500°K, and a spectral emission profile with a single large peak at 453 nm (close to peak MP spectral absorbance).

5.2.5 Retinotopic ocular sensitivity

ROS was measured by performing microperimetry, using the Microperimeter MP 1® (Nidek Technologies Srl, 6/A - 35020 Albignasego, Padova, Italy). ROS performs a similar function to visual field analysis, but has the added advantage of being able to test at the site of a lesion.

ROS was measured monocularly and without correction, at a constant room illuminance of 1.5 lux. The study eye was pharmacologically dilated with one drop of guttae Tropicamide BP 1% w/v minims® (Chauvin Pharmaceuticals Ltd, Ashton Road, Harold Hill, Routond, Essex, RM3 8SL, UK) fifteen minutes prior to the test. The other eye was covered for the duration of the test. The patient was given a hand-button, and was instructed to fixate at the presented fixation target, a red cross spanning three degrees from fixation. They were informed that they will see small lights appear, either on the fixation target or close to it, and were instructed to press the hand-button every time they see such a light, no matter how dim or bright it may appear.

The central 16 degrees of fixation were examined. The examination pattern comprised 21 stimuli, presented under mesopic background illumination of 1.27 cd/m2 (4 asb). The stimulus size was Goldmann III (26 minutes of arc), of white colour and of 200 msec presentation duration. Stimulus intensity ranged from 20 dB (dimmest [4 asb]) to 0 dB (brightest [400 asb]); an increase of 1 dB equates to 0.1 log reduction in stimulus intensity (asb). In order to reduce testing time, the threshold values were determined for four paracentral loci (one in each quadrant of the visual field being examined); the initial attenuation of the stimulus was set at 10 dB, in this case. The thresholds for these four test loci were then used as the starting intensities for testing sensitivities of the remaining loci in each of the corresponding quadrants. This protocol has been previously utilised.[348] Thresholds were determined using a 4-2 linear staircase strategy (which uses one reversal to determine threshold). At any given locus, the initial (pre-test) intensity value was presented, and depending on whether this stimulus was seen or not seen, the intensity of the stimulus was then either decreased or increased by 4 dB, respectively. At reversal, the intensity of the stimulus then increased/decreased in increments corresponding to 2dB, until such a point as the stimulus was no longer

detectable. The eye tracking function was used for the duration of the test. Once all 21 stimuli had been presented, a fundus colour photograph of 45 degrees of the field of view was taken using the built-in colour camera (resolution 1392x1038) using a flash intensity of 10 W/second. ROS was calculated for three areas: fixation (one stimulus); within central 5 degrees (including fixation) using an average of nine stimuli; and within 16 degrees of fixation (average of 21 stimuli).

5.2.6 Reading performance

Reading speed and near VA were measured with an English version of the standardised Radner Reading Chart (can assess both reading acuity and reading speed), the reliability and reproducibility of which have been established, both for subjects with normal vision, and for those with visual impairment.[349] The reading chart consists of "sentence optotypes" that are highly comparable in terms of number of words (14 words), word length, position of words, lexical difficulty and syntactical complexity. Language specific characteristics were taken into account as were the number of letters and syllables per word, line, and sentence. Reading ability was tested monocularly with the patient's reading correction on. The patient was instructed to hold the chart at a distance of 40cm, which was measured by the examiner, advised not to alter it during the examination, and was monitored for compliance by the examiner throughout the procedure. The sentences were covered with a piece of paper, and the patient was asked to uncover sentence after sentence, reading each one aloud as quickly and as accurately as possible. Reading acuity (the sentence of smallest print that was read with a fluency i.e. in less than 20 seconds) was expressed in logRAD (logarithm of the reading acuity; the angular subtense of these letters at the fixation distance used; the reading equivalent of logMAR). Reading errors were calculated by noting the number of missed or misspoken word(s) in the sentence. Errors were counted, even when immediately

corrected, and those of the last sentence were then included in the calculation of the reading acuity score (logRAD score = logRAD + 0.005, for each incorrectly read word of a subject's last sentence). For example, a subject reads the 0.3 logRAD line and incorrectly reads two words, his/her score is 0.3 + (2 x 0.005) = 0.310.

Reading speed was measured in seconds using a stopwatch (www.online-stopwatch.com). Reading speed (wpm) was calculated for each sentence (acuity level) based on the number of words in a sentence and the time required to read the sentence (14 words x 60 seconds divided by the reading time in seconds). Reading speed was calculated at each visit based on the highest level of logRAD acuity achieved at baseline i.e. even if the logRAD value improved at the following visit, the best baseline logRAD value was used to calculate the reading speed to maintain continuity. Mean reading speed was calculated using the reading speed of each of the sentences read (across the range of print sizes) at any given study visit.

5.2.7 Preferential hyperacuity

Preferential Hyperacuity was measured using the Reichert Foresee PHP® Preferential Hyperacuity Perimeter (Figure 5.2). The PHP exploits the principle of visual attention being attracted to a more prominent stimulus[350] and uses this to determine the size of any pathological distortion (PD) that may be present. The stimulus generated is a linear series of horizontal or vertical white dots (on a black background), and utilises the technique of dot misalignment to create a discontinuity, or artificial distortion (AD), in the line contour (see example on monitor in Figure 5.2). Depending on the size of the AD the patient may see one of four things: only the AD (if there is no PD or the PD is small), only the PD (if the AD is small), both the AD and the PD (if the two are of similar size), or neither (if the AD falls on a region of scotoma).[351]

Figure 5.2 The Reichert Foresee Preferential Hyperacuity Perimeter®. *Image obtained from Grafton Optical.*

An explanatory tutorial and trial run were performed before each test (according to the standardized protocol) and the patients were supervised for the duration of the test. The patient's chin was placed on an adjustable rest at a fixed distance from the screen (50cm), so that the patient's line of sight was perpendicular to the centre of the screen. The normal reading correction was worn and the fellow eye was occluded. A trial frame was also available to provide the appropriate refractive correction, if required i.e. in cases where the subject presented with bifocals or multifocals, or without his/her reading spectacles.

The device assesses approximately a total of 500 data points within the central 14° of the subject's visual field, each data point at a spatial resolution of 0.75°. Each stimulus was flashed (for 160ms) and the patient was asked to identify the location of perceived misalignments at each stimulus presentation, using a pen, on the touch-sensitive screen. The technique relies on the presumption that when photoreceptors are anatomically undisturbed, no extra misalignment is perceived, other than the AD

presented. However, if the subject's photoreceptors are slightly misaligned due to, for example, the presence of fluid as a result of CNV, or due to the elevation of the RPE as a result of drusen, additional pathological misalignments can be perceived by the patient and recorded by the PHP.

The PHP output is presented in Figure 5.3 and its features are described, as follows:

- Within/Outside normal limits: Within normal limits means that similar visual field findings are found in the normal population of intermediate AMD patients (dry AMD) in the normative database. Outside normal limits means that similar visual field findings are found in the population of CNV patients in the normative database. If the deviation is outside normal limits, a "p" value is given. For example, $p < 1\%$ means that the visual field defect in this test is found among 1% of the intermediate AMD population. Categories for p values are: $p < 10\%$, $p < 5\%$, $p < 1\%$.
- Reliability is determined by two indices, 1) False negative errors: the frequency with which the patient failed to respond to stimuli expected to be visible and 2) False positive errors: the frequency with which the patient responded to stimuli that could not have been seen. The test is reliable only if both reliability indices are reliable, which is reflected in an overall reliability result ("Yes" or "No").
- Hyperacuity deviation map: displays a spatial representation of the patient's metamorphopsia (compiled using all the test parameters). The cross in the centre represents fixation. Each point in the map has a colour corresponding to the level of disturbance at this point. A metamorphopsia scale legend is provided, where darker colours correspond to larger disturbances.
- A test score: an arbitrary score generated by the algorithm used to compare the presenting results to the normative database of intermediate and CNV patients.

The score is interpreted by looking at the p-value, which gives an indication of the chance of a test with this score of being an intermediate AMD (non-CNV) patient. In the example below (Figure 5.3), there is a 0.93% chance that this subject does not have intermediate AMD and is, therefore, very (99.07%) likely to have CNV. Of note, more recent versions of the PHP have removed the test score, which was deemed to be causing confusion.

- Numbers of clusters: the number of detected metamorphopsia clusters in the data.
- Total Integrated Intensity: Displays the progression in time of the integrated intensity of the distortion detected over all clusters in the test.
- Total Area: Displays the progression in time of the total areas of distortion (area of all clusters in the test) in square degrees.

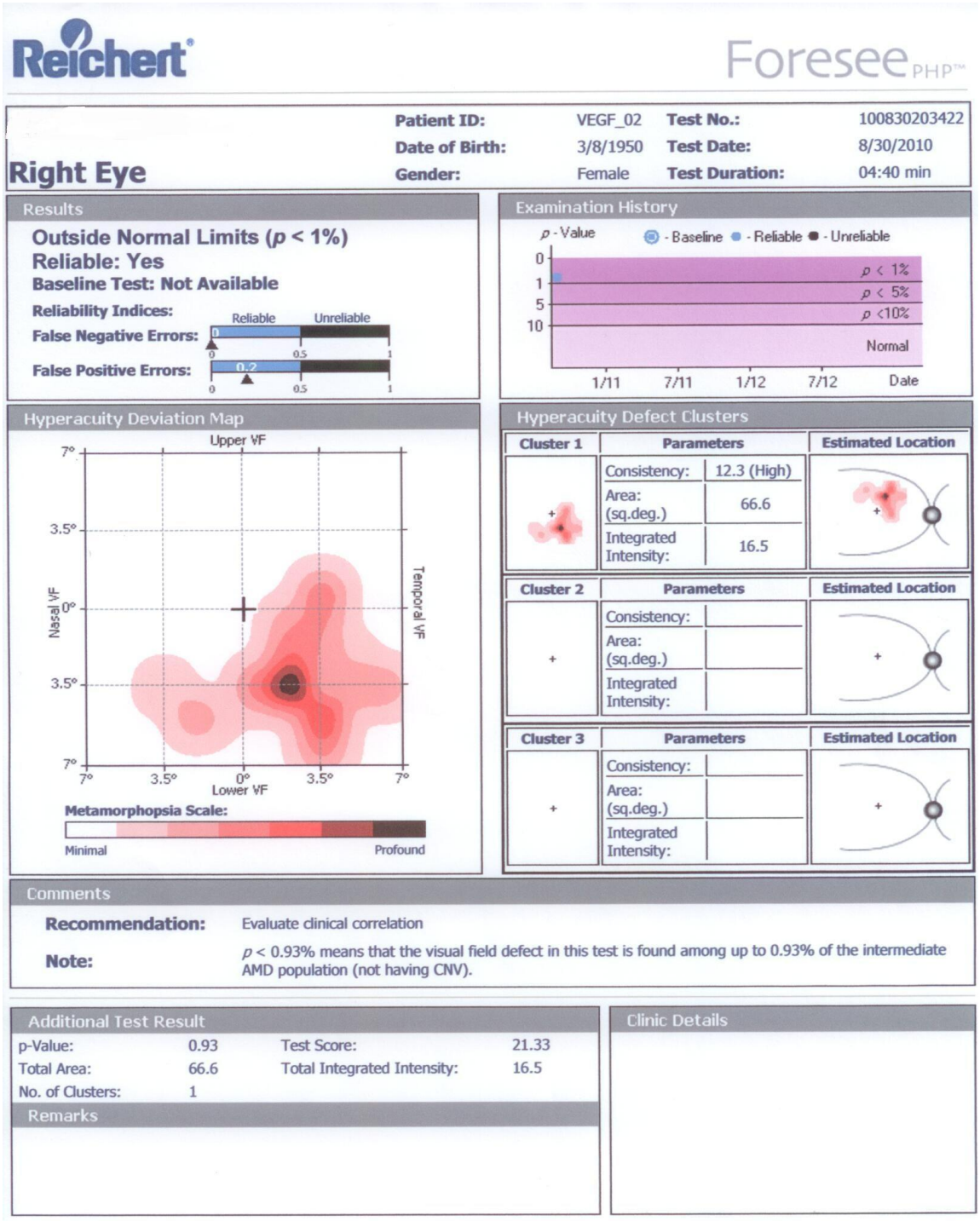


Figure 5.3 The Reichert Foresee Preferential Hyperacuity Perimeter® output.

5.2.8 Assessment of retinal thickness

Optical Coherence Topography (OCT) was performed using a Topcon 3D OCT-1000® (version 3.01, Mark I; Topcon Corporation, Tokyo, Japan) on each patient at each visit, as part of their normal pre- and post-injection assessment.

OCT is a non-invasive, cross sectional imaging technique, which uses low-coherence interferometry to produce a high resolution, two-dimensional image of optical scattering from the internal microstructure of the eye.[352] Image resolutions of 1–15 µm (twice the magnitude of conventional ultrasound) can be achieved.

Approximately twenty minutes following pupil dilation with Tropicamide (as described in ROS section, above), OCT was performed on each eye, separately. The patient placed his/her chin on a chinrest, was asked to look ahead at the centre of a large cross and keep their eyes open for five seconds.

The central 1 mm mean foveal thickness (MFT), which has also been described as central subfield thickness in previous studies, was obtained from typical ETDRS macular thickness maps.[353-356] Foveal thickness was defined as the distance between the inner and outer boundaries of the scanned image, identified using a validated internal algorithm, and did not include any fluid under the RPE.

5.2.9 Subjective Visual Function (NEI VFQ)

Subjectively perceived visual impairment in everyday life was evaluated using the NEI VFQ-25, Version 2000 (Appendix 3). The NEI VFQ-25 was developed to measure patients' perception of vision-related function[357-359] and is a reliable and valid vision-specific quality-of-life instrument.[358, 360] It is also the most frequently used measure of patient-reported, vision-related function in studies of nv-AMD.[359, 361, 362] It has been validated by a study which confirms the responsiveness of the NEI VFQ-25 to changes in VA over time and the benefit of using it for a nv-AMD population receiving pharmacologic therapy.[363] Further studies have shown its effectiveness in detecting differences in patients' reading speed and CS.[364, 365]

The NEI VFQ-25 contains 25 questions that measure different components of visual function, with thirteen additional optional items that enhance the reliability of

certain activities, all grouped within subscales. The 38-question version was used in this study. Scores range from 0 (worst) to 100 (best, perfect vision-related function). The 38 questions fall within 12 subscales: one general health subscale and eleven related to vision including: general vision, near- and distance-vision activities, driving, vision-specific dependency, social functioning, role difficulties, peripheral and colour vision, ocular pain, and mental health issues related to vision. The overall composite score (a number between 0 and 100) is calculated by taking the mean of all the subscales, excluding the general health subscale (see www.nei.nih.gov/resources/visionfunction/manual_cm2000.pdf).

Two versions of the questionnaire are available; one, a self-administered version, the other, examiner administered (used in this study). The questions, and the possible answers, were read aloud by the examiner, and the patient was required to verbally indicate their choice. The questionnaire was administered at baseline, six months, and at the final study visit (either when their treatment concluded or the study period ended). In cases where a patient had less than six study visits, the questionnaire was administered at baseline and when the treatment concluded. Rasch analysis was applied to the questionnaire data, according to a recently developed protocol,[366] using commercial software (WINSTEPS Rasch measurement computer program, version 3.70.0.2; Beaverton, Oregon [http://www.Winsteps.com]), thus calibrating item difficulty and patient ability on the same scale. Values for the long-form visual function score (LFVFS), near vision score (NVS) and overall composite score are reported in this study. The overall composite score (a score between 0 and 100) combines the scores of 11 subscales encompassing items related to socio-emotional state, and items related to visual functioning. However, simply combining them in one common score will not accurately reflect the contribution of each to this overall score. This has largely been remedied through recently derived scales (socioemotional and visual functioning

scales) in combination with Rasch analysis.[229] Rasch analysis transforms raw nominal numeric questionnaire values into a continuous scale, reducing noise and allowing for parametric statistical analyses of the data.[230]

Since a clinically meaningful change in NEI VFQ is difficult to quantify, a additional supplementary question was asked at every visit (excluding baseline), where the patients were simply asked if their vision had "improved", "deteriorated" or exhibited "no change" since their most recent injection (supplementary questionnaire; see Appendix 4). These results were used to generate an individual's overall description of his/her experience over the course of the study period. Eligibility for inclusion in this aspect of the analysis required that an individual did not report both improvement and deterioration over the course of the study.

5.2.10 Statistical Analysis

Descriptive statistics were calculated for all measured variables, including demographic, ocular, psychophysical and morphological data, as well as data on subjective visual functioning (the questionnaire). VAR scores were used for the statistical analysis of CDVA data. Statistical analysis was performed using the software package PASW Statistics 18.0 (IBM Corp., Somers, NY, USA).

Baseline and exit visit measures were compared using the paired-samples *t* test. Correlations between observed changes in MFT (and MFV) and observed changes in psychophysical measures following serial anti-VEGF therapy were investigated using Pearson correlations. Power analysis, for the sample size of 43 subjects (following dropouts), yielded the following results: for detecting a correlation of 0.5, the power of a sample of this size is 0.94; for detecting a change of half a standard deviation on a paired t test, the power is 0.89. Tests were 2-sided in all analyses and the 5% level of

significance was used throughout. Bonferroni adjustment was incorporated when multiple tests were performed.

5.3 Results

5.3.1 Baseline data

Forty-seven patients (47 study eyes) met the inclusion criteria and were recruited into this study. Of the study eyes, 26 (55%) where pseudophakic and 21 (45%) were phakic, with no eyes with visually important lens opacities included. Baseline data were typically collected one or two days prior to intravitreal injection of ranibizumab in those cases where a course of anti-VEGF therapy was commencing or recommencing. The demographic, ocular, psychophysical and morphological data, as well as data on subjective visual difficulty, are given in Table 5.1. Of the 47 eyes, it was possible to obtain baseline measurements prior to the first injection (of that course of injections) in 16 participants who were commencing or recommencing treatment, one of whom did not continue beyond baseline. It was hypothesised that data from the subgroup might differ from study eyes where serial intravitreal treatment was already underway, because of the recent (re)activation of nv-AMD in this subgroup, and therefore warranted separate analyses. The remaining 31 study eyes were already undergoing treatment when recruited into the study (mean [±sd] and range of duration of prior treatment: 7 [±5] and 1-20 months, respectively). Eight of the 47 study patients were concurrently undergoing serial intravitreal ranibizumab treatment in their fellow eye, at enrolment. A total of 248 injections of ranibizumab were administered to the study eyes over the course of the investigation. The mean (±sd) and range number of injections per patient was 5.4 (±2.8) and 1-10, respectively, over the course of the study period.

Table 5.1 Baseline demographic, ocular, psychophysical, morphological and subjective visual difficulty data, in subjects with nv-AMD.

Variable	n (%)	Mean (±sd)	Range
Demographic			
Age (years)	47 (100%)	72.02 (9.61)	53 – 97
Gender			
Male	11 (23.4%)		
Female	36 (76.6%)		
Ocular			
Eye			
Right	24 (51.1%)		
Left	23 (48.9%)		
Psychophysical			
CDVA			
Study eye	47 (100%)	87.64 (9.04)	64 – 104
Fellow eye	47 (100%)	74.57 (31.15)	2 – 103
Reading performance			
LogRad	47 (100%)	0.320 (0.2)	0.005 – 0.905
Reading speed (wpm)	47 (100%)	85 (27)	31 – 142
Mean reading speed (wpm)	47 (100%)	136 (35)	32 – 216
PHP			
Total area	35 (74.5%)†	54.53 (44.60)	0.00 – 166.4
Total integrated intensity	35 (74.5%)†	11.10 (14.16)	0.00 – 54.60
CS (mesopic conditions)			
Frequency (cpd)			
1.5	46 (97.9%)†	20.43 (9.68)	3.50 – 36.00
3	46 (97.9%)†	29.28(18.81)	5.00 – 80.00
6	46 (97.9%)†	11.24 (9.45)	6.00 – 45.00
12	46 (97.9%)†	4.15 (1.03)	4.00 – 11.00
18	46 (97.9%)†	2.00 (0.00)	2.00 – 2.00
CS (photopic conditions)			
Frequency (cpd)			
1.5	46 (97.9%)†	22.10 (13.24)	3.50 – 71.00
3	46 (97.9%)†	35.74 (22.35)	10.00 – 114.00
6	46 (97.9%)†	20.59 (18.80)	6.00 – 64.00
12	46 (97.9%)†	5.67 (4.22)	4.00 – 22.00
18	46 (97.9%)†	2.48 (2.50)	2.00 – 18.00
GD (mesopic conditions)			
Frequency (cpd)			
1.5	46 (97.9%)†	11.46 (7.83)	3.50 – 36.00
3	46 (97.9%)†	16.54 (12.32)	5.00 – 57.00
6	46 (97.9%)†	7.65 (5.78)	6.00 – 33.00
12	46 (97.9%)†	4.09 (0.59)	4.00 – 8.00
18	46 (97.9%)†	2.00 (0.00)	2.00 – 2.00

GD (photopic conditions)			
Frequency (cpd)			
1.5	46 (97.9%)†	20.31 (12.09)	3.50 – 71.00
3	46 (97.9%)†	35.98 (24.67)	5.00 – 114.00
6	46 (97.9%)†	19.28 (19.23)	6.00 – 90.00
12	46 (97.9%)†	5.78 (5.73)	4.00 – 30.00
18	46 (97.9%)†	2.39 (1.61)	2.00 – 12.00
Mean retinotopic ocular sensitivity (dB)			
Fixation	45 (95.7%)†	8.71 (5.92)	0.00 – 20
Central 5°	45 (95.7%)†	9.70 (4.85)	0.44 – 19.56
Central 16°	45 (95.7%)†	11.02 (4.53)	1.40 – 19.10
Morphological (OCT)			
MFT (µm)	47 (100%)	232 (57)	126 – 403
MFV (µm)	47 (100%)	0.18 (0.05)	0.10 – 0.32
Subjective visual disability (questionnaire)			
Composite score	47 (100%)	89.92 (8.12)	65.91 – 100.00
Rasch-scaled LFVFS	47 (100%)	-1.71 (1.37)	-6.07 – -0.07
Rasch-scaled NVS	47 (100%)	-2.19 (1.29)	-4.38 – 0.10

Abbreviations: nv-AMD=neovascular age-related macular degeneration; n=number of subjects; CDVA=corrected-distance visual acuity; LogRad=log reading acuity; Reading speed (at best baseline LogRad value); mean reading speed (for range of LogRad values); wpm=words per minute; PHP=preferential hyperacuity perimeter; CS=contrast sensitivity; cpd=cycles per degree; GD=glare disability; dB=decibel; OCT=optical coherence tomography; MFT=mean foveal thickness; MFV=mean foveal volume; LFVFS=long-form visual functioning score;

†n≠47 as certain tests/measures could not be obtained or were unreliable

5.3.2 Follow-up data

Of the 47 patients recruited at baseline, four did not participate further. Of these, two fell ill (one patient was immobile due to a car accident, the other [aged 97] was not well enough to attend for further injections, and clinical review deemed that these events were unrelated to intravitreal injections of ranibizumab). A further two withdrew from the study for personal reasons. The mean (±sd) number of visits for the remaining 43 subjects was 6 (±2.6), with a range of 2-10 study visits. Patients were assessed, and data collected, approximately two weeks following each monthly injection of ranibizumab.

An analysis (2-tailed paired *t* test) was performed to investigate which, if any, of the measured parameters exhibited significant change over the course of the study

period (Table 5.2). The two time points chosen for this purpose were baseline and exit visits, respectively. An exit visit was defined as the patient's final study visit (two weeks after the preceding and final intravitreal injection in the study period). Therefore, in some cases, the exit visit was associated with cessation of treatment (n=23) as it was deemed, clinically, that maximum realisable benefits of treatment had been reached i.e. no evidence of active CNV (on FFA). The remainder (n=20) coincided with the termination of the study period (11 months; and these patients may have, therefore, continued with further intravitreal injections of ranibizumab following closure of the study).

Table 5.2 Baseline and exit data for study eyes in the entire study group and subgroup, between baseline and exit study visits.

	Entire group				**Subgroup**			
	Baseline	**Exit**			**Baseline**	**Exit**		
Variable	**Mean (±sd)**	**Mean (±sd)**	**p**	**Bon.?**	**Mean (±sd)**	**Mean (±sd)**	**p**	**Bon.?**
CDVA study eye	87.9 (9.3)	88.7 (10.3)	0.480	N	89.1 (12.1)	91.3 (13.1)	0.387	N
CDVA non-study eye	74.7 (30.2)	75.6 (30.1)	0.419	N	80.8 (23.3)	84.3 (21.9)	0.134	N
Reading performance								
logRAD	0.33 (0.20)	0.28 (0.22)	**0.032†**	N	0.25 (0.18)	0.19 (0.19)	0.139	N
Reading speed	85 (28)	103 (46)	**0.019**	N	85 (26)	118 (56)	**0.037**	N
Mean reading speed	136 (36)	146 (42)	**0.005**	N	148 (28)	166 (36)	**0.005**	N
Preferential Hyperacuity Perimetry								
Ta	51.2 (41.5)	60.3 (45.4)	0.165	N	45.1 (32.8)	48.7 (46.0)	0.810	N
Tii	9.8 (12.4)	11.9 (14.0)	0.250†	N	6.9 (6.2)	9.6 (11.4)	0.674†	N
CS (mesopic)*								
Frequency (cpd)								
1.5	19.47 (10.1)	30.22 (21.00)	**0.003**	N	20.30 (12.15)	39.43 (26.12)	**0.008**	N
3	28.93 (19.72)	42.02 (34.04)	**0.004†**	N	32.47 (24.01)	53.13 (33.87)	**0.036**	N
6	11.26 (9.78)	15.91 (17.32)	**0.002†**	Y	12.80 (11.19)	26.67 (25.14)	0.070	N
12	4.09 (1.17)	4.88 (2.27)	**p<0.001†**	Y	3.80 (0.77)	6.27 (3.35)	**0.001†**	Y
18	1.98 (0.15)	2.37 (1.25)	**p<0.001†**	Y	1.93 (0.26)	3.07 (1.98)	**0.001†**	Y
CS (photopic)*								
Frequency (cpd)								
1.5	21.50 (14.03)	27.22 (18.69)	0.163†	N	25.20 (18.18)	34.50 (23.06)	0.347	N
3	35.05 (23.68)	47.63 (30.12)	**0.005†**	N	40.00 (30.72	61.07 (32.54)	**0.020**	N
6	19.56 (19.28)	28.44 (30.98)	**0.001†**	Y	25.93 (22.52)	47.13 (42.60)	0.221	N
12	5.72 (4.00)	10.16 (15.29)	**p<0.001†**	Y	7.40 (6.53)	19.47 (23.08)	**0.025**	N
18	2.49 (2.59)	3.58 (4.01)	**p<0.001†**	Y	2.33 (1.59)	6.53 (5.82)	**0.016**	N
GD (mesopic)*								
Frequency (cpd)								

1.5	11.10 (7.88)	18.76 (15.03)	**0.002†**	Y	12.40 (9.79)	27.87 (17.80)	**0.019†**	N
3	16.47 (12.63)	29.28 (23.67)	**p<0.001**	Y	17.93 (14.80)	42.13 (30.51)	**0.001**	Y
6	7.51 (6.02)	11.49 (11.71)	**p<0.001†**	Y	10.33 (9.77)	19.87 (16.83)	0.134	N
12	4.02 (0.77)	4.70 (2.89)	**p<0.001†**	Y	3.80 (0.77)	6.00 (4.72)	**0.001†**	Y
18	1.98 (0.15)	2.09 (0.43)	**p<0.001†**	Y	1.93 (0.26)	2.27 (0.70)	**p<0.001†**	Y
GD (photopic)*								
Frequency (cpd)								
1.5	19.50 (12.57)	24.77 (14.71)	0.081†	N	20.40 (11.44)	30.80 (15.82)	0.220†	N
3	35.16 (25.82)	47.33 (33.20)	**p<0.001†**	Y	43.13 (30.86)	67.67 (39.65)	**0.039**	N
6	19.04 (19.46)	28.42 (31.20)	**0.001†**	Y	26.13 (25.76)	46.93 (40.53)	0.069	N
12	5.84 (5.96)	7.86 (9.21)	**p<0.001†**	Y	7.53 (9.21)	13.80 (13.66)	**0.022**	N
18	2.40 (1.68)	3.63 (4.05)	**p<0.001†**	Y	2.73 (2.63)	6.67 (5.83)	**0.021†**	N
Mean ROS (dB)								
Fixation	8.56 (5.91)	10.20 (5.71)	**0.026**	N	8.36 (7.32)	11.64 (6.72)	0.056	N
Central 5°	9.63 (4.83)	11.18 (4.48)	**0.003**	N	9.27 (6.46)	12.34 (5.17)	**0.013**	N
Central 16°	11.03 (4.49)	12.11 (4.00)	**0.005†**	N	10.32 (5.51)	12.55 (4.42)	**0.017†**	N
Optical Coherence Tomography								
MFT (µm)	233 (59)	205 (40)	**0.001†**	Y	275 (64)	208 (25)	**0.002**	Y
MFV (µm)	0.18 (0.05)	0.16 (0.03)	**p<0.001†**	Y	0.22 (0.05)	0.16 (0.02)	**0.002†**	Y
Subjective visual function								
Rasch-scaled LFVFS	-1.70 (1.41)	-1.86 (1.61)	0.222†	N	-2.21 (1.85)	-2.38 (1.77)	0.414	N
Rasch-scaled NVS	-2.21 (1.32)	-2.49 (1.48)	0.210†	N	-2.48 (1.28)	-3.10 (1.28)	**0.041†**	N

Abbreviations: Bon.=significant following Bonferroni correction?; Y=yes; N=no; CDVA=corrected distance visual acuity; reading speed (at best baseline LogRad value); wpm=words per minute; mean reading speed (for range of LogRad values); CS=contrast sensitivity; mesopic=under mesopic conditions; cpd=cycles per degree; photopic=under photopic conditions; GD=glare disability; ROS=retinotopic ocular sensitivity; dB=decibel; OCT=optical coherence tomography; MFT=mean foveal thickness; MFV=mean foveal volume; NVS=near vision score.

*all tests were performed on log-transformed data

†non-parametric tests were used as data was not normally distributed.

For the study group (n=43), a statistically significant improvement over time was observed for the following parameters: reading acuity (p=0.03); mean reading speed (p<0.01); reading speed at best baseline reading acuity (p=0.019); mesopic CS, at all spatial frequencies (p<0.01 for all values); photopic CS at 3, 6, 12 and 18 cpd (p<0.01, for all); mesopic GD, at all spatial frequencies (p<0.01, for all); photopic GD at 3, 6, 12 and 18 cpd (p<0.01, for all); ROS at fixation (p=0.026) and within the central 5 and central 16 degrees of fixation (p<0.01) (Figure 5.4); MFT and MFV (p<0.01, for both). Of note, there was no significant change in CDVA in the study group (Figure 5.5) or subgroup (p>0.05, for all).

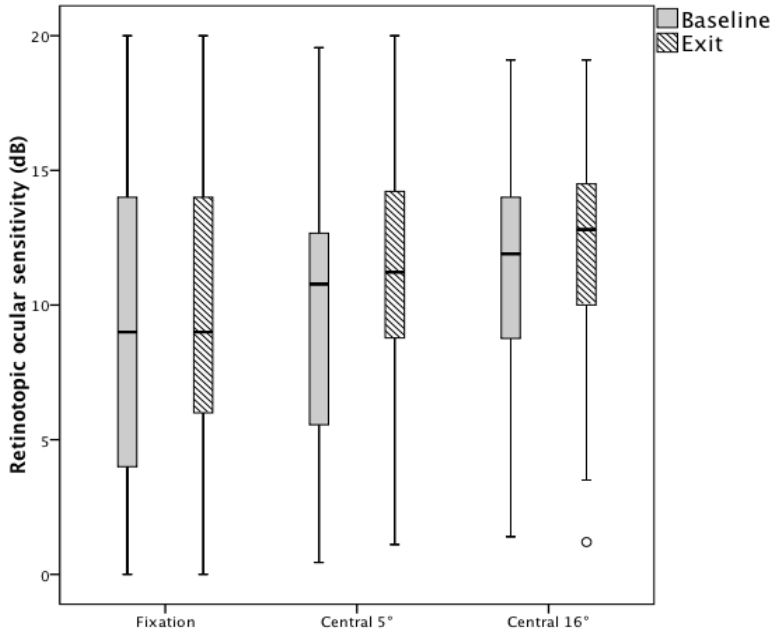


Figure 5.4 Box plot values for measures of mean retinotopic ocular sensitivity at baseline and exit study visits at fixation, within the central 5 degrees of fixation and within the central 16 degrees of fixation, for the entire study group (paired *t* test: p= 0.026, p=0.003 and p=0.005, respectively).

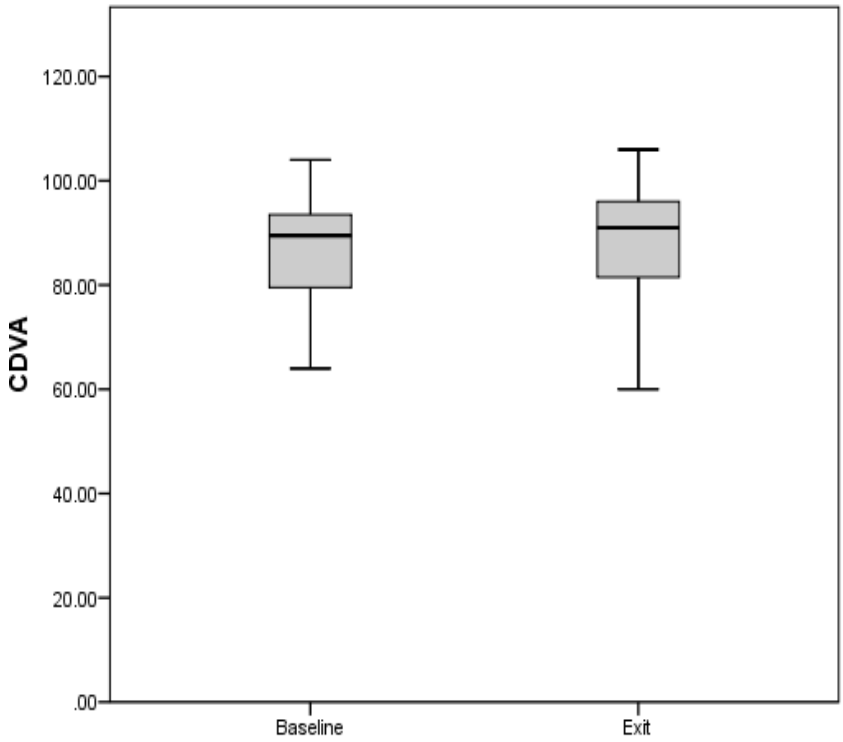


Figure 5.5 Corrected distance visual acuity (CDVA) at baseline and at exit study visits in the study group.

Possible correlations were investigated between baseline data and observed changes in MFT over the course of the study period for the study group and subgroup to investigate prognostic indicators for change in MFT. Significant associations are given in Table 5.3. All other parameters were non-significant ($p>0.05$, for all). Figure 5.6 graphically represents the relationship between baseline ROS (within the central 5°) and change in MFT.

Table 5.3 Statistically significant correlations between baseline psychophysical measures of visual function and change in mean foveal thickness (baseline to exit) for the study group and subgroup.

	Entire study group		Sig. after Bonferroni?	Subgroup		Sig. after Bonferroni?
Variable	**r**	**p**		**r**	**p**	
CDVA	0.353*	0.020	No	-	-	-
LogRad	-	-	-	-0.762**	0.001	Yes
LogCSmesopic_3cpd	-	-	-	0.644*	0.013	No
LogCSmesopic_6cpd	-	-	-	0.716**	0.004	No
LogGDmesopic_1.5cpd	-	-	-	0.639*	0.014	No
LogGDmesopic_3cpd	-	-	-	0.685*	0.007	No
LogGDphotopic_6cpd	-	-	-	0.735**	0.003	No
ROS fixation	0.494**	0.001	Yes	0.808**	<0.001	Yes
ROS central 5°	0.472**	0.002	Yes	0.708**	0.005	No
ROS central 16°	0.370*	0.017	No	0.623*	0.017	No

Abbreviations: CDVA=corrected-distance visual acuity; LogRad=log reading acuity; CS=contrast sensitivity; cpd=cycles per degree; GD=glare disability; ROS=retinotopic ocular sensitivity

ROS=retinotopic ocular sensitivity; -=not significant

*correlation significant at the 0.05 level (2 tailed)

**correlation significant at the 0.01 level (2 tailed)

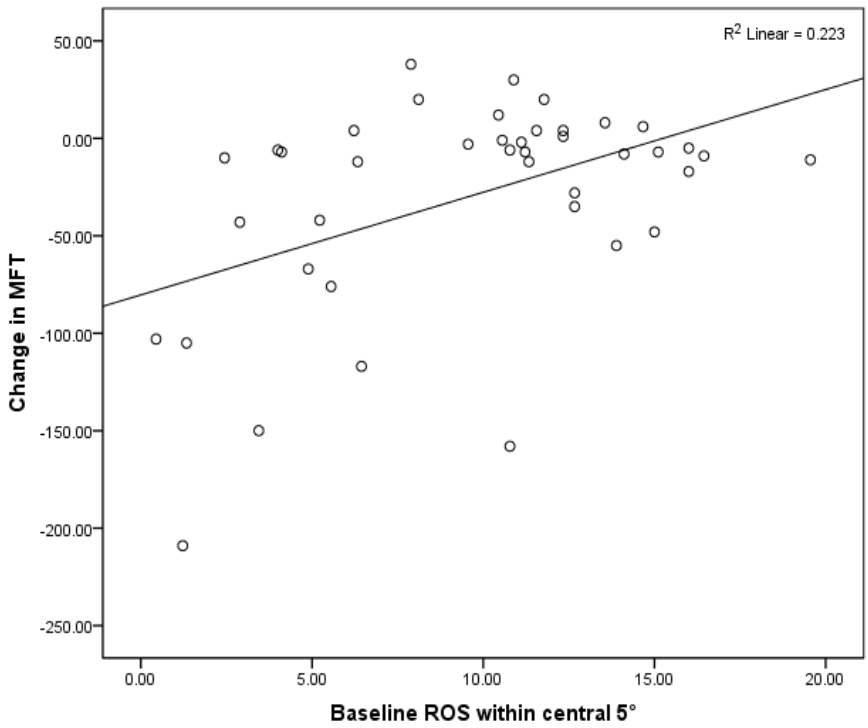


Figure 5.6 Relationship between change in mean foveal thickness (MFT) and baseline measures of retinotopic ocular sensitivity (ROS) within the central 5° of fixation.

An analysis was performed to investigate the relationship between observed changes in MFT and observed changes in other parameters for the entire study group and subgroup, and statistically significant findings are displayed in Table 5.4. Figure 5.7 graphically represents the relationship between change in ROS (within the central 5°) and change in MFT, and Figure 5.8 displays the relationship between change in CDVA and change in MFT (following the removal of three outliers).

Table 5.4 Significant correlations between observed changes in mean foveal thickness and observed changes in other parameters for the entire study group and subgroup.

Variable	Entire group		Sig. after Bonferroni?	Subgroup		Sig. after Bonferroni?
	r	p		r	p	
CDVA†	-0.311*	0.042	No	-0.569*	0.027	No
LogGDmesopic_1.5cpd	-0.334*	0.031	No	-	-	-
LogGDmesopic_3cpd	-0.344*	0.026	No	-	-	-
LogGDmesopic_18cpd	-0.348*	0.024	No	-	-	-
ROS fixation	-0.411**	0.008	No	-	-	-
ROS central 5°	-0.592**	<0.001	Yes	-0.611*	0.020	No
ROS central 16°	-0.536**	<0.001	Yes	-0.554*	0.040	No

Abbreviations: CDVA=corrected-distance visual acuity; CS=contrast sensitivity; mesopic/photopic=under mesopic/photopic conditions; cpd=cycles per degree; GD=glare disability; ROS=retinotopic ocular sensitivity; MFV=mean foveal thickness.

*correlation significant at the 0.05 level (2 tailed)

**correlation significant at the 0.01 level (2 tailed)

†becomes non-significant with the removal of 3 outliers (r=-0.271; p=0.091); an outlier was defined as having a change in CDVA of more than 2 standard deviations from the mean.

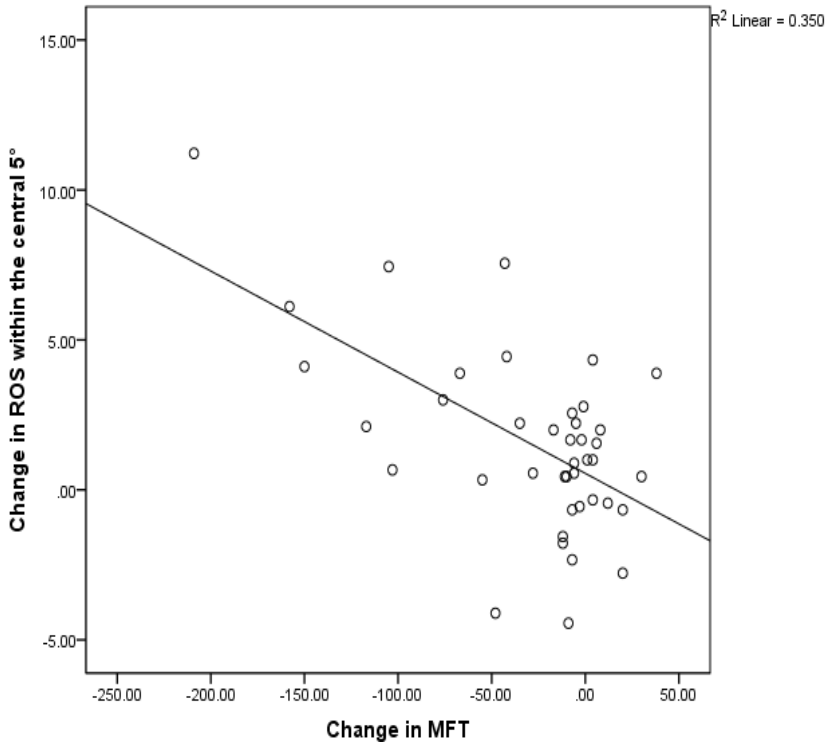


Figure 5.7 Relationship between change in mean foveal thickness (MFT) and change in retinotopic ocular sensitivity (ROS) within the central 5° of fixation

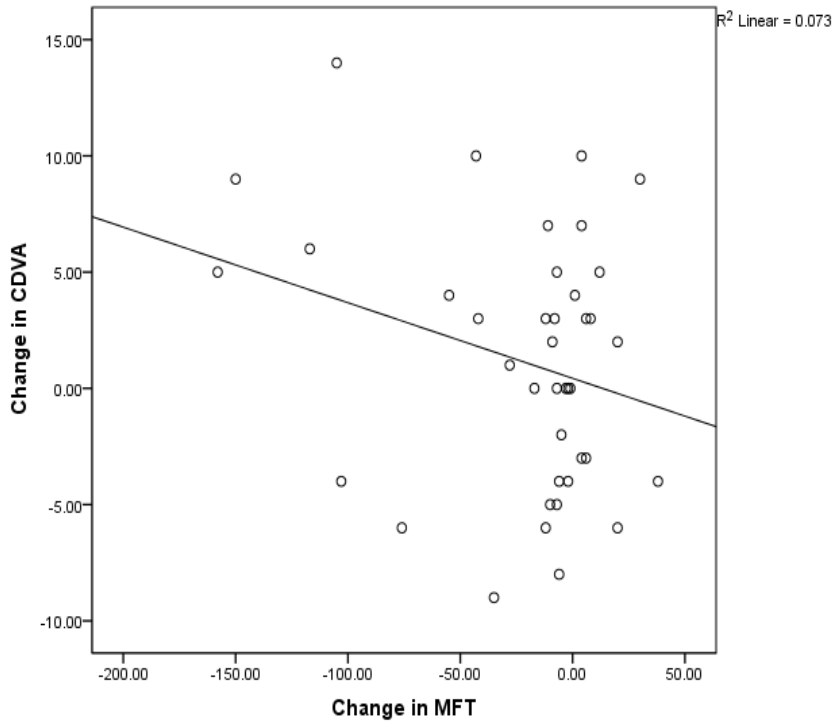


Figure 5.8 Relationship between change in mean foveal thickness (MFT) and change in corrected distance visual acuity (CDVA) (following the removal of three outliers).

Analysis was then performed to investigate the relationship between observed changes in Rasch-scaled LFVFS and NVS over the study period, and observed changes in other parameters (psychophysical and measures of retinal thickness), and statistically significant correlations are given in Table 5.5. Of note, there was no significant correlation between observed changes in LFVFS and observed changes in CDVA (r=-0.278; p=0.082).

Table 5.5 Statistically significant correlations between observed changes in Rasch-scaled LFVFS and NVS, and observed changes in psychophysical measures over the study period for the entire study group and subgroup.

Variable	Entire group		Sig. after Bonferroni?	Subgroup		Sig. after Bonferroni?
LFVFS	**r**	**p**		**r**	**p**	
Reading acuity	-	-	-	0.537	0.039	No
LogCSphotopic_3cpd	-0.400*	0.012	No	-	-	-
LogCSphotopic_6cpd	-0.407*	0.010	No	-	-	-
LogGDphotopic_6cpd	-0.380*	0.017	No	-	-	-
ROS fixation	-0.392*	0.015	No	-	-	-
NVS	**r**	**p**		**r**	**p**	
CDVA	-0.372*	0.018	No	-0.653**	0.008	No
Reading acuity	-	-	-	0.706**	0.003	No
LogCSphotopic_3cpd	-0.324*	0.044	No	-	-	-
ROS fixation	-0.464**	0.003	No	-0.754**	0.002	Yes
ROS central 5°	-	-	-	-0.663**	0.010	No

Abbreviations: LFVFS=long-form visual function score; reading acuity=best LogRad value; CS=contrast sensitivity: photopic=under photopic conditions; cpd=cycles per degree; GD=glare disability; ROS=retinotopic ocular sensitivity; CDVA=corrected distance visual acuity.

*correlation significant at the 0.05 level (2 tailed)

** correlation significant at the 0.01 level (2 tailed)

Thirty-seven patients met the criteria for the supplementary questionnaire analysis; 24 (65%) indicated an overall improvement, 9 (24%) noted no change and 4 (11%) reported deterioration in their vision, over the course of the study. Analysis of variance was then used to investigate the relationship between study eyes that were self-reported by participants as exhibiting an “improvement”, “no change” or “deterioration” with respect to changes in parameters of visual function and macular thickness over the course of the study period. Significant relationships were identified between changes in mesopic CS (and GD) at 6 cpd and self-reported improvement in visual function in the study eye ($p=0.030$ and $p=0.043$, respectively), but for no other parameter of visual function ($p >0.05$ for all).

5.4 Discussion

This study investigated visual function, and its response to treatment, through a range of psychophysical tests, in patients with nv-AMD undergoing serial intravitreal injections of ranibizumab, and explored whether alternative measures of visual function are more appropriate than, or complimentary to, CDVA in reflecting the patient's visual experience. In addition, I investigated if any such alternative parameters were more sensitive to changes in retinal thickness than CDVA and whether or not they were of prognostic value.

Values for MFT and MFV decreased in response to treatment, between first and final visits, consistent with previous studies (see example Figure 5.11).[153, 344, 367] In this analysis, CDVA did not change significantly for the study group or subgroup, between baseline and exit visits. Parameters of visual function that did improve for the study group and subgroup, however, included: reading acuity and reading speed, CS under mesopic and photopic conditions, GD under mesopic and photopic conditions, and ROS. The study eyes in subgroup exhibited an additional and statistically significant improvement in the Rasch-scaled NVS. Interestingly, this observed improvement in Rasch-scaled NVS within this subgroup was significantly associated with an observed improvement in reading acuity (LogRAD), over the course of the study period.

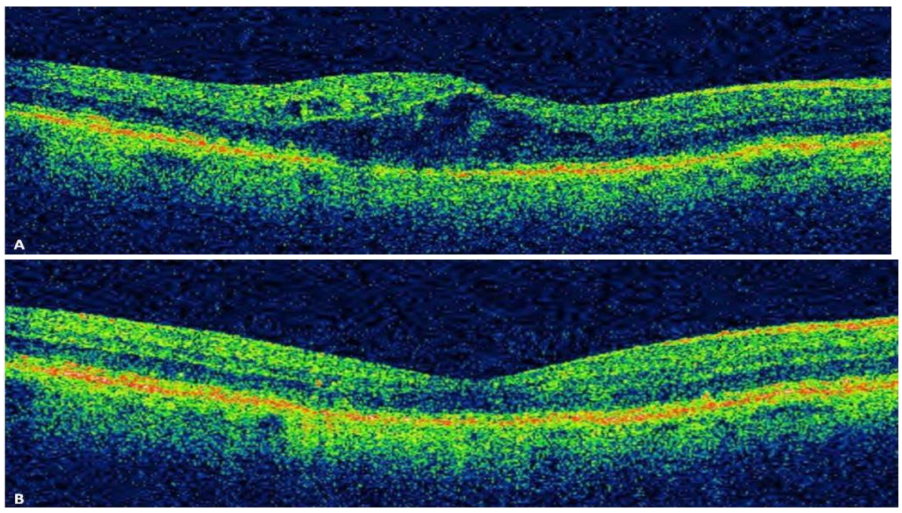


Figure 5.9 OCT macular scans at A) baseline with presence of intraretinal fluid and intraretinal cysts and, B) at exit, representing normal macular architecture.

Mean CDVA did not improve significantly over the course of the study for the group or subgroup. This finding may be attributable to our inclusion criteria and to the short period of follow-up in this study. Studies have shown that poor baseline CDVA is associated with a greater benefit of treatment in terms of this outcome measure,[342, 343] and these observations are consistent with the findings of another study, which reported no significant improvement in CDVA in patients with nv-AMD in patients where baseline CDVA was 6/12 (logMAR 0.3) or better.[368] Given that, in the current study, all study eyes had baseline CDVA of logMAR 0.7 or better (indeed, 42 of the 43 eyes had baseline CDVA of logMAR 0.6 or better), and given the ceiling effect previously discussed, it is perhaps unsurprising that no statistically significant improvement in CDVA was observed in our study, especially in light of the short period of follow-up. In addition, improvements in CDVA may not have been observed due to the fact that a proportion of the subjects in our study were already undergoing anti-VEGF therapy at the study onset.

It has been shown that CS may be an important measure of visual function in patients with subfoveal CNV due to AMD, providing additional information that cannot be obtained from visual acuity.[175] Studies have suggested that, when compared with

visual acuity, CS better measures the ability to perform tasks accurately and efficiently, as well as the ability to discriminate between objects[177] and judge distances.[178] Also, GD is a clinically important problem in AMD, and impacts adversely on mobility performance.[369] Interestingly, Sandberg et al have proposed that a slow recovery from glare may be an independent risk factor for the development of CNV.[370] In this study, all measures of CS and GD for all spatial frequencies improved significantly over the course of the study period and only two of the twenty parameters measured did not reach statistical significance (for the entire study group). It is also interesting to note, when testing CS under photopic conditions, 81% and 96% of study eyes could not see any target at either 12 or 18 cpd, respectively, at baseline, and these proportions decreased by exit visit (to 75% and 84%, respectively).

A progressive improvement in ROS in response to ranibizumab therapy for nv-AMD, as far as 24 months following the initiation of treatment and in spite of stabilisation of VA after six months, has been previously demonstrated.[7] In the current study, ROS, within the central 5 degrees and within the central 16 degrees, improved significantly for the study group and subgroup, whereas ROS at fixation improved significantly only when the entire study group was analysed. Microperimetry has also proved useful in monitoring response to other modes of treatment, such as verteporfin therapy.[371]

For the study group and subgroup, a significant improvement in reading speed was observed between baseline and exit visits over the course of the study, but this observed improvement did not correlate with a change in CDVA. Such a disparity has been previously observed by Frennesson et al, who suggested that a change in CDVA does not necessarily relate to a change in near vision.[372] However, it should be stated that, in the current study, the repetitive nature of the test (every month), and the consequential patient familiarity with the test texts (despite comprehensive patient

training at baseline) may have contributed to the observed improvement in reading speed over the course of the study period.

PHP values (TA and TII) exhibited no change over the course of the study period, nor did observed changes in these PHP values correlate with observed changes in MFT or LFVFS. A study by Das et al did report a significant association between TA (of distortion recorded) and reduction in subretinal fluid following anti-VEGF therapy.[351] It has failed, however, at least according to the results of the current study, to exhibit significant change in study eyes with nv-AMD which are undergoing successful serial monthly intravitreal ranibizumab therapy. Indeed, self-reported improvement in visual function over the course of the study period was not associated with changes in PHP. These findings may be due to the fact this test was designed specifically to detect recent-onset CNV and distinguish it from intermediate AMD,[213] and only 15 (of 43) subjects in this study could be classified as having recent-onset CNV. The PHP, then, may not have been sensitive enough to detect a change in subjects with nv-AMD who were already undergoing serial intravitreal anti-VEGF therapy, particularly when one considers that the greatest visual benefit (in response therapy) occurs within the first three months of treatment.[150, 343]

Measurements of subjective visual difficulty did not change over time, either when graded according to NEI guidelines, or when questionnaire scores were subjected to Rasch analysis for the study group or subgroup (with the exception of improvement in the NVS for study eyes in subgroup). This finding is at odds with previous reports, where subjectively perceived visual improvement following anti-VEGF therapy has been detected using validated questionnaires.[363, 373] Possible reasons for this finding may include the relatively short duration of the current study, given that treatment for this condition may last for years; only subjects with relatively good CDVA were assessed, who are likely to be less symptomatic compared to subjects with poor CDVA,

thus reducing the scope of potential visual improvement amongst the current study's recruits. Also, the confounding effect of the status of the fellow eye on overall visual experience could be influential in respect of this aspect of our investigation. Further, and particularly at baseline, it may be difficult for a patient to have yet gauged, much less quantified, the impact of his/her condition on aspects of their daily quality of life (e.g. playing golf, night driving). In the current study, this latter point may be particularly important, as an intravitreal injection of ranibizumab was administered within one week of diagnosis for all study eyes. Also, decision-to-treat, in cases of nv-AMD, and in contrast with more insidious conditions such as cataract, is not made solely on the basis of patient symptoms, and may even be recommended when the patient is asymptomatic, thus rendering the usefulness of a questionnaire such as the NEI VFQ questionable for this purpose. Finally, it should be emphasised that stabilisation of visual function can be deemed to be a successful outcome of intravitreal anti-VEGF therapy in cases of nv-AMD. Although the NEI VFQ did not exhibit improvement over the course of the study period for the study group or subgroup, it is important to note that deterioration was not observed either for the study group or subgroup, deterioration being the expected outcome of the natural history of nv-AMD.

Of note, 66% of patients reported an overall improvement in vision over the course of the study, when using a supplementary questionnaire. Bearing in mind this was not a validated questionnaire and had no means of quantifying the level of improvement or deterioration experienced by the patient, it could be considered, at least in the context of this study, and because of its within-subject and temporal comparative nature, to represent a truer reflection of patient experience. The results of this particular analysis suggest that subjectively perceived improvement in visual function in cases of nv-AMD undergoing intravitreal anti-VEGF therapy has a closer association with CS and GD at medium spatial frequencies, than with CDVA.

Of note, and although the study group or subgroup did not exhibit significant change in terms of CDVA or LFVFS over the course of the study, some subjects did exhibit change in the above parameters, thus facilitating analysis of relationships between such changes and other outcome measures. These relationships are discussed with full appreciation of the fact that correlations, while identifying relationships between variables, may or may not represent causal relationships.

Given the importance of OCT in the diagnosis and decision-to-treat/decision-to-discontinue treatment in cases of nv-AMD, the relationship between observed changes in MFT and observed changes in the psychophysical parameters over the course of the study period was analysed. Although there was a significant correlation between observed changes in MFT and observed changes in CDVA (which, notably, became non-significant with the removal of outliers) and also with observed changes in GD under mesopic conditions at low and high spatial frequencies, the strongest such association was with observed change in ROS, both at fixation, but more robustly, within the central 5 and 16 degrees of fixation. Changes in microperimetry have also been shown to reflect changes in macular thickness for other eye conditions, such as diabetic macular oedema.[206, 374] Microperimetry examines the light differential threshold at the retina, and in this respect differs from VA, a measure of the angular resolution limits of the eye at high contrast. The advantage of ROS is that it is retinotopic, a function which allows it to probe visual function more deeply than would CDVA. Intuitively, therefore, one would expect that measures of ROS are more appropriate than CDVA when attempting to correlate function and morphological changes at the macula for conditions such as AMD. On the basis of this rationale, and on the basis of the findings of the current study, I believe that ophthalmologists should consider incorporating measures of ROS into the routine assessment and monitoring of patients with AMD.

For the entire study group, significant correlations were detected between changes in patient-reported experience for the entire study group and changes in CS (under photopic conditions at 3 and 6 cpd), GD (under photopic conditions) at 6 cpd, and ROS at fixation, but not with CDVA. In other words, such measures of visual function appear to better reflect changes in subjectively perceived visual function than does CDVA. These results should be interpreted with full appreciation of the fact that they were not significant at the 1% level, in the absence of correction for multiple testing. Of note, change in CDVA was associated with change in the NVS.

The psychophysical prognostic indicators for reduction in macular thickness for the entire study group following treatment were CDVA and ROS, ROS displaying significance at the 1% level. Analysis on study eyes in subgroup demonstrated further parameters of potential prognostic value, including: reading acuity, CS and GD under mesopic conditions at 3 cpd, CS and CS and GD under photopic conditions at 6 cpd. In other words, a greater reduction in MFT over the course of the study period was associated with worse measures of these aspects of visual function at baseline, a finding that is unsurprising as these measures of visual function will be grossly and adversely affected where MFT is greater, thus allowing for a more substantial reduction in MFT (and a parallel improvement in these parameters) over time. However, it should be noted that this finding will have been affected in the current study by confounding attributable to the fact that CDVA of logMAR 0.7 or better was an inclusion criterion. While CDVA does not appear to be either the most robust or most sensitive outcome measure in patients undergoing intravitreal anti-VEGF therapy for nv-AMD, this study has shown that it does have some prognostic value for eyes undergoing such treatment for this condition, at least where baseline acuity is relatively good (logMAR 0.7, or better).

In an attempt to achieve best outcomes without overtreating patients with nv-AMD, the posology for intravitreal ranibizumab for this condition has recently been revised (http://www.medicines.ie/medicine/11837/SPC/Lucentis+10mg+ml+Solution+for+Injection/#POSOLOGY). This revision of posology was informed by the evolving body of literature since the publication of the phase III MARINA and ANCHOR trials, where monthly injections were given for a period of two years. In brief, it is now recommended that monthly injections are given until best CDVA is achieved and maintained for three consecutive injections, when interruption of treatment is recommended with monthly monitoring. Where deterioration in CDVA, attributable to activity of nv-AMD, is observed, recommencement of treatment is recommended under the same regime. In light of this revised posology, however, the results of our study strongly suggest that CS-guided or ROS-guided re-treatments are likely to be more sensitive indicators of functional deterioration, and would, therefore, prompt recommencement of treatment at an earlier stage than would a deterioration in CDVA, thereby reducing the risk of irrecoverable loss of visual function prior to re-treatment.[375]

5.5 Conclusion

This study has demonstrated improvements in many parameters of visual function in eyes with nv-AMD undergoing monthly intravitreal ranibizumab injections. Outcome measures other than CDVA, such as CS, GD and ROS, should not only be considered in the design of studies investigating nv-AMD, but also in treatment and retreatment strategies for patients with the condition, at least in eyes where baseline CDVA is logMAR 0.7 or better.

Chapter 6. Supplementation with three different macular carotenoid formulations in subjects with early age-related macular degeneration.

6.1 Study rationale, aims and objectives

Although anti-VEGF therapy has resulted in better outcomes for patients with nv-AMD,[376] this treatment is cumbersome to the patient and to the healthcare provider, often requiring many months/years of monthly intravitreal injections. In addition, there is, as yet, no effective treatment for atrophic AMD, which has similarly detrimental effects on a patient's quality of life.[21]

Investigators interested in exploring ways of preventing or delaying the onset of AMD, or at least retarding its progression, have directed their attention towards the possible protective role of MP, and its constituent components: L, Z and MZ. There is also a strong rationale to suggest that MP can enhance visual performance in subjects with the condition.

While L and Z can be obtained from many foods,[377] MZ is not present in a conventional western diet, although it has been identified in certain types of seafood.[378] However, it should be noted that there is a paucity of studies conducted to test foods for the presence of MZ, and further study in this area is needed. Interestingly, MZ has been found, albeit in trace amounts, in serum of subjects who have not been supplemented with this carotenoid.[379] Certain properties of MZ render this carotenoid of particular interest to those exploring ways of preventing or delaying the onset of AMD, or ameliorating the course of the condition, or studying the contribution MP makes to visual performance and experience (in subjects with and without ocular disease), and these include: MZ is generated from L in the primate retina;[380] it is the dominant

carotenoid at the epicentre of the macula (Figure 6.1);[381] MZ accounts for about one third of total MP;[253] it appears to be the most powerful antioxidant of the macular carotenoids in the presence of the xanthophyll binding proteins;[382] the presence of all three macular carotenoids is required if MP is to maximally exert its antioxidant effects;[319] the presence of MZ facilitates a wider range of pre-receptoral blue light filtration by MP.[317, 318]

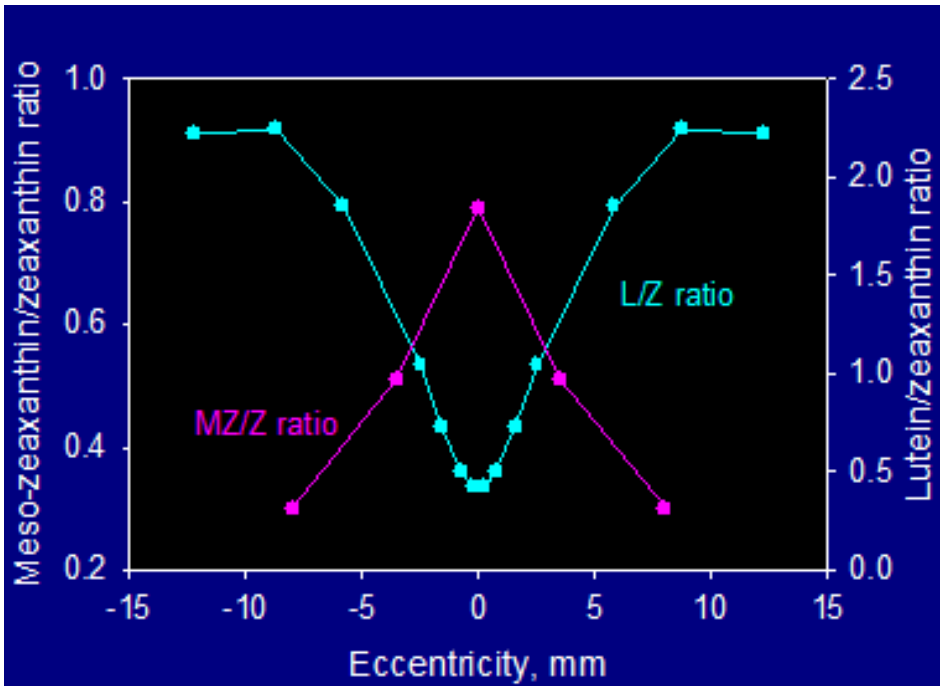


Figure 6.1 The ratio between MZ and Z concentrations (pink line) and the ratio between L and Z concentrations (turquoise line), at the macula (*image courtesy of Prof. Richard Bone*).

Interestingly, an atypical central dip in the spatial profile of MP, characterised by the lack of a central peak in MPOD, is associated with risk for AMD.[321] It is reasonable to hypothesise that such atypical profiles may be attributable, at least in part, to an inability to convert retinal L to retinal MZ, and a consequential lack of MP at the site of dominance of this carotenoid (i.e. at the foveal centre). Interestingly, supplementation with a formulation containing MZ has the ability, to rebuild MP

centrally and confer a typical central peak on its spatial profile.[322, 379] It has also been shown that, in normal healthy subjects, a formulation containing all three macular carotenoids (including MZ) has the greatest impact, in terms of enhancing visual performance, as measured by CS (both in the presence and absence of glare), when compared to placebo or when compared to a formulation that contains only L (and lesser amounts of Z).

While the role of MZ for visual performance in normal subjects has been explored,[170] no trial to date has yet investigated the impact of a supplement containing MZ on visual performance (or disease progression), in subjects with early AMD. In addition, the majority of clinical trials that have focused on visual outcomes following macular carotenoid supplementation have focused, for the most part, on CDVA. Evidence suggests that other psychophysical measures of visual performance should be considered when attempting to quantify visual changes in response to supplementation. Further, no trial has yet investigated the impact of a supplement containing MZ on the progression of AMD.

MOST AMD (a sister trial to MOST Vision, discussed in section 4.5.3.2) was designed to investigate the effect of three different macular carotenoid formulations (one of which contains all three macular carotenoids [L, Z and MZ]), on MP enhancement, on visual performance (taking into consideration a range of psychophysical measures) and on disease progression, in subjects with early AMD (MOST Vision assessed normal subjects).

6.2 Methods

6.2.1 Study design

This study was conducted at the Institute of Vision Research and Institute of Eye Surgery, Waterford, Ireland. Early AMD was defined as the presence of drusen and pigmentary changes and the absence of any signs of late AMD (GA or nv-AMD). Recruitment was mediated through the Institute of Eye Surgery (ophthalmology clinic) and Institute of Vision Research, through advertisement in local media and through leaflet, poster and flyer distribution to optometrist practices locally and nationally. Inclusion criteria were: early AMD in at least one eye (confirmed by an ophthalmologist at a screening clinic and subject to subsequent corroboration by an accredited reading centre); CDVA of ≥ 6/12 (logMAR 0.3) in the study eye. Exclusion criteria were: a recent history (within three months of baseline visit) of macular carotenoid supplementation; diabetes mellitus; any visually consequential ocular co-morbidity. Ethics approval was granted by the Waterford Regional Hospital Ethics Committee (Appendix 5), and written and informed consent was secured from each subject (Appendix 6). The research was conducted in accordance with the principles of the Declaration of Helsinki. All subjects were naive to the tests involved (with the exception of CDVA).

This study, titled the *Meso*-zeaxanthin Ocular Supplementation Trial [MOST]: (trial registration number: ISRCTN60816411) is a randomised single-blind clinical trial of oral supplementation with one of three different macular carotenoid formulations. The supplements were prepared in a soft-gel capsule. Subjects were randomly assigned to one of three supplementation groups, as follows: Group 1: 20mg of L and 2mg of Z (Ultra Lutein™; supplied by Nature's Plus, Europe); Group 2: 10mg of MZ, 10mg of L and 2mg of Z (Macushield™; supplied by MacuVision, Europe); Group 3: 17 mg MZ, 3 mg of L and 2mg of Z (supplied by Industrial Organica, Mexico). Study subjects were

required to consume one tablet per day with a meal. Compliance was monitored by tablet counting at each study visit and encouraged by regular phone calls. Study visits were carried out at baseline, six and 12 months.

Visual acuity, and CS and GD using the FVA™ were carried out as outlined in Chapter 6 (sections 6.1.2 and 6.1.3, respectively). Of note, the unavailability of the FVA™ at the study outset resulted in a reduced number of complete (baseline and 12 month) datasets for measurements using this device (n=39).

ROS was carried out as described in Chapter 6 (section 6.1.4), the exception being the number of retinal loci tested and the extent of the visual field that was examined. This change in method was as follows: The central 12 degrees were examined, using a total of 29 stimuli. ROS was calculated for the following three areas: within the central 4 degrees of fixation (using an average of 13 stimuli), within the central 8 degrees of fixation (average of 21 stimuli), and within the central 12 degrees of fixation (average of 29 stimuli).

6.2.2 Contrast sensitivity by letter chart

CS was measured (in the study eye only) at five separate spatial frequencies (letter sizes) using the logMAR chart provided by Test Chart 2000 PRO® at a test distance of 4m and at a constant room illuminance of 870 lux (photopic conditions), using distance spectacle correction, if required.

Contrast of a letter is defined as follows (Weber contrast):

$$\frac{I - I_{\mathrm{b}}}{I_{\mathrm{b}}},$$

where I represents the luminance of the character and I_b represents the luminance of the background.

Each patient was asked to identify (ETDRS) letters, presented in one isolated row (spatial frequency) at a time. For each spatial frequency tested, the contrast of the letters was reduced systematically using the software's contrast adjustment function (calibrated prior to commencement of the study using a light meter) until the contrast threshold of the patient was reached i.e. the patient could not distinguish any more letters. The software's letter randomisation program selected five random letters each time a different contrast was tested. The patients were encouraged to take their time whilst trying to identify the letters (for adaptation purposes), particularly approaching their threshold contrast level, and to blink regularly.

The average of three different readings was taken for the lowest contrast at which letters were legible to the patient, in a similar manner to that used in CDVA testing. The percentage contrast level (of the target), CS, and logCS for each spatial frequency (row of letters) were recorded. Table 6.1 shows the contrast levels tested and their corresponding CS and logCS values. Any missed or any additional correctly identified letters i.e. on a subsequent line, were each assigned a (logCS) value of -0.03 and +0.03, respectively, and the total was added to the final logCS score.

Table 6.1 Contrast levels tested using letter chart, and corresponding CS and logCS values.

% Contrast	CS*	logCS
100	1.00	0.00
71.0	1.41	0.15
50.1	2.00	0.30
35.5	2.82	0.45
25.1	3.98	0.60
17.8	5.62	0.75
12.6	7.94	0.90
8.9	11.24	1.05
6.3	15.87	1.20
4.5	22.22	1.35
3.2	31.62	1.50
2.2	44.67	1.65
1.6	62.50	1.80
1.1	89.13	1.95
0.8	125.00	2.10
0.6	178.57	2.25

Abbreviations: CS=contrast sensitivity

*CS=100 divided by % contrast

Five different letter sizes (spatial frequencies) were tested, each having the following angular subtense in cycles per degree (cpd): 6/120 (1.2cpd), 6/60 (2.4cpd), 6/24 (6cpd), 6/15 (9.6cpd) and 6/9.5 (15.2cpd). It should be noted that letters, by nature, can contain many different spatial frequencies, so it is not possible to assign a precise spatial frequency to a particular letter. However, it has been suggested that the most important frequency for letter identification is two cycles per letter width.[383] Take the letter **F**, for example; it contains approximately two cycles (two dark bands and two white bands). The spatial frequencies cited above represent those calculated on this basis (see Appendix 7).

6.2.3 Macular pigment optical density

Each subject's MP spatial profile was obtained using the Macular Densitometer™, a device that has been slightly modified from that developed and described by Wooten et

al.[384] The device uses heterochromatic flicker photometry (HFP) to obtain a measure of MPOD at five retinal eccentricities.

HFP is based on the principle that MP (a yellow pigment), located anterior to the photoreceptor layer in the retina, absorbs incident blue light before it reaches the photoreceptors. MP's peak absorption is at approximately 460nm. The test requires the subject to make iso-luminance matches between two flickering lights, which alternate between a wavelength band absorbed by MP (blue) and one that is not (green). The radiance of the blue light (absorbed by MP) is adjusted until the subject's perception of flicker is minimised or eliminated. The higher the individual MP level at a particular retinal eccentricity, the more blue light that will be required to match the luminance of the green light to minimise the flicker (Figure 6.2). The log ratio of the amount of blue light absorbed centrally, where MP peaks, to that absorbed at a peripheral retinal locus (in this case, 7°) where MP is optically undetectable, gives a measure of the individual's MPOD.

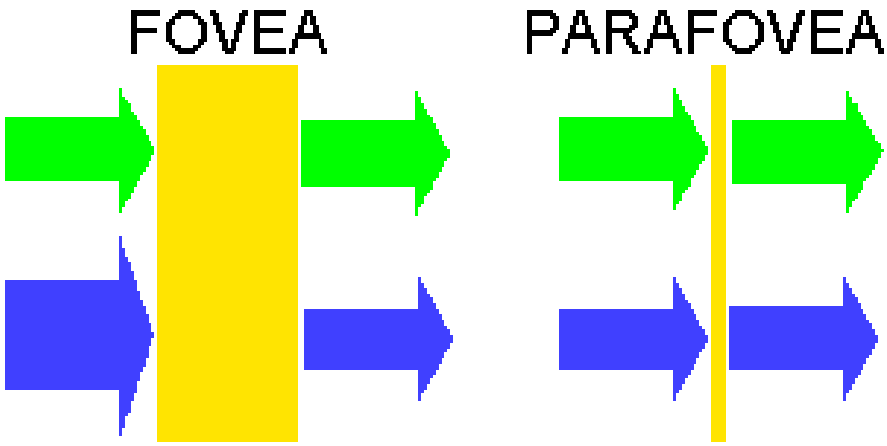


Figure 6.2 Principle of heterochromatic flicker photometry. At the fovea, more blue light (large blue arrow) is required to match the luminance of the green light than at the parafovea (reference point), as MP (indicated in yellow) is concentrated at the fovea and is optically undetectable at the parafovea (reference point). (*Image courtesy of the MPRG, Waterford*)

Typically, there is an inter-individual range of alternation rates where flicker is not perceived and this range is called the null zone. Customised HFP (cHFP)[385] was designed to accommodate for these differences in flicker sensitivity amongst individuals, which are known to vary with age and disease.[386, 387] If a fixed flicker frequency is used, a subject with low flicker sensitivity (i.e., low critical flicker fusion frequency) will most likely experience a large null flicker zone. On the other hand, a subject with a high critical flicker frequency may not be able to eliminate flicker from the test target, which would make the task difficult to complete. Therefore, predicted optimal flicker frequency rates for the targets at each eccentricity (determined using an age-guided algorithim; see Table 6.2) were used to customise the test for each subject to facilitate accurate subject performance and reduce measurement error (see also publication by Connolly et al[379]).

Table 6.2 Age-guided optimal flicker frequencies for Densitometer™ targets.

Age*	**OFF for each retinal eccentricity**		
	0.25°	**0.5°, 1°, 1.75°**	**7°**
18-20	18	19	13
21-20	18	19	12
31-40	17	18	11
41-50	15	16	10
51-60	13	14	9
61-70	12	13	8
71-80	11	12	7
81+	10	11	6

OFF = optimal flicker frequency;

*years

The spatial profile of MP was measured at four different retinal eccentricities: 0.25°, 0.5°, 1°, and 1.75°, with a reference point at 7°. The targets and fixation points used for each retinal eccentricity measured are displayed in Figure 6.3 and were as follows: the 0.25° and 0.5° eccentricities were measured using a 0.5° and 1° diameter disc, respectively, with a 5min black fixation point at the centre; the 1° and 1.75°

eccentricities were measured using a 20' wide annuli with mean radii corresponding to those eccentricities used with a centrally fixated 5min black fixation point. Values at the reference point were obtained using a 2° diameter disc located 7° nasal to a 5min red fixation point.

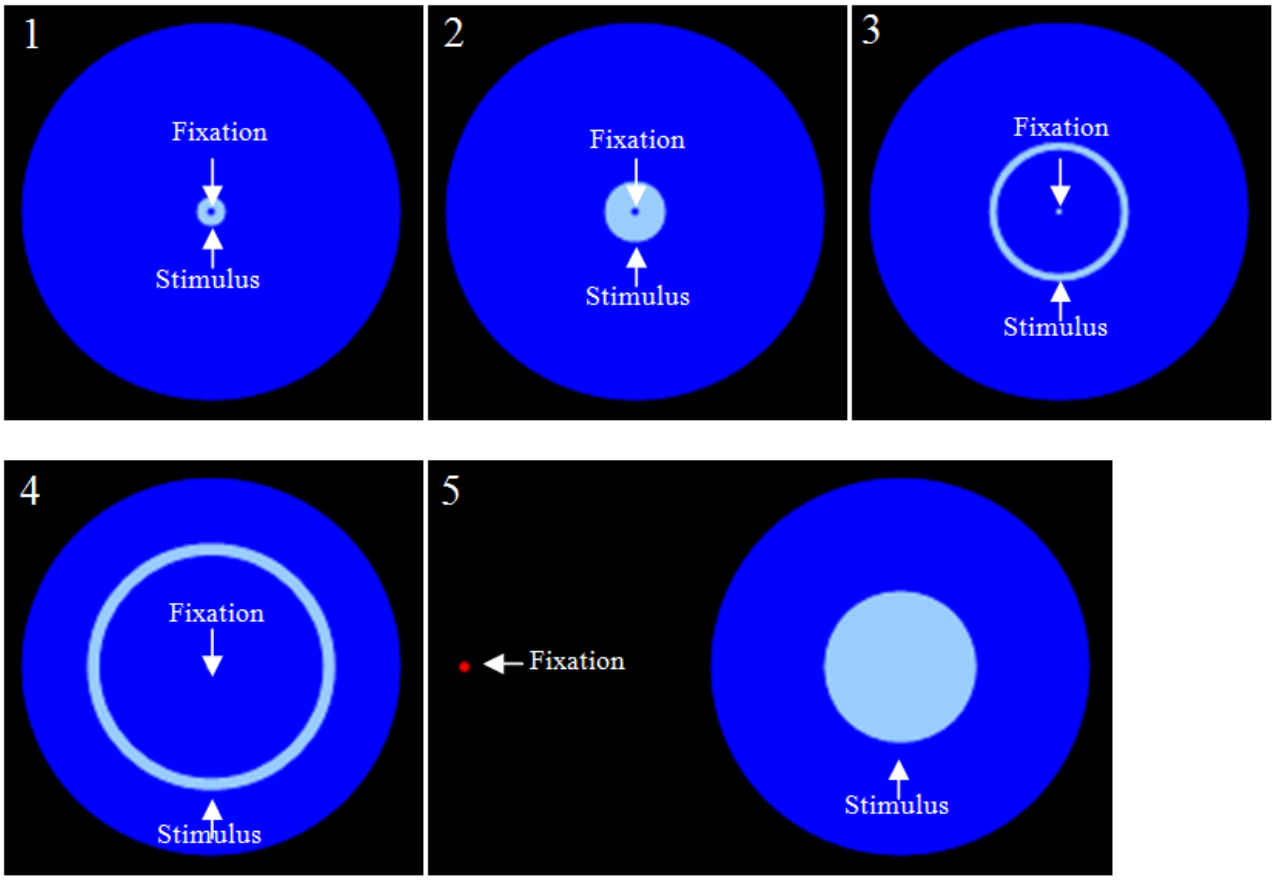


Figure 6.3 Stimuli and fixation points for each of the five targets of the Macular Densitometer™. (*Image courtesy of the MPRG, Waterford*)

Room lights were dimmed for the recording of MPOD (room illuminance was 1.5 lux). The "bracketing method" previously described by Connolly et al was employed for the measurement of MPOD in this study, as it has been found to be more suitable for assessing older subjects than the original "method of adjustment".[379] These two methods are described as follows:

<u>The Method of Adjustment:</u> The radiance button is set (by the examiner) to either the lowest or highest blue light intensity. The subject then pushes one of the (two) radiance

control buttons, which alters the blue/green ratio, until the beginning of the null flicker zone is reached. They are encouraged to continue holding down the button until the end of the null flicker zone is reached. The subject then uses the other radiance control button to go back through the zone of no flicker, and then to continue to go back and forth through this zone in smaller increments until they feel they have identified the centre of this zone (i.e., their null flicker point). The radiance value at this point is recorded. The examiner then offsets the radiance button randomly and the test is repeated four additional times, as above. The entire procedure is then repeated for the remaining eccentricities. MPOD is calculated using the log ratio of the measurement radiance values with respect to the reference radiance values (obtained at 7°), using an appropriate MPOD calculator provided by Macular Metrics (Providence, Rhode Island, USA).

If the subject cannot identify a null flicker zone, the flicker frequency is typically increased the by two Hz. Similarly, if the subject reports a very wide null zone, the flicker frequency is reduced by two Hz. Further such adjustments are made if required.

The Bracketing Method: The examiner sets the radiance button all the way to lowest blue light intensity. The examiner then pushes the radiance button, increasing the blue/green ratio until the subject reports no flicker. This radiance value is recorded and this procedure repeated an additional four times. The examiner then sets the radiance button all the way to highest blue light intensity and the procedure is repeated, as above. For any given retinal eccentricity, a total of ten radiance values are obtained; five approaching from the lowest blue light intensity and five approaching from highest blue light intensity. The same procedure is repeated for the remaining eccentricities. MPOD is calculated using, in this case, a bracketing procedure MPOD calculator.

All subjects were trained how to perform the HFP task at their first study visit. MPOD data was not recorded until subjects demonstrated a high level of understanding of the task. Reliability and reproducibility of MPOD measurements obtained using the Macular Metrics Densitometer™ have previously been reported.[388, 389]

6.2.4 Blood extraction

Non-fasting blood samples were collected at baseline and 12 months by standard venepuncture techniques for the purposes of assessing the safety (using pathology analysis) of macular carotenoid supplementation, in particular, MZ, as it is currently the least explored macular carotenoid. The blood was collected in two plastic collection tubes as follows: Tube 1 (glucose) contained sodium fluoride, and tube 2 (hematology) contained the anticoagulant dipotassium ethylene diamine tetra-acetic acid (K_2EDTA). All collection tubes were labelled with the subject's number, visit number and date and were inverted a minimum of eight times to ensure appropriate mixing of the blood with each additive in the tubes.

6.2.5 Clinical pathology analysis

Clinical pathology analysis was performed on all subjects at baseline and at 12 months, to test for any change in renal and liver function, lipid profile, hematologic profile, and markers of inflammation, following supplementation with the macular carotenoids.

The serum tube was centrifuged within two hours of collection, and a 1mL sample was aliquotted into a clean, labelled, plastic tube that was then transported with the other two tubes to Biomnis Ireland (Dublin, Ireland; Irish National Accreditation Board certified), for independent analysis. Serum levels of the following parameters

were measured at baseline and at 12 months: sodium; potassium; chloride; urea; creatinine; bilirubin; alanine aminotransferase (ALT); alkaline phosphatase (ALP); aspartate aminotransferase (AST); gamma-glutamyl transferase (GGT); total protein; albumin; globulin; calcium; magnesium; phosphate; uric acid; cholesterol-HDL (high density lipoprotein); cholesterol-LDL; cholesterol-total; triglycerides; glucose; full blood count + 5-part Diff; C-reactive protein (CRP) - high sensitivity (hsCRP).

Analysis at Biomnis Laboratories was conducted using one of two integrated diagnostic immunoassay systems (Abbott Architect ci8200; Abbott Labs, Abbott Park, IL, or Advia 120; Siemens Healthcare Diagnostics, Deerfield, IL), as appropriate. The reference ranges for this study were obtained from the insert kits for the instrumentation used by Biomnis Laboratories. Exceptions were the reference ranges for lipids (HDL, LDL, total cholesterol and triglycerides), which were obtained from the European Guidelines on Cardiovascular Disease Prevention,[390] and for glucose, which were obtained from the World Health Organisation.[391]

6.2.6 L/Z diet screener

A subject's weekly intake of carotenoid rich foods (eggs, broccoli, corn, dark leafy vegetables) was inputted into the L/Z screener (courtesy of Dr. Elizabeth Johnson, Tufts University, Boston, USA) to give a carotenoid-based diet score. The screener was used for control purposes, i.e. to ensure that there was no difference between supplementation groups at baseline with respect to dietary intake of the carotenoids. In the excel-based screener, values are weighted for frequency of intake of the food and for bioavailability of L and Z within these foods. A ranking score reflecting the relative intakes was generated. Evaluation of the L/Z screener against the Willet food frequency questionnaire yielded a positive correlation that was strongly significant ($p<0.01$).[322] The range of scores on the L/Z screener is 0 to 75. After adding foods with known

concentrations of the L and Z into the screener, the following estimates were made: a low dietary carotenoid intake score ranges from 0-15 (≤2mg/day); a medium dietary carotenoid intake score ranges from 16-30 (3-13mg/day); and a high dietary carotenoid intake score ranges from 31-75 (>13mg/day).

6.2.7 Subjective visual function (VFNq30)

An adapted version of the (Visual Function in Normals Questionnaire), a non-validated questionnaire designed to assess subjective visual function in normal subjects, was used in this study (Appendix 8), in an attempt to investigate the subjective response, if any, to supplementation with the macular carotenoids. The design was loosely based on a previously validated visual activities questionnaire.[392] The questionnaire allowed the subject to quantify their visual performance using three separate metrics: situational analysis (SA), which required the subject to rate their visual performance in specified daily life situations; comparative analysis (CAn), which required the subject to compare their perceived visual performance to that of their peers/family/friends; subjective satisfaction score (SSS), which required the subject to provide an overall estimate of their perceived quality of vision. Each of the three metrics described was computed to give a performance score for four different functional aspects of vision: acuity/spatial vision, glare disability, light/dark adaptation and daily visual tasks.

6.2.8 Stereo fundus imaging and grading

6.2.8.1 Imaging

Two sets of 30° stereoscopic colour photographs, centred on the disc, centred on the macula, and a non-stereoscopic colour fundus photograph centred temporal to, but including, the fovea, were obtained. This was followed by the acquisition of a single anterior segment image centred on the pupil of each eye. All images were anonymised and sent to the Ocular Epidemiology Reading Centre at the University of Wisconsin, USA, for grading.

6.2.8.2 AMD lesion grading (procedure described by Klein et al)[393]

Early AMD is characterised by retinal drusen and pigmentary abnormalities (increased retinal pigment and RPE depigmentation). Late AMD is characterised by areas of GA or signs of CNV. AMD grading involves measuring different characteristics of each (e.g. size, type and area of drusen and area of pigmentary abnormalities).

Each photo was fitted with a grid consisting of three concentric circles (central, inner and outer subfields) and four radial lines so that the fovea is contained within the centre circle (Figure 6.4). The radius of the innermost circle corresponds to 500µm in the fundus of an average eye and the radii of the middle and outer circles to 1500µm and 3000µm, respectively. This grid divides the photo into nine separate subfields (centre circle + four inner subfields + four outer subfields). Three sets of open circles of differing sizes were used to estimate the size of drusen, the area involving drusen and the area involving pigmentary changes (Figure 6.5) Circles, C_0, C_1 and C_2 have diameters corresponding to 63µm, 125µm and 250µm, respectively. Drusen area was quantified using the circles C_1, I_1 and O_1, which represent 1.6% of the area of the central, inner, and outer subfields, respectively, and by C_2, I_2 and O_2, which represent 6.3% of the area of the same subfields.

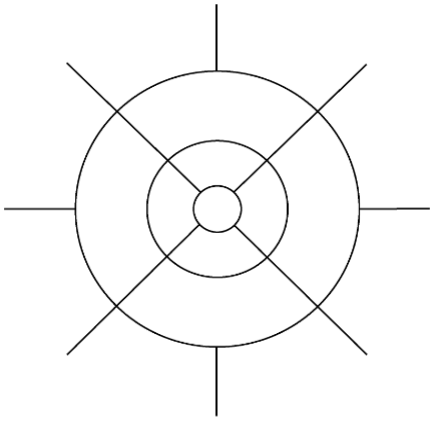


Figure 6.4 Grid used to define subfields at the macula.

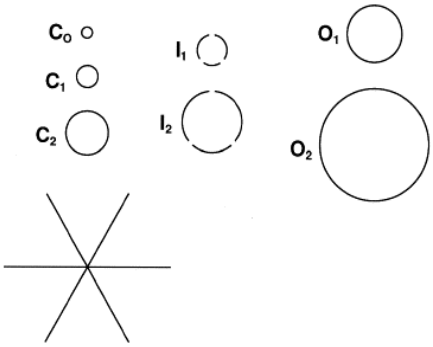


Figure 6.5 Grid used to estimate drusen size, drusen area and area of pigmentary changes.

Overall findings were reported on an 11-step AREDS AMD-severity scale (see Appendix 9) The levels of increasing severity in the 11-step AREDS scale were defined by drusen area, increased pigment, RPE depigmentation and the late AMD lesions (signs of nv-AMD or GA). For the purposes of this study, a change of two or more steps along the AREDS severity scale was defined as being clinically significant.[394] Fundus photographs were graded at baseline and at 12-month study visits.

6.2.9 Statistical analysis

Descriptive statistics were calculated for the entire group and for each supplementation group, for all measured variables, including demographic, ocular, psychophysical and

morphological data, as well as data on subjective visual functioning. Statistical analysis was performed using the software package PASW Statistics 18.0 (IBM Corp., Somers, NY, USA). Power and sample size calculations were performed using PASS 2008 (NCSS, LLC. Kaysville, Utah, USA).

Baseline differences between treatment groups e.g. in measures of visual performance, age, gender etc. were assessed using analysis of variance and contingency table analysis, as appropriate. Baseline and 12-month visit measures were compared using the paired-samples *t* test. One-way ANOVA was used to test for statistically significant differences between the three groups. Pearson correlations were used to investigate the relationship between MPOD and a range of psychophysical measures, at baseline. Change in AMD severity grade between the three intervention groups was assessed using the Pearson chi-square test for contingency tables.

Following dropouts and exclusions, data from 52 subjects remained for longitudinal analysis. A sample of this size has power of 0.85 for detecting a correlation of 0.4 and power of 0.97 for detecting a change of half of one standard deviation on a paired *t* test, assuming a 5% level of significance and a two-tailed test. For a contingency table analysis designed to detect changes of two or more steps on the AMD severity scale, the power of a sample of this size is 0.78 for detecting a large effect size (W=0.5 using Cohen's classification).[395]

6.3 Results

6.3.1 Baseline data

Seventy-nine eyes (of 79 subjects) were recruited into the study. Following recruitment and a baseline visit, a total of 12 subjects were excluded following image grading (at the University of Wisconsin); five were excluded on the basis of not having AMD and a further seven were excluded on the basis of signs of late AMD. Following these

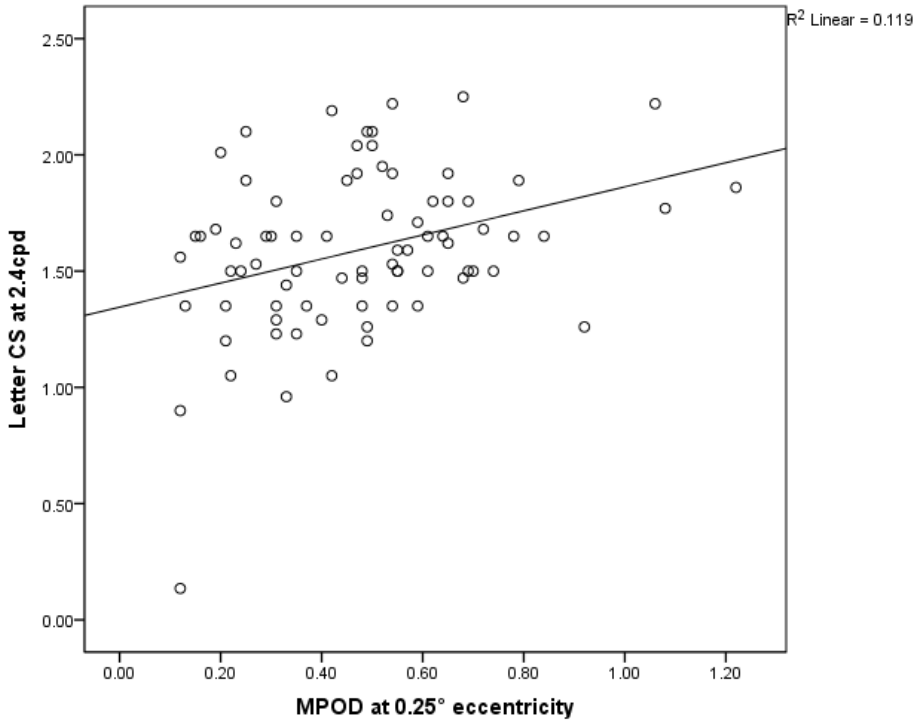
exclusions, 67 eyes (of 67 subjects) remained for analysis. Twenty-three subjects were recruited to Group 1, 25 to Group 2 and 19 to Group 3. Positive and statistically significant relationships between MPOD and visual function, at baseline, are presented in Table 6.3 (see also Figure 6.6 for the graphical representation of the relationships between letter CS 2.4 cpd and MPOD at 0.25° eccentricity).

Table 6.3. The relationship between MPOD (at 0.25° eccentricity) and psychophysical parameters of visual function, at baseline.

Variable	r	p
CDVA	0.282	**0.022**
Letter CS at 2.4cpd	0.295	**0.017**
Letter CS at 15.2cpd	0.300	**0.015**
CS ms 3cpd	0.313	**0.025**
CS pt 3cpd	0.345	**0.013**
GD pt 3cpd	0.281	**0.045**
GD pt 6cpd	0.286	**0.042**
GD pt 12cpd	0.277	**0.049**
Letter CS at 1.2cpd	0.105	0.404
Letter CS at 6.0cpd	0.205	0.102
Letter CS at 9.6cpd	0.235	0.059
CS ms 1.5cpd	0.267	0.059
CS ms 6cpd	0.141	0.323
CS ms 12cpd	0.096	0.505
CS ms 18cpd	0.028	0.843
CS pt 1.5cpd	0.230	0.104
CS pt 6cpd	0.237	0.094
CS pt 12cpd	0.106	0.460
CS pt 18cpd	-0.003	0.984
GD ms 1.5cpd	0.069	0.631
GD ms 3cpd	0.223	0.115
GD ms 6cpd	0.249	0.078
GD ms 12cpd	0.125	0.383
GD ms 18cpd	0.036	0.802
GD pt 1.5cpd	0.177	0.214
GD pt 18cpd	0.169	0.235
ROS central 4°	0.134	0.287
ROS central 8°	0.158	0.209
ROS central 12°	0.096	0.444
Diet	0.081	0.524

Abbreviations: CDVA=corrected distance visual acuity; CS=contrast sensitivity; cpd=cycles per degree; ms=under mesopic conditions; pt=under photopic conditions; GD=glare disability; ROS=retinopic ocular sensitivity.

Figure 6.6 The relationship between MPOD at 0.25° and letter CS at 2.4 cpd (6/60) at baseline.



Abbreviations: MPOD=macular pigment optical density; CS=contrast sensitivity; cpd=cycles per degree.

Of the 67 subjects, eight subjects discontinued for personal reasons, three were not available to attend for the 12-month visit, two discontinued for health reasons (deemed to be unrelated to intervention), one had cataract surgery on the study eye prior to the 12-month visit, and one patient developed nv-AMD and did not re-attend, leaving 52 subjects with complete datasets for the 12-month analyses; 17 in Group 1, 21 in Group 2, and 14 in Group 3. Baseline demographic, lifestyle, anthropometric, and visual data for the remaining 52 subjects are presented in Table 6.4. Of note, there was no significant difference between the groups with respect to any baseline data variables (with the exceptional of one questionnaire variable [daily situation analysis]; p=0.046), nor with respect to AMD grade (p=0.994; Table 6.5).

Table 6.4 Baseline demographic, lifestyle, anthropometric and visual data.

	Entire group	Group 1	Group 2	Group 3	Sig.
Gender	n (%)	n (%)	n (%)	n (%)	
Male	18 (35%)	5 (29%)	8 (38%)	5 (36%)	0.851
Female	34 (65%)	12 (71%)	13 (62%)	9 (64%)	
Eye					
Right	33 (63%)	9 (53%)	14 (67%)	10 (71%)	0.525
Left	19 (37%)	8 (47%)	7 (33%)	4 (29%)	
Smoking status					
Current	4 (8%)	2 (12%)	2 (10%)	0 (0%)	0.224
Past	25 (48%)	8 (47%)	7 (33%)	10 (71%)	
Never	23 (44%)	7 (41%)	12 (57%)	4 (29%)	
Education					
Primary	10 (19%)	3 (18%)	2 (10%)	5 (36%)	0.270
Secondary	23 (44%)	6 (35%)	12 (57%)	5 (36%)	
Third level	19 (37%)	8 (47%)	7 (33%)	4 (28%)	
Variable	**mean ±(sd)** (n=52)	**mean ±(sd)** (n=17)	**mean ±(sd)** (n=21)	**mean ±(sd)** (n=14)	
Age	66 (8)	65 (7)	64 (9)	70 (8)	0.117
BMI (kg/m^2)	26.1 (5.5)	25.5 (4.1)	27.1 (3.6)	25.2 (8.6)	0.562
CDVA (study eye)	99 (7)	99 (7)	99 (8)	98 (6)	0.868
CDVA (non-study eye)	95 (10)	96 (9)	96 (12)	94 (8)	0.834
Macular Pigment Optical Density					
0.25° eccentricity	0.50 (0.25)	0.50 (0.25)	0.50 (0.24)	0.47 (0.21)	0.925
0.5° eccentricity	0.39 (0.22)	0.38 (0.27)	0.41 (0.22)	0.36 (0.19)	0.797
1.0° eccentricity	0.26 (0.15)	0.27 (0.18)	0.27 (0.13)	0.24 (0.17)	0.851
1.75° eccentricity	0.14 (0.11)	0.16 (0.11)	0.15 (0.11)	0.11 (0.12)	0.554
Letter CS (photopic conditions)					
1.2 cpd	68.3 (46.4)	73.0 (49.1)	61.2 (41.3)	73.2 (52.3)	0.674
2.4 cpd	57.1 (41.2)	59.7 (45.3)	56.8 (40.5)	54.3 (41.4)	0.938
6.0 cpd	25.6 (14.8)	29.0 (14.9)	24.3 (14.0)	23.6 (16.0)	0.530
9.6 cpd	13.7 (8.6)	16.0 (9.1)	12.3 (7.3)	12.9 (9.7)	0.399
15.2 cpd	6.5 (4.9)	7.1 (4.5)	6.2 (4.8)	6.4 (5.7)	0.827
FVA CS (mesopic conditions) (n=39)*					
Frequency (cpd)					
1.5	47.8 (28.1)	45.6 (29.8)	46.3 (24.5)	44.6 (30.4)	0.885
3	57.1 (36.4)	47.1 (26.5)	58.6 (33.0)	58.0 (44.9)	0.905
6	25.9 (18.1)	21.0 (17.1)	25.9 (17.5)	24.3 (20.4)	0.762
12	6.0 (4.2)	4.8 (2.1)	5.6 (3.9)	6.9 (5.4)	0.380
18	2.6 (2.0)	3.1 (3.0)	2.1 (0.5)	2.4 (1.2)	0.483
FVA CS (photopic conditions) (n=39)*					
Frequency (cpd)					
1.5	36.0 (23.0)	34.9 (24.3)	41.1 (22.0)	31.8 (21.4)	0.657
3	63.3 (29.8)	56.6 (27.3)	66.1 (33.6)	65.4 (27.5)	0.492
6	45.5 (32.0)	40.3 (25.0)	45.1 (32.1)	41.7 (36.4)	0.965
12	15.2 (13.6)	13.4 (11.0)	14.8 (14.3)	15.2 (13.7)	0.993
18	7.2 (7.6)	6.4 (6.3)	5.8 (5.5)	8.5 (9.8)	0.691
FVA GD (mesopic conditions) (n=39)*					
Frequency (cpd)					

1.5	37.3 (27.0)	39.9 (25.2)	37.5 (28.8)	26.8 (24.1)	0.285
3	48.6 (37.9)	54.07 (43.5)	42.4 (26.8)	40.4 (40.3)	0.491
6	20.4 (16.0)	18.0 (17.3)	19.8 (14.3)	18.6 (15.9)	0.832
12	5.9 (3.7)	5.1 (3.0)	5.1 (3.1)	6.9 (4.3)	0.228
18	2.3 (1.1)	2.4 (1.6)	2.2 (0.7)	2.1 (0.5)	0.904
FVA GD (photopic conditions) (n=39)*					
Frequency (cpd)					
1.5	39.8 (22.8)	45.9 (26.9)	36.8 (16.1)	37.9 (23.4)	0.280
3	65.0 (27.1)	58.1 (28.0)	67.2 (31.2)	59.0 (22.1)	0.786
6	51.5 (35.3)	45.3 (27.5)	52.3 (40.5)	45.5 (38.7)	0.946
12	16.1 (14.3)	16.2 (16.7)	12.9 (11.0)	15.8 (14.2)	0.876
18	7.9 (8.0)	7.7 (9.5)	4.8 (4.1)	9.4 (8.8)	0.410
ROS					
Central 4°	18.0 (2.2)	18.3 (1.9)	18.4 (1.7)	17.1 (2.9)	0.194
Central 8°	18.3 (1.8)	18.5 (1.5)	18.5 (1.6)	17.6 (2.5)	0.345
Central 12°	18.0 (2.2)	18.4 (1.7)	18.2 (2.1)	17.2 (2.7)	0.303
Subjective vision questionnaire (n=49)*					
Glare SA	59 (20)	59 (20)	63 (21)	54 (19)	0.465
Glare CAn	55 (18)	50 (19)	58 (19)	55 (15)	0.425
Glare SSS	61 (25)	55 (27)	67 (25)	58 (24)	0.384
Acuity SA	66 (20)	69 (20)	68 (20)	61 (19)	0.501
Acuity CAn	56 (15)	58 (16)	57 (13)	52 (15)	0.593
Acuity SSS	68 (20)	66 (22)	72 (21)	63 (18)	0.499
Light SA	66 (17)	66 (20)	70 (17)	60 (11)	0.295
Light CAn	56 (14)	55 (17)	58 (11)	54 (13)	0.662
Light SSS	67 (19)	69 (17)	70 (21)	60 (17)	0.303
Daily SA	77 (15)	75 (13)	83 (14)	71 (16)	**0.046**
Daily CAn	58 (12)	55 (12)	60 (13)	58 (13)	0.489
Daily SSS	70 (15)	66 (14)	75 (16)	66 (13)	0.123
Diet score (n=50)	18.7 (11.2)	17.3 (10.9)	21.9 (12.7)	16.0 (8.4)	0.267

Abbreviations: BMI=body mass index; CDVA=corrected distance visual acuity; CS=contrast sensitivity; cpd=cycles per degree; GD=glare disability; FVA=functional vision analyzer; ROS=retinotopic ocular sensitivity; SA=situational analysis; CAn=comparative analysis; SSS=subjective satisfaction score.

Group 1: 20mg L, 2mg Z; Group 2: 10mg MZ, 10mg L, 2mg Z; Group 3: 17 mg MZ, 3 mg L, 2mg Z

* n≠52 (for entire study groups) due to either a) the absence of the test in question at study outset, b) the patient had difficulty/could not perform the test, or c) the data was unreliable.

Table 6.5 AMD-severity grading for entire group and intervention subgroups at baseline.

Grade	Entire group (n=52)	Group 1 (n=17)	Group 2 (n=21)	Group 3 (n=14)	Sig.
1-2	9 (17.3%)	3 (17.6%)	4 (19.0%)	2 (14.3%)	0.994
3-4	22 (42.3%)	8 (47.1%)	8 (38.1%)	6 (42.9%)	
5-6	13 (25.0%)	4 (23.5%)	5 (23.8%)	4 (28.6%)	
7-8	8 (15.4%)	2 (11.8%)	4 (19.0%)	2 (14.3%)	

Group 1: 20mg L, 2mg Z; Group 2: 10mg MZ, 10mg L, 2mg Z; Group 3: 17 mg MZ, 3 mg L, 2mg Z

6.3.2 Longitudinal data

Values for MPOD at each eccentricity, at baseline and 12 months, and their corresponding p values with respect to change over time, are summarised in Table 6.6. Of note, there were no statistically significant differences between the three supplementation groups with respect to change in MPOD, at any eccentricity ($p>0.5$, for all; determined using one-way ANOVA).

Table 6.6 Mean (±sd) MPOD at baseline and twelve months.

	Group 1			Group 2			Group 3		
Eccentricity	**Baseline**	**12 months**	**p**	**Baseline**	**12 months**	**p**	**Baseline**	**12 months**	**p**
0.25°	0.50 ±0.25	0.59 ±0.30	0.077	0.50 ±0.25	0.60 ±0.21	**0.005**	0.46 ±0.21	0.59 ±0.20	**0.010**
0.5°	0.38 ±0.27	0.47 ±0.27	0.055	0.42 ±0.22	0.50 ±0.19	**0.005**	0.36 ±0.19	0.46 ±0.21	**0.020**
1°	0.27 ±0.18	0.34 ±0.16	0.083	0.27 ±0.13	0.34 ±0.17	**0.005**	0.24 ±0.17	0.33 ±0.16	**0.019**
1.75°	0.16 ±0.11	0.21 ±0.09	**0.018**	0.14 ±0.11	0.22 ±0.12	**0.002**	0.11 ±0.12	0.19 ±0.10	**0.006**

Abbreviations: MPOD=macular pigment optical density; n=number of subjects

Group 1: 20mg L, 2mg Z; Group 2: 10mg MZ, 10mg L, 2mg Z; Group 3: 17 mg MZ, 3 mg L, 2mg Z

Mean values (at baseline and 12 months) for parameters measured in the study, and their respective p values in relation to change over time, are displayed in Table 6.7. Letter CS values at baseline and at 12 months, for the three intervention groups, for each spatial frequency, is displayed in Figure 6.7. One-way ANOVA (and subsequent post-hoc analysis) showed statistically significant differences between Groups 1 and 3 with respect to change in letter CS at 6.0, 9.6 and 15.2 cpd, and between Groups 2 and 3 for letter CS at 6.0 cpd ($p<0.05$, for all). There were no significant differences between groups for the remaining visual performance parameters ($p>0.5$, for all).

Table 6.7 Mean (±sd) values for measures of visual function and subjective visual experience, at baseline and at 12 months.

	Group 1			Group 2			Group 3		
	Baseline	**12 months**	**p**	**Baseline**	**12 months**	**p**	**Baseline**	**12 months**	**p**
CDVA (SE)	99 (7)	98 (6)	0.408	99 (8)	98 (5)	0.554	98 (6)	98 (7)	0.799
CDVA (nSE)	96 (9)	95 (9)	0.486	96 (12)	95 (11)	0.675	94 (8)	97 (4)	0.201
Letter CS (photopic)									
1.2	73.0 (49.1)	91.8 (48.5)	**0.021***	61.2 (41.3)	91.9 (53.6)	**0.014***	73.2 (52.3)	92.2 (55.0)	0.081*
2.4	59.7 (45.3)	86.7 (54.2)	**0.006***	56.8 (40.5)	77.8 (51.6)	**0.008***	54.3 (41.4)	86.2 (52.1)	**0.002***
6.0	29.0 (14.9)	38.1 (26.7)	0.098*	24.3 (14.0)	30.9 (18.8)	0.058*	23.6 (16.0)	42.2 (25.9)	**0.002***
9.6	16.0 (9.1)	16.4 (9.0)	0.939*	12.3 (7.3)	17.5 (12.3)	0.066*	12.9 (9.7)	20.1 (11.9)	**0.016***
15.2	7.1 (4.5)	7.8 (5.5)	0.408*	6.2 (4.8)	7.8 (6.4)	0.189*	6.4 (5.7)	8.7 (5.8)	**0.005***
CS (mesopic)†									
Frequency (cpd)									
1.5	45.6 (29.8)	69.6 (28.2)	**0.007***	46.3 (24.5)	65.5 (30.1)	**0.047***	44.6 (30.4)	61.6 (29.0)	0.292*
3	47.1 (26.5)	73.9 (63.3)	**0.007***	58.6 (33.0)	73.2 (38.0)	0.058*	58.0 (44.9)	90.9 (47.2)	0.175*
6	21.0 (17.1)	44.2 (91.8)	0.521*	25.9 (17.5)	38.5 (38.7)	0.278*	24.3 (20.4)	46.6 (38.6)	0.123*
12	4.8 (2.1)	13.9 (30.8)	0.109*	5.6 (3.9)	7.2 (4.2)	0.137*	6.9 (5.4)	11.3 (12.0)	0.498*
18	3.1 (3.0)	2.1 (0.5)	0.207*	2.1 (0.5)	3.2 (5.1)	0.317*	2.4 (1.2)	3.0 (2.8)	0.655*
CS (photopic)†									
Frequency (cpd)									
1.5	34.9 (24.3)	47.7 (23.5)	**0.007***	41.1 (22.0)	47.3 (26.3)	0.241*	31.8 (21.4)	48.9 (27.0)	**0.023***
3	56.6 (27.3)	75.0 (19.9)	**0.002***	66.1 (33.6)	80.2 (32.6)	0.108*	65.4 (27.5)	78.0 (34.9)	0.169*
6	40.3 (25.0)	48.4 (33.5)	0.310*	45.1 (32.1)	63.5 (49.1)	0.064*	41.7 (36.4)	55.9 (39.8)	0.192*
12	13.4 (11.0)	13.1 (11.6)	0.709*	14.8 (14.3)	28.5 (34.0)	0.118*	15.2 (13.7)	26.6 (28.7)	0.314*
18	6.4 (6.3)	5.6 (6.5)	0.498*	5.8 (5.5)	8.0 (9.5)	0.687*	8.5 (9.8)	10.6 (12.3)	0.866*
GD (mesopic)†									
Frequency (cpd)									
1.5	39.9 (25.2)	40.9 (23.5)	0.753*	37.5 (28.8)	42.9 (27.5)	0.289*	26.8 (24.1)	48.3 (33.1)	**0.021***
3	54.07 (43.5)	47.9 (24.1)	0.439*	42.4 (26.8)	63.3 (35.9)	**0.010***	40.4 (40.3)	52.9 (35.9)	0.161*
6	18.0 (17.3)	14.8 (13.0)	0.564*	19.8 (14.3)	27.6 (31.2)	0.553*	18.6 (15.9)	27.3 (29.8)	0.345*
12	5.1 (3.0)	5.6 (2.7)	0.273*	5.1 (3.1)	13.9 (28.9)	0.144*	6.9 (4.3)	5.8 (5.0)	0.115*
18	2.4 (1.6)	2.0 (0.0)	0.336*	2.2 (0.7)	5.7 (15.3)	0.655*	2.1 (0.5)	2.7 (2.7)	0.317*
GD (photopic)†									
Frequency (cpd)									
1.5	45.9 (26.9)	64.1 (30.1)	**0.006***	36.8 (16.1)	61.8 (27.9)	**0.002***	37.9 (23.4)	64.6 (34.0)	0.058*
3	58.1 (28.0)	89.1 (42.2)	**0.002***	67.2 (31.2)	99.7 (37.7)	**0.006***	59.0 (22.1)	85.4 (40.3)	0.330*
6	45.3 (27.5)	58.8 (50.3)	**0.000***	52.3 (40.5)	69.1 (78.7)	**0.012***	45.5 (38.7)	68.6 (49.5)	0.120*
12	16.2 (16.7)	16.8 (20.6)	0.953*	12.9 (11.0)	18.7 (16.8)	0.169*	15.8 (14.2)	20.5 (22.7)	0.320*
18	7.7 (9.5)	8.8 (11.9)	0.674*	4.8 (4.1)	13.4 (21.9)	0.071*	9.4 (8.8)	10.6 (11.8)	0.993*
Retinotopic ocular sensitivity									
Central 4°	18.3 (1.9)	18.5 (1.5)	0.271	18.4 (1.8)	18.3 (2.3)	0.640	17.5 (2.7)	17.7 (3.0)	0.718
Central 8°	18.5 (1.5)	18.7 (1.3)	0.510	18.5 (1.7)	18.4 (2.1)	0.850	18.0 (2.3)	18.0 (2.7)	0.928
Central 12°	18.4 (1.7)	18.5 (1.4)	0.442	18.1(2.2)	18.3 (2.1)	0.673	17.5 (2.6)	17.2 (3.2)	0.611
Questionnaire									

Glare SA	59 (21)	58 (17)	0.513	65 (20)	69 (25)	0.512	54 (19)	56 (15)	0.723
Glare CAn	51 (19)	50 (15)	0.655	60 (18)	60 (19)	1.000	55 (15)	51 (16)	0.257
Glare SSS	55 (28)	62 (21)	0.268	69 (23)	69 (18)	1.000	58 (24)	58 (20)	1.000
Acuity SA	70 (20)	70 (22)	0.880	68 (21)	73 (16)	0.130	61 (19)	71 (13)	**0.041**
Acuity CAn	59 (17)	53 (10)	0.157	57 (14)	60 (13)	0.180	52 (15)	54 (13)	0.753
Acuity SSS	66 (23)	70 (20)	0.373	73 (21)	70 (17)	0.450	63 (18)	68 (16)	0.549
Light SA	65 (21)	59 (18)	**0.047**	70 (18)	74 (15)	0.216	60 (11)	68 (11)	**0.037**
Light CAn	56 (18)	54 (9)	0.705	58 (12)	59 (16)	0.790	53 (13)	57 (8)	0.504
Light SSS	69 (17)	68 (19)	0.634	73 (19)	69 (18)	0.479	60 (17)	70 (10)	0.139
Daily SA	75 (14)	73 (15)	0.706	83 (15)	82 (19)	0.706	71 (16)	77 (12)	0.144
Daily CAn	56 (12)	57 (11)	0.655	60 (13)	62 (9)	0.527	58 (13)	58 (16)	1.000
Daily SSS	66 (15)	73 (16)	**0.043**	76 (16)	72 (14)	0.191	66 (13)	73 (9)	0.108
Diet score	17.3 (10.9)	23.3 (11.3)	**0.044**	20.6 (11.7)	26.7 (16.1)	**0.020**	15.3 (8.5)	27.5 (11.2)	**0.006**

Abbreviations: CDVA=corrected distance visual acuity; SE=study eye; nSE=non-study eye; CS=contrast sensitivity; cpd=cycles per degree; GD=glare disability; SA=situational analysis; CAn=comparative analysis; SSS=subjective satisfaction score

Group 1: 20mg L, 2mg Z; Group 2: 10mg MZ, 10mg L, 2mg Z; Group 3: 17 mg MZ, 3 mg L, 2mg Z

†as measured by the Functional Vision Analyzer™; n=13 for Group 1, n=14 for Group 2, n=12 for Group 3.

*the statistical tests were based on log-transformed data.

Note: The p values reported are for the paired t test (or the corresponding non-parametric test when the data distribution was non-normal).

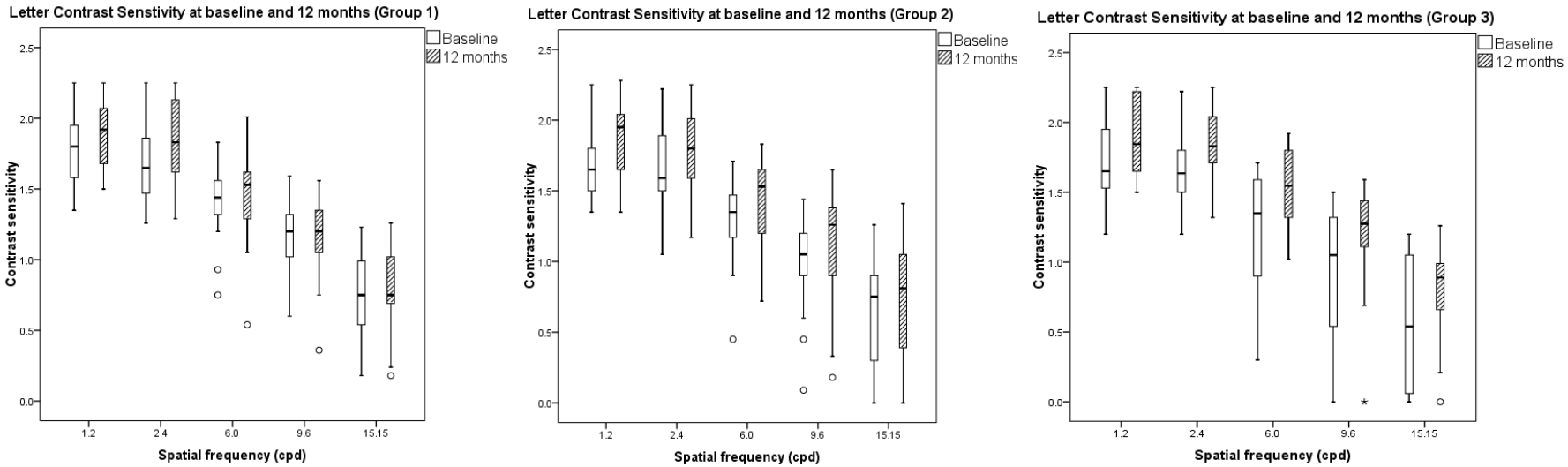


Figure 6.7 Letter CS at baseline and at 12 months, for each group.

Group 1: 20mg L, 2mg Z; Group 2: 10mg MZ, 10mg L, 2mg Z; Group 3: 17 mg MZ, 3 mg L, 2mg Z

The proportion of subjects in each intervention group exhibiting a change in severity scale grade of two or more, considered clinically meaningful for the purpose of this study,[394] is given in Table 6.8. A change in the negative direction (i.e. -1, -2) indicates a progression along the AMD severity scale, whereas positive integers indicate regression (improvement). Between baseline and 12 months, there was no statistically significant difference between treatment groups with respect to change in AMD severity scale (p=0.455, Pearson chi-square test).

In brief, 79% of subjects exhibited no clinically meaningful change in AMD severity grade between baseline and 12 months, with approximately 11% exhibiting deterioration and 9% exhibiting an improvement.

Table 6.8 Change in AMD grade (11-step scale) between baseline and 12 months.

Group	n	-2	-1	0	+1	+2	+3	Sig.
1	17	1 (5.9%)	1 (5.9%)	10 (58.8%)	3 (17.6%)	1 (5.9%)	1 (5.9%)	0.455
2	21	3 (14.3%)	2 (9.5%)	11 (52.4%)	3 (14.3%)	2 (9.5%)	0	
3	14	2 (14.3%)	5 (35.7%)	4 (28.6%)	2 (14.3%)	1 (7.1%)	0	
Total	**52 (100%)**	**6 (11.5%)**	**8 (15.4%)**	**25 (48.1%)**	**8 (15.4%)**	**4 (7.7%)**	**1 (1.9%)**	

Abbreviations: n=number of subjects; negative value indicates disease progression; a positive value indicates disease regression; 0=no change in grade

Group 1: 20mg L, 2mg Z; Group 2: 10mg MZ, 10mg L, 2mg Z; Group 3: 17 mg MZ, 3 mg L, 2mg Z

Clinical pathology analysis results are reported in Table 6.9. Of note, two variables in Group 1, two variables in Group 2 and two variables in Group 3 demonstrated statistically significant changes from baseline (in both positive and negative directions). All variables, however, remained within their respective and normal reference ranges.

Table 6.9 Clinical pathology variables following supplementation with the macular carotenoids assessed at baseline and at 12 months for each of the three intervention groups.

Pathology variable	Function of test	Reference Range (Unit)	Group 1 (n=9)*			Group 2 (n=20)*			Group 3 (n=12)*		
			Baseline	12 months	p	Baseline	12 months	p	Baseline	12 months	p
Sodium	Renal profile	135-145 (mmol/L)	139±3	138±3	0.312	141±3	138±2	**0.001**	136±3	137±4	0.371
Potassium	Renal profile	3.3-5.3 (mmol/L)	4.6±0.3	4.7±0.2	0.366	4.6±0.4	4.7±0.4	0.475	4.7±0.3	4.8±0.2	0.709
Chloride	Renal profile	98-107 (mmol/L)	104±2	106±2	0.073	104±3	104±3	0.922	103±4	103±4	0.612
Urea	Renal profile	2.5-7.7 (mmol/L)	7.2±2.4	6.5±1.4	0.174	6.1±1.1	6.6±1.5	0.073	6.7±1.5	6.0±1.7	0.053
Creatinine	Renal profile	40-90 (μmol/L)	81±13	74±10	0.086	78±14	77±15	0.299	76±19	75±17	0.681
Total protein	Liver profile	64-83 (g/L)	69±3	68±3	0.499	71±4	70±3	0.415	70±5	70±5	0.558
Albumin	Liver profile	37-52 (g/L)	41±2	40±3	0.444	43±2	42±2	0.134	41±2	42±2	0.410
Globulins	Liver profile	21-36 (g/L)	28±4	28±3	1.000	28±4	29±3	0.737	29±5	28±4	0.272
Total bilirubin	Liver profile	3.4-21.0 (μmol/L)	6.2±2.0	7.8±2.2	**0.050**	9.1±4.7	9.9±5.3	0.293	8.0±3.6	10.1±4.0	**0.001**
AAT	Liver profile	0-55 IU/L	23±8	21±8	0.426	22±6	22±6	0.752	19±3	20±5	0.279
ASA	Liver profile	5-36 IU/L	24±3	24±4	0.782	22±4	22±4	0.903	21±3	22±4	0.083
Alkaline phosphate	Liver profile	40-150 IU/L	79±27	87±31	**0.013**	78±20	79±20	0.501	76±11	82±17	0.114
GGT	Liver profile	9-36 IU/L	39±40	40±41	0.668	27±11	28±14	0.395	27±16	32±23	0.075
Cholesterol total	Lipid profile	<5.0 (mmol/L)	5.2±1.0	5.2±1.1	0.708	4.7±1.3	4.5±0.9	0.231	4.8±1.0	4.8±0.9	1.000
Triglycerides	Lipid profile	0.60-1.70 (mmol/L)	1.47±0.61	1.34±0.66	0.185	1.44±0.49	1.39±0.60	0.700	1.51±1.31	1.29±0.82	0.236
HDL	Lipid profile	1.00-1.55 (mmol/L)	1.51±0.37	1.43±0.31	0.063	1.31±0.33	1.24±0.28	**0.044**	1.46±0.47	1.46±0.51	0.942
Direct LDL	Lipid profile	<3.0 (mmol/L)	3.1±1.0	3.2±1.0	0.419	2.8±1.1	2.7±0.8	0.317	2.8±0.9	2.7±0.9	0.671
Calcium	Bone profile	2.10-2.60 (mmol/L)	2.31±0.10	2.32±0.14	0.661	2.35±0.07	2.35±0.07	0.825	2.31±0.06	2.40±0.11	**0.005**
Phosphate	Bone profile	0.80-1.56 (mmol/L)	1.13±0.17	1.20±0.24	0.292	1.17±0.17	1.19±0.19	0.672	1.07±0.25	1.10±0.21	0.414
Magnesium	Bone profile	0.65-1.10 (mmol/L)	0.99±0.05	0.94±0.09	0.159	0.97±0.08	0.98±0.06	0.573	0.93±0.12	0.94±0.08	0.599
Uric Acid	Bone profile	155-394 (μmol/L)	290±54	280±62	0.579	315±65	312±66	0.724	305±65	322±89	0.260
Glucose	Bone profile	3.1-6.1 (mmol/L)	5.3±0.7	5.3±1.2	0.910	5.0±0.6	5.0±0.7	0.867	5.0±0.9	5.1±0.7	0.273
HSRP	Inflammation marker	<5.0 (mg/L)	1.2±0.5	1.6±1.0	0.097	2.2±2.4	2.3±2.1	0.864	4.0±5.1	4.3±6.7	0.728

Full blood count											
White cell count	Haematology	3.88-10.49 (10e9/L)	6.54±2.00	5.88±1.03	0.331	6.74±1.53	6.81±1.78	0.830	6.13±1.56	5.95±1.10	0.661
Red cell count	Haematology	3.73-5.02 (10e12/L)	4.51±0.42	4.36±0.33	0.367	4.50±0.41	4.47±0.39	0.377	4.44±0.45	4.46±0.45	0.858
Haemoglobin	Haematology	11.3-15.2 (g/dL)	13.6±1.1	13.6±0.9	0.622	13.7±1.1	13.7±1.2	0.596	13.6±1.2	13.6±1.1	0.969
Haematocrit	Haematology	0.323-0.462 (L/L)	0.407±0.032	0.405±0.020	0.769	0.413±0.031	0.413±0.031	0.939	0.409±0.031	0.412±0.031	0.779
MCV	Haematology	83.1-99.1 (fL)	90.5±3.1	93.1±5.1	0.222	92.0±4.0	92.6±4.0	0.414	92.4±3.7	92.6±3.7	0.778
MCH	Haematology	28.3-33.9 (pg)	30.1±1.2	31.3±1.8	0.134	30.4±1.3	30.8±1.2	0.167	30.7±1.2	30.5±1.4	0.632
MCHC	Haematology	32.1-36.6 (g/dL)	33.3±1.0	33.6±0.8	0.357	33.1±0.8	33.3±1.2	0.523	33.2±1.1	33.0±1.0	0.468
Platelets	Haematology	164-382 (10e9/L)	332±249	249±123	0.196	258±88	250±118	0.527	244±46	254±61	0.369
Differential white cell count											
Neutrophils	Haematology	1.91-7.16 (10e9/L)	3.80±1.27	3.44±0.82	0.423	4.09±1.20	4.23±1.38	0.580	3.92±1.23	3.84±1.21	0.809
Lymphocytes	Haematology	1.01-3.13 (10e9/L)	1.82±0.54	1.66±0.39	0.309	1.81±0.43	1.70±0.42	0.128	1.42±0.36	1.38±0.39	0.704
Monocytes	Haematology	0.19-0.68 (10e9/L)	0.47±0.18	0.40±0.13	0.322	0.43±0.11	0.45±0.16	0.495	0.41±0.12	0.35±0.09	0.132
Eosinophils	Haematology	0.05-0.51 (10e9/L)	0.22±0.05	0.18±0.06	0.195	0.18±0.09	0.23±0.13	0.055	0.17±0.10	0.17±0.08	1.000
Basophils	Haematology	0.02-0.15 (10e9/L)	0.05±0.03	0.05±0.02	0.505	0.05±0.02	0.07±0.04	0.063	0.04±0.02	0.06±0.03	0.042
Large unstained cells	Haematology	0.00-0.30 (10e9/L)	0.17±0.07	0.14±0.03	0.222	0.18±0.07	0.16±0.05	0.122	0.16±0.05	0.14±0.02	0.177

Abbreviations: AAT=alanine aminotransferase; ASA= aspartate aminotransferase; GGT=gamma glytamyl transpeptidase; HDL=high density lipoprotein; LDL=low density lipoprotein; HSCP=high sensitive reactive protein; MCV=mean corpuscular volume; MCH=mean corpuscular haemoglobin; MCHC=mean corpuscular haemoglobin concentration

Group 1: 20mg L, 2mg Z; Group 2: 10mg MZ, 10mg L, 2mg Z; Group 3: 17 mg MZ, 3 mg L, 2mg Z

*total n≠52 as data on pathology analysis was not available for all subjects at both baseline and 12 months

6.3.3 A subsidiary six-month analysis

While the study's primary end-point was 12 months, it was felt that the six-month data, when viewed separately, may be of interest in terms of changes in measures of MPOD and visual performance, particularly if one wants to consider the possible differences in response time to the supplements. Repeated measures that would include baseline, 6 and 12 month data were not sufficiently powered due to the change in the composition of the study cohort between baseline and 12 months (primarily due to dropouts).

Twenty-one subjects from Group 1, 22 subjects from Group 2, and 15 subjects from Group 3 were eligible for this analysis i.e. had datasets at baseline and six months. There were no significant differences between groups with respect to any variable at baseline ($p>0.05$, for all). Values for MPOD at each eccentricity, at baseline and six months, are summarized in Table 6.10. Of note, there were statistically significant increases in MPOD at all eccentricities for each of the three groups (with the exception of 0.5° in Group 3).

Table 6.11 reports those parameters of visual performance that changed significantly between baseline and six months. In brief, two variables in Group 1, nine variables in Group 2, and two variables in Group 3, improved significantly between baseline and six months. Of note, mean CDVA reduced significantly between baseline and six months in Group 1.

Table 6.10 Mean (±sd) MPOD at baseline and six months.

	Group 1			**Group 2**			**Group 3**		
Eccentricity	**Baseline**	**6 months**	**p**	**Baseline**	**6 months**	**p**	**Baseline**	**6 months**	**p**
0.25°	0.47 ±0.25	0.58 ±0.27	**0.001**	0.50 ±0.24	0.63 ±0.20	**<0.001**	0.46 ±0.20	0.55 ±0.21	**0.028**
0.5°	0.36 ±0.25	0.46 ±0.23	**0.004**	0.42 ±0.21	0.52 ±0.19	**0.002**	0.36 ±0.16	0.42 ±0.17	0.089
1°	0.25 ±0.17	0.35 ±0.17	**0.001**	0.28 ±0.13	0.38 ±0.15	**<0.001**	0.23 ±0.15	0.30 ±0.12	**0.023**
1.75°	0.15 ±0.11	0.21 ±0.12	**0.010**	0.15 ±0.11	0.23 ±0.11	**0.004**	0.11 ±0.10	0.19 ±0.10	**0.006**

Abbreviations: MPOD=macular pigment optical density; n=number of subjects

Group 1: 20mg L, 2mg Z; Group 2: 10mg MZ, 10mg L, 2mg Z; Group 3: 17 mg MZ, 3 mg L, 2mg Z

Table 6.11 Visual performance parameters which displayed significant changes between baseline and 6 months (for each study group).

Variable	Baseline Mean (±sd)	6 months Mean (±sd)	p
Group 1			
CDVA (SE)	99 (7)	96 (7)	0.019*
GD pt 1.5cpd	40.29 (26.51)	47.94 (24.61)	0.013
GD pt 3cpd	53.94 (25.67)	79.29 (40.91)	0.019
Group 2			
Letter CS 1.2cpd	59.09 (38.71)	85.50 (48.71)	0.018
Letter CS 9.6cpd	12.32 (6.75)	22.59 (20.70)	0.018
CS ms 3cpd	57.72 (33.79)	71.50 (42.39)	0.012
CS pt 3cpd	62.78 (31.06)	80.78 (33.70)	0.026
CS pt 18cpd	5.33 (5.39)	10.70 (12.55)	0.029
GD ms 3cpd	39.61 (26.81)	52.00 (37.06)	0.031
GD pt 1.5cpd	34.83 (16.81)	42.14 (20.85)	0.005
GD pt 3cpd	60.44 (30.20)	79.94 (36.11)	0.009
Group 3			
CS ms 6cpd	22.07 (21.22)	30.33 (33.61)	0.026
GD ms 6cpd	16.60 (15.91)	27.80 (34.91)	0.035

Abbreviations: CDVA=corrected distance visual acuity; SE=study eye; GD=glare disability; pt=under photopic conditions; cpd=cycles per degree; CS=contrast sensitivity; ms=under mesopic conditions.
*p value relates to a significant decrease in visual acuity
Group 1: 20mg L, 2mg Z; Group 2: 10mg MZ, 10mg L, 2mg Z; Group 3: 17 mg MZ, 3 mg L, 2mg Z
Note: CS and GD p values are based on log-transformed data.

6.4 Discussion

MOST AMD is a randomised single-blind clinical trial comparing the effect of supplementation with three different macular carotenoid formulations on MPOD, visual performance and AMD grade, over a period of twelve months, in subjects with early AMD.

This study found a positive and statistically significant correlation, at baseline, between MPOD and measures of visual function, including CDVA, letter CS, grating CS under mesopic and photopic conditions at low spatial frequencies and GD under photopic conditions at low and mid-range spatial frequencies. In other words, and in the absence of supplementation, high MPOD is associated with better vision. Our findings

are consistent with those of another study, which found a significant and positive association between MPOD and both CDVA and CS (under mesopic and photopic conditions at intermediate spatial frequencies) in subjects without disease.[29] It has been shown that, in subjects with atrophic AMD, lower MP levels are associated with poorer CS function (the minimum amount of contrast needed to detect visual stimuli at a range of spatial frequencies) for low spatial frequencies, although that observation did not reach statistical significance.[34]

In the current study, MPOD was significantly greater at one year than at baseline at all eccentricities for subjects supplemented with all three macular carotenoids (Group 2) and for subjects supplemented with high doses of MZ (17mg; Group 3). Although the observed augmentation in mean MPOD at 12 months did not reach statistical significance for subjects supplemented with high doses of L (20mg; Group 1), except at 1.75 ° eccentricity, it should be noted that the increases observed for this group at other eccentricities were not dissimilar in magnitude to those observed for Groups 2 and 3 (standard deviations in Group 1 were, however, larger). In addition, there was no statistically significant difference between groups with respect to change in MPOD over the study period.Of note, the L/Z diet score increased significantly between baseline and 12 months, for all three groups, which may have also been a contributing factor in the observed increase in MPOD.

The significant rise in MPOD across the spatial profile when all three macular carotenoids (Group 2) are included in the formulation or when supplemented with 17mg of MZ and small amounts of L and Z (Group 3), and especially the augmentation of MP centrally, is neither surprising nor counter-intuitive, given the known distribution of MP's individual constituent carotenoids. The inclusion of MZ in the formulation is likely to result in augmentation of MP centrally (demonstrated in Groups 2 and 3 here),

as this is the site of dominance of this carotenoid. The inclusion of L in the formulation (as in all groups) will result in MP augmentation at the more peripheral site of that carotenoid's natural dominance (1.75°), attested to by the significance of the augmentation of MP at this locus in the high L group (Group 1). These observations are also consistent with recently published findings,[322] which revealed that supplementation with a formulation containing MZ can (re)generate the typical central peak of MP at the foveal centre in subjects who lack such a central peak at baseline. Interestingly, and in addition, that study showed that subjects supplementing with all three macular carotenoids exhibited augmentation in MPOD across their spatial profiles. Indeed, this atypical profile (the lack of a central peak, sometimes referred to a "central dip"), is of particular interest, as such atypical profiles, putatively attributable to an inability to convert retinal L to retinal MZ, are associated with increased risk of AMD.[321] It would appear, therefore, that supplementation with all three macular carotenoids results in the greatest augmentation of MPOD across its spatial profile, thereby putatively affording the greatest protection against the (photo-) oxidative processes known to be important in the pathogenesis of AMD. Interestingly, *in vitro* work has concluded that the antioxidant capacity of the macular carotenoids is maximised when all three macular carotenoids are present.[396]

In this study, supplementation with the macular carotenoids resulted in the demonstrable improvements in CS in subjects with early AMD, but the inclusion of MZ in the formulation was required to achieve improvements at low *and* high spatial frequencies. Again, it is unsurprising that CS would improve following augmentation of MP, especially as such augmentation was demonstrated centrally, given the consequential enhancement of pre-receptoral filtration of blue light and attenuation of CA and light scatter. This is particularly important for subjects with AMD, as CS is an

important measure of visual function in patients afflicted with the condition. Studies have shown that, when compared with VA, CS better relates to the ability to perform tasks accurately and efficiently, to discriminate between objects[177] and to judge distances.[178] A statistically significant improvement (decrease) in contrast acuity thresholds (comparable with the reciprocal of CS) has been shown in normal subjects supplemented with L under mesopic conditions.[186] Furthermore, the observation that supplementation with high doses of L (in the absence of MZ) resulted in improved CS at low spatial frequencies only (and no observed change in mean CS at high spatial frequencies) is consistent with the fact that visual function at low spatial frequencies will be mediated by slightly eccentric retinal loci. Of note, concentrations of L are higher in the peripheral macula, compared to the fovea.[397]

Previous studies have investigated the impact of macular carotenoid supplementation on CS in subjects with AMD, with the majority of studies reporting improvements in CS following supplementation (with L and Z),[34, 169, 280, 292, 293] although no study to date has tested a formulation containing MZ. A recent study has shown significant increases in CS at low spatial frequencies following supplementation with either 10mg L, 20mg L, or 10mg L and 10mg Z (combined), in subjects with early AMD, over a 48-week study period. [398] Although significant improvements were found in CS for higher spatial frequencies (by 48 weeks), the magnitude of the differences were less than those found at the lower spatial frequencies (across all intervention groups). Of interest, the authors report no improvement in CS at 18 cpd in any of the groups. This relatively poorer response at higher spatial frequencies could be attributable to the absence of MZ in their formulation. These findings are in agreement with those reported in the current study, which found demonstrable improvements in CS at high spatial frequencies, but only amongst subjects who were supplemented with a

formulation containing MZ, and not amongst subjects supplementing with high doses of L alone.

There were observed improvements across all three groups, between baseline and 12 months, in mean CS and GD values measured using the FVA™ for the majority of spatial frequencies tested, although not all reached statistical significance, nor was the distribution of the significant parameters consistent. This may have been due, at least in part, to the reduced number of datasets collected for this particular device (n= 39). It is interesting to note, however, that mean increases in CS and GD of at least 20% (from baseline to 12 months) were seen in 10 (of 20) variables in Group 1, in 19 variables in Group 2 and in 17 variables in Group 3. This does seem to suggest that subjects taking a carotenoid formulation that includes MZ are more likely to have improvements in CS and GD, compared to a formulation which does not contain this carotenoid. These results, although speculative, do correlate with the findings of a recent study in normals.[170]

MP's capacity to filter short wavelength (blue) light at a pre-receptoral level render it capable, in theory at least, of reducing the effect of a number of optical aberrations. CA is the most important aberration affecting visual quality[399] and primarily relates to the defocus of short wavelength light (up to 1.2 dioptres compared to mid wavelength light [550nm]),[400] the attenuation of which is achieved by the pre-receptoral absorption of blue light.[401] In 1866, Max Schultze was the first to propose the theory that the absorption of short wavelength light by MP, before it was incident upon the underlying photoreceptors, would reduce CA, putatively improving VA,[402] and this theory has been extended to include CS at a range of spatial frequencies.[403, 404] The impact of CA on CS is well documented; improvements have been reported in CS and CDVA when both chromatic and monochromatic aberrations are minimised.[405] A

study investigated CS in three different groups of pseudophakic subjects, each group having an intra-ocular lens (IOL) implant of differing Abbe number (the lower the Abbe number, the greater the CA produced). There was no difference in CS between the three groups when CS was measured under 549nm monochromatic light, whereas there were statistically significant differences in CS between groups when CS was measured under broadband white light; the group with the IOL of lower Abbe number had poorer CS under white light compared with the group with the IOL of higher Abbe number. These observations suggest that CA (in this case, caused by IOLs) can degrade the quality of the retinal image, as measured by CS.[406]

The impact of light scatter on CS and visibility has been eloquently described by Wooten and Hammond[33] in a review where they explore the effects of scatter by air particles, in particular scatter caused by haze aerosols (a dispersed system of small particles suspended in a gas,[190] the most common component of the atmosphere), on visibility i.e. "how far one can see and how well details can be resolved." Light scatter is wavelength-dependent for small particles (e.g. 0.2μm), such as those found in haze aerosols. As light passes through the atmosphere, shorter wavelengths are more prone to scatter than longer wavelengths. The scattering of short-wavelength light creates a bluish veiling luminance, often termed "blue haze", which, when superimposed on the retinal image, reduces the contrast of targets being observed. The authors propose that having MPOD of e.g. 0.5 OD units (compared with having MPOD of zero OD units) can attenuate the veiling luminance of a short-wavelength dominant background by 17%, thereby increasing the visibility and discriminability of objects in natural viewing conditions.

Results of the subsidiary six-month analysis has shown that, over this time period, all three groups had comparable increases in MPOD at all eccentricities

following supplementation with the macular carotenoids. Statistically significant improvements in vision in this initial six-month period favoured Group 2, where there were a total of nine separate parameters of visual performance exhibiting improvements during this period, compared with two parameters in both Groups 1 and 3. These findings suggest that, within a six-month supplementation period, a formulation containing all three macular carotenoids potentially has the greatest impact on visual performance. This finding is supported by a similar study in normal subjects, which has shown that supplementation with a preparation containing all three macular carotenoids (L, Z and MZ) results in demonstrable improvements in visual performance, improvements that are not observed amongst subjects supplementing with L and Z alone (i.e. in the absence of MZ).[170]

These supplementary six-month findings, however, should only be interpreted in conjunction with the 12-month results with full appreciation of the fact that it is not a comparison of like with like. The apparent inconsistencies between these six-month findings and those reported for 12 months (previously discussed), can be deemed attributable to differences in the cohort composition at each of these time points. For example, not only were there were dropouts between six and 12 months (n=6), there were an additional 11 subjects who had a 12-month visit who did not attend for a six-month visit. It is not, therefore, possible to compare the changes observed at six months with those observed at 12 months as the same subjects are not being analysed at each of these time points. This is the most likely reason for the inconsistencies observed here.

There was no statistically significant change over time (either at six or 12 months) in measures of ROS in this study. This may be in part due to the relatively high mean ROS scores at baseline (18.1 dB on average; maximum score achievable is 20dB, thus creating a ceiling effect), the small numbers in the study and the relatively short

follow-up (12 months). Only one study (prior to the current one) has investigated the impact of macular carotenoid supplementation on ROS in subjects with early AMD. This study reports a mean±sd improvement of 7.3%±13.2% in mean ROS following supplementation with L for six months (20mg for first three months and 10mg for the remaining three months), although the observed improvements did not reach statistical significance. Of note, the authors did report a significant correlation between the increase in MPOD and the increase in ROS over the study period.[35] ROS has been shown to be an effective gauge of macular function compared to, for example, CDVA.[204] However, the (albeit limited) evidence to date suggests that microperimetry may not be capable of detecting functional improvements following macular carotenoid supplementation, at least not within a 12 month time frame and with a relatively small study population and with the protocol employed. Further study over a longer period (particularly considering the chronic nature of the condition) and with a larger cohort of subjects is warranted. Again, it should be noted that deterioration in ROS was also not observed for any of the supplementation groups, which is important considering the natural degenerative course of the condition. It is interesting to note that ROS was the primary outcome measure affected by intravitreal ranibizumab in cases of nv-AMD (see Chapter 5) and, therefore, may be more appropriate for appreciation of change in later stages of the condition.

Another study[407] has demonstrated improvements in retinal function (measured using the multifocal elecroretinogram [mfERG]),[81] following supplementation with the macular carotenoids, in patients with early AMD over a 48-week study period. Retinal function was assessed for six separate concentric rings of retinal eccentricity, ring 1 being closest to fixation. Following 48 weeks of supplementation with either 10mg L, 20mg L, or 10mg L and 10mg Z (combined), the authors reported statistically

significant increases in the mfERG densities within ring 1 for all intervention groups, with no observed changes in the placebo group. Furthermore, increases in MP were associated with increases in mfERG densities in the central retina (rings 1 and 2, but not in rings 3-6). Their findings are consistent with those of a previous study that reported significant improvements in mfERG densities in the central retina amongst early AMD patients receiving supplementation (10mg L and 1mg Z, in combination with co-antioxidants) compared to placebo.[408] In brief, these studies, therefore, provide objective evidence that supplementation with the macular carotenoids benefits visual function, and are consistent with the findings of the current study.

This study has shown that, from a morphological perspective, AMD remains stable for at least 12 months following supplementation with the macular carotenoids (regardless of intervention type). However, the findings presented here must be interpreted with full appreciation of the study's weaknesses, namely the small numbers of subjects involved, the study's short duration, and the absence of a placebo group. For purposes of discussion, it is reasonable to compare our findings to the placebo group in the recently published CARMA, which was a randomised, double blind, placebo controlled clinical trial of L (12mg) and Z (0.6mg) supplementation with co-antioxidants versus placebo in patients with early AMD.[294] The study population of CARMA is comparable with that of the current study, in terms of inclusion and exclusion criteria, methodology of AMD grading, and demographic and geographic considerations.[294] In CARMA, at 12 months, 47.4% of eyes in the placebo arm (108/228 eyes) exhibited any degree of progression (an increase in grade of one or more increments) compared with 41.7% of eyes in the active arm (96/230 eyes). While there were a slightly higher proportion of eyes in the placebo arm that progressed when compared to the active arm, the authors report no statistically significant difference

between these event rates (i.e. no intervention effect). Interestingly, however, in the current study, only 27% of subjects (all of whom were supplementing with the macular carotenoids) showed progression by one or more steps at 12 months (data on file).

Clinical pathology analysis has confirmed that, in subjects with early AMD, all variables remained with their respective normal reference ranges following supplementation with any of the three carotenoid formulations over a 12-month period, contributing further to the evidence concerning the safety of these supplements. These results follow on from those of a recent report, which found no adverse clinical implications in young healthy subjects following six months of supplementation with a formulation containing MZ (10.6mg), Z (1.2mg) and L (5.9mg).[409]

6.5 Conclusion

MP can be augmented, and CS enhanced, in subjects with early AMD who receive supplemental macular carotenoids. A formulation containing MZ appears to offer advantages over a formulation that does not contain MZ, in terms of improvements in psychophysical function and in terms of MP augmentation across its spatial profile, the latter putatively affording greater protection against (photo-)oxidative injury. However, the results of the current study should prompt and inform a well-designed, placebo-controlled clinical trial (ideally of longer duration) of supplementation with L, Z and MZ in subjects with AMD, where outcome measures should include visual function and disease progression.

Chapter 7. The relationship between augmentation of central MP and visual performance, in subjects with low MP at baseline

7.1 Study rationale, aims and objectives

It is important to consider baseline MPOD in any sample when investigating the impact of supplementation on MPOD augmentation and/or visual performance. MPOD values can range from 0.0 to over 1.0 OD units,[235] and are related to a range of variables, such as ethnicity, iris colour and diet.[410-412] It has been shown that MPOD response to supplementation is related to baseline MPOD levels.[323, 413]

Whilst studies have commented on the response of MP to macular carotenoid supplementation amongst subjects with low MP, the impact of macular carotenoid supplementation on visual performance in this specific group of subjects has not been investigated.

The lack of a central peak (commonly referred to as a "central dip") in the MPOD spatial profile has generated interest in recent years. A study has found this variation in MPOD spatial profile amongst 12% of its study population.[321]

The purpose of these subsidiary analyses of MOST was to investigate the relationship between central MPOD and visual performance, in early AMD subjects, who present (at baseline) with differing levels of central MPOD. In addition, I explored MPOD response and change in visual performance following supplementation in subjects with a central dip in MPOD spatial profile at baseline.

7.2 Methods

Subjects recruited for MOST AMD (Chapter 6) were further divided into tertiles based on their baseline MPOD at 0.25° (note: MPOD was not reassessed), as follows: highest tertile = MPOD ≥0.54; middle tertile = MPOD >0.32 and <0.54; lowest tertile = MPOD ≤ 0.32. These three groups were analysed with respect to change in central MP and with respect to change in vision following supplementation.

An atypical central dip MPOD spatial profile was defined as MPOD at 0.25° not exceeding MPOD at 0.5° eccentricity by more than 0.04 OD units (previously described[322]), which is divergent from the more common spatial profile of a central peak that declines from the foveal centre. Four of the five central dip subjects were also in the low baseline MPOD tertile and these may, therefore, be considered a subgroup of the low MP tertile group.

The three groups (low, medium and high baseline MP) were compared, with respect to observed changes in MPOD and letter CS, using one-way ANOVA.

Methods of MPOD measurement and visual performance assessment are described in Chapter 6.

7.3 Results

The relationship between baseline MPOD and response to supplementation, in terms of change in MPOD and letter CS, is given in Table 7.1.

Table 7.1 Comparing change in MPOD and letter CS between low, mid-range and high baseline MPOD groups.

	MP group[1]	Change[2]	Sig.
MPOD at 0.25°	Low	0.22 (0.13)	**<0.001**
	Med	0.01 (0.15)	
	High	0.08 (0.13)	
Letter CS at 1.2cpd	Low	33.4 (59.2)	0.435
	Med	10.7 (31.7)	
	High	29.9 (42.1)	
Letter CS at 2.4cpd	Low	41.2 (44.0)	0.186
	Med	32.2 (32.8)	
	High	14.6 (29.2)	
Letter CS at 6.0cpd	Low	17.7 (19.4)	0.176
	Med	9.0 (16.7)	
	High	8.7 (15.0)	
Letter CS at 9.6cpd	Low	8.7 (9.9)	0.211
	Med	3.1 (9.1)	
	High	2.8 (6.2)	
Letter CS at 15.2cpd	Low	3.4 (3.5)	0.062
	Med	0.8 (3.9)	
	High	1.1 (4.1)	

Abbreviations: MPOD=macular pigment optical density; MP=macular pigment; CS=contrast sensitivity; cpd=cycles per degree; GD=glare disability; ms=mesopic conditions.

[1]MP group = tertile groups according to MPOD at 0.25° eccentricity: high=top tertile; med=middle tertile; low=bottom tertile. The low groups consisted of 15 subjects with baseline MPOD ≤ 0.32 and the high MPOD group had 22 subjects with baseline MPOD ≥ 0.54, leaving 15 subjects in the medium tertile.

[2]Change=mean change between baseline and 12 months.

Considering the increasing interest in MPOD spatial profiles,[321, 322, 414, 415] change in MPOD and change in measures of visual function was explored, in subjects who presented with a central dip at baseline. Five subjects had an atypical MPOD spatial profile at baseline. Four of these patients were in Group 2 and one was in Group

3. MPOD spatial profiles (at 0.25° and 0.50° eccentricity) at baseline and at twelve months following supplementation, for each of the five subjects, are displayed in Table 7.2. The atypical MPOD spatial profile was no longer present at 12 months in four of the five cases (the remaining subject exhibited an increase in MPOD at both 0.25° and at 0.50°, but the central dip was not, as such, rebuilt).

Table 7.2 Central MPOD values at baseline and twelve months in subjects with an atypical profile at baseline.

Subject	Group	Baseline		12 months	
		MPOD 0.25	MPOD 0.5	MPOD 0.25	MPOD 0.5
MZAMD001	2	0.22	0.26	0.28	0.37
MZAMD006	2	0.31	0.31	0.39	0.32
MZAMD039	3	0.21	0.20	0.55	0.37
MZAMD042	2	0.19	0.33	0.44	0.31
MZP2006	2	0.55	0.54	0.60	0.55

Abbreviations: MPOD=macular pigment optical density

In this group of subjects (n=5), the change in MPOD at 0.25° correlated significantly with a change in letter CS at all spatial frequencies (Table 7.3; see Figure 7.1 for graphical representations of these relationships), but with no other parameter of visual function ($p > 0.05$; data not shown). In contrast, these relationships (between change in MPOD and change in letter CS) were not significant amongst subjects with typical MP spatial profiles at baseline ($r < 0.1$; $p > 0.05$, for all).

Table 7.3 Significant associations between change in MPOD (at 0.25° eccentricity) and change in other parameters, in patients with an atypical MPOD profile at baseline.

Variable	r	p
CS 1.2 cpd	0.960	0.010
CS 2.4 cpd	0.966	0.008
CS 6.0 cpd	0.930	0.022
CS 9.6 cpd	0.922	0.026
CS 15.2 cpd	0.914	0.030

Abbreviations: CS=contrast sensitivity; cpd=cycles per degree

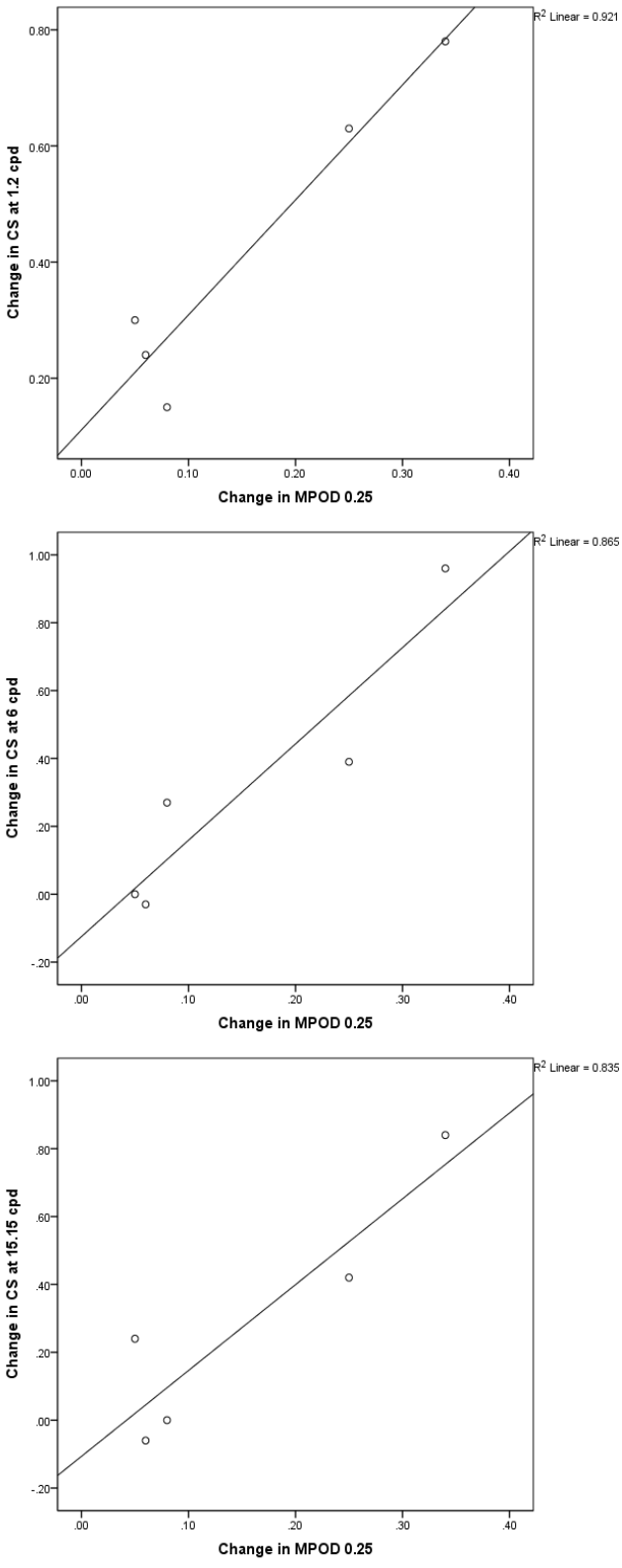


Figure 7.1 The relationship between the change in MPOD at 0.25° and change in letter contrast sensitivity at 1.2, 6.0 and 15.2 cpd, respectively.

Note: values for CS are presented in log form

7.4 Discussion

The extent to which MP augments in response to carotenoid supplementation is related to baseline MP levels,[323, 413] where subjects with relatively low baseline MP are more likely to exhibit increases in MP compared to subjects with high baseline MP (perhaps due to a ceiling effect; of note, peak MP levels typically range between 0.0 and 1.0 OD units,[235] and are subject to dietary modification[329, 340]). This has been confirmed in the current study, where there was a statistically significant difference between subjects in the lowest baseline MPOD tertile (≤ 0.32 OD units) compared to subjects in the middle or highest baseline MPOD tertiles, with respect to change in central MPOD, where the greatest augmentation of MP was seen amongst subjects with low baseline MPOD. However, the very low p-value reported here (0.01) should be interpreted with caution; statistically there is nearly always a negative correlation between X (MPOD at baseline) and Y-X (difference in MPOD between baseline and 12 months), which may account, at least in part, for this finding.

Of interest, the data indicate that subjects with low MPOD at baseline are likely to have the greatest improvements in CS following supplementation (with any of the three macular carotenoid formulations), albeit not statistically significant in this small sample. In other words, MP enrichment and detectable improvements in vision are of greatest relevance to subjects who have poor/low MP to begin with. This has important implications for any anticipated improvements in vision following supplementation, and may account for the variability in the results of studies investigating the impact of carotenoid supplementation on MPOD and vision. Interestingly, Wooten and Hammond observed, combining data from a number of (USA-based) studies, that 43% of subjects in the sample (n=846) had MP levels of <0.2.[33] This suggests that a significant percentage of the (Western) population may not have optimised vision because of low

MP, which may have important implications, not only for the current population with early AMD and reduced vision, but possibly also for professionals such as pilots, sportspeople, drivers and soldiers. Further study in a normal population is warranted, in this respect. In such and similar cases, measurement of MP and, where appropriate, a carotenoid-rich diet and/or supplements, may be important to consider, even in the absence of disease.

The spatial profile of MP and its variability has been of interest to those investigating the role of MP, both for vision and for protection against AMD. Initial studies reporting on the distribution of MP describe it declining exponentially with increasing retinal eccentricity.[234, 416] However, variations in its distribution have also been reported,[416, 417] one of which has been coined a "central dip". A study (previously discussed in section 4.5.3.3) showed that, prior to disease onset, the known risk factors for AMD (tobacco use, family history of the condition, increasing age) are independently associated with a relative lack of MP.[320] A proportion of that study population (12%) exhibited a central dip in their MPOD spatial profile and, interestingly, this central dip was associated with tobacco use and increasing age,[321] suggesting that such atypical MP spatial profiles may independently represent risk for AMD. It has been hypothesised that these central dips in the MP spatial profile are attributable to a relative lack of MZ, considering MZ is the dominant carotenoid in the foveal centre, and may be the result of an inability among subjects with such spatial profiles to convert retinal L to MZ (retinal MZ is formed from retinal L, but not retinal Z). Such subjects may require this carotenoid in supplement form in order to achieve a typical and desirable spatial profile.

Our analysis has found an association between observed changes in central MPOD and observed changes in measures of visual performance as measured by letter

CS, amongst subjects with a central dip at baseline. The data from this analysis has shown normalisation of atypical MPOD spatial profiles (in 80% of cases) following supplementation with an MZ-based formulation (I cannot comment on the response of central dip profiles to a high L-based supplement using the results of this study). This finding has been previously demonstrated in a study designed to investigate the effect of supplementation on a group of subjects (normal) that exhibited a central dip in their MP spatial profile (see section 5.5.3.3).[322] The authors concluded that MP spatial profiles characterised by central dips, can be normalised following supplementation with a formulation containing MZ, but not with a formulation that is lacking this carotenoid (at least not in an eight-week period). In addition, augmentation across MP's spatial profile required supplementation with a formulation containing all three macular carotenoids. Interestingly, a recent study has shown that "ring-like structures" in MPOD, observed using autoflourescence, and which are representative of central dips observed using HFP,[415] were not attenuated following supplementation with 12mg L and 2mg Z (but no MZ).[414]

While the attenuation of central dips has been demonstrated through (MZ) supplementation, the implications of this for vision have not yet been investigated. The data from this analysis putatively suggest that subjects with atypical spatial profiles, such as have been described, are more likely to benefit visually following supplementation. While I fully acknowledge the obvious weakness of small numbers in this case, the outcome is intriguing and warrants further study.

It has already been shown that central MP levels are positively related to visual performance.[29-33] However, the extent to which MP augments in response to carotenoid supplementation has been shown to be related to baseline MP levels,[323, 413] where subjects with relatively low baseline MP are more likely to exhibit increases in MP

compared to subjects with high baseline MP (probably due to a "ceiling effect"). Subjects with a central dip may be similar to subjects with low central MP (and normal MP spatial profiles) i.e. both having relatively low central MP, a condition which facilitates a heightened uptake of these carotenoids at the macula following supplementation. In contrast, one would expect a ceiling effect, in terms of MP (and possibly vision), amongst subjects with relatively high amounts of MP. Therefore, MP augmentation is of particular importance and relevance for subjects with low MP or with a central dip in their MPOD profile. As has been previously discussed (Chapter 6), Wooten and Hammond observed, combining data from a number of (USA-based) studies, that 43% of subjects in the sample (n=846) had MP levels of <0.2. Kirby et al reported that 12% of their study sample (normal, healthy individuals; study based in Ireland) had a central dip in their MPOD spatial profile.[321] Considering these observations, the results of this study (in additional to the findings reported in section 6.3.2) suggest that there may be a significant percentage of the (Western) population that may not have optimised vision because of low MP or because of an atypical MP spatial profile (see Discussion, Chapter 6). This suggests that MPOD measurement should be considered in subjects who present with poor CS and/or who present with symptoms of glare, even in the absence of disease, the cause of which may be attenuated with macular carotenoid supplementation, particularly in cases where atypical profiles are observed.

The obvious weakness of this subsidiary study is the small number of subjects involved, which prevent us drawing firm conclusions. Further trials to confirm these findings are necessary, amongst subjects with and without AMD.

7.5 Conclusion.

In the current study, subjects with low baseline central MPOD had the greatest increases in MPOD and the greatest improvements in CS, when compared with subjects in the mid-range or high baseline MPOD categories. Eighty per cent of subjects who presented with a central dip at baseline had their MPOD spatial profile normalised following supplementation with the macular carotenoids. This normalisation was strongly associated with an improvement in CS at each spatial frequency. These findings indicate that the optimisation of CS is putatively dependent on central MP levels, which should be given due consideration when investigating the impact of macular carotenoid supplementation on visual performance.

Chapter 8. The relationship between retinal morphology and visual performance, in subjects with age-related macular degeneration.

8.1 Study rationale, aims and objectives

The diagnosis of AMD is currently determined by the clinical appearance of the macula i.e. signs of drusen (size, form and number), pigmentary and atrophic changes. A range of AMD grading scales exist, which grade a given individual's risk of progression to later, more visually consequential, forms of the condition. Additional measures, such as OCT and FFA have provided a further understanding of AMD, facilitating identification of the presence of fluid or cysts in, or under, the retina.

In addition to clinical examination, valid evaluation of the visual consequences of AMD is essential, not only for accurate documentation of disease status and progression, but also to inform ophthalmologists of the impact of disease severity on visual function and on quality of life. CDVA has been used as the primary measure of vision to quantify disease severity in cases of AMD (and other ocular conditions), most likely because of its ease of use, low cost and familiarity (for both patient and eye care practitioner).[14, 157] There is, however, a general consensus that it is neither a true reflection of daily visual experience, nor of disease severity.[418-420] The limitation of CDVA is that it measures the angular resolution limits of the eye at high contrast only, the real world presenting a myriad of different visual experiences, affected by things such as lighting conditions, colour, colour contrast levels, which cannot be assessed by CDVA. It has already been shown that the use of VA charts in isolation can hinder the interpretation of patients' functional visual difficulty in AMD,[418] as well as other eye conditions such as glaucoma, cataract and diabetic retinopathy.[419, 420]

The purpose of this study was to investigate the relationship between measures of visual performance (including CDVA) and MFT in cases of nv-AMD, and between measures of visual performance and AMD-severity grade, in cases of early AMD, and to explore whether other psychophysical parameters should be considered instead of, or in addition to, CDVA, in an attempt to better understand AMD, and its impact on visual function, and also in the design of clinical research studies.

8.2 Methods

8.2.1 Subjects

Data collected for the subjects recruited for the studies outlined in Chapters 6 and 7 were used for this cross-sectional analysis. Forty-seven subjects (with nv-AMD) were recruited for Study 1 (VEGF) and were assessed at baseline for measures of visual performance and measures of MFT, as outlined in Chapter 5. Sixty-six subjects (with early AMD) were recruited for Study 2 (MOST) and were assessed at baseline with respect to measures of visual performance and AMD-severity grade, as outlined in Chapter 6. The psychophysical and morphological assessments utilised in the respective studies are described in Chapters 5 and 6.

8.2.2 Statistical analysis

Pearson correlations were used to investigate bivariate relationships between measures of foveal thickness and measures of visual performance. Multivariate analysis was used to investigate the relationship between AMD-severity grade and measures of visual performance.

Assuming a 5% level of significance, and a two-tailed test, a sample of 47 has power of 0.81 for detecting a correlation of 0.4 and a sample of 66 has power of 0.92 for detecting a correlation of 0.4.

8.3 Results

8.3.1 Study 1

Data collected from forty-seven subjects (47 eyes) with active nv-AMD were available for this cross sectional analysis. Statistically significant moderate correlations were found between measures of MFT and measures of ROS at fixation, within the central 5° and within the central 16° of fixation (Table 8.1; see Figures 8.1, 8.2 and 8.3 for graphical representation of these respective relationships). All other measures of visual performance were not significantly correlated with MFT ($p>0.05$, for all; Table 8.1). Of note, CDVA was not significantly correlated with MFT, ($r = -0.247$; $p = 0.094$).

Table 8.1 The relationship between MFT and measures of visual performance.

Variable	r	p
ROS fixation	-0.325	**0.029**
ROS central 5°	-0.344	**0.021**
ROS central 16°	-0.298	**0.047**
CDVA	-0.247	0.094
logRAD	0.047	0.752
Reading speed	0.144	0.334
Mean reading speed	0.088	0.555
PHP ta	-0.005	0.976
PHP tii	-0.047	0.789
FVA CS (mesopic conditions)		
Frequency (cpd)		
1.5	-0.050	0.742
3	-0.088	0.559
6	0.002	0.987
12	-0.181	0.229
18	-	-
FVA CS (photopic conditions)		
Frequency (cpd)		
1.5	0.134	0.374
3	-0.006	0.969
6	-0.023	0.879
12	0.024	0.875
18	-0.019	0.898
FVA GD (mesopic conditions)		
Frequency (cpd)		
1.5	-0.019	0.900
3	-0.080	0.597
6	0.060	0.692
12	-0.087	0.567
18	-	-
FVA GD (photopic conditions)		
Frequency (cpd)		
1.5	0.147	0.329
3	0.176	0.242
6	0.026	0.865
12	-0.069	0.650
18	-0.052	0.733

Abbreviations: MFT=mean foveal thickness; ROS=retinotopic ocular sensitivity; CDVA=corrected distance visual acuity; PHP=preferential hyperacuity perimetry; ta=total area; tii=total integrated intensity; FVA=Functional Vision Analyser; CS=contrast sensitivity; cpd=cycles per degree; GD=glare disability.

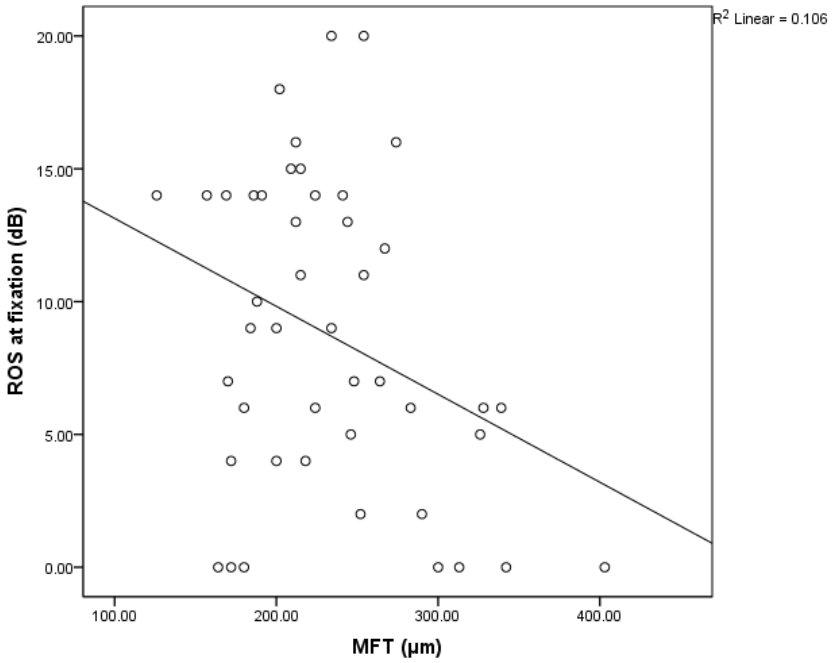


Figure 8.1 The relationship between mean foveal thickness (MFT) and retinotopic ocular sensitivity (ROS) at fixation in subjects with neovascular age-related macular degeneration.

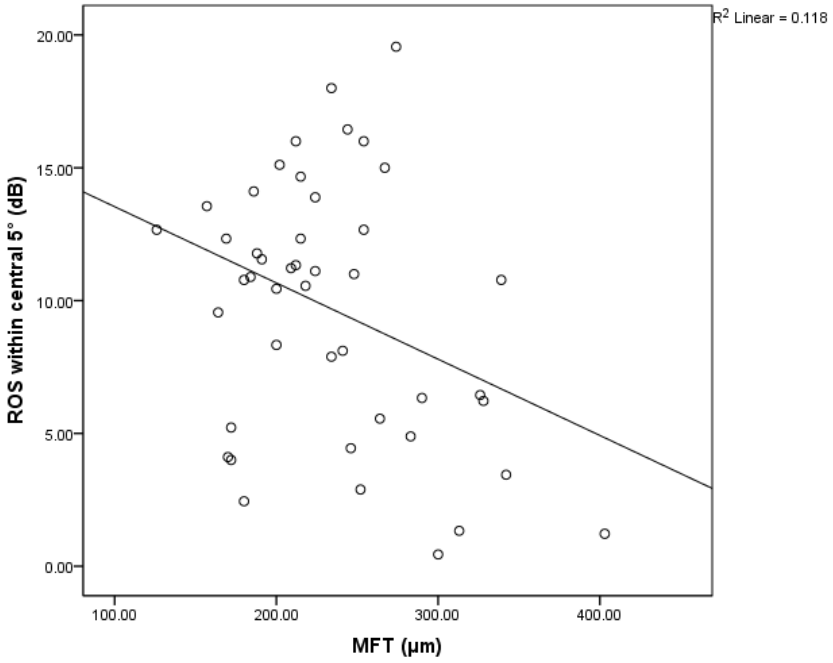


Figure 8.2 The relationship between mean foveal thickness (MFT) and retinotopic ocular sensitivity (ROS) within the central 5° in subjects with neovascular age-related macular degeneration.

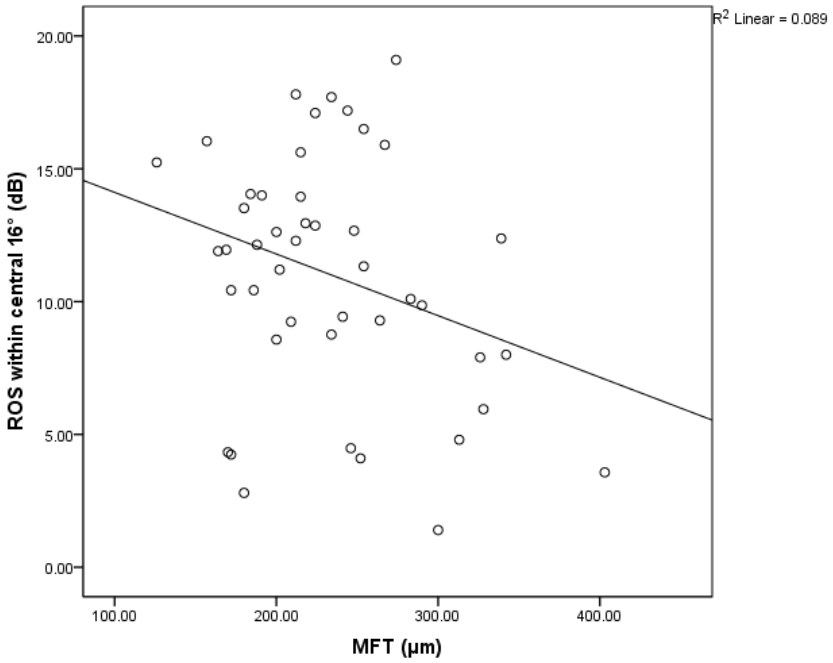


Figure 8.3 The relationship between mean foveal thickness (MFT) and retinotopic ocular sensitivity (ROS) within the central 16° in subjects with neovascular age-related macular degeneration.

8.3.2 Study 2

Data collected from 66 subjects (66 eyes) with early AMD were available for analysis. The eight early AMD grades were grouped as follows: Group 1 = grades 1 and 2 (n=12); Group 2 = grades 3 and 4 (n=25); Group 3 = grades 5 and 6 (n=18); Group 4 = grades 7 and 8 (n=11). There was an inversely significant relationship between measures of ROS and AMD-severity grade (Table 8.2 and Figure 8.4), such that, as AMD severity increased, ROS deteriorated. No other parameters of visual function were significantly related to AMD severity, including CDVA (p=0.37; Figure 8.5).

Table 8.2 The relationship between AMD severity and measures of visual performance ROS.

Variable	Group 1	Group 2	Group 3	Group 4	p
ROS central 4°	18.91 (0.75)	18.64 (1.77)	16.74 (4.01)	16.00 (3.13)	**0.010**
ROS central 8°	18.98 (0.62)	18.89 (1.48)	17.43 (2.89)	16.34 (2.68)	**0.003**
ROS central 12°	18.79 (0.87)	18.61 (1.60)	17.10 (2.73)	15.89 (2.91)	**0.002**
CDVA	98 (8)	100 (6)	96 (9)	99 (5)	0.373
Letter CS (photopic conditions)					
1.2 cpd	71.5 (44.7)	81.5 (52.4)	47.3 (25.4)	39.7 (22.1)	**0.006**
2.4 cpd	58.1 (40.6)	65.2 (43.2)	45.3 (40.3)	40.1 (22.4)	0.126
6.0 cpd	26.9 (12.7)	29.6 (15.6)	19.9 (13.5)	23.6 (14..4)	0.242
9.6 cpd	15.5 (8.4)	16.2 (9.2)	10.2 (7.2)	12.3 (6.6)	0.079
15.2 cpd	6.5 (4.3)	7.6 (4.9)	4.5 (4.3)	6.1 (4.1)	0.064
FVA CS (mesopic conditions)					
Frequency (cpd)					
1.5	41.4 (21.1)	47.7 (26.9)	40.2 (31.2)	47.4 (33.2)	0.428
3	50.8 (33.7)	56.8 (37.3)	41.4 (26.9)	60.0 (39.5)	0.395
6	27.3 (15.4)	24.3 (19.9)	23.1 (18.7)	20.7 (16.1)	0.822
12	4.6 (2.9)	6.0 (4.5)	6.6 (4.4)	5.1 (2.0)	0.620
18	2.7 (2.0)	2.7 (2.3)	2.3 (0.7)	2.0 (2.0)	0.760
FVA CS (photopic conditions)					
Frequency (cpd)					
1.5	29.4 (9.6)	34.7 (21.6)	31.9 (21.2)	45.0 (30.0)	0.618
3	57.7 (26.0)	59.1 (28.8)	60.0 (32.1)	61.3 (31.7)	0.914
6	42.9 (27.5)	47.5 (34.9)	37.6 (37.4)	37.6 (23.0)	0.396
12	12.2 (10.8)	15.1 (15.7)	12.1 (10.1)	11.7 (8.2)	0.946
18	5.9 (7.3)	7.6 (8.8)	5.4 (4.2)	4.9 (4.0)	0.926
FVA GD (mesopic conditions)					
Frequency (cpd)					
1.5	48.2 (34.6)	32.8 (22.6)	27.6 (22.8)	27.1 (19.0)	0.391
3	38.1 (23.8)	51.0 (37.8)	38.4 (40.7)	37.7 (28.8)	0.403
6	20.2 (16.9)	19.5 (16.5)	16.6 (14.5)	21.7 (16.0)	0.901
12	6.4 (4.9)	5.8 (3.7)	5.1 (2.2)	4.0 (0)	0.522
18	2.7 (2.0)	2.2 (0.6)	3.0 (2.7)	2.0 (0)	0.467
FVA GD (photopic conditions)					
Frequency (cpd)					
1.5	35.7 (17.3)	39.8 (19.6)	36.0 (29.0)	39.4 (29.4)	0.721
3	66.0 (25.4)	66.6 (27.4)	50.4 (28.8)	65.3 (28.6)	0.113
6	39.7 (26.1)	53.8 (35.5)	35.6 (36.1)	51.9 (34.0)	0.211
12	15.4 (18.5)	16.5 (13.8)	10.9 (9.7)	13.9 (10.7)	0.649
18	7.7 (10.1)	8.4 (8.2)	5.4 (5.7)	7.0 (5.6)	0.733

Abbreviations: AMD=age-related macular degeneration; ROS=retinotopic ocular sensitivity; CDVA=corrected distance visual acuity; CS=contrast sensitivity; cpd=cycles per degree; FVA=Functional Vision Analyser; GD=glare disability.

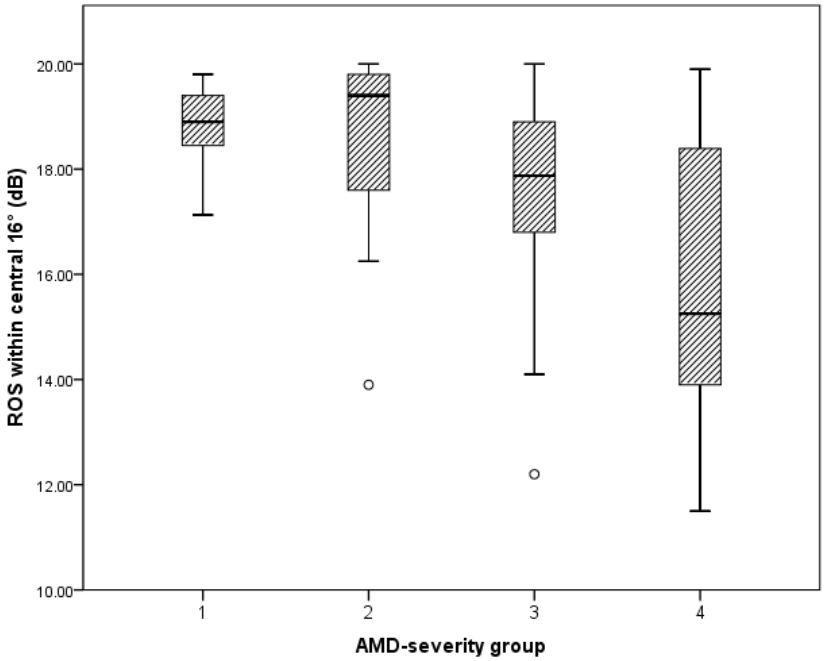


Figure 8.4 The relationship between AMD-severity and ROS within the central 16°.
Abbreviations: AMD=age-related macular degeneration; ROS=retinotopic ocular sensitivity.
AMD severity group defined as: group 1=grades 1 and 2; group=grades 3 and 4; group 3=grades 5 and 6; group 4=grades 7 and 8.

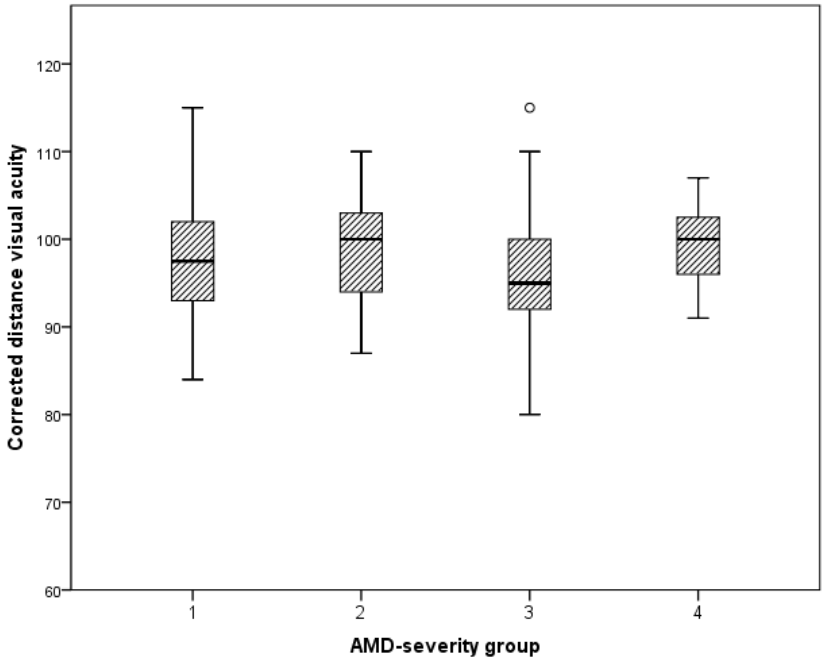


Figure 8.5 The relationship between AMD-severity and CDVA. Abbreviations: CDVA=corrected distance visual acuity; AMD=age-related macular degeneration; AMD severity group defined as: group 1=grades 1 and 2; group=grades 3 and 4; group 3=grades 5 and 6; group 4=grades 7 and 8.

Baseline VA and FVA measures were available from a study involving normal subjects (n=36)[170] and were compared with the corresponding data for both the early and nv-AMD groups. There was a statistically significant difference in age between the three groups and, hence, age was controlled for in these analyses. As the three studies had different inclusion criteria for levels of CVDA (6/6, 6/12 and 6/30 for the normal, early AMD and nv-AMD studies, respectively), subjects with relatively good CDVA (≥ 6/9, to allow for comparable numbers in the three groups) were selected and analysed, across the three studies. One-way ANOVA was used to test for differences between three groups of subjects with respect CS and GD, controlling for age and CDVA (Table 8.3).

Table 8.3 Comparing measures of visual performance for eyes without disease, eyes with early AMD and eyes with nv-AMD, all with relatively good CDVA

Variable	Normals (n=36)	Early AMD (n=47)	Nv-AMD (n=22)	p
Age (years)	*51 (15)*	*67 (8)*	*71 (11)*	*<0.001*
ROS central 5°†	-	18.0 (2.2)	11.9 (3.9)	**<0.001**
ROS central 16°‡	-	17.9 (2.2)	12.7 (3.7)	**<0.001**
FVA CS (mesopic conditions)				
Frequency (cpd)				
1.5	58.9 (25.3)	48.0 (26.7)	24.8 (9.0)	**<0.001**
3	68.1 (34.5)	56.3 (33.2)	39.1 (20.1)	0.122
6	48.1 (29.3)	25.9 (17.9)	14.3 (11.5)	**0.033**
12	9.5 (8.6)	6.1 (4.0)	4.0 (0)	0.548
18	2.6 (2.7)	2.6 (1.8)	2.0 (0)	0.157
FVA CS (photopic conditions)				
Frequency (cpd)				
1.5	47.6 (25.0)	36.0 (21.6)	28.6 (15.4)	0.113
3	85.8 (32.7)	62.5 (28.0)	44.2 (21.1)	**0.017**
6	99.0 (39.6)	46.3 (32.2)	30.5 (20.4)	**0.005**
12	37.7 (26.8)	14.3 (12.8)	7.2 (5.6)	**0.002**
18	12.5 (11.2)	6.8 (7.1)	3.0 (3.6)	0.211
FVA GD (mesopic conditions)*				
Frequency (cpd)				
1.5	31.2 (21.3)	35.6 (25.2)	14.7 (8.7)	**<0.001**
3	41.8 (23.5)	47.4 (34.8)	22.0 (13.3)	**0.010**
6	27.4 (21.5)	20.6 (15.8)	9.2 (8.1)	**0.036**
12	5.4 (3.1)	5.6 (3.5)	4.2 (0.9)	0.157
18	2 (0.5)	2.5 (1.7)	2 (0)	**0.044**
FVA GD (photopic conditions)*				
Frequency (cpd)				
1.5	54.3 (25.5)	39.6 (23.4)	25.0 (13.9)	**0.002**
3	89.4 (28.7)	65.6 (26.5)	44.0 (25.7)	**<0.001**
6	102.1 (42.1)	50.3 (33.2)	28.2 (22.4)	**<0.001**
12	33.2 (18.3)	15.5 (13.5)	7.7 (7.9)	0.055
18	12.9 (8.6)	7.8 (7.8)	2.8 (2.3)	0.230

Abbreviations: AMD=age-related macular degeneration; nv-neovascular; CDVA=corrected distance visual acuity; ROS=retinotopic ocular sensitivity; FVA=Functional Vision Analyser; CS=contrast sensitivity; cpd=cycles per degree; GD=glare disability; - = ROS data not available (test not performed in this study)

†central 4° assessed in early AMD subjects

‡central 12° assessed in early AMD subjects

Note: Tests were performed on log-transformed data

*a higher lux glare source was used in the normal study compared to that used in the early and nv-AMD studies

Further analysis was performed to test the assumption that early AMD is of little visual consequence. Eyes in the normal and early AMD groups, which had CDVA of ≥ 6/7.5 were included in the analysis, again controlling for age (but not CDVA). The results are presented in Table 8.4

Table 8.4 Measures of CS and GD in normal and in early AMD subjects, all with CDVA ≥ 6/7.5

Variable	Normals (n=36)	Early AMD (n=35)	p
Age (years)	*51 (15)*	*65 (9)*	*<0.001*
CDVA (study eye)	*108 (5)*	*105 (5)*	*<0.001*
FVA CS (mesopic conditions)			
Frequency (cpd)			
1.5	58.9 (25.3)	48.6 (27.9)	**0.048**
3	68.1 (34.5)	56.2 (30.1)	0.143
6	48.1 (29.3)	27.0 (18.2)	**0.013**
12	9.5 (8.6)	6.5 (4.5)	0.357
18	2.6 (2.7)	2.7 (2.1)	0.096
FVA CS (photopic conditions)			
Frequency (cpd)			
1.5	47.6 (25.0)	34.9 (21.0)	0.193
3	85.8 (32.7)	63.8 (28.2)	**0.034**
6	99.0 (39.6)	50.1 (31.5)	**<0.001**
12	37.7 (26.8)	16.0 (13.9)	**<0.001**
18	12.5 (11.2)	7.6 (7.8)	0.266
FVA GD (mesopic conditions)*			
Frequency (cpd)			
1.5	31.2 (21.3)	34.4 (23.4)	0.185
3	41.8 (23.5)	50.5 (38.1)	0.234
6	27.4 (21.5)	22.1 (16.0)	0.370
12	5.4 (3.1)	6.2 (3.9)	0.155
18	2 (0.5)	2.4 (1.2)	**0.046**
FVA GD (photopic conditions)*			
Frequency (cpd)			
1.5	54.3 (25.5)	38.7 (23.8)	0.115
3	89.4 (28.7)	64.1 (24.5)	**0.007**
6	102.1 (42.1)	54.9 (35.3)	**0.001**
12	33.2 (18.3)	18.1 (14.3)	**0.029**
18	12.9 (8.6)	8.9 (8.3)	0.597

Abbreviations: AMD=age-related macular degeneration; CDVA=corrected distance visual acuity; FVA=Functional Vision Analyser; CS=contrast sensitivity; cpd=cycles per degree; GD=glare disability;

Note: Tests were performed on log-transformed data

*a higher lux glare source was used in the normal study compared to that used in the early AMD study

8.4 Discussion

This analysis was designed to investigate the relationship between disease severity related features/measures of macular morphology and a number of measures of visual function in subjects with AMD. The results of this study have shown that disease severity (defined by MFT in cases of nv-AMD, or AMD-severity grade in cases of early AMD) is best reflected psychophysically by measures of ROS. This is consistent with the hypothesis that CDVA alone is not an appropriate psychophysical test to evaluate the visual impact of AMD, in general. This is of clinical importance as CDVA is still the most widely used test of visual function for patients with this condition and is often the determining measure of disease impact on quality of life.

The presence of early AMD lesions are associated with a decrease in CDVA of up to two letters or fewer when compared with eyes without AMD.[163] However, the test-retest variability of CDVA can be up to as much as two lines of letters on a logMAR chart,[164] indicating that a difference of two letters cannot be reliably measured. Late AMD, on the other hand, is associated with a more significant decrease in CDVA (approximately seven lines of letters), but only when signs of advanced AMD involved the central subfields of the macula.[163] It has been shown, however, that there is no statistical difference in acuity between subjects with nv-AMD and subjects with GA.[163] CDVA is, therefore, unlikely to be a sensitive psychophysical measure to quantify disease severity in cases of AMD. A wide acuity range has also been demonstrated despite similar areas of atrophy in AMD, although, unsurprisingly, foveal involvement was the key predictor of VA.[165] Another study has shown that for the same level of VA, eyes with GA have worse function, particularly for dark-adapted vision tests and reading speed, than eyes with drusen.[167] Similarly, lesion size in subfoveal nv-AMD cannot explain the wide variations in VA.[166]

Microperimetry is a thresholding technique that tests light sensitivity incrementally at specific retinal loci (VA is testing spatial resolution of targets). The fact that ROS is a retinotopic test allows for a finer probing of macular function that that achieved by CDVA. Therefore, one might expect that measures of ROS are more appropriate than CDVA, particularly for obtaining a morphology-related functional assessment, in cases of AMD.

AMD-severity grade and visual performance: Clinical manifestations of AMD are typically categorised into a grading scale to determine disease severity and risk of progression to later, more visually consequential, stages of the condition. A number of grading systems exist which define and classify the signs of AMD from fundus photographs. The most widely used systems are the Wisconsin Age-related Maculopathy Grading System,[393] the International Classification and Grading System[41] and the grading scale used in AREDS.[421] In this respect, the current analysis has involved subjects diagnosed with the early stages of the condition, based on an 11-step AREDS grading scale. The levels of increasing severity in the 11-step AREDS scale are defined by drusen area, increased pigment, RPE depigmentation and the late AMD lesions (signs of nv-AMD [grade 11] or GA [grades 9 and 10]). Our study has shown that disease severity, in cases of early AMD across its range of stages (1-8), is best reflected by measures of ROS.

No study, to my knowledge, has reported on the relationship between ROS (or CS or GD) with respect to a classified AMD grade, such as the AREDS scale. However, a range of studies have looked at the relationship between clinical signs at the macula and ROS in cases of AMD. It has been shown that in subjects with early AMD, ROS diminishes in areas overlying drusen and/or pigment abnormalities, in the presence of

good VA (6/6), and this reduction in sensitivity was greater when both lesions were present.[204]

Another study reported a significant reduction in ROS in subjects with early AMD compared with age-matched controls.[422] The use of specialised software to overlay the microperimetry infrared image onto the OCT retinal image facilitated the assessment of ROS over individual druse, which was found to be significantly reduced when compared to adjacent retinal ROS values. The integrity of the inner segment/outer segment (IS/OS) junction, which has been shown to be a significant predictor of VA in macular diseases,[423] was also observed to correlate significantly with ROS.[422] In addition, and compared with drusen height, diameter, volume, the integrity of the IS/OS junction was the strongest predictor of ROS overlying drusen. Other studies have also reported on the correlation between the integrity of the IS/OS junction and ROS for a range of eye conditions.[424-427] Although it has been shown that the IS/OS junction is related to drusen volume, it is interesting to note that the IS/OS junction is not a feature gradable from fundus images (and, therefore, does not contribute to any grading scale, such as AREDS), as it can only be detected using OCT.

It has also been reported that drusen diameter, drusen height, and drusen volume do not offer additional predictive value if the IS/OS junction integrity grading is known.[422] Indeed, Sunness et al have reported comparable sensitivity values between drusen and non-drusen areas.[428] Also, Midena et al found that the number of drusen, the presence of focal hyperpigmentation, and the presence of RPE atrophy, did not influence mean sensitivity values,[180] suggesting that drusen alone may not account fully for functional deficits. However, differences in methodology may account for the discrepancies between these results and those previously discussed, particularly since the latter studies (Sunness and Midena) utilised traditional, and considerably more

limited, perimetry (the Fundus Camera Stimulator and the Humphrey Field Analyzer™, respectively). The latter, when compared with microperimetry, does not allow a point to point correlation of function and morphology, cannot facilitate real-time fundus tracking, nor provide an appreciation of fixation stability. The Fundus Camera Stimulator, on the other hand, facilitates visualisation of the posterior pole and retinal location of the targets, but unlike microperimtery, does not automatically correct for a subject's eye movements to ensure, for example, that the desired retinal areas are being tested.

Considering the relevance of the IS/OS junction and the fact that it is not measurable using traditional fundus imagery, and yet is related to ROS, ROS (by microperimetry) may, then, offer additional, and possibly more useful, information with respect to understanding disease severity in AMD than would be provided by a classified grading scale in isolation. A limitation of the current study is that OCT measurements were not taken on the early AMD cohort so that the IS/OS junction could, therefore, not be assessed.

Other methods of measuring retinal function (although not ROS) have included the measurement of rod and cone sensitivities in isolation. Two particular studies are of interest, involving subjects with early AMD; Remky et al investigated cone sensitivity using short-wavelength automated perimetry,[429] and Scholl et al reported on both rod and cone sensitivity (through a technique called fine matrix mapping in scotopic and photopic conditions, respectively).[430] In both studies, retinal functional loss was evident, even in cases of good VA. The fine matrix mapping technique (Scholl et al) detected more rod than cone sensitivity decreases, which supports the notion that deterioration in the rod system precedes that of the cone system in AMD.[431] Both

studies found that functional deficits correlated with fundus abnormalities (large soft drusen and pigmentary RPE changes).

AMD-grading scales have been developed for purposes of determining disease severity, tracking disease progression, and predicting progression to late AMD.[41, 393, 421] It is interesting to note that, while the presence of high-risk drusen has been shown to be a sensitive indicator of the risk of disease progression (i.e. if someone is going to progress, they will be correctly identified), the specificity is relatively low (i.e. if someone is not going to progress, there is a high chance that the scale will incorrectly predict progression).[432] Therefore, there are people who are at greater risk of progression who are classified (according to grading scales) similarly to those who have lower risk; a notable limitation of the clinical grading system. It is important to identify the difference, so that subjects who are at greatest risk are those included, for example, in trials investigating factors related to disease progression. In fact, a recent publication has suggested that clinical vision measures (in combination with gene testing, which cannot detect disease) may increase the power of prediction models for AMD.[433] Measures of visual performance, such as ROS, could potentially be used as functional biomarkers in AMD. Long-term prospective studies are needed in this respect.

Macular thickness and visual performance: The AREDS scale defines the stage of AMD, from early (grades 1-8) through to late (9-11), and distinguishes nv-AMD (grade 11) from GA (grades 9 and 10) but does not subdivide these later stages. In cases of nv-AMD, one way in which severity can be further quantified morphologically, is through measures of OCT. Nv-AMD is characterised by the presence of CNV, which is associated with the leakage of fluid (from neovascular vessels) into and/or under the neurosensory retina in the macular region, disrupting the normal structure of the

photoreceptors and increasing retinal thickness through the presence of fluid/cysts, as observed on OCT. Anti-VEGF therapy functions to inhibit the action of VEGF, thereby arresting the development of CNV and thus reducing fluid/cysts at the macula, thus normalising retinal thickness. Therefore, one of the most important measurable morphological features of nv-AMD is retinal thickness at the fovea. Consequently, outcome measures that can efficiently quantify morphological as well as functional damage (or status) are of critical importance in determining the most effective treatment or treatment strategies.

A range of studies have previously investigated the relationship between OCT parameters and visual function in subjects with AMD[205, 425, 434-436] and other pathological conditions of the macula, such as diabetic macular oedema.[207, 437, 438] A recent study found a statistically significant negative correlation between central retinal thickness and central ROS in patients with nv-AMD.[436] Others have also postulated that the measurement of ROS may be a more appropriate method to assess central visual function than conventional VA, following a study where there were significant improvements in ROS (within the central 10° of fixation), significant decreases in foveal thickness, but no significant improvements in CDVA, following PDT in subjects with nv-AMD.[439]

In another study, a statistically significant relationship was found between RPE lesion area and central ROS (but not CDVA) in patients with nv-AMD undergoing anti-VEGF therapy, at every study visit (baseline, one week, one, two and three months).[205] In that particular study, however, while the authors did not find a correlation between measures of retinal thickness (although it decreased significantly) and visual function, they did report a correlation between RPE lesion size and ROS. The absence of a correlation between retinal thickness and ROS in that case may be due, at least in part,

to the relatively small sample size (n=23) for which correlations were performed. However, the authors postulate that the condition of the RPE may be more relevant in terms of understanding impact on function.

There was no statistically significant correlation between measures of CS or GD and retinal thickness in this study. However, a previous study has reported on the inverse relationship between CS and the subretinal fluid in cases of nv-AMD.[440] The latter study involved a larger sample size (n=122), it only included previously untreated nv-AMD subjects and also, the retinal outcome measure was the extent of subretinal fluid (compared to MFT in the current study, although these are somewhat related). These differences in methodology (particularly in sample size) may be the cause of the disparity between the results of the two studies.

Considering the functional criterion for retreatment in cases of nv-AMD is based on a change in CDVA (defined as a loss of five letters),[441] the results of this particular analysis, in combination with the results presented in Chapter 5, are of particular importance. Treatment strategies that depend on change in a measure as crude and insensitive as CDVA, may mean that patients are not treated early enough, timely intervention being paramount to successful outcomes.[151, 155] This hypothesis is supported by a recent study, which explored the relationship between macular thickness, VA and ROS in patients undergoing intravitreal ranibizumab for nv-AMD. In brief, intravitreal ranibizumab was administered if VA or OCT showed signs of active disease. Five (of a total of 21) eyes showed no change in VA or OCT findings, and, therefore, required no intravitreal injections. In these eyes, mean ROS decreased by 13% during the study period, indicating that ROS can deteriorate in eyes with stable VA and stable retinal thickness.[435] This is also of relevance to the results reported in Chapter 5.

This study reports significant differences between normal, early and nv-AMD subjects with respect to measures of CS and GD, under both photopic and mesopic conditions, in spite of relatively good CDVA (≥6/9). A statistically significant difference in ROS was also observed between the early and nv-AMD groups. This further highlights the fact that CDVA alone cannot account for visual performance in AMD. Another study has reported differences in foveal dark-adapted sensitivity between eyes with GA and eyes with early AMD, and VA of 6/7.5 or better.[167] Also, poor (patient-reported) visual function in dim illumination, specifically poor dark adaptation and need for more light when reading, has been shown in subjects with GA, despite good VA.[442]

A number of studies have also reported on psychophysical function in subjects with (early and late) AMD, highlighting a range of functional abnormalities associated with the condition, including S-cone sensitivity, flicker sensitivity, dark adaptation, colour-match area, and photostress recovery time, which are often either undetected or poorly quantified by traditional CDVA measures.[160, 443-445] The majority of these tests, however, have limitations in clinical or even research settings, for one or more of the following reasons: time-consuming; reliant on significant operator expertise; necessitate expensive equipment; require reasonably high concentration levels on the part of the subject, which may be challenging for the population in question (AMD subjects) who can present with poor cognitive function and/or other sensory limitations, hindering optimal test performance.

The results of this study have also shown that measures of CS and GD differ between normal, early and nv-AMD subjects, when CDVA is relatively good. However, in terms of detecting change *within* the early and nv-AMD categories, respectively, measures of ROS displayed the strongest correlations with retinal morphology, most

probably due to the fact that ROS is retinotopic, which probes visual function more deeply, compared to CS and GD (and CDVA). ROS may be considered a relatively familiar test for patients as it resembles (in terms of patient instruction and operation) visual field testing, which is commonly used by ophthalmologists/optometrists amongst this study population, thus rendering it potentially more patient-friendly. In terms of duration, the procedure for measuring ROS according to our protocol lasted approximately five minutes per eye. In this respect, I suggest that it be considered as measurement of visual function in a clinical setting as well as in the design of studies investigating visual function in AMD.

Additional results, comparing normal and early AMD subjects with respect to measures of CS and GD, have shown that, despite good CDVA, measures of CS and GD (particularly at low-mid range spatial frequencies) are significantly worse in eyes with early AMD compared to normals. Previous studies have shown similar results; for example, CS functions have been shown to be depressed in subjects with early AMD, compared with age-matched controls, both at the fovea and paracentrally (at two, five, and ten degrees from the fovea), demonstrating that sensitivity loss is not confined to the fovea (which is the retinal locus assessed primarily assessed by CDVA).[446] It has also been shown that subjects with early AMD have significant loss of CS at low spatial frequencies, before the loss of high contrast VA, across a range of eccentricities, including at the fovea itself.[447] The results of the current study contribute to the literature that challenges the general assumption that early AMD is of little visual consequence, and suggests that CDVA alone cannot account for the impact of the condition on visual performance. ROS data from normal subjects were not available for our analysis; however, a significant reduction in ROS in subjects with early AMD

compared with age-matched controls has been previously shown in eyes with CDVA of ≥6/9.[422]

8.5 Conclusion

Our study has contributed to the evidence germane to the relationship between disease severity and psychophysical function. This study has shown that measures of CS and GD differ between normal, early and nv-AMD subjects, in cases of good CDVA. ROS, in addition to this, can also reflect macular morphology, in cases of early, and in cases of nv-AMD, an outcome that cannot be achieved by conventional CDVA. Measures of ROS may, in fact, provide information complementary to morphological assessment, further highlighting the need for appropriate functional, as well as structural evaluation in patients with this condition. This is important in terms of understanding disease status (and its functional impact), monitoring disease progression, and also assessing the efficacy of emerging therapies, both in clinical practice and for the purposes of research trials. The findings of this study add to those presented in Chapter 5, where it was shown that ROS is a useful tool in the monitoring of subjects undergoing intravitreal ranibizumab for nv-AMD, and may be usefully incorporated into progression models for the condition.

Chapter 9. Conclusions and future considerations

This work was designed to investigate visual performance status and its response to intervention, in cases of early and in cases of nv-AMD, using a range of psychophysical tests beyond conventional CDVA.

The results reported and the conclusions drawn herein are based on the outcomes of, a) a literature review of the evidence pertaining to the macular carotenoids, AMD and visual performance, and b) two clinical trials, one which investigated visual function in response to ranibizumab treatment in subjects with nv-AMD, and the other, which has explored MPOD levels, visual function and AMD progression following supplementation with three different macular carotenoid formulations. The conclusions and the future considerations proposed as a result of the outcomes of this work are as follows:

1. The evidence germane to the role of MP for visual performance and its putative protective function against AMD has been reviewed. Appraising the totality of currently available evidence, it would appear that supplementation with the macular carotenoids offers the best means of fortifying the antioxidant defenses of the macula, thus putatively reducing the risk of AMD and/or its progression, and of optimising visual performance. I hope that this review of the literature will assist eyecare professionals to make well-informed decisions with respect to the prevention and/or delay of AMD onset and/or its progression (in anticipation of the results of RCTs), in addition to visual performance optimisation.

2. This study has investigated the impact of three different macular carotenoid formulations on the augmentation of MP, on visual performance and on disease progression, in subjects with early AMD. This study has shown that MP can be augmented, and CS enhanced, in subjects with early AMD who receive supplemental macular carotenoids. This is of particular interest considering the progressively degenerative nature of AMD. No trial to date has investigated the potential of MZ with respect to development or progression of AMD (or on visual performance in subjects with the conditions), as it has only recently become available in supplement form. A formulation containing MZ appears to offer advantages over a formulation that does not contain MZ, in terms of improvements in psychophysical function and in terms of MP augmentation across its spatial profile. However, I do believe that a supplement containing equal concentrations (1:1:1) of the three carotenoids (L, Z and MZ) warrants investigation, both with respect to AMD progression and visual performance.

The results of this study should prompt and inform a well-designed, placebo-controlled clinical trial (ideally of longer duration) of supplementation with L, Z and MZ in subjects with AMD, where outcome measures should include, MPOD augmentation, visual function and disease progression.

While the rationale suggests that MP is protective against the onset of AMD, there have been no published trials that have investigated the potential of macular carotenoids in this respect. This would involve recruiting subjects who are not afflicted with the condition and evaluating macular health over time with respect to intake of the carotenoids (compared to placebo) and with respect to MPOD. Such a trial would need to be no less than 15 years in duration following completion of recruitment. Of note, a unique observational study is currently underway in Ireland, entitled "The Irish Longitudinal Study of Ageing (TILDA),"[448] and is investigating health, lifestyles and

financial status of circa 8,000 randomly selected people aged 50 years and older. A major component of this prospective cohort study is the investigation into the relationship between baseline MP levels and the prevalence and incidence of AMD.[449] MP measurements and retinal photographs are being obtained at three separate study waves: year one, year four and year eight. This study will investigate, for the first time, whether baseline MP levels relate to the ultimate risk of developing AMD. However, the gold standard interventional trial to investigate the role of macular carotenoids in AMD prevention is still warranted.

3. This study has explored the relationship between central MPOD and visual performance, amongst early AMD subjects who present (at baseline) with differing levels of central MPOD. While recent studies have commented on the response of MP to macular carotenoid supplementation amongst subjects with low MP, the impact of macular carotenoid supplementation on visual performance in this specific group of subjects was never previously investigated. Subjects with low baseline central MPOD had the greatest increases in MPOD and the greatest improvements in CS, when compared with subjects with medium or high baseline MPOD. The impact of macular supplementation on visual performance in subjects with central dips is provocative and warrants further study. The findings suggest that the optimisation of CS (and putatively visual performance in general) is somewhat dependent on central MP levels. These results should prompt further investigation amongst subjects with low MP and/or with atypical spatial profiles (with or without disease), in particular to explore the impact of macular carotenoid supplementation on visual performance. This may have implications, not only for subjects afflicted with AMD, but also for subjects who present with symptoms of glare/reduced vision, particularly those who work in

professions where optimised vision is particularly important, such as pilots, sportspeople, and drivers.

If MP levels are playing a role in visual performance, this then warrants the measurement of MP in clinical practice, particularly in cases where visual symptoms cannot be explained by refractive error or disease. The limitation associated with reliably measuring MP, presently, is testing duration, which is of particular relevance for a clinical setting. For example, MP measurement using the gold standard Densitometer™ takes roughly ten minutes per eye. Other devices, such as VisuCam® 200, which may employ a shorter testing period, are limited by other factors, such as one- (rather than dual-) wavelength technology, and the fact that measurement of MPOD at a peripheral reference point is not considered. This field still awaits an easy-to-use, patient friendly device that can reliably measure MPOD, particularly if MP measurement is to be incorporated into routine clinical practice.

In addition, and given the growing interest in MPOD spatial profiles, a device that can yield a spatial profile of MP (similar to that provided by the Densitometer™) warrants consideration. Currently available commercial devices, such as the MacuScope™ and the MPS 900, measure MP at a single retinal locus (often 0.5° eccentricity), and cannot, therefore, detect the presence of e.g. a central dip.

4. In eyes with nv-AMD undergoing monthly intravitreal ranibizumab injections, there have been demonstrable improvements in a range of parameters of visual function, but no significant change in CDVA, despite a reduction in mean MFT. This finding has important implications when attempting to understand the effect of this treatment on a subject's visual performance and also, for a clinician's decision to treat/retreat/cease treatment in patients with the condition. This work suggests that outcome measures

other than CDVA, such as CS, GD and ROS, should not only be considered in the design of studies investigating nv-AMD, but also in treatment and retreatment strategies for patients with the condition, at least in eyes where baseline CDVA is relatively good.

5. This work also challenges the assumption that early AMD is not visually consequential and suggests the use of other tests to determine visual performance and experience, in subjects with the condition. While CDVA may not be greatly affected by early stages of the condition, it is clear that measures such as CS and GD are depressed compared to normal subjects and, therefore, should be considered in the diagnosis and monitoring of patients with AMD.

Glare is an appreciable and common complaint amongst subjects, not only with eye disease such as AMD, but also amongst those without disease. However, there is currently no "true" GD test, which makes it difficult to conclusively comment on the effect of glare. What has been reported is a measure of CS in the presence of glare, which is not a measure of glare, per se. Devices are limited even in this respect. Further investigation into the measurement of glare is warranted.

6. In terms of understanding disease severity using measures of visual function, this study has shown that CDVA poorly reflects retinal morphology in cases of early AMD and in cases of nv-AMD. ROS, however, appears to be a measure which is more reflective of disease severity in these conditions, where it correlates well with AMD-severity grade (in cases of early AMD) and also with MFT (in cases of nv-AMD). It has also been shown that, where CDVA is good, CS and GD differ between normal, early and nv-AMD subjects. ROS, in addition, has been shown to be impacted to a different

extent depending on whether you have early- or nv-AMD, something that is not observed when using conventional CDVA. Measures of ROS may, in fact, provide information complementary to morphological assessment, further highlighting the need for appropriate functional, as well as structural evaluation in patients with this condition. This is important in terms of understanding disease status (and its functional impact), monitoring disease progression, and also assessing the efficacy of emerging therapies, both in clinical practice and for the purposes of research trials. If a measure such as CDVA is a primary outcome measure in an interventional trial, and yet it is showing to be incapable of reflecting disease status or detecting changes in visual performance over time, we must, therefore, question its use in such circumstances.

Also, any intervention that endeavours to improve visual function must seek to do so from a patient's perspective, and not just from observing increases on any given device/chart. Currently available subjective vision questionnaires have limitations, which have been previously discussed. While they may be capable of distinguishing between, e.g. early and late AMD, they have not been sensitive enough, at least with respect to the current study, to detect change following intervention. There is a need to develop a more refined method of determining patient-perceived change in visual performance over time.

7. This study has attempted to probe more deeply the functioning of the visual system in subjects with AMD. However, the tests used and discussed here may or may not be appropriate for other eye conditions, which would warrant further investigation. In addition, the visual function measures reported here are by no means exhaustive. Other tests and other devices should also be explored. For example, the mfERG has yielded interesting results in other studies, where improvements were observed following

macular carotenoid supplementation in subjects with early AMD.[407, 408] The advantage of this technique over e.g. ROS, is that it is objective, not requiring a patient response. It is, however, relatively cumbersome. Further exploration of this method, and perhaps how it relates to measures ROS in subjects with AMD and additionally, its response following supplementation with an MZ-based supplement (which has not been previously explored), may be interesting.

In general, this thesis advocates the incorporation of tests, complimentary to CDVA, such as CS, GD, and particularly ROS, when attempting to understand disease severity in cases of AMD. In terms of monitoring change over time, the results of this study do seem to indicate that measures of ROS may be of particular benefit in monitoring subjects with nv-AMD, while measures of CS and GD may be more apt in monitoring change in subjects with early AMD. These tests should not only be considered in clinical practice settings (optometric, ophthalmological) but also when considering vision-related research, such that it may provide better insight into the impact on vision in AMD and on its response to intervention, particularly when new therapies are being investigated.

References

1. Bressler NM. Age-related macular degeneration is the leading cause of blindness. *JAMA*. 2004;291:1900-1901.

2. Owsley C. Contrast sensitivity. *Ophthalmol Clin North Am*. 2003;16:171-177.

3. Feigl B, Greaves A, Brown B. Functional outcomes after multiple treatments with ranibizumab in neovascular age-related macular degeneration beyond visual acuity. *Clin Ophthalmol*. 2007;1:167-175.

4. Loshin DS, White J. Contrast sensitivity. The visual rehabilitation of the patient with macular degeneration. *Arch Ophthalmol*. 1984;102:1303-1306.

5. Bellmann C, Unnebrink K, Rubin GS, Miller D, Holz FG. Visual acuity and contrast sensitivity in patients with neovascular age-related macular degeneration. Results from the Radiation Therapy for Age-Related Macular Degeneration (RAD-) Study. *Graefes Arch Clin Exp Ophthalmol*. 2003;241:968-974.

6. Sandberg MA, Gaudio AR. Slow photostress recovery and disease severity in age-related macular degeneration. *Retina*. 1995;15:407-412.

7. Parravano M, Oddone F, Tedeschi M, et al. Retinal functional changes measured by microperimetry in neovascular age-related macular degeneration treated with ranibizumab: 24-month results. *Retina*. 2010;30:1017-1024.

8. Kiss CG, Geitzenauer W, Simader C, Gregori G, Schmidt-Erfurth U. Evaluation of ranibizumab-induced changes in high-resolution optical coherence tomographic retinal morphology and their impact on visual function. *Invest Ophthalmol Vis Sci*. 2009;50:2376-2383.

9. Midena E, Radin PP, Pilotto E, Ghirlando A, Convento E, Varano M. Fixation pattern and macular sensitivity in eyes with subfoveal choroidal neovascularization secondary to age-related macular degeneration. A microperimetry study. *Semin Ophthalmol*. 2004;19:55-61.

10. Ergun E, Maar N, Radner W, Barbazetto I, Schmidt-Erfurth U, Stur M. Scotoma size and reading speed in patients with subfoveal occult choroidal neovascularization in age-related macular degeneration. *Ophthalmology*. 2003;110:65-69.

11. Stifter E, Weghaupt H, Benesch T, Thaler A, Radner W. Discriminative power of reading tests to differentiate visual impairment caused by cataract and age-related macular degeneration. *J Cataract Refract Surg*. 2005;31:2111-2119.

12. Kloos P, Bernasconi P, Estermann S, Bachmann B, Rutishauser Y, Tholen A. [Visual acuity and magnification requirement after ranibizumab in patients with wet age-related macular degeneration]. *Klin Monbl Augenheilkd*. 2008;225:385-391.

13. Ozdemir H, Karacorlu M, Senturk F, Karacorlu SA, Uysal O. Microperimetric changes after intravitreal bevacizumab injection for exudative age-related macular degeneration. *Acta Ophthalmol*. 2012;90:71-75.

14. Charalampidou S, Loughman J, Nolan J, et al. Prognostic Indicators and Outcome Measures for Surgical Removal of Symptomatic Nonadvanced Cataract. *Arch Ophthalmol*. 2011;129:1155-1161.

15. Bhutto IA, McLeod DS, Hasegawa T, et al. Pigment epithelium-derived factor (PEDF) and vascular endothelial growth factor (VEGF) in aged human choroid and eyes with age-related macular degeneration. *Exp Eye Res*. 2006;82:99-110.

16. Wong TY, Chakravarthy U, Klein R, et al. The natural history and prognosis of neovascular age-related macular degeneration: a systematic review of the literature and meta-analysis. *Ophthalmology*. 2008;115:116-126.

17. Klein R, Klein BE, Linton KL. Prevalence of age-related maculopathy. The Beaver Dam Eye Study. *Ophthalmology*. 1992;99:933-943.

18. Resnikoff S, Pascolini D, Etya'ale D, et al. Global data on visual impairment in the year 2002. *Bull World Health Organ*. 2004;82:844-851.

19. Owen CG, Fletcher AE, Donoghue M, Rudnicka AR. How big is the burden of visual loss caused by age related macular degeneration in the United Kingdom? *Br J Ophthalmol*. 2003;87:312-317.

20. Kelliher C, Kenny D, O'Brien C. Trends in blind registration in the adult population of the Republic of Ireland 1996-2003. *Br J Ophthalmol*. 2006;90:367-371.

21. Sunness J, Massof RW, Rubin GS, Appelgate CA, Smolen H. Quality Of Life Measures In Age-related Geographic Atrophy Of The Macula . *ARVO*. 2011;A226.

22. Ferris FL, Davis MD, Clemons TE, et al. A simplified severity scale for age-related macular degeneration: AREDS Report No. 18. *Arch Ophthalmol*. 2005;123:1570-1574.

23. . Risk factors for choroidal neovascularization in the second eye of patients with juxtafoveal or subfoveal choroidal neovascularization secondary to age-related macular degeneration. Macular Photocoagulation Study Group. *Arch Ophthalmol*. 1997;115:741-747.

24. Tomany SC, Cruickshanks KJ, Klein R, Klein BEK, Knudtson MD. Sunlight and the 10-year incidence of age-related maculopathy - The Beaver Dam eye study. *Arch Ophthalmol*. 2004;122:750-757.

25. Beatty S, Koh HH, Henson D, Boulton M. The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Survey of Ophthalmology*. 2000;45:115-134.

26. Bone RA, Landrum JT, Hime GW, Cains A, Zamor J. Stereochemistry of the Human Macular Carotenoids. *Investigative Ophthalmology & Visual Science*. 1993;34:2033-2040.

27. Snodderly DM, Brown PK, Delori FC, Auran JD. The Macular Pigment .1. Absorbance Spectra, Localization, and Discrimination from Other Yellow Pigments in Primate Retinas. *Investigative Ophthalmology & Visual Science*. 1984;25:660-673.

28. Kassoff A, Kassoff J, Buehler J, et al. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss - AREDS Report No. 8. *Arch Ophthalmol*. 2001;119:1417-1436.

29. Loughman J, Akkali MC, Beatty S, et al. The relationship between macular pigment and visual performance. *Vision Res*. 2010;50:1249-1256.

30. Engles M, Wooten B, Hammond B. Macular pigment: a test of the acuity hypothesis. *Invest Ophthalmol Vis Sci*. 2007;48:2922-2931.

31. Kvansakul J, Rodriguez-Carmona M, Edgar DF, et al. Supplementation with the carotenoids lutein or zeaxanthin improves human visual performance. *Ophthalmic Physiol Opt*. 2006;26:362-371.

32. Stringham JM, Hammond BR, Jr. The glare hypothesis of macular pigment function. *Optom Vis Sci*. 2007;84:859-864.

33. Wooten BR, Hammond BR. Macular pigment: influences on visual acuity and visibility. *Progress in Retinal and Eye Research*. 2002;21:225-240.

34. Richer S, Stiles W, Statkute L, et al. Double-masked, placebo-controlled, randomized trial of lutein and antioxidant supplementation in the intervention of atrophic age-related macular degeneration: the Veterans LAST study (Lutein Antioxidant Supplementation Trial). *Optometry*. 2004;75:216-230.

35. Weigert G, Kaya S, Pemp B, et al. Effects of lutein supplementation on macular pigment optical density and visual acuity in patients with age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2011;52:8174-8178.

36. Stringham JM, Hammond BR. Macular pigment and visual performance under glare conditions. *Optom Vis Sci*. 2008;85:82-88.

37. Hirsch J, Curcio CA. The spatial resolution capacity of human foveal retina. *Vision Res*. 1989;29:1095-1101.

38. Legge GE, Ross JA, Isenberg LM, LaMay JM. Psychophysics of reading. Clinical predictors of low-vision reading speed. *Invest Ophthalmol Vis Sci*. 1992;33:677-687.

39. Mangione CM, Gutierrez PR, Lowe G, Orav EJ, Seddon JM. Influence of age-related maculopathy on visual functioning and health-related quality of life. *Am J Ophthalmol*. 1999;128:45-53.

40. Ebert EM, Fine AM, Markowitz J, Maguire MG, Starr JS, Fine SL. Functional vision in patients with neovascular maculopathy and poor visual acuity. *Arch Ophthalmol*. 1986;104:1009-1012.

41. Bird AC, Bressler NM, Bressler SB, et al. An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group. *Survey of Ophthalmology*. 1995;39:367-374.

42. Kanski JL. Clinical Ophthalmology: a systemic approach (4th Edition). In: Butterworth Heinemann; 1999:404.

43. HARMAN D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol*. 1956;11:298-300.

44. HARMAN D. The biologic clock: the mitochondria? *J Am Geriatr Soc*. 1972;20:145-147.

45. Stone WL, Farnsworth CC, Dratz EA. A reinvestigation of the fatty acid content of bovine, rat and frog retinal rod outer segments. *Exp Eye Res*. 1979;28:387-397.

46. Beatty S, Koh H, Phil M, Henson D, Boulton M. The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Surv Ophthalmol*. 2000;45:115-134.

47. Machlin LJ, Bendich A. Free radical tissue damage: protective role of antioxidant nutrients. *FASEB J*. 1987;1:441-445.

48. Halliwell B. Antioxidants and human disease: a general introduction. *Nutr Rev*. 1997;55:S44-S49.

49. Sies H. Oxidative stress: from basic research to clinical application. *Am J Med*. 1991;91:31S-38S.

50. Borish ET, Pryor WA, Venugopal S, Deutsch WA. DNA synthesis is blocked by cigarette tar-induced DNA single-strand breaks. *Carcinogenesis*. 1987;8:1517-1520.

51. McCord JM. The evolution of free radicals and oxidative stress. *Am J Med*. 2000;108:652-659.

52. Bok D. The retinal pigment epithelium: a versatile partner in vision. *J Cell Sci Suppl*. 1993;17:189-95.:189-195.

53. Steinberg RH. Interactions between the retinal pigment epithelium and the neural retina. *Doc Ophthalmol*. 1985;60:327-346.

54. GEERAETS WJ, WILLIAMS RC, CHAN G, Ham WT, Jr., GUERRY D, III, SCHMIDT FH. The loss of light energy in retina and choroid. *Arch Ophthalmol*. 1960;64:606-15.:606-615.

55. Curcio CA, Medeiros NE, Millican CL. Photoreceptor loss in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 1996;37:1236-1249.

56. Katz ML, Robison WG, Jr. What is lipofuscin? Defining characteristics and differentiation from other autofluorescent lysosomal storage bodies. *Arch Gerontol Geriatr*. 2002;34:169-184.

57. Kennedy CJ, Rakoczy PE, Constable IJ. Lipofuscin of the retinal pigment epithelium: a review. *Eye (Lond)*. 1995;9:763-771.

58. Rozanowska M, Jarvis-Evans J, Korytowski W, Boulton ME, Burke JM, Sarna T. Blue light-induced reactivity of retinal age pigment. In vitro generation of oxygen-reactive species. *J Biol Chem*. 1995;270:18825-18830.

59. Sundelin S, Wihlmark U, Nilsson SE, Brunk UT. Lipofuscin accumulation in cultured retinal pigment epithelial cells reduces their phagocytic capacity. *Curr Eye Res*. 1998;17:851-857.

60. Schalch W. Carotenoids in the retina--a review of their possible role in preventing or limiting damage caused by light and oxygen. *EXS*. 1992;62:280-98.:280-298.

61. Ham WT, Jr., Mueller HA, Sliney DH. Retinal sensitivity to damage from short wavelength light. *Nature*. 1976;260:153-155.

62. Pitts DG. A comparative study of the effects of ultraviolet radiation on the eye. *Am J Optom Arch Am Acad Optom*. 1970;47:535-546.

63. Algvere PV, Marshall J, Seregard S. Age-related maculopathy and the impact of blue light hazard. *Acta Ophthalmol Scand*. 2006;84:4-15.

64. Noell WK, Walker VS, Kang BS, Berman S. Retinal damage by light in rats. *Invest Ophthalmol*. 1966;5:450-473.

65. Wu J, Seregard S, Algvere PV. Photochemical damage of the retina. *Surv Ophthalmol*. 2006;51:461-481.

66. Ruffolo JJ, Jr., Ham WT, Jr., Mueller HA, Millen JE. Photochemical lesions in the primate retina under conditions of elevated blood oxygen. *Invest Ophthalmol Vis Sci*. 1984;25:893-898.

67. Boulton M, Dontsov A, Jarvis-Evans J, Ostrovsky M, Svistunenko D. Lipofuscin is a photoinducible free radical generator. *J Photochem Photobiol B*. 1993;19:201-204.

68. Shaban H, Borras C, Vina J, Richter C. Phosphatidylglycerol potently protects human retinal pigment epithelial cells against apoptosis induced by A2E, a compound suspected to cause age-related macula degeneration. *Exp Eye Res*. 2002;75:99-108.

69. Sparrow JR, Vollmer-Snarr HR, Zhou J, et al. A2E-epoxides damage DNA in retinal pigment epithelial cells. Vitamin E and other antioxidants inhibit A2E-epoxide formation. *J Biol Chem*. 2003;278:18207-18213.

70. Taylor HR, Munoz B, West S, Bressler NM, Bressler SB, Rosenthal FS. Visible light and risk of age-related macular degeneration. *Trans Am Ophthalmol Soc*. 1990;88:163-73; discussion 173-8.:163-173.

71. Tomany SC, Cruickshanks KJ, Klein R, Klein BE, Knudtson MD. Sunlight and the 10-year incidence of age-related maculopathy: the Beaver Dam Eye Study. *Arch Ophthalmol*. 2004;122:750-757.

72. Wiegand RD, Giusto NM, Rapp LM, Anderson RE. Evidence for rod outer segment lipid peroxidation following constant illumination of the rat retina. *Invest Ophthalmol Vis Sci*. 1983;24:1433-1435.

73. Ham WT, Jr., Ruffolo JJ, Jr., Mueller HA, Clarke AM, Moon ME. Histologic analysis of photochemical lesions produced in rhesus retina by short-wave-length light. *Invest Ophthalmol Vis Sci*. 1978;17:1029-1035.

74. Lam S, Tso MO, Gurne DH. Amelioration of retinal photic injury in albino rats by dimethylthiourea. *Arch Ophthalmol*. 1990;108:1751-1757.

75. Fletcher AE, Bentham GC, Agnew M, et al. Sunlight exposure, antioxidants, and age-related macular degeneration. *Arch Ophthalmol*. 2008;126:1396-1403.

76. Suzuki M, Kamei M, Itabe H, et al. Oxidized phospholipids in the macula increase with age and in eyes with age-related macular degeneration. *Mol Vis*. 2007;13:772-8.:772-778.

77. Lu L, Hackett SF, Mincey A, Lai H, Campochiaro PA. Effects of different types of oxidative stress in RPE cells. *J Cell Physiol*. 2006;206:119-125.

78. Seddon JM, Gensler G, Milton RC, Klein ML, Rifai N. Association between C-reactive protein and age-related macular degeneration. *JAMA*. 2004;291:704-710.

79. Donoso LA, Kim D, Frost A, Callahan A, Hageman G. The role of inflammation in the pathogenesis of age-related macular degeneration. *Surv Ophthalmol*. 2006;51:137-152.

80. Ferrero-Miliani L, Nielsen OH, Andersen PS, Girardin SE. Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1beta generation. *Clin Exp Immunol*. 2007;147:227-235.

81. Johnson LV, Leitner WP, Staples MK, Anderson DH. Complement activation and inflammatory processes in Drusen formation and age related macular degeneration. *Exp Eye Res*. 2001;73:887-896.

82. Hageman GS, Luthert PJ, Victor Chong NH, Johnson LV, Anderson DH, Mullins RF. An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration. *Prog Retin Eye Res*. 2001;20:705-732.

83. Anderson DH, Mullins RF, Hageman GS, Johnson LV. A role for local inflammation in the formation of drusen in the aging eye. *Am J Ophthalmol*. 2002;134:411-431.

84. Li CM, Clark ME, Chimento MF, Curcio CA. Apolipoprotein localization in isolated drusen and retinal apolipoprotein gene expression. *Invest Ophthalmol Vis Sci*. 2006;47:3119-3128.

85. Umeda S, Suzuki MT, Okamoto H, et al. Molecular composition of drusen and possible involvement of anti-retinal autoimmunity in two different forms of macular degeneration in cynomolgus monkey (Macaca fascicularis). *FASEB J*. 2005;19:1683-1685.

86. Dentchev T, Milam AH, Lee VM, Trojanowski JQ, Dunaief JL. Amyloid-beta is found in drusen from some age-related macular degeneration retinas, but not in drusen from normal retinas. *Mol Vis*. 2003;9:184-90.:184-190.

87. Francis PJ, Schultz DW, Hamon S, Ott J, Weleber RG, Klein ML. Haplotypes in the complement factor H (CFH) gene: associations with drusen and advanced age-related macular degeneration. *PLoS One*. 2007;2:e1197.

88. Doney AS, Leese GP, Olson J, Morris AD, Palmer CN. The Y402H variant of complement factor H is associated with age-related macular degeneration but not with diabetic retinal disease in the Go-DARTS study. *Diabet Med*. 2009;26:460-465.

89. Hollyfield JG, Bonilha VL, Rayborn ME, et al. Oxidative damage-induced inflammation initiates age-related macular degeneration. *Nat Med*. 2008;14:194-198.

90. Hollyfield JG, Bonilha VL, Rayborn ME, et al. Oxidative damage-induced inflammation initiates age-related macular degeneration. *Nat Med*. 2008;14:194-198.

91. Klein R, Klein BEK, Linton KLP. Prevalence of Age-Related Maculopathy - the Beaver Dam Eye Study. *Ophthalmology*. 1992;99:933-943.

92. Klein R, Klein BEK, Jensen SC, Meuer SM. The five-year incidence and progression of age-related maculopathy - The Beaver Dam eye study. *Ophthalmology*. 1997;104:7-21.

93. Smith W, Assink J, Klein R, et al. Risk factors for age-related macular degeneration: Pooled findings from three continents. *Ophthalmology*. 2001;108:697-704.

94. Seddon JM, Ajani UA, Mitchell BD. Familial aggregation of age-related maculopathy. *American Journal of Ophthalmology*. 1997;123:199-206.

95. Seddon JM, Francis PJ, George S, Schultz DW, Rosner B, Klein ML. Association of CFH Y402H and LOC387715 A69S With Progression of Age-Related Macular Degeneration. *JAMA: The Journal of the American Medical Association*. 2007;297:1793-1800.

96. Schaumberg DA, Hankinson SE, Guo Q, Rimm E, Hunter DJ. A prospective study of 2 major age-related macular degeneration susceptibility alleles and interactions with modifiable risk factors. *Arch Ophthalmol*. 2007;125:55-62.

97. Schork NJ. Genetics of complex disease: approaches, problems, and solutions. *Am J Respir Crit Care Med*. 1997;156:S103-S109.

98. Mares JA, Voland RP, Sondel SA, et al. Healthy Lifestyles Related to Subsequent Prevalence of Age-Related Macular Degeneration. *Arch Ophthalmol*. 2010.

99. Cai J, Nelson KC, Wu M, Sternberg P, Jr., Jones DP. Oxidative damage and protection of the RPE. *Prog Retin Eye Res*. 2000;19:205-221.

100. Klein R, Klein BEK, Moss SE. Relation of smoking to the incidence of age-related maculopathy - The Beaver Dam Eye Study. *Am J Epidemiol*. 1998;147:103-110.

101. Khan JC, Thurlby DA, Shahid H, et al. Smoking and age related macular degeneration: the number of pack years of cigarette smoking is a major determinant of risk for both geographic atrophy and choroidal neovascularisation. *Br J Ophthalmol*. 2006;90:75-80.

102. Chakravarthy U, Wong TY, Fletcher A, et al. Clinical risk factors for age-related macular degeneration: a systematic review and meta-analysis. *BMC Ophthalmol*. 2010;10:31.:31.

103. Thornton J, Edwards R, Mitchell P, Harrison RA, Buchan I, Kelly SP. Smoking and age-related macular degeneration: a review of association. *Eye (Lond)*. 2005;19:935-944.

104. SanGiovanni JP, Chew EY, Clemons TE, et al. The relationship of dietary carotenoid and vitamin A, E, and C intake with age-related macular degeneration in a case-control study: AREDS Report No. 22. *Arch Ophthalmol*. 2007;125:1225-1232.

105. Margrain TH, Boulton M, Marshall J, Sliney DH. Do blue light filters confer protection against age-related macular degeneration? *Prog Retin Eye Res*. 2004;23:523-531.

106. Bone RA, Landrum JT, Mayne ST, Gomez CM, Tibor SE, Twaroska EE. Macular pigment in donor eyes with and without AMD: A case-control study. *Invest Ophthalmol Vis Sci*. 2001;42:235-240.

107. Nolan JM, Stack J, O' DO, Loane E, Beatty S. Risk factors for age-related maculopathy are associated with a relative lack of macular pigment. *Exp Eye Res*. 2007;84:61-74.

108. Kirby ML, Beatty S, Loane E, et al. A central dip in the macular pigment spatial profile is associated with age and smoking. *Invest Ophthalmol Vis Sci*. 2010;51:6722-6728.

109. Virgili G, Bini A. Laser photocoagulation for neovascular age-related macular degeneration. *Cochrane Database Syst Rev*. 2007:CD004763.

110. Fingar VH. Vascular effects of photodynamic therapy. *J Clin Laser Med Surg*. 1996;14:323-328.

111. Zarbin M, Szirth B. Current treatment of age-related macular degeneration. *Optom Vis Sci*. 2007;84:559-572.

112. Bressler NM, Arnold J, Benchaboune M, et al. Verteporfin therapy of subfoveal choroidal neovascularization in patients with age-related macular degeneration: additional information regarding baseline lesion composition's impact on vision outcomes-TAP report No. 3. *Arch Ophthalmol*. 2002;120:1443-1454.

113. Kaiser PK. Verteporfin PDT for subfoveal occult CNV in AMD: two-year results of a randomized trial. *Curr Med Res Opin*. 2009;25:1853-1860.

114. Bressler NM, Bressler SB, Hawkins BS, Marsh MJ, Sternberg P, Jr., Thomas MA. Submacular surgery trials randomized pilot trial of laser photocoagulation versus surgery for recurrent choroidal neovascularization secondary to age-related macular degeneration: I. Ophthalmic outcomes submacular surgery trials pilot study report number 1. *Am J Ophthalmol*. 2000;130:387-407.

115. Gass JD. Biomicroscopic and histopathologic considerations regarding the feasibility of surgical excision of subfoveal neovascular membranes. *Am J Ophthalmol*. 1994;118:285-298.

116. Machemer R, Steinhorst UH. Retinal separation, retinotomy, and macular relocation: II. A surgical approach for age-related macular degeneration? *Graefes Arch Clin Exp Ophthalmol*. 1993;231:635-641.

117. Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. *Science*. 1983;219:983-985.

118. Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science*. 1989;246:1306-1309.

119. Kerbel RS. Tumor angiogenesis. *N Engl J Med*. 2008;358:2039-2049.

120. Takahashi H, Shibuya M. The vascular endothelial growth factor (VEGF)/VEGF receptor system and its role under physiological and pathological conditions. *Clin Sci (Lond)*. 2005;109:227-241.

121. Pieramici DJ, Avery RL. Ranibizumab: treatment in patients with neovascular age-related macular degeneration. *Expert Opin Biol Ther*. 2006;6:1237-1245.

122. Holmes K, Roberts OL, Thomas AM, Cross MJ. Vascular endothelial growth factor receptor-2: structure, function, intracellular signalling and therapeutic inhibition. *Cell Signal*. 2007;19:2003-2012.

123. Adamis AP, Miller JW, Bernal MT, et al. Increased vascular endothelial growth factor levels in the vitreous of eyes with proliferative diabetic retinopathy. *Am J Ophthalmol*. 1994;118:445-450.

124. Green WR, Chan CC, Hutchins GM, Terry JM. Central retinal vein occlusion: a prospective histopathologic study of 29 eyes in 28 cases. *Trans Am Ophthalmol Soc*. 1981;79:371-422.:371-422.

125. Blaauwgeers HG, Holtkamp GM, Rutten H, et al. Polarized vascular endothelial growth factor secretion by human retinal pigment epithelium and localization of vascular endothelial growth factor receptors on the inner choriocapillaris. Evidence for a trophic paracrine relation. *Am J Pathol*. 1999;155:421-428.

126. Michaelson IC. The mode of development of the retinal vessels and some observations of its significance in certain retinal diseases. *Trans Ophthalmol Soc UK*. 1948;68:137-180.

127. ASHTON N, WARD B, SERPELL G. Effect of oxygen on developing retinal vessels with particular reference to the problem of retrolental fibroplasia. *Br J Ophthalmol*. 1954;38:397-432.

128. Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. *Science*. 1983;219:983-985.

129. Ferrara N, Houck K, Jakeman L, Leung DW. Molecular and biological properties of the vascular endothelial growth factor family of proteins. *Endocr Rev*. 1992;13:18-32.

130. Shweiki D, Itin A, Soffer D, Keshet E. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature*. 1992;359:843-845.

131. Krzystolik MG, Afshari MA, Adamis AP, et al. Prevention of experimental choroidal neovascularization with intravitreal anti-vascular endothelial growth factor antibody fragment. *Arch Ophthalmol*. 2002;120:338-346.

132. Akiyama H, Mohamedali KA, RL ES, et al. Vascular targeting of ocular neovascularization with a vascular endothelial growth factor121/gelonin chimeric protein. *Mol Pharmacol*. 2005;68:1543-1550.

133. Adamis AP, Shima DT, Tolentino MJ, et al. Inhibition of vascular endothelial growth factor prevents retinal ischemia-associated iris neovascularization in a nonhuman primate. *Arch Ophthalmol*. 1996;114:66-71.

134. Wells JA, Murthy R, Chibber R, et al. Levels of vascular endothelial growth factor are elevated in the vitreous of patients with subretinal neovascularisation. *Br J Ophthalmol*. 1996;80:363-366.

135. Grossniklaus HE, Ling JX, Wallace TM, et al. Macrophage and retinal pigment epithelium expression of angiogenic cytokines in choroidal neovascularization. *Mol Vis*. 2002;8:119-26.:119-126.

136. Gragoudas ES, Adamis AP, Cunningham ET, Jr., Feinsod M, Guyer DR. Pegaptanib for neovascular age-related macular degeneration. *N Engl J Med*. 2004;351:2805-2816.

137. Adams GP, Weiner LM. Monoclonal antibody therapy of cancer. *Nat Biotechnol*. 2005;23:1147-1157.

138. Moshfeghi AA, Rosenfeld PJ, Puliafito CA, et al. Systemic bevacizumab (Avastin) therapy for neovascular age-related macular degeneration: twenty-four-week results of an uncontrolled open-label clinical study. *Ophthalmology*. 2006;113:2002-2012.

139. Avery RL, Pieramici DJ, Rabena MD, Castellarin AA, Nasir MA, Giust MJ. Intravitreal bevacizumab (Avastin) for neovascular age-related macular degeneration. *Ophthalmology*. 2006;113:363-372.

140. Costa RA, Jorge R, Calucci D, Cardillo JA, Melo LA, Jr., Scott IU. Intravitreal bevacizumab for choroidal neovascularization caused by AMD (IBeNA Study): results of a phase 1 dose-escalation study. *Invest Ophthalmol Vis Sci*. 2006;47:4569-4578.

141. Spaide RF, Laud K, Fine HF, et al. Intravitreal bevacizumab treatment of choroidal neovascularization secondary to age-related macular degeneration. *Retina*. 2006;26:383-390.

142. Aisenbrey S, Ziemssen F, Volker M, et al. Intravitreal bevacizumab (Avastin) for occult choroidal neovascularization in age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol*. 2007;245:941-948.

143. Lazic R, Gabric N. Intravitreally administered bevacizumab (Avastin) in minimally classic and occult choroidal neovascularization secondary to age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol*. 2007;245:68-73.

144. Goff MJ, Johnson RN, McDonald HR, Ai E, Jumper JM, Fu A. Intravitreal bevacizumab for previously treated choroidal neovascularization from age-related macular degeneration. *Retina*. 2007;27:432-438.

145. Bashshur ZF, Bazarbachi A, Schakal A, Haddad ZA, El Haibi CP, Noureddin BN. Intravitreal bevacizumab for the management of choroidal neovascularization in age-related macular degeneration. *Am J Ophthalmol*. 2006;142:1-9.

146. Chen CY, Wong TY, Heriot WJ. Intravitreal bevacizumab (Avastin) for neovascular age-related macular degeneration: a short-term study. *Am J Ophthalmol*. 2007;143:510-512.

147. Emerson MV, Lauer AK, Flaxel CJ, et al. Intravitreal bevacizumab (Avastin) treatment of neovascular age-related macular degeneration. *Retina*. 2007;27:439-444.

148. Tufail A, Patel PJ, Egan C, et al. Bevacizumab for neovascular age related macular degeneration (ABC Trial): multicentre randomised double masked study. *BMJ*. 2010;340:c2459. doi: 10.1136/bmj.c2459.:c2459.

149. Rosenfeld PJ, Brown DM, Heier JS, et al. Ranibizumab for neovascular age-related macular degeneration. *N Engl J Med*. 2006;355:1419-1431.

150. Brown DM, Michels M, Kaiser PK, Heier JS, Sy JP, Ianchulev T. Ranibizumab versus verteporfin photodynamic therapy for neovascular age-related macular degeneration: Two-year results of the ANCHOR study. *Ophthalmology*. 2009;116:57-65.

151. Abraham P, Yue H, Wilson L. Randomized, double-masked, sham-controlled trial of ranibizumab for neovascular age-related macular degeneration: PIER study year 2. *Am J Ophthalmol*. 2010;150:315-324.

152. Regillo CD, Brown DM, Abraham P, et al. Randomized, double-masked, sham-controlled trial of ranibizumab for neovascular age-related macular degeneration: PIER Study year 1. *Am J Ophthalmol*. 2008;145:239-248.

153. Fung AE, Lalwani GA, Rosenfeld PJ, et al. An optical coherence tomography-guided, variable dosing regimen with intravitreal ranibizumab (Lucentis) for neovascular age-related macular degeneration. *Am J Ophthalmol*. 2007;143:566-583.

154. Singer MA, Awh CC, Sadda S, et al. HORIZON: An Open-Label Extension Trial of Ranibizumab for Choroidal Neovascularization Secondary to Age-Related Macular Degeneration. *Ophthalmology*. 2012;119:1175-1183.

155. Schmidt-Erfurth U, Eldem B, Guymer R, et al. Efficacy and safety of monthly versus quarterly ranibizumab treatment in neovascular age-related macular degeneration: the EXCITE study. *Ophthalmology*. 2011;118:831-839.

156. Holz FG, Amoaku W, Donate J, et al. Safety and efficacy of a flexible dosing regimen of ranibizumab in neovascular age-related macular degeneration: the SUSTAIN study. *Ophthalmology*. 2011;118:663-671.

157. Martin DF, Maguire MG, Fine SL, et al. Ranibizumab and Bevacizumab for Treatment of Neovascular Age-related Macular Degeneration: Two-Year Results. *Ophthalmology*. 2012;119:1388-1398.

158. Schmucker C, Ehlken C, Agostini HT, et al. A safety review and meta-analyses of bevacizumab and ranibizumab: off-label versus goldstandard. *PLoS ONE*. 2012;7:e42701.

159. Gescheider G. *Psychophysics: the fundamentals*. Third ed.: Lawrence Erlbaum Associates, Inc., New Jersey; 1997.

160. Neelam K, Nolan J, Chakravarthy U, Beatty S. Psychophysical function in age-related maculopathy. *Surv Ophthalmol*. 2009;54:167-210.

161. Bailey IL, Lovie JE. New design principles for visual acuity letter charts. *Am J Optom Physiol Opt*. 1976;53:740-745.

162. Rubin GS, Bandeen-Roche K, Huang GH, et al. The association of multiple visual impairments with self-reported visual disability: SEE project. *Invest Ophthalmol Vis Sci*. 2001;42:64-72.

163. Klein R, Wang Q, Klein BEK, Moss SE, Meuer SM. The Relationship of Age-Related Maculopathy, Cataract, and Glaucoma to Visual-Acuity. *Invest Ophthalmol Vis Sci*. 1995;36:182-191.

164. Rosser DA, Cousens SN, Murdoch IE, Fitzke FW, Laidlaw DA. How sensitive to clinical change are ETDRS logMAR visual acuity measurements? *Invest Ophthalmol Vis Sci*. 2003;44:3278-3281.

165. Sarks JP, Sarks SH, Killingsworth MC. Evolution of geographic atrophy of the retinal pigment epithelium. *Eye (Lond)*. 1988;2:552-577.

166. . Visual outcome after laser photocoagulation for subfoveal choroidal neovascularization secondary to age-related macular degeneration. The influence of initial lesion size and initial visual acuity. Macular Photocoagulation Study Group. *Arch Ophthalmol*. 1994;112:480-488.

167. Sunness JS, Rubin GS, Applegate CA, et al. Visual function abnormalities and prognosis in eyes with age-related geographic atrophy of the macula and good visual acuity. *Ophthalmology*. 1997;104:1677-1691.

168. Jentsch S, Schweitzer D, Hammer M, Lang G.E, Dawczynski J. The Lutega-Study: Lutein And Omega- 3- Fatty Acids And Their Relevance For Macular Pigment In Patients With Age-related Macular Degeneration (AMD). In: 2011.

169. Piermarocchi S, Saviano S, Parisi V, et al. Carotenoids in Age-related Maculopathy Italian Study (CARMIS): two-year results of a randomized study. *Eur J Ophthalmol*. 2011;22:216-225.

170. Loughman J, Nolan JM, Howard AN, Connolly E, Meagher K, Beatty S. The impact of macular pigment augmentation on visual performance using different carotenoid formulations. *Invest Ophthalmol Vis Sci*. 2012;53:7871-7880.

171. Beatty S, Chakravarthy U, Nolan JM, et al. Secondary Outcomes in a Clinical Trial of Carotenoids with Coantioxidants versus Placebo in Early Age-Related Macular Degeneration. *Ophthalmology*. 2012:10.

172. Nolan JM, Loughman J, Akkali MC, et al. The impact of macular pigment augmentation on visual performance in normal subjects: COMPASS. *Vision Res*. 2011.

173. van den Berg TJ. Importance of pathological intraocular light scatter for visual disability. *Doc Ophthalmol*. 1986;61:327-333.

174. Owsley C. Contrast sensitivity. *Ophthalmol Clin North Am*. 2003;16:171-177.

175. Mones J, Rubin GS. Contrast sensitivity as an outcome measure in patients with subfoveal choroidal neovascularisation due to age-related macular degeneration. *Eye (Lond)*. 2005;19:1142-1150.

176. Scott IU, Feuer WJ, Jacko JA. Impact of visual function on computer task accuracy and reaction time in a cohort of patients with age-related macular degeneration. *Am J Ophthalmol*. 2002;133:350-357.

177. Scott IU, Feuer WJ, Jacko JA. Impact of visual function on computer task accuracy and reaction time in a cohort of patients with age-related macular degeneration. *Am J Ophthalmol*. 2002;133:350-357.

178. Rubin GS, Roche KB, Prasada-Rao P, Fried LP. Visual impairment and disability in older adults. *Optom Vis Sci*. 1994;71:750-760.

179. Hyvarinen L, Laurinen P, Rovamo J. Contrast sensitivity in evaluation of visual impairment due to macular degeneration and optic nerve lesions. *Acta Ophthalmol (Copenh)*. 1983;61:161-170.

180. Midena E, Degli AC, Blarzino MC, Valenti M, Segato T. Macular function impairment in eyes with early age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 1997;38:469-477.

181. Korobelnik J. Consistency of Effect of Ranibizumab at 2 Doses on Contrast Sensitivity in 3 Phase III and IIIB Studies of Patients with CNV Secondary to

AMD. *American Society of Retina Specialists and European VitreoRetinal Society Annual Meeting*. 2006.

182. Feigl B, Greaves A, Brown B. Functional outcomes after multiple treatments with ranibizumab in neovascular age-related macular degeneration beyond visual acuity. *Clin Ophthalmol*. 2007;1:167-175.

183. Chakravarthy U, Harding SP, Rogers CA, et al. Ranibizumab versus Bevacizumab to Treat Neovascular Age-related Macular Degeneration: One-Year Findings from the IVAN Randomized Trial. *Ophthalmology*. 2012.

184. Kumar A, Gopalakrishnan K, Sinha S. Combination photodynamic therapy and intravitreal ranibizumab in neovascular AMD in a north Indian population: a pilot study. *Retina*. 2008;28:1132-1137.

185. Moussa S, Ansari-Shahrezaei S, Smretschnig E, et al. Contrast sensitivity after intravitreal antivascular endothelial growth factor therapy for myopic choroidal neovascularization. *Graefes Arch Clin Exp Ophthalmol*. 2010;248:1087-1090.

186. Kvansakul J, Rodriguez-Carmona M, Edgar DF, et al. Supplementation with the carotenoids lutein or zeaxanthin improves human visual performance. *Ophthalmic Physiol Opt*. 2006;26:362-371.

187. Richer S, Stiles W, Statkute L, et al. Double-masked, placebo-controlled, randomized trial of lutein and antioxidant supplementation in the intervention of atrophic age-related macular degeneration: the Veterans LAST study (Lutein Antioxidant Supplementation Trial). *Optometry*. 2004;75:216-230.

188. Fry GA, King VM. The pupillary response and discomfort glare. *Journal of the Illuminating Engineering Society*. 1975;4:307.

189. Vos JJ. Disability glare - A state of the art report. In. 2 ed.; 1984:39.

190. McCartney EJ. *Optics of the atmosphere scattering by molecules and particles* New York: Wiley; 1976.

191. Snyder AW, Pask C. The Stiles-Crawford effect--explanation and consequences. *Vision Res*. 1973;13:1115-1137.

192. Olmedilla B, Granado F, Blanco I, Vaquero M. Lutein, but not alpha-tocopherol, supplementation improves visual function in patients with age-related cataracts: a 2-y double-blind, placebo-controlled pilot study. *Nutrition*. 2003;19:21-24.

193. Legein CP, Bouma H. Reading and the ophthalmologist. An introduction into the complex phenomenon of ordinary reading as a guideline for analysis and treatment of disabled readers. *Doc Ophthalmol*. 1982;53:123-157.

194. Hazel CA, Petre KL, Armstrong RA, Benson MT, Frost NA. Visual function and subjective quality of life compared in subjects with acquired macular disease. *Invest Ophthalmol Vis Sci*. 2000;41:1309-1315.

195. Legge GE, Rubin GS, Pelli DG, Schleske MM. Psychophysics of reading--II. Low vision. *Vision Res*. 1985;25:253-265.

196. Calabrese A, Bernard JB, Hoffart L, et al. Wet versus dry age-related macular degeneration in patients with central field loss: different effects on maximum reading speed. *Invest Ophthalmol Vis Sci*. 2011;52:2417-2424.

197. Legge GE, Mansfield JS, Chung ST. Psychophysics of reading. XX. Linking letter recognition to reading speed in central and peripheral vision. *Vision Res*. 2001;41:725-743.

198. Crossland MD, Culham LE, Rubin GS. Fixation stability and reading speed in patients with newly developed macular disease. *Ophthalmic Physiol Opt*. 2004;24:327-333.

199. Bullimore MA, Bailey IL. Reading and eye movements in age-related maculopathy. *Optom Vis Sci*. 1995;72:125-138.

200. Frennesson C, Nilsson UL, Peebo BB, Nilsson SE. Significant improvements in near vision, reading speed, central visual field and related quality of life after ranibizumab treatment of wet age-related macular degeneration. *Acta Ophthalmol*. 2010;88:420-425.

201. Koch KR, Muether PS, Hermann MM, Hoerster R, Kirchhof B, Fauser S. Subjective perception versus objective outcome after intravitreal ranibizumab for exudative AMD. *Graefes Arch Clin Exp Ophthalmol*. 2012;250:201-209.

202. Ray NJ, Fowler S, Stein JF. Yellow filters can improve magnocellular function: motion sensitivity, convergence, accommodation, and reading. *Ann N Y Acad Sci*. 2005;1039:283-93.:283-293.

203. Eperjesi F, Fowler CW, Evans BJ. Effect of light filters on reading speed in normal and low vision due to age-related macular degeneration. *Ophthalmic Physiol Opt*. 2004;24:17-25.

204. Midena E, Vujosevic S, Convento E, Manfre' A, Cavarzeran F, Pilotto E. Microperimetry and fundus autofluorescence in patients with early age-related macular degeneration. *Br J Ophthalmol*. 2007;91:1499-1503.

205. Kiss CG, Geitzenauer W, Simader C, Gregori G, Schmidt-Erfurth U. Evaluation of ranibizumab-induced changes in high-resolution optical coherence tomographic retinal morphology and their impact on visual function. *Invest Ophthalmol Vis Sci*. 2009;50:2376-2383.

206. Vujosevic S, Midena E, Pilotto E, Radin PP, Chiesa L, Cavarzeran F. Diabetic macular edema: correlation between microperimetry and optical coherence tomography findings. *Invest Ophthalmol Vis Sci*. 2006;47:3044-3051.

207. Okada K, Yamamoto S, Mizunoya S, Hoshino A, Arai M, Takatsuna Y. Correlation of retinal sensitivity measured with fundus-related microperimetry to visual acuity and retinal thickness in eyes with diabetic macular edema. *Eye (Lond)*. 2006;20:805-809.

208. Squirrell DM, Mawer NP, Mody CH, Brand CS. Visual outcome after intravitreal ranibizumab for wet age-related macular degeneration: a comparison between best-corrected visual acuity and microperimetry. *Retina*. 2010;30:436-442.

209. Westheimer G. Spatial frequency and light-spread descriptions of visual acuity and hyperacuity. *J Opt Soc Am*. 1977;67:207-212.

210. Lakshminarayanan V, Enoch JM. Vernier acuity and aging. *Int Ophthalmol*. 1995;19:109-115.

211. Williams RA, Enoch JM, Essock EA. The resistance of selected hyperacuity configurations to retinal image degradation. *Invest Ophthalmol Vis Sci*. 1984;25:389-399.

212. Lakshminarayanan V, ENOCH JM. Vernier acuity and aging. *Int Ophthalmol*. 1995;19:109-115.

213. Alster Y, Bressler NM, Bressler SB, et al. Preferential Hyperacuity Perimeter (PreView PHP) for detecting choroidal neovascularization study. *Ophthalmology*. 2005;112:1758-1765.

214. Loewenstein A, Malach R, Goldstein M, et al. Replacing the Amsler grid: a new method for monitoring patients with age-related macular degeneration. *Ophthalmology*. 2003;110:966-970.

215. Goldstein M, Loewenstein A, Barak A, et al. Results of a multicenter clinical trial to evaluate the preferential hyperacuity perimeter for detection of age-related macular degeneration. *Retina*. 2005;25:296-303.

216. Querques G, Berboucha E, Leveziel N, Pece A, Souied EH. Preferential hyperacuity perimeter in assessing responsiveness to ranibizumab therapy for exudative age-related macular degeneration. *Br J Ophthalmol*. 2011;95:986-991.

217. Querques G, Querques L, Rafaeli O, Canoui-Poitrine F, Bandello F, Souied EH. Preferential hyperacuity perimeter as a functional tool for monitoring exudative age-related macular degeneration in patients treated by intravitreal ranibizumab. *Invest Ophthalmol Vis Sci*. 2011;52:7012-7018.

218. Stein JD, Brown MM, Brown GC, Hollands H, Sharma S. Quality of life with macular degeneration: perceptions of patients, clinicians, and community members. *Br J Ophthalmol*. 2003;87:8-12.

219. . Guidance for industry: patient-reported outcome measures: use in medical product development to support labeling claims: draft guidance. *Health Qual Life Outcomes*. 2006;4:79.:79.

220. Mitchell J, Wolffsohn JS, Woodcock A, et al. Psychometric evaluation of the MacDQoL individualised measure of the impact of macular degeneration on quality of life. *Health Qual Life Outcomes*. 2005;3:25.:25.

221. Rovner BW, Casten RJ. Activity loss and depression in age-related macular degeneration. *Am J Geriatr Psychiatry*. 2002;10:305-310.

222. Moore LW, Miller M. Older men's experiences of living with severe visual impairment. *J Adv Nurs*. 2003;43:10-18.

223. Williams RA, Brody BL, Thomas RG, Kaplan RM, Brown SI. The psychosocial impact of macular degeneration. *Arch Ophthalmol*. 1998;116:514-520.

224. McNair DM, Lorr M, Droppleman LF. Manual for the profile of mood states. In: San Diego, Educational and Industrial Testing Services.; 1971.

225. Tolman J, Hill RD, Kleinschmidt JJ, Gregg CH. Psychosocial adaptation to visual impairment and its relationship to depressive affect in older adults with age-related macular degeneration. *Gerontologist*. 2005;45:747-753.

226. Brody BL, Gamst AC, Williams RA, et al. Depression, visual acuity, comorbidity, and disability associated with age-related macular degeneration. *Ophthalmology*. 2001;108:1893-1900.

227. Mangione CM, Lee PP, Pitts J, Gutierrez P, Berry S, Hays RD. Psychometric properties of the National Eye Institute Visual Function Questionnaire (NEI-VFQ). NEI-VFQ Field Test Investigators. *Arch Ophthalmol*. 1998;116:1496-1504.

228. Suner IJ, Kokame GT, Yu E, Ward J, Dolan C, Bressler NM. Responsiveness of NEI VFQ-25 to changes in visual acuity in neovascular AMD: validation studies from two phase 3 clinical trials. *Invest Ophthalmol Vis Sci*. 2009;50:3629-3635.

229. Pesudovs K, Gothwal VK, Wright T, Lamoureux EL. Remediating serious flaws in the National Eye Institute Visual Function Questionnaire. *J Cataract Refract Surg*. 2010;36:718-732.

230. Wright BD, Mok M. Rasch models overview. *J Appl Meas*. 2000;1:83-106.

231. Lamoureux EL, Ferraro JG, Pallant JF, Pesudovs K, Rees G, Keeffe JE. Are standard instruments valid for the assessment of quality of life and symptoms in glaucoma? *Optom Vis Sci*. 2007;84:789-796.

232. Velozo CA, Lai JS, Mallinson T, Hauselman E. Maintaining instrument quality while reducing items: application of Rasch analysis to a self-report of visual function. *J Outcome Meas*. 2000;4:667-680.

233. Pesudovs K, Garamendi E, Elliott DB. The Contact Lens Impact on Quality of Life (CLIQ) Questionnaire: development and validation. *Invest Ophthalmol Vis Sci*. 2006;47:2789-2796.

234. Trieschmann M, van Kuijk FJ, Alexander R, et al. Macular pigment in the human retina: histological evaluation of localization and distribution. *Eye (Lond)*. 2008;22:132-137.

235. Hammond BR, Wooten BR, Snodderly DM. Individual variations in the spatial profile of human macular pigment. *Journal of the Optical Society of America A-Optics Image Science and Vision*. 1997;14:1187-1196.

236. Sackett DL. The Cochrane Collaboration. *ACP J Club*. 1994;120 Suppl 3:A11.:A11.

237. Black N. Why we need observational studies to evaluate the effectiveness of health care. *BMJ*. 1996;312:1215-1218.

238. Stephenson J, Imrie J. Why do we need randomised controlled trials to assess behavioural interventions? *BMJ*. 1998;316:611-613.

239. Centre for Evidence Based Medicine UoO. Levels of Evidence for therapy. In: 2009.

240. Buzzi F. *Nuove sperienze fatte sulli occhio umano*; 1792.

241. Soemmering S. De foramina centrali limbo luteo cincto retinae humanae. *Comment Soc Reg Sci Goetting*. 1799;13.

242. Home E. An account of the orifice in the retina of the human eye, discovered by Professor Soemmering: to which are added proofs of this appearance being extended to the eyes of other animals. *Philos Trans R Soc Lond*. 1798;2:332.

243. Nussbaum JJ, Pruett RC, Delori FC. Historic perspectives. Macular yellow pigment. The first 200 years. *Retina*. 1981;1:296-310.

244. Walls GL, Judd HD. The Intra-Ocular Colour-Filters of Vertebrates. *Br J Ophthalmol*. 1933;17:705-725.

245. Schultze M. *Ueber den gelben Fleck der Retina, seinen Einfluss auf normales Sehen und auf Farbenblindheit*; 1866.

246. Walls GLJHD. The Intra-Ocular Colour-Filters of Vertebrates. *Br J Ophthalmol*. 1933;17:641-675.

247. Chevalleraeu A. De la colouration jaune de la macula. *Ann Oculist*. 1907:241.

248. Henning H. Optische Versuche an Vogeln und Schildkroten uber die Bedeutung der roten Oelkugeln im Auge. *Arch Gesphysiol*. 1920:91-123.

249. Erhard H. Messende Untersuchungen ueber den Farbensinn der Vogel. *Zool Jahrb (Zool Physiol)*. 1924:489-552.

250. Wald G. HUMAN VISION AND THE SPECTRUM. *Science*. 1945;101:653-658.

251. Bone RA, Landrum JT, Tarsis SL. Preliminary identification of the human macular pigment. *Vision Res*. 1985;25:1531-1535.

252. Landrum JT, Bone RA, Moore LL, Gomez CM. Analysis of zeaxanthin distribution within individual human retinas. *Methods Enzymol*. 1999;299:457-67.:457-467.

253. Bone RA, Landrum JT, Hime GW, Cains A, Zamor J. Stereochemistry of the human macular carotenoids. *Invest Ophthalmol Vis Sci*. 1993;34:2033-2040.

254. Neuringer M, Sandstrom MM, Johnson EJ, Snodderly DM. Nutritional manipulation of primate retinas, I: effects of lutein or zeaxanthin supplements on serum and macular pigment in xanthophyll-free rhesus monkeys. *Invest Ophthalmol Vis Sci*. 2004;45:3234-3243.

255. Bone RA, Landrum JT, Friedes LM, et al. Distribution of lutein and zeaxanthin stereoisomers in the human retina. *Exp Eye Res*. 1997;64:211-218.

256. Johnson EJ, Neuringer M, Russell RM, Schalch W, Snodderly DM. Nutritional manipulation of primate retinas, III: Effects of lutein or zeaxanthin supplementation on adipose tissue and retina of xanthophyll-free monkeys. *Invest Ophthalmol Vis Sci*. 2005;46:692-702.

257. Whitehead AJ, Mares JA, Danis RP. Macular pigment: a review of current knowledge. *Arch Ophthalmol*. 2006;124:1038-1045.

258. Sujak A, Gabrielska J, Grudzinski W, Borc R, Mazurek P, Gruszecki WI. Lutein and zeaxanthin as protectors of lipid membranes against oxidative damage: the structural aspects. *Arch Biochem Biophys*. 1999;371:301-307.

259. Landrum JT, Bone RA. Lutein, zeaxanthin, and the macular pigment. *Arch Biochem Biophys*. 2001;385:28-40.

260. Billsten HH, Bhosale P, Yemelyanov A, Bernstein PS, Polivka T. Photophysical properties of xanthophylls in carotenoproteins from human retinas. *Photochem Photobiol*. 2003;78:138-145.

261. Krinsky NI. Possible biologic mechanisms for a protective role of xanthophylls. *J Nutr*. 2002;132:540S-542S.

262. Barker FM, Snodderly DM, Johnson EJ, et al. Nutritional Manipulation of Primate Retinas. V: Effects of Lutein, Zeaxanthin and n--3 Fatty Acids on Retinal Sensitivity to Blue Light Damage. *Invest Ophthalmol Vis Sci*. 2011.

263. Sundelin SP, Nilsson SE. Lipofuscin-formation in retinal pigment epithelial cells is reduced by antioxidants. *Free Radic Biol Med*. 2001;31:217-225.

264. Trevithick-Sutton CC, Foote CS, Collins M, Trevithick JR. The retinal carotenoids zeaxanthin and lutein scavenge superoxide and hydroxyl radicals: a chemiluminescence and ESR study. *Mol Vis*. 2006;12:1127-35.:1127-1135.

265. Kirschfeld K. Carotenoid pigments: their possible role in protecting against photooxidation in eyes and photoreceptor cells. *Proc R Soc Lond B Biol Sci*. 1982;216:71-85.

266. Khachik F, Bernstein PS, Garland DL. Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. *Invest Ophthalmol Vis Sci*. 1997;38:1802-1811.

267. Jorgensen K, Skibsted LH. Carotenoid scavenging of radicals. Effect of carotenoid structure and oxygen partial pressure on antioxidative activity. *Z Lebensm Unters Forsch*. 1993;196:423-429.

268. Snodderly DM, Auran JD, Delori FC. The macular pigment. II. Spatial distribution in primate retinas. *Invest Ophthalmol Vis Sci*. 1984;25:674-685.

269. Trieschmann M, van Kuijk FJ, Alexander R, et al. Macular pigment in the human retina: histological evaluation of localization and distribution. *Eye (Lond)*. 2008;22:132-137.

270. Beatty S, Boulton M, Henson D, Koh HH, Murray IJ. Macular pigment and age related macular degeneration. *Br J Ophthalmol*. 1999;83:867-877.

271. Wrona M, Rozanowska M, Sarna T. Zeaxanthin in combination with ascorbic acid or alpha-tocopherol protects ARPE-19 cells against photosensitized peroxidation of lipids. *Free Radic Biol Med*. 2004;36:1094-1101.

272. Siems WG, Sommerburg O, van Kuijk FJ. Lycopene and beta-carotene decompose more rapidly than lutein and zeaxanthin upon exposure to various pro-oxidants in vitro. *Biofactors*. 1999;10:105-113.

273. Cantrell A, McGarvey DJ, Truscott TG, Rancan F, Bohm F. Singlet oxygen quenching by dietary carotenoids in a model membrane environment. *Arch Biochem Biophys*. 2003;412:47-54.

274. Bhosale P, Bernstein PS. Synergistic effects of zeaxanthin and its binding protein in the prevention of lipid membrane oxidation. *Biochim Biophys Acta*. 2005;1740:116-121.

275. Yemelyanov AY, Katz NB, Bernstein PS. Ligand-binding characterization of xanthophyll carotenoids to solubilized membrane proteins derived from human retina. *Experimental Eye Research*. 2001;72:381-392.

276. Thomson LR, Toyoda Y, Langner A, et al. Elevated retinal zeaxanthin and prevention of light-induced photoreceptor cell death in quail. *Invest Ophthalmol Vis Sci*. 2002;43:3538-3549.

277. Chucair AJ, Rotstein NP, SanGiovanni JP, During A, Chew EY, Politi LE. Lutein and zeaxanthin protect photoreceptors from apoptosis induced by oxidative stress: relation with docosahexaenoic acid. *Invest Ophthalmol Vis Sci*. 2007;48:5168-5177.

278. Li B, Ahmed F, Bernstein PS. Studies on the singlet oxygen scavenging mechanism of human macular pigment. *Arch Biochem Biophys*. 2010;504:56-60.

279. Hemenger RP. Dichroism of the macular pigment and Haidinger's brushes. *J Opt Soc Am*. 1982;72:734-737.

280. Richer SP, Stiles W, Graham-Hoffman K, et al. Randomized, double-blind, placebo-controlled study of zeaxanthin and visual function in patients with atrophic age-related macular degeneration: the Zeaxanthin and Visual Function Study (ZVF) FDA IND #78, 973. *Optometry*. 2011;82:667-680.

281. Nebeling LC, Forman MR, Graubard BI, Snyder RA. The impact of lifestyle characteristics on carotenoid intake in the United States: the 1987 National Health Interview Survey. *Am J Public Health*. 1997;87:268-271.

282. Sommerburg O, Keunen JE, Bird AC, van Kuijk FJ. Fruits and vegetables that are sources for lutein and zeaxanthin: the macular pigment in human eyes. *Br J Ophthalmol*. 1998;82:907-910.

283. Perry A, Rasmussen H, Johnson E. Xanthophyll (lutein, zeaxanthin) content in fruits, vegetables and corn and egg products. *Journal of Food Composition and Analysis*. 2009;22:9-15.

284. Chung HY, Rasmussen HM, Johnson EJ. Lutein bioavailability is higher from lutein-enriched eggs than from supplements and spinach in men. *J Nutr*. 2004;134:1887-1893.

285. Thurnham DI. Macular zeaxanthins and lutein -- a review of dietary sources and bioavailability and some relationships with macular pigment optical density and age-related macular disease. *Nutr Res Rev*. 2007;20:163-179.

286. Maoka T, Arai A, Shimizu M, Matsuno T. The first isolation of enantiomeric and meso-zeaxanthin in nature. *Comp Biochem Physiol B*. 1986;83:121-124.

287. Connolly EE, Beatty S, Thurnham DI, et al. Augmentation of macular pigment following supplementation with all three macular carotenoids: an exploratory study. *Curr Eye Res*. 2010;35:335-351.

288. Hennekens CH, Buring JE. Observational evidence. *Ann N Y Acad Sci*. 1993;703:18-24; discussion 24.:18-24.

289. Rychetnik L, Frommer M, Hawe P, Shiell A. Criteria for evaluating evidence on public health interventions. *J Epidemiol Community Health*. 2002;56:119-127.

290. Richer S. ARMD--pilot (case series) environmental intervention data. *J Am Optom Assoc*. 1999;70:24-36.

291. Olmedilla B, Granado F, Blanco I, Vaquero M, Cajigal C. Lutein in patients with cataracts and age-related macular degeneration: a long-term supplementation study. *Journal of the Science of Food and Agriculture*. 2001;81:904-909.

292. Sasamoto Y, Gomi F, Sawa M, Tsujikawa M, Nishida K. Effect of 1-year lutein supplementation on macular pigment optical density and visual function. *Graefes Arch Clin Exp Ophthalmol*. 2011;249:1847-1854.

293. Bartlett HE, Eperjesi F. Effect of lutein and antioxidant dietary supplementation on contrast sensitivity in age-related macular disease: a randomized controlled trial. *Eur J Clin Nutr*. 2007;61:1121-1127.

294. Neelam K, Hogg RE, Stevenson MR, et al. Carotenoids and co-antioxidants in age-related maculopathy: design and methods. *Ophthalmic Epidemiol*. 2008;15:389-401.

295. Hammond BR, Jr., Wooten BR. CFF thresholds: relation to macular pigment optical density. *Ophthalmic Physiol Opt*. 2005;25:315-319.

296. Davison P, Akkali M, Loughman J, Scanlon G, Nolan J, Beatty S. Macular pigment: its associations with color discrimination and matching. *Optom Vis Sci*. 2011;88:816-822.

297. Arundale K. An investigation into the variation of human contrast sensitivity with age and ocular pathology. *Br J Ophthalmol*. 1978;62:213-215.

298. Gittings NS, Fozard JL. Age related changes in visual acuity. *Exp Gerontol*. 1986;21:423-433.

299. Klein R, Klein BE, Linton KL, De Mets DL. The Beaver Dam Eye Study: visual acuity. *Ophthalmology*. 1991;98:1310-1315.

300. Bernstein PS, Ahmed F, Liu A, et al. Macular Pigment Imaging in AREDS2 Participants: An Ancillary Study of AREDS2 Subjects Enrolled at the Moran Eye Center. *Invest Ophthalmol Vis Sci*. 2012;53:6178-6186.

301. Seddon JM, Ajani UA, Sperduto RD, et al. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case-Control Study Group. *JAMA*. 1994;272:1413-1420.

302. VandenLangenberg GM, Mares-Perlman JA, Klein R, Klein BE, Brady WE, Palta M. Associations between antioxidant and zinc intake and the 5-year

incidence of early age-related maculopathy in the Beaver Dam Eye Study. *Am J Epidemiol*. 1998;148:204-214.

303. Mares-Perlman JA, Fisher AI, Klein R, et al. Lutein and zeaxanthin in the diet and serum and their relation to age-related maculopathy in the third national health and nutrition examination survey. *Am J Epidemiol*. 2001;153:424-432.

304. Flood V, Smith W, Wang JJ, Manzi F, Webb K, Mitchell P. Dietary antioxidant intake and incidence of early age-related maculopathy: the Blue Mountains Eye Study. *Ophthalmology*. 2002;109:2272-2278.

305. Moeller SM, Parekh N, Tinker L, et al. Associations between intermediate age-related macular degeneration and lutein and zeaxanthin in the Carotenoids in Age-related Eye Disease Study (CAREDS): ancillary study of the Women's Health Initiative. *Arch Ophthalmol*. 2006;124:1151-1162.

306. Snellen EL, Verbeek AL, Van Den Hoogen GW, Cruysberg JR, Hoyng CB. Neovascular age-related macular degeneration and its relationship to antioxidant intake. *Acta Ophthalmol Scand*. 2002;80:368-371.

307. Tan JS, Wang JJ, Flood V, Rochtchina E, Smith W, Mitchell P. Dietary antioxidants and the long-term incidence of age-related macular degeneration: the Blue Mountains Eye Study. *Ophthalmology*. 2008;115:334-341.

308. . Antioxidant status and neovascular age-related macular degeneration. Eye Disease Case-Control Study Group. *Arch Ophthalmol*. 1993;111:104-109.

309. Mares-Perlman JA, Brady WE, Klein R, et al. Serum antioxidants and age-related macular degeneration in a population-based case-control study. *Arch Ophthalmol*. 1995;113:1518-1523.

310. Simonelli F, Zarrilli F, Mazzeo S, et al. Serum oxidative and antioxidant parameters in a group of Italian patients with age-related maculopathy. *Clin Chim Acta*. 2002;320:111-115.

311. Gale CR, Hall NF, Phillips DI, Martyn CN. Lutein and zeaxanthin status and risk of age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2003;44:2461-2465.

312. Cardinault N, Abalain JH, Sairafi B, et al. Lycopene but not lutein nor zeaxanthin decreases in serum and lipoproteins in age-related macular degeneration patients. *Clin Chim Acta*. 2005;357:34-42.

313. Delcourt C, Carriere I, Delage M, Barberger-Gateau P, Schalch W. Plasma lutein and zeaxanthin and other carotenoids as modifiable risk factors for age-related maculopathy and cataract: the POLA Study. *Invest Ophthalmol Vis Sci*. 2006;47:2329-2335.

314. Michikawa T, Ishida S, Nishiwaki Y, et al. Serum antioxidants and age-related macular degeneration among older Japanese. *Asia Pac J Clin Nutr*. 2009;18:1-7.

315. Zhou H, Zhao X, Johnson EJ, et al. Serum carotenoids and risk of age-related macular degeneration in a chinese population sample. *Invest Ophthalmol Vis Sci*. 2011;52:4338-4344.

316. Nussbaum JJ, Pruett RC, Delori FC. Historic perspectives. Macular yellow pigment. The first 200 years. *Retina*. 1981;1:296-310.

317. Landrum JT, Bone RA. Lutein, zeaxanthin, and the macular pigment. *Archives of Biochemistry and Biophysics*. 2001;385:28-40.

318. Billsten HH, Bhosale P, Yemelyanov A, Bernstein PS, Polivka T. Photophysical properties of xanthophylls in carotenoproteins from human retinas. *Photochem Photobiol*. 2003;78:138-145.

319. Li B, Ahmed F, Bernstein PS. Studies on the singlet oxygen scavenging mechanism of human macular pigment. *Arch Biochem Biophys*. 2010.

320. Nolan JM, Stack J, O' DO, Loane E, Beatty S. Risk factors for age-related maculopathy are associated with a relative lack of macular pigment. *Exp Eye Res*. 2007;84:61-74.

321. Kirby ML, Beatty S, Loane E, et al. A Central Dip in the Macular Pigment Spatial Profile is Associated with Age and Smoking. *Invest Ophthalmol Vis Sci*. 2010;5:6722-6728.

322. Nolan JM, Akkali MC, Loughman J, Howard AN, Beatty S. Macular carotenoid supplementation in subjects with atypical spatial profiles of macular pigment. *Exp Eye Res*. 2012;101:9-15.

323. Richer S, Devenport J, Lang JC. LAST II: Differential temporal responses of macular pigment optical density in patients with atrophic age-related macular degeneration to dietary supplementation with xanthophylls. *Optometry*. 2007;78:213-219.

324. Hammond BR, Jr., Johnson EJ, Russell RM, et al. Dietary modification of human macular pigment density. *Invest Ophthalmol Vis Sci*. 1997;38:1795-1801.

325. Johnson EJ, Hammond BR, Yeum KJ, et al. Relation among serum and tissue concentrations of lutein and zeaxanthin and macular pigment density. *Am J Clin Nutr*. 2000;71:1555-1562.

326. Landrum JT, Bone RA, Joa H, Kilburn MD, Moore LL, Sprague KE. A one year study of the macular pigment: the effect of 140 days of a lutein supplement. *Exp Eye Res*. 1997;65:57-62.

327. Berendschot TT, Goldbohm RA, Klopping WA, van de KJ, van NJ, van ND. Influence of lutein supplementation on macular pigment, assessed with two objective techniques. *Invest Ophthalmol Vis Sci*. 2000;41:3322-3326.

328. Aleman TS, Duncan JL, Bieber ML, et al. Macular pigment and lutein supplementation in retinitis pigmentosa and Usher syndrome. *Invest Ophthalmol Vis Sci*. 2001;42:1873-1881.

329. Bone RA, Landrum JT, Guerra LH, Ruiz CA. Lutein and Zeaxanthin Dietary Supplements Raise Macular Pigment Density and Serum Concentrations of these Carotenoids in Humans. *J Nutr*. 2003;133:992-998.

330. Koh HH, Murray IJ, Nolan D, Carden D, Feather J, Beatty S. Plasma and macular responses to lutein supplement in subjects with and without age-related maculopathy: a pilot study. *Exp Eye Res*. 2004;79:21-27.

331. Bernstein PS, Zhao DY, Sharifzadeh M, Ermakov IV, Gellermann W. Resonance Raman measurement of macular carotenoids in the living human eye. *Arch Biochem Biophys*. 2004;430:163-169.

332. Bone RA, Landrum JT, Cao Y, Howard AN, varez-Calderon F. Macular pigment response to a supplement containing meso-zeaxanthin, lutein and zeaxanthin. *Nutr Metab (Lond)*. 2007;4:12.:12.

333. Wenzel AJ, Sheehan JP, Gerweck C, Stringham JM, Fuld K, Curran-Celentano J. Macular pigment optical density at four retinal loci during 120 days of lutein supplementation. *Ophthalmic Physiol Opt*. 2007;27:329-335.

334. Schalch W, Cohn W, Barker FM, et al. Xanthophyll accumulation in the human retina during supplementation with lutein or zeaxanthin - the LUXEA (LUtein Xanthophyll Eye Accumulation) study. *Arch Biochem Biophys*. 2007;458:128-135.

335. Johnson EJ, Chung HY, Caldarella SM, Snodderly DM. The influence of supplemental lutein and docosahexaenoic acid on serum, lipoproteins, and macular pigmentation. *Am J Clin Nutr*. 2008;87:1521-1529.

336. Stringham JM, Hammond BR. Macular pigment and visual performance under glare conditions. *Optom Vis Sci*. 2008;85:82-88.

337. Nolan JM, Loughman J, Akkali MC, et al. The impact of macular pigment augmentation on visual performance in normal subjects: COMPASS. *Vision Res*. 2011;51:459-469.

338. Trieschmann M, Beatty S, Nolan JM, et al. Changes in macular pigment optical density and serum concentrations of its constituent carotenoids following supplemental lutein and zeaxanthin: the LUNA study. *Exp Eye Res*. 2007;84:718-728.

339. Richer S, Devenport J, Lang JC. LAST II: Differential temporal responses of macular pigment optical density in patients with atrophic age-related macular degeneration to dietary supplementation with xanthophylls. *Optometry*. 2007;78:213-219.

340. Loughman J, Nolan JM, Beatty S. Impact of dietary carotenoid deprivation on macular pigment and serum concentrations of lutein and zeaxanthin. *Br J Nutr*. 2012:1-2.

341. Mones J, Biarnes M, Trindade F, Casaroli-Marano R. FUSION regimen: ranibizumab in treatment-naive patients with exudative age-related macular degeneration and relatively good baseline visual acuity. *Graefes Arch Clin Exp Ophthalmol*. 2012.

342. Singh RP, Fu EX, Smith SD, Williams DR, Kaiser PK. Predictive factors of visual and anatomical outcome after intravitreal bevacizumab treatment of neovascular age-related macular degeneration: an optical coherence tomography study. *Br J Ophthalmol*. 2009;93:1353-1358.

343. Kaiser PK, Brown DM, Zhang K, et al. Ranibizumab for predominantly classic neovascular age-related macular degeneration: subgroup analysis of first-year ANCHOR results. *Am J Ophthalmol*. 2007;144:850-857.

344. Lalwani GA, Rosenfeld PJ, Fung AE, et al. A variable-dosing regimen with intravitreal ranibizumab for neovascular age-related macular degeneration: year 2 of the PrONTO Study. *Am J Ophthalmol*. 2009;148:43-58.

345. Bailey IL, Lovie JE. New design principles for visual acuity letter charts. *Am J Optom Physiol Opt*. 1976;53:740-745.

346. SLOAN LL. New test charts for the measurement of visual acuity at far and near distances. *Am J Ophthalmol*. 1959;48:807-13.:807-813.

347. Hohberger B, Laemmer R, Adler W, Juenemann AG, Horn FK. Measuring contrast sensitivity in normal subjects with OPTEC 6500: influence of age and glare. *Graefes Arch Clin Exp Ophthalmol*. 2007;245:1805-1814.

348. Chen FK, Patel PJ, Xing W, et al. Test-retest variability of microperimetry using the Nidek MP1 in patients with macular disease. *Invest Ophthalmol Vis Sci*. 2009;50:3464-3472.

349. Stifter E, Konig F, Lang T, et al. Reliability of a standardized reading chart system: variance component analysis, test-retest and inter-chart reliability. *Graefes Arch Clin Exp Ophthalmol*. 2004;242:31-39.

350. Wylegala EA, Pilat J, Teper SJ, Wroblewska-Czajka E, Bartusek M. Monitoring of photodynamic therapy results in age-related macular degeneration by means of preferential hyperacuity perimeter. *Eur J Ophthalmol*. 2007;17:768-775.

351. Das R, Shi Y, Silvestri G, Chakravarthy U. Distortion maps from preferential hyperacuity perimetry are helpful in monitoring functional response to Lucentis therapy. *Retina*. 2009;29:1013-1018.

352. Huang D, Swanson EA, Lin CP, et al. Optical coherence tomography. *Science*. 1991;254:1178-1181.

353. Grover S, Murthy RK, Brar VS, Chalam KV. Comparison of retinal thickness in normal eyes using Stratus and Spectralis optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2010;51:2644-2647.

354. Taban M, Sharma S, Williams DR, Waheed N, Kaiser PK. Comparing retinal thickness measurements using automated fast macular thickness map versus six-radial line scans with manual measurements. *Ophthalmology*. 2009;116:964-970.

355. Polito A, Del BM, Isola M, Zemella N, Bandello F. Repeatability and reproducibility of fast macular thickness mapping with stratus optical coherence tomography. *Arch Ophthalmol*. 2005;123:1330-1337.

356. Massin P, Vicaut E, Haouchine B, Erginay A, Paques M, Gaudric A. Reproducibility of retinal mapping using optical coherence tomography. *Arch Ophthalmol*. 2001;119:1135-1142.

357. Mangione CM, Berry S, Spritzer K, et al. Identifying the content area for the 51-item National Eye Institute Visual Function Questionnaire: results from focus groups with visually impaired persons. *Arch Ophthalmol*. 1998;116:227-233.

358. Mangione CM, Lee PP, Gutierrez PR, Spritzer K, Berry S, Hays RD. Development of the 25-item National Eye Institute Visual Function Questionnaire. *Arch Ophthalmol*. 2001;119:1050-1058.

359. Mangione CM, Lee PP, Pitts J, Gutierrez P, Berry S, Hays RD. Psychometric properties of the National Eye Institute Visual Function Questionnaire (NEI-VFQ). NEI-VFQ Field Test Investigators. *Arch Ophthalmol*. 1998;116:1496-1504.

360. Revicki DA, Rentz AM, Harnam N, Thomas VS, Lanzetta P. Reliability and validity of the National Eye Institute Visual Function Questionnaire-25 in patients with age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2010;51:712-717.

361. Miskala PH, Bressler NM, Meinert CL. Relative contributions of reduced vision and general health to NEI-VFQ scores in patients with neovascular age-related macular degeneration. *Arch Ophthalmol*. 2004;122:758-766.

362. Berdeaux GH, Nordmann JP, Colin E, Arnould B. Vision-related quality of life in patients suffering from age-related macular degeneration. *Am J Ophthalmol*. 2005;139:271-279.

363. Suner IJ, Kokame GT, Yu E, Ward J, Dolan C, Bressler NM. Responsiveness of NEI VFQ-25 to changes in visual acuity in neovascular AMD: validation studies from two phase 3 clinical trials. *Invest Ophthalmol Vis Sci*. 2009;50:3629-3635.

364. Cusick M, SanGiovanni JP, Chew EY, et al. Central visual function and the NEI-VFQ-25 near and distance activities subscale scores in people with type 1 and 2 diabetes. *Am J Ophthalmol*. 2005;139:1042-1050.

365. Orr P, Rentz AM, Margolis MK, et al. Validation of the National Eye Institute Visual Function Questionnaire-25 (NEI VFQ-25) in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2011;52:3354-3359.

366. Pesudovs K, Gothwal VK, Wright T, Lamoureux EL. Remediating serious flaws in the National Eye Institute Visual Function Questionnaire. *J Cataract Refract Surg*. 2010;36:718-732.

367. Kaiser PK, Blodi BA, Shapiro H, Acharya NR. Angiographic and optical coherence tomographic results of the MARINA study of ranibizumab in neovascular age-related macular degeneration. *Ophthalmology*. 2007;114:1868-1875.

368. Williams TA, Blyth CP. Outcome of ranibizumab treatment in neovascular age related macula degeneration in eyes with baseline visual acuity better than 6/12. *Eye (Lond)*. 2011;25:1617-1621.

369. Kuyk T, Elliott JL. Visual factors and mobility in persons with age-related macular degeneration. *J Rehabil Res Dev*. 1999;36:303-312.

370. Sandberg MA, Weiner A, Miller S, Gaudio AR. High-risk characteristics of fellow eyes of patients with unilateral neovascular age-related macular degeneration. *Ophthalmology*. 1998;105:441-447.

371. Schmidt-Erfurth UM, Elsner H, Terai N, Benecke A, Dahmen G, Michels SM. Effects of verteporfin therapy on central visual field function. *Ophthalmology*. 2004;111:931-939.

372. Frennesson C, Nilsson UL, Peebo BB, Nilsson SE. Significant improvements in near vision, reading speed, central visual field and related quality of life after ranibizumab treatment of wet age-related macular degeneration. *Acta Ophthalmol*. 2010;88:420-425.

373. Chang TS, Bressler NM, Fine JT, Dolan CM, Ward J, Klesert TR. Improved vision-related function after ranibizumab treatment of neovascular age-related macular degeneration: results of a randomized clinical trial. *Arch Ophthalmol*. 2007;125:1460-1469.

374. Okada K, Yamamoto S, Mizunoya S, Hoshino A, Arai M, Takatsuna Y. Correlation of retinal sensitivity measured with fundus-related microperimetry

to visual acuity and retinal thickness in eyes with diabetic macular edema. *Eye (Lond)*. 2006;20:805-809.

375. Holz FG, Korobelnik JF, Lanzetta P, et al. The effects of a flexible visual acuity-driven ranibizumab treatment regimen in age-related macular degeneration: outcomes of a drug and disease model. *Invest Ophthalmol Vis Sci*. 2010;51:405-412.

376. Rosenfeld PJ, Rich RM, Lalwani GA. Ranibizumab: Phase III clinical trial results. *Ophthalmol Clin North Am*. 2006;19:361-372.

377. Sommerburg O, Keunen JEE, Bird AC, van Kuijk FJGM. Fruits and vegetables that are sources for lutein and zeaxanthin: the macular pigment in human eyes. *Br J Ophthalmol*. 1998;82:907-910.

378. Maoka T, Arai A, Shimizu M, Matsuno T. The first isolation of enantiomeric and meso-zeaxanthin in nature. *Comp Biochem Physiol B*. 1986;83:121-124.

379. Connolly EE, Beatty S, Thurnham DI, et al. Augmentation of macular pigment following supplementation with all three macular carotenoids: an exploratory study. *Curr Eye Res*. 2010;35:335-351.

380. Johnson EJ, Neuringer M, Russell RM, Schalch W, Snodderly DM. Nutritional manipulation of primate retinas, III: effects of lutein or zeaxanthin supplementation on adipose tissue and retina of xanthophyll-free monkeys. *Investigative Ophthalmology Visual Science*. 2005;46:692-702.

381. Bone RA, Landrum JT, Friedes LM, et al. Distribution of lutein and zeaxanthin stereoisomers in the human retina. *Experimental Eye Research*. 1997;64:211-218.

382. Bhosale P, Bernstein PS. Synergistic effects of zeaxanthin and its binding protein in the prevention of lipid membrane oxidation. *Biochim Biophys Acta*. 2005;1740:116-121.

383. Alexander KR, Xie W, Derlacki DJ. Spatial-frequency characteristics of letter identification. *J Opt Soc Am A Opt Image Sci Vis*. 1994;11:2375-2382.

384. Wooten BR, Hammond BR, Land RI, Snodderly DM. A practical method for measuring macular pigment optical density. *Investigative Ophthalmology & Visual Science*. 1999;40:2481-2489.

385. Stringham JM, Hammond BR, Nolan JM, et al. The utility of using customized heterochromatic flicker photometry (cHFP) to measure macular pigment in patients with age-related macular degeneration. *Exp Eye Res*. 2008;87:445-453.

386. Falsini B, Fadda A, Iarossi G, et al. Retinal sensitivity to flicker modulation: reduced by early age-related maculopathy. *Invest Ophthalmol Vis Sci*. 2000;41:1498-1506.

387. Tyler CW. Two processes control variations in flicker sensitivity over the life span. *J Opt Soc Am A*. 1989;6:481-490.

388. Loane E, Stack J, Beatty S, Nolan JM. Measurement of macular pigment optical density using two different heterochromatic flicker photometers. *Curr Eye Res*. 2007;32:555-564.

389. Kirby ML, Galea M, Loane E, Stack J, Beatty S, Nolan JM. Foveal anatomic associations with the secondary peak and the slope of the macular pigment spatial profile. *Invest Ophthalmol Vis Sci*. 2009;50:1383-1391.

390. Graham I, Atar D, Borch-Johnsen K, et al. European guidelines on cardiovascular disease prevention in clinical practice: executive summary: Fourth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (Constituted by representatives of nine societies and by invited experts). *Eur Heart J*. 2007;28:2375-2414.

391. World Health Organisation. Definition of diabetes mellitus and intermediate hyperglycemia. Geneva. *WHO report*. 2006.

392. Sloane ME, Ball K, Owsley C, Bruni JR, Roenker DL. The Visual Activities Questionnaire: Developing an instrument for assessing problems in everyday visual tasks. *Technical Digest, Noninvasive Assessment of the Visual System, Topical Meeting of the Optical Society of America*. 1992.

393. Klein R, Davis MD, Magli YL, Segal P, Klein BE, Hubbard L. The Wisconsin age-related maculopathy grading system. *Ophthalmology*. 1991;98:1128-1134.

394. Klein R, Klein BE, Knudtson MD, Meuer SM, Swift M, Gangnon RE. Fifteen-year cumulative incidence of age-related macular degeneration: the Beaver Dam Eye Study. *Ophthalmology*. 2007;114:253-262.

395. Cohen J. *Statistical power analysis for the behavioral sciences*. 2nd ed. Hillsdale, New Jersey: Lawrence Erlbaum Associates; 1988.

396. Li B, Ahmed F, Bernstein PS. Studies on the singlet oxygen scavenging mechanism of human macular pigment. *Arch Biochem Biophys*. 2010;504:56-60.

397. Landrum JT, Bone RA, Moore LL, Gomez CM. Analysis of zeaxanthin distribution within individual human retinas. *Methods Enzymol*. 1999;299:457-67.:457-467.

398. Ma L, Yan SF, Huang YM, et al. Effect of Lutein and Zeaxanthin on Macular Pigment and Visual Function in Patients with Early Age-Related Macular Degeneration. *Ophthalmology*. 2012;Epub ahead of print.

399. van Meeteren A. Calculations on the Optical Modulation Transfer Function of the Human Eye for White Light. *Optica Acta: International Journal of Optics*. 1974;21.

400. Howarth PA, Bradley A. The longitudinal chromatic aberration of the human eye, and its correction. *Vision Res*. 1986;26:361-366.

401. Wald G. Human Vision and the Spectrum. *Science*. 1945;101:653-658.

402. Schultze M. *Ueber den gelben Fleck der Retina, seinen Einfluss auf normales Sehen und auf Farbenblindheit*: Cohen & Sohn; 1866.

403. Campbell FW, Gubisch RW. The effect of chromatic aberration on visual acuity. *J Physiol*. 1967;192:345-358.

404. DeValois RL, DeValois KK. *Spatial Vision* Oxford: Oxford Science Publications; 1988.

405. Yoon GY, Williams DR. Visual performance after correcting the monochromatic and chromatic aberrations of the eye. *J Opt Soc Am A Opt Image Sci Vis*. 2002;19:266-275.

406. Negishi K, Ohnuma K, Hirayama N, Noda T. Effect of chromatic aberration on contrast sensitivity in pseudophakic eyes. *Arch Ophthalmol*. 2001;119:1154-1158.

407. Ma L, Dou HL, Huang YM, et al. Improvement of Retinal Function in Early Age-Related Macular Degeneration After Lutein and Zeaxanthin Supplementation: A Randomized, Double-Masked, Placebo-Controlled Trial. *Am J Ophthalmol*. 2012;154:625-634.

408. Parisi V, Tedeschi M, Gallinaro G, Varano M, Saviano S, Piermarocchi S. Carotenoids and antioxidants in age-related maculopathy italian study: multifocal electroretinogram modifications after 1 year. *Ophthalmology*. 2008;115:324-333.

409. Connolly EE, Beatty S, Loughman J, Howard AN, Louw MS, Nolan JM. Supplementation with all three macular carotenoids: response, stability, and safety. *Invest Ophthalmol Vis Sci*. 2011;52:9207-9217.

410. Wolf-Schnurrbusch UE, Roosli N, Weyermann E, Heldner MR, Hohne K, Wolf S. Ethnic differences in macular pigment density and distribution. *Invest Ophthalmol Vis Sci*. 2007;48:3783-3787.

411. Hammond BR, Fuld K, Snodderly DM. Iris color and macular pigment optical density. *Experimental Eye Research*. 1996;62:293-297.

412. Burke JD, Curran-Celentano J, Wenzel AJ. Diet and Serum Carotenoid Concentrations Affect Macular Pigment Optical Density in Adults 45 Years and Older. *J Nutr*. 2005;135:1208-1214.

413. Trieschmann M, Beatty S, Nolan JM, et al. Changes in macular pigment optical density and serum concentrations of its constituent carotenoids following supplemental lutein and zeaxanthin: the LUNA study. *Exp Eye Res*. 2007;84:718-728.

414. Zeimer M, Dietzel M, Hense HW, Heimes B, Austermann U, Pauleikhoff D. Profiles of macular pigment optical density and their changes following supplemental lutein and zeaxanthin: new results from the LUNA study. *Invest Ophthalmol Vis Sci*. 2012;53:4852-4859.

415. Nolan J, Beatty S. Profiles of macular pigment optical density and their changes following supplemental lutein and zeaxannthin. *Invest Ophthalmol Vis Sci*. 2012;19;53:6303.

416. Berendschot TTJM, van Norren D. Macular Pigment Shows Ringlike Structures. *Investigative Ophthalmology Visual Science*. 2006;47:709-714.

417. Delori FC, Goger DG, Keilhauer C, Salvetti P, Staurenghi G. Bimodal spatial distribution of macular pigment: evidence of a gender relationship. *J Opt Soc Am A Opt Image Sci Vis*. 2006;23:521-538.

418. Dubuc S, Wittich W, Gomolin JE, Kapusta M, Overbury O. Beyond visual acuity: functional outcome and patient satisfaction following treatment for age-related macular degeneration. *Can J Ophthalmol*. 2009;44:680-685.

419. Broman AT, Munoz B, Rodriguez J, et al. The impact of visual impairment and eye disease on vision-related quality of life in a Mexican-American population: proyecto VER. *Invest Ophthalmol Vis Sci*. 2002;43:3393-3398.

420. Charalampidou S, Nolan J, Loughman J, et al. Psychophysical impact and optical and morphological characteristics of symptomatic non-advanced cataract. *Eye (Lond)*. 2011;25:1147-1154.

421. Davis MD, Gangnon RE, Lee LY, et al. The Age-Related Eye Disease Study severity scale for age-related macular degeneration: AREDS Report No. 17. *Arch Ophthalmol*. 2005;123:1484-1498.

422. Hartmann KI, Bartsch DU, Cheng L, et al. Scanning laser ophthalmoscope imaging stabilized microperimetry in dry age-related macular degeneration. *Retina*. 2011;31:1323-1331.

423. Oster SF, Mojana F, Brar M, Yuson RM, Cheng L, Freeman WR. Disruption of the photoreceptor inner segment/outer segment layer on spectral domain-optical coherence tomography is a predictor of poor visual acuity in patients with epiretinal membranes. *Retina*. 2010;30:713-718.

424. Mitamura Y, Aizawa S, Baba T, Hagiwara A, Yamamoto S. Correlation between retinal sensitivity and photoreceptor inner/outer segment junction in patients with retinitis pigmentosa. *Br J Ophthalmol*. 2009;93:126-127.

425. Landa G, Su E, Garcia PM, Seiple WH, Rosen RB. Inner segment-outer segment junctional layer integrity and corresponding retinal sensitivity in dry and wet forms of age-related macular degeneration. *Retina*. 2011;31:364-370.

426. Ojima Y, Tsujikawa A, Hangai M, et al. Retinal sensitivity measured with the micro perimeter 1 after resolution of central serous chorioretinopathy. *Am J Ophthalmol*. 2008;146:77-84.

427. Yamaike N, Tsujikawa A, Sakamoto A, et al. Retinal sensitivity after intravitreal injection of bevacizumab for the treatment of macular edema secondary to retinal vein occlusion. *Retina*. 2009;29:757-767.

428. Sunness JS, Johnson MA, Massof RW, Marcus S. Retinal sensitivity over drusen and nondrusen areas. A study using fundus perimetry. *Arch Ophthalmol*. 1988;106:1081-1084.

429. Remky A, Lichtenberg K, Elsner AE, Arend O. Short wavelength automated perimetry in age related maculopathy. *Br J Ophthalmol*. 2001;85:1432-1436.

430. Scholl HP, Bellmann C, Dandekar SS, Bird AC, Fitzke FW. Photopic and scotopic fine matrix mapping of retinal areas of increased fundus autofluorescence in patients with age-related maculopathy. *Invest Ophthalmol Vis Sci*. 2004;45:574-583.

431. Curcio CA, Owsley C, Jackson GR. Spare the rods, save the cones in aging and age-related maculopathy. *Invest Ophthalmol Vis Sci*. 2000;41:2015-2018.

432. Sunness JS, Massof RW, Johnson MA, Bressler NM, Bressler SB, Fine SL. Diminished foveal sensitivity may predict the development of advanced age-related macular degeneration. *Ophthalmology*. 1989;96:375-381.

433. Feigl B, Morris CP. The challenge of predicting macular degeneration. *Curr Med Res Opin*. 2011;27:1745-1748.

434. Shin HJ, Chung H, Kim HC. Association between foveal microstructure and visual outcome in age-related macular degeneration. *Retina*. 2011;31:1627-1636.

435. Alexander P, Mushtaq F, Osmond C, Amoaku W. Microperimetric changes in neovascular age-related macular degeneration treated with ranibizumab. *Eye (Lond)*. 2012;26:678-683.

436. Sulzbacher F, Kiss C, Kaider A, et al. Correlation of SD-OCT Features and Retinal Sensitivity in Neovascular Age-related Macular Degeneration. *Invest Ophthalmol Vis Sci*. 2012.

437. Karacorlu M, Ozdemir H, Senturk F, Karacorlu SA, Uysal O. Correlation of retinal sensitivity with visual acuity and macular thickness in eyes with idiopathic epimacular membrane. *Int Ophthalmol*. 2010;30:285-290.

438. Charbel IP, Helb HM, Holz FG, Scholl HP. Correlation of macular function with retinal thickness in nonproliferative type 2 idiopathic macular telangiectasia. *Am J Ophthalmol*. 2008;145:169-175.

439. Okada K, Kubota-Taniai M, Kitahashi M, Baba T, Mitamura Y, Yamamoto S. Changes in visual function and thickness of macula after photodynamic therapy for age-related macular degeneration. *Clin Ophthalmol*. 2009;3:483-488.

440. Keane PA, Patel PJ, Ouyang Y, et al. Effects of retinal morphology on contrast sensitivity and reading ability in neovascular age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2010;51:5431-5437.

441. Lalwani GA, Rosenfeld PJ, Fung AE, et al. A variable-dosing regimen with intravitreal ranibizumab for neovascular age-related macular degeneration: year 2 of the PrONTO Study. *Am J Ophthalmol*. 2009;148:43-58.

442. Steinmetz R.L., Walker D., Fitzke F.W., ird A.C. Prolonged Dark Adaptation in Patients with Age-Related Macular Degeneration. *The ARVO Annual Meeting Sarasota, FL*. 1991.

443. Hogg RE, Chakravarthy U. Visual function and dysfunction in early and late age-related maculopathy. *Prog Retin Eye Res*. 2006;25:249-276.

444. Dimitrov PN, Robman LD, Varsamidis M, et al. Visual function tests as potential biomarkers in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2011;52:9457-9469.

445. Dimitrov PN, Robman LD, Varsamidis M, et al. Relationship between Clinical Macular Changes and Retinal Function in Age-Related Macular Degeneration. *Invest Ophthalmol Vis Sci*. 2012;53:5213-5220.

446. Brown B., Lovie-Kitchin J.E. Contrast sensitivity in central and paracentral retina in age-related maculopathy. *Clinical and Experimental Optometry*. 1988;70:145-148.

447. Qiu F, Leat SJ. Functional deficits in early stage age-related maculopathy. *Clin Exp Optom*. 2009;92:90-98.

448. Kenny R, Whelan B, Cronin H, et al. The Design of the Irish Longitudinal Study on Ageing (TILDA). *www.tcd.ie/tilda/assets/pdf/DesignReport2010 pdf*. 2010.

449. Nolan JM, Kenny R, O'Regan C, et al. Macular pigment optical density in an ageing Irish population: The Irish Longitudinal Study on Ageing. *Ophthalmic Res*. 2010;44:131-139.

Publications and presentations

First author publications

Sabour-Pickett S, Nolan JM, Loughman J, Beatty S. **A review of the evidence germane to the putative protective role of the macular carotenoids for age-related macular degeneration.** *Mol Nutr Food Res*. 2011:10

A manuscript entitled, "**Visual performance in patients with neovascular age-related macular degeneration undergoing treatment with intravitreal ranibizumab**", is currently under peer-review.

A manuscript entitled, "**Supplementation with three different macular carotenoid formulations in patients with early age-related macular degeneration**", is currently under peer review.

A Book Chapter entitled "**Clinical trials investigating the macular carotenoids**" is currently under editorial review.

Oral presentations

1. "**Visual performance in patients with neovascular age-related macular degeneration undergoing treatment with intravitreal ranibizumab.**" *Ophthalmologists' Conference. Whitfield Clinic, Waterford; January 2012.*
2. "**Prognostic indicators and outcome measures in patients neovascular age-related macular degeneration undergoing treatment with intravitreal ranibizumab.**" *European Academy of Optometry and Optics conference, Dublin; April 2012*
3. "**The role of Macular Pigment in age-related macular degeneration and for visual performance.**" *Boots Opticians educational event, Birmingham, UK; March 21st 2012.*

Lectured at additional accredited educational events for Optometrists and General Practitioners at Whitfield clinic, Waterford, during my postgraduate study.

Poster presentations

1. **A review of the evidence germane to the putative protective role of the macular carotenoids for age-related macular degeneration.**

   Sabour-Pickett S, Nolan JM, Loughman J, Beatty S. *International Macular Carotenoids conference, Cambridge, UK; July 2011.*

2. **Prognostic indicators and outcome measures for patients with neovascular age-related macular degeneration undergoing treatment with intravitreal ranibizumab.**

   Sabour-Pickett S, Loughman J, Nolan JM, Stack J, Pesudovs K, Beatty S. *DIT research symposium, Dublin, Ireland; November 2011.*

3. **Prognostic indicators and outcome measures for patients with neovascular age-related macular degeneration undergoing treatment with intravitreal ranibizumab.**

   Sabour-Pickett S, Loughman J, Nolan JM, Stack J, Pesudovs K, Beatty S. *ARVO , Fort Lauderdale, Florida, USA; May 2012.*

4. **Supplementation with three different macular carotenoid formulations in patients with early age-related macular degeneration.**

   Nolan JM, Connolly E, **Sabour-Pickett S**, Loughman J, Howard A, Beatty S. *ARVO , Fort Lauderdale, Florida, USA; May 2012.*

Appendices