Visual Function in Aging and Age-Related Macular Degeneration Including Subretinal Drusenoid Deposits

Manjot Kaur Grewal

University College London
Supervised by:
Prof. Sobha Sivaprasad
Prof. Glen Jeffery

This dissertation is submitted for the degree of
Doctor of Philosophy

Declaration

I, Manjot Kaur Grewal confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Acknowledgements

I would like to express my sincere gratitude to my supervisors Professor Sobha Sivaprasad and Professor Glen Jeffery for their guidance and encouragement which they generously gave me throughout these past four years.

I am grateful to Professor Sobha Sivaprasad for her mentorship, inexhaustible patience and continuous motivation for this work.

A huge thank you to everyone at the NIHR Moorfields Clinical Research Facility (CRF) for all of the hard work you have contributed to this PhD. In particular, I would like to thank Dr Piyali Sen, Dr Deepthy Menon, Dr Shruti Chandra, nurses April, Aldrich, Nafeesa and Jenny, and the wonderful admin staff Julie and Sarah. I would also like to extend my gratitude to all of the participants that agreed to take part in my studies.

A heartfelt thanks to my colleagues in the lab, Dr Jaimie Hoh Kam, Harpreet Sangha and Tobias Weinrich who have been a constant source of encouragement and friendship throughout the occasionally stressful, but overall memorable past 4 years.

Finally, I would like to express my love and gratitude to my parents, especially my mother Tejinder for her enduring support and encouragement. You have always believed in me, for which I will forever be grateful. To my siblings, Harsharn, Sukhmani and Manvir for their continuous optimism and encouragement. To my husband Amar, who has encouraged me during my studious endeavours for the past 15 years, thank you. Lastly, to my daughter Mahi, who has brought along so much love and happiness in our lives.

Abstract

Age-related macular degeneration (AMD) is the leading cause of visual impairment in the developed world among people over 50 years of age. Although AMD is clinically characterised by the presence of drusen, subretinal drusenoid deposits (SDD) have also been recognized as a distinct morphological feature that confers increased risk of developing advanced AMD. To date, there has been a lack of validated biomarkers that can capture early changes in visual function that strongly correlate to the anatomical alterations which also include SDD phenotype.

This thesis aimed to explore functional and structural markers to differentiate between healthy eyes (n=11) and intermediate AMD (iAMD) with SDD (n=11) and without SDD (n=17) and non-foveal atrophic AMD (n=11). Firstly, I assessed scotopic thresholds using a novel dark-adapted chromatic (DAC) perimeter, in healthy aging and in varying AMD disease. Individuals with SDD had depressed retinal sensitivity centrally, particularly inferiorly and nasally. Functionally, eyes with SDD were comparable to eyes with non-foveal atrophy, but structurally differed in outer nuclear layer (ONL) and total retinal volumes and thicknesses. Importantly, only rod-mediated tests were able to distinguish iAMD with and without SDD.

Another aim of this thesis was to explore the efficacy of 670nm light on aging and AMD. Although an improvement in scotopic thresholds was observed in healthy aged eyes (n=4) compared to younger eyes (n=5), a pilot study conducted in 40 participants over the age 55 years (12 control, 28 with intermediate AMD) refuted any clinical benefit.

In conclusion, this thesis supports the need to re-classify the AMD severity scale by incorporating eyes with SDD as a separate group. This phenotype should be sub-analysed in clinical trials evaluating potential prophylactic agents to delay the progression. Scotopic sensitivity offers diagnostic value, but rod intercept time offers both prognostic and diagnostic value as candidate biomarkers.

Impact statement

Age-related macular degeneration (AMD) is the leading cause of visual impairment in the developed world among people over 50 years of age and can lead to irreversible loss of central vision due to either extensive atrophy or scarring subsequent to a neovascular lesion. This has detrimental impact on the individual's quality of life and independence, and is becoming a growing public health care concern and socio-economic burden. In dry AMD, the anatomical changes are slow to develop and progress and as such makes it challenging to understand the exact causes of a switch from aging to AMD. Clinical trials in AMD tend to fail partly because of unreliable endpoints that are unable to monitor subtle changes. In this thesis, I demonstrate that participants with iAMD can be functionally stratified into those with drusen only and those with drusen and SDD. Additionally, I show that the standard dark adaptometry protocol is able to detect disease progression in iAMD without SDD.

This thesis also shows that patients with SDD lesions secondary to AMD have worse visual function compared to those without but not dissimilar to those who have already developed non-foveal atrophy. This is indicative of different functional pathophysiology and therefore, this work supports the need for re-classification of AMD incorporating and defining SDD phenotype. This has implications in future clinical trials as patients with this phenotype may respond differently to a treatment and may even require different targeted therapies. Furthermore, the magnitude of 670nm light treatment effect on aging was explored. I demonstrate in a pilot study that daily exposure of 670nm red-light therapy for two minutes daily has modest improvement in scotopic sensitivity in healthy older individuals when compared to younger participants. However, functionally, there was no visual gain in participants with AMD over a course of 12 months.

Therefore, this thesis has implications for both AMD patients and the general aging population as well as for researchers for the development and design of future AMD clinical trials evaluating potential prophylactic agents.

Table of Contents

List of Figures

List of Tables

List of abbreviations

AMD	Age-related macular degeneration
ATP	Adenosine triphosphate
ARMS	Age-related maculopathy susceptibility protein 2
BCVA	Best-corrected visual acuity
CRA	Central retinal artery
CT	Choroidal thickness
CFH	Complement factor H
cGMP	Cyclic guanosine monophosphate
COX	Cytochrome oxidase c
DA	Dark adaptation
ETDRS	Early Treatment Diabetic Retinopathy Study
ERG	Electroretinogram
ELM	External limiting membrane
ffERG	Full field electroretinograms
GA	Geographic atrophy
GDP	Guanosine diphosphate
HTRA-1	High-temperature requirement A serine peptidase 1
HIF	Hypoxia-inducible factors
IRBP	Interphotoreceptor retinoid-binding protein
LLD	Low luminance deficit
LLQ	Low luminance questionnaire
LLVA	Low luminance visual acuity
MAC	Membrane attack complex
mtDNA	Mitochondrial DNA
NIR	Near Infra-red
nAMD	Neovascular AMD
OCT	Optical coherence tomography
ONL	Outer Nuclear Layer
PDE	Phosphodiesterase
PED	Pigment epithelial detachment
PBM	Photobiomodulation
PUFA	Polyunsaturated fatty acids
ROS	Reactive oxygen species
RGC	Retinal ganglion cell
RNFL	Retinal Nerve Fibre Layer
RPE-BM	Retinal pigment epithelium - Bruch's membrane
RPE	Retinal pigment epithelium
RPEDC	Retinal pigment epithelium-drusen complex
RIT	Rod intercept time
SDD	Subretinal drusenoid deposit
TRT	Total retinal thickness
TRV	Total retinal volume

Chapter 1. Introduction

This introductory chapter begins by providing an overview of normal retinal structure and function, retinal physiology and how these are altered in aging and AMD. This is followed by a description of the risk factors, pathophysiology, classification, treatment and investigation of AMD. The latter part of the chapter provides an overview of the emerging field of photobiomodulation and how this treatment may benefit retinal function. This chapter concludes with an outline of the specific aims of this thesis.

1.1. Overview of normal retinal structure and function

The eye consists of three coats enclosing the transparent refractive media. The outermost coat consisting mostly of collagen fibres, contains the sclera and the cornea. The middle layer is vascular and pigmented, also knowns as the uvea, encompasses the choroid, iris and the ciliary body (Snell and Lemp, 2013). Lastly, the innermost layer consists of the retina which is the light-sensitive layer covering approximately 65% of the eye's interior surface, situated between the choroid and the vitreous humour (Oyster, 2006). It is the most complex structure of the eye and acts as the primary stage of the visual processing pathway (Oyster, 2006). As the light enters the eye through the cornea and falls on the retina, electrical impulses are generated by the photoreceptors which are modified, integrated, and relayed to the retinal ganglion cells (RGC) by various interneurons. These signals are then transmitted to the brain via the optic nerve.

Structurally, the human retina under a light microscope appears to be defined by 10 different layers. The basic organisation of principal retinal neurons is shown in Figure 1.1. (Kolb, 2013). All vertebrate retinae have an outer pigmented layer, three nuclear layers housing neuronal cell bodies (outer, inner and ganglion), and two layers of synaptic connections also termed plexiform layers (Kolb, 2013). The outer nuclear layer contains cell bodies of the photoreceptors.

Proximal to the outer plexiform layer is the inner nuclear layer containing the cell bodies of bipolar, horizontal and amacrine cells which convey information from the photoreceptors to retinal ganglion cells (RGCs). Lastly, the ganglion cell layer consists of ganglion cell bodies. The projected axons from RGCs running across the inner surface of the retina form the nerve fibre layer and converge at the optic nerve (Snell and Lemp, 2013). The outer plexiform layer is the area where the neural impulse from photoreceptors at cone pedicles and rod spherules is transmitted to bipolar and horizontal cells via synaptic connections. In the inner plexiform layer, bipolar cells axons synapse with the dendrites of ganglion cells whilst amacrine cells modulate neural impulse between the bipolar cells and ganglion cells (Kolb, 2013).

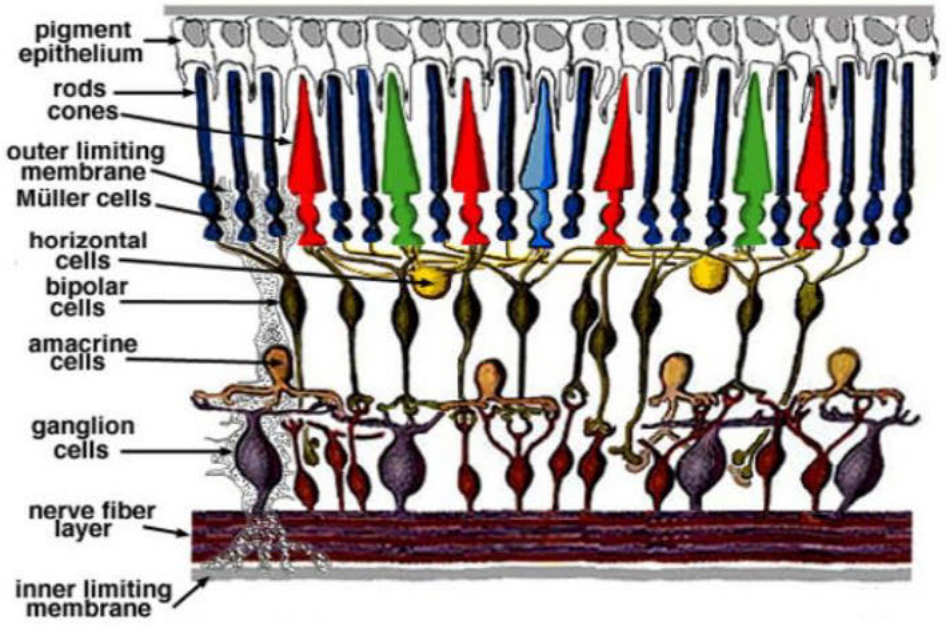

Figure 1.1.Schematic representation of the main retinal neurons (Kolb, 2011).

1.1.1. Blood supply to the retina and the choroid

The retina is supplied by dual vasculature; the inner retina is supplied by the inner circulation formed by the central retinal artery (CRA) whilst the outer retina is nourished by vasculature external to the pigment epithelium, the choroidal capillaries. Both vasculatures originate from the ophthalmic artery which is the first branch of internal carotid artery (Hayreh, 2006).

The CRA receives 20-30% of the total ocular blood flow while the choroidal vasculature accounts for 65-85% of the blood supply (Kolb, 2013; Henkind, 1979).

The CRA enters the eye through the optic disc and immediately divides into superior and inferior branches (Hayreh, 1962). These branches further bifurcate into nasal and temporal divisions within the nerve fibre layer below the internal limiting membrane (Snell and Lemp, 2013). Continued bifurcation and branching of retinal vessels form a diffuse network of capillaries located within the nerve fibre layer or ganglion cell layer (superficial capillary network) or within the inner nuclear layer (deep network) (Iwasaki and Inomata, 1986). These capillaries are lined with unfenestrated endothelial cells surrounded by endothelial basement membrane and pericytes (Snell and Lemp, 2013). The inner retinal circulation does not anastomose with other vascular system which leaves the inner retina vulnerable to ischemia in an event of an artery occlusion causing retinal infarction (Hayreh, 1975). However, the presence of a cilioretinal artery derived from choroidal vessels can spare the central retina in 15-30% of the population (Lorentzen, 1970).

Choroidal circulation nourishes the overlying outer retina to a depth of around 130µm (Hayreh, 1975). Choroidal vasculature feeds off short posterior ciliary arteries. These enter through the sclera proximal to the optic nerve and anastomose with branches of long posterior ciliary arteries, anterior ciliary arteries, and major arterial circle of the iris (Snell & Lemp, 2013). The choroid is a vascular layer situated between the sclera and the Bruch's membrane and is anatomically divided into three layers. From the sclera towards the Bruch's membrane, these are known as Haller's layer, Sattler's layer and the choriocapillaris. Haller's layer contains the largest blood vessels which decrease in diameter towards the choriocapillaris (Hayreh, 1975). The thin layer of choriocapillaris apposed to Bruch's membrane, forms a dense network of fenestrated capillaries making it permeable to the metabolites which are supplied to the outer retina. Choriocapillaris also nourishes the avascular area of the macula, where it is found at its greatest density (Nickla and Wallman, 2010). In addition to nourishing the retina, the choroid is also responsible for thermoregulation, focusing the eye and secretion of growth factors (Nickla and Wallman, 2010).

1.1.2. Bruch's membrane

Although not considered to be a part of the retina itself, Bruch's membrane is a pentalaminar layer providing structural support and anchorage to the retinal pigment epithelium (RPE) and acts as a barrier between the fenestrated choroidal capillaries and the metabolically active photoreceptors and RPE (Guymer, Luthert and Bird, 1999; Booij *et al.*, 2010). It consists of (from the choroid towards the RPE); 1) choriocapillaris basal lamina 2) outer collagenous layer 3) elastin layer 4) inner collagenous layer and 5) RPE basal lamina (Booij *et al.*, 2010). Together, the collagen and elastic fibres form a sieve-like structure, facilitating passive transport of biomolecules between choriocapillaris and the RPE (Booij *et al.*, 2010). It allows nutrients, oxygen and retinoids from the choroidal circulation to the outer retina and collects metabolic waste from the photoreceptors and RPE towards the choroid (Booij *et al.*, 2010). Any disturbance in the structure or composition in this layer may hinder its diffusion capabilities affecting the normal function of the RPE and photoreceptors (Guymer, Luthert and Bird, 1999).

1.1.3. Retinal Pigment Epithelium

The RPE is a single cuboidal layer forming a barrier between the neural retina and choriocapillaris. It consists of an average of between 3 and 4 million hexagonal cells organised in a regular pattern (Panda-Jonas, Jonas and Jakobczyk-Zmija, 1996). The cells which are columnar in the macula, become larger and less regular with increasing eccentricity (Boulton and Dayhaw-Barker, 2001). The polarised RPE cells are densely packed with pigmented granules called melanosomes which are critical in absorbing excess light (Bhutto and Lutty, 2012). The cell cytoplasm also contains abundant smooth endoplasmic reticulum and lysosomes dispersed throughout the cell, whilst mitochondria are concentrated towards the basal side of the cell (Boulton and Dayhaw-Barker, 2001). The apical surface of the RPE is covered with numerous microvilli which interdigitate with the photoreceptor outer segments (Bonilha *et al.*, 2006). Laterally, the RPE cells membranes are joined to adjacent cells via tight junctions (zonulae occludentes) forming the outer blood-retinal barrier between the choroid and neural retina (Cunha-Vaz, Bernardes and Lobo, 2011).

Additionally, these cells are metabolically interconnected to neighbouring cells through gap junctions (Stalmans and Himpens, 1997). Collectively, this morphology and all the organelles contained within the RPE are responsible for maintaining the many functions of this layer which are illustrated in Figure 1.2 (Strauss, 2005). RPE cells play a critical role in supporting photoreceptors by phagocytosis of the tips of shed photoreceptor outer segments, regeneration of visual pigment and facilitation of transepithelial transport of metabolites between the choroid and photoreceptors (Strauss, 2005). Furthermore, RPE cells secrete growth factors required for choroidal and retinal maintenance and are partly responsible for spatial buffering of ions in the subretinal space (Strauss, 2005).

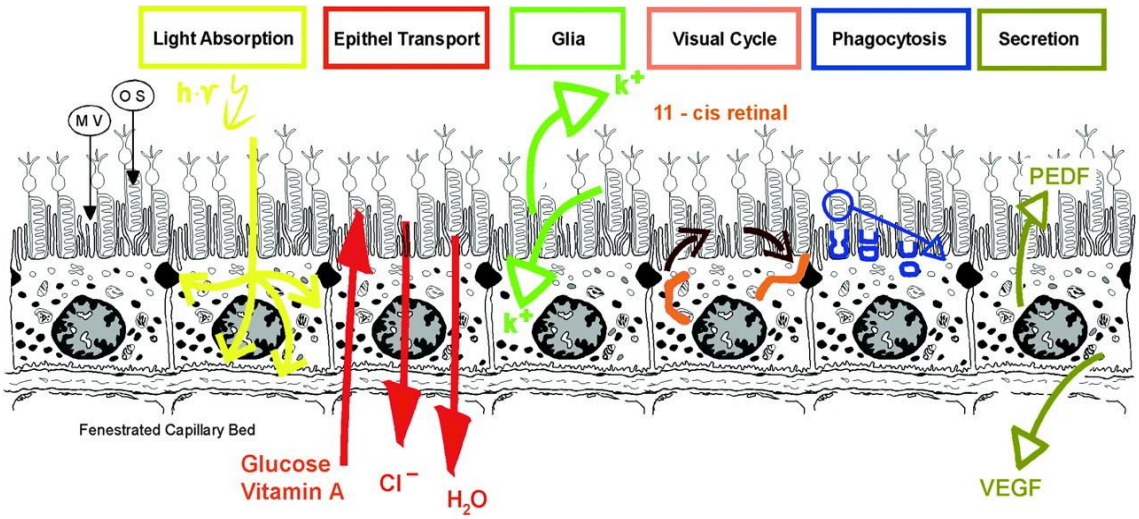

Figure 1.2. Schematic diagram of various retinal pigment epithelium (RPE) functions (from Strauss, 2005).

1.1.4. Photoreceptors

Humans have a duplex retina, with two specialised neurons capable of performing phototransduction known as rod and cone photoreceptors. These are highly specialised and polarised cells which convert light energy into a neuronal impulse, initiating the visual processing pathway (Bhutto and Lutty, 2012). They are distinguished by their shape, retinal distribution and their photopigment content (Mustafi, Engel and Palczewski, 2009) .

The human retina houses approximately 4.6 million cones with a peak density of approximately 199 000 cones/mm^2 at the fovea (Curcio *et al.*, 1990). Cone density decreases steeply with increasing retinal eccentricity as shown in Figure 1.3. Cones operate under photopic (day light; above 0.03 cd/m^2) conditions, requiring over a hundred photons to generate a response and are responsible for high acuity vision at the expense of sensitivity (Purves *et al.,* 2001; Kolb, 2011). Humans have three subtypes of cone photoreceptors; red (L cones), green (M cones) and blue (S cones) distinguished by their response to different peak wavelengths; at 563nm, 534nm and 420 respectively (Bowmaker and Dartnall, 1980). Collectively, these form the basis of colour vision.

By contrast, there are 92 million rod photoreceptors dominating the peripheral retina and absent from the fovea (Curcio *et al.*, 1990). Rod density peaks at approximately 18 degrees eccentricity, slowly decreasing towards the far-peripheral retina (Osterberg, 1935; Kolb, 2013). These cells are highly sensitive to light, having the ability to respond to a single photon of light, albeit at the expense of spatial resolution (Purves *et al.,* 2001). They provide maximum sensitivity in scotopic (night) conditions and have a spectral sensitivity peaking at 498nm (Bowmaker and Dartnall, 1980). When both rod and cone photoreceptors are active in low but not quite dark lighting conditions is termed mesopic vision (Kolb, 2011).

Both photoreceptors share similar micro-anatomy, consisting of outer segments, connecting cilium, inner segments, cell nucleus and a synaptic ending (Mustafi, Engel and Palczewski, 2009). However, the outer segment morphology differs between the photoreceptors; the rods have longer and slender outer segments whilst the cone outer segments are typically conical in shape (Mustafi, Engel and Palczewski, 2009). The outer segments contain stacks of membranous discs, embedded with visual pigment known as rhodopsin in the rods and iodopsins in the cones. These discs are free floating in the rods whilst in the cones these remain attached to the surrounding cell membrane (Young, 1967; Anderson, Fisher and Steinberg, 1978). As the new discs are produced at the base of the outer segments, the old ones are displaced to the tip where they are shed to be phagocytised by the RPE (Young and Bok, 1969).

The inner segments are rich in mitochondria (in the ellipsoid portion) and provide the metabolic machinery required for the ionic pumps fundamental to the dark current described in section 1.2.3. (Eells, 2019). The myoid portion of the inner segments contain the machinery required for protein synthesis (Hoang *et al.*, 2002). The main features of photoreceptors are illustrated in Figure 1.4.

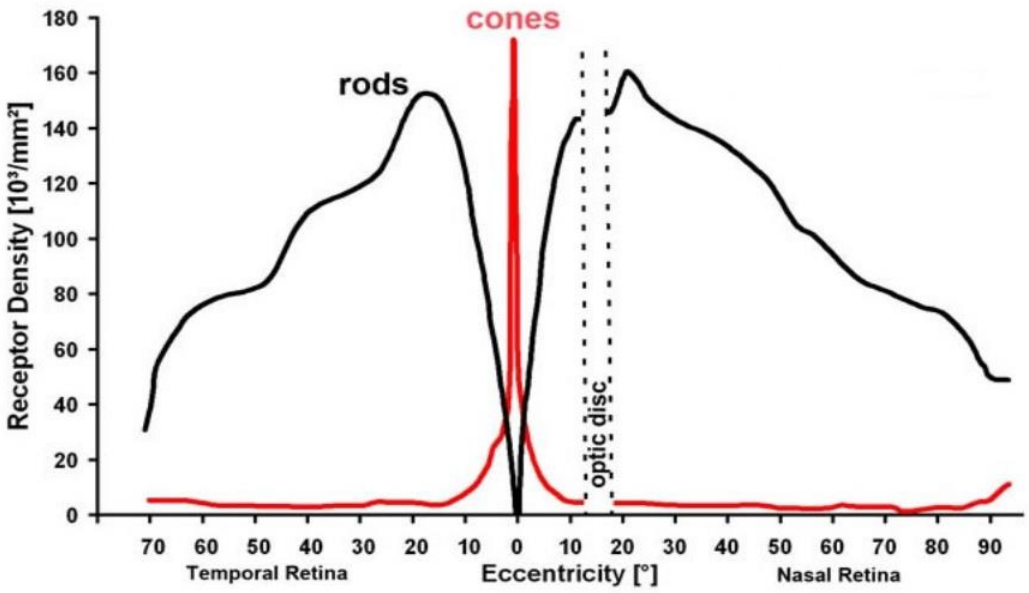

Figure 1.3. A graph displaying rod and cone photoreceptor densities across the horizontal meridian (Kolb et al., 2013; from http://webvision.med.utah.edu/).

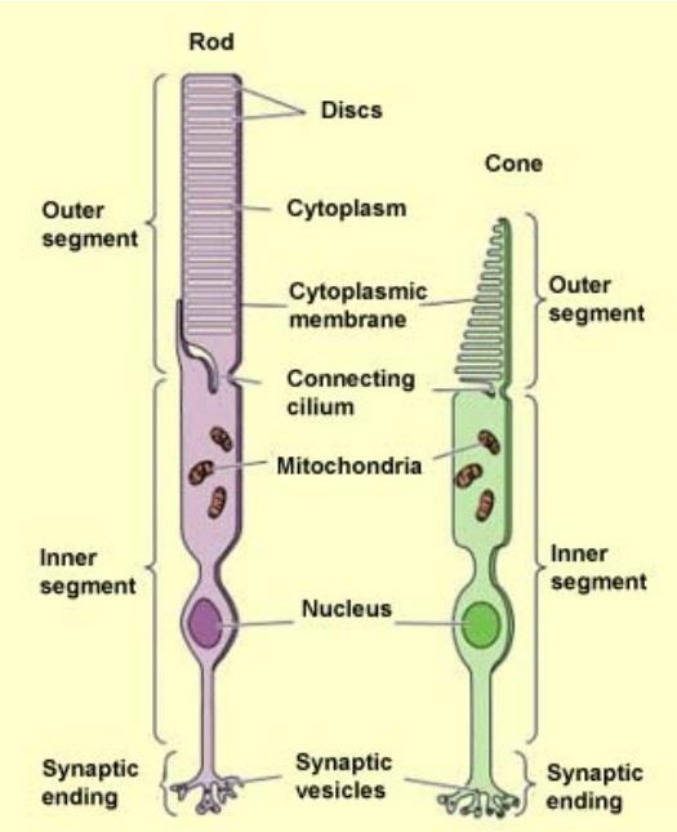

Figure 1.4. Schematic diagram of rod and cone photoreceptor structure (Bruno Dubuc, copyright permission from http://thebrain.mcgill.ca).

1.1.5. Macula

The macula is clinically defined as the central region of the retina spanning 6mm in diameter, centred on the fovea and responsible for 21.5° visual angle (Curcio *et al.*, 1990). It is located within the major temporal vascular arcades and consists of the perifovea, parafovea, and fovea which appears darker than the surrounding retina (Tsang and Sharma, 2018; Snell and Lemp, 2013). Within the fovea is the foveal pit, an area with the highest cone density devoid of rods and S-cones, with maximum resolving power. This depression is formed by the lateral displacement of the interneurons and inner retinal layers leaving only the photoreceptors in the middle (Snell and Lemp 2013). In addition to the photoreceptors, the parafovea consists of bipolar and ganglion cells, displaced from the foveal pit. Surrounding these regions is the perifovea which eventually merges with the peripheral retina (Tsang and Sharma, 2018).

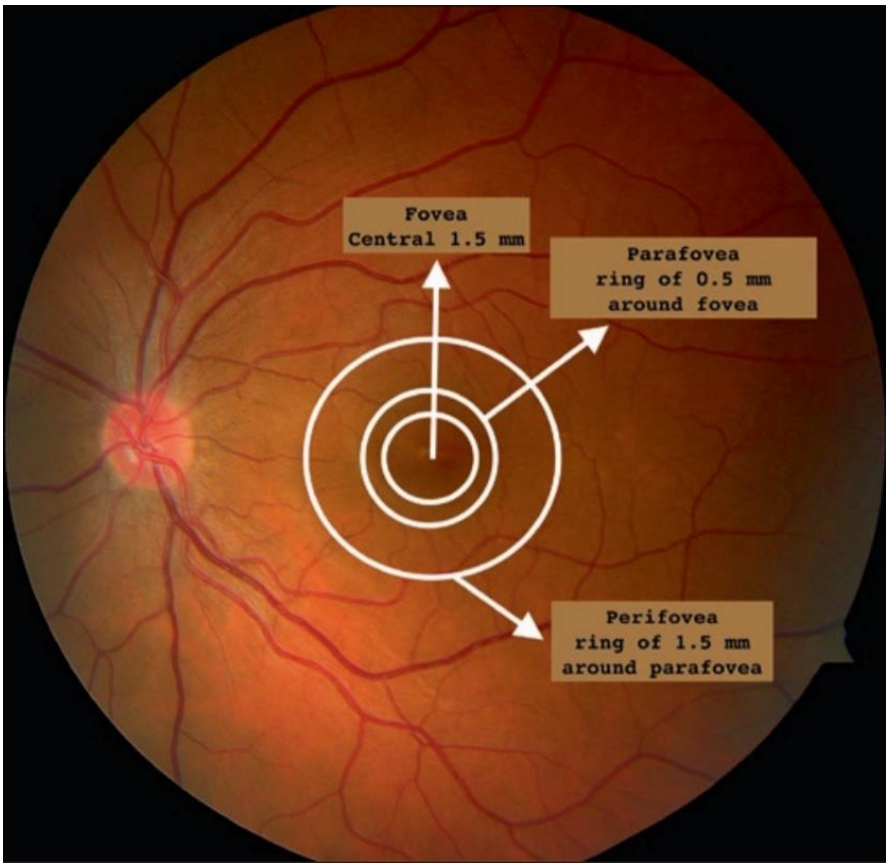

Figure 1.5. Anatomical landmarks of the macula. The macular regions are circled with corresponding typical measurements of diameter (Tsang and Sharma 2018; copyright permission from Springer Nature [2021] license 4981130113743).

1.2. Retinal physiology

Light under photopic and scotopic conditions initiates specific changes in physiological processing in the retina. Visual perception is initiated through the activation of the visual pigment in the outer segment discs by absorbing photons. Photochemical changes associated with rising light levels are termed as light adaptation whilst decreases in illumination are called dark adaptation.

1.2.1. Phototransduction

The conversion of light (photons) into electric signals, known as phototransduction, involves a chain of photochemical reactions (Wang and Kefalov, 2011). The process is summarised as follows. Firstly, a photon is absorbed by the visual pigment.

Upon the capture of this light energy, the retinal chromophore bound to the visual pigment undergoes structural alteration from 11-cis retinal to an all-trans retinal configuration known as photoisomerization (Wong-Riley, 2010). The opsin which is unable to bind to all -trans retinal, converts to active form of metarhodopsin II leading to the dissociation of the chromophore from the opsin (Lamb and Pugh, 2004, 2006). Then, the freed activated opsin binds to transducin already coupled with guanosine diphosphate (GDP). The activated transducin-GTP complex activates phosphodiesterase (PDE), a protein responsible for breaking down cyclic guanosine monophosphate (cGMP). The decreasing concentration of cGMP causes photoreceptor sodium ion (Na^+) channels to close, reducing the Na+ current and resulting in hyperpolarisation of photoreceptors (Wong-Riley, 2010). As the neurotransmitter release is reduced at the photoreceptor synaptic end, physiological nerve signal is produced and propagated down the visual pathway (Lamb and Pugh, 2004, 2006).

1.2.2. Regeneration of the visual pigment

The regeneration of the visual pigment to its original state is achieved through a series of biochemical processes known as the visual cycle. This is initiated where all-trans retinal is split from opsin and is reduced by retinol dehydrogenase to all-trans-retinol. All-trans retinol is then transferred to the RPE through photoreceptor outer segments by the interphotoreceptor binding protein (IRBP) and transformed into all-trans retinyl via the addition of an ester bond leading to isomerization of 11-cis retinol by RPE65. There, it is converted back 11-cis retinal and diffused back to the photoreceptor by IRBP. Once it has reached the outer segment, visual pigment is regenerated as 11-cis retinal and recombines with rhodopsin (Lamb and Pugh, 2004, 2006; Fu, 2018).

In cones, an additional pathway exists for pigment regeneration where all-trans retinol can be transported from the outer segments to Müller cells located within the inner retina, to be isomerized to 11-cis retinol (Wang and Kefalov, 2011). Following this, 11-cis retinol is delivered back to the inner segment to be oxidised to 11-cis retinal before transferring back to the outer segment to be coupled with opsin to form the visual pigment (Wang and Kefalov, 2011).

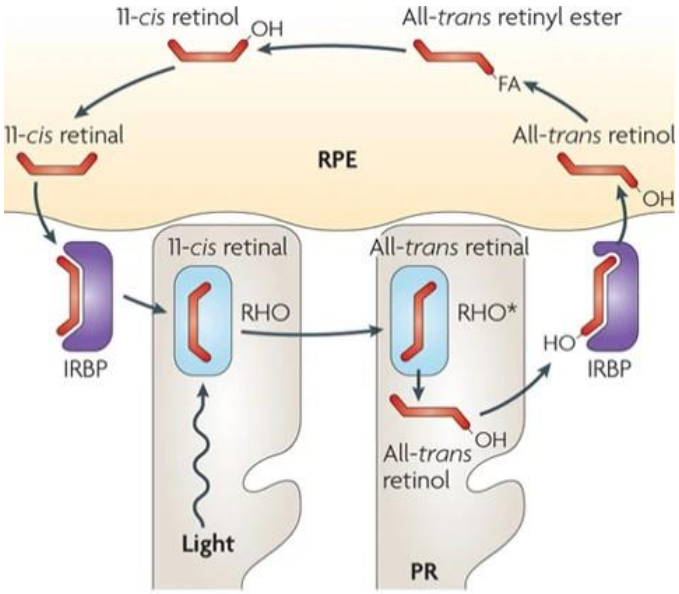

Figure 1.6. Phototransduction in rod photoreceptor. RHO = Rhodopsin, IRBP = Interphotoreceptor retinoid-binding protein (Wright et al., 2010; copyright permission from Elsevier [2020] licence 4978851407359).

1.2.3. The dark current

The current flowing between the inner and outer segments of the photoreceptors under scotopic conditions is known as the dark current (Hagins, Penn and Yoshikami, 1970). In the dark, cGMP-gated cation channels remain open allowing the influx of Na^+ to enter and travel to the inner segment where they are actively pumped out by ATP dependant Na+/K+ pumps (Hagins, Penn and Yoshikami, 1970). This process is metabolically demanding with an estimated 50% of mitochondrial ATP generated by the photoreceptors used to maintain the dark current through these pumps (Wong-Riley, 2010). This dark current leads to depolarization which causes the release of glutamate from photoreceptors and finally resulting in neurotransmission to synapsing bipolar cells (Wong-Riley, 2010; Reuter, 2011). Conversely, in photopic conditions, the cation channels close causing hyperpolarization of photoreceptors, leading to a reduced release of glutamate from photoreceptor synaptic terminal (Hagins, Penn and Yoshikami, 1970; Reuter, 2011).

1.3. The aging outer retina and choroid

Aging is a complex and multifactorial process which remains poorly understood. Many theories have attempted to clarify this progression, which are broadly categorised as: 1) programmed theories, where aging follows a tightly regulated biological timetable leading to progressive alteration in gene expression impacting biological maintenance, repair, and defence mechanisms, or 2) cumulative damage or errors theories, where environmental assaults accumulate over time thus inducing aging (Jin, 2010). Broadly, these include but are not limited to general wear and tear of cellular machinery (Jin, 2010), accumulation of cross-linked proteins slowing down physiological processes (Brys, Vanfleteren and Braeckman, 2007), build-up of DNA damage leading to progressive genetic mutations (Gensler and Bernstein, 1981) and accumulation of free radicals causing oxidative damage (Harman, 1956). The retina is not immune to aging and undergoes various physiological and structural changes. Here, the age-related changes in the choroid, Bruch's membrane, RPE and photoreceptors are described.

1.3.1. Aging of choroid

With age, the choroid decreases in thickness by up to 57% and contains fewer blood vessels. The remaining choroidal vessels also have narrower diameters, culminating to a 34% reduction of the choriocapillaris (Ramrattan *et al.* 1994). As a result, the choroidal blood volume and flow also decrease, but the choroidal blood velocity remains stable (Grunwald, Hariprasad and Dupont, 1998). In addition to the structural changes of the choroid itself, it has been suggested that the drop in choroidal and retinal blood flow may also be a consequence of reduced metabolic demand from the retina due to photoreceptor cell loss and a decrease in RPE cell volume that occurs with normal aging (Grunwald, Hariprasad and Dupont, 1998).

1.3.2. Aging of Bruch's membrane

Bruch's membrane thickens with age by 2.7µm, representing a 135% increase (Ramrattan *et al.*, 1994). Loss of hydraulic conductivity, accumulation of lipids within the inner collagenous layer and cross-linking of collagen fibres all contribute to this thickening (Booij *et al.*, 2010; Curcio *et al.*, 2011). These alterations eventually lead to the loss of elasticity and impeded permeability of water across Bruch's membrane (Booij *et al.*, 2010; Nickla and Wallman, 2010). Together with changes to choroidal blood flow, these layers have the potential to curb the normal provision of oxygen and metabolites to the outer retina and disrupt the removal of waste products.

1.3.3. Aging of the RPE

With aging, the RPE cell density decreases at a rate of 0.3% per year (Panda-Jonas, Jonas and Jakobczyk-Zmija, 1996). The cells become larger and less regular in shape. Functionally, aging RPE cells also lose melanin granules, lessening light absorption capacity and increasing the possibility of phototoxicity (Boulton and Dayhaw-Barker, 2001). These cells also continuously accumulate lipofuscin granules which consist of lipid and proteins from incomplete degradation of phagocytosed outer segment discs (Boulton *et al.*, 1989; Boulton and Dayhaw-Barker, 2001). These granules occupy only 1% of the cytoplasmic volume in the first decade of life, increasing to 19% by the age of 80 (Feeney-Burns, Hilderbrand and Eldridge, 1984). Importantly, accumulation of lipofuscin has been associated with RPE dysfunction and photoreceptor death (Dorey *et al.*, 1989).

1.3.4. Aging of photoreceptors

Histopathological studies have shown that rods are more vulnerable to deterioration in aging than cones (Gao and Hollyfield, 1992; Curcio *et al.*, 1993). Curcio et al. (1993) analysed 24 eyes aged between 27 and 90 years and measured photoreceptor spatial densities within the central 43 degrees of the retina. They reported a 30% decrease in rod density within the central 28.5-30 degrees of vision whilst cones appeared to be unaffected (Curcio *et al.*, 1993).

Spatially, the outer segment diameter of remaining rods enlarge, filling in the gaps from dying rods (Curcio *et al.*, 1993).

1.4. Age-related macular degeneration

Age-related macular degeneration (AMD) is the leading cause of irreversible central vision loss in the elderly in the developed world (Klein *et al.,* 2013, Lim *et al.,* 2012), typically over the age of 55 (Klein *et al.,* 2006). It is the third commonest cause of blindness worldwide following cataracts and glaucoma (Pascolini and Mariotti, 2012). An estimated 196 million people are affected by this disease, which is projected to increase to 288 million in 2040 (Wong *et al.*, 2014). AMD can be clinically classified by disease severity as early, intermediate or late AMD. There is little impact on visual acuity (VA) in the first two stages. Late AMD can either be of dry form, also known as geographic atrophy (GA) which results in slow progressive loss of photoreceptors and subsequently central vision loss, or wet form, referred to as neovascular or exudative AMD, which can lead to a rapid deterioration of central vision if left untreated. Atrophic AMD accounts for 85-90% of the cases whereas wet AMD accounts for 10-15% (Bhutto and Lutty, 2012).

In England, approximately 49% of sight impaired certifications in 2011 to 2012 were due to AMD, of which 53% were from GA, 31% from wet AMD and 16% from other subtypes of AMD (Bunce *et al.*, 2015). Approximately 1.5 million people are estimated to suffer from AMD disease in the UK (Owen *et al.*, 2012). Due to aging population, the prevalence of AMD is projected to increase globally and poses a significant public health burden for the cost of management, treatment and provision of social care (Owen *et al.*, 2012).

1.4.1. Risk factors

AMD is a complex disease with a multifactorial aetiology. Many risk factors have been associated with the development and progression of AMD. These can be categorised as 1) non-modifiable risk factors such as age, ethnicity, gender and genetic makeup, or 2) modifiable risk factors including smoking, obesity, diet, hypertension, hyperlipidaemia and light exposure.

1.4.1.1. Age

Age has been widely recognized as the greatest non-modifiable risk factor for the prevalence and progression of all AMD types (Klein, Klein and Linton, 1992; Age Related Eye Disease Study Group (AREDS), 2000; Buch *et al.*, 2005; Owen *et al.*, 2012; Rudnicka *et al.*, 2012). In a meta-analysis study of AMD in Europe, Owen et al. (2012) reported the increasing prevalence of late AMD normalised to the UK population, from 2.4% in those aged 50 years of more to 4.8% in 65-year-olds or more and 12.2% for those over the age of 80. Similarly, the estimated prevalence of late AMD was reported to increase from 0% in those under the age of 55 to 18.5% in those 85 years or older in the Blue Mountains Eye Study (Mitchell *et al.*, 1995). Therefore, increasing age remains a strong and consistent risk factor for AMD (Chakravarthy *et al.*, 2010).

1.4.1.2. Gender

There has been inconsistent evidence in the literature regarding the association of gender and the risk of developing AMD. Owen et al. (2012) reported higher incidence of neovascular AMD and GA cases in females compared to males. Retinal features such as intermediate drusen and pigmentary abnormalities were also found to be more common in females (Age Related Eye Disease Study Group (AREDS), 2000). Combined data from Blue Mountains Eye Study, the Beaver Dam Eye Study and the Rotterdam Study found an increased risk of early AMD in females compared to males, but not in late stages of the disease (Smith, Mitchell and Wang, 1997). Conversely, some studies described higher incidence of early AMD in men than women (Miyazaki *et al.*, 2005; Kawasaki *et al.*, 2008). A meta-analysis study, however, did not find any gender imbalance in the prevalence of early or late AMD (Wong *et al.*, 2014). Nevertheless, as women tend to live longer and AMD is strongly associated with age, it is difficult to draw unequivocal conclusions based on gender and risk of AMD. More studies are needed to elucidate the association of gender and AMD on the basis of gender alone, excluding the effect of age.

1.4.1.3. Ethnicity

Multiple studies have consistently reported higher prevalence of AMD in those with European ancestry than other ethnic groups (Friedman *et al.*, 1999; Bressler *et al.*, 2008; Varma *et al.*, 2010). The prevalence of large drusen and focal retinal pigment was found to be more common in white population compared with those of African ancestry (Friedman *et al.*, 1999). Similarly, the Salisbury Eye Evaluation study observed that these features were more commonly found in the central macular zone of white participants compared the black participants (Bressler *et al.*, 2008). While the prevalence of early AMD seems to be similar to white population, those of African ancestry, Asian and Hispanic ethnicities are less likely to develop late AMD (Friedman *et al.*, 1999; Chang *et al.*, 2008; Varma *et al.*, 2010; Vanderbeek *et al.*, 2011). A meta-analysis study also described higher prevalence of early AMD in those of European ancestry than those of Asian ancestry while those of African ancestry had lower prevalence of either early or late AMD compared to European ancestry (Wong *et al.*, 2014). One study in discordance with these findings is the multi-ethnic study of atherosclerosis (MESA) which did not report significant differences between the white, Asians (largely Chinese), Hispanics and black populations in the incidence of late AMD (Fernandez *et al.*, 2018). However, the authors discussed the limitation of only capturing a small proportion of AMD patients in their cohort. Although the cause of substantial disparity in the incidence of the disease among different ethnic groups remains elusive, it has been suggested that higher concentrations of melanin may offer a protective mechanism against AMD (Jampol and Tielsch, 1992). Genetic variation within different ethnic populations is also likely to influence the development of this disease (Klein, 2011).

1.4.1.4. Hypertension

Metelitsina and colleagues (2006) have noted decreased choroidal blood flow in AMD patients with systemic hypertension than those without, suggesting that hypertension may be linked to the pathogenesis of AMD, particularly neovascular AMD.

A strong association has been found between diastolic blood pressure and drusen (Vidaurri *et al.*, 1984) and neovascular AMD (Sperduto and Hiller, 1986). High systolic blood pressure has been associated with RPE hypopigmentation and neovascular disease (Klein *et al.*, 2003). The prevalence of AMD was also significantly associated with the duration of hypertensive disease (Sperduto and Hillier, 1986). Another study with conflicting results reported strong associations for AMD and systolic blood pressure and high pulse pressure but no association for diastolic BP (Van Leeuwen *et al.*, 2003). However, other studies did not find an association between AMD and hypertension such as the Beaver Dam Eye Study (Klein, Klein and Franke, 1993) and the Atherosclerosis Risk in Communities Study (Klein *et al.*, 1999).

1.4.1.5. Body Mass Index (BMI)

There has been conflicting evidence regarding the association of BMI and risk of AMD development. Higher body weight has been linked to increased oxidative stress and inflammatory processes, both of which have been suggested to be involved in AMD pathogenesis (Klein *et al.*, 2014; Haas *et al.*, 2015). Several studies have found an association between obesity and an increased risk of AMD (Klein *et al.*, 2003; Seddon *et al.*, 2003; Buch *et al.*, 2005). Among these, a study by Seddon and colleagues (2003) reported a statistically significant relative risk for AMD of 2.32 among overweight subjects and 2.35 in obese participants (Seddon *et al.*, 2003). Interestingly, another study found increased incidence of AMD not only with increasing BMI (rate ratio of 1.24 for overweight and 2.15 for obese), but also in lean men with a rate ratio of 1.24 (Schaumberg *et al.*, 2001). However, other studies were unable to demonstrate any association between BMI and AMD (Tan *et al.*, 2007; Mares *et al.*, 2011).

1.4.1.6. Diabetes

Several studies have investigated the relationship between diabetes and incidence of AMD, with varying results. The Blue Mountains Eye Study found a strong association with GA at baseline with odds ratio of 4.0 (95% CI, 1.6-10.3), but no correlation with early disease or with neovascular AMD with odd ratio of 1.0 (95% CI, 0.5-1.8) and 1.2 (95% CI, 0.4-3.5) respectively (Mitchell and Wang, 1999). These findings remained consistent at 10-year incident report where diabetes was reported to predict incident GA with a relative risk ratio of 3.89 (95% CI, 1.36-11.08) whereas no relationship was elicited with neovascular AMD (Tan *et al.*, 2007). Conversely, the EUREYE study found a positive relationship between diabetes and neovascular AMD as opposed to with GA (Topouzis *et al.*, 2009). However, no association was found between diabetes and AMD in other studies (Fraser-Bell *et al.*, 2008; Xu *et al.*, 2009).

1.4.1.7. Hypercholesterolemia

The relationship between serum cholesterol levels and AMD has also been explored and remains equivocal. Mares-Perlman and colleagues (1995) observed a positive association between the two; subjects with cholesterol intake in the highest quintile had 60% higher odds of early AMD compared with those in lowest quintile. Similar odds ratio did not reach statistical significance in subjects with late AMD (Mares-perlman *et al.*, 1995). A correlation with higher serum high-density lipoprotein (HDL) cholesterol and GA was found in the Beaver Dam Eye Study, with a risk ratio of 1.29 (95% CI, 1.05-1.58) (Klein *et al.*, 2003). Conversely, higher total and low-density lipoprotein (LDL) cholesterol were linked to increased risk of both GA and nAMD while higher HDL was found to be protective against the disease (Reynolds, Rosner and Seddon, 2010). However, others found no relationship with serum cholesterol and AMD (Smith *et al.*, 1998; Klein *et al.*, 1999). Lipid lowering agents were also not found to be beneficial against the development of AMD (McGwin *et al.*, 2006).

1.4.1.8. Smoking

Cigarette smoking is the strongest and most consistently described modifiable risk factor for AMD (Christen *et al.*, 1996; Seddon *et al.*, 1996; Chakravarthy *et al.*, 2010). It has been estimated that smokers are 2-3 times more likely to develop AMD than non-smokers (Thornton *et al.*, 2005) and risk developing the disease on average 10 years earlier than those who do not smoke (Mitchell *et al.*, 2002). Smoking cessation for 15 years has been found to decrease the risk of AMD (Seddon *et al.*, 1996), however it may take up to 20 years to return to the level of a person who has never smoked (Khan *et al.*, 2006). Although the mechanism underlying the relationship between smoking and AMD is not fully understood, smoking has been associated with the reduction of levels of serum antioxidants which could be extended to a decrease in retinal antioxidants (Evans, 2001). Smoking has also been suggested to reduce choroidal blood flow prompting hypoxia and ischemia (Solberg, Rosner and Belkin, 1998).

1.4.1.9. Light exposure

Sunlight exposure has been associated with increased risk of developing AMD (Taylor *et al.*, 1990; Mitchell, Smith and Wang, 1998). Lifetime exposure also increases the risk (Cruickshanks, Klein and Klein, 1993). Extended ultraviolet (UV) exposure may cause changes to the RPE similar to those found in AMD patients (West *et al.*, 1989). Given this, wearing of sunglasses and hats have been suggested to prevent UV light damage to the retina. However, other studies failed to find any relationship between the two (Age Related Eye Disease Study Group (AREDS), 2000). This inconsistency reflects the challenges in quantifying sunlight exposure and measuring the cumulative effect over time.

1.4.1.10. Genetics

The role of genetics in the development of AMD has been widely studied. A study by Hammond and colleagues (2002) with 506 twin pairs revealed that the presence of large drusen and 20 or more hard drusen were the most heritable phenotypes. A higher concordance of AMD in monozygotic twins (37%) compared to dizygotic twins (19%) was indicative genetic susceptibility (Hammond *et al.*, 2002). Currently, approximately 40 genes have been identified which may be implicated in the development of AMD (Lambert *et al.*, 2016). These are involved in many biological pathways including the complement system, cholesterol metabolism, neovascularization, retinal-specific function and oxidative stress (Lambert *et al.*, 2016). Genome-wide association studies (GWAS) have identified common risk variants, known as single nucleotide polymorphisms (SNPs), for AMD risk loci across various biological pathways however, approximately 57% of known variants to disease risk are from complement genes such as complement factor H (CFH), complement factor I (CFI), complement factor B (CFB), complement components such as C2 and C3 (Fritsche *et al.*, 2014). Specifically, CFHY402H polymorphism has been shown to be present in 50% of AMD patients (Edwards *et al.*, 2005). Polymorphisms of CFH and age-related maculopathy susceptibility 2 (ARMS2)/ HTRA-1 have the greatest odds ratios for developing AMD (Fritsche *et al.*, 2013).

Additionally, CFH risk variants were reported to be preferentially associated with geographic atrophy whereas ARMS2/HTRA1 risk variant has been shown to favour progression towards neovascular AMD (Seddon *et al.*, 2007; Sobrin *et al.*, 2011; Fritsche *et al.*, 2013, 2014).

1.4.2. Changes to outer retina and choroid in AMD

Structural changes to choroidal circulation, Bruch's membrane, RPE and photoreceptors have all been implicated in the development of AMD. Many of the alterations observed in these retinal layers can be considered of as an accelerated or exaggerated adaptation to aging.

Importantly, these layers are interdependent and any perturbation in one layer can adversely impact another. This section describes the main structural changes to the aforementioned layers.

1.4.2.1. Changes to the choroid in AMD

Decrease in choroidal thickness, density of choriocapillaris and narrower choroidal vessels occurs with normal aging (Ramrattan *et al.*, 1994) invariably affecting vascular output of the choroid (Grunwald *et al.,* 1998; Ravalico *et al.,* 1996). However, these deficits are more pronounced in AMD disease. Choriocapillaris density and choriocapillary diameter were reduced in eyes with basal laminar deposits, geographic atrophy and disciform scarring compared to normal aged maculae (Ramrattan *et al.*, 1994). Grunwald and colleagues (1998) measured choroidal blood flow, volume and velocity in 20 eyes with at least 10 large drusen and compared with 10 eyes of 10 age- and blood pressure-matched controls without large drusen. They reported a decrease of 37% in choroidal blood flow in AMD eyes attributed to a 33% reduction in choroidal volume as velocity was unaffected (Grunwald *et al.*, 1998). Additionally, choroidal perfusion abnormalities and delayed choroidal filling have been associated with AMD (Pauleikhoff *et al.*, 1990; Holz *et al.*, 1994; Ciulla *et al.*, 2002).

1.4.2.2. Changes to Bruch's membrane in AMD

The evident presence of drusen between Bruch's membrane and RPE which is the hallmark of AMD disease, along with the excessive accumulation of lipids, increased calcification of the elastic fibres and further basal diffuse depositions all contribute to its thickening (Curcio *et al*., 2011; Guymer *et al.,* 1999; Starita *et al.,* 1995; Booij *et al*., 2010). Consequently, it forms a diffusion barrier against metabolic exchange between the choriocapillaris and RPE. It is unclear when these age-related changes become abnormal and predispose to AMD. However, the thicker Bruch's membrane physically increases the distance from the choriocapillaris to the oxygen consuming rods and cones which may be a source of hypoxia (Stefánsson *et al*. 2011).

1.4.2.3. Changes to the RPE in AMD

Changes to the RPE include gradual loss of melanin granules, atrophy of microvilli, disarray in the organization of basal infoldings (Bonilha, 2008). Additionally, the accumulation of deposits between the RPE and Bruch's membrane and an increase in the accumulation of lipofuscin are all observed in normal aging (Bonilha, 2008). However, these changes are exacerbated in AMD. Lipofuscin is difficult to break down and progressively engorges RPE cells, extruding onto Bruch's membrane leading to the formation of basal laminar deposits and drusen (Katz *et al.,* 1996; Boulton *et al*., 1989; Young, 1987). In addition to structural distortion, lipofuscin has been associated with RPE dysfunction causing apoptosis (Holz *et al.*, 1999).

1.4.2.4. Changes to the photoreceptors in AMD

Following the findings of a 30% loss in rod density in aging, Curcio and colleagues (1996) investigated whether this loss continues in AMD. They examined thirteen donor eyes with signs of mid to late AMD and found that the foveal cone mosaic appeared normal. However, they observed cone inner segments lied adjacent to each other lacking the typical intervening rods in the parafovea, a pattern not observed in healthy eyes.

As the disease progresses towards later stages, only small islands of cones remain, allowing for sufficient to vision to be maintained for some time until all photoreceptors are lost as a consequence of the development of geographic atrophy or the formation of disciform scar (Curcio, Medeiros and Millican, 1996). The authors suggested that in some of the older eyes, RPE cells become dysfunctional which causes further rod loss, beyond the threshold noted in healthy normal eyes. Furthermore, this study highlighted that the greatest cell loss occurs within 1.5-10° eccentricity from fixation corresponding to visual function deficits observed in psychophysical studies (Sturr *et al.*, 1997; Owsley *et al.*, 2000; Fraser *et al.*, 2016).

1.4.3. AMD pathogenesis

The exact mechanism of AMD is unknown. It is believed to be a multifactorial disease where both environmental factors and genetic predisposition contribute to AMD pathogenesis. Although several pathways have been proposed, oxidative stress, hypoxia and the complement system remain the most widely accepted mechanisms for the development of this disease. This section provides an overview of these mechanisms.

1.4.3.1 Oxidative stress

Oxidative stress refers to the mechanism of cellular damage caused by reactive oxygen intermediates (ROI) also called reactive oxygen species (ROS) (Beatty *et al.*, 2000; Fanjul-Moles and López-Riquelme, 2016). These include free radicals, hydrogen peroxide or singlet oxygen and are generated from metabolic processes during aerobic respiration or to cope with stress reactions such as hypoxia (Fanjul-Moles and López-Riquelme, 2016). Free radicals such as superoxide (when oxygen gains a single electron) cause oxidative damage to lipids, proteins, dietary carbohydrates and nucleic acids, by robbing their electrons (Beatty *et al.*, 2000). The cumulative build up during lifetime of oxidised macromolecules from reactions involving ROS, forms the basis of the free radical theory of aging and age-related disorders originally proposed by Harman (Harman, 1956).

ROS are produced during normal metabolic activity in the mitochondria (oxidative phosphorylation) and also from photochemical reactions (Beatty et al. 2000; Spaide et al. 2003). The retina is particularly vulnerable to the production of ROS due to its high oxygen consumption, exposure to cumulative irradiation, high proportion of readily oxidised polyunsaturated fatty acids (PUFA) from photoreceptor outer segments, presence of photosensitisers such as rhodopsin and lipofuscin and the process of phagocytosis itself in the RPE (Beatty *et al.*, 2000). Other factors associated with increased generation of ROS include aging, inflammation and smoking (Beatty *et al.*, 2000).

Lipids are highly susceptible to oxidation (Fanjul-Moles and López-Riquelme, 2016). Lipid peroxides can further oxidise other lipids and bind to many proteins causing unusual structures rendering them more difficult to breakdown within the RPE (Spaide et al. 2003). This lipid-protein aggregate, called lipofuscin is stored in the RPE and high level of this material has been associated with RPE and photoreceptor degeneration (Sarks, Sarks and Killingsworth, 1988; Dorey *et al.*, 1989). Furthermore, the presence of oxidative protein modifications and peroxidised lipids within drusen (Spaide *et al.*, 1999; Crabb *et al.*, 2002) and delayed rate of disease progression with antioxidant oral supplements intake (AREDS, 2001; Chew *et al.*, 2014) provide evidence for this mechanism.

1.4.3.2. Hypoxia

Another mechanism thought to be involved in AMD pathogenesis is hypoxia. The outer retina is predominantly oxygenated by the choroidal circulation and this supply to photoreceptor inner segments just about meets the metabolic demands to sustain the dark current (reaching 0 mm Hg at proximal end of the inner segments) in a healthy retina (Wangsa-Wirawan, 2003). This indicates that any biophysical changes to the choroidal vasculature may render it susceptible to oxygen deficiency causing outer retinal hypoxia. Unlike retinal circulation, choroidal vasculature is not autoregulated, has much higher blood flow and is susceptible to systemic changes (Delaey and Van De Voorde, 2000; Metelitsina *et al.*, 2006; Feigl, 2009).

Changes to the haemodynamic profile of the choroidal circulation including reduced perfusion have been found to be exaggerated in AMD (Pauleikhoff *et al.*, 1990; Ramrattan *et al.*, 1994; Grunwald *et al.*, 1998), can occur in earlier in the disease (Feigl, 2009). Reduced choroidal perfusion may inevitably result in hypoxia. In addition to structural changes, hypoxia inducible factor (HIF) has been found to be upregulated in AMD eyes, implicated in choroidal neovascular membrane in AMD and cell apoptosis in GA (Arjamaa *et al.*, 2009). Hypoxia may also be induced by the thickening of Bruch's membrane and presence of drusen or other deposits which may impair oxygen diffusion to the outer retina due to increased distance from the choriocapillaris to oxygen consuming photoreceptors (Stefánsson, Geirsdóttir and Sigurdsson, 2011).

1.4.3.3. The complement system

The contributory role of complement system in AMD pathogenesis is derived from histopathological and genome-wide studies in AMD. The complement system is a core component of the innate immune system consisting of over 30 proteins (Maugeri *et al.*, 2018). It is activated via three pathways: classical, alternative and lectin pathways all leading to the activation of C3 convertase and if left unregulated, culminating into the formation of membrane attack complex (MAC) responsible for cell lysis and death (Whitmore *et al.*, 2015; Maugeri *et al.*, 2018). Histological work has demonstrated the presence of inflammatory molecules, choroidal dendritic cells and immunological proteins within drusen (Hageman *et al.*, 2001; Penfold *et al.*, 2001). In addition to oxidative stress which causes upregulation of the complement pathway (Maugeri *et al.*, 2018), there is strong genetic evidence of genetic polymorphisms in genes which govern complement pathway activity involved in increased risk of AMD as described in section 1.4.1.10.

1.4.4. Clinical features of AMD

The presence of drusen is considered to be the clinical hallmark of AMD (Sivaprasad *et al.*, 2016). These deposits are formed from accumulation of extra-cellular material typically located between Bruch's membrane and the RPE.

Drusen can be further morphologically differentiated into subtypes, including hard, soft, calcified, cuticular (previously known as basal laminar deposits) and subretinal drusenoid deposits (SDD) also called reticular pseudodrusen (RPD) (Khan *et al.*, 2016). They differ in relation to their size, quantity, location, and chemical composition. In addition to drusen, the presence of clinical features such as RPE pigmentary abnormalities, geographic atrophy and neovascular AMD also define AMD (Ferris *et al.*, 2013). For the purpose of this thesis, the focus will be on soft drusen and SDD, with brief descriptions of the other subtypes.

1.4.4.1. Drusen

Hard drusen are small (<63µm) yellow-white deposits with well-defined borders consisting of hyaline material and can be found both in the periphery and macula (Khan *et al.*, 2016). The appearance of hard drusen is generally considered to be a normal finding with aging, although large areas of these small drusen has been associated with AMD onset (Klein, *et al*. 1992; Klein *et al.* 2015). Calcified drusen contain higher amount of hydroxyapatite spherules giving them their refractile appearance though calcification may occur in all drusen (Suzuki *et al.*, 2015; Khan *et al.*, 2016). Cuticular drusen, previously called basal laminar deposits, are numerous (at least 50) small dot-like lesions which normally first appear in the periphery but are also found at the macula (Russell *et al.*, 2000; Boon *et al.*, 2013). They are formed from aggregations of carbohydrates, lipid and proteins and in addition to AMD, are also associated with dense deposit disease which is a kidney disorder (Boon *et al.*, 2013).

Soft drusen are usually larger (> 125 µm in diameter) than hard drusen with less distinct edges and appear as low mounds of yellow or white colour (Sarks, Sarks and Killingsworth, 1994). They are located between the RPE basement membrane and Bruch's membrane and are present exclusively at the posterior pole and supero-temporally (Wang *et al.,* 1996). These can enlarge and coalesce to form confluent drusen and may result in pigment epithelial detachment (PED) (Bressler *et al.*, 1994). The presence of soft drusen is suggestive of diffuse changes to Bruch's membrane (Sarks, Sarks and Killingsworth, 1994).

They consist mainly of lipids (estimated to be at least 40%), vitronectin, components of the complement system and various proteins (Wang *et al.*, 2010). The exact pathogenesis of drusen has not been established. One theory is that drusen arise from basally secreted metabolic waste product from within the RPE cells, which accumulates as a consequence of dysregulation of physiological systems of lipid transfer and lipid cycling pathways within the RPE (Chen *et al.*, 2020). Another view is that drusen have dual origin of lipids, dietary lipids and cholesterol which may also be implicated in addition to lipids derived from photoreceptor outer segments (Curcio, 2018).

1.4.4.2. Subretinal drusenoid deposit

In contrast to drusen described above, SDDs are found between the photoreceptors and the RPE (Zweifel *et al.*, 2010). These were first described by Mimoun et al., (1990) and consists of yellowish interlacing networks of drusen of variable diameter which, although did not fluoresce on fluorescein angiography, were more discernible in blue light (Mimoun *et al.,* 1990; Arnold *et al.*, 1995; Sivaprasad *et al.*, 2016) Their content is similar to that found in drusen, including apolipoprotein E, CFH, and vitronectin, however absence of immune cells within or near SDD has also been recently reported (Rudolf *et al.*, 2008; Chen *et al.*, 2020). SDD lesions are predominantly found in the superior perifovea (Zweifel *et al.*, 2010; Curcio *et al.*, 2013). They can be seen with or without the presence of drusen, but more frequently observed in intermediate stage rather than early AMD (Domalpally *et al.*, 2019). Additionally, longitudinal studies have found SDD to be a strong predictor for progression to nAMD and GA (Schmitz-Valckenberg *et al.*, 2011; Marsiglia *et al.*, 2013; Finger *et al.*, 2014, 2016; Shijo *et al.*, 2017; Lee *et al.*, 2019). Furthermore, a study found that compared to those with large drusen, those with SDD were older, consisted more frequently of female gender and developed AMD later (Boddu *et al.*, 2014).

Although the pathogenesis of SDD remains elusive, it has been suggested that SDD may arise following the fibrosis of the choroidal stroma and decline in choroidal vascularity (Arnold *et al.*, 1995). The apparent presence of these lesions in areas of choroidal water shed zones is also suggestive of reduced choroidal perfusion supporting the notion of hypoxic origins of SDD pathogenesis (Alten *et al.*, 2013).

However, the causal relationship has not been determined. From histopathological studies, it has been suggested that the RPE is a polarized and bidirectional secretor of lipids and any alteration to the physiological polarization may lead to misdirection of materials into the subretinal space. Alternatively, it has recently been proposed that molecular blockage at the RPE, perhaps in physiological lipid cycling, could result in aggregation of materials growing upwards towards the photoreceptors (Chen *et al.*, 2020). As the deposits grow, the external limiting membrane (ELM) becomes compromised causing focal deflection and shortening of photoreceptors and eventual clearance by Müller glia (Chen *et al.*, 2020).

1.4.4.3. RPE abnormalities

In AMD, both hyper-and hypopigmentary abnormalities can be present (Ferris *et al.*, 2013). Hyperpigmentary changes are clinically seen as small areas of pigment clumping appearing darker than the surrounding healthy RPE, resulting from increased melanin concentration, cell proliferation or migration of RPE cells into subretinal space or outer retina (Bressler *et al.*, 1994). In contrast, areas with reduced melanin appear as mottled pigmentation called hypopigmentation arising from RPE atrophy or thinning (Bressler *et al.*, 1994; Bonilha, 2008). Hyperpigmentary changes are considered to be a risk factor for the development for neovascular AMD (Klein *et al.*, 2002).

1.4.4.4. Geographic atrophy

Geographic atrophy of the RPE is the end stage of dry AMD and is clinically apparent as a well demarcated area of hypopigmented RPE, in which choroidal vasculature underneath becomes visible (Bressler *et al.*, 1994). Initially, focal RPE atrophic lesions occur in the parafovea and perifovea, spreading as either distinct patches that expand and coalesce or by forming a horseshoe-like pattern, sparing the fovea (Sarks, Sarks and Killingsworth, 1988). The slow progression of GA also involves attenuation of submacular choriocapillaris and degeneration of overlying photoreceptors (Lutty *et al.*, 2020). As the disease progresses, the fovea eventually becomes compromised causing central loss of vision.

1.4.4.5. Neovascular AMD

Neovascular AMD (nAMD), also referred to as wet AMD is the other late stage of this disease characterised by choroidal neovascularisation. This feature develops due to an imbalance between anti- and pro-angiogenic growth factors responsible for choroidal maintenance in healthy eyes (Schlingemann, 2004). New blood vessels from the choroid grow through Bruch's membrane, usually at multiple sites, into the sub-RPE space (Green and Enger, 1993).

In some cases, neovascularization can also penetrate through the RPE into the subretinal space and fluid and/or blood can accumulate (Campochiaro *et al.*, 1999). This retinal elevation causes metamorphopsia, where straight line may appear bent or have a kink, the classic symptom reported by patients (Campochiaro *et al.*, 1999). Diagnosis and classification of this feature is based on optical coherence tomography (OCT) and fluorescein angiography.

1.4.5. Classification

Over the decades, various definitions and severity scales were devised and utilized in epidemiological studies into AMD based on colour fundus photography or structural measures such as OCT. Earlier studies relied on visual acuity and ophthalmological opinion. The Wisconsin Age-Related Maculopathy Grading Scale was devised to provide consistency in the grading of pathological features using stereo colour fundus photographs and allow comparability of results between research studies (Klein *et al.*, 1991; Hageman *et al.*, 2001). This grading system was used for longitudinal AMD studies, including the Beaver Dam, Rotterdam and Blue Mountains studies (Klein, Klein and Linton, 1992; Mitchell *et al.*, 1995; Vingerling *et al.*, 1995). Further refinement was collaboratively proposed creating International AMD Classification and Grading System (Bird *et al.*, 1995). The latter was divided into 3 conditions: early ARM consisting of drusen with or without associated pigment and/or focal hyperpigmentation of the RPE and late AMD further classified as either dry (with area of GA greater that 175µm) or wet (any of RPE detachment, sub-retinal neovascular membrane, disciform scar, subretinal haemorrhage or hard exudates) (Bird *et al.*, 1995).

More recent grading scales include the Age-Related Eye Disease Study System (AREDS, 2001b) and the Beckman Clinical Classification of AMD system (Ferris *et al.*, 2013). For the purpose of this thesis, the latter was used as it is simpler and more clinically useful for predicting the risk of late AMD. Of note, small drusen (<63 µm) are termed drupelets and confer little risk for progression to late AMD (0.5% risk over 5 years). The features are summarized in Table 1.1.

Table 1.1. Beckman Initiative for AMD Classification System (from Ferris et al., 2013)

Beckman Initiative for AMD Classification System

Classification of AMD	**Features (lesions assessed within 2-disc diameters of fovea of either eye)**
No apparent aging changes	No drusen and No AMD pigmentary abnormalities
Normal aging changes	Only drupelets (small drusen ≤63 µm) and No AMD pigmentary abnormalities
Early AMD	Medium drusen >63 µm and ≤125 µm and No AMD pigmentary abnormalities
Intermediate AMD	Large drusen > 125 µm and/or Any AMD pigmentary abnormalities
Late AMD	Neovascular AMD and/or Any geographic atrophy
AMD pigmentary abnormalities = any definite hyper- or hypopigmentary abnormalities associated with medium or large drusen but not associated with known disease entities.	

Similar to other classification systems, the Beckman Initiative classification also has a limitation in that it does not include SDD lesions secondary to AMD. These are important as they confer an increased risk to progress to advanced disease. The inclusion of this feature and other types of nAMD has been previously advocated (Spaide, 2018).

1.4.6. Current treatments

Currently, there are no potential therapeutic interventions to prevent or slow down the progression of intermediate AMD to advanced stages of the disease; geographic atrophy or neovascular AMD.

Although there is treatment available for neovascular AMD by means of intravitreal anti-VEGF injections, patients with dry AMD or geographic atrophy do not have recourse to any treatment. Currently, the only preventative option available to patients with dry AMD remains the dietary supplementation from the AREDS study, which found that a supplementation of zinc oxide, cupric oxide, b-carotene, Vitamin C and Vitamin E reduced the relative risk of AMD progression to advanced stage in patients with intermediate or advanced AMD in one eye to the other eye by 25% (Age-Related Eye Disease Study Research, 2001b).

It did not provide evidence in halting development of new GA or progression of existing atrophy (Age-Related Eye Disease Study Research, 2001b). AREDS2 Study added carotenoids lutein and zeaxanthin, omega-3 PUFA (Age-Related Eye Disease Study 2 Research, 2013). It is important to mention that both of these trials did not demonstrate any evidence of any effect on other AMD groups nor on drusen subtypes. The study was also conducted in an educated and well-nourished population, a limitation reported by the authors (Chew *et al*., 2014).

Recent experimental therapies for dry AMD have aimed to supress chronic inflammatory response with intravitreal C5 inhibitors (Yehoshua *et al.*, 2014) rapamycin (Wong *et al.*, 2013), reduce lipid accumulation at Bruch's membrane via statins (Barbosa *et al.*, 2014), enhance choroidal perfusion with vasodilator (Augustin *et al.*, 2013), preserve RPE and photoreceptor with Fenretinide (Mata *et al.*, 2013) and visual cycle inhibitors (Rosenfeld *et al.*, 2018). All of these have had none to very modest effects. Stem cell therapies (Da Cruz *et al.*, 2018) and gene therapy (Clinicaltrials.gov, 2019), are currently being investigated for advanced stages of AMD.

1.4.7. Clinical investigation of AMD

In clinical setting, typical tests used to diagnose AMD include measurement of visual acuity, fundus colour photography, central visual field assessment with the Amsler Chart and fluorescein angiography. Technological advancements in retinal imaging such as OCT, autofluorescence and near infrared imaging have contributed to increased sensitivity at detecting AMD disease phenotypes (Talks *et al.*, 2017).
Furthermore, there is a body of evidence supporting other visual function tests may detect visual function deficit earlier than the visual acuity assessment and as such may be more suitable clinical trial end points. These include low-luminance visual acuity (LLVA), scotopic microperimetry, dark adaptation and possibly electroretinograms (ERGs).

1.4.7.1. Visual acuity

Currently, best-corrected visual acuity (BCVA) is the only validated endpoint that is accepted as a visual function clinical endpoint in clinical trials in retinal diseases. However, it relatively unaffected until the fovea has become compromised. In fact, early AMD has been associated with a decrease of two letters which although statistically significant is not clinically useful given the test-retest variability of 1-2 lines difference (Rosser *et al.*, 2003; Patel *et al.*, 2008). This variability increases with increasing disease severity (Patel *et al.*, 2008). Additionally, clinical studies often preclude participants on the basis of minimum visual acuity in their inclusion criteria therefore preventing to capture, if any, relationship between visual acuity and disease. Furthermore, BCVA which is typically measured with high contrast letters may fail to detect any cone dysfunction, as only an estimated 44% of normal foveal cones are required to achieve 6/6 visual acuity (Frisén and Frisén, 1979).

LLVA can be measured by placing a neutral density filter in front of the testing eye. It is predominantly a test that measures cone function in reduced illumination which has been shown to be a strong predictive marker across all disease severities in AMD (Sunness *et al.*, 1997, 2008; Puell *et al.*, 2012; Wu *et al.*, 2014; Cocce *et al.*, 2018). Lastly, low-luminance deficit (LLD), defined as the difference between BCVA and LLVA, has been reported as early as the early AMD stage (Puell *et al.*, 2012). It has also been found to better capture visual difficulties in low luminance conditions in participants with intermediate AMD (Wu, Guymer and Finger, 2016).

1.4.7.2. Scotopic Thresholds

Scotopic thresholds (or absolute thresholds) measure the minimum light that can be detected once the retina is fully adapted in the dark which can take more than 40 min to attain (Lamb and Pugh, 2004). Given the histopathologic evidence of rod vulnerability in aging and AMD (Curcio *et al.*, 1993; Curcio, Medeiros and Millican, 1996), multiple psychophysical studies were undertaken and have corroborated these findings (Jackson *et al.*, 1998; Jackson and Owsley, 2000; Owsley *et al.*, 2000).

Although the presence of hard drusen is generally not associated with AMD, reduced scotopic sensitivity has been reported to be compromised in that phenotype compared to healthy controls, but mesopic sensitivity remained unaffected (Nebbioso, Barbato and Pescosolido, 2014). More recent studies have shown reduced scotopic function in individuals with intermediate AMD, particularly in those with SDD at multiple loci (Fraser *et al.*, 2016; Flynn, Cukras and Jeffrey, 2018; R. Tan, Guymer and Luu, 2018). Another study evaluating mesopic function, where both photoreceptors are involved, reported reduced function in eyes with SDD secondary to AMD which was correlated with the number of SDD lesions and their distribution (Ooto *et al.*, 2013). Therefore, dark-adapted scotopic sensitivity has been shown to be reduced in individuals at the earliest stages of the disease, declining further with increasing disease severity and may be a suitable functional biomarker for clinical trials.

1.4.7.3. Dark adaptation

The human visual system is generally able to adapt very rapidly to changes in illumination over a wide range of light intensities. However, this physiological principle is not upheld when we return to darkness following prolonged retinal exposure to an intense light source where a high concentration of visual pigment is “bleached”. In this case, the visual sensitivity recovers slowly. This slow, biphasic recovery of visual sensitivity in termed as dark adaptation (DA) (Lamb and Pugh, 2004). Dark adaptation forms the basis of the Duplicity Theory which refers to the ability of the photoreceptors and rods to operate under different illumination (Weale, 1961). Whilst the cone system is involved above roughly 0.03 cd/m2 illuminance level, rod-mediated mechanism is initiated in conditions below this illumination (Kalloniatis and Luu, 2007). The threshold intensity required to detect a visual stimulus over time can be plotted graphically, generating the dark adaptation curve. Visual thresholds rise following light exposure after which they decrease in two distinct phases. The initial several minutes of rapid recovery reflects the recovery of cones leading to a rod-cone break where rod thresholds fall below that of cones. The second phase reflects the steady followed by slower recovery of rods taking a further 30 minutes.

It can take more than 40 minutes to reach complete recovery of sensitivity to attain fully dark adapted state after near-total bleach. (Lamb and Pugh, 2004; Kalloniatis and Luu 2007). These features are shown in the classical dark adaptation curves obtained by Hecht in the 1930s (Hecht et al., 1937) as illustrated in Figure 1.7.

Several factors can affect dark adaptation including the size of the stimulus, retinal eccentricity tested, the percentage of photopigment bleached (from the intensity and duration of the pre-adapting light), the wavelength of light used and the rate of rhodopsin regeneration (Hecht, Haig and Wald, 1935; Hecht and Shlaer, 1936; Hecht, 1937; Lamb and Pugh, 2004; Dimitrov *et al.*, 2008).

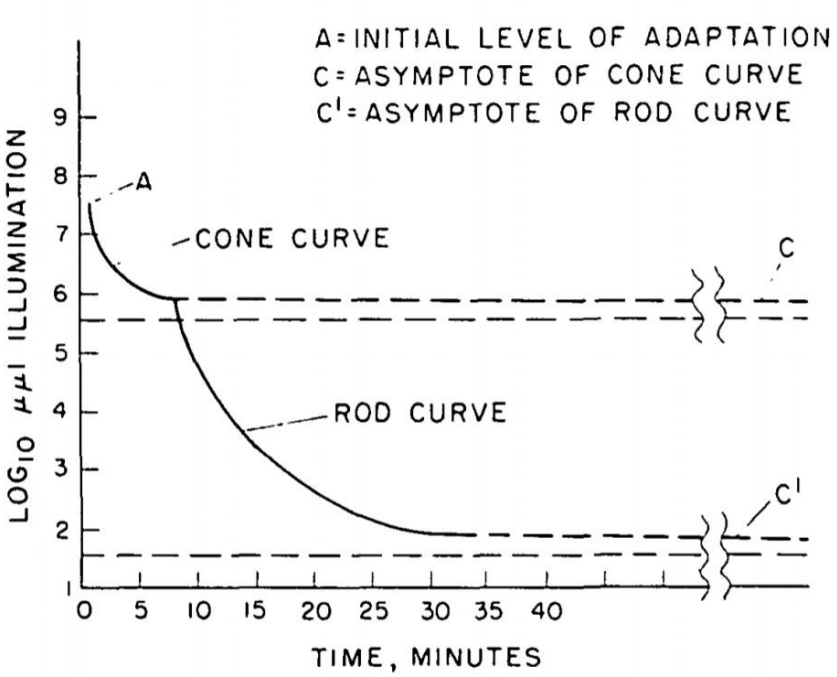

Figure 1.7. Dark adaptation curve (from McFarland, 1960)

Dark adaptation in inherently dependant on the rate of rhodopsin regeneration through the retinoid cycle (Rushton, 1965; Lamb and Pugh, 2004). Fundamentally, DA can be used as a proxy to measure the integrity of RPE-photoreceptor complex, a system supported by the choroidal vascularity via Bruch's membrane. Therefore, any disruptions to these structures may impact DA. Although there is a natural prolongation of DA as a consequence of aging (Jackson, Owsley and Mcgwin, 1999; Tahir *et al.*, 2017), it is further impaired in AMD and has been suggested as a potential biomarker for the disease (Owsley *et al.*, 2001, 2014, 2016; Dimitrov *et al.*, 2008, 2011).

Moreover, dark adaptation is severely delayed in the presence of subretinal drusenoid deposits in AMD (Fraser *et al.*, 2016; Flynn, Cukras and Jeffrey, 2018; Luu *et al.*, 2018). The precise mechanism causing the delay in DA in AMD eyes is not known. The choroid is responsible for supplying retinoids and metabolites needed by the RPE for regeneration of rhodopsin (Lamb and Pugh, 2006). Therefore, the presence of drusen may prevent the adequate diffusion of nutrients to the RPE via Bruch's membrane (Starita, Hussain and Marshall, 1995; Booij *et al.*, 2010). Also, thinning of the choroid and choroidal hypoperfusion could limit the quantity of substrates reaching the RPE (Ramrattan *et al.*, 1994; Ciulla *et al.*, 2002).

1.4.7.4. Full Field Electroretinograms

Full field electroretinograms (ffERGs) is an objective test which measures the electric responses of the photoreceptors. The ERG is recorded in response to a flash presented within a large dome-shaped Ganzfeld bowl. This allows for uniform illumination the central 120° of the retina by a flash simulator. It therefore measures pan-retinal electrical response from photoreceptors, but provides no spatial information, i.e., no focal decline can be elicited (Berrow *et al.*, 2010). The literature on the impact of AMD on full field ERGs is sparse and remains equivocal. A study with 21 participants with AMD and visual acuity of at least 6/18 found no abnormality in ffERGs regardless of disease state of the tested eye (Sunness *et al.*, 1985). Another study supported these findings where participants with early AMD and healthy control did not differ in scotopic and photopic ERGs nor in flicker ERGs suggesting peripheral vision remains unaffected (Holopigian *et al.*, 1997).

In contrast, Ladewig and colleagues (2003) observed a decrease in 30Hz flicker amplitude in a cohort of 20 AMD subjects compared to healthy controls. Another study retrospectively investigated ffERGs on 52 AMD patients, which identified a subgroup of AMD patients with pan-retinal cone dysfunction (Ronan *et al.*, 2006).

1.4.7.5. Optical coherence tomography (OCT)

Optical coherence tomography is a contactless non-invasive imaging device that allows the cross-sectional assessment of retinal structures at microscopic level (Huang *et al.*, 1991). It is based on the principle of interferometry where a beam of light (near infrared wavelength ranging from 800-1400nm) is shone on the retina and the system analyses the intensity and echo time delay of back scattered light reflected off different cell types (Costa *et al.*, 2006; Schuman, 2008). The delay in light reflected back from deeper tissues is longer to than that of superficial tissue (Costa *et al.*, 2006). The difference between a series of reflective measurements at varying depths within the retina allows to generate axial a-scans which can be gathered linearly to form a cross-sectional image called a B-scan and a combination of these parallel scans produces a 3-D image of the retina (Schuman, 2008).

The advent of OCT has allowed clinicians to study outer retinal structures and clinical characteristics of interest in AMD in greater resolution. This modality can be used to diagnose nAMD, monitoring of clinical feature of nascent GA, identify anatomical risk factors such as intraretinal hyperreflective foci, drusen phenotypes, and RPE abnormalities (Guymer and Wu, 2020). Additionally, individuals with early and intermediate AMD can be distinguished from healthy- aged eyes by quantifying total retina volumes and abnormal RPE drusen complex thickening and thinning volumes (Farsiu *et al.*, 2014; Lamin *et al.*, 2019).

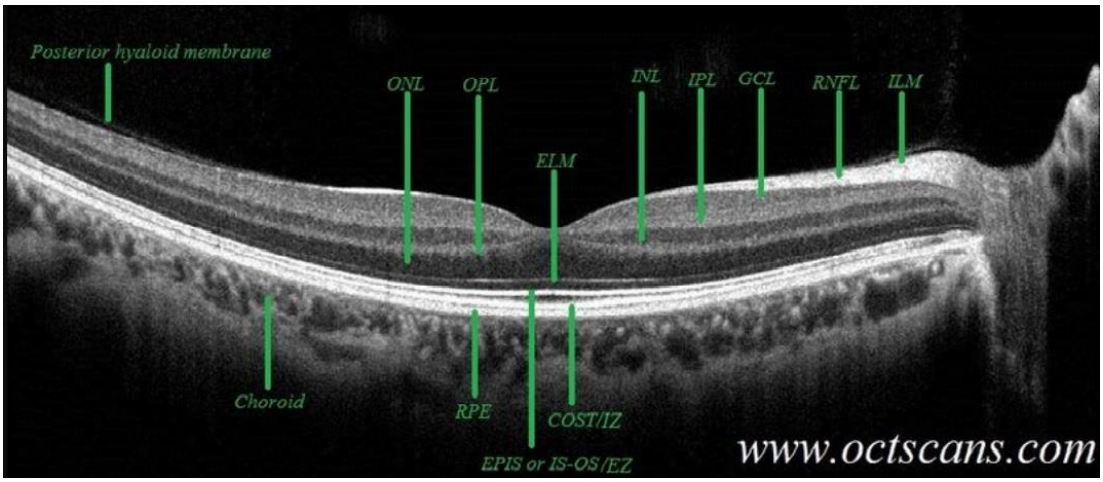

Figure 1.8. SD-OCT image of a normal right eye (from Optical Coherence Tomography Scans Website, http://www.octscans.com).

1.5. Photobiomodulation

There is a growing body of evidence that mitochondrial function may be modulated with exposure to light termed photobiomodulation. As mitochondrial dysfunction is implicated in aging and AMD, photobiomodulation may be a practical and inexpensive treatment option to improve the affected biological processes. First, a brief overview of mitochondrial dysfunction is described followed by an introduction, mechanism and therapeutic outcomes of photobiomodulation.

1.5.1. Mitochondrial dysfunction

The most important function of mitochondria is the generation of adenosine triphosphate (ATP) via oxidative phosphorylation. This is the process where mitochondria break down organic molecules into carbon dioxide and water. However, this procedure also generates reactive oxidative species (ROS). The latter play an important role in redox signalling from the mitochondria to the rest of the cell but are also a source oxidative damage. Implications of oxidative stress in the retina are described in section 1.4.3.1. In retinal aging, the efficiency of the electron transport chain is reduced which causes substantial rise in the production of ROS (Eells, 2019). This further promotes mitochondrial DNA (mtDNA) damage and leads to accumulation of mtDNA (Beatty *et al.*, 2000; Barron *et al.*, 2001; Eells, 2019). Studies from AMD donor eyes have reported a decline in mitochondrial density, structural alterations and an increase in RPE mtDNA damage (Nordgaard *et al.*, 2008; Karunadharma *et al.*, 2010; Lin *et al.*, 2011).

1.5.2. Introduction to photobiomodulation

Photobiomodulation (PBM), also known as low-level light therapy (LLLT) and near infra-red (NIR) light therapy, was re-discovered in the 1960's by Endre Mester in Hungary where he investigated the ability of 694nm laser in causing cancer in shaved mice (Mester *et al.*, 1971). He reported that light therapy did not induce cancer and it also stimulated faster hair growth in the light-treated group compared to the control group (Mester *et al.*, 1971).

Previously, Niels Finsen was awarded a Nobel prize for successful treatment of cutaneous tuberculosis with UV light (Grzybowski and Pietrzak, 2012). PBM can be defined as the use of light to modulate biological function to induce therapeutic effect without damaging cells and emitting heat. Essentially, it is the conversion of light energy to metabolic energy in view of inducing a biological response. The red to NIR wavelengths (600 – 1000nm) have been shown to be most effective in eliciting a beneficial biological effect in cells lacking specialised photopigment due to deeper penetration of long wavelength and decreased absorption by water and other chromophores (Gonzalez-Lima and Rojas, 2011; Chung *et al.*, 2012). It has also been well established that PBM yields a biphasic dose response curve whereas when the dose increases, the beneficial biological response increases gradually as it reaches a maximum value. If the dose keeps increasing passed that maximum level, the biological response decreases until baseline is reached, and if pushed further an inhibitory response is observed which can lead to cell damage or apoptosis (Hamblin, Huang and Heiskanen, 2019).

Multiple studies have investigated the power and energy levels in the red and NIR spectrum for therapeutic effect. These have been variable and ranged from 5-50mW/cm² (Huang *et al.*, 2011). Although a great range of the red NIR spectrum has been used in laboratory studies, 670nm wavelength is the most commonly used (Begum *et al.*, 2013; Calaza *et al.*, 2015; Powner *et al.*, 2016; Merry *et al.*, 2017; Sivapathasuntharam *et al.*, 2017a, 2019; Cheng *et al.*, 2018).

1.5.1. Mechanism

It is widely accepted that cytochrome oxidase c (COX), which is the terminal enzyme of the mitochondrial electron transport chain (a series of 5 main enzyme complexes, shown in Figure 1.9.), is the main biological photoacceptor of red to NIR light (Karu, 1999; Karu *et al.*, 2005; Hamblin and Demidova, 2006; Karu, 2008; Chung *et al.*, 2012). Essentially, COX contains four redox metal centres: CuA, CuB, Hem a and Hem a3. The electrons travel from cytochrome CuA to Hem a, from Hem a to Hem a3-CuB, and lastly to molecular oxygen (Karu, 2008). This is aided by decreased absorption of long wavelength by water and other chromophores as shown in Figure 1.10.

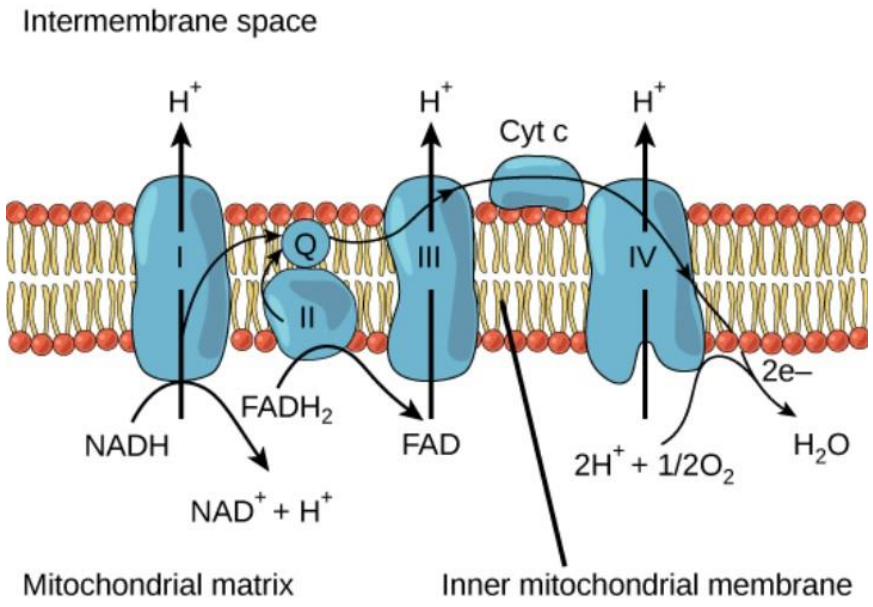

Figure 1.9. Electron transport chain (from https://courses.lumenlearning.com/ivytech-bio1-1/chapter/reading-electron-transport-chain/)

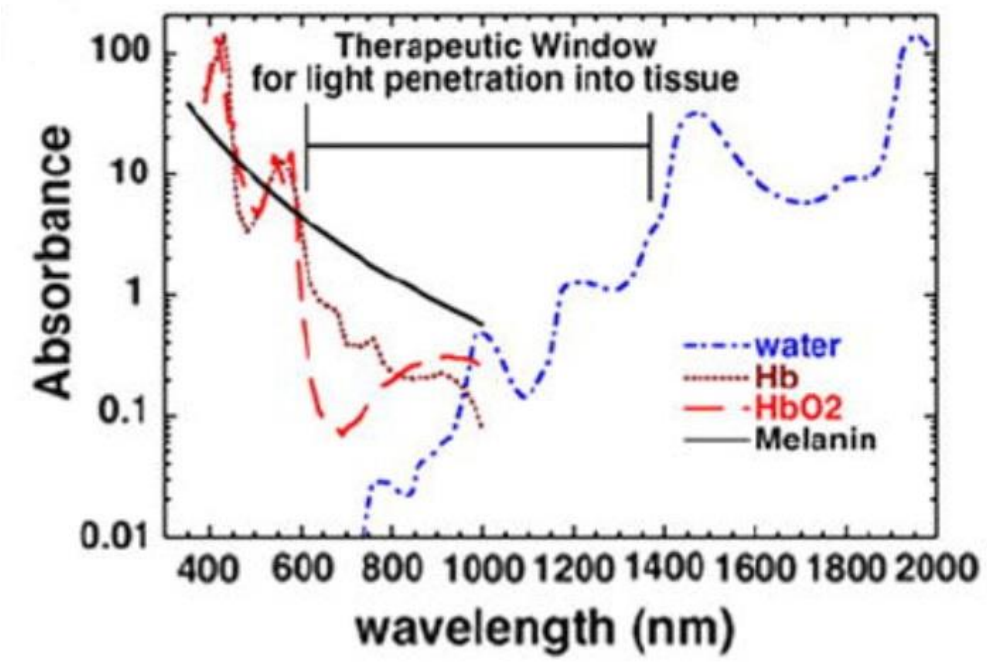

Figure 1.10. Therapeutic window for photobiomodulation illustrating decreased absorption of water, haemoglobin and melanin (figure adapted from Chung et al., 2012).

PBM increases the oxidation of COX, boosting the transfer of electrons and consequentially increasing oxygen consumption and mitochondrial membrane potential (Desmet *et al.*, 2006; Gonzalez-Lima and Rojas, 2011). This has been further associated with increased COX activity and ATP levels (Desmet *et al.,* 2006). Following these changes, secondary downstream signalling cascades are initiated; there's an increase in NAD^+/NADH ratio, mitochondrial membrane potential and displacement of the inhibitory nitric oxide from COX complex leading to an increase in ATP (Karu *et al.*, 2005; Karu, 2008). It has also been hypothesized that photon capture may activate light-sensitive ion channels causing an influx of calcium ions in the cell (De Freitas & Hamblin, 2016). These secondary effects initiate retrograde signalling between the mitochondria and nuclei with consequential changes in enzyme activity and gene expression (Karu, 2008). However, it is very likely that PBM may affect other cellular pathways (Xu *et al.*, 2014; Zhang *et al.*, 2016; Lima *et al.*, 2019).

1.5.2. 670nm treatment in animal models and humans

Much evidence has been gathered in efforts to describe beneficial biological effects from PBM therapy in animal studies. Exposure of 670nm therapy has been shown to improve cellular respiration, mobility and cognition in aged Drosophila Melanogaster (Weinrich *et al.*, 2017). PBM also increased COX activity, ATP and mtDNA content, retinal function and decreased ROS (Begum *et al.*, 2013; Gkotsi *et al.*, 2014; Weinrich *et al.*, 2017). In bumblebees exposed to neonicotinoid pesticides, 670nm light therapy was able to reduce death rates and improved ATP levels, mobility and visual function (Powner *et al.*, 2016). Along with improved mitochondrial function, retinal function (ERGs) in murine model retinal disease has also been documented (Begum *et al.*, 2013; Sivapathasuntharam *et al.*, 2017b). Diabetes induced RGC death can be reduced and ERG amplitude increased post red light treatment (Tang *et al.*, 2013). Another study has shown that PBM treatment over 8 months inhibited retinal capillary leakage and degeneration of retinal capillaries prevented reduction in visual function in diabetic rodents (Cheng *et al.*, 2018).

In humans, the use of PBM therapy has also been investigated for a wide range of neurological and psychological conditions such as Alzheimer's Disease and Parkinson's Disease (Peoples *et al.*, 2012; Johnstone *et al.*, 2014), traumatic brain injury (Ando *et al.*, 2011), stroke (Yip *et al.*, 2011), clinical depression and anxiety (Schiffer *et al.*, 2009). Photobiomodulation has been translated in human retinal trials. Tang and colleagues (2014) reported a reduction in non-centre involving diabetic macular oedema in a case series of 4 patients following daily exposure to 670nm light for 160 seconds for 2-9 months. LLLT has further been investigated in AMD. A brief exposure to 780nm light, twice a week for two weeks lead to significant visual acuity improvement in patients with both dry and wet AMD which was sustained months after treatment (Ivandic & Ivandic, 2008). Merry et al. (2017) also reported improved VA, contrast sensitivity and reduced drusen volume. Patients were exposed to light 3 times per week for a duration of 3 weeks, using 3 different wavelengths (590nm (4mW/cm^2), 670nm (at 50-80mW/cm^2) and 790nm (0.6mW/cm^2) with view of affecting different cellular targets (Merry et al., 2017).

There is a growing body of evidence suggesting potential benefits of PBM therapy in retinal disease and particularly in AMD where mitochondrial dysfunction has been implicated.

1.6. Rationale and PhD objectives

Development of potential treatments for dry AMD is challenging due to phenotypical heterogeneity of the disease and the lack the validated clinical biomarkers. Visual acuity, the only validated clinical endpoint for trial in retinal diseases, does not correlate with disease nor its progression until the end stage of AMD where the fovea has become compromised. The anatomical changes take years to develop and as such there is an unmet need to identify biomarkers that can detect disease progression and identify individuals that are at higher risk of progressing to advanced stages. Additionally, there is growing evidence that patients with SDD confer greater risk to develop advanced AMD, however, this phenotype does not appear in any clinical classification system.

Therefore, this thesis aimed to investigate the diagnostic and prognostic capacity of potential functional and structural clinical endpoints and to evaluate tests that may be able to discriminate intermediate AMD into those with and without SDD. This work also aimed to evaluate the impact of 670nm light therapy in aging and in AMD.

The objectives of this thesis included:

- Establish test-retest variability and measure the mean and pointwise scotopic sensitivity in aging and across varying AMD severity groups using a novel dark-adapted chromatic perimeter.
- Identify potential functional and structural biomarkers which can stratify intermediate AMD into those with and without SDDs.
- Evaluate function-function and function-structure relationships in AMD.
- Investigate the capability of potential functional and quantitative OCT clinical endpoints to detect disease progression at 1 year follow up visit in AMD.
- Evaluate the magnitude of effect of photobiomodulation in aging and across different stages of AMD on visual function and structural parameters.
- Evaluate the impact of photobiomodulation over time in intermediate AMD compared with a control arm.

Chapter 2. Methodology

2.1. General methods

Participants were recruited for two exploratory clinical studies covered in this thesis:

1) FUnction and Structure Correlation in Healthy aging and In Age-related macular degeneration (FUSCHIA), was a single centre, prospective observational study to assess visual function and structural changes with age and AMD. Cross-sectional (Chapter 4) and longitudinal (12 months, Chapter 6) results were collected and analysed.

2) A pilot study to investigate the effect of 670 nm light on visual function in aging and AMD (670nm trial). This was a single centre, prospective, interventional study to assess the effect of 670 nm light exposure over 12 months on visual function in subjects aged 55 or older with normal macular health or AMD (chapter 5).
The aims and objectives are described in the introduction and in detail in their respective chapters.

2.1.2. Recruitment

Potential subjects aged 55 years or above were recruited from AMD registers maintained in Moorfields Eye Hospital, medical retina clinics or referral from GP or optometrists. The study was discussed with potential participants and they were provided with the Patient Information Sheet.

2.1.3. Inclusion criteria

Inclusion criteria included subjects aged 55 years or over with normal fundus or with dry AMD with a minimum BCVA of 50 ETDRS letter in the study eye, good media clarity (Grade ≤ 3 on the LOCS III cataract grading scale), adequate pupillary dilation, and subject cooperation sufficient for satisfactory imaging and functional tests.

2.1.4. Exclusion criteria

Potential subjects were excluded if there was any ocular condition that could affect or alter VA during the course of the study (nAMD, central GA, glaucoma or diabetic retinopathy in the study eye), significant systemic disease or history of medication known to affect visual function, epilepsy, history of major ocular surgery in the last 3 months or anticipated within the next 6 months following enrolment in the study eye. Participants were also precluded if they had any allergies to adhesives or any other component used.

2.1.5. Ethical approval

The Ethical Review Board of Moorfields Eye Hospital approved the data collection for both studies that were conducted according to the International Conference on Harmonization Guidelines for Good Clinical Practice and the tenets of the Declaration of Helsinki. For the FUSCHIA trial, study procedures took place at Moorfields Eye Hospital from May 2017 to 2020. The study was approved by the Camden and Kings Cross National Research Ethics Committee (NRES) Committee London REC 16/LO/1317. The 670nm trial protocol was approved by the NRES (16/LO/2022) and data was collected from March 2017 to January 2019.

2.1.6. Data collection

All participants were aged between 55 and 85 years of age and had a BCVA in the study-eye of 50 ETDRS letters or better. A diagnosis of dry AMD was made in one eye only (study eye). At baseline visit, demographic data including age, sex, smoking status, relevant systemic disease, concomitant medication and nutrient supplementation (AREDS or equivalent) was recorded. Study procedures were carried out annually for the FUSCHIA study and repeated at 1 month, 3 months, 6 months and 12 months for the 670nm interventional study. The collected data was tabulated into an Excel spreadsheet before being transferred to statistical programs for analysis.

2.2. Trial procedures

2.2.1. Informed consent

Participant invitation letter and participant information sheet were given to the patient prior to their attendance to a screening or baseline visit. The study investigator was responsible for taking the informed consent of each participant at the beginning of any trial procedure. Following a full explanation of the study and opportunity to ask questions, participants were invited to take part. If participants were keen and had confirmed eligibility on that day, the screening visit was used as a baseline visit. Once the consent form was signed, the study investigator took a thorough and relevant ocular and medical history, followed by an undilated eye exam in order to ensure the eligibility criteria had been met.

2.2.2. Sequence of study assessments and Imaging

Participants underwent a structured ocular and medical history. Best corrected visual acuity and low luminance visual acuity were measured using the EDTRS chart at 4 meters. Following pupil dilation with 2.5% phenylephrine and 1% tropicamide, scotopic thresholds, dark adaptation and hand-held electrophysiology tests were carried out. Following these, retinal imaging of both eyes including colour fundus photographs, spectral-domain optical coherence tomography (SD-OCT) and optical coherence tomography angiography (OCT-A), qualitative autofluorescence images were taken.

2.2.3. Visual Acuities

For all participants, a refraction protocol was carried out at baseline visit prior to assessing BCVA. The latter was measured for each eye using an ETDRS test chart (number of letters seen recorded). LLVA was measured by placing a 2.0-log neutral density filter over the eye and have participants read the same chart, with the aim of the filter to lower background luminance by 100-fold.

These tests were performed monocularly (right eye tested first, followed by the left eye) with alternating charts for each eye to avoid repetition and memorisation at 4 metre distance. Subsequently, the difference between BCVA and LLVA in ETDRS letters was defined as the LLD score. The scores for the study eye only were used in the analysis and collected on an EXCEL spreadsheet. Visual acuities were recorded by multiple trained optometrists for the 670nm study whereas a single research optometrist carried out visual acuity assessment for the FUSCHIA study.

2.2.4. Low Luminance Questionnaire (LLQ)

The LLQ was also administered for the FUSCHIA cohort. This questionnaire consists of 32-items with six subscales related to low luminance settings: 1) extreme lighting, 2) mobility, 3) general dim lighting, 4) peripheral vision, 5) driving and 6) emotional distress. Each question was scored on a scale ranging from 0, or maximal difficulty, to 100, or no difficulty. The questions were categorized into different subscales and averaged to generate one score per subscale. The weighted subscales were then averaged to produce a composite LLQ score. The LLQ is publicly accessible at: https://www.uab.edu/medicine/ophthalmology/images/research/Low_Luminance.pdf.

2.2.5. Photopic flicker ERGs

Cone function was assessed by measuring full field flicker ERGs using a small handheld portable commercial device called the RETeval system (LKC Technologies, Inc., Gaithersburg, MD, USA) shown in Figure 2.1. The device uses self-adhesive skin electrode arrays, which are placed 2mm below the lower lid margins. The device was placed in front of the right eye which was tested first while the left eye was covered. It emitted a fixed flash (stimulus strength, 3.0 cd·s/m2) with a background of 30 cd/ m2 at 28.3 Hz frequency and was conducted in both eyes prior to pupillary dilation for FUSCHIA study (post pupillary dilation for 670nm study).

This instrument was operated using proprietary software which calculated the implicit time (mS) and amplitude (μV) using aggregated values rather than actual values. An example of the results sheet from this device is illustrated in Figure 2.2.

To evaluate the reliability of this test, inter-eye correlation in healthy eyes was measured (see below section 2.2.5.1.).

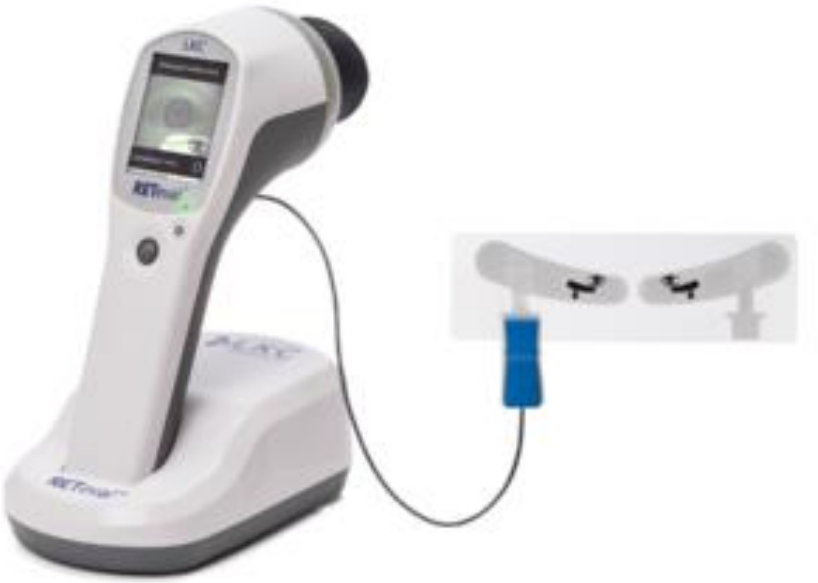

Figure 2.1. RETeval, hand-held stimulator and recording apparatus in docking station. The pupillary area is measured by a pupilometer, to deliver uniform retinal illumination. This device is attached to skin electrode which is placed at the mid-pupillary line to beyond the lateral canthus. The electrode array contains three electrodes: positive, negative, and ground. Image obtained from (https://www.lkc/product/reteval).

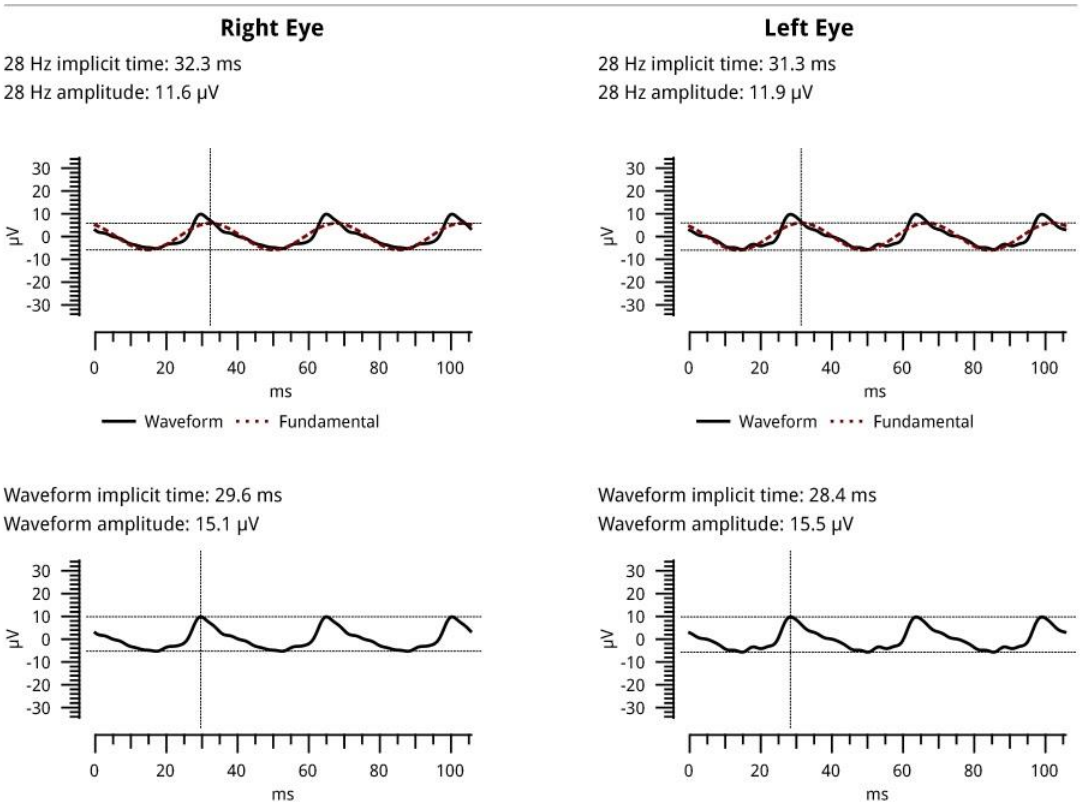

Figure 2.2. Representative image of 28.3Hz frequency results sheet from RETeval. The top two plots represent the implicit time and amplitude from the best-fitted sine wave to the waveform. The bottom two plots represent the implicit time and amplitude directly from the waveform (raw data).

2.2.5.1. Reproducibility of photopic flicker ERGs: inter-eye correlation in healthy eyes

To assess the variability of this instrument, inter-eye correlation was evaluated in healthy individuals from the FUSCHIA study cohort consisting of 10 participants (data missing for one patient due to mechanical failure of device) with a mean age of 65.1 ± 6.2 years. The protocol has been described in section 2.2.5. The right eye was highly correlated with the left eye for implicit time (normal distribution, Pearson correlation r=0.96, 95% CI 0.84 to 0.99, p<0.0001) whereas the correlation was insignificant for the amplitude parameter between the two eyes (non-normal distribution, Spearman correlation r= 0.54, p=0.1091). These results are displayed in Figure 2.3.

Linear regression analysis was also performed of implicit time and amplitude against age, which revealed a significant relationship between implicit time and age ($r^2 = 0.43$, p=0.041), and a nonsignificant relationship between amplitude and age ($r^2 = 0.22$, p= 0.175) displayed in Figure 2.4.

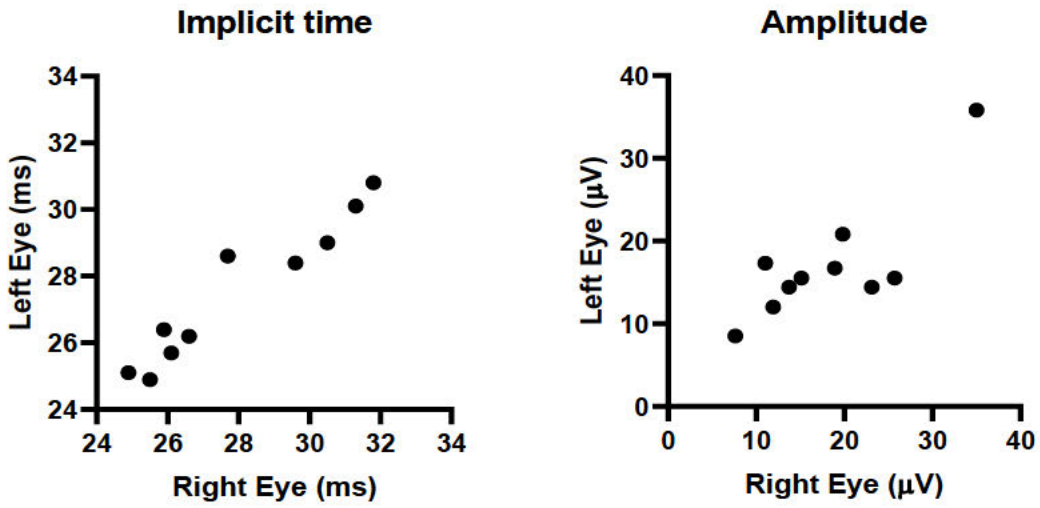

Figure 2.3. Scatter plots showing the inter eye correlation in individuals with normal retinal health (N=10). The eyes were significantly correlated for the implicit timing parameter (Pearson correlation r=0.96, p<0.0001) whereas no significant correlation was found between the eyes for amplitude (Spearman correlation r= 0.54, p=0.1091).

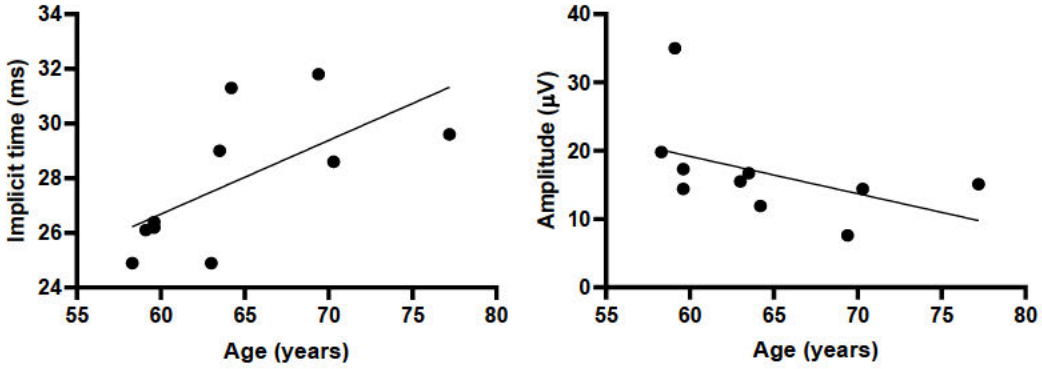

Figure 2.4. Scatter plots showing the relationship between implicit time ($r^2 = 0.43$, p=0.041) and amplitude ($r^2 = 0.22$, p= 0.175) against the age of the healthy cohort from FUSCHIA study (N=10).

The results from photopic flicker ERGs suggested that implicit timing was a reliable parameter whereas the amplitude showed non-statistically significant inter eye correlation and association with age. The amplitude measurement was found to be less reliable and more significantly affected affecting by the positioning of the electrode strips (Hobby *et al.*, 2018). Results from linear regression models between implicit timing and age showing a delayed response with increasing age is in agreement with most published data using conventional methods for ERG assessment (Birch and Anderson, 1992). Although there was a trend of decreasing amplitude with age, this failed to reach statistical significance which was expected from previously published data. Despite having the desirable characteristic of easier clinical use and more comfortable (compared to corneal electrodes) ERG recording for the patient, it is possible that the amplitude measurement with this handheld device may be more vulnerable and less accurate than conventional methods.

2.2.6. Scotopic Thresholds

Medmont Dark-Adapted Chromatic perimeter is a novel instrument to measure static and dynamic rod function. It consists of a black-coloured perimeter, with test locations covering a field up to 144° horizontally and 72° vertically shown in Figure 2.5.

Following pupillary mydriasis to at least 6mm, the participant was seated in the dark for 40 minutes with a black sleeping mask on their eyes to avoid any light reaching the eye. Appropriate corrective lenses were placed in the lens holder to account for participant's refraction for a viewing distance of 30 cm. Following dark adaptation, scotopic thresholds were measured monocularly in the study eye within the central 24° of the retina using 505nm (cyan) stimulus at 17 retinal locations, 4°, 8°, 12° eccentricity to the fovea with one added location at 6° inferior in the vertical meridian. This was repeated with a 625nm (red) stimulus. Participants were instructed to focus on the central red fixation light at all times and to press the response button when a light stimulus was seen. Fixation was monitored using an infrared camera built in the perimeter. The light stimulus was 1.73 ° in size (Goldmann size V) and was presented for 110 msec in a random order across all retinal locations using 3dB steps.

The test was done twice and the average of the two tests was used to calculate the mean retinal sensitivity. The reliability of the tests was also monitored using false positive and false negative assessment and therefore tests exceeding 30% errors in these outputs were excluded from analysis. Validation assessment for this instrument was performed and is described in detail in Chapter 3.

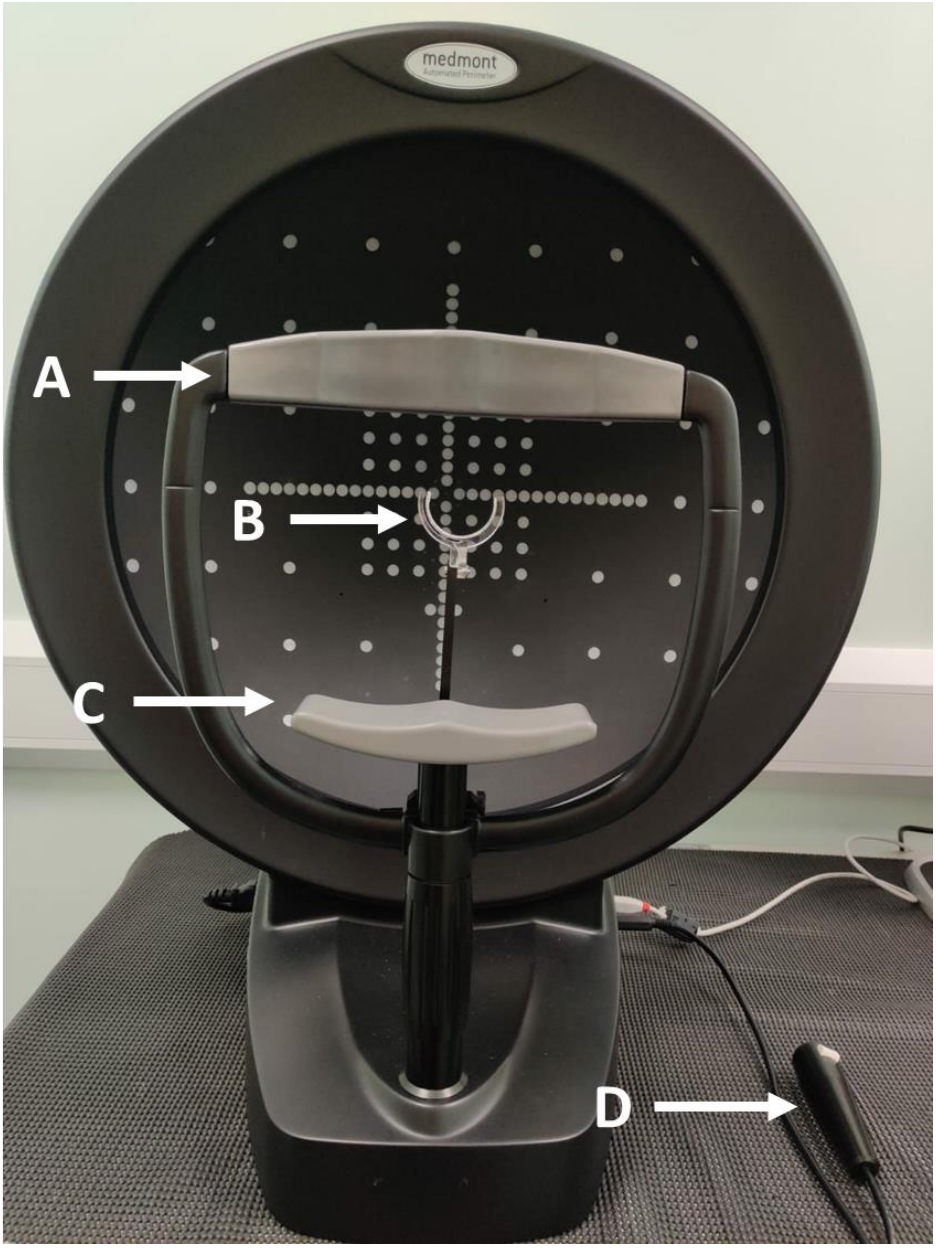

Figure 2.5. Photograph of Medmont dark adapted (DAC) perimeter (Medmont, Melbourne, Australia). The DAC consists of a black-coloured perimeter, with test locations covering a field up to 144° horizontally and 72° vertically. (A) forehead rest (B) lens holder (C) chin rest (D) response button.

2.2.7. Dark adaptometry

Dark adaptation was measured using the AdaptDx (MacuLogix, Hummelstown, PA), a computer-automated dark adaptometer. Patients were light adapted in the room for 3-5 minutes and pupil size was measured (at least 6mm) before being instructed to place their chin and forehead on the instrument, focusing on the red central light. Corrective lenses were inserted in the lens holder for a viewing distance of 30 cm. The test eye monocularly was exposed to the equivalent rhodopsin bleach of 82% with the delivery of a 505 nm photoflash subtending 4° and centred at 5° on the inferior vertical meridian (~ 0.80 ms duration). Light stimuli were presented for 200 ms using a 3-down/1-up staircase and thresholds were measured until the rod-intercept (time taken to recover 5.0 × 10−3 scotopic cd/m2 or 3.1 log units of stimulus attenuation) was reached, or up to 20 minutes post-bleach, which ever was shorter. The participant was instructed to press on the response button when light stimuli were seen and had 15 seconds rest between each threshold measurement. Fixation was monitored using an infrared camera and through the instrument's fixation error output. When fixation error exceeded 30%, the tests were deemed unreliable and were not included in the analysis. No validation assessment was performed for the dark adaptometry functional outcome as it has previously been shown to have good reliability with the diagnostic sensitivity of 88% and 100% specificity (Jackson and Edwards, 2008).

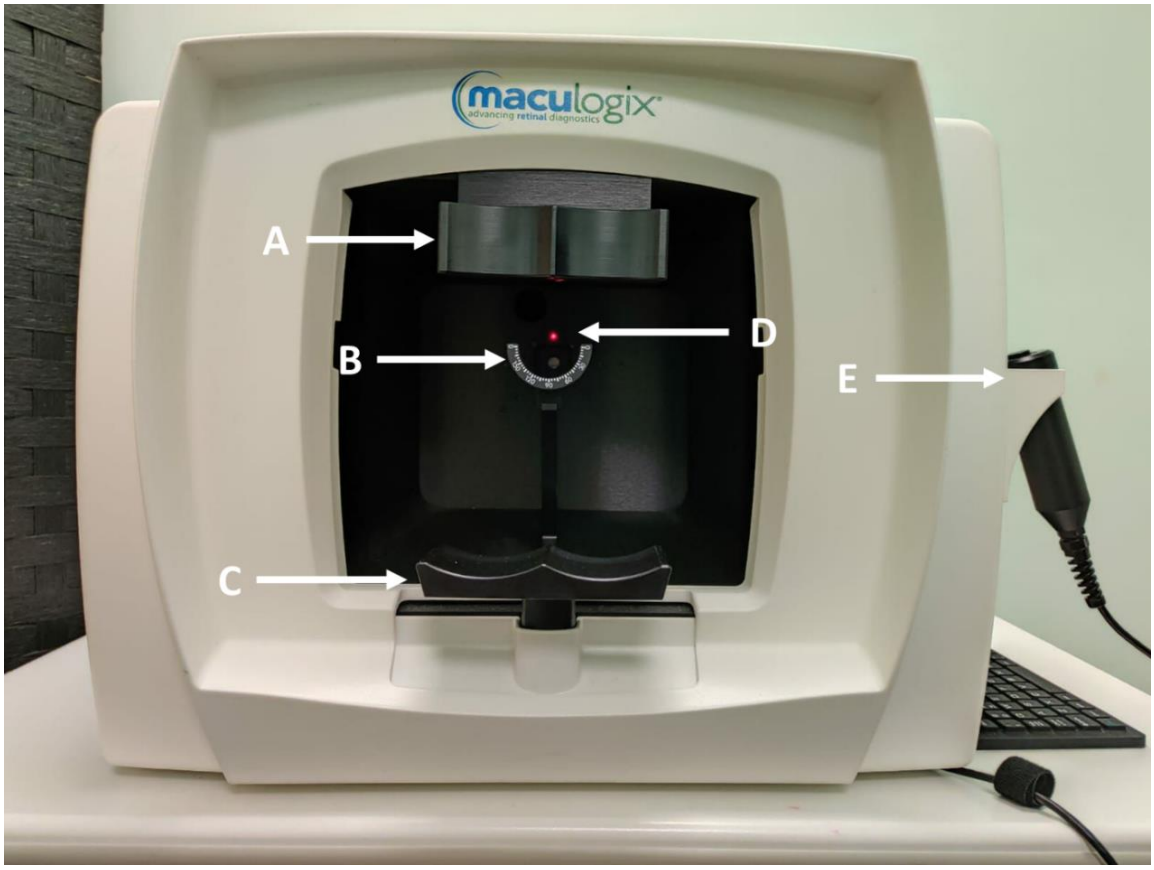

Figure 2.6. Photograph of dark adaptometer AdaptDx (MacuLogix, Pennsylvania, USA). (A) forehead rest (B) trial lens holder (C) chin rest (D) red fixation light (E) response button.

2.2.8. Fundus photography

Fundus photography was performed with the Topcon TRC-50DX (Topcon Corporation, Tokyo, Japan). The imaging protocol included a 35° stereo pair centred on the fovea and a fundus reflex photograph (anterior segment) to document media opacities.

2.2.9. OCT scan acquisition

SD-OCT and infrared imaging was acquired with a Spectralis HRA system (Heidelberg Engineering, Heidelberg, Germany). This instrument was used to acquire dense raster volume scans covering a retinal area of 6 x 6 mm^2 (20° x 20° visual angle) comprising of 49 parallel OCT B-scans for both eyes. Using the automated real-time (ART) function, 15 images were averaged for each scan. Images were taken by trained ophthalmic technicians from the NIHR Clinical Research Facility Unit at Moorfields Eye Hospital who also assessed the quality of the scan during its acquisition.

2.2.10. Retinal thickness and volumetric measurement

In the FUSCHIA study, for thickness analysis of retinal layers, volumetric SD-OCT data were initially automatically segmented with the Heidelberg Eye Explorer software, then each segmentation of the multiple B-scans was reviewed carefully and manually corrected if required. The following layer volumes and thicknesses were measured: the total retinal volume (TRV), total retinal thickness (TRT), retinal pigment epithelium drusen complex (RPEDC) thickness and volume, outer nuclear layer (ONL) thickness and volume. Enhanced depth imaging optical coherence tomography (EDI-OCT) was performed for all patients. Choroidal thickness (CT) was measured manually with the help of built-in callipers in the OCT software. Measurements were made from the outer portion of hyperreflective line corresponding to retinal pigment epithelium to the inner portion of hyperreflective zone corresponding to the choroidoscleral junction. They were obtained at the subfoveal point (SFCT), and also at a distance of 1500µm and 3000µm from the locus of measurement of SFCT in the nasal and temporal quadrants. The mean of these 5 values was taken as the mean CT and used for analysis.

For the 670nm trial, volumetric analysis was performed using the automated layer segmentation software (Orion, Voxeleron LLC). Retinal macular layer volumes were derived from a circular area around the foveal centre at 6mm in diameter.

The following layer volumes were derived: retinal nerve fibre layer (RNFL), ganglion cell-inner plexiform layer (GCIPL), inner nuclear layer (INL), ONL, photoreceptors (PR), retinal pigment epithelium-Bruch's membrane complex (RPE-BM) and total retinal volume (TRV). The data was generated by the software and exported to an EXCEL spreadsheet. The layer boundaries are shown for a typical participant's OCT scan in Figure 2.7.

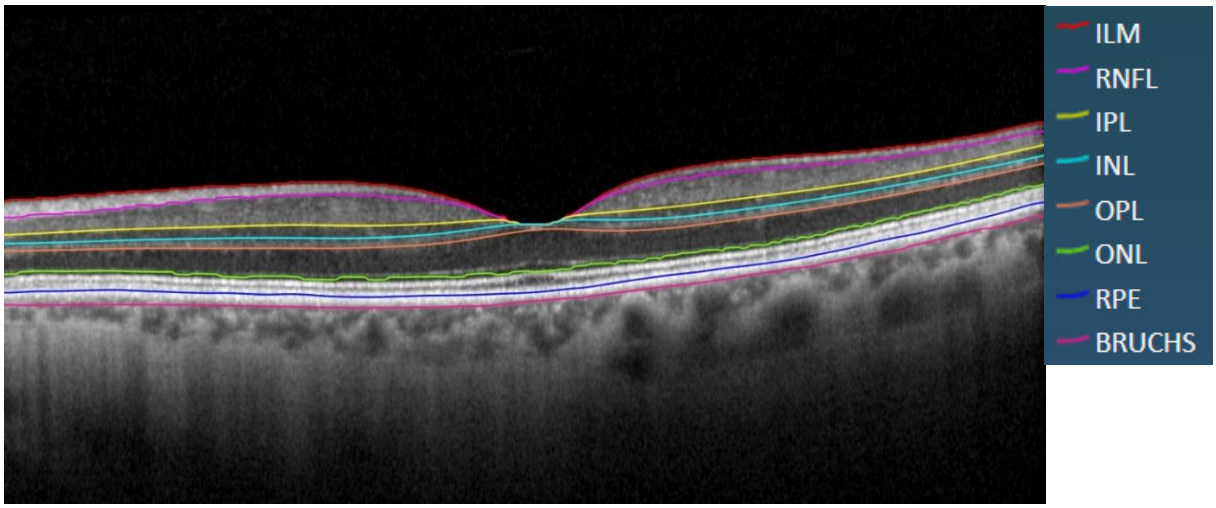

Figure 2.7. Retinal layer segmentation with layer boundaries in a normal eye as analysed by the Orion software. Retinal layers are defined as: Layers 1= ILM, 2=RNFL, 3= IPL, 4=INL, 5=OPL, 6=ONL, 7=RPE, 8=Bruch's membrane. Retinal layer volumes are defined as layers 1-2 = Retinal Nerve Fibre Layer (RNFL); layers 2-3 = Ganglion Cell Ganglion Cell and Inner Plexiform Layer (GCIPL); layers 3-4 = Inner Nuclear Layer (INL); layers 4-5 = Outer Plexiform Layer (OPL); layers 5-6 = Outer Nuclear Layer (ONL); layers 6-7 = Photoreceptor complex (PR); layers 7-8 = Retinal Pigment Epithelium-Bruch's Membrane complex (RPE-BM); layers 1-8 = Total Retinal Volume (TRV).

2.2.11. Grading

The colour fundus images were graded according to Beckman Initiative for AMD Classification System (Ferris *et al.,* 2013) by two independent graders both of which were familiar with the clinical presentation of AMD.

Where disagreement arose between the two graders, the results were adjudicated independently by the chief investigator for both studies (SS). SD-OCT and fundus autofluorescence and infrared reflectance (IR) images were also double graded for presence of any new areas of hyper or hypoautofluorescence and to detect the presence of SDDs.

The 5 stages of the Beckman classification scale were used to classify AMD severity (Table 1.1., Section 1.4.5). Subretinal drusenoid deposits do not appear in any AMD classification system, but have been classified by Zweifel et al., (2010) as shown in Table 2.1.

Table 2.1. Subretinal drusenoid deposit classification by Zweifel et al. (2010).

Stage	Description
1	diffuse deposition of granular hyperreflective material in the interdigitation zone
2	mounds of accumulated material sufficient to alter the contour of the inner segment- outer segment boundary
3	material with conical appearance and breaking through the inner segment-outer segment boundary

In both studies undertaken in this thesis, only participants with SDDs at stage 3 were included in the SDD group. The participants were categorized as the following for both studies:

- Healthy aging group: includes normal aging changes and early AMD as the latter has a low risk of conversion to late stages of AMD.
- iAMD without SDD group: includes all patients with more than one large drusen (>125 µm) with or without pigmentary abnormalities.
- iAMD with SDD group: in addition to above features, includes patients with presence of at least 5 visible SDD on OCT B-scans that interrupted the ellipsoid zone. Patients were included in this group if SDD was present in both IR imaging and on OCT B-scans
- Non-foveal atrophic AMD: non-foveal atrophy (at least 175 µm) with or without SDD (only for FUSCHIA study)

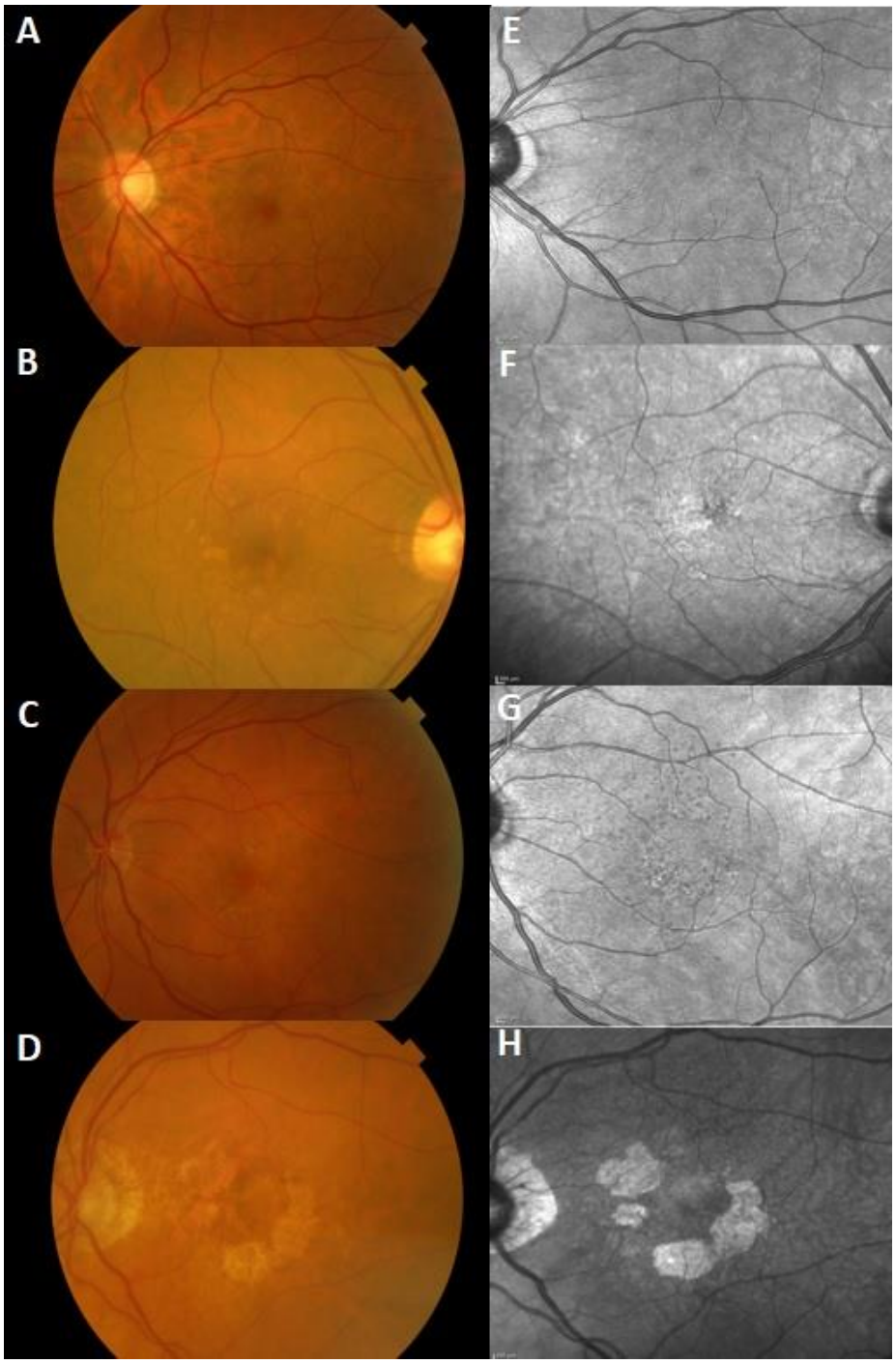

Figure 2.8. Representative fundus photographs and corresponding infrared images of different groups: healthy aging (A and E), intermediate AMD without SDD (B and F), intermediate AMD with SDD (C and G) and non-foveal GA (D and H).

Chapter 3. Scotopic thresholds on dark-adapted chromatic (DAC) perimetry in healthy aging and age-related macular degeneration

Evidence suggests that scotopic retinal sensitivity decreases with age but is further reduced in AMD disease and markedly in eyes with SDD. One of the objectives of this PhD was to investigate scotopic sensitivity using a novel dark-adapted chromatic perimeter (DAC) perimeter. This instrument was of particular interest as it allows to measure static and dynamic rod function without being subject to flooring and ceiling effects due to the increased dynamic testing range (0-75dB). This chapter begins by providing an overview of the instrument and its utility in the evaluation of scotopic sensitivity in AMD and aging. The chapter goes on to describe the effect of retinal eccentricity in various AMD phenotypes.

3.1. Introduction

Histological evidence of preferential rod susceptibility in aging and AMD (Curcio *et al.*, 1993; Curcio, Medeiros and Millican, 1996) has been corroborated by several functional studies (Eisner *et al.*, 1987; Sturr *et al.*, 1997; Jackson *et al.*, 1998; Jackson, Owsley and Mcgwin, 1999; Jackson and Owsley, 2000; Owsley *et al.*, 2000, 2001, 2014). These include psychophysical tests by dark adaptation that measures the rate of recovery of retinal sensitivity following exposure to a bright light, and by scotopic thresholds, which measure the minimum light that can be detected once the retina is fully adapted in the dark. Interpretation of earlier studies measuring scotopic sensitivity in aging is difficult due to different instrumentation and methodologies. Few studies reported no difference in scotopic function in individuals from the age of 19-20 to early 60s (Pulos, 1989; Hammond *et al.*, 1998). Pulos (1989) measured scotopic sensitivity at 6 temporal retinal loci (2.5° to 30°) across the horizontal meridian using a Goldmann-Weekers dark adaptometer whereas Hammond and colleagues (1998) only

assessed it at 6° nasally with a Maxwellian-view system. In contrast, Sturr et al., (1997) reported 0.39 log units change in aging using a custom-built perimeter at 10° in the temporal visual field. Additionally, a cross sectional study by Jackson and Owsley (2000) reported a diffuse and gradual decrease of mean scotopic sensitivity (27 loci across central 18°) obtained on a modified Humphrey Field Analyser (HFA; Zeiss Humphrey Systems, Dublin, CA) by 0.08 log units per decade, a decline twice as fast compared with photopic sensitivity in otherwise healthy retinae (Jackson and Owsley, 2000). Of interest, they found no variation in sensitivity by retinal eccentricity. Scotopic thresholds are further reduced in individuals with AMD particularly in the parafovea compared to age-matched controls (Steinmetz *et al.*, 1993; Jackson *et al.*, 2005). However, some individuals with varying AMD severity retain normal scotopic function (Owsley *et al.*, 2000).

Scotopic sensitivity can be measured by standard automated perimetry. It has traditionally been assessed using adapted perimetry systems including the HFA or subsequently, by scotopic microperimetry, such as the scotopic Nidek microperimeter (MP- 1S; Nidek Technologies, Padova, Italy) and the scotopic Macular Integrity Assessment (S-MAIA; CenterVue, Padova, Italy) using the in-built 4-2 staircase strategy (Jackson *et al.*, 1998; Jackson and Owsley, 2000; Owsley *et al.*, 2000; Crossland *et al.*, 2011; Nebbioso, Barbato and Pescosolido, 2014; Steinberg *et al.*, 2017). Although the S-MAIA has an improved dynamic range over the MP-1S, both of these devices have a limited dynamic range of luminance implicating floor and ceiling effects (Steinberg *et al.*, 2017).

More recently, a dark-adapted chromatic perimeter (DAC) was developed (Medmont Pty Ltd International, Victoria, Australia), with large dynamic range (0 -75dB for cyan stimulus, 0-50db for red stimulus) with 135-fixed test locations covering a field up to 144° horizontally and 72° vertically. This also allows the ability to perform two-color dark-adapted perimetry in which sensitivity to cyan (505 nm) is compared with sensitivity to a red (625 nm) stimulus at the same location further quantifying the integrity of rod function (Jacobson, Apathy and Parel, 1991; Bennett *et al.*, 2017).

The rods are highly sensitive to the 505nm (cyan) stimuli by at least 2 log units more than cones and insensitive to 625nm (red) stimuli. Therefore, a difference in thresholds between the cyan and red stimuli greater than 20db in dark-adapted healthy eyes is deemed to be rod mediated. A smaller difference between the two stimuli is indicative of rod dysfunction as the response is cone-mediated (Bennett *et al.*, 2017, 2019). Previous studies using this device have shown reduced scotopic function in individuals with iAMD, particularly in those with SDD, following dark adaptation for 20 minutes and using the 4-2 staircase strategy (Fraser *et al.*, 2016; Flynn, Cukras and Jeffrey, 2018; Tan, Guymer and Luu, 2018). However, as this instrument does not have an in-built normative perimetric database, the effect of aging has only been evaluated in one study to our knowledge (Bennett *et al.*, 2019). Although the test-retest repeatability has been previously published, it showed high variability of rod function (Tan, Guymer and Luu, 2018; Bennett et al., 2019; Uddin et al., 2020).

The work presented in this chapter sought to investigate whether taking the mean of retinal sensitivity and increasing decibel steps decreased this variability. Furthermore, spatial variation of scotopic sensitivity was examined, as only limited studies have investigated this across AMD severity phenotypes and none included the non-foveal atrophy stage.

3.2. Aims

The aims of this chapter were to investigate:

- Intrasession and intersession agreement in 9 healthy individuals.
- Effect of age on scotopic sensitivity in 20 healthy individuals.
- Comparison of 2 dB-step and 3 dB-step strategies were studied on five individuals with iAMD and five healthy age- matched individuals.
- Effect of retinal location on scotopic sensitivity (with cyan and red stimuli) in a total of 50 individuals consisting of 11 healthy individuals and 39 AMD participants with varying severity of AMD.

3.3. Methods

The study protocol for scotopic sensitivity testing with DAC perimeter and classification of AMD participants has been describe in chapter 2. Additional analysis used in this study is described below. Images were double graded by Dr Shruti Chandra (medical retina fellow) for the purpose of participant classification as per the Beckman Initiative for AMD Classification System.

3.3.1. Ethical approval

Participants were provided with a patient information sheet and informed consent was obtained from each participant. The study conformed to the standards set by the International Conference on Harmonization Guidelines for Good Clinical Practice and the tenets of the Declaration of Helsinki. The procedures were approved by the local ethics committee at University College London Research Ethics Committee (UCL Ethics Approval Application #: 11013/001) for young and older individuals. Participants from FUSCHIA study were also included, which was approved by the Camden and Kings Cross NRES Committee London REC 16/LO/1317.

3.3.2. Test-retest on healthy individuals

Intrasession reliability consisted of participants repeating the same assessment immediately; two consecutive tests were used as test 1 and test 2 for both stimuli. All tests were conducted at 3dB steps. For intersession test-retest repeatability, measurements were repeated on a separate visit at approximately 7 days (upto 14 days) from the initial visit. Intersession reliability was measured as the agreement between the average of the two tests carried out at the first visit against the average of the two consecutive tests at the second visit for both stimuli. The same group was used to evaluate the age-related changes in retinal sensitivity.

3.3.3. Effect of age on scotopic sensitivity

To assess the change in retinal sensitivity with age, results were pooled from 9 healthy participants from test-retest study and 11 healthy controls from the FUSCHIA trial using linear regression on the mean scotopic sensitivity from 20 healthy individuals with age ranging from 25 to 77.2 years.

3.3.4. Comparison of 2dB vs 3dB testing strategy

The 3dB step strategy was carried out and the 2dB strategy was performed either on the same day or within the next 2 days.

3.3.5. Effect of retinal location on scotopic sensitivity

Detailed protocol has been described in chapter 2. Briefly, participants were dark adapted for 40 minutes after which scotopic thresholds were measured monocularly in the study eye with a 505nm (cyan) and 625nm (red) stimuli at 17 retinal locations encompassing central 24 ° of the central retina. The mean retinal sensitivity was calculated with the average of two consecutive tests and pointwise sensitivities were computed to investigate whether a particular area of the retina was more vulnerable to scotopic dysfunction.

3.3.6. Statistical analysis

Statistical analyses were performed with GraphPad Prism (GraphPad, San Diego, CA). The normal distribution of the data was verified using the Shapiro-Wilk test. To examine the intrasession and intersession repeatability and accuracy in healthy aging, Bland-Altman plots, Intraclass correlation coefficient (ICC) and Spearman's correlation coefficient (r) were computed. Coefficient of repeatability (CoR) was calculated as 1.96 times the standard deviation (SD) of the difference between the two measurements. The agreement between 2 dB-step and 3 dB-step was also analysed similarly.

The relationship between age and mean retinal sensitivity was evaluated using linear regression analysis. Mean retinal sensitivity for both stimuli (and the difference between them) was calculated and compared among the study groups using Kruskal

Wallis test and post hoc multiple comparisons Dunn's uncorrected test. The significance level was set at $p< 0.05$.

3.4. Results

3.4.1. Test-retest variability in healthy aging

A total of 9 participants were recruited for the evaluation of repeatability for Medmont DAC perimeter: 5 younger (range 25.0 to 38.5 years) and 4 older participants (range 59 to 64 years). Bland-Altman plots were generated to assess agreement between assessments shown in Figure 3.1. Intraclass correlation coefficient (ICC) and Spearman correlation were calculated to quantify the observed agreement and is described in Table 1. The overall intrasession and intersession had excellent reliability (ICC > 0.90) and tests were highly correlated (Spearman r = 0.75 to 0.86). There was no definite indication of improvement in performance (learning effect) for both the cyan and red stimulus from the either first session (mean ± SD; 60.76 ± 3.58dB and 35.10 ± 2.26dB respectively) to the second (61.57± 2.28dB and 35.11± 2.73dB respectively) or from visit one (cyan 61.17 ± 2.89dB; red 35.14 ± 2.48dB) to visit two (cyan 61.31±2.88Db; red 35.36 ± 2.78dB) as illustrated in Figure 3.2. Therefore, this data suggests that scotopic thresholds measurement with Medmont DAC perimeter have high reproducibility.

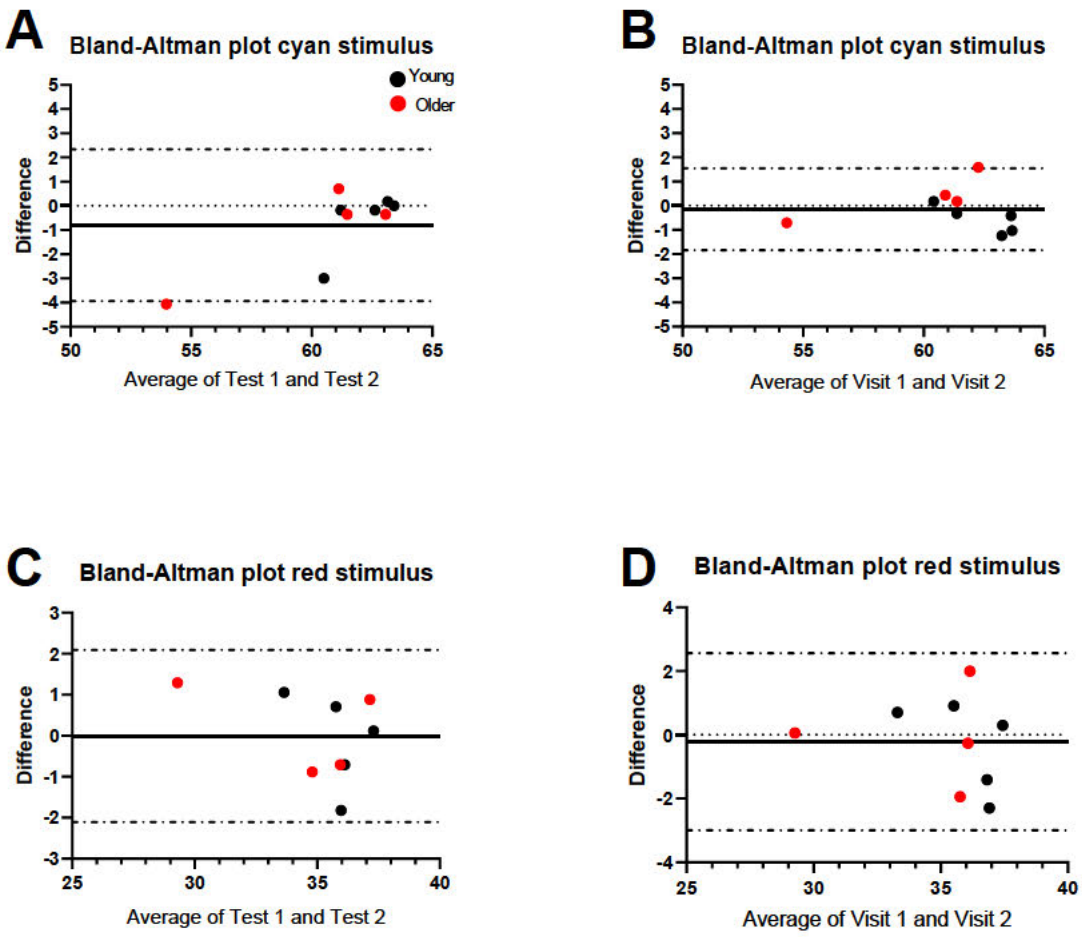

Figure 3.1. Bland-Altman plots with 95% limits of agreement (dashed lines) illustrating the difference in scotopic thresholds between intrasession measurements (A) cyan stimulus, (C) red stimulus, and between intersession measurements (B) cyan stimulus and (D) red stimulus. Black dotted line represents the mean between the two measurements. Black circles represent young participants (N=5) and red circles represent older participants (N=4).

Table 3.1. Intraclass correlation coefficient (ICC) with 95% confidence intervals, Spearman correlation (r) and coefficient of repeatability (CoR) for mean sensitivity (MS) and pointwise sensitivity (PWS).

Outcome	ICC	Lower confidence limit	Upper confidence limit	Spearman correlation coefficient (r)	p-value	CoR MS (dB)	CoR PWS (dB)
Intrasession (cyan)	0.91	0.64	0.98	0.83	0.008	3.35	5.96
Intersession (cyan)	0.98	0.91	0.995	0.85	0.006	1.62	4.47
Intrasession (red)	0.96	0.80	0.99	0.75	0.026	2.03	5.09
Intersession (red)	0.93	0.68	0.98	0.86	0.003	2.66	4.65
3db vs 2dB cyan	0.86	0.47	0.96	0.77	0.012	-	-
3db vs 2dB red	0.77	0.07	0.96	0.86	0.024	-	-

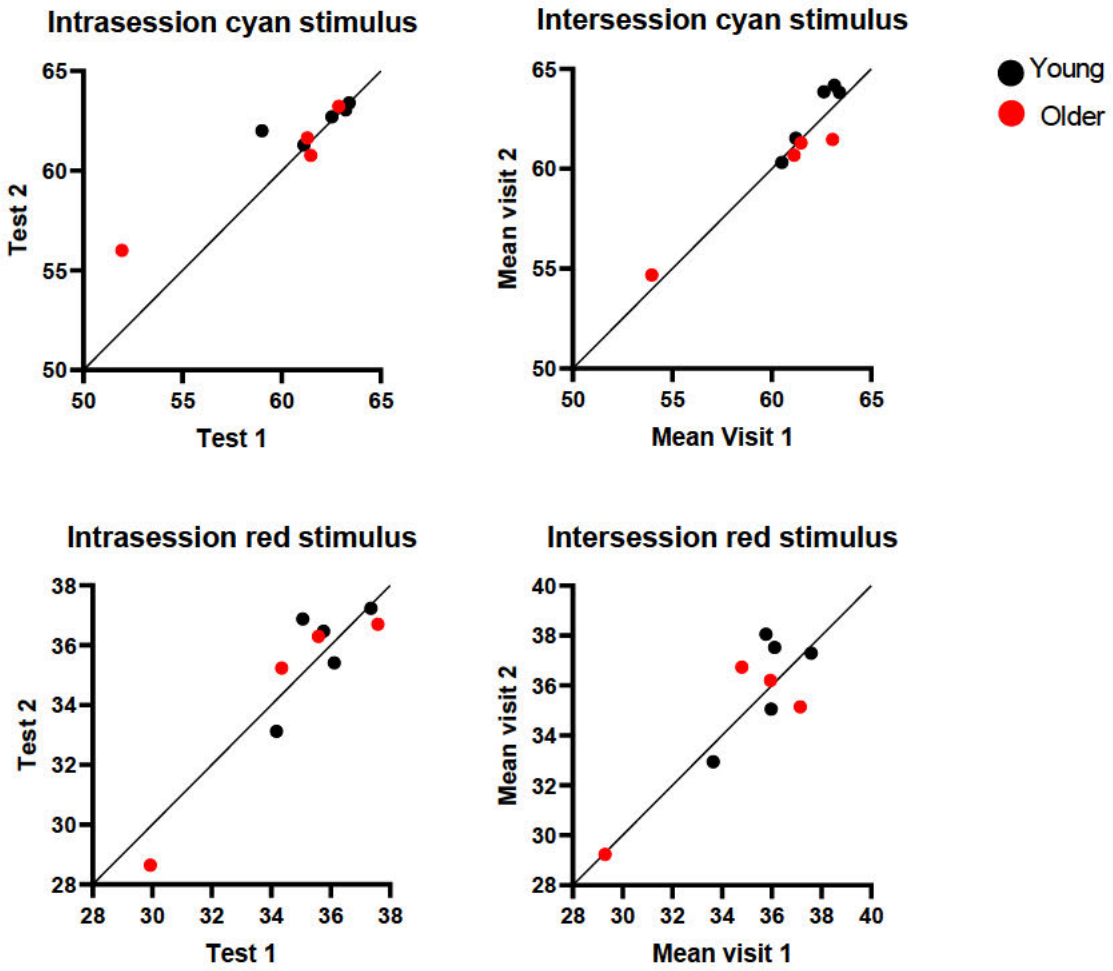

Figure 3.2. Scatter plots showing mean retinal sensitivity for both tests (cyan and red stimuli) within one session and between two visits (young participants N=5; older participants N=4). The dots above the line of identity indicate improvement in in the second session (test 2 or visit 2) and the dots below the line of identity indicate a decline in the second session.

3.4.2. Age-related change in retinal sensitivity

Change in retinal sensitivity with age was assessed in 20 healthy participants of various ages (range 25 to 77.2 years). Linear regression analysis revealed significant association between age and retinal sensitivity for the cyan stimulus (β coefficient= -0.126, R2 = 0.302, p=0.012) whereas no statistically significant association was found for the red stimulus (β coefficient= -0.088, R2 = 0.191, p=0.054).

3.4.3. Reliability between 2db and 3db step strategies

Five healthy controls and five AMD participants completed these tests. The reliability between the two strategies was assessed with Bland-Altman plots which revealed good agreement between both strategies. On average, 2dB step strategy measured 0.86 dB less than 3dB step strategy for the cyan stimulus and 1.23 dB less for the red stimulus. Both strategies were strongly correlated for the cyan ($r = 0.77$, $p = 0.012$) and red ($r = 0.86$, $p= 0.024$) stimuli as displayed in Figure 3.3. Intraclass correlation coefficient indicated good reliability (ICC > 0.75). These accuracy and reliability estimates are summarised in Table 3.1. The mean testing time for 2db and 3db steps for the cyan stimulus in healthy participants was 2.55 and 2.46 minutes respectively ($p=0.576$) and in AMD individuals, 2.13 and 1.95 minutes respectively ($p=0.066$). However, tests performed with red stimulus were significantly longer with 2db step than 3db step in the AMD group at an average test time of 2.19 and 1.95 minutes respectively ($p=0.042$). No statistical analysis was performed in the healthy participants for the red stimulus as only data for 2 participants was available.

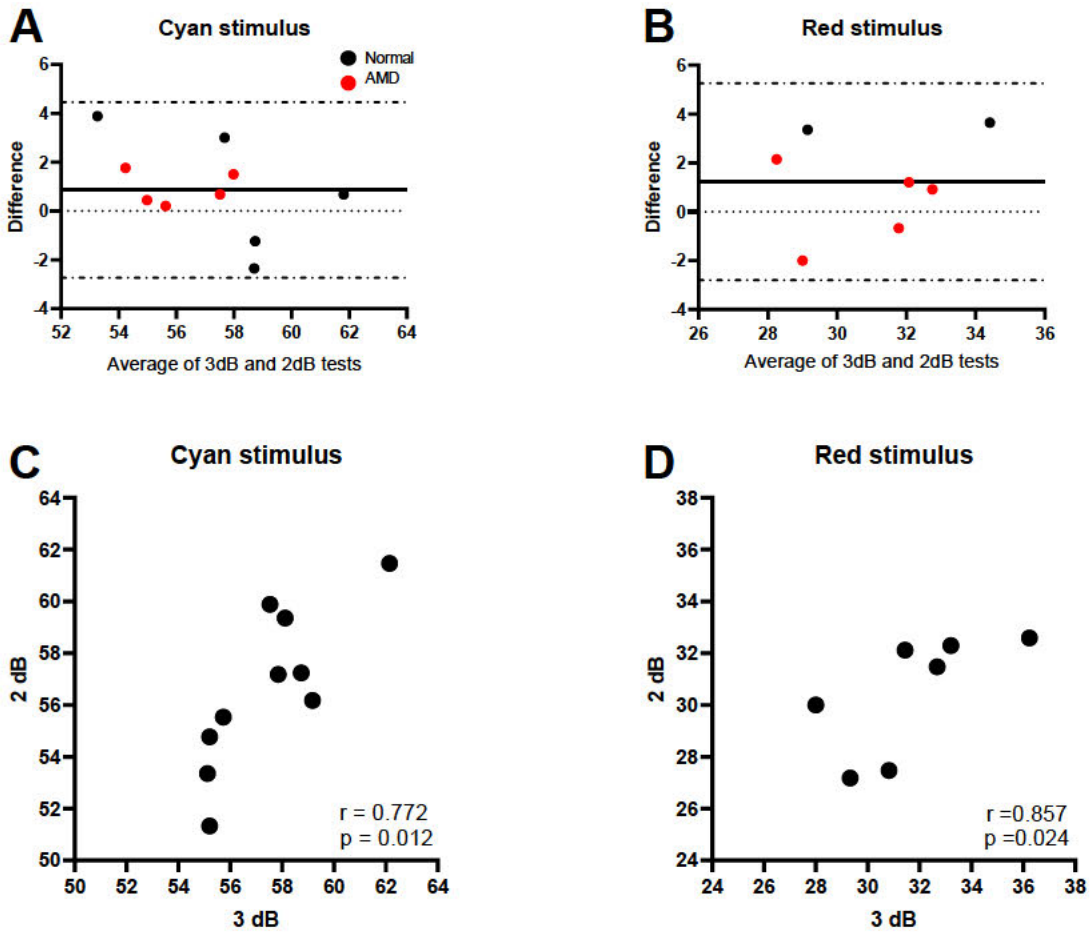

Figure 3.3. Bland-Altman plots with 95% limits of agreement (dashed lines) illustrating the difference in mean scotopic thresholds between 3dB and 2dB test strategies for both cyan (A) and red stimuli (B). Black dotted line represents the mean between the two measurements. Black circles represent healthy participants (N=5 for cyan stimulus and N=2 for red stimulus due to mechanical problems with the instrument) and red circles represent iAMD participants (N=5 for both stimuli). The bottom two graphs show the correlations between 3dB and 2dB stimulus for the cyan (C) and red (D) stimuli.

3.4.4. Retinal sensitivity in healthy aging and varying AMD severity groups

There was no statistically significant difference between either healthy controls and iAMD with no SDD nor between individuals with iAMD with SDD and those with non-foveal atrophy. Mean retinal sensitivity measured with cyan and red stimuli was significantly reduced in the non-foveal atrophic AMD group compared with healthy aging participants (cyan, p=0.003; red, p= 0.0004) and compared to those with iAMD no SDD (cyan, p=0.0008; red, p= 0.002).

Individuals with SDD had reduced rod function compared with healthy participants for both stimuli (cyan, p= 0.014; red, p=0.008). Participants with SDD also had lower thresholds when compared with iAMD without SDD (cyan, p=0.005; red, p=0.038). The difference between both stimuli was only statistically different between iAMD no SDD and participants with non-foveal atrophy (p=0.003). Significant differences in mean retinal scotopic thresholds are illustrated in Figure 3.4.

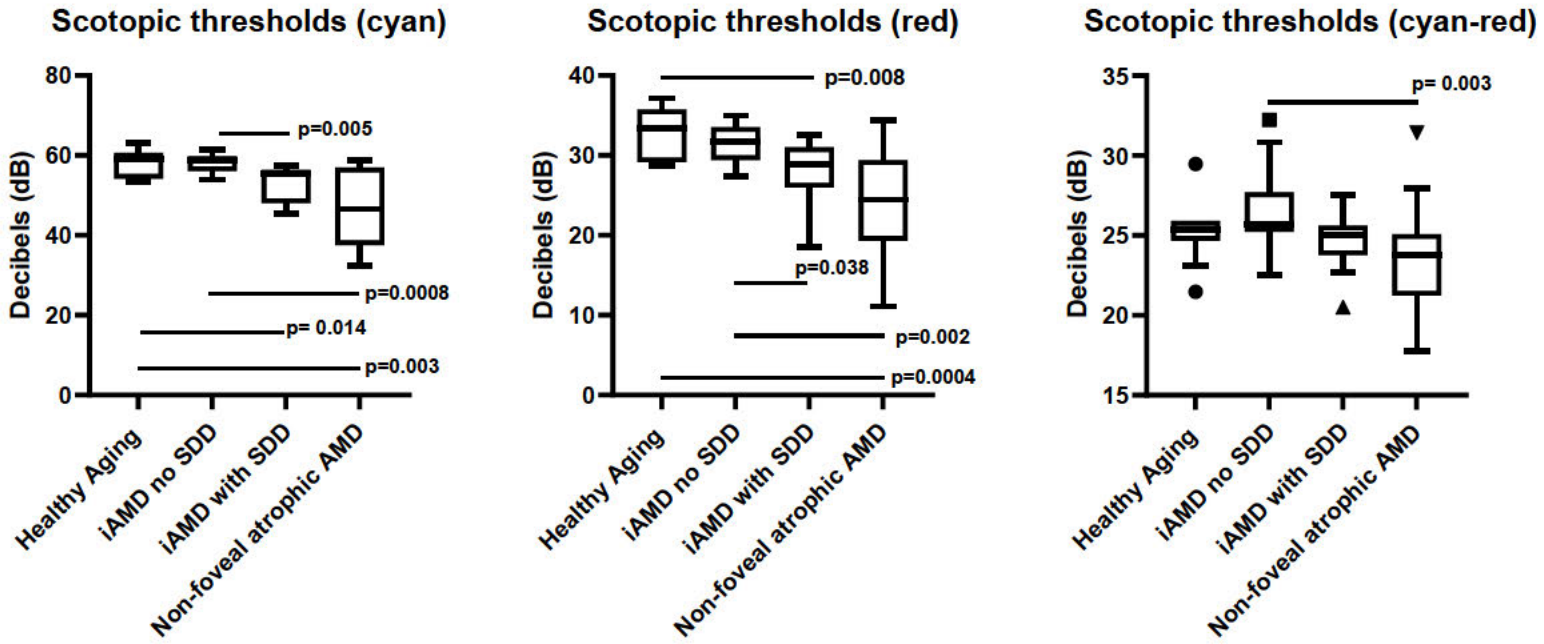

Figure 3.4. Boxplots for scotopic thresholds for cyan, red and difference between the two, showing distribution of data across graded AMD severities. Black circles, squares and triangles show outliers (where data exceeds distance from the median by 1.5 times the interquartile range). Significant differences between groups (P<0.05) are displayed.

Scotopic responses at specific loci for each AMD severity group are shown graphically in Figure 3.5. and described in Table 3.2-3.4. Using the principle of two-color dark-adapted perimetry, the range of threshold difference (cyan minus red) at each retinal location was calculated to determine whether the responses were mediated by rods, cones, or a mix of both. All data obtained was mediated by rods.

There was no statistically significant difference between healthy participants and participants with iAMD without SDD nor between subjects with iAMD with SDD and late AMD at any retinal location for both cyan and red stimuli. Relative to healthy aging group, participants with non-foveal atrophy had reduced thresholds at all locations apart from more peripheral locations (8° and 12° superior retina, 12° temporal and supero-temporally). Similarly, individuals with advanced disease also had lower thresholds across all retinal locations compared to iAMD without SDD except for 4 locations (8° temporal; 12° temporal, superior and supero-nasal retinal eccentricities). Individuals with SDD had lower thresholds within the central 4 degrees and more pronounced inferiorly, but no significant difference in the superior retina compared to healthy controls. A representative case is shown in Figure 3.6. Relative to individuals with iAMD no SDD, participants with SDD had reduced rod function at inferior and nasal eccentricities and 12° supero-temporal location, whereas no difference was found at temporal eccentricities. Pairwise comparisons between groups for all 17 retinal loci of both cyan and red stimuli with statistical significance are tabulated in Table 3.5.

Table 3.2. Mean scotopic thresholds to cyan stimuli across all retinal eccentricities per AMD severity group.

	Group, Mean ± SD			
Retinal eccentricity	**Healthy aging (N = 11)**	**iAMD no SDD (N = 17)**	**iAMD with SDD (N = 11)**	**Late AMD (N = 11)**
4° superior	55.05 ± 5.58	55.74 ± 6.05	50.27 ± 6.07	42.64 ± 14.93
8° superior	57.27 ± 4.82	59.18 ± 3.09	55.18 ± 4.99	51.64 ± 7.60
12° superior	58.82 ± 4.03	59.26 ± 3.98	57.77 ± 4.29	53.95 ± 9.42
12° supero-nasal	58.14 ± 3.91	59.53 ± 2.85	55.86 ± 5.75	53.68 ± 9.45
12° supero-temporal	58.86 ± 3.11	59.71 ± 2.49	55.73 ± 5.27	56.27 ± 4.18
4° inferior	57.59 ± 5.51	57.06 ± 2.64	46.73 ± 7.28	41.27 ± 13.36
6° inferior	58.86 ± 4.80	58.38 ± 3.19	48.64 ± 7.24	43.73 ± 11.75
8° inferior	60.18 ± 4.94	58.38 ± 2.49	50.82 ± 8.20	46.59 ± 11.04
12° inferior	57.05 ± 8.89	59.62 ± 2.49	54.91 ± 4.45	51.77 ± 8.45
12° infero-nasal	57.59 ± 4.64	58.82 ± 2.54	54.09 ± 4.75	46.05 ±12.28
12° infero-temporal	58.05 ± 4.32	58.56 ± 3.08	54.77 ± 5.10	51.50 ± 7.53
4° nasal	57.41 ± 4.04	56.62 ± 4.08	47.95 ± 9.66	40.55 ± 20.90
8° nasal	58.50 ± 4.60	59.44 ± 2.84	53.82 ± 7.21	42.77 ± 15.04
12° nasal	55.45 ± 5.56	55.12 ± 4.69	47.68 ± 8.36	39.77 ± 11.92
4° temporal	57.68 ± 5.67	54.76 ± 6.26	50.68 ± 8.73	44.00 ± 12.35
8° temporal	59.36 ± 3.96	58.03 ± 2.48	56.14 ± 4.42	51.91 ± 8.51
12° temporal	59.59 ± 3.56	58.85 ± 2.63	56.68 ± 5.59	54.36 ± 8.60

Table 3.3. Mean scotopic thresholds to red stimuli across all retinal eccentricities per AMD severity group.

	Group, Mean ± SD			
Retinal eccentricity	Healthy aging (N = 11)	iAMD no SDD (N = 17)	iAMD with SDD (N = 11)	Late AMD (N = 11)
4° superior	32.18 ± 3.39	29.41 ± 5.77	26.23 ± 3.47	20.25 ± 8.13
8° superior	33.41 ± 3.75	32.24 ± 3.53	30.32 ± 2.80	27.45 ± 6.30
12° superior	32.77 ± 3.34	32.59 ± 2.93	30.32 ± 3.70	28.68 ± 5.67
12° supero-nasal	32.68 ± 3.45	33.12 ± 2.32	30.82 ± 4.42	28.45 ± 5.95
12° supero-temporal	32.64 ± 3.64	33.38 ± 1.68	30.18 ± 4.52	29.09 ± 5.20
4° inferior	31.27 ± 3.90	30.82 ± 3.51	24.18 ± 6.13	19.80 ± 10.66
6° inferior	33.18 ± 4.81	31.53 ± 3.75	24.23 ± 8.87	20.00 ± 10.69
8° inferior	33.91 ± 4.61	31.88 ± 3.94	27.95 ± 6.31	23.77 ± 8.29
12° inferior	31.95 ± 8.00	33.12 ± 4.25	31.05 ± 4.33	26.05 ± 8.88
12° infero-nasal	33.73 ± 4.34	31.44 ± 3.74	28.95 ± 5.94	23.50 ± 8.93
12° infero-temporal	33.05 ± 4.04	31.97 ± 3.60	29.41 ± 5.44	26.41 ± 7.65
4° nasal	32.50 ± 3.22	30.38 ± 2.19	26.09 ± 5.70	20.25 ± 11.56
8° nasal	34.14 ± 3.18	33.03 ± 2.54	29.23 ± 5.96	21.36 ± 10.92
12° nasal	29.05 ± 5.48	29.15 ± 4.45	22.77 ± 7.75	20.35 ± 6.54
4° temporal	32.45 ± 3.88	28.62 ± 4.89	26.45 ± 4.96	21.68 ± 9.72
8° temporal	33.95 ± 4.37	31.44 ± 3.78	30.05 ± 4.04	26.23 ± 8.24
12° temporal	33.59 ± 4.30	31.26 ± 3.61	30.32 ± 3.99	27.86 ± 7.69

Table 3.4. Mean scotopic thresholds difference (cyan-red) stimuli across all retinal eccentricities per AMD severity group.

	Group, Mean ± SD			
Retinal eccentricity	**Healthy aging (N = 11)**	**iAMD no SDD (N = 17)**	**iAMD with SDD (N = 11)**	**Late AMD (N = 11)**
4° superior	22.86 ± 4.20	26.32 ± 3.71	24.05 ± 4.80	24.65 ± 10.30
8° superior	23.86 ± 4.50	26.94 ± 3.22	24.86 ± 5.43	24.18 ± 3.31
12° superior	26.05 ± 4.06	26.68 ± 3.22	27.45 ± 3.69	25.27 ± 5.88
12° supero-nasal	25.45 ± 3.83	26.41 ± 2.02	25.05 ± 3.42	25.23 ± 4.80
12° supero-temporal	26.23 ± 3.13	26.32 ± 2.65	25.55 ± 5.62	27.18 ± 4.81
4° inferior	26.32 ± 4.07	26.24 ± 3.94	22.55 ± 2.79	23.75 ± 5.81
6° inferior	25.68 ± 3.70	26.85 ± 2.93	24.41 ± 5.40	23.73 ± 5.69
8° inferior	26.27 ± 2.81	26.50 ± 2.90	22.86 ± 2.28	22.82 ± 4.71
12° inferior	25.09 ± 2.81	26.50 ± 4.53	23.86 ± 2.84	25.73 ± 3.19
12° infero-nasal	23.86 ± 2.65	27.38 ± 3.40	25.14 ± 4.27	22.55 ± 5.70
12° infero-temporal	25.00 ± 2.58	26.59 ± 2.34	25.36 ± 3.32	25.09 ± 1.67
4° nasal	24.91 ± 2.21	26.24 ± 3.32	21.86 ± 5.22	22.95 ± 11.77
8° nasal	24.36 ± 3.86	26.41 ± 2.98	24.59 ± 4.40	21.41 ± 7.13
12° nasal	26.41 ± 6.58	25.97 ± 3.35	24.91 ± 3.16	20.80 ± 7.48
4° temporal	25.23 ± 4.30	26.15 ± 5.97	24.23 ± 7.34	22.32 ± 7.60
8° temporal	25.41 ± 4.03	26.59 ± 3.65	26.09 ± 2.43	25.68 ± 5.30
12° temporal	26.00 ± 2.83	27.59 ± 3.42	26.36 ± 3.51	26.50 ± 4.24

Table 3.5. Pairwise comparisons between groups for all 17 retinal loci to both cyan and red stimuli (unpaired, non-parametric Kruskal Wallis test with post hoc Dunn's uncorrected test)

Retinal eccentricity	Stimuli	Healthy aging vs iAMD no SDD	Healthy aging vs iAMD with SDD	Healthy aging vs Late AMD	iAMD no SDD vs iAMD with SDD	iAMD no SDD vs Late AMD	iAMD with SDD vs Late AMD
4° superior	cyan	0.708	0.111	**0.039**	**0.033**	**0.008**	0.634
	red	0.197	**0.005**	**0.0002**	0.067	**0.004**	0.309
8° superior	cyan	0.293	0.382	0 095	**0.044**	**0.004**	0.427
	red	0.467	**0.029**	**0.007**	0.094	**0.025**	0.604
12° superior	cyan	0.594	0.797	0 208	0.414	0 055	0.316
	red	0.972	0.171	**0.048**	0.123	**0.027**	0.544
12° supero-nasal	cyan	0.476	0.365	0 272	0.087	0 055	0.848
	red	0.745	0.302	**0.034**	0.144	**0.008**	0.275
12° supero-temporal	cyan	0.517	0.160	0.156	**0.028**	**0.027**	0.988
	red	0.487	0.220	0.157	**0.041**	**0.024**	0.852
4° inferior	cyan	0.870	**0.001**	**0.0003**	**0.0005**	**0.0001**	0.725
	red	0.900	**0.006**	**0.002**	**0.004**	**0.001**	0.707
6° inferior	cyan	0.793	**0.001**	**0.0004**	**0.0009**	**0.0003**	0.752
	red	0.437	**0.004**	**0.0005**	**0.016**	**0.002**	0.572
8° inferior	cyan	0.600	**0.004**	**0.0006**	**0.009**	**0.001**	0.552
	red	0.242	**0.011**	**0.0006**	**0.010**	**0.010**	0.390
12° inferior	cyan	0.572	0.056	**0.026**	**0.008**	**0.003**	0.746
	red	0.879	0.223	**0.024**	0.234	**0.020**	0.298
12° infero-nasal	cyan	0.593	0.085	**0.006**	**0.015**	**0.0003**	0.293
	red	0.210	**0.047**	**0.0006**	0.351	**0.011**	0.146
12° infero-temporal	cyan	0.627	0.181	**0.029**	0.050	**0.004**	0.398
	red	0.485	0.084	**0.010**	0.228	**0.034**	0.404
4° nasal	cyan	0.533	**0.008**	**0.011**	**0.022**	**0.029**	0.918
	red	0.135	**0.002**	**0.0006**	0.054	**0.020**	0.682
8 °nasal	cyan	0.503	0.086	**0.004**	**0.010**	**0.0001**	0.240
	red	0.410	**0.012**	**0.0001**	0.052	**0.0008**	0.196
12° nasal	cyan	0.950	0.071	**0.002**	**0.040**	**0.0005**	0.189
	red	0.851	**0.030**	**0.002**	**0.028**	**0.002**	0.347
4° temporal	cyan	0.301	**0.035**	**0.002**	0.197	**0.017**	0.323
	red	0.074	**0.009**	**0.0004**	0.273	**0.032**	0.344
8° temporal	cyan	0.315	0.090	**0.009**	0.389	0 059	0.353
	red	0.174	0.051	**0.004**	0.432	0 069	0.349
12° temporal	cyan	0.517	0.172	0 055	0.392	0.143	0.580
	red	0.224	0.094	**0.026**	0.529	0 218	0.585

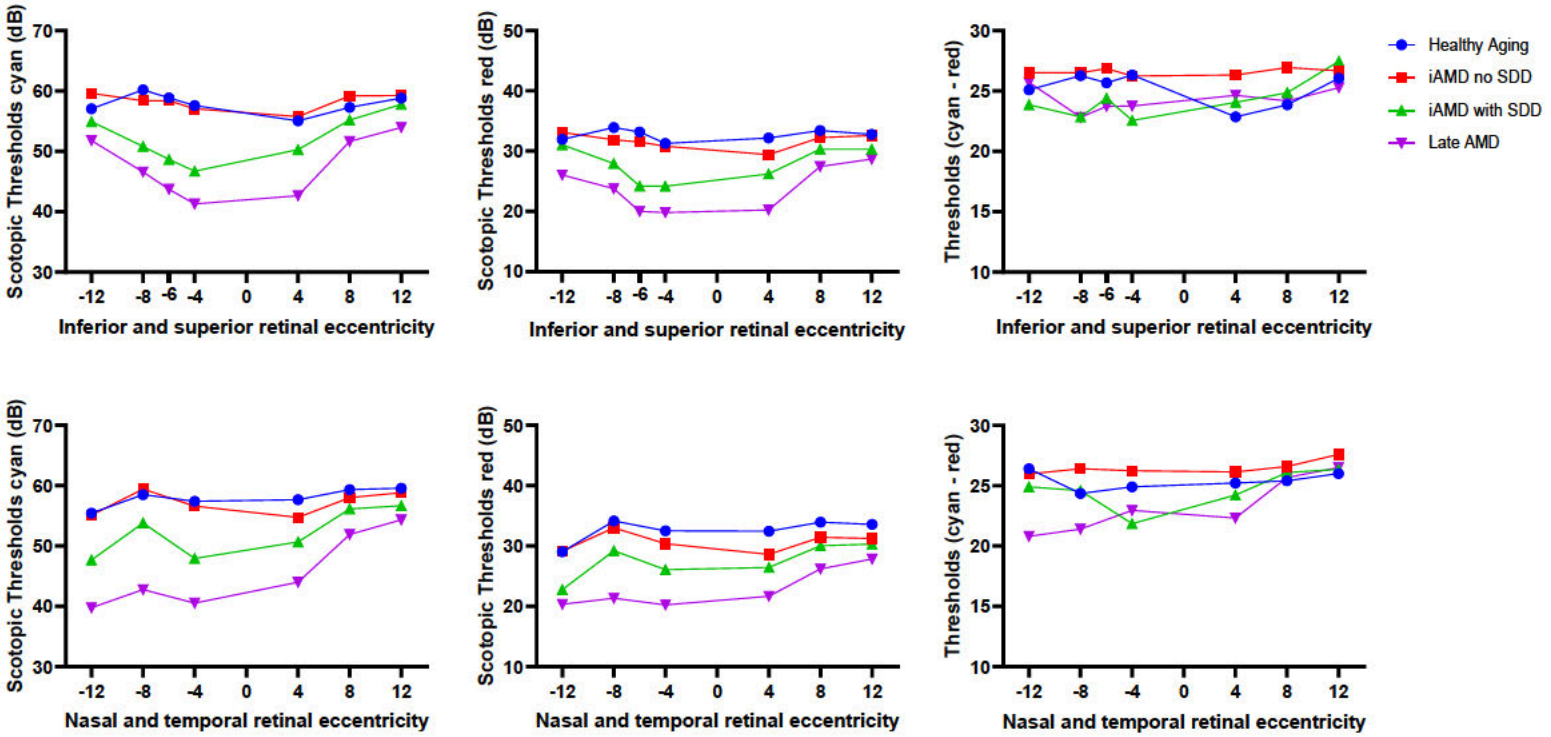

Figure 3.5. Scotopic thresholds at 4°, 8° and 12° eccentricity superior, inferior, temporal and nasal to the fovea) plus one further location at 6° inferior in the vertical meridian are shown for cyan and red stimulus. Four additional locations covering 12° eccentricities in all the four retinal quadrants not shown for illustrative purposes. The panels on the right show the difference between cyan and red thresholds. Negative eccentricities correspond to the inferior and nasal retina, positive eccentricities to the superior and temporal retina. Healthy aging (N= 11), iAMD no SDD (N=17), iAMD with SDD (N=11) and Late AMD (N=11).

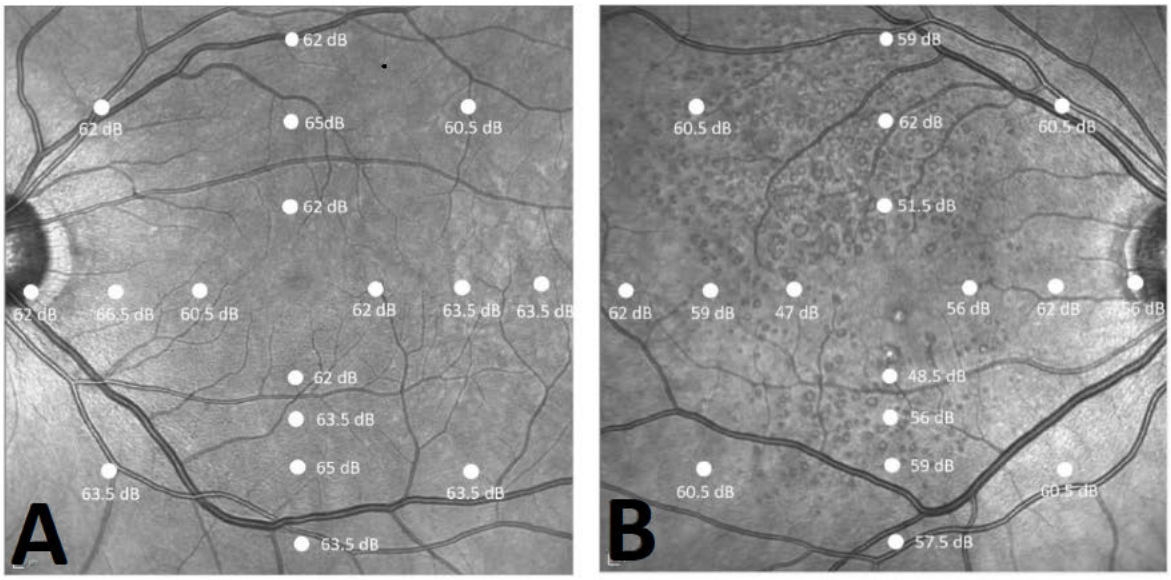

Figure 3.6. Examples of pointwise sensitivities in a participant with a healthy retina (A) and iAMD with SDD (B) with the scotopic thresholds topographically displayed over the infrared image across all 17 loci shown (4°,8°,12° eccentricities nasal, temporal, superior and inferior to the fovea and one additional location at 6° inferiorly). The sensitivities were decreased in the paracentral loci compared to peripheral loci in panel B compared to panel A.

3.5. Discussion

This study demonstrated that it is feasible to utilize 3db-step strategy which is comparable to the commonly used 2 dB step strategy (4-2 staircase) with Medmont DAC perimeter. Both strategies were strongly correlated, and ICC indicated good reliability. The mean scotopic thresholds obtained via 3 dB strategy (58.4 ± 2.5dB) yielded comparable values to the 2 dB strategy for the healthy cohort reported by Bennett and colleagues (2019) in older participants (55.7 ± 4.7dB). Although current perimetric strategies appear to be at near optimal levels, 3 dB strategy can reduce the mean number of trials (number of stimulus presentations) to reach optimal threshold, consequently reducing the testing time. Present findings did not show statistically significant difference in the length of tests conducted between 2db and 3db for the cyan stimulus despite the trend of decreasing time with 3db step strategy. However, tests performed with 3 dB-step with the red stimulus were shorter than 2 dB step.

Therefore, 3 dB test strategy is likely to be more efficient for wider retinal eccentricity testing as opposed to central macular testing without compromising accuracy.

The overall intrasession and intersession for mean retinal sensitivity had excellent reliability (ICC > 0.90), tests were highly correlated (Spearman r = 0.75 to 0.86) and there was no significant learning effect between sessions in our cohort in health aging. Our intrasession pointwise sensitivity CoR for both stimuli (cyan 5.96db and red 5.09 dB) was lower than the controls in the study by Tan and colleagues (2018b) cyan: 8.4 dB, red: 6.7 dB and comparable to the central PWS of 5.4 dB by Bennett and colleagues (2019) for the 505nm stimulus. Our CoR between visits (cyan: 4.47 dB, red: 4.65 dB) was lower than Tan and colleagues (2018b) with cyan: 8.2 dB and red 6.2 dB and by Bennett and colleagues (2019) of 6.0 dB in our respective healthy cohorts. It was also lower than the overall CoR of 7.2 dB by Uddin et al. (2020) which comprised of 3 controls, 4 iAMD and 5 patients with SDD. Therefore, taking the average of two of retinal sensitivity measurements between visits improves coefficient of repeatability and the evaluation of scotopic sensitivity with Medmont DAC perimeter showed high reproducibility. Current results also validated previous findings on the effect of aging in participants with normal retina on DAC perimetry. Bennett and colleagues (2019) found an overall decrease in scotopic thresholds of 1.2 dB in aging. Similarly, we observed 1.26 dB decrease per decade.

The principle of two-color dark-adapted perimetry, which exploits the differential spectral sensitivities between rods and cones, was used to determine whether the responses were mediated by rods, cones, or a mix of both (Jacobson, Apathy and Parel, 1991). Based on this approach, all data obtained in our study was mediated by rods as the difference was greater than 20 dB. Current study also shows particularly reduced mean retinal sensitivity in individuals with SDD which is in concordance with previous reports (Flynn, Cukras and Jeffrey, 2018; Tan, Guymer and Luu, 2018). Interestingly, there was no difference in mean scotopic sensitivity in individuals with iAMD without SDD when compared with healthy controls. This finding is substantiated by Tan et al. (2018a), where they found that scotopic thresholds were indistinguishable between controls and iAMD without SDD when there was no preceding photobleach.

This is in contrast to other studies that have shown reduced thresholds following dark adaptation between these two groups (Flynn, Cukras and Jeffrey, 2018; Fraser *et al.*, 2016). However, this is likely due to prior exposure of pre-adapting light and shorter length of time of dark adaptation of 20 minutes in both of these studies whereas we used 40 minutes. It can take 30 to 45 minutes for rods to reach to their maximum sensitivity for healthy dark-adapted eyes and therefore some AMD individuals may still have been dark adapting at 20 minutes (Tipton, 1984). In addition, the duration and intensity of the preadapting light affects the level of thresholds (Hecht and Shlaer, 1936). Therefore, the photobleach preceding scotopic thresholds measurements in other studies may have inadvertently affected absolute scotopic sensitivity. Hence, under optimal conditions, rod scotopic sensitivity in iAMD eyes is similar to healthy eyes.

Current findings were also unable to elicit any difference in mean scotopic sensitivity between individuals with SDD and those with non-foveal atrophy, suggesting that the presence of SDD is an indicator of severe disease with functional outcome comparable to those with non-foveal atrophy. Considering present analysis of pointwise sensitivities, there was an overall decrease in sensitivity for almost all locations for atrophic individuals compared with individuals with healthy retinae and those with iAMD without SDD, but no significant difference at any retinal loci when compared to individuals with iAMD with SDD. Consistent with our mean retinal sensitivity results, there was no difference in pointwise sensitivity at any retinal location between iAMD without SDD and healthy controls. Importantly, these findings suggest that the overall loss of rod function is not directly correlated with drusen load or extent of GA.

Present data also revealed that individuals with SDD not only have reduced thresholds centrally but are more depressed at inferior and nasal retinal locations compared with controls and iAMD without SDD. This is an interesting finding, as SDD are usually more abundant in the superior perifovea (Curcio *et al.*, 2013). In addition, scotopic thresholds were able to differentiate iAMD eyes with and without SDD in 11 out of 17 loci. Thus, the presence of SDD is a structural sign of rod dysfunction which is independent of SDD location.

Strengths of our study include methodical and consistent procedures and validated retinal grading by two graders with multimodal imaging. Dark adaptation of 40 minutes allowed for rod photoreceptors to reach maximum sensitivity. We also included the non-foveal GA phenotype which has not been investigated in similar studies previously. The main limitation of this study was the small sample sizes for all groups. Despite this, statistically significant rod function deficits were found in SDD eyes comparable to non-foveal atrophic eyes. A larger AMD cohort and longitudinal analysis would further validate our findings.

In conclusion, this study demonstrated the feasibility of using 3 dB step protocol for measuring scotopic thresholds and using the mean of two tests reduced intrasession and intersession variability. Eyes with SDD have reduced rod function compared to iAMD without SDD and healthy eyes, but similar to eyes with non-foveal atrophy. Results from this study highlight that rod dysfunction is not directly correlated with drusen load, SDD location or extent of GA.

Chapter 4. FUSCHIA Study: Function-Structure Correlation in Healthy Aging and AMD

Currently, there are no preventive or treatment options for intermediate AMD. The lack of validated functional endpoints is a challenge in conducting clinical trials for this condition. Phenotypic heterogeneity of this disease also increases the difficulty of developing targeted treatments. The overarching objective of this PhD was to carry out cross-sectional evaluation, alongside the longitudinal assessment (Chapter 6), to identify functional and structural trial outcome measures as biomarkers for AMD severity, including eyes with SDD. This chapter describes the baseline results from the investigation of the prognostic capacity of a series of psychophysical tests, all of which have shown some evidence supporting their association to AMD severity.

4.1. Introduction

AMD is clinically characterised by the presence of drusen (Feigl, 2009). However, subretinal drusenoid deposits (SDD) have also been recognized as a distinct morphological feature that confers increased risk of developing advanced AMD (Finger *et al.*, 2014; Steinberg *et al.*, 2015). Over a quarter of people with intermediate AMD have SDD on multimodal imaging (Wu *et al.*, 2016). Clinical trials that evaluated interventions to prevent progression of intermediate AMD to advanced AMD had not stratified the eligibility criteria into eyes with and without SDD (Guymer *et al.,* 2019; Yehoshua *et al.,* 2014). For example, secondary analysis of the LEAD trial showed that eyes with iAMD with SDD did not benefit from nanosecond laser intervention suggesting that future trials in intermediate AMD should re-define the selection criteria (Guymer *et al.*, 2019). A major challenge in conducting preventive trials in AMD is the lack of validated functional endpoints. BCVA is the only validated functional endpoint that is accepted by regulatory authorities as a clinical trial endpoint in retinal diseases.

However, changes in BCVA do not necessarily parallel disease progression from intermediate to late AMD or the progression of non-central geographic atrophy (GA) to fovea-involving GA as BCVA is only affected when the disease involves the fovea (Beirne *et al.*, 2006; Hogg and Chakravarthy, 2006). Therefore, there is an unmet need to capture other changes in retinal function that are experienced by subjects that may be used as an independent endpoint or can be correlated with anatomical changes at the macula so that the structural change can be used as a surrogate marker of functional decline (Dimitrov *et al.*, 2011). Developing and validating these endpoints will enable the evaluation of novel therapeutic agents to prevent or delay the progression to foveal involving advanced AMD better, a disease of paramount public health importance with significant societal burden (Beirne *et al.*, 2006).

There are limited studies evaluating visual function changes in aging and eyes that are phenotypically at risk of development or progression of advanced AMD (Tan *et al.,* 2019; Finger *et al.,* 2013; Nguyen *et al*., 2018; Rogala *et al.,* 2015). With the recent evidence that photoreceptors may be affected early in some eyes with intermediate AMD, it is also important to stratify intermediate AMD into sub-groups based on visual function changes (Schmitz-Valckenberg *et al*., 2010; Tan, Guymer, and Luu 2018). There is strong evidence that rod dysfunction is affected in eyes with intermediate AMD, particularly in those with SDD (Flynn, Cukras and Jeffrey, 2018; Saßmannshausen *et al.*, 2020).

The work in this chapter sought to evaluate functional and structural differences and their correlations between varying AMD severity groups when eyes with intermediate AMD and those with SDD are considered a separate phenotype.

4.2. Aims

The aims of this chapter were to:

- Determine visual function deficits in aging and people who are phenotypically at risk of developing or progressing to advanced AMD. The primary objective of this study was to evaluate visual function deficit in low luminance (ETDRS letters). Secondary objectives included investigation of visual function deficit in photoreceptor function as per rod-mediated tests and visual acuities, pan-retinal cone function with 28.3 Hz flicker on RETeval device.
- Investigate structural deficits in healthy aging eyes and those with varying AMD severity.
- Explore the relationship between the functional and structural outcome measures.

4.3. Methods

Detailed methodology relating to the functional and structural assessments along with patient classification is described in detail in Chapter 2. The following clinical assessments were completed at baseline visit:

Slit lamp examination (anterior and posterior segment)
Medical history
Vital signs (blood pressure, height and weight)
Visual acuity (post refraction)
Low luminance acuity
Low luminance questionnaire
Electroretinography measured using RETeval (LKC Technologies, Gaithersburg, MD
Scotopic thresholds using Medmont DAC perimeter (Melbourne, Australia)
Dark adaptation using AdaptDx (MacuLogix, Middletown, Pennsylvania, USA)
Fundus photography (Topcon, Tokyo, Japan)
Optos wide field photography (Optos PLC, Dunfermline, UK)
Optical coherence tomography (OCT) (Heidelberg Spectralis OCT, Germany)

Quantitative fundus autofluorescence (qFAF) (Heidelberg Spectralis OCT, Germany)
OCT-A (Heidelberg Spectralis OCT, Germany)

**Drs Shruti Chandra, Rajna Rasheed, Piyali Sen, Deepthy Menon (medical retina fellows) helped with clinical management of the patients. Dr Shruti Chandra also helped with grading of AMD participants as the second grader. Sarega Gurudas (statistician) confirmed statistical analysis.*

4.3.1 Statistical analysis

All statistical analysis was done using SPSS software (SPSS Statistics 23.0, SPSS Statistics for Windows, R2011; IBM Corp., Armonk, NY, USA) and GraphPad Prism (Version 8.2.1). Graphs were generated with the latter software. The normal quantile-quantile plots (Q-Q plots) and Shapiro-Wilk test were used to assess whether the data was normally distributed. Patient characteristics were analysed using chi squared or Fisher exact tests for categorical variables and ANOVA or Kruskal-Wallis tests for continuous data. Linear regression analysis was used to model the relationship between each functional parameter and age within the healthy aging group.

Pairwise differences between AMD groups were calculated using the unpaired one-way ANOVA or nonparametric Kruskal Wallis followed by post hoc uncorrected Dunn's multicomparison test. Non-parametric bootstrap was performed on 1000 bootstrap replicates, using a pooled method to obtain adjusted p-values for the ANOVA test, validating one-way ANOVA in normal and non-normal data and overcoming limitations due to small sample size. Due to the exploratory nature of this study and small sample size, p-values were not adjusted for multiple comparisons. ROUT method was performed to detect any outliers in the data. Inter-rater agreement for patient classification was assessed using Cohen's kappa coefficient. The nominal level of statistical significance was set at $\alpha=0.05$.

Lastly, linear regression analysis was performed to assess relationships between various psychometric tests and Spearman correlation coefficient was calculated to evaluate function-structure relationships.

4.4. Results

4.4.1. Patient characteristics

A total of 50 participants were recruited, comprising of healthy aging group (n=11), iAMD no SDD (n=17), AMD with SDD (n= 11) and late AMD (n= 11). Descriptive statistics of each group at baseline visit is shown in Table 4.1. The mean age for healthy aging group, iAMD without SDD, AMD with SDD, and non-foveal atrophic AMD participants at baseline was 65.1 ± 6.2 years (63.3% female), 66.3 ± 8.1 years (52.9% female), 74.2 ± 5.6 years (54.5% female) and 73.0 ± 6.0 years (63.6 % female) respectively. Significant difference was only found between the mean age of the healthy aging group and the iAMD with SDD group (p=0.029). There were no differences in smoking status, blood pressure, diabetes, or lipid profile between groups but body mass index (BMI) was significantly higher in the group with non-foveal atrophy compared to individuals in the healthy aging group (p= 0.013) and those in AMD no SDD group (p=0.0068).

Functional outcome measures were not age-adjusted as SDD are associated with advancing disease and linear regression showed no significant relationship between age in the healthy aging group and study-eye best-corrected visual acuity (p= 0.317), low luminance acuity (p=0.112) , low luminance deficit (p= 0.617), low luminance questionnaire (p=0.793), scotopic thresholds with cyan stimulus (p= 0.400), scotopic thresholds with red stimulus (p= 0.686), rod-intercept time (p=0.822) , ERG amplitude (p=0.175). By contrast, the ERG timing was found to be significantly related to age(p=0.041), although was not adjusted for analysis. Linear regression analysis is displayed in Figure 4.1. The inter-rater agreement was high (Cohen's kappa coefficient =0.96) for evaluation and grading of colour fundus images for AMD classification.

Table 4.1. Participant characteristics for each graded AMD severity group as per Beckman Initiative classification

	Group 1 Healthy aging (N=11)	**Group 2 iAMD no SDD (N=17)**	**Group 3 iAMD with SDD (N=11)**	**Group 4 Non-foveal atrophic AMD (N=11)**	**P value**
Age (mean ± SD)	65.1 (± 6.2)	66.3 (± 8.1)	74.2 (± 5.6)	73.0 (± 6.0)	0.003
Gender balance (% female)	63.6	52.9	54.5	63.6	0.939
Body Mass Index (BMI)	25.7	26.0	27.1	31.6	0.005
Smoking status Current or former smoker (%)	27.3	47.1	63.6	70	0.207
Systolic BP	125.5	136.8	145.2	136.7	0.111
Diastolic BP	76.5	82.7	83.0	76.9	0.208
Hypertension (%)	27.3	18.8	63.6	63.6	0.032
Hyperlipedemia (%)	54.6	35.3	81.8	45.5	0.113
Diabetes (%)	0	0	18.2	9.1	0.210

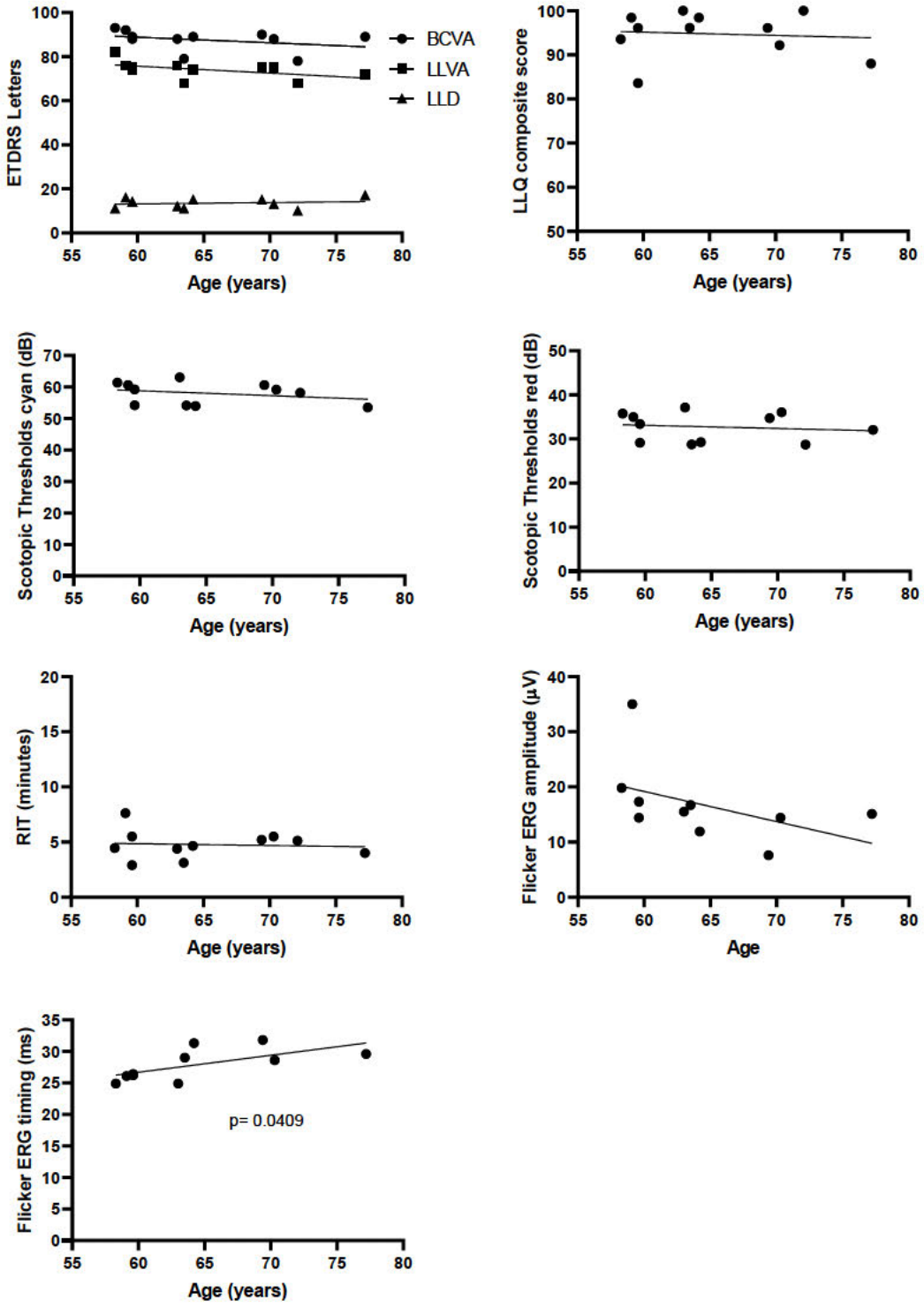

Figure 4.1. Scatter plot for the healthy aging group showing raw data for each functional outcome measure. Linear regression analysis was used to model the relationship between each functional outcome measure and age. P-value displayed for statistically significant relationship only. BCVA = best corrected visual acuity, LLVA= low luminance visual acuity, LLD= low luminance deficit, LLQ = low luminance questionnaire, RIT = rod-intercept time, ERG = electroretinogram.

4.4.2. Retinal function and disease severity

Study-eye visual acuities (BCVA, LLVA, LLD), low luminance questionnaire (LLQ), scotopic thresholds, RIT and photopic function observations were recorded. Applying the Shapiro-Wilk testing and using normal Q-Q plots revealed the data were not normally distributed within each severity grade and therefore non-parametric tests were used for analysis. All data for functional outcome measures is summarised in Table 4.2.

Table 4.2.Cross-sectional analysis for all functional outcome measures for all groups.

	Group, Mean ± SD, Median (Minimum, Maximum)						**Pairwise comparisons Post hoc uncorrected Dunn's test p value**					
Test	**Healthy aging (n=11)** †	**iAMD no SDD (n=17)** †	**iAMD with SDD (n=11)** †	**Non-foveal atrophic AMD (n=11)** †	**Overall p value** *	**Bootstrap Adjusted p-value****	**Healthy aging vs iAMD no SDD**	**Healthy aging vs iAMD with SDD**	**Healthy aging vs non-foveal atrophic AMD**	**iAMD no SDD vs iAMD with SDD**	**iAMD no SDD vs non-foveal atrophic AMD**	**iAMD with SDD vs non-foveal atrophic AMD**
BCVA (ETDRS letters)	87.6 (± 4.8) 78, 89, 93	85.7 (± 6.1) 67, 87, 93	83.6 (± 4.3) 77, 83, 90	74.2 (± 11.2) 50, 76.5, 87	**0.0005**	**<0.001**	0.237	**0.028**	**<0.0001**	0.218	**0.001**	0.065
LLVA (ETDRS letters)	74.1 (± 3.89) 68, 75, 82	71.1 (± 8.2) 47, 73, 80	67.3 (± 8.2) 56, 67, 79	60.8 (± 12.1) 40, 64.5, 74	**0.008**	**0.005**	0.385	**0.045**	**0.002**	0.181	**0.009**	0.230
LLD (ETDRS letters)	13.5 (± 2.3) 10, 14, 17	14.6 (± 4.3) 9, 14, 22	16.3 (± 4.6) 11, 15, 26	13.4 (± 3.7) 9, 12.5, 20	0.424	0.274	-	-	-	-	-	-
LLQ composite score	94.8 (± 5.2) 96.1 (83.6, 100.0)	87.3 (± 9.1) 90.6 (71.9, 99.2)	87.7 ± (12.9) 93.8 (66.4, 100.0)	72.8± (24.6) 71.1 (27.3, 98.0)	**0.032**	**0.005**	**0.044**	0.203	**0.004**	0.543	0.240	0.106
Scotopic Thresholds cyan (dB)	58.02 (± 3.45) 53.53, 59.18, 63.06	58.05 (± 2.29) 53.97, 58.68, 61.47	52.90 (± 4.31) 45.41, 55.38, 57.50	47.68 (± 9.47) 32.35, 46.56, 58.74	**0.0006**	**<0.001**	0.913	**0.014**	**0.003**	**0.005**	**0.0008**	0.6241
Rod intercept time (minutes)	4.77 (± 1.29) 2.90, 4.65, 7.62	7.42 (± 4.51) 3.68, 6.17, 20.00	20.00 (± 0.00) 20.00, 20.00, 20.00	19.51 (± 1.49) 15.26, 20.00, 20.00	**<0.0001**	**<0.001**	0.240	**<0.0001**	**<0.0001**	**<0.0001**	**0.0003**	0.717
Photopic ERG Amplitude (µV)	16.8 (± 7.2) 7.6, 15.3, 35.0	17.8 (± 5.6) 11.1, 17.8, 31.5	14.0 (± 5.4) 7.5, 13.55, 25.1	13.3 (± 5.6) 5.9, 13.1, 25.0	0.142	0.258	-	-	-	-	-	-
Photopic ERG Time (ms)	27.9 (± 2.5) 24.9, 27.5, 31.8	27.7 (± 2.0) 25.2, 27.2, 30.9	28.2 (± 1.0) 27.1, 28.05, 30.0	30.2 (± 2.5) 26.2, 30.15, 34.4	0.067	**0.043**	-	-	-	-	-	-

* Overall P value based on Kruskal-Wallis test of difference among medians

† Outliers excluded following ROUT analysis; one participant for BCVA, LLVA, LLD (non-foveal atrophy group) and 2 for RIT (1 AMD with SDD and 1 from non-foveal atrophy group).
Photopic flicker ERGs were measured in only 42 participants of the total 50 recruited due to a temporary mechanical fault with instrumentation (healthy aging group N=10, iAMD no SDD N=14, iAMD with SDD N=8, Non-foveal atrophy N=10.

4.4.2.1. Study-eye visual acuities

One participant was excluded for analysis in the non-foveal atrophic AMD group as the LLD score was an outlier as calculated with the ROUT method (described in methods chapter). Table 4.2 shows that there was a statistical difference between groups in BCVA and LLVA. However, there was no difference among groups in low luminance deficit assessment (LLD). Pairwise comparison showed there were no differences in either BCVA or LLVA between the healthy aging group and iAMD without SDD. BCVA and LLVA scores were significantly reduced in non-foveal atrophic AMD group compared with the healthy group ($p<0.0001$ and $p=0.002$ respectively). Patients with atrophy also exhibited poorer BCVA ($p=0.001$) and LLVA ($p=0.009$) scores compared with patients with intermediate disease without SDD. In patients with SDD, BCVA and LLVA scores were lower compared with healthy participants ($p=0.028$ and $p= 0.045$ respectively). There was no significant difference between iAMD with SDD and non-foveal atrophic AMD. All visual acuity measurements (BCVA, LLVA and LLD) for each participant are illustrated in Figure 4.2.

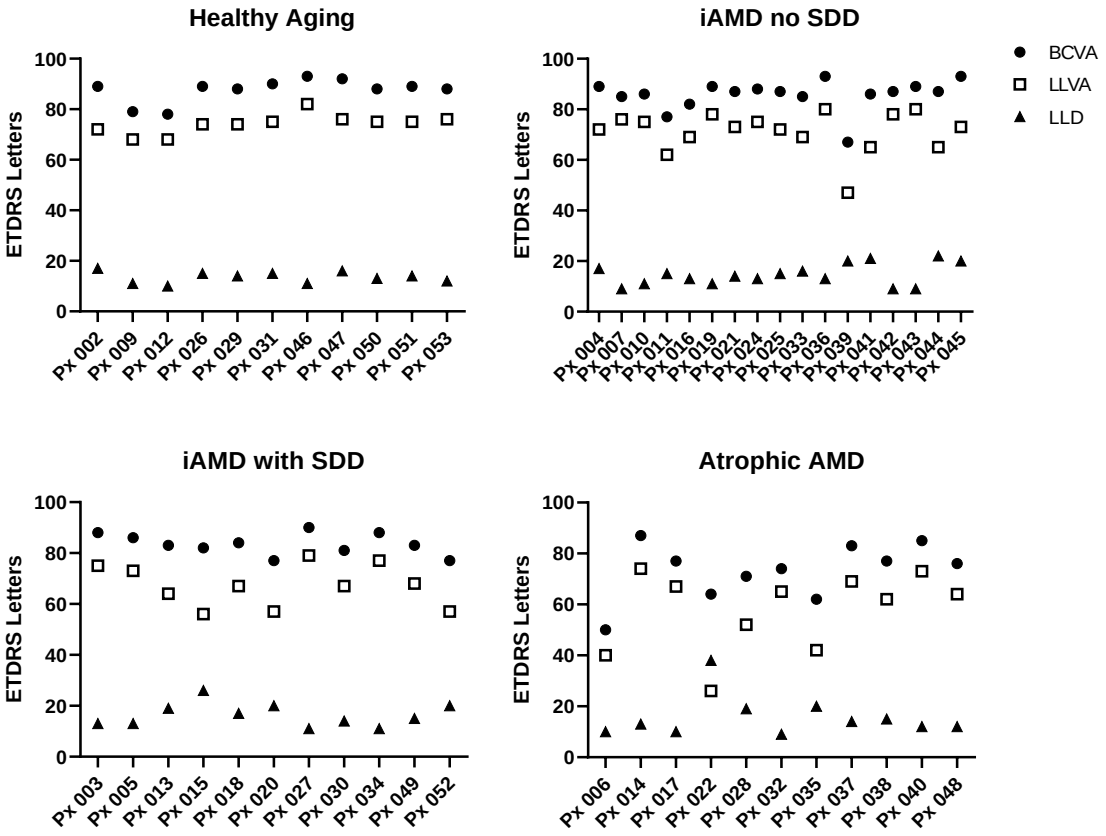

Figure 4.2. Scatter plots for best-corrected visual acuity (BCVA), low luminance visual acuity (LLVA) and low luminance deficit (LLD) for all participants across all graded AMD severity groups. Dark circles, squares and triangles represent BCVA, LLVA and LLD respectively.

4.4.2.2. Low luminance questionnaire

Significant association between LLQ composite score and age of the full cohort was observed (p=0.038), however it only explained 8.6% of the variance. This was, however, statistically non-significant when adjusted for disease status as shown in Figure 4.3 (β coefficient = -0.390, R^2 = 0.035, p=0.211).

There was considerable variability in LLQ composite scores in eyes with non-foveal atrophic AMD compared with other groups, with participants obtaining very high scores (maximum 98.0) whereas moderate variability was observed in iAMD groups with or without SDD as illustrated in Figure 4.4.

LLQ composite scores were significantly different in non-foveal atrophic AMD from healthy aging (p= 0.004) and modestly different between iAMD without SDD and healthy aging (p=0.044).

There were significant differences in the LLQ subscale scores between groups as shown in Table 4.3. Patients with non-foveal atrophic AMD had consistently lower median subscale scores compared with other groups. The subscale scores were significantly lower for patients with atrophy when compared with healthy aging group for extreme lighting (p= 0.002), emotional distress (p =0.003) and overall LLQ composite score (p= 0.004). The scores were also lower for the iAMD without SDD compared with healthy participants for extreme lighting, emotional distress and composite score (p = 0.030, p=0.047 and p= 0.044 respectively).

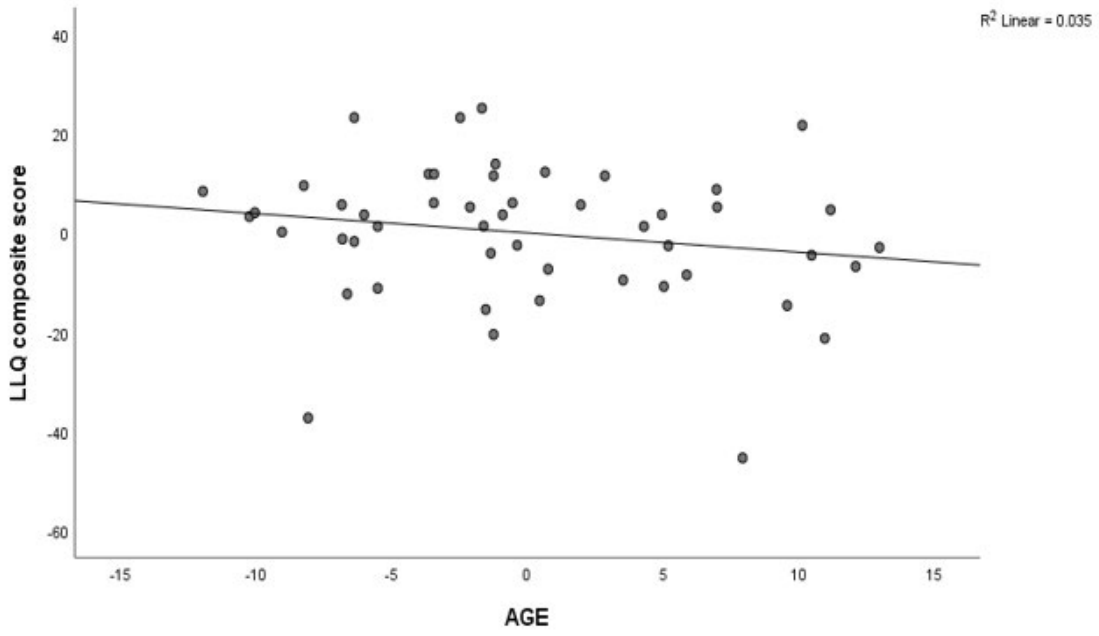

Figure 4.3. Linear regression showing relationship of age against LLQ composite score in the full cohort (N=50) after adjusting for disease (β coefficient = -0.390, R2 = 0.035, p= 0.211).

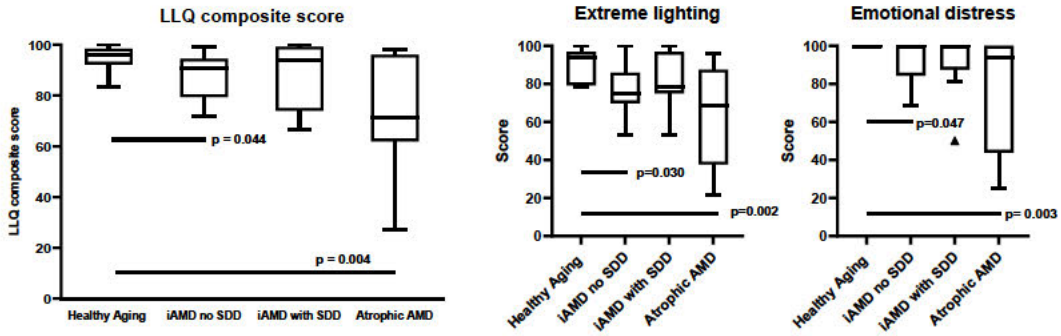

Figure 4.4. LLQ composite score (left) and statistically different subscale categories showing distribution of data across graded AMD severities. Only statistically significant differences ($p < 0.05$) between groups are displayed on the graph. Error bars represent standard deviation. Healthy aging (N= 11), iAMD no SDD (N=17), iAMD with SDD (N=11) and non-foveal atrophic AMD (N=11).

Table 4.3.The LLQ subscale and composite scores between each subject group.

	Group, Mean ± SD, Median (Minimum, Maximum)					Pairwise comparisons Post hoc uncorrected Dunn's test p value †					
LLQ Categories	**Healthy Aging (N=11)**	**iAMD no SDD (N=17)**	**iAMD with SDD (N= 11)**	**Atrophic AMD (N= 11)**	**Overall P-value ***	**Healthy aging vs iAMD no SDD**	**Healthy aging vs iAMD with SDD**	**Healthy aging vs non-foveal atrophic AMD**	**iAMD no SDD vs iAMD with SDD**	**iAMD no SDD vs non-foveal atrophic AMD**	**iAMD with SDD vs non-foveal atrophic AMD**
Extreme lighting	90.5 ± 9.0, 93.8 (78.1, 100.0)	77.5 ± 13.1, 75.0 (53.1, 100.0)	81.3 ± 15.4, 78.1 (53.1, 100.0)	61.8 ± 26.9, 68.8 (21.9, 95.8)	0.015	**0.030**	0.210	**0.002**	0.432	0.197	0.060
Mobility	97.0 ± 4.6, 100.0 (87.5, 100.0)	93.6 ± 7.1, 95.8 (79.2, 100.0)	92.4 ± 10.7, 100.0 (75.0, 100.0)	86.0 ± 18.0, 91.7 (45.8, 100.0)	0.451	-	-	-	-	-	-
General dim lighting	95.5 ± 6.6, 100.0 (83.3, 100.0)	89.5 ± 10.7, 91.7 (66.7, 100.0)	93.2 ± 9.9, 100.0 (75.0, 100.0)	76.1 ± 23.0, 79.2 (41.7, 100.0)	0.051	-	-	-	-	-	-
Peripheral vision	96.2 ± 8.6, 100.0 (75.0, 100.0)	94.1 ± 8.7, 100.0 (75.0, 100.0)	96.2 ± 8.6, 100.0 (75.0. 100.0)	80.3 ± 26.7, 91.7 (25.0, 100.0)	0.092	-	-	-	-	-	-
Driving	93.0 ± 10.3, 95.0 (65.0, 100.0)	83.5± 17.0, 90.0 (45.0, 100.0)	79.5 ± 29.7, 90.0 (5.0, 100.0)	58.6 ± 36.5, 50.0 (0.0, 100.0)	0.157	-	-	-	-	-	-
Emotional distress	100.0 ± 0.0, 100.0 (100.0, 100.0)	92.7 ± 10.4, 100.0 (68.8, 100.0)	91.5 ± 15.4, 100.0 (50.0, 100.0)	75.6 ± 29.5, 93.8 (25.0, 100.0)	0.032	**0.047**	0.100	**0.003**	0.861	0.216	0.200
Composite	94.8 ± 5.2, 96.1 (83.6, 100.0)	87.3 ± 9.1, 90.6 (71.9, 99.2)	87.7 ± 12.9, 93.8 (66.4, 100.0)	72.8± 24.6 71.1 (27.3, 98.0)	0.032	**0.044**	0.203	**0.004**	0.543	0.240	0.106

*P-values specified relate to the significance in mean rank difference between study groups (non-parametric Kruskal Wallis test); †Post hoc pairwise comparisons for subscales with overall p-value < 0.05.

4.4.2.3. Scotopic thresholds

Scotopic thresholds were reduced in participants with iAMD with SDD (p=0.014) and in patients with extrafoveal atrophic AMD (p=0.003) when compared to healthy controls. Mean retinal sensitivity was also significantly depressed between both iAMD with SDD (p= 0.005) and non-foveal AMD (p=0.0008) groups compared to iAMD without SDD participants. There was no difference in mean retinal sensitivity between healthy controls and eyes with iAMD without SDD. In addition, eyes with SDD and non-foveal atrophy did not differ functionally.

4.4.2.4. Dark adaptation

For rod intercept time, one outlier in each of the iAMD with SDD and late AMD groups were removed from analysis using the ROUT method. Tests with fixation errors over 33% were excluded from analysis. RIT was significantly higher, indicating slower recovery in the iAMD with SDD and late AMD groups compared to healthy aging (p <0.0001) and iAMD no SDD (p= 0.0004, p= 0.002 respectively). There was no statistical difference between the healthy aging group and iAMD without SDD group (p>0.9999).

To investigate the relationship of dark adaptation on patient characteristics and other functional outcomes, participants were classified on the basis of normal or abnormal RIT; defined as a rod-intercept time of ≥12.3 minutes (Owsley *et al.*, 2014). Table 4.4. provides patient demographics and lifestyle of the entire cohort by dark adaptation status. Participants with impaired dark adaptation were older (p=0.002), had higher BMI (p=0.010) and significantly reduced visual acuities, scotopic thresholds and ERG amplitude, although increased ERG timing as described in Table 4.5.

Table 4.4. Patient demographics and lifestyle of the entire cohort by dark adaptation status.

	Group, Mean ± SD		
	Normal DA (N = 27)	Abnormal DA (N=21)	P-value
Age (years ± SD)	66.0 ± 7.5	73.1 ± 6.2	0.002
Gender N (± SD) **Male** **Female**	 11 (40.7) 16 (59.3)	 9 (42.9) 12 (57.1)	0.883
BMI (± SD)	25.9 ± 4.0	29.4 ± 4.9	0.010
Systolic BP (± SD)	133.0 ± 19.3	140.4 ± 17.2	0.178
Diastolic BP (± SD)	80.6 ± 10.3	79.6 ± 9.3	0.730
Smoking status N (± SD) **Current/former** **Never**	 11(40.7) 16 (59.3)	 13 (42.9) 8 (57.1)	0.146

Table 4.5.Functional outcome measures by dark adaptation status of all 50 participants at baseline visit.

	Group, Mean ± SD		
Functional outcome measure	Normal DA (N = 27)	Abnormal DA (N=21)	P-value
BCVA (ETDRS Letters)	86.6 ± 5.6	78.8 ± 9.8	0.0001
LLVA (ETDRS Letters)	72.4 ± 7.0	63.6 ± 13.2	0.002
LLD (ETDRS Letters)	14.2 ± 3.7	15.2 ± 6.2	0.884
Scotopic thresholds cyan (dB)	57.91 ± 2.71	50.46 ± 8.15	0.0003
Photopic flicker ERG amplitude (μV)	17.36 ± 6.16	13.62 ± 5.51	0.027
Photopic flicker ERG timing (ms)	27.76 ± 2.19	29.19 ± 2.20	0.047

4.4.2.5. Photopic function

Photopic flicker ERGs were measured in only 42 participants of the total 50 recruited due to a temporary mechanical fault with instrumentation. No significant difference was found between groups for flicker ERG amplitude and implicit time at baseline visit

4.4.3. Retinal structure and disease severity

No data were omitted from the analysis of retinal layer volumes and thicknesses. Shapiro-Wilk testing revealed the data were normally distributed for total retinal and ONL volumes and thicknesses and non-normally distributed for RPEDC volume and thickness and CT. All structural outcome measures and pairwise comparisons among groups are displayed in Table 4.6. The means of total retinal volume, retinal thickness, ONL volume, ONL thickness and CT were significantly different between groups ($p=0.030$, $p=0.0097$, $p=0.0004$, $p<0.0001$ and $p=0.007$ respectively).

Participants with non-foveal atrophy had significantly reduced mean total retinal thickness, ONL volume and ONL thickness compared with healthy subjects ($p = 0.029$, $p= 0.0009$ and $p<0.0001$ respectively). Eyes with atrophy also had reduced ONL volume and thinner total retinal and ONL thicknesses compared with iAMD without SDD ($p=0.002$, $p<0.0001$ and $p<0.0001$ respectively) and eyes with SDD ($p=0.004$, $p=0.001$ and $p<0.0001$ respectively). No difference was found in RPEDC volume or thickness between groups ($p=0.704$ and $p=0.225$ respectively). Thinner choroids were observed in participants with non-foveal atrophy compared to healthy eyes ($p=0.0007$) and eyes with SDD ($p= 0.023$), but not in iAMD eyes without SDD ($p=0.092$). Eyes with SDD did not differ significantly from healthy eyes nor from iAMD eyes without SDD on any morphological parameter. Furthermore, bootstrap analysis (1000 bootstrap replicates) was in agreement to statistical outcome in all structural parameters compared to one-way ANOVA tests.

Table 4.6. Cross-sectional quantitative OCT parameters analysis between all groups.

	Group, Mean ± SD						Pairwise comparisons Post hoc uncorrected Dunn's test p value					
Layer	**Healthy Aging (N=11) mean (±SD)**	**iAMD no SDD (N=17) mean (±SD)**	**iAMD with SDD (N=11 mean (±SD)**	**Non-foveal GA (N=11) mean (±SD)**	**Overall p-value**	**Bootstrap adjusted p-value**	**Healthy aging vs iAMD no SDD**	**Healthy aging vs iAMD with SDD**	**Healthy aging vs non-foveal atrophic AMD**	**iAMD no SDD vs iAMD with SDD**	**iAMD no SDD vs non-foveal atrophic AMD**	**iAMD with SDD vs non-foveal atrophic AMD**
Total retinal volume	8.57 (± 0.31)	8.71 (± 0.49)	8.69 (± 0.28)	8.14 (± 0.78)	**0.030**	**0.027**	0.465	0.575	0.055	0.909	**0.006**	**0.015**
Total retinal thickness	311.10 (± 9.84)	316.85 (±18.87)	317.99 (±12.74)	291.67 (± 32.26)	**0.0097**	**0.007**	0.464	0.426	**0.029**	0.883	**0.002**	**0.004**
ONL volume	1.73 (± 0.27)	1.77 (± 0.14)	1.73 (± 0.13)	1.41 (± 0.30)	**0.0004**	**0.001**	0.675	0.968	**0.0009**	0.643	**<0.0001**	**0.001**
ONL thickness	67.76 (± 9.65)	67.54 (± 6.28)	66.06 (± 4.61)	50.17 (± 11.02)	**<0.0001**	**<0.001**	0.943	0.622	**<0.0001**	0.637	**<0.0001**	**<0.0001**
RPEDC volume	2.24 (± 0.05)	2.33 (± 0.24)	2.44 (± 0.38)	2.35 (±0.48)	0.704	0.521	0.532	0.289	0.901	0.586	0.626	0.349
RPEDC thickness	80.75 (1.98)	86.00 (±11.62)	90.89 (17.57)	87.63 (±30.59)	0.225	0.652	0.387	0.145	0.645	0.470	0.170	0.057
Choroidal thickness	250.3 (±101.3)	168.9 (± 58.9)	209.6 (±104.2)	123.0 (± 50.5)	**0.007**	**0.004**	**0.042**	0.266	**0.0007**	0.417	0.092	**0.023**

4.4.4 Relationship between functional parameters

In this section, the relationship between visual acuities (BCVA and LLVA) and patient-perceived vision (LLQ) was investigated. In addition, the association between rod-mediated tests and other functional outcome measures was evaluated.

4.4.4.1. Relationship between visual acuities and patient-perceived vision

To determine the relationship between BCVA and LLVA, linear regression analysis of BCVA was performed against LLVA. There was strong linear association between the two; BCVA was a significant predictor of LLVA in all disease groups as shown in Figure 4.5. According to the R^2 parameter, BCVA was able to explain 78% ($p=0.0003$), 74% ($p<0.0001$), 84% ($p<0.0001$) and 91% ($p<0.0001$) of the variance in LLVA for the healthy aging, iAMD no SDD, iAMD with SDD and atrophic AMD groups respectively. Linear regression of visual acuities and low luminance deficit against LLQ composite score is shown in Table 4.7. There was no significant association between BCVA, LLVA or LLD and LLQ composite score in healthy aging, iAMD without SDD and atrophic AMD participants. However, LLQ composite score was weakly associated with BCVA ($p = 0.047$) in patients with SDD.

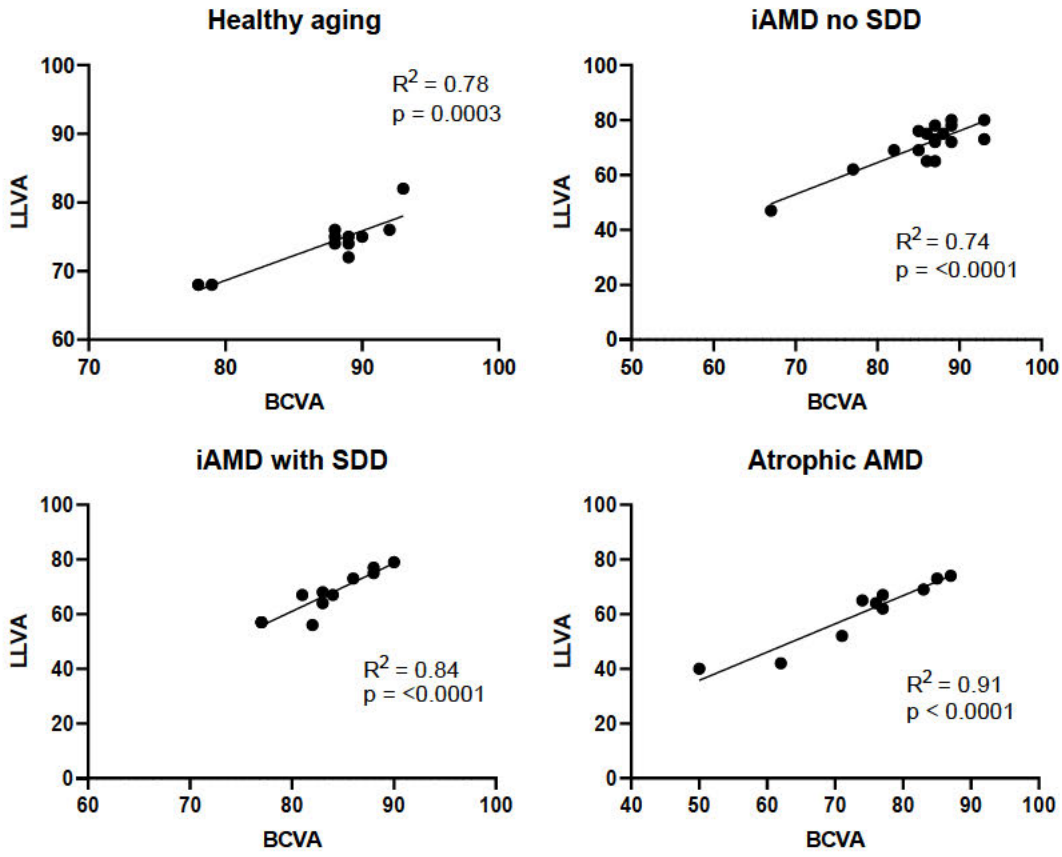

Figure 4.5. Scatter plots showing significant relationships between best-corrected visual acuity (BCVA) and low luminance visual acuity (LLVA) in healthy aging (n=11, R^2 = 0.78, p= 0.0003), iAMD no SDD (n=17, R^2 = 0.74, p<0.0001), iAMD with SDD (n=11, R^2 = 0.84, p <0.0001) and atrophic AMD (n=10, one subject was excluded due to high discrepancy between BCVA and LLVA, R^2 = 0.91, p<0.0001). iAMD = intermediate AMD, subretinal drusenoid deposits = SDD.

Table 4.7. Linear regression of functional tests against LLQ Composite Score in all AMD severity groups.

Outcome measure	Healthy aging			AMD no SDD			AMD with SDD			Non-foveal Atrophic AMD		
	B coefficient	**Standardized Coefficient**	**P value**	**B coefficient**	**Standardized Coefficient**	**P value**	**B coefficient**	**Standardized Coefficient**	**P value**	**B coefficient**	**Standardized Coefficient**	**P value**
BCVA	-0.299	-0.276	0.411	0.551	0.368	0.146	1.828	0.608	0.047	0.076	0.041	0.910
LLVA	-0.203	-0.153	0.654	0.186	0.166	0.523	0.927	0.592	0.055	0.239	0.141	0.697
LLD	-0.734	-0.321	0.337	0.444	0.208	0.424	-1.359	-0.489	0.127	-1.859	-0.337	0.341

* One participant was excluded in the non-foveal atrophic AMD group due to unusually high LLD score.

4.4.4.2. Correlations between rod-mediated and other functional outcome measures

The relationships between rod-mediated functional outcome measures (scotopic thresholds and rod-intercept time) and other psychometric tests were investigated and shown in Figure 4.6. Individuals who failed to reach the rod criterion level were assigned a maximum value of 20 minutes by default, which undoubtedly underestimated the mean RIT values. Linear regression analysis was therefore inappropriate in examining relationships with this outcome measure. Thus, Spearman correlation was performed. BCVA, LLVA and scotopic thresholds were significantly and negatively correlated with RIT ($r= -0.51$, $p=0.002$; $r= -0.42$, $p=0.003$; and $r= -0.42$, $p=0.003$ respectively). However, LLQ was not associated with RIT ($r=-0.21$, $p= 0.146$). By contrast, BCVA ($r=0.55$), LLVA ($r=0.64$) and LLQ ($r=0.39$) correlated well with scotopic thresholds.

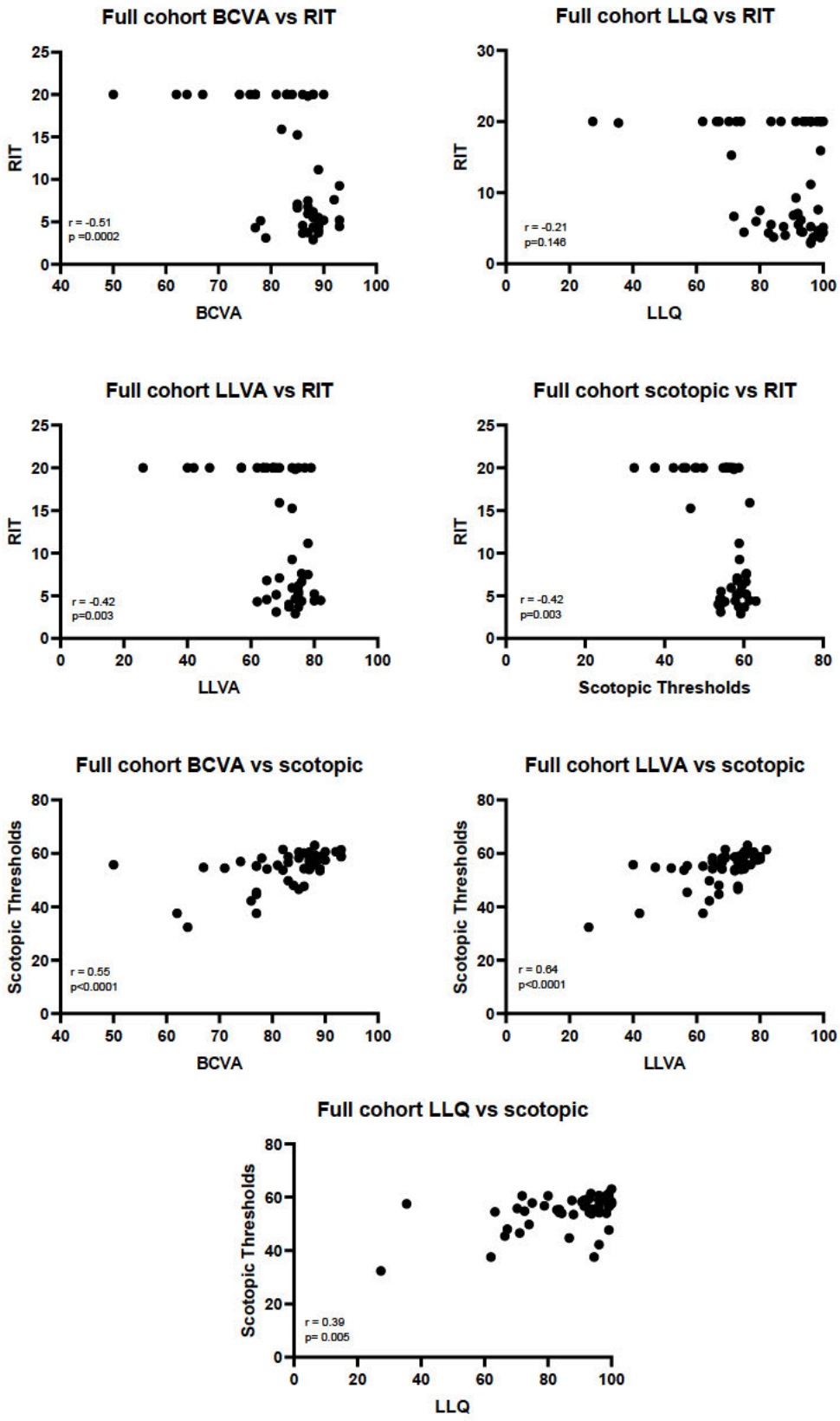

Figure 4.6. Scatterplots examining the relationship between rod-mediated function and other visual function outcome measures amongst all subjects.

4.4.5. Correlation between functional and structural outcome measures

Table 4.8 displays the structure-function correlations of outcome measures. BCVA, LLVA, scotopic thresholds showed moderate correlation with mean ONL volume ($r=0.42$, $p=0.003$; $r=0.48$, $p=0.0004$ and $r=0.46$, $p=0.0009$ respectively). BCVA and scotopic thresholds were also moderately associated with mean ONL thickness ($r=0.55$, $p<0.0001$ and $r=0.52$, $p=0.0001$ respectively) whereas LLVA showed a slightly stronger correlation ($r=0.61$, $p<0.0001$). BCVA and LLVA also correlated with mean CT ($r=0.43$, $p=0.002$ and $r=0.42$, $p=0.003$ respectively) while scotopic thresholds showed a weaker association ($r=0.34$, $p=0.015$). Furthermore, a weak negative correlation was found between the implicit time for photopic ERG and mean ONL volume ($r=-0.33$, $p=0.033$) and ONL thickness ($r=-0.37$, $p=0.016$) as well as a moderate negative association with mean CT ($r=-0.49$, $p=0.001$). No significant association was found between LLD, LLQ, RIT and photopic ERG amplitude and quantitative OCT parameters. There was also no association between RPEDC parameters and visual function outcome measures.

Table 4.8. Correlation between functional and structural outcome measures (Spearman's correlation: r)

Parameters	Mean total retinal volume	Mean total retinal thickness	Mean ONL volume	Mean ONL thickness	Mean RPEDC volume	Mean RPEDC thickness	Choroidal thickness
BCVA (n= 49)	r=0.21 p=0.148	r=0.16 p=0.266	**r=0.42 p=0.003**	**r=0.55 p=<0.0001**	r=-0.02 p=0.919	r=0.04 p=0.805	**r=0.43 p=0.002**
LLVA (n= 49)	r=0.25 p=0.083	r=0.22 p=0.126	**r=0.48 p=0.0004**	**r=0.61 p<0.0001**	r=-0.06 p=0.666	r=-0.01 p=0.970	**r=0.42 p=0.003**
LLD (n= 49)	r=-0.08 p=0.602	r=-0.10 p=0.502	r=-0.16 p=0.259	r=-0.24 p=0.099	r=0.09 p=0.530	r=0.09 p=0.555	r=-0.11 p=0.441
LLQ (n= 50)	r=0.02 p=0.892	r=0.11 p=0.431	r=0.10 p=0.490	r=0.25 p=0.082	r=0.07 p=0.608	r=0.07 p=0.606	r=0.25 p=0.078
Scotopic thresholds (n= 50)	r=0.24 p=0.094	r=0.18 p=0.215	**r=0.46 p=0.0009**	**r=0.52 p=0.0001**	r=0.10 p=0.501	r=0.08 p=0.569	**r=0.34 p=0.015**
RIT (n= 48)	r=-0.03 p=0.830	r=-0.08 p=0.603	r=-0.11 p=0.453	r=-0.25 p=0.082	r=0.10 p=0.507	r=0.06 p=0.685	r=-0.24 p=0.103
Photopic ERG amplitude (n= 42)	r=0.29 p=0.066	r=0.31 p=0.048	r=0.23 p=0.146	r=0.30 p=0.054	r=0.08 p=0.618	r=0.12 p=0.454	r=0.23 p=0.149
Photopic ERG time (n=42)	r=-0.21 p=0.174	r=-0.20 p=0.204	**r=-0.33 p=0.033**	**r=-0.37 p=0.016**	r=-0.12 p=0.445	r=-0.15 p=0.335	**r=-0.49 p=0.001**

4.5. Discussion

In this present study, there was no significant difference in BCVA or LLVA between iAMD with no SDD and healthy aging. However, BCVA and LLVA were reduced significantly in eyes with iAMD with SDD compared to healthy aging. This discrimination is important as it stratifies iAMD into those with and without functional loss based on the presence or absence of SDD.

Although previous studies have reported reduced LLVA in AMD patients with drusen >125 µm compared with healthy participants (Puell *et al.*, 2012; Wu *et al.*, 2014; Cocce *et al.*, 2018), these used clinical severity scales of AMD based on traditional classification, which does not include presence of SDD as a distinct entity.

By categorizing eyes with SDD as a separate group, this study revealed that the reduced LLVA in iAMD eyes may indeed be driven by the subgroup of patients with SDD. Similarly, eyes with non-foveal atrophy were found to have significantly worse BCVA and LLVA when compared with controls (healthy aging) and eyes with iAMD without SDD. This is in accordance with previous studies by Sunness et al which have shown LLVA to be reduced in eyes with non-foveal GA and also predict subsequent vision loss (Sunness *et al.*, 1997, 2008).

There is discordance in literature regarding LLD measure. Current findings from this exploratory study found no statistically significant difference in LLD between groups which contrasts with results from Puell et al. (2012) who found significant difference in LLD in the non-advanced AMD groups. Wu et al. (2014) noted a difference in LLD between participants with non-foveal GA and control group only, but no significant difference in LLD between control and in the non-atrophic AMD groups. However, Cocce and colleagues (2018) reported no mean difference in LLD between groups in their cohort (Cocce *et al.*, 2018). This may be due to the fact that we divided our groups to include SDD as a separate group and the baseline VA was good across all groups in our study. The heterogeneity in outcomes indicate that retinal function changes may be independent of the currently used clinical grading scales based on structure. This finding may in part be influenced by presence of SDD which is not currently included in the classification (Querques *et al.*, 2014). In our study, LLD ranged from 13.5 to 16.3 letters, a difference of 2.8 letters between groups which is clinically not meaningful when VA variability ranges from 5 to 10 letters (Rosser *et al.*, 2003; Patel *et al.*, 2008). Therefore, our study suggests that LLD is not a biomarker of increasing disease severity. Given the variability and discrepancies regarding LLVA and LLD, present findings indicate that LLVA and LLD do not detect greater extent of visual dysfunction than BCVA.

This study also evaluated whether LLQ composite scores deteriorate with age and disease severity. Although current results showed a decrease in LLQ composite score with age, this was insignificant when adjusted for disease severity. These results substantiate previous findings that age is not associated with LLQ (Yazdanie *et al.*, 2017; Thompson *et al.*, 2018). However, LLQ composite score was found to be an accurate marker for disease severity, although it could not differentiate between iAMD with and without SDD. The subscales that were most affected were extreme lighting and emotional distress in iAMD no SDD and the non-foveal atrophy group. The subscale on difficulty in dim lighting did not reach statistical significance. Interestingly, there was no difference in composite score between healthy controls and iAMD with SDD. This is difficult to interpret but it may be that eyes with SDD eyes are not affected by extreme lighting. There were large standard deviations in LLQ subscales in this small sample sized study which could confound our findings.

In the present study, all eyes with SDD had delayed RIT of 20 minutes (the maximum limit placed in this study), indicating that RIT is a good test that can accurately discriminate eyes with intermediate AMD with and without SDD. Studies that have not previously stratified intermediate AMD by the presence of SDD reported delayed rod function tests in iAMD and these results may have been driven by those with SDD (Cocce *et al.*, 2018; Hsu *et al.*, 2019). Our results support more recent work showing significant impaired rod function in SDD eyes (Nguyen *et al.*, 2018; R. Tan, Guymer and Luu, 2018; Tan *et al.*, 2019; Mcguinness *et al.*, 2020). However, the length of RIT test was automatically terminated at 20 minutes in our study, undoubtedly underestimating the actual RIT and this may be a factor in not capturing any difference between patients with SDD and those with atrophy. When comparing dark adaptation status among the entire cohort, those with impaired dark adaptation were more likely to be older and have significantly reduced visual acuities, consistent with findings with that of Flamendorf et al. (2015). Furthermore, delayed dark adaptation was associated with reduced scotopic thresholds, photopic flicker ERG amplitude and an increased latency. All patients with SDD and non-foveal GA and one patient iAMD without SDD had delayed dark adaptation also in agreement with Flamendorf et al. (2015).

Flamendorf and colleagues also reported that participants with higher blood pressure were more likely to have longer rod-intercept time. In our study, participants with impaired dark adaptation did have higher systolic BP but did not reach statistical significance.

When evaluating the mean scotopic threshold in the different groups, it was found that eyes with SDD and non-foveal atrophy did not differ functionally nor did healthy subjects compared with iAMD without SDD. Eyes with SDD were functionally similar to those with parafoveal atrophy rather than those with iAMD without SDD. However, some eyes with SDD and parafoveal atrophy achieved scotopic thresholds comparable with iAMD without SDD and healthy eyes respectively. Therefore, scotopic threshold is not as accurate as RIT in terms of differentiating eyes with SDD from the other groups.

Photopic flicker ERGs were also used to assess cone function. There is contentious evidence of decline in pan-retinal cone function with AMD (Ronan *et al.*, 2006). As full electrophysiology assessments were time consuming and impractical in this study, a handheld flicker ERG device was used. There was no significant difference in photopic amplitude or latency across AMD severity groups. As the foveal cone mosaic remains fairly stable in aging and survives until the very late stages of the disease, this assessment may not be able to adequately capture cone dysfunction owing to its pan-retinal response. Other tests have been more successful at elucidating cone dysfunction in AMD such as cone thresholds (Dimitrov *et al.*, 2011; Rodriguez *et al.*, 2018) and cone adaptation (Dimitrov *et al.*, 2012).

Relationships between vision and patient-perceived vision were also investigated. LLVA is a measure of mesopic function, predominantly cone-mediated in reduced illumination (Sunness *et al.*, 2008; Connolly and Barbur, 2009; Puell *et al.*, 2012). We found a strong correlation between BCVA and LLVA across all groups and likely explanation for this would be that both tests are inherently dependent on foveal cone function consequently resulting in similar functional mechanism. Stockman and Sharpe explained that the visual acuity in mesopic setting requires integrated cone function mediated by post-receptoral pathways and a disruption of this would lead to a drop in LLVA worsening the LLD (Stockman and Sharpe, 2006).

A similar reduction in both BCVA and LLVA could be secondary to mechanical disruption, disorientation of photoreceptors thereby reducing spatial resolution at both illuminance levels (Eckmiller, 2004; Gao *et al.*, 2008; Hartmann *et al.*, 2012). As LLD is a difference of BCVA and LLVA, any improvement or worsening in LLD has to be interpreted in the context of actual values of BCVA and LLVA (Sunness *et al.*, 2008). For example, an improved LLD might be a result of worsening foveal photopic vision, thereby lowering the BCVA, but LLVA may not be affected to the same degree as parafoveal cones are less responsive to changes in illumination than foveal cones (Sunness *et al.*, 2008). Similarly, a parallel reduction in BCVA and LLVA could result in a non-significant difference in LLD which probably explains the indiscriminate LLD result between groups.

Our present findings also showed that rod mediated tests correlated well with visual acuities and LLQ. The rod-intercept time negatively and moderately related to BCVA and LLVA and only weakly associated with LLQ. Mcguinness and colleagues (2020) have also shown similar moderate relationships between RIT and visual acuity as did Flamendorf and colleagues (2015) between BCVA and RIT. Nevertheless, other studies have found stronger correlation between LLQ and RIT (Yazdanie *et al.*, 2017; Thompson *et al.*, 2018). Visual acuities, RIT and LLQ were also significantly associated with scotopic thresholds. This consistency in psychometric tests supports the validity of lack functional differences between healthy eyes and those with AMD without SDD and between SDD eyes and those with atrophic changes.

This study showed that BCVA, LLVA, scotopic threshold and RIT were significantly reduced in iAMD with SDD, and the values were comparable to eyes with parafoveal atrophy, suggesting that SDD is a marker of advanced disease despite no noticeable atrophic changes on multimodal imaging. Parafoveal atrophy indicates localised tissue loss. However, those with SDD have generalised and equal loss of function whether or not there is manifest atrophy as shown on reflection images. Sunness et al. (1997) have previously established delayed dark adaptation as a predictor of progression of geographic atrophy. Present findings are compatible with the concept that both SDD and parafoveal atrophy show similar magnitude of impairment of dark adaptation suggesting that SDD is as strong a predictor of disease progression as parafoveal atrophy.

In this study, increasing thickness and volume of the ONL was associated with better photopic and scotopic function suggesting that SDD may be an indicator of outer retinal cell loss. However, larger sample size in each group is required to evaluate whether there are group-wise differences. Histopathological and clinical studies have also suggested a possible role for choroid in the pathogenesis of SDD (Knudtson *et al.*, 2004; Grewal *et al.*, 2014) but no association was found between CT and RIT in this study. SDD has been previously linked to choroidal vascular insufficiency (Grewal *et al.*, 2014), however further research is required to evaluate whether RIT correlates with changes in choroidal vasculature whether there is a threshold of choroidal thinning below which RIT may be affected. The Beijing Eye Study showed that visual acuity impairment correlated with SFCT of 30µm or less (Shao *et al.*, 2014).

Present findings also suggest that eyes with SDD may be already on track to irreversible disease progression, and so clinical trials on prevention or treatment of progression of geographic atrophy should exclude them or undertake a sub analysis of this group. The Subthreshold Nanosecond Laser Intervention in Age-Related Macular Degeneration (LEAD) randomized controlled trial is an example where this was done and the SDD group did less well than those eyes without SDD (Guymer *et al.*, 2019), reinforcing the need for their segregation.

The strengths of this study include detailed retinal grading of AMD patients with varying disease severity including stratification of those with and without SDD from two graders based on multimodal imaging with high inter-rater agreement (Cohen's kappa coefficient = 0.96). In addition to deep phenotyping, all functional assessments were carried out systematically and consistently by a single observer limiting interobserver variability. Full dark adaptation of the subjects for 40 minutes prior to scotopic testing allowed rods to reach absolute thresholds. Despite the small sample size, we observed significant and consistent differences between AMD groups, substantiated with non-parametric bootstrap analysis, and significant associations between clinical measures, validating the findings of our cohort. Although our findings can be extrapolated with caution, they cannot be directly applied in clinical practice due to small sample size but would be useful for designing future clinical trial endpoints.

Some limitations of this pilot study include the small sample size in each group. The length of RIT test was automatically terminated at 20 minutes in our study, and this may be a factor in not capturing any difference between patients with SDD and those with atrophy. We also classified the SDD group based on a small number of SDDs (at least 5 SDD that show interruption of the EZ zone) as against recent studies that have used area of SDD as classification criteria (Grewal *et al.*, 2014). For the purpose of correlating function with structure in our study, we evaluated 6mm region which may have resulted in poor association with RPEDC. It is also important to note that visual functional parameters reflect uniocular function where as LLQ represents binocular function and therefore the results could be affected depending on the status of the non-study eye.

In conclusion, this exploratory work supports the need to re-classify AMD severity scale by incorporating SDD based on visual function tests. Although current findings showed that BCVA, LLVA, scotopic threshold and RIT were significantly reduced in iAMD with SDD, only rod-mediated tests were able to meaningfully discriminate iAMD eyes with and without SDD. The prognostics capacity to monitor disease progression is further explored in longitudinal assessment in chapter 6. Present findings also provide strong evidence that eyes with SDD are surrogate markers of photoreceptor abnormalities, although not necessarily cell death that are as significant as eyes with non-central atrophy and so are unlikely to show an improvement in visual functions with potential novel prophylactic agents that are evaluated to decrease the rate of progression of AMD.

Chapter 5. Evaluation of the effects of 670nm photobiomodulation in healthy aging and AMD

This chapter describes the results of two exploratory studies undertaken to investigate the effect of photobiomodulation with 670nm light delivered daily by means of a hand-held LED light source in healthy aging and in AMD. Firstly, a proof-of-concept study was conducted in healthy young and older participants where the effect of red-light therapy over two weeks was measured on scotopic thresholds. Secondly, an exploratory prospective clinical trial was undertaken to evaluate the safety and effectiveness of 670nm photobiomodulation in healthy aging and patients with intermediate AMD over a period of 12 months. Functional tests included in the clinical trial included BCVA, LLVA, scotopic thresholds, dark adaptometry and photopic flicker ERGs. Structural evaluations included retinal layer volumetric analysis of ONL and RPE-BM complex at baseline and 12-month visit.

In the first instance, the results from the small pilot study are described. This is followed by the analysis of changes in healthy aging, iAMD without SDD and iAMD with SDD groups at 12 months for functional and structural outcome measures. Lastly, the effect of red-light therapy over time is evaluated for each functional parameter by adjusting for disease at all study visits (baseline, 1 month, 3 months, 6 months and 12 months).

5.1. Introduction

Currently, there are no potential therapeutic interventions to prevent or slow down the progression of intermediate AMD to advanced stages of the disease; geographic atrophy or neovascular AMD. Despite anti-VEGF treatment being available for neovascular AMD, the advanced stages of neovascular AMD and geographic atrophy are best avoided due to the sequelae of irreversible functional and structural changes at the macula. Therefore, treatment options targeting the intermediate AMD stage are likely to be more valuable than those focused on advanced AMD.

Recent literature supports photobiomodulation as a potential therapeutic approach to treat retinal diseases. The efficacy of light therapy has been previously explored using 505nm wavelength in proof of concept studies and showed a decrease in macular oedema and improved cone function in patients with diabetic retinopathy (Arden *et al.*, 2011; Sahni *et al.*, 2017). As the retina is most metabolically active under scotopic conditions, it was hypothesised that light exposure could prevent full dark adaptation and decrease oxygen demand by supressing rods during sleep, consequently delaying the rate of progression of diabetic macular oedema (Arden *et al.*, 2011; Sahni *et al.*, 2017). Nonetheless, these observations were refuted by larger Phase 3 trial for non-centre involving diabetic macular oedema in the CLEOPATRA study (Sivaprasad *et al.*, 2018). This intervention was also explored in participants with early AMD in the study eye and nAMD in the fellow eye, however this phase I/IIa did not find any substantial clinical benefit (Robinson *et al.*, 2018).

Recently, the application of red to infrared light (= 600–1000 nm) has also been investigated in retinal disorders. In contrast to low level light therapy which targeted retinal hypoxia, red light therapy can be used to modulate mitochondrial function. It has been proposed that light at this wavelength is absorbed by cytochrome c oxidase, the rate-limiting enzyme in mitochondrial respiration, increasing its activity, ATP output and mitochondrial membrane potential efficiency (Karu, 1999; Karu *et al.*, 2005; Hamblin and Demidova, 2006; Karu, 2008). With aging, the membrane potential and function of mitochondria decline, causing a fall in ATP production (Kokkinopoulos *et al.*, 2013; Gkotsi *et al.*, 2014; Eells, 2019). These changes can signal cell death. As the retina has the greatest energy demand in the body, it is particularly vulnerable to mitochondrial dysfunction. Studies have suggested this mitochondrial compromise may in partly contribute to AMD pathogenesis (Nordgaard *et al.*, 2008; Karunadharma *et al.*, 2010; Terluk *et al.*, 2015; Ferrington *et al.*, 2016; Ferrington, Sinha and Kaarniranta, 2016; Datta *et al.*, 2017). Therefore, targeting mitochondria may be an option to delay retinal aging and prevent the switch to AMD or possibly delaying its progression. A case series on daily red-light therapy on four patients with non-centre-involving diabetic macular oedema resulted in a reduction in macular fluid (Tang, Herda and Kern, 2014).

Similarly, clinical investigations have been undertaken to assess the benefit of red-light therapy in AMD. A study by Ivandic and Ivandic reported improvement in visual acuity and colour vision lasting up to 36 months, using a low-level laser therapy with a 780 nm wavelength exposure delivered on four occasions (two treatments per week) over a two-week period in patients with dry and wet AMD (Ivandic and Ivandic, 2008). In 2017, Merry et al. reported improved visual acuity, contrast sensitivity and regression of drusen in patients with early and intermediate AMD (iAMD) using multi-wavelength LED light therapy (590 nm, 670 nm and 790 nm) with three treatments per week over a course of three weeks (Merry *et al.*, 2017). Both studies may however be over-estimating the effect of treatment by using visual acuity as the primary outcome measure as it is relatively unaffected in early or iAMD. Furthermore, a reduction in drusen volume may be associated with the natural dynamics of drusen formation and spontaneous regression (or impending GA) and therefore this outcome may not necessarily be linked to light therapy. An important factor that has not been taken into account in any of these studies is that iAMD is a heterogeneous disease. Patients with iAMD may have associated SDD and these eyes tend to progress to advanced form faster than those without SDD. Therefore, studies should ensure that these sub-phenotypes are taken into account when evaluating the effect of any potential treatment.

In this chapter, I sought to explore the magnitude of any effect of 670 nm light exposure on scotopic thresholds in healthy aging. Investigations carried out in this chapter also aimed to establish the possible therapeutic effect of photobiomodulation on multiple visual functions and anatomical structures in healthy aging and AMD with and without SDD to assess whether any of the tests could be reliably used in a future definitive randomized controlled trial on photobiomodulation in AMD.

5.2. Pilot study: Effect of 670nm photobiomodulation on mean scotopic sensitivity in healthy aging

5.2.1. Aim

Evaluate the effect of 670nm light on scotopic thresholds in a healthy young and older cohort without known retinal pathology.

5.2.2. Methods

The study protocol was approved by the UCL Research Ethics Committee (UCL Ethics Approval Application #: 11013/001). In accordance with the principles of Good Clinical Practice, participants were given at least 24 hours to decide if they wished to take part in the study. Due to the exploratory nature of this study, no formal sample size calculation was applied.

5.2.2.1. Participant population

Twelve subjects between the age of 29 and 72 were recruited for this pilot study, including young (n=7) and old (n=5) from past or present staff members or their friends. These were all healthy individuals with no known ocular pathology.

5.2.2.2. 670nm LED light device and procedure

The light device was a small hand-held light source housed in a 2.5cm diameter and 8.7cm long steel tube producing diffuse red light ranging from 650 - 700 nm, emitting energy equivalent to 40mW/cm^2 or 4.8J/cm^2 (in 120 seconds) at the viewing aperture. The viewing aperture comprised 9 light-emitting diodes (LED) which were covered by a diffuser to ensure patient comfort and tolerance. The distal end of the device is closed with a push button switch which activates by the application of power, the light source.

The device in shown in Figure 5.1 and the spectral range of light emitted is illustrated in Figure 5.2. Participants applied the 670 nm light to the front of the study eye (looking at the red light) for two minutes at a time, every morning for 2 weeks.

Figure 5.1. Picture of red-light device consisting of the light source with 9 LED diodes and the power supply (in red) and a diffuser (in white) to ensure uniform and comfortable illumination.

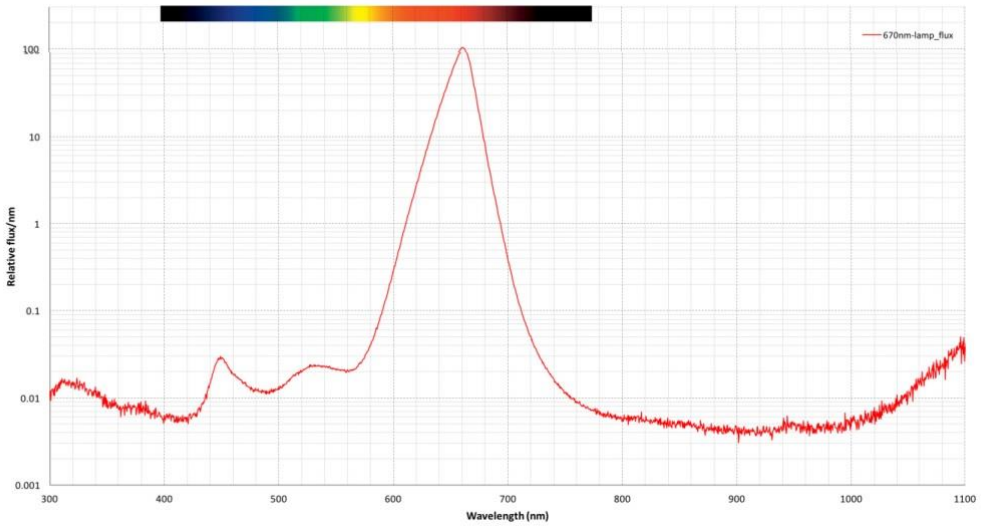

Figure 5.2. Representative spectral output ranging from 650 – 700nm of the device used in both exploratory trials described in this chapter, measured with a spectrophotometer (Ocean Optics, Florida, USA).

5.2.2.3. Statistical Analysis

Mann-Whitney statistical test was used to analyse the difference in baseline thresholds between the young and older subjects. Wilcoxon matched-pairs signed rank test was used, to assess comparative change between baseline and post 2-week treatment with groups. Statistical analysis was performed in GraphPad Prism (version 6, San Diego, CA). Graphs were also generated by the same statistical software.

5.2.3. Results

Results of scotopic thresholds for all subjects are shown in Figure 5.3. Subjects were ordered by age, arbitrarily separated in two groups, young (mean 35.3 ± 5.9 years) from older (65.4 y ± 5.7 years) at 50 years. At baseline, mean retinal sensitivities were significantly lower in the older (55.16 ± 1.73 dB) cohort compared with the younger (60.09 ± 2.59 dB) cohort (p=0.005). Both groups had improved thresholds after treatment, an increase of 1.83 dB (p=0.06) and 2.76 dB (p=0.03) were observed in younger and older participants respectively. These results are displayed in Figure 5.4 and 5.5.

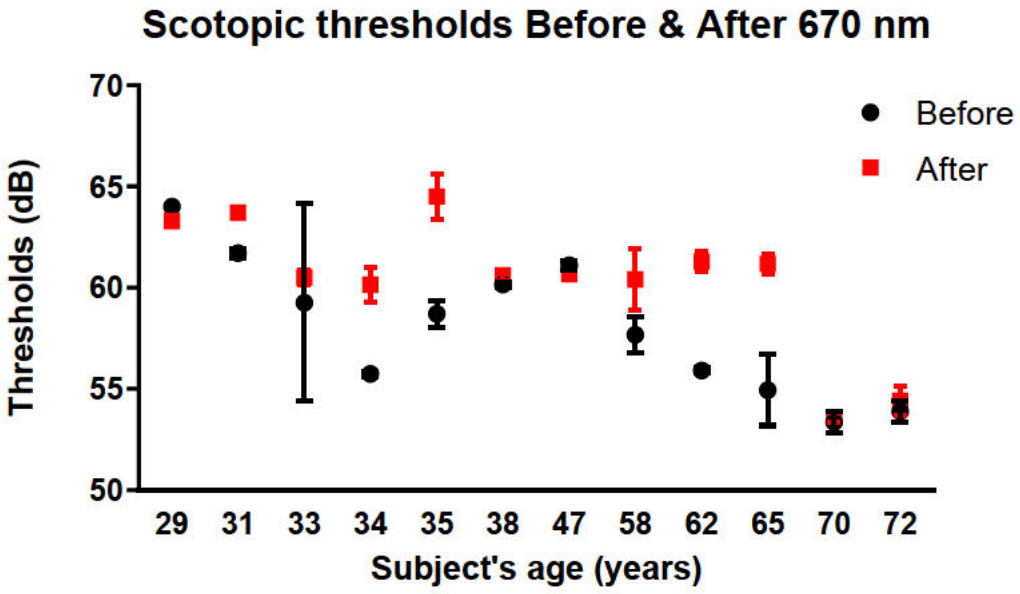

Figure 5.3. Mean scotopic thresholds (raw data) for all 12 subjects: young (N=7) and older (N=5) from before and post 2-week 670nm light exposure in ascending age order. Black dots represent the thresholds before exposure and the red squares after 670nm therapy. The error bars represent SD.

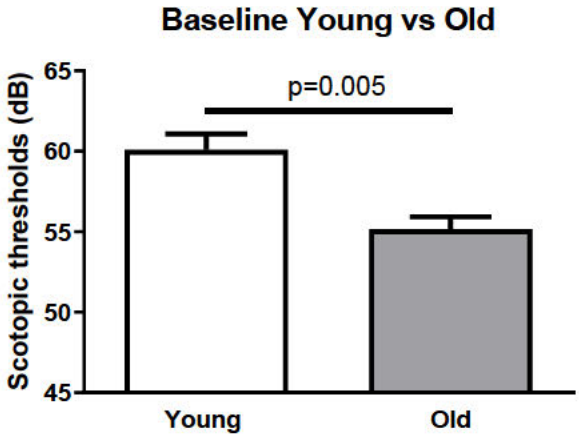

Figure 5.4. Baseline scotopic thresholds for young and old subjects. Mean retinal sensitivities were significantly reduced in the older group (55.16 ± 1.73 dB, N=5) compared to the younger group (60.09 ± 2.59 dB, N=7), p=0.005. Error bars = SEM.

Figure 5.5. Mean scotopic thresholds from baseline and after 2-week treatment in young (N=7) and older subjects (N=5). Retinal sensitivity increased significantly in the older cohort (p=0.034). Error bars = SEM.

5.2.4. Discussion

Photoreceptor inner segments, which are highly enriched with mitochondria, require large quantities of ATP to fuel the ionic pumps needed to maintain the dark current (Wong-Riley, 2010; Eells, 2019). However, in aged human retina, there is a progressive and disproportionate parafoveal loss of rod photoreceptors compared to relatively stable cone photoreceptors (Curcio *et al.*, 1993). Scotopic retinal sensitivity also declines at a faster pace than photopic sensitivity (Jackson and Owsley, 2000). The findings in this pilot study show improved aged rod photoreceptor function (increase of 2.76dB) following brief exposure to 670-nm light daily over the course of two weeks only in the older cohort.

It is possible the younger cohort did not significantly benefit from the improvement because their mitochondrial function has not yet been compromised. A mean difference change assessment performed in the lab revealed the intersession and intrasession mean difference change of 2.16 dB and 2.42 dB respectively (unpublished). Although a small improvement, the increase in scotopic thresholds obtained was above that of the variability measured for this test on the Medmont DAC perimeter. However, these findings need to be further validated in longitudinal and larger sample size study. The effect of this treatment is further explored in healthy aging and AMD in the following section.

5.3. Effect of 670nm photobiomodulation in healthy aging and AMD

5.3.1. Aims

The aims of this prospective, interventional clinical trial were:
Assess the primary outcome measure of change in visual function as determined by rod recovery time after exposure to bright flash of light in the study eye from baseline to 12 months in healthy aging and iAMD, measured by AdaptDx dark adaptometer.
Secondary aims of the clinical trial were to investigate the effect of 670nm light on:

- Rod function (static thresholds)
- Best corrected visual acuity, low-luminance acuity and low luminance deficit
- Cone function with photopic flicker ERGs
- Anatomical changes using SD OCT, autofluorescence and colour photographs

5.3.2. Methods

This study was conducted according to the International Conference on Harmonization Guidelines for Good Clinical Practice and the tenets of the Declaration of Helsinki. The study protocol was approved by the National Research Ethics Committee (16/LO/2022). All study participants provided written informed consent.

Part of the data in this chapter was collected by Dr Chrishnepriya Sivapathasuntharam (Baseline and 1-month visits for 670nm trial). Dr Shruti Chandra was the second grader for classification of AMD participants. Sarega Gurudas (statistician) helped/confirmed statistical analysis.

5.3.2.1. Study population

Forty-two individuals aged over 55 years with healthy fundus or with intermediate AMD (with and without SDD) were directly recruited from retinal clinics or by invitation from hospital or local optometrist or GP. Healthy aging group comprised mainly of spouses of participants with AMD. Participant classification is described in chapter 2.

5.3.2.2. Intervention; 670nm light device

The handheld 670 nm light device used in this trial is described in section 5.2.2.2

5.3.2.3. Procedure

Participants applied the 670 nm light to the front of the study eye (looking at the red light) for two minutes at a time, every morning for 12 months.

5.3.2.4. Safety and compliance

The safety of each participant during the trial was monitored by the documentation and appropriate management of any adverse events (AEs) or serious adverse event (SAEs). Participants were invited to attend an unscheduled visit if they had any visual concerns or via the telephone for any other concerns. Relevant information was gathered and the relationship of any SAE to the intervention was determined by the chief investigator. Compliance to the intervention was also recorded by the participants.

They were instructed to record their adherence to the trial regimen daily on a monthly diary provided and record any observations related to the device such as dimming of the light output due to reduced battery and any malfunction of the device. These were collected at every visit.

5.3.2.5. Statistical analysis

Statistical methodology was conservative, without assumption of normality. Statistical analysis and graphs were generated using GraphPad Prism (Version 8.2.1). Linear mixed effects modelling was performed with IBM SPSS Statistics (SPSS Inc. SPSS for Windows, Version 25.0. Chicago, USA). Given the phase I/II nature of the trial, the main analysis comprised of descriptive statistics to summarize the demographic characteristics and outcome measures of healthy aging and the AMD group. As SDD is a poor prognostic indicator and may suggest more advanced disease, the AMD group was subdivided into those with and without SDD. Change from baseline to 12 months was analysed using paired t-tests (for normally distributed data) or Wilcoxon matched-pairs signed rank test (for non-normally distributed data). Mixed effects modelling was performed for analysis of repeated measures at each visit (baseline, 1 month, 3 months, 6 months and 12 months) for all functional outcome measures which allows for robust statistical assessment even with missing data. Multiple group comparative analysis between outcome measures were conducted using the one-way analysis of variance (ANOVA) and p-values were adjusted in accordance with the Bonferroni correction.

5.3.3. Results

5.3.3.1. Patient characteristics

Of the 42 participants recruited, 35 completed the trial (healthy aging group n=12, iAMD no SDD group n=15 and iAMD with SDD group n=8).

Five withdrew consent due to the intensity of the study visit assessments and two developed neovascular AMD and were excluded. The flow diagram of subject enrolment and classification is displayed in Figure 5.6.

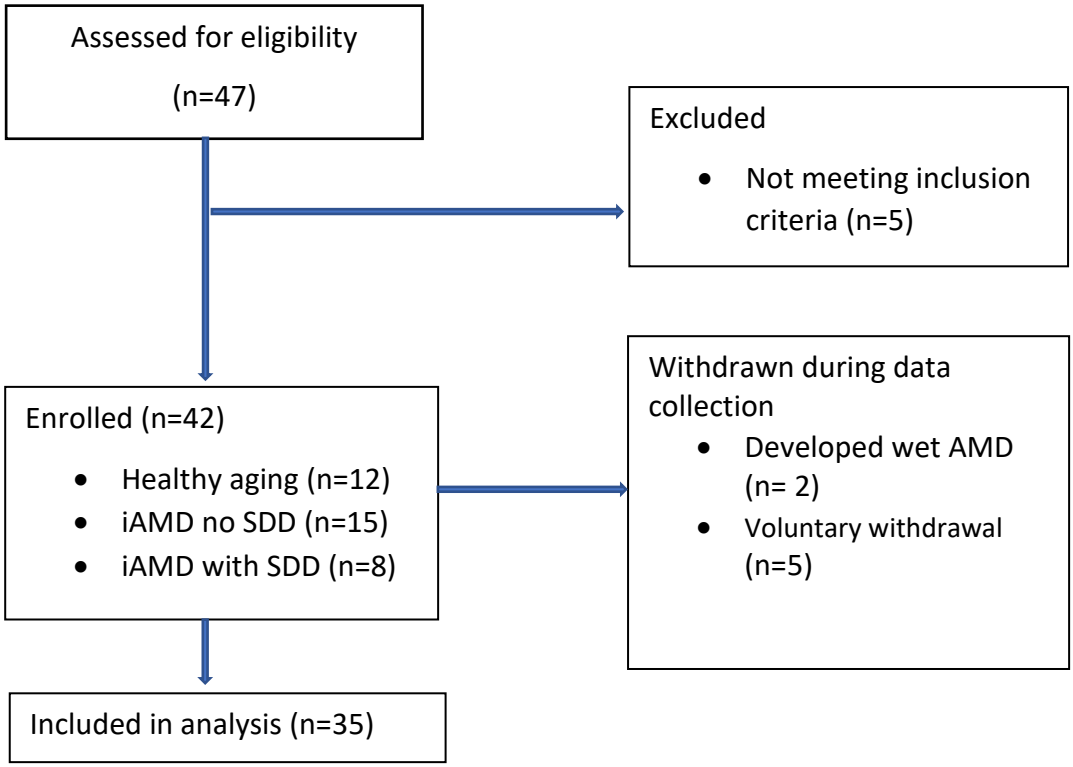

Figure 5.6. Flow diagram of study participants enrolment in 670nm clinical trial over 12-month period.

The mean age for healthy aging and iAMD and iAMD with SDD participants at baseline was 69.9 ± 2.5 years (range: 66.3-74.2 years, 58% female), 69.5 ± 7.2 years (range: 55.6-79.9 years, 67% female) and 69.3 ± 6.8 years (range 60.3-78.7, 63% female) respectively. The healthy-aged and AMD groups were well matched in age between all three groups. There was a higher prevalence of females within all groups and 95% of all participants were Caucasians (5% were Asian, N=2). Descriptive statistics of each group at baseline is shown in Table 5.1.

Table 5.1. Participant characteristics in each group at baseline visit who completed 12-month study duration.

	Healthy Aging (N= 12)	iAMD no SDD (N=15)	iAMD with SDD (N=8)
Mean Age (± SD)	69.9 (± 2.5)	69.4 (±7.2)	69.3 (±6.8)
Ethinicity (N, %)			
Asian	1 (8%)	1 (7%)	0 (0%)
Caucasian	11 (92%)	14 (93%)	8 (100%)
Gender balance			
N (% female)	7 (58%)	10 (67%)	5 (63%)
Smoking status			
Current or former smoker (N, %)	4 (33%)	8 (53%)	2 (25%)
Diabetes history (N, %)	0%	0%	0%
Hypertension (N, %)	4 (33%)	3 (20%)	3 (38%)
Hyperlipidemia ((N, %)	6 (50%)	4 (27)%	1 (13%)
Ocular supplements (N, %)	0 %	8 (56%)	5 (63%)

5.3.3.2. Primary Outcome measure

In healthy eyes, rod-intercept time reduced by 3.76 min (p = 0.03) at 12-month follow up. No reduction in rod-intercept time was observed in iAMD with no SDD (p = 0.49) or iAMD with SDD (p > 0.99) groups. A comparison of RIT data was performed with inclusion and exclusion of assessments with more than 30% fixation error. Although this did not affect the statistical outcome, we opted to exclude 17 tests that did not meet this criterion as per previous studies.

5.3.3.3. Effect of 670nm light therapy on functional secondary outcomes: baseline vs 12 months

The changes from baseline to 12 months for all functional measure outcomes is summarized in table 5.2. There was no improvement in visual acuities in any group over time. Statistically significant decrease of 3.6 letters in low luminance was found in iAMD group (p= 0.005).

A significant increase in low-luminance deficit of 4.5 letters (p= 0.006) and 3.6 letters (p=0.05) was observed in iAMD no SDD and iAMD with SDD groups respectively. These are presented in Figure 5.7.

Scotopic thresholds improved by 1.77 dB (p=0.03) in healthy aging, however no change was observed for iAMD no SDD (p=0.30) or iAMD without SDD (p=0.58). None of the noticeable outliers were removed from analysis as they were plausible data and did not affect the statistical outcome of the parameters. Results for rod-mediated tests are shown in Figure 5.8.

Analysis of mean change in photopic outcomes revealed no significant difference over the 12-month period in photopic 28.3Hz flicker ERGs amplitude or timing in the iAMD without SDD group. A decrease in the amplitude in the healthy aging group of 3.21 µV (p=0.02) was observed. A modest increase in timing in the iAMD with SDD group (p=0.04) was found whereas other groups remained unchanged. Photopic function outcomes are represented in Figure 5.9.

Table 5.2. Twelve-month change from baseline in functional outcome measures for each study group. Means alongside the standard deviation (bracketed) values are shown. The P-values specified relate to the significance of the difference in mean change within study groups.

Outcomes	**Healthy Aging Mean (SD); N**			**iAMD no SDD Mean (SD); N**			**iAMD with SDD Mean (SD); N**		
	Baseline	**12 Months**	**P value**	**Baseline**	**12 Months**	**P value**	**Baseline**	**12 Months**	**P value**
BCVA (ETDRS letters)	87.1 (6.5); 12	85.7 (6.4); 12	0.356	84.8 (7.6); 15	85.7 (6.6); 15	0.456	81.6 (3.7); 8	83.0 (3.9); 8	0.211
LLVA (ETDRS letters)	73.5 (6.0); 12	70.4 (6.2); 12	0.182	70.8 (8.1); 15	67.2 (9.1); 15	0.005	68.1 (6.3); 8	65.9 (8.7); 8	0.188
LLD (ETDRS letters)	13.6 (3.7); 12	15.3 (4.3); 12	0.363	14.0 (5.5); 15	18.5 (6.3); 15	0.006	13.5 (5.9); 8	17.1 (8.3); 8	0.050
Scotopic Thresholds (dB)	56.18 (2.94); 12	57.96 (1.75); 12	0.030	54.69 (4.59); 15	55.58 (6.20); 15	0.303	54.92 (4.39); 8	53.93 (4.64); 8	0.576
RIT (minutes)	9.35 (5.21); 7	5.59 (2.58); 7	0.031	14.92 (6.12); 8	13.24 (5.17); 8	0.438	20.00 (0.00); 5	19.21 (1.77); 5	>0.999
Photopic 28.3Hz flicker ERG amplitude (μV)	14.21 (5.92); 11	11.00 (4.21); 11	0.020	16.82 (7.74); 12	14.54 (6.30); 12	0.583	14.59 (4.67); 7	12.43 (5.88); 7	0.443
Photopic 28.3Hz flicker ERG timing (ms)	27.80 (1.28); 11	28.05 (0.82); 11	0.338	28.31 (1.34); 12	28.88 (1.28); 12	0.192	28.13 (1.23); 7	29.43 (1.14); 7	0.038

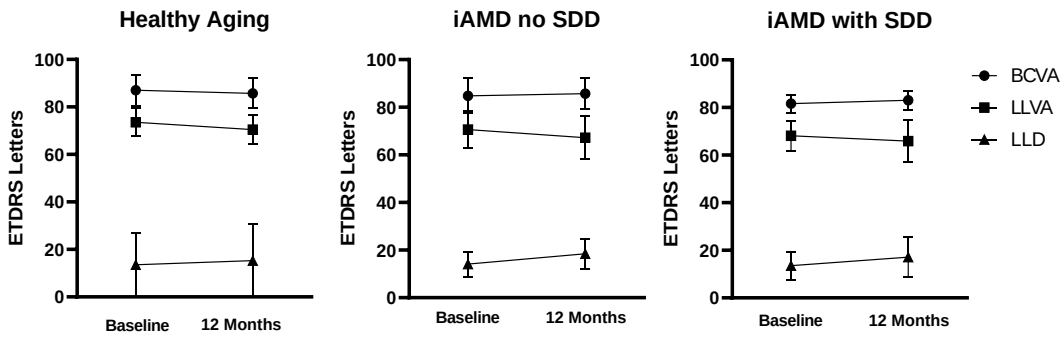

Figure 5.7. Means plots showing 12-month change in best-corrected visual acuity (BCVA), low-luminance visual acuity (LLVA) and low-luminance deficit (LLD) within healthy aging, iAMD without SDD and iAMD with SDD groups. Whiskers denote standard deviation. There was no change in the healthy aging group in any parameter. Statistically significant decrease in LLVA (3.6 letters, p=0.005) and an increase in LLD (4.5 letters, p=0.006) was detected in the iAMD without SDD group. A low-luminance deficit of 3.6 letters (p=0.050) was found in the AMD with SDD group while there was no difference in BCVA or LLVA.

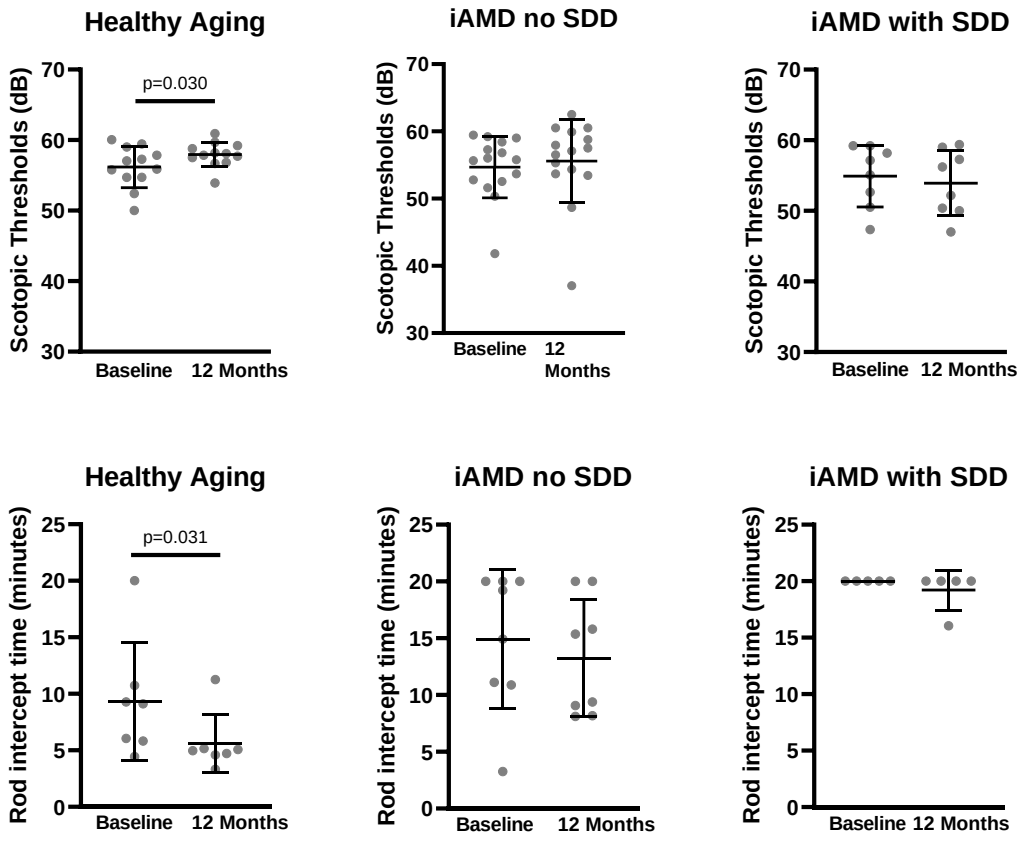

Figure 5.8. Scatter plots showing change in scotopic thresholds and rod-intercept time (RIT) from baseline to 12 months for 670nm trial participants. Error bars represent the mean with standard deviation. Only assessments with less than 33% fixation error are displayed in RIT plots. There was an improvement in rod-mediated function for the healthy aging group only (scotopic thresholds p=0.030, RIT p=0.031).

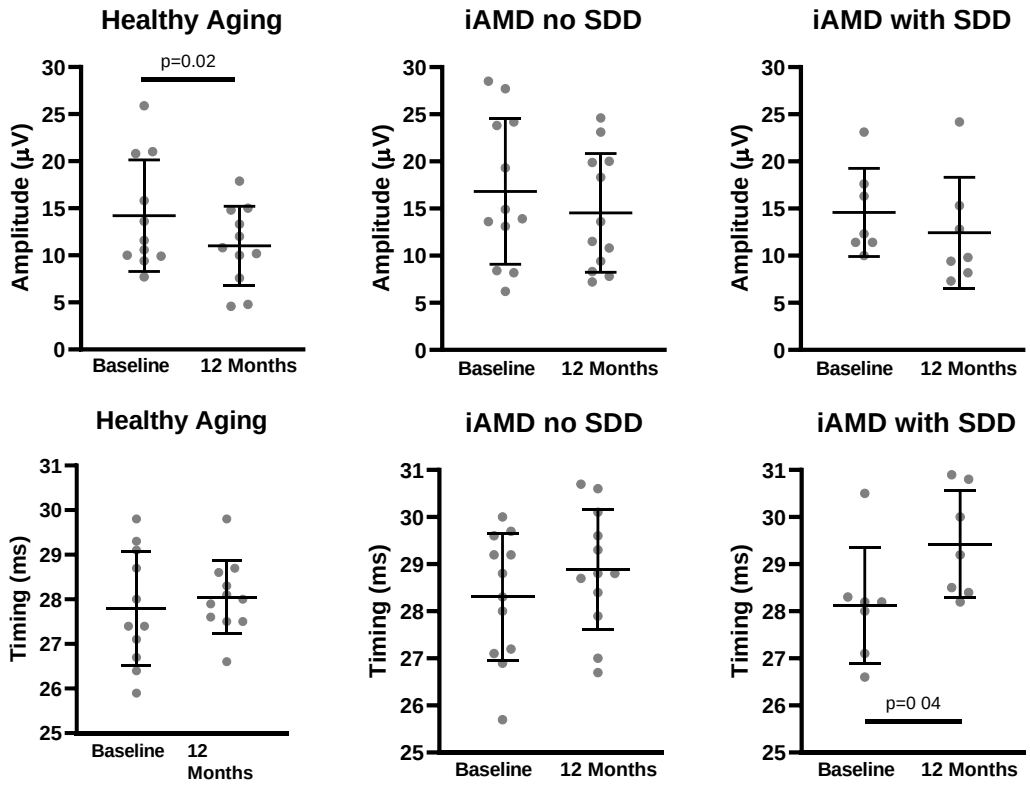

Figure 5.9. Scatter plots comparing change in photopic 28.3Hz flicker ERGs over 12-month period in each group. Error bars represent the mean with standard deviation. There was statistically significant difference in amplitude in healthy aging group p= 0.02 and no change in the other two groups (iAMD no SDD p=0.58, iAMD with SDD p= 0.44). An increase in time was recorded in the iAMD with SDD group (p=0.04) whilst the healthy aging or iAMD no SDD groups remained stable over 12 months (p=0.34 and p=0.19 respectively).

5.3.3.4. Effect of 670nm light therapy on structural outcomes within groups

This study focused on two structural outcome measures: volumetric analysis over time of the ONL and RPE-BM complex within the central 6mm diameter of the macula. Differences in volumetric layers between groups at baseline and 12 months and changes within groups over time were assessed. All data was normally distributed with the exception of RPE-BM complex volume outcome measure the iAMD group. An evident outlier in the dataset (Px 67014) was confirmed by applying the ROUT method (Motulsky and Brown, 2006) and therefore that particular observation was omitted in statistical evaluation.

No significant difference was observed in mean ONL volumes between groups at baseline and 12 months (p=0.679, p=0.091 respectively). A substantial variation was found at baseline (p=0.019) and 12 months (p=0.006) between groups in RPE-BM complex mean volumes. This is shown graphically in Figure 5.10. Eyes with SDD had higher RPE-BM complex mean volume compared to healthy eyes at baseline (p=0.035) and 12 months (p=0.023). Increased RPE-BM volume was observed in participants with iAMD no SDD at annual review (p=0.014).

Changes within groups were also evaluated with paired-t-tests and are displayed in Figure 5.11. A significant decrease in ONL volume was observed in disease groups (p= 0.0002, p=0.005 in iAMD no SDD and iAMD with SDD respectively) while the healthy aging group remained unchanged (p=0.936) over 12 months. No considerable difference was observed in the RPE-BM complex thickness over time in any of the groups (healthy aging p=0.971, iAMD no SDD p=0.097 and iAMD with SDD p=0.197).

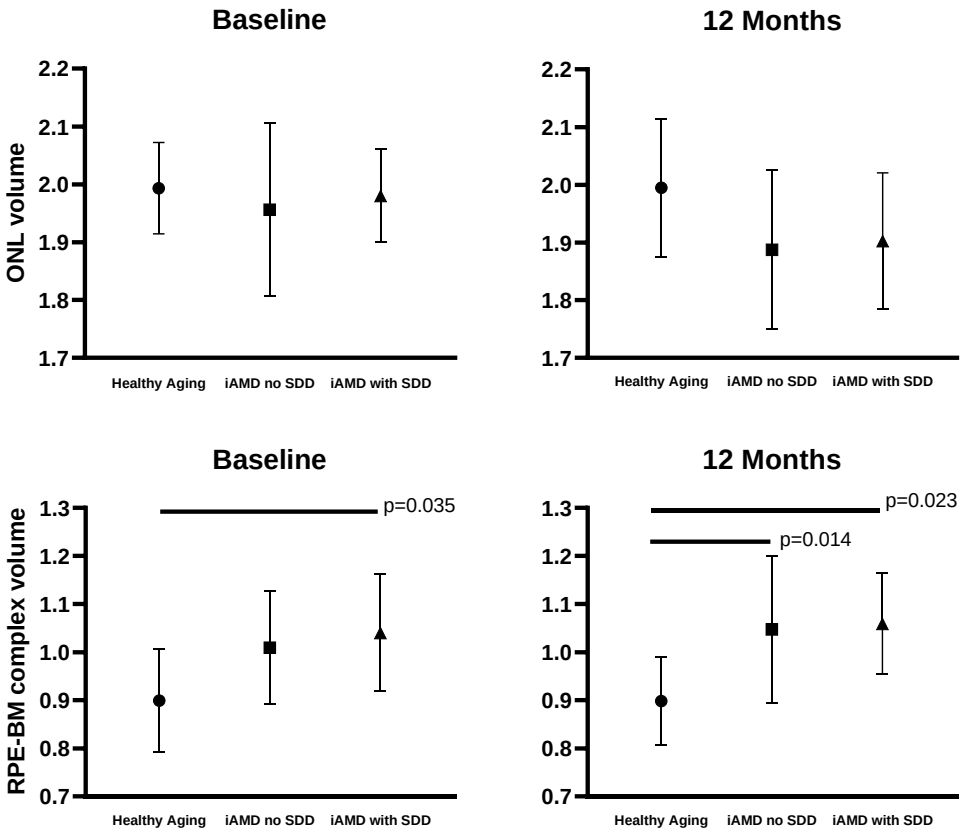

Figure 5.10. Mean plots showing differences at baseline and 12 months in outer nuclear layer (ONL) and retinal pigment epithelium and Bruch's membrane complex (RPE-BM) layer volumes between healthy aging (n=12), iAMD no SDD (n=16) and iAMD with SDD (n=8) groups. Statistically significant differences are displayed on the plots. Error bars represent SD.

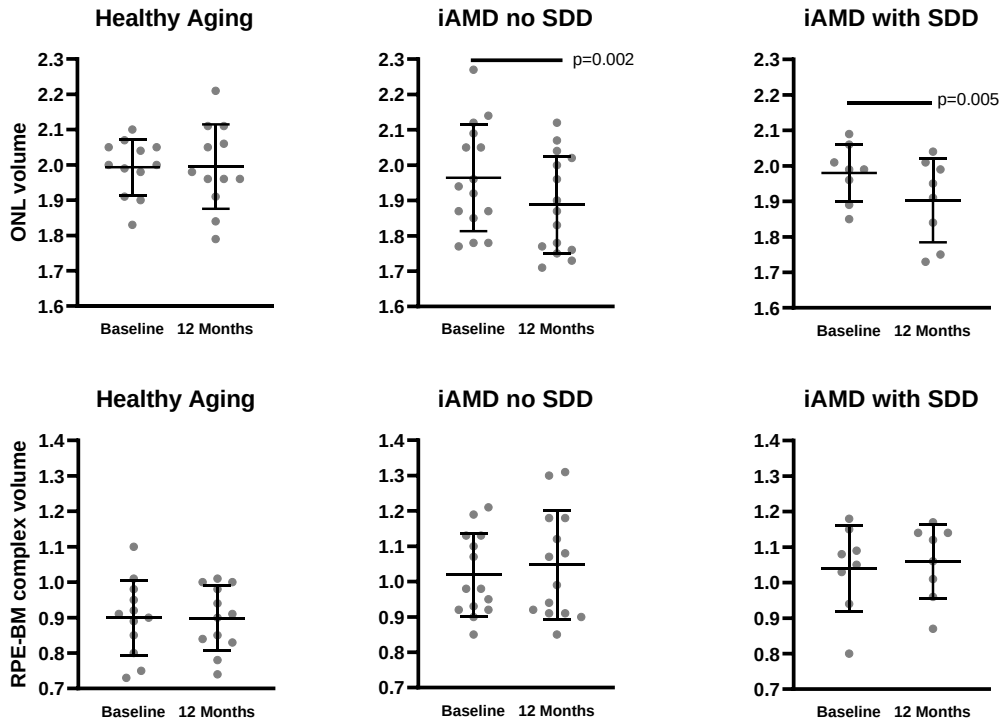

Figure 5.11. Scatter plots showing individual data and mean change within groups from baseline to 12 months. Statistically significant differences are displayed on the plots. Error bars represent SD. ONL volume decreased in both disease groups (iAMD no SDD p=0.002 and iAMD with SDD, p=0.005) whereas RPE-BM complex volume remained unchanged from baseline to annual review in all groups.

5.3.3.5. Effect of 670nm light therapy over time on functional measures

Previous light therapies in AMD studies have only reported post-treatment final outcome results (Ivandic & Ivandic 2008, Merry et al., 2017). This proof-of-concept study also sought to investigate potential changes of 670nm light exposure at multiple time periods on functional outcome measures which are detailed in Table 5.3-5.5. After adjusting for disease group, the RIT in healthy aging was significantly reduced from 10.67 minutes at baseline by 5.61 minutes (95% CI = -9.05 to -2.17; p= 0.002) at 3 months, by 6.37 minutes (95% CI = -9.87 to -2.87; p<0.001) at 6 months and by 5.80 minutes (95% CI = -9.24 to -2.36; p=0.001) at 12 months.

The adjusted difference between iAMD no SDD group and healthy aging group was statistically significant (at 3 months, 5.36 minutes, 95% CI = 0.89 to 9.83; p=0.019; at 6 months, 6.19 minutes, 95% CI = 1.55 to 10.83; p=0.009 whereas the change between the iAMD no SDD group and healthy aging subjects was not significant at any time point. In contrast, the adjusted difference was non-significant for scotopic thresholds. The changes over 5 visits for rod-mediated tests are illustrated in Figure 5.12. None of the predicted differences in any of the other functional outcome measures were statistically significant as represented in Figure 5.13 and 5.14.

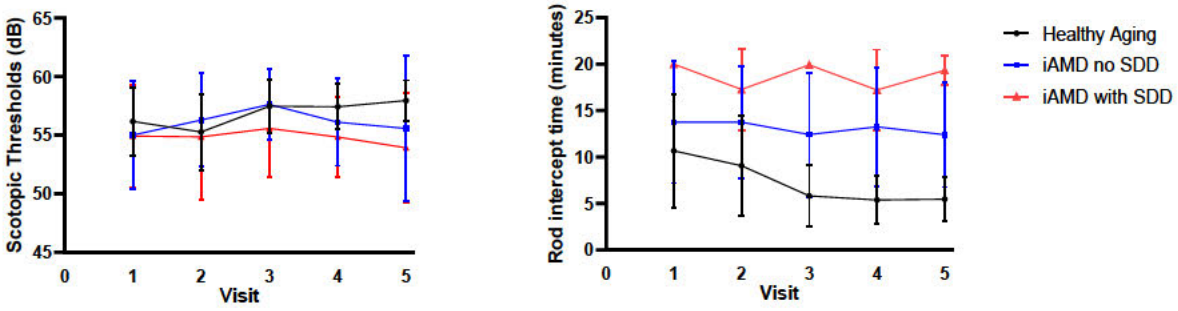

Figure 5.12. Mean plots representing change over 5 visits (1=Baseline, 2=1 month, 3= 3 months, 4= 6 months and 5 = 12 months) between healthy aging, iAMD no SDD and iAMD with SDD groups for scotopic thresholds and rod intercept time (RIT). There was no statistically significant change for scotopic thresholds in any group over time. RIT was reduced in the healthy aging group at visit 3 by 5.61 minutes (95% CI = -9.05 to -2.17; p= 0.002), at visit 4 by 6.37 minutes (95% CI = -9.87 to -2.87; p<0.001) and at visit 5 by 5.80 minutes (95% CI = -9.24 to -2.36; p=0.001) from baseline. There was no significant improvement in RIT for the disease groups. Error bars represent SD.

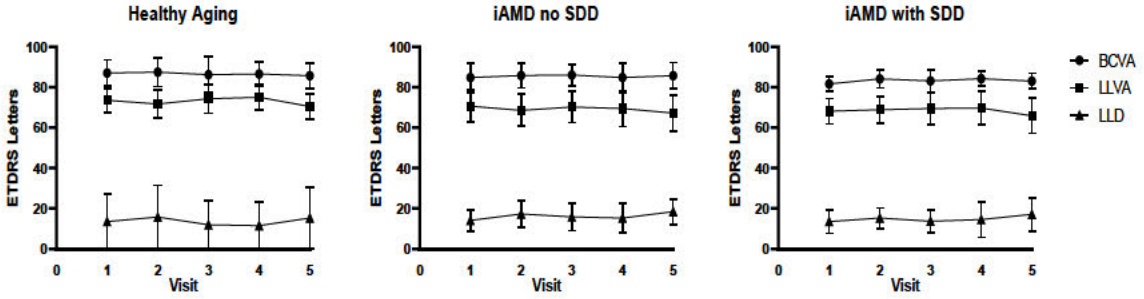

Figure 5.13. Mean plots showing best-corrected visual acuity (BCVA), low-luminance visual acuity (LLVA) and low-luminance deficit (LLD) change across all 5 visits between healthy aging, iAMD with and without SDD groups (Visit 1=Baseline, 2=1 month, 3= 3 months, 4= 6 months and 5 = 12 months). There was no difference in BCVA, LLVA or LLD at any visit compared to baseline for any group. Error bars represent SD. No significant change found after adjusting for disease at any visit within groups.

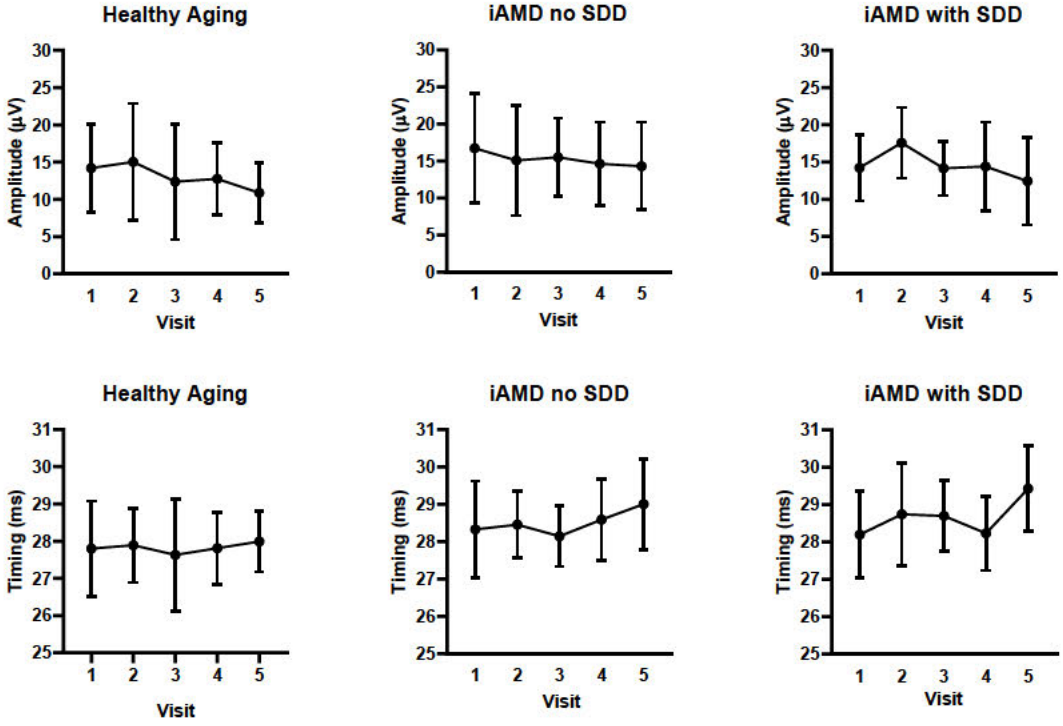

Figure 5.14. Mean plots representing change over 5 visits (1=Baseline, 2=1 month, 3= 3 months, 4= 6 months, and 5 = 12 months) between healthy aging, iAMD with and without SDD groups for photopic 28.3Hz flicker ERGs amplitude and timing obtained from the best-fitted sine wave to the waveform. There was no significant change over time for any group. Error bars represent SD

Table 5.3. Table 5.3 Change in visual acuity outcomes; best-corrected visual acuity (BCVA), low-luminance visual acuity (LLVA) and low luminance deficit (LLD).

BCVA (ETDRS Letters)	**Mean (SD); N**			**Change from baseline Mean (SE)**			**Adjusted difference between groups (95% CI)**			
	Healthy aging	iAMD with no SDD	iAMD with SDD	Healthy aging	iAMD with no SDD	iAMD with SDD	iAMD with no SDD vs Healthy aging	p-value	iAMD- with SDD vs Healthy aging	p-value
Baseline	87.1 (6.5); 12	84.8 (7.6); 15	81.6 (3.7); 8	-	-	-	-	-	-	-
1 Month	87.4 (7.2); 12	85.9 (6.4); 15	84.1 (4.5); 8	0.33 (1.05)	1.13 (0.93)	2.50 (1.28)	0.80 (-1.97- 3.58)	0.569	2.17 (-1.10- 5.43)	0.192
3 Months	86.2 (8.8); 12	86.0 (5.5); 15	83.1 (5.6); 7	-0.92 (1.05)	1.20 (0.93)	1.42 (1.34)	2.12 (-0.66- 4.89)	0.134	2.34 (-1.02- 5.70)	0.170
6 Months	86.5 (6.3); 10	84.8 (7.3); 13	84.2 (3.7); 6	0.57 (1.11)	0.89 (0.98)	1.40 (1.41)	0.32 (-2.58- 3.22)	0.829	0.84 (-2.69- 4.37)	0.640
12 Months	85.7 (6.4); 12	85.7 (6.6); 15	83.0 (3.9); 8	-1.42 (1.05)	0.87 (0.93)	1.38 (1.28)	2.28 (-0.47- 5.03)	0.104	2.79 (-0.47- 6.05)	0.092
LLVA (ETDRS Letters)	**Mean (SD); N**			**Change from baseline Mean (SE)**			**Adjusted difference between groups (95% CI)**			
	Healthy aging	iAMD with no SDD	iAMD with SDD	Healthy aging	iAMD with no SDD	iAMD with SDD	iAMD with no SDD vs Healthy aging	p-value	iAMD- with SDD vs Healthy aging	p-value
Baseline	73.5 (6.0); 12	70.8 (8.1); 15	68.1 (6.3); 8	-	-	-	-		-	-
1 Month	71.7 (7.0); 12	68.6 (8.2); 15	68.9 (6.5); 8	-1.83 (1.50)	-2.20 (1.34)	0.75 (1.84)	-0.37 (-4.35- 3.62)	0.856	2.58 (-2.12- 7.28)	0.279
3 Months	74.3 (7.2); 12	70.3 (8.0); 15	69.4 (7.7); 7	0.75 (1.50)	-0.53 (1.34)	0.90 (1.92)	-1.28 (-5.27- 2.70)	0.525	0.15 (-4.69- 4.98)	0.950
6 Months	75.0 (6.1); 10	69.5 (8.9); 13	69.7 (8.4); 6	2.25 (1.59)	-0.05 (1.40)	2.05 (2.02)	-2.30 (-6.50- 1.90)	0.281	-0.20 (-5.29- 4.89)	0.939
12 Months	70.4 (6.2); 12	67.2 (9.0); 15	65.9 (8.7); 8	-3.08 (1.50)	-3.60 (1.34)	-2.25 (1.84)	-0.52 (-4.50- 3.47)	0.798	0.83 (-3.87- 5.53)	0.726
LLD (ETDRS Letters)	**Mean (SD); N**			**Change from baseline Mean (SE)**			**Adjusted difference between groups (95% CI)**			
	Healthy aging	iAMD with no SD	iAMD with SDD	Healthy aging	iAMD with no SDD	iAMD with SDD	iAMD with no SDD vs Healthy aging	p-value	iAMD with SDD vs Healthy aging	p-value
Baseline	13.6 (3.7); 12	14.0 (5.5); 15	13.5 (5.9); 8	-	-	-	-		-	-
1 Month	15.6 (5.8); 12	17.3 (7.1); 15	15.3 (5.0); 8	2.17 (1.57)	3.33 (1.40)	1.75 (1.92)	1.17 (-2.99- 5.32)	0.580	-0.42 (-5.32- 4.49)	0.867
3 Months	11.9 (5.7); 12	15.7 (7.0); 15	13.7 (5.6); 7	-1.67 (1.57)	1.73 (1.40)	2.13 (1.92)	3.40 (-0.76- 7.56)	0.108	3.79 (-1.11- 8.69)	0.128
6 Months	11.5 (3.9); 10	15.3 (7.3); 13	14.5 (8.9); 6	-1.74 (1.66)	0.94 (1.46)	-0.62 (2.10)	2.68 (-1.70- 7.06)	0.229	1.12 (-4.19- 6.42)	0.678
12 Months	15.3 (4.3); 12	18.5 (6.3); 15	17.1 (8.3); 8	1.67 (1.57)	4.47 (1.40)	3.63 (1.92)	2.80 (-1.36- 6.96)	0.185	1.96 (-2.94- 6.86)	0.431

Table 5.4. Change in rod function outcome measures; scotopic threshold and rod-intercept time (RIT).

Rod-intercept time (RIT, minutes)	**Mean (SD); *n***			**Change from baseline Mean (SE)**			**Adjusted difference between groups (95% CI)**			
	Healthy aging	iAMD with no SDD	iAMD with SDD	Healthy aging	iAMD with no SDD	iAMD with SDD	iAMD with no SDD vs Healthy aging	*p*-value	iAMD with SDD vs Healthy aging	*p*-value
Baseline	10.67 (6.09); 8	14.52 (6.41); 10	20.00 (0.00); 5	-	-	-	-	-	-	-
1 Month	9.06 (5.33); 8	14.19 (6.08); 12	17.29 (4.36); 5	−1.89 (1.72)	0.93 (1.55)	−0.42 (2.45)	2.82 (−1.78–7.42)	0.227	1.47 (−4.47–7.40)	0.625
3 Months	5.83 (3.33); 11	12.51 (6.89); 15	19.92 (0.22); 7	−5.61 (1.74)	−0.62 (1.50)	−0.27 (2.40)	4.98 (0.43–9.54)	0.032	5.33 (−0.55–11.21)	0.075
6 Months	5.39 (2.60); 10	13.27 (6.42); 13	17.21 (4.36); 5	−6.37 (1.77)	−0.44 (1.57)	−3.93 (2.45)	5.92 (1.24–10.61)	0.014	2.44 (−3.56–8.43)	0.422
12 Months	5.48 (2.40); 11	12.40 (5.64); 13	19.34 (1.61); 6	−5.80 (1.74)	−1.20 (1.57)	−0.46 (2.40)	4.60 (−0.04–9.25)	0.052	5.34 (−0.54–11.23)	0.075
Scotopic Thresholds (dB)	**Mean (SD); *n***			**Change from baseline Mean (SE)**			**Adjusted difference between groups (95% CI)**			
	Healthy aging	iAMD with no SDD	iAMD with SDD	Healthy aging	iAMD with no SDD	iAMD with SDD	iAMD with no SDD vs Healthy aging	*p*-value	iAMD with SDD vs Healthy aging	*p*-value
Baseline	56.18 (2.94); 12	54.70 (4.60); 15	54.92 (4.40); 8	-	-	-	-		-	-
1 Month	55.27 (3.23); 12	56.05 (4.01); 15	54.85 (5.42); 8	−0.91 (1.09)	1.36 (0.98)	−0.07 (1.34)	2.27 (−0.63–5.18)	0.124	0.84 (−2.58–4.27)	0.628
3 Months	57.47 (2.32); 12	57.46 (3.10); 15	55.58 (4.15); 7	1.29 (1.09)	2.77 (0.98)	0.83 (1.40)	1.48 (−1.42–4.39)	0.314	−0.46 (−3.97–3.06)	0.797
6 Months	57.42 (1.93); 10	56.11 (3.73); 13	54.84 (3.39); 6	1.21 (1.16)	1.89 (1.02)	0.09 (1.47)	0.67 (−2.39–3.73)	0.664	−1.12 (−4.82–2.58)	0.550
12 Months	57.95 (1.75); 12	55.58 (6.20); 15	53.93 (4.64); 8	1.77 (1.09)	0.89 (0.98)	−0.99 (1.34)	−0.87 (−3.78–2.03)	0.553	−2.76 (−6.18–0.67)	0.114

Table 5.5. Change in cone function outcome measures; photopic 28.3Hz flicker ERGs amplitude and timing.

Photopic flicker Timing (ms)	**Mean (SD); *n***			**Change from baseline Mean (SE)**			**Adjusted difference between groups (95% CI)**			
	Healthy aging	iAMD with no SDD	iAMD with SDD	Healthy aging	iAMD with no SDD	iAMD with SDD	iAMD with no SDD vs Healthy aging	*p*-value	iAMD with SDD vs Healthy aging	*p*-value
Baseline	27.80 (1.28); 11	28.33 (1.29); 13	28.20 (1.16); 8	-	-	-	-	-	-	-
1 Month	27.89 (1.00); 11	28.44 (0.92); 14	28.74 (1.37); 7	0.03 (0.29)	−0.20 (0.27)	0.13 (0.35)	−0.22 (−1.01–0.56)	0.569	0.10 (−0.80–1.00)	0.827
3 Months	27.63 (1.50); 12	28.22 (0.78); 14	28.70 (0.95); 7	−0.30 (0.28)	−0.20 (0.26)	−0.09 (0.35)	0.09 (−0.67–0.86)	0.805	0.20 (−0.69–1.09)	0.649
6 Months	27.81 (0.97); 10	28.59 (1.09); 12	28.23 (0.99); 6	−0.20 (0.30)	0.22 (0.27)	−0.16 (0.37)	0.42 (−0.38–1.22)	0.302	0.04 (−0.90–0.98)	0.937
12 Months	27.99 (0.82); 12	29.01 (1.22); 14	29.43 (1.14); 7	0.06 (0.28)	0.55 (0.26)	0.86 (0.35)	0.48 (−0.28–1.24)	0.210	0.80 (−0.09–1.69)	0.077
Photopic flicker Amplitude (µV)	**Mean (SD); *n***			**Change from baseline Mean (SE)**			**Adjusted difference between groups (95% CI)**			
	Healthy aging	iAMD with no SDD	iAMD with SDD	Healthy aging	iAMD with no SDD	iAMD with SDD	iAMD with no SDD vs Healthy aging	p-value	iAMD with SDD vs Healthy aging	P-value
Baseline	14.21 (5.92); 11	16.75 (7.41); 13	14.24 (4.44); 8	-	-	-	-	-	-	-
1 Month	15.04 (7.86); 11	15.64 (7.36); 14	17.59 (4.77); 7	1.18 (1.65)	−0.08 (1.53)	2.64 (1.99)	−1.26 (−5.72–3.10)	0.575	1.46 (−3.66–6.59)	0.573
3 Months	12.39 (7.74); 12	15.42 (5.47); 14	14.16 (3.64); 7	−1.61 (1.60)	0.72 (1.49)	−0.19 (1.99)	2.33 (−2.01–6.68)	0.289	1.42 (−3.65–6.49)	0.580
6 Months	12.77 (4.84); 10	14.63 (5.66); 12	14.40 (5.98); 6	−0.29 (1.70)	−0.90 (1.55)	−0.29 (2.10)	−0.61 (−5.17–3.95)	0.791	0.00 (−5.35–5.35)	1.000
12 Months	10.88 (4.03); 12	14.32 (5.91); 14	12.43 (5.88); 7	−3.12 (1.60)	−1.21 (1.50)	−1.87 (2.00)	1.91 (−2.43–6.26)	0.385	1.25 (−3.82–6.33)	0.626

5.3.3.6. Compliance

Participants recorded their own compliance daily. If participants did not regularly use the device in the mornings, they were deemed non-compliant for that criteria. The overall compliance was high in all groups with over 85% of participants declaring using the light device consistently at the same time every morning with less than 5 missed days a month as shown in Table 5.6.

5.3.3.7. Adverse events

There were no device-related serious adverse events in this study. There was no difference between the 2 groups in the proportion of participants with systemic or ocular (in the study eye) serious adverse events or adverse events, unrelated to the progression to late AMD. Two patients in the AMD group progressed to neovascular AMD during the study, both of these patients presented with asymptomatic subretinal fluid.

Table 5.6. Compliance data for 12 month (± 1 month) for all 35 intervention participants who completed the trial from participant's monthly diary.

Groups	Subject ID	Days missed	Total days device used	Compliance (%)	Average days missed/month	Device used every morning same time?
Healthy aging	6702	85	280	76.71	7.08	N
	6705	3	362	99.18	0.25	Y
	67017	26	339	92.88	2.17	N
	67018	57	308	84.38	4.75	N
	67020	11	354	96.99	0.92	Y
	67024	1	364	99.73	0.08	Y
	67030	6	359	98.36	0.50	Y
	67032	1	364	99.73	0.08	Y
	67033	1	364	99.73	0.08	Y
	67037	5	360	98.63	0.42	Y
	67040	5	360	98.63	0.42	Y
	67043	2	363	99.45	0.17	Y
iAMD with no SDD	6706	0	365	100.00	0.00	Y
	6708	21	344	94.25	1.75	N
	67014	9	356	97.53	0.75	Y
	67015	0	365	100.00	0.00	Y
	67016	0	365	100.00	0.00	Y
	67019	10	355	97.26	0.83	Y
	67021	0	365	100.00	0.00	Y
	67029	20	345	94.52	1.67	N
	67034	9	356	97.53	0.75	Y
	67038	13	352	96.44	1.08	Y
	67041	14	351	96.16	1.17	Y
	67042	15	350	95.89	1.25	Y
	67044	1	364	99.73	0.08	Y
	67045	1	364	99.73	0.08	Y
	67047	0	365	100.00	0.00	Y
iAMD with SDD	6701	0	365	100.00	0.00	Y
	67012	3	362	99.18	0.25	Y
	67022	5	360	98.63	0.42	Y
	67023	5	360	98.63	0.42	Y
	67025	2	363	99.45	0.17	Y
	67027	8	357	97.81	0.67	Y
	67031	0	365	100.00	0.00	Y
	67036	4	361	98.90	0.33	Y
Cohort average		**9.8**	**355.2**	**97.32**	**0.82**	**85.7%**

5.3.4. Discussion

The findings of this proof-of-concept study suggest that 670nm light therapy had no beneficial improvement on intermediate AMD with or without SDD over 12 months. It is important to note that due to a lack of a control group, we are unable to ascertain if this treatment was able to delay progression in intermediate AMD compared to those who do not receive the intervention. This is challenging as the red light is evidently visible and therefore it is difficult to get an appropriate sham device. Nonetheless, whether photobiomodulation therapy was able to prevent further disease progression is examined using a control arm (healthy aging cohort from the observational FUSCHIA study) in chapter 7 of this thesis.

This study is not in agreement with previous results reported by Merry at al. (2017) and Ivandic & Ivandic (2008) relating to increased BCVA following light therapy. An increase on average of 2.3 ETDRS letters in the iAMD no SDD group and 2.8 ETDRS letters in the AMD with SDD group when compared to the healthy aging group was found but was not statistically significant and fall much below the improved visual acuities reported in the aforementioned trials. These could represent inter-test variations. The variability of BCVA has been previously reported (Patel *et al.*, 2008). There was also a negative trend in low luminance outcome measure although this was not deemed statistically significant.

The primary aim of this proof of concept study was to evaluate dark adaptometry which has been previously been investigated as a potential biomarker in AMD due to disproportionate decline in rod function in this disease (Owsley *et al.*, 2016). Interestingly, 670nm light exposure had no effect on disease groups, but did reduce rod recovery time in the healthy aging group. This may be due investigator error at baseline and 1 month study visit, who had in some instances performed this assessment following OCT imaging and fundus photography due to patient fatigue, suggesting some of the patients may not have recovered fully from retinal bleaching effect of the imaging instruments and therefore resulted in delayed rod recovery.

Another rod-mediated test evaluated was the scotopic thresholds. There was an increase of 2.63 dB at 3 months and 1.77 dB at 6 months before being reduced to 0.77 dB at 12 months in healthy in participants with iAMD no SDD when compared to baseline results. There was a fairly small increase of 1.77 dB in the healthy aging group at 12 months. A test-retest variability assessment on the Medmont DAC perimeter was performed on young, older and AMD patients and the mean difference change was found to be 2.16 dB and 2.42dB respectively (unpublished). The results obtained in this study fall short to detect meaningful change post intervention.

None of the other cone function outcome measures revealed any positive effect post-treatment. The peri-orbital skin electrodes of the handheld device, RETeval, are very sensitive to correct positioning and much more critical to the recording of the amplitude parameter and less so for time latency parameter (Hobby *et al.*, 2018). This is consistent with our results where amplitudes were highly variable though there was a tendency towards an average decrease at 12 months (not significant) whereas the time parameter remained stable throughout the trial.

While this trial did not investigate drusen regression due to its naturally dynamic formation and spontaneous regression (or impending GA), structural volumetric analysis of the RPE-BM complex layer was assessed to evaluate the effect of 670nm light therapy. This is assuming a decrease in RPE-BM complex layer volume if drusen regression had occurred. After 12 months, there was no difference from baseline in any of the groups. There was also no beneficial impact of 670nm light exposure on ONL after 12 months. A protective effect was described in treated aged CFH mice where controls had thinner ONL compared to the treated group (Sivapathasuntharam *et al.*, 2019), although appreciating in humans the treatment may simply have not been long enough when taking into account the lifespan of a murine model. Also, in Merry et al, (2017) trial, subjects were given light therapy in both eyes, and this begs the question whether or not there was an additive effect due to the abscopal effect. This implies the application of the light to a body-part far from the intended point of effect, still had an effect (Johnstone *et al.*, 2014; Saliba *et al.*, 2015).

Multiple studies have investigated the power and energy levels in the red and NIR spectrum for therapeutic effect. These have been variable and ranged from 5-50mW/cm² (Huang, et al., 2011). The dosage used in this study was equivalent to 40 mW/ cm^2 or 4.8 J/ cm^2 (in 120 s) daily for up to 12 months. This was primarily derived from previous successful outcomes in aged murine retinae and murine models of AMD studies (Kokkinopoulos *et al.*, 2013; Gkotsi *et al.*, 2014; Calaza *et al.*, 2015; Sivapathasuntharam *et al.*, 2017b). It was also in alignment with previously published clinical AMD trials which used photobiomodulation regimen of 780 nm semiconductor laser delivered twice a week for 2 weeks for 40 s at 0.3 J/ cm^2 (equivalent to 7.5 mW/ cm^2) from Ivandic and Ivandic (2008) and at 670 nm, for 88 s, delivering 4–7.7 J/ cm^2 (equivalent to 50-80mW/ cm^2) during a total of 9 sessions over 3 weeks, yielding positive outcomes (Merry *et al.*, 2017). However, the optimal dosage and energy therapy remains elusive. This needs to be established to allow for comparison between trials. Nonetheless, the safety and feasibility of 670nm light therapy was established in neonates in the prevention of retinopathy of prematurity albeit at reduced dose of 9 J/ cm^2, equivalent to 10mW/ cm^2 (Kent *et al.*, 2015). It is unclear if other wavelengths could have similar effect in increasing ATP output, which could be absorbed within COX's absorbance spectrum.

This is the first proof of concept study in discordance with the limited studies published investigating therapeutic benefit of photobiomodulation in AMD. A strength of this study was a robust and established assessment protocol of all parameters. There was also, a very good overall compliance of more than 85% amongst all participants with the exclusion of one subject. The major advantage of the current approach was that these measures were evaluated in the same eyes over a period of time allowing the analysis of the impact of 670 nm on both rods and cones in a well-characterised group of subjects whose fundi were evaluated by a Beckman Initiative Scale classification system.

Some limitations of the clinical trial include a small sample size. Due to the exploratory nature of these exploratory studies, no power calculation was made, and as such may not have been powered to meaningfully detect a small effect.

Compliance is another limitation, which relied only on subjective recording of consistency and adherence to treatment regimen. Some patients may have overemphasized their compliance and unfortunately this was not monitored objectively. This was not a concern in the other light therapy trials as particpants were given the treatment via the research team as opposed to self-usage of the device at home. The red-light devices were also not routinely verified for continuous emission of power required. Some patients advised us that the light intensity from the device would occasionally start to dim with prolonged use of the batteries. We replenished these batteries every month to ensure consistent light output throughout the trial. In addition, AMD is a heterogeneous disease and the treatment may not have been successful due to the advanced nature of the intermediate stage of the disease in the present cohort. A direct comparison of study population between studies is challenging as different AMD classification systems were used and due to different dosage and light intensity given this may yield different overall results.

In conclusion, the 670nm light treatment offered in this study was not effective in treating or improving functional or structural outcome measures in intermediate AMD. Future trials should aim to establish clinical efficacy of 670nm light treatment for AMD and appropriate biomarkers to assess therapeutic effect of photobiomodulation. As the therapy works widely in animal models, normal aging and induced pathology, it is likely that the window of treatment opportunity, dosing and timing of intervention need to be investigated further. It is possible that there may be an age-dependent time frame where rods can be rescued to enhance their function. As the therapy is suggestive of rod function improvement in healthy aging, further research is required to establish the optimal age window that would benefit from treatment.

Chapter 6. Longitudinal assessment of functional and structural parameters in AMD

In this chapter, longitudinal changes in functional and structural parameters over a 12-month period from the FUSCHIA study cohort (cross-sectional analysis presented in chapter 4) are described. Participants returned at 12-month interval (+3 months) and all outcome measures were re-evaluated.

6.1 Introduction

It is imperative to identify potential functional and structural markers that may be useful to monitor the progression of dry AMD and to detect changes following novel therapeutic agents to prevent or delay the progression of AMD. Previous studies have highlighted rod dysfunction as a candidate marker (Owsley *et al.*, 2001, 2014, 2016; Dimitrov *et al.*, 2011; Jackson *et al.*, 2014). In Chapter 3, we showed that significant differences existed between groups for BCVA, LLVA, LLQ, scotopic thresholds and RIT and that only rod-mediated tests (scotopic thresholds and RIT) were able to distinguish between iAMD with and without SDD. More importantly, we showed that participants with SDD exhibited visual function deficit comparable to those with non-foveal atrophic AMD. In this prospective longitudinal study, we sought to validate these baseline findings. Another aim was to characterise the natural progression of varying AMD severity over 12 months with potential psychometric tests that have been reported to capture visual function deficit in this disease and whether or not these tests could also detect their progression.

6.2 Aims

The primary objective of this study was to investigate the capability of potential functional clinical endpoints to detect disease progression at 1 year follow up visit.

Secondary objective was to evaluate quantitative OCT parameters change in varying severity of AMD disease, specifically ONL, RPEDC complex and choroidal thicknesses and volumetric change.

6.3. Methods

The methods to obtain data for functional and structural outcome measures and cross-sectional analysis are discussed in detail in chapter 2. In addition, a 1-way ANOVA or Kruskal Wallis tests were performed for between group comparisons at baseline and 1-year follow-up visit. The mean change of all functional and structural outcome measures was analysed using paired t-tests for normally distributed data and Wilcoxon matched pairs signed rank test was used for non-parametric data.

6.4. Results

6.4.1. Demographic and clinical characteristics of the participants

Of the 50 patients enrolled in the FUSCHIA trial, 44 completed the 12 -month study visit (9 healthy participants, 17 iAMD without SDD, 10 iAMD with SDD and 8 non-foveal atrophy patients). Six were withdrawn throughout the course of the study; two were from the healthy aging group, one from the iAMD with SDD and 3 from the late AMD group. The reasons for withdrawal are shown in Table 6.1. None of the participants with healthy eyes developed AMD and no apparent regression or new SDD were observed in the cohort. However, 3 individuals with non-foveal atrophy converted to central GA (n=1) and neovascular AMD (n=2). The mean age (±SD) of this longitudinal study cohort was 70.1 (± 7.5) years. Participants with iAMD with SDD were statistically modestly older (75.2 ± 5.9 years) than the iAMD without SDD (67.4 ± 8.2 years) participants (p=0.046) and non-significantly older than healthy aging (67.1 ± 6.2 years) and non-foveal atrophic AMD (72.6 ± 6.0 years) groups. There were 23 females (52%) and 21 males (48%) in this cohort. Participant characteristics are summarized in Table 6.2.

Table 6.1. Reasons for withdrawal at 12-month follow-up visit.

Study ID	Study Classification	Reason for withdrawal
FUSCHIA 017	Non-foveal atrophic AMD	Conversion to wet AMD
FUSCHIA 022	Non-foveal atrophic AMD	Conversion to central GA
FUSCHIA 027	iAMD with SDD	Assessments too long/uncomfortable
FUSCHIA 029	Healthy Aging	Assessments too long/uncomfortable
FUSCHIA 035	Non-foveal atrophy AMD	Conversion to wet AMD
FUSCHIA 047	Healthy aging	Assessments too long/uncomfortable

Table 6.2. Patient characteristics for all subjects that completed 12-month study visit.

	Group 1 Healthy aging (N=9)	**Group 2 iAMD no SDD (N=17)**	**Group 3 iAMD with SDD (N=10)**	**Group 4 Non-foveal atrophic AMD (N=8)**
Age (mean ± SD)	67.5 (± 6.2)	67.4 (± 8.1)	75.2 (± 5.9)	72.6 (± 6.0)
Gender balance				
N (% female)	5 (56%)	9 (53%)	5 (50%)	4 (50%)
Smoking status				
Current or former smoker (%)	3 (33%)	8 (47%)	7 (70%)	5 (71%)
Hypertension (%)	3 (33%)	3 (19%)	6 (60%)	5 (63%)
Hyperlipedemia (%)	5 (56%)	6 (35%)	8 (80%)	3 (38%)
Diabetes (%)	0	0	1 (10%)	1 (13%)

6.4.2. Functional outcome measures

The mean and median values at baseline and 12 months for all function outcome measures along with overall differences between groups are shown in Table 6.2. There was a statistically significant decrease in BCVA of 4.5 ETDRS letters from baseline to the annual review in the iAMD with SDD (p= 0.035) and atrophy groups (0.008). However, there was no significant change in LLVA and LLD in any group at 12 months. Patients with atrophy also had lower composite scores on LLQ questionnaire compared to the previous year (mean change of -7.40 ± 8.35, p= 0.016), however this was not observed in any other group. On average, patients with intermediate disease without SDD had increased rod intercept time by 1.90 minutes from baseline visit (p=0.043) as illustrated in Figure 6.1.

In contrast, healthy eyes showed only minimal change in RIT in 12 months. There was also no change in the SDD and atrophy groups as they had already reached 20 minutes at baseline (the maximum time set for the test). Furthermore, there was no change in scotopic thresholds in any group by 12 months. Relationships between significant functional outcome measures for the whole cohort are displayed in Figure 6.3. A decline in visual function was observed for BCVA (N=28), LLVA (N=25), scotopic thresholds (N=25) and composite LLQ scores (N=32). However, 16 of the 44 eyes had improved or exhibited no change in BCVA whereas 18 of the 44 eyes had improved or remained unchanged in LLVA score. At 12 months, 18 eyes had performed better on scotopic perimetry and 12 out of 44 participants had improved or unchanged LLQ scores. Differences between groups remained significant for BCVA, LLVA, scotopic thresholds and RIT over time ($p=0.0008$, $p=0.008$, $p<0.0001$ and $p< 0.0001$ respectively), but not for composite LLQ scores ($p=0.542$) and pairwise comparisons between groups for these outcomes at 12 months are described in Table 6.3. None of the functional parameters differed between healthy eyes and those with iAMD no SDD nor between eyes with iAMD with SDD and non- foveal atrophic AMD at annual follow up visit. Compared to healthy eyes, eyes with SDD and non-foveal atrophy had reduced BCVA, LLVA, scotopic thresholds and RIT. Similarly, significant visual function deficits were observed across the same outcome measures when eyes with SDD and non-foveal atrophy were compared to eyes with iAMD no SDD. Participants with iAMD with SDD had reduced BCVA ($p=0.036$), scotopic thresholds ($p<0.0001$) and RIT ($p=0.0002$) when compared to iAMD without SDD. There were no significant differences over time for photopic flicker ERGs amplitude and time within or between any group.

Table 6.3. Functional outcome measures at baseline and 12-month visit for all groups

Test	Visit	Normal + Early AMD	iAMD without SDD	iAMD with SDD	Non-foveal GA	Overall p-value [b]
BCVA (letters)	**Baseline**					
	Number	9	17	10	8	**0.0002**
	Mean (SD)	87.0 (5.1)	85.7 (6.1)	82.9 (3.9)	75.4 (11.7)	
	Minimum, median, maximum	78, 89, 93	67, 87, 93	77, 83, 88	50, 76.5, 87	
	12 Months					
	Number	9	17	10	8	**0.0008**
	Mean (SD)	87.3 (6.1)	85.2 (7.0)	78.4 (8.5)	70.9 (15.0)	
	Minimum, median, maximum	77, 87, 95	66, 86, 94	57, 81, 86	36, 73.5, 83	
	p-value[a]	0.922	0.545	**0.035**	**0.008**	
LLVA (letters)	**Baseline**					
	Number	9	17	10	8	**0.004**
	Mean (SD)	73.9 (4.3)	71.1 (8.2)	66.1 (7.6)	62.4 (11.4)	
	Minimum, median, maximum	68, 75, 82	47, 72, 80	56, 67, 77	40, 64.5, 74	
	12 Months					
	Number	9	17	10	8	**0.005**
	Mean (SD)	73.0 (8.7)	70.4 (8.4)	63.1 (8.4)	57.5 (13.8)	
	Minimum, median, maximum	61, 72, 87	51, 73, 80	44, 63.5, 73	29, 60, 72	
	p-value[a]	0.563	0.451	0.125	0.102	
LLD (letters)	**Baseline**					
	Number	9	17	10	8	0.599
	Mean (SD)	13.1 (2.3)	14.6 (4.3)	16.8 (4.5)	13.0 (3.1)	
	Minimum, median, maximum	10, 13, 17	9, 14, 22	11, 16, 26	9, 12.5, 19	
	12 Months					
	Number	9	17	10	8	0.468
	Mean (SD)	14.3 (4.6)	14.8 (4.2)	15.3 (2.5)	13.4 (6.2)	
	Minimum, median, maximum	7, 15, 23	10, 14, 24	11, 15.5, 20	7, 12, 27	
	p-value[a]	0.586	0.660	0.445	0.813	
LLQ	**Baseline**					
	Number	9	17	10	8	
	Mean (SD)	94.22 (5.57)	87.32 (9.10)	86.47 (12.85)	78.11 (22.24)	**0.032**
	Minimum, median, maximum	83.59, 96.09, 100.0	71.88, 90.63, 99.22	66.41, 92.58, 99.22	35.45, 82.81, 98.00	
	12 Months					
	Number	9	17	10	8	0.542
	Mean (SD)	90.50 (6.36)	84.74 (11.68)	85.80 (11.18)	70.70 (27.60)	
	Minimum, median, maximum	81.00, 89.84, 100.00	57.81, 87.50, 98.44	61.72, 87.82, 99.22	25.78, 78.13, 97.00	
	p-value[a]	0.086	0.065	0.410	**0.016**	

Table 6.3. Functional outcome measures at baseline and 12-month visit for all groups (Continued)

Test	Visit	Normal + Early AMD	iAMD without SDD	iAMD with SDD	Non-foveal GA	Overall p-value [b]
Scotopic Thresholds (dB)	**Baseline**					
	Number	9	17	9	8	**0.0006**
	Mean (SD)	57.59 (3.69)	58.05 (2.29)	52.23 (3.67)	51.23 (8.02)	
	Minimum, median, maximum	53.53, 58.21, 63.06	53.97, 58.68, 61.47	47.71, 55.38, 56.62	37.56, 55.15, 58.74	
	12 Months					
	Number	9	17	**9**	8	**<0.0001**
	Mean (SD)	57.85 (2.39)	58.37 (2.34)	50.92 (3.94)	49.66 (6.94)	
	Minimum, median, maximum	55.03, 58.12, 62.15	54.06, 58.74, 61.65	43.26, 51.59, 55.21	38.47, 51.99, 56.18	
	p-value[a]	0.820	0.311	0.359	0.078	
Rod intercept time (minutes)	**Baseline**					
	Number	9	16	**8**	**6**	**<0.0001**
	Mean (SD)	4.66 (0.78)	6.63 (3.24)	20.00 (0.00)	19.18 (1.92)	
	Minimum, median, maximum	3.12, 4.65, 5.50	3.68, 6.06, 15.91	20.00, 20.00, 20.00	15.26, 20.00, 20.00	
	12 Months					
	Number	9	16	8	6	**<0.0001**
	Mean (SD)	5.87 (4.84)	8.54 (3.94)	20.00 (0.00)	18.29 (4.19)	
	Minimum, median, maximum	2.75, 3.76, 18.24	3.43, 7.53, 14.86	20.00, 20.00, 20.00	9.75, 20.00, 20.00	
	p-value[a]	>0.999	**0.043**	n/a	>0.999	
Photopic amplitude (µV)	**Baseline**					
	Number	8	13	7	7	0.142
	Mean (SD)	14.8 (3.7)	17.3 (5.4)	13.7 (5.8)	12.0 (3.6)	
	Minimum, median, maximum	7.6, 15.3, 19.8	12.0, 17.9, 31.5	7.5, 13.0, 25.1	5.9, 12.3, 16.4	
	12 Months					0.189
	Number	8	13	7	7	
	Mean (SD)	15.4 (5.9)	15.9 (3.4)	13.5 (4.1)	12.2 (3.4)	
	Minimum, median, maximum	6.7, 17.0, 24.5	11.5, 16.9, 23.0	7.0, 12.9, 19.7	6.9, 12.7, 16.3	
	p-value[a]	0.844	0.151	>0.999	0.594	
Photopic Time (ms)	**Baseline**					
	Number	8	13	7	7	0.067
	Mean (SD)	28.3 (2.7)	27.7 (2.1)	28.2 (1.0)	29.2 (1.9)	
	Minimum, median, maximum	24.9, 28.8, 31.8	25.2, 27.1, 30.9	27.1, 28.2, 30.0	26.2, 29.3, 31.5	
	12 Months					0.285
	Number	8	13	7	7	
	Mean (SD)	28.1 (2.6)	27.9 (2.1)	28.5 (0.7)	29.4 (2.2)	
	Minimum, median, maximum	25.1, 27.1, 32.7	25.8, 26.9, 32.6	27.5, 28.8, 29.2	26.4, 29.45, 31.5	
	p-value[a]	0.742	0.209	0.453	0.953	

p-value[a] Wilcoxon matched pairs signed rank test within each group, baseline versus 12 months
p-value[b] One-way ANOVA or Kruskal Wallis test

Table 6.4. Pairwise comparisons for functional outcomes with overall significant differences between groups at 12-month follow-up visit.

Outcome	Healthy aging vs iAMD no SDD	Healthy aging vs iAMD with SDD	Healthy aging vs non-foveal atrophic AMD	iAMD no SDD vs iAMD with SDD	iAMD no SDD vs non-foveal atrophic AMD	iAMD with SDD vs non-foveal atrophic AMD
BCVA	0.468	**0.013**	**0.0005**	**0.036**	**0.001**	0.248
LLVA	0.517	**0.030**	**0.002**	0.063	**0.003**	0.226
Scotopic	0.619	**0.002**	**0.0097**	**<0.0001**	**0.0006**	0.662
RIT	0.179	**<0.0001**	**0.0003**	**0.0002**	**0.005**	0.668

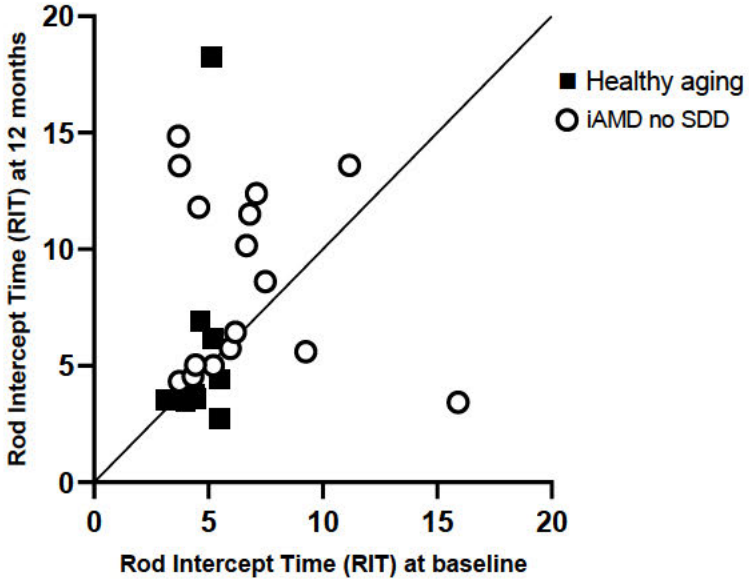

Figure 6.1. Scatterplot showing the relationship between RIT at baseline and RIT at 12 months for healthy aging (N= 9) and AMD no SDD groups (N= 16). Both AMD with SDD and non-foveal atrophic AMD groups were omitted as no adequate comparison of change was possible. Data above the line of identity indicates prolonged dark adaptation whereas the data below signifies shortened (improved) dark adaptation. This shows that the majority of the eyes (64%) had greater RIT at 12 months compared to baseline. This change over 12 months was significant for AMD no SDD group (p=0.043) and non-significant for the healthy aging group (p>0.999).

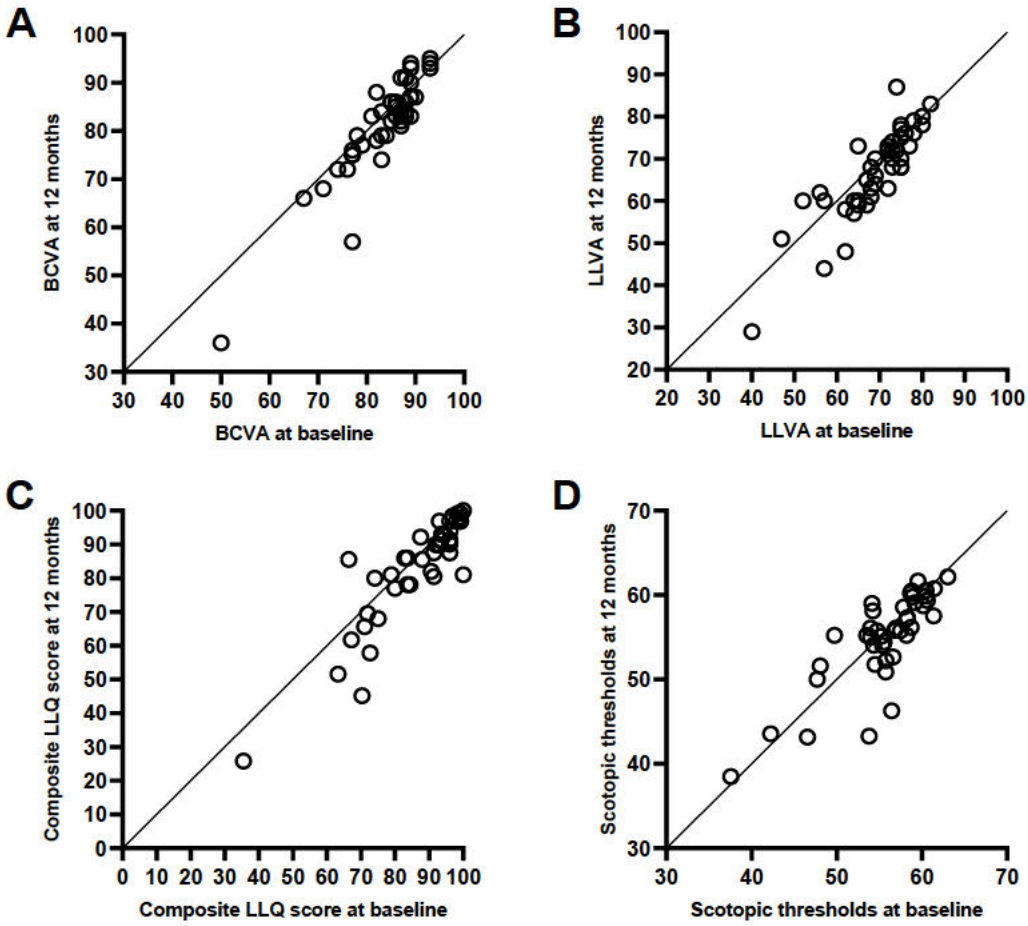

Figure 6.2. Scatterplot showing the relationship between A) BCVA, B) LLVA, C) composite LLQ scores for the entire cohort (N=44) and D) scotopic thresholds (N=43) at baseline and at 12 months. Data plotted above the line of identity signify improvement in function and data below the line indicate a decline in function. The panels show most of the eyes had reduced BCVA (N=28, 64%), LLVA (N=25, 57%) composite LLQ score (N=32, 73%) and scotopic thresholds (N=25, 58%) at 12 months compared to baseline.

6.4.3. Structural outcome measures

Mean retinal layer thickness and volume were measured for total retina, ONL and RPEDC along with CT and are described in Table 6.4. Mean change over 12 months and difference between groups is also included. At 1-year follow-up visit, participants with non-foveal atrophic AMD had reduced total retinal thickness (p=0.027), ONL volume (p=0.039), ONL thickness (p=0.016). In the same group, choroids were also thinner at 12 months than baseline(p=0.030).

There was also a reduction in ONL volume (p=0.005), ONL thickness (p=0.003) and CT (p=0.010) and a modest increase in RPEDC volume (p=0.043) in iAMD participants with SDD at 12-month visit compared to baseline visit. None of the structural OCT parameters changed significantly in eyes with iAMD without SDD over 12 months. In contrast, healthy eyes had modestly thicker ONL (p=0.048) and thinner choroids (p=0.040) at 12 months compared to baseline measurements.

Table 6.5. Baseline to 12-month visit quantitative OCT parameters across varying AMD severity.

Layer	Visit	Healthy Aging (N=9)	iAMD no SDD (N=17)	iAMD with SDD (N=10)	Non-foveal GA (N=8)	Overall p – value[b]
Total retinal volume Mean (SD)	Baseline	8.49 (0.27)	8.71 (0.49)	8.70 (0.30)	8.14 (0.77)	**0.049**
	12months	8.50 (0.30)	8.59 (0.40)	8.67 (0.27)	8.08 (0.76)	**0.042**
	Change	0.01 (0.06)	-0.12 (0.30)	-0.03 (0.11)	-0.06 (0.11)	
	p value[a]	0.679	0.108	0.428	0.188	
Total retinal thickness Mean (SD)	Baseline	308.77 (8.78)	316.85 (18.87)	318.40 (13.35)	291.30 (27.17)	**0.0095**
	12months	309.90 (9.62)	313.98 (16.82)	315.81 (9.48)	286.05 (25.33)	**0.0012**
	Change	1.13 (2.32)	-2.86 (14.74)	-2.59 (6.90)	-5.25 (5.34)	
	P value[a]	0.182	0.435	0.266	**0.027**	
ONL volume Mean (SD)	Baseline	1.69 (0.27)	1.77 (0.14)	1.73 (0.13)	1.45 (0.34)	**0.013**
	12months	1.71 (0.26)	1.75 (0.13)	1.66 (0.10)	1.38 (0.35)	**0.016**
	Change	0.02 (0.04)	-0.01 (0.05)	-0.08 (0.06)	-0.07 (0.07)	
	P value[a]	0.086	0.222	**0.005**	**0.039**	
ONL thickness Mean (SD)	Baseline	65.95 (9.62)	67.54 (6.28)	65.89 (4.82)	53.59 (11.07)	**0.009**
	12month	67.26 (8.98)	67.00 (6.12)	62.56 (4.79)	49.16 (11.53)	**<0.0001**
	Change	1.31 (1.69)	-0.54 (1.87)	-3.32 (2.64)	-4.43 (3.76)	
	P value[a]	**0.048**	0.253	**0.003**	**0.016**	
RPEDC volume Mean (SD)	Baseline	2.23 (0.05)	2.33 (0.24)	2.47 (0.39)	2.29 (0.33)	0.574
	12months	2.26 (± 0.05)	2.29 (0.15)	2.53 (0.44)	2.22 (0.40)	0.446
	Change	0.03 (0.05)	-0.05 (0.18)	0.07 (0.08)	-0.08 (0.14)	
	P value[a]	0.091	0.498	**0.043**	0.164	
RPEDC thickness Mean (SD)	Baseline	80.38 (1.80)	86.00 (11 62)	90.98 (18.13)	81.87 (15.44)	0.171
	12months	81.54 (2.51)	83.84 (6.15)	93.35 (17.86)	76.38 (17.77)	0.238
	Change	1.16 (1.70)	-2.16 (10.65)	1.37 (3.44)	-5.49 (5.98)	
	P value[a]	0.074	0.827	0.264	**0.036**	
Choroidal thickness Mean (SD)	Baseline	223.22 (76.54)	168.87 (58.90)	216.50 (107.19)	139.95 (47.55)	0.063
	12months	201.40 (70.20)	163.19 (64.28)	195.06 (96.22)	117.03 (28.25)	0.103
	Change	-21.82 (26.72)	-5.68 (14.75)	-21.44 (20.27)	-22.93 (23.81)	
	P value[a]	**0.040**	0.132	**0.010**	**0.030**	

P-value[a] Paired t-test or Wilcoxon matched pairs signed rank test within each group, baseline vs 12 months

p-value[b] One-way ANOVA or Kruskal Wallis test between groups at baseline and 12 months

6.5. Discussion

Longitudinal studies investigating visual function deficit whilst incorporating eyes with SDD in the clinical classification of AMD are lacking. In this exploratory study, results of functional and structural measures from baseline to 12 months in participants with varying AMD severity were compared to determine if these could detect disease progression. A decline in BCVA of 4.5 letters in both iAMD with SDD and non-foveal atrophic AMD groups at annual review was observed. Tan and colleagues (2019) also reported a decrease in BCVA (2.3 ± 2.6, p=0.01) in participants with SDD (referred to as RPD group). BCVA has been reported to be affected in eyes with late AMD. Our results show no change in BCVA in the healthy aging or eyes with iAMD without SDD, consistent with previous studies (Jackson *et al.*, 2014; Tan *et al.*, 2019). Whether this deterioration in visual acuity in eyes with SDD is progressive beyond annual review remains to be determined. Nonetheless, albeit statistically significant, these changes are not clinically meaningful as visual acuity variability ranges from 5 to 10 letters (Rosser *et al.*, 2003; Patel *et al.*, 2008). Therefore, visual acuities remain poor functional markers for dry AMD progression and in those where the fovea has been spared.

Present findings show no significant change in LLVA and LLD at 12 months. This is in agreement with previous studies (Hsu *et al.*, 2019; Tan *et al.*, 2019). In contrast to these findings, Nguyen et al. (2018) reported a significant increase in LLD of 3 and 5 letters in AMD and control groups respectively. We have in the baseline chapter, showed that LLVA is predominantly a foveal function and the LLD did not change between groups. In this longitudinal study, we have similar results in terms of change in LLVA. In fact, the change in LLVA was not even as large as BCVA, highlighting that it is not an effective endpoint for intermediate AMD trials. The change in LLD is indeed driven by BCVA, rather than LLVA and the change in LLD was similar between arms.

The test-retest reliability for LLQ scores over 1-month interval has been previously reported to be high (>0.74) for all subscales with the exception of peripheral vision in a cohort of participants with normal retinal aging and varying AMD severity (Owsley, Mcgwin, *et al.*, 2006).

However, Owsley and McGwin (2016) defined clinically significant decrease in LLQ score to be a decrease of at least 5 points over time in their normal retinal aging cohort. Our results showed a trend in decreasing LLQ composite scores for all groups over 12 months however, only results from the non-foveal atrophic AMD group reached statistical significance with a mean decrease of 7.41 points over 12 months. This is in concordance with previous literature where lower composite scores have been associated with increasing disease severity (Owsley, McGwin, *et al.*, 2006).

Limited studies have evaluated scotopic thresholds with DAC perimeter. Nguyen et al. (2018) reported no difference from baseline to 12-month follow-up visit in the average pointwise sensitivity (PWS) in controls and participants with and without SDD. However, they did report an increase in the proportion of test points with declining function over 1 year. Similarly, Tan and colleagues (2019) showed no deterioration in PWS difference in controls, AMD and RPD groups. Although in our study participants with AMD with SDD and non-foveal atrophic AMD had reduced scotopic sensitivities (-2.30 ± 5.66dB and -1.56 ± 1.88dB respectively), this decline was not statistically significant. While these findings are subclinical, it has been estimated that scotopic sensitivity declines by 1.2dB per decade in healthy retinae due to aging (Bennett *et al.*, 2019). A comparable projection was found in our cross-sectional data of 0.126dB decline per year discussed in chapter 3. Thus, should the decreasing trend continue beyond annual review, this could become more clinically significant and suggestive of worsening disease. Therefore, 12-months may be too short of time frame to be able to convincingly draw conclusions and evidence disease progression for scotopic sensitivities as measured by DAC perimeter.

In contrast to scotopic sensitivities, dark adaptation impairment has been investigated more extensively and suggested as a potential functional marker of AMD by various authors from cross-sectional studies (Eisner *et al.*, 1987; Owsley *et al.*, 2001, 2016; Dimitrov *et al.*, 2008, 2011, 2012; Flamendorf *et al.*, 2015). Likewise, in chapter 4, we showed that both scotopic thresholds and RIT were able to differentiate iAMD eyes with and without SDD. However, there is limited literature on longitudinal assessment of dark adaptometry which evaluate the sensitivity of the test to disease progression. Additionally, some of the longitudinal data did not

categorize SDD phenotype within the clinical classification of AMD. As such, earlier natural history studies by Jackson *et al.*, (2014) who observed severely delayed rod intercept time in 19% of the participants with varying AMD severity at annual review, and Owsley et al., (2017) showed prolonged RIT in 75% of iAMD eyes at 2 year follow up visit may have inadvertently included some SDD eyes.

Our results showed no change over time in the healthy aging group which is in keeping with previous work (Nguyen *et al.*, 2018). In the present study, the only functional outcome measure to show a significant change over 12 months in AMD without SDD is dark adaptation, with an average prolongation of 1.90 minutes. This result is similar to that of Chen et al., (2019) who found a mean change of 1.4 minutes per year in their 4-year follow-up study. In contrast, Tan and colleagues (2019) did not find any significant change over time in their AMD without SDD or with SDD groups but did find an improvement in their control group after 1 year. The latter is likely due to within patient variability or learning effect as opposed to a clinical improvement. Unfortunately, the RIT for SDD eyes in the present study was unable to detect any changes due to the limitation of the test automatically terminating at 20 minutes if the participant failed to reach RIT. None of the patients within that group reached RIT within the 20 minutes at baseline and as such we were unable to capture any worsening over time. It has been previously shown that rod function takes many hours to recover in SDD eyes (Luu *et al.*, 2018). This implies that longer testing times are required to elicit any change over time in this group which presents a clinical challenge. Furthermore, we found no change in RIT in the non-foveal atrophic AMD group, however three participants had to withdraw due to the development of central atrophy and neovascular AMD at annual follow up. It is unclear how rod function is affected in this group and how quickly it may deteriorate, but as with SDD eyes, 20 minutes testing time may be too short to detect meaningful change. It is important to note that the presence of SDDs have been reported to confer increased risk of developing advanced AMD (Finger *et al.*, 2014; Steinberg *et al.*, 2015) and we cannot rule that SDD had regressed in our cohort of non-foveal atrophy. Therefore, segregation or exclusion of eyes with and without SDD as well those with non-foveal atrophic AMD is important for longitudinal analysis of retinal function and potential therapeutic targets.

In the present study, no difference was observed within groups at baseline and 12 months and no change was detected within groups over time for either the photopic ERG amplitude or implicit time. As cones seem to evade damage until the end stage of the disease, pan-retinal ERGs are unlikely to offer any diagnostic or predictive value in the progression of AMD.

For quantifiable OCT structural outcome measures, current study showed significant decrease in ONL volume in eyes with SDD and those with non-foveal atrophy from baseline to 12 month- follow up whereas no change was found in healthy eyes nor in eyes with iAMD without SDD. Additionally, ONL was also thinner in eyes with SDD and non-foveal atrophy but not in iAMD without SDD at annual review. This is in agreement with recent longitudinal study which has shown minimal thinning in drusen-only eyes and more pronounced thinning in SDD eyes over the course of 2 years (Ramon *et al.*, 2019). Decrease in ONL thickness is indicative of progressive degeneration or damage of the photoreceptor nuclei. Whether or not this ONL thinning is implicated in reduced BCVA and rod-mediated visual dysfunction in SDD eyes and those with non-foveal atrophy remains to be determined. Investigation of photoreceptor inner and outer segment integrity may better describe structure-function relationship.Eyes with SDD also had decreased RPEDC volume and thinner choroid from the previous year. Although we did not observe any SDD regression in our participants, this has been associated with underlying choroidal thinning and outer retinal atrophy (Spaide, 2013). Also, while participants in our non-foveal atrophy group showed a decreasing trend in RPEDC and CT, these failed to reach statistical significance ($p=0.055$). This is in contrast to previous findings which found thinner ONL, RPEDC and choroid to be associated with future geographic atrophy progression (Pfau *et al.*, 2020).

Interestingly, our results did not show any difference between groups nor change in RPEDC volume or thickness over a 12month period in healthy eyes and eyes with iAMD without SDD. A modest increase in RPEDC volume for SDD eyes ($p=0.043$) and RPEDC thinning in eyes with atrophy were observed.

This contrasts previous findings which have reported increasing RPEDC volume in AMD eyes over 2 years (Lamin *et al.*, 2019) and RPEDC thickening over the course of 1 year (Saßmannshausen *et al.*, 2020) in eyes with iAMD. In the report by Lamin et al., (2019), the mean increase in RPEDC was observed a year prior to conversion to neovascular AMD. In contrast, our patient group is predominantly non-converters, therefore the disease progression is slow.

In conclusion, our current study suggests that changes in ONL volume and thickness can be detected in iAMD eyes with SDD and non-foveal atrophy at 12-month follow up, but not in iAMD eyes with drusen only. Although ONL, RPEDC and total retinal thicknesses and volumes have been suggested as prognostic structural markers for AMD, their efficacy to identify disease onset or progression has not been established in longitudinal studies. The inconclusive prognostic performance of functional outcomes over 12 months may be due small cohort or that 1 year may not be long enough to meaningfully detect disease progression. Nonetheless, RIT was the only outcome measure to show quantifiable visual function deficit in iAMD eyes without SDD. Due to the limitation of the test automatically terminating at 20 minutes if the participant failed to reach RIT, our findings were unable to elicit any worsening in eyes with SDD and non-foveal atrophy. Of note, no eyes with SDD improved RIT at 12 months. These observations are important when evaluating potential prophylactic agents to delay the progression of AMD as severe rod dysfunction may require different therapeutic interventions than eyes with drusen only. We know from a previous study that high-dose vitamin A supplementation significantly improved dark adaptation which implies that dark adaptation can be modulated. This exploratory study provided foundational knowledge on potential clinical endpoints however, larger longitudinal confirmatory studies are required to effectively establish prognostic capacity of each visual function and structural outcome measures.

Chapter 7. Longitudinal impact of photobiomodulation on disease progression

In this chapter, the longitudinal effect of photobiomodulation in halting disease progression in intermediate AMD with and without SDD is investigated. Visual function and structural changes over a 12-month period from the FUSCHIA study cohort as the control arm and 670nm trial cohort as the interventional cohort are compared.

7.1. Introduction

In chapter 5, the effect of 670nm red light therapy was explored on visual and structural deficits in healthy aging and AMD. Photobiomodulation was shown to improve scotopic thresholds in older participants when compared to younger participants following 2-week exposure for 120 seconds. Although there was a trend in increasing scotopic retinal sensitivity in the participants with healthy retinae in the 670nm clinical trial, this was not statistically significant. In fact, only RIT improved over time in healthy participants. However, the intervention failed to improve any visual function deficits in iAMD. Nonetheless, the intervention's capacity to delay disease progression was not evaluated due to a lack of a control group. Here, we investigate effect of 670nm light therapy in preventing disease progression with the FUSCHIA study cohort as the control arm.

7.2. Aims

The primary aim of this chapter was to investigate the efficacy of 670nm light therapy in delaying further disease progression as measured by both cone and rod photoreceptor function compared to a non-interventional group. The secondary aim was to investigate the effect of photobiomodulation on ONL and RPE-BM complex over time compared to those without the intervention.

7.3. Methods

Detailed methodology for trial procedures for functional and structural outcome measures and patient classification are described in chapter 2. Ethical approval for both FUSCHIA and 670nm clinical trials are termed in chapter 4 and 5 respectively. *Baseline data for the intervention cohort was collected by Dr Chrishnepriya Sivapathasuntharam.*

7.3.1. Statistical analysis

All statistical analyses were performed with GraphPad Prism. Differences between control and intervention study groups from the same phenotypic classification were analysed. Normal distribution of the data was assessed using Shapiro-Wilk test. Unpaired t-tests were performed for normally distributed data and Mann-Whitney U test were used to analyse non-normally distributed data. The ROUT method was used to detect any outliers in the data.

7.4. Results

7.4.1. Participant characteristics

Only participants who had completed the full study period of 12 months in both trials were included in this analysis. A total of 36 participants comprised the control arm from the observational FUSCHIA trial classified as healthy aging (N=9), iAMD without SDD (N=17) and iAMD with SDD (N=10). The cohort receiving the intervention from the 670nm trial included 35 participants categorized as healthy aging (N=12), iAMD without SDD (N= 15) and iAMD with SDD (N=8) as illustrated in Figure 7.1. As the 670nm trial did not include participants with non-foveal atrophic AMD, data from this group in the FUSCHIA trial were omitted for analysis in this chapter. None of the groups differed significantly in age between controls and those exposed to 670nm light: healthy aging (p=0.093), AMD without SDD (p=0.263) and AMD with SDD (p=0.118). Patient characteristics are described in Table 7.1.

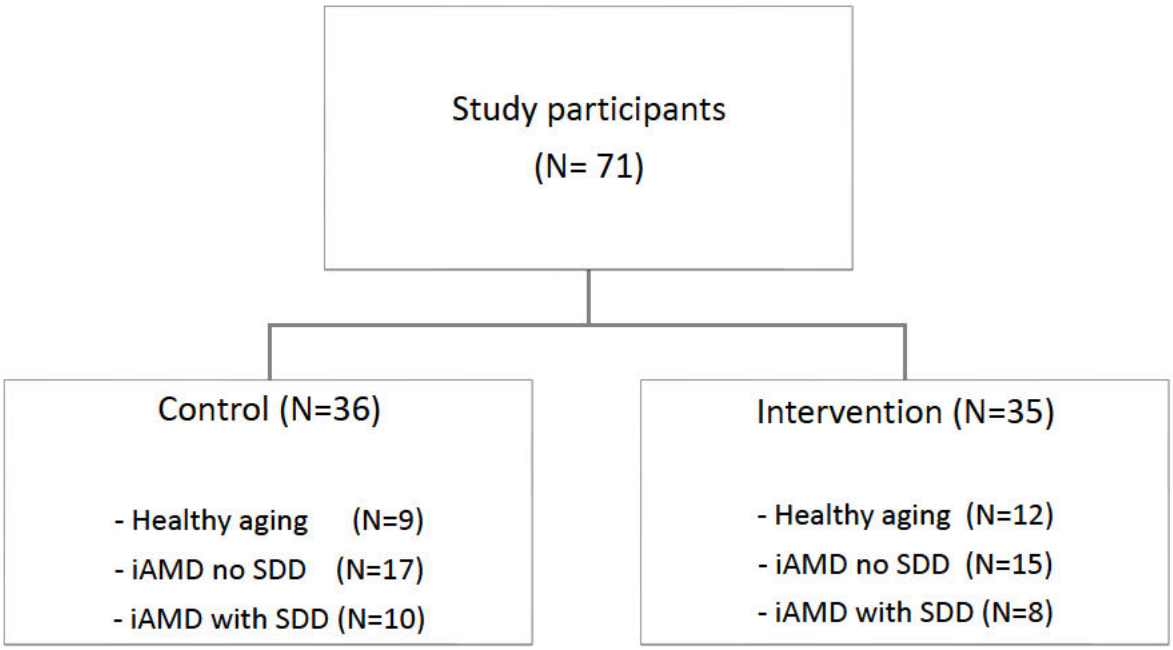

Figure 7.1. Flow chart of the study participants forming the control (FUSCHIA study) and intervention (670nm Trial) arm.

Table 7.1. Participant characteristics in each group at baseline visit who completed 12-month study duration for control and intervention cohorts.

	Healthy Aging		iAMD no SDD		iAMD with SDD	
	Control (N=9)	Intervention (N=12)	Control (N=17)	Intervention (N=15)	Control (N=10)	Intervention (N=8)
Age, Mean (SD)	66.4 (6.2)	69.9 (2.5)	63.3 (8.1)	69.5 (7.2)	74.2 (5.9)	69.3 (6.8)
Gender balance N (% female)	5 (56%)	7 (58%)	9 (53%)	10 (67%)	5 (50%)	5 (63%)
Ethnicity						
Caucasian	9 (100%)	11 (92%)	17 (100%)	14 (93%)	10 (100%)	8 (100%)
Asian	0	1 (8%)	0	1 (7%)	0	0
Smoking status	3 (33%)	4 (33%)	8 (47%)	8 (53%)	7 (70%)	2 (25%)

7.4.2. Disease progression on the basis of retinal function

Results relating to the changes in functional outcome measures for both the control and intervention arms are described in Table 7.2. A number of outliers were identified using the ROUT method across BCVA, LLVA, LLD and scotopic sensitivity parameters. If the outlier values were plausible and there was no obvious indication of errors, analysis was carried out on the entire dataset. The exception to this was one participant in the iAMD with SDD in the control arm who had unusually and severely reduced scotopic thresholds. However, for the rod-intercept time test, the plausibility of the outlier obtained was compared to the results of previous or subsequent visits. For example, a patient who had poor recovery on the dark adaptation test at initial visit, but normal recovery at subsequent visits was deemed to be an outlier. Therefore, two tests were excluded from the control arm (1 for each iAMD no SDD and iAMD with SDD) and 15 for intervention arm (5 of healthy aging group, 7 of AMD no SDD group and 3 of AMD with SDD group). This exclusion did not affect statistical outcome.

There was no significant difference between controls and those receiving the intervention at baseline or 12 months across all disease severities for BCVA, LLVA or LLD. Despite this, analysis showed the mean overall change of BCVA (±SD) was significantly reduced (p=0.008) in the iAMD with SDD control group (-4.5 ±6.4) compared to the iAMD with SDD receiving the intervention (1.4 ±2.8). Additionally, the mean change over time in LLD score increased significantly in the AMD without SDD (p=0.013) and AMD with SDD intervention groups (p=0.030) compared to control groups.

Scotopic thresholds were significantly lower in participants with iAMD without SDD being treated with 670nm light compared to controls at baseline (p=0.019), but similar at 12 months (p=0.173). Retinal sensitivities were stable in healthy aging groups at baseline (p=0.341) and at final visit (0.918). Similarly, SDD eyes with and without treatment were indistinguishable at baseline (p=0.357) and at annual review (p=0.169). Analysis of mean change showed no significant difference between groups.

Although eyes with iAMD without SDD had longer RIT then controls at baseline (p=0.007) and at 12 months (p=0.021), the mean overall change in RIT did not differ between groups (p= 0.168). In the healthy aging cohorts, those in the intervention arm also had significantly delayed RIT compared to non-intervention group at baseline (p=0.018), however the results at annual review showed no significant difference (p=0.470). Nonetheless, the mean RIT improved significantly (p=0.008) by 3.77 (±2.98) minutes in the intervention group compared to the control group in which RIT was prolonged by 1.21 (±4.68) minutes. There was also no change in the SDD groups as they had already reached 20 minutes at baseline (the maximum time set for the test) except for one participant in the intervention arm.

7.4.3. Disease progression on the basis of retinal structure

Volumetric analysis of ONL and RPE-BM complex retinal layers was also performed and are described in Table 7.3. There was no significant difference at baseline, 12 months or change over time for ONL across any of the study groups. In contrast, the RPE-BM complex volume was significantly higher at baseline for both AMD no SDD (p=0.0002) and AMD with SDD (p=0.008) groups in the intervention arm compared to control groups. The mean change in RPE-BM complex volume over 12 months was greater in all control groups (healthy aging, p= 0.002; iAMD no SDD, p<0.0001 and AMD with SDD p= 0.004) compared to the groups receiving the red-light therapy. Therefore, RPE-BM complex layer volume increased considerably more in the control groups over 12 months than it did for the intervention groups.

Table 7.2. Functional outcome measure characteristics between study groups in control and intervention arms.

	Healthy Aging			iAMD no SDD			iAMD with SDD		
	Control (N=9)	**Intervention (N=12)**	**P value**	**Control (N=17)**	**Intervention (N=15)**	**P value**	**Control (N=10)**	**Intervention (N=8)**	**P value**
BCVA									
Baseline	87.6 (4.8)	86.6 (5.9)	0.794	85.7 (6.1)	84.8 (7.6)	0.715	82.9 (3.9)	81.6 (3.7)	0.493
12 Months	87.3 (6.1)	85.7 (6.4)	0.555	85.2 (7.0)	85.7 (6.6)	0.830	78.4 (8.5)	83.0 (3.9)	0.137
Change	0.3 (2.7)	-0.9 (8.8)	0.686	-0.5 (3.5)	0.9 (3.2)	0.253	-4.5 (6.4)	1.4 (2.8)	**0.008**
LLVA									
Baseline	73.9 (4.3)	73.5 (6.0)	0.871	71.1 (8.2)	70.8 (8.1)	0.744	66.1 (7.6)	68.1 (6.3)	0.555
12 Months	73.0 (8.7)	70.4 (6.2)	0.435	70.4 (8.4)	67.2 (9.1)	0.307	63.1 (8.4)	65.9 (8.7)	0.301
Change	-0.9 (6.3)	-3.1 (7.5)	0.486	-0.7 (4.0)	-3.6 (4.2)	0.056	-3.0 (5.5)	-2.3 (4.4)	0.759
LLD									
Baseline	13.1 (2.3)	13.6 (3.7)	0.741	14.6 (4.3)	14.0 (5.5)	0.735	16.8 (4.5)	13.5 (5.9)	0.195
12 Months	14.3 (4.6)	15.3 (4.3)	0.645	14.8 (4.2)	18.5 (6.3)	0.057	15.3 (2.5)	17.1 (8.3)	0.519
Change	1.2 (5.3)	1.7 (6.1)	0.863	0.2 (4.4)	4.5 (5.4)	**0.013**	-1.5 (4.7)	3.6 (4.3)	**0.030**
Scotopic thresholds									
Baseline	57.59 (3.69)	56.18 (2.94)	0.341	58.05 (2.29)	54.69 (4.59)	**0.019**	52.23 (3.67)	54.92 (4.39)	0.357
12 Months	57.85 (2.39)	57.96 (1.75)	0.918	58.37 (2.34)	55.58 (6.20)	0.173	50.92 (3.94)	53.93 (4.64)	0.169
Change	0.26 (2.92)	1.77 (2.46)	0.215	0.32 (1.20)	0.89 (5.57)	0.684	-2.30 (5.66)	-0.99 (4.77)	0.673
RIT									
Baseline	4.66 (0.78)	9.35 (5.21)	**0.018**	6.63 (3.24)	14.92 (6.12)	**0.007**	20.00 (0.00)	20.00 (0.00)	1.000
12 Months	5.87 (4.84)	5.59 (2.58)	0.470	8.54 (3.94)	13.24 (5.17)	**0.021**	20.00 (0.00)	19.21 (1.77)	0.385
Change	1.21 (4.68)	-3.77 (2.98)	**0.008**	1.91 (5.51)	-1.69 (6.42)	0.168	0.00 (0.00)	-0.79 (1.77)	0.385

Table 7.3. Structural outcome measure characteristics between study groups in control and intervention arms.

	Healthy Aging			iAMD no SDD			iAMD with SDD		
	Control (N=9)	**Intervention (N=12)**	**P value**	**Control (N=17)**	**Intervention (N=15)**	**P value**	**Control (N=10)**	**Intervention (N=8)**	**P value**
ONL									
Baseline	1.99 (0.18)	1.99 (0.08)	0.940	2.02 (0.12)	1.96 (0.15)	0.267	1.93 (0.11)	1.98 (0.08)	0.323
12 Months	1.96 (0.18)	2.00 (0.12)	0.562	1.93 (0.08)	1.89 (0.14)	0.331	1.89 (0.11)	1.90 (0.12)	0.760
Change	-0.03 (0.06)	0.00 (0.07)	0.121	-0.09 (0.08)	-0.08 (0.08)	0.599	-0.05 (0.10)	-0.08 (0.05)	0.448
RPE-BM									
Baseline	0.85 (0.10)	0.90 (0.11)	0.279	0.87 (0.09)	1.09 (0.31)	**0.0002**	0.87 (0.06)	1.04 (0.12)	**0.008**
12 Months	1.03 (0.24)	0.90 (0.09)	0.096	1.04 (0.13)	1.13 (0.34)	0.716	1.16 (0.25)	1.06 (0.10)	0.778
Change	0.18 (0.20)	0.00 (0.08)	**0.002**	0.17 (0.09)	0.03 (0.06)	**<0.0001**	0.29 (0.22)	0.02 (0.04)	**0.004**

7.5. Discussion

Only two studies have previously investigated the effect of photobiomodulation in AMD eyes (Ivandic and Ivandic, 2008; Merry *et al.*, 2017). The findings of this present study showed that 670nm light therapy had no beneficial effect on disease progression in patients with iAMD over 12 months as measured by BCVA, LLVA, LLD and scotopic sensitivity parameters. In fact, LLD score was worse at annual visit for both iAMD with and without SDD groups compared to control group. In contrast, BCVA was significantly worse in eyes with SDD that did not receive the intervention compared to those that had 670nm light therapy. These changes, however, are likely to represent the variability of visual acuity as opposed to definite worsening (Rosser *et al.*, 2003; Patel *et al.*, 2008). This is at odds with previous studies which reported significantly improved BCVA post treatment (Ivandic and Ivandic, 2008; Merry *et al.*, 2017).

Only RIT in the healthy aging group receiving the treatment improved significantly over 12 months whilst the mean recovery time increased in the control group. This result needs to be interpreted with caution, as some of the assessments in the intervention groups done at baseline visit were performed after imaging tests which could have invariably caused retinal bleaching and contributed to delayed RIT. Although similar pattern was observed in the iAMD without SDD group, this did not reach statistical significance. It is important to note that this was an exploratory study and as such was not powered to detect small differences between study groups. Unfortunately, no meaningful analysis of change over time could be performed on the AMD with SDD phenotype as the majority of the eyes did not reach the rod criterion at 20 minutes, a cut off at which the test terminated automatically.

Volumetric analysis as opposed to the evaluation of thicknesses was chosen as it is less likely to be affected by focal changes from either photoreceptor loss or retinal alterations caused by the emergence or regression of drusen or SDD. Structurally, the mean change in ONL volume over 12 months stayed the same when compared between control and intervention arms.

However, ONL volume decreased significantly in both iAMD with and without SDD in the intervention cohort over time (from chapter 5). This result was also observed in the natural history study of SDD eyes, but not in iAMD without SDD (chapter 4). This reflects the loss of photoreceptor nuclei and therefore suggests disease progression regardless of light intervention. However, the opposite effect was observed with the RPE-BM complex volume. Control groups across all disease severity groups had a statistically significant increase in RPE-BM complex volume compared to those in the intervention groups. Although the RPE-BM complex volumes of control groups for both iAMD with and without SDD were lower at baseline visit than the intervention arm, the volume at 12 months exceeded that of intervention arms. These results suggest a possible delay of disease progression following red-light therapy. Mitochondrial dysfunction in the RPE of AMD is well documented in literature (Nordgaard *et al.*, 2008; Karunadharma *et al.*, 2010; Terluk *et al.*, 2015; Ferrington *et al.*, 2016; Ferrington, Sinha and Kaarniranta, 2016; Datta *et al.*, 2017) and would be most receptive to photobiomodulation which increases mitochondrial membrane potential and ATP production (Tiina I Karu, 2008; Kokkinopoulos *et al.*, 2013; Gkotsi *et al.*, 2014; Calaza *et al.*, 2015) . It is unclear which functional mechanism is improved in the RPE, but improved phagocytosis of shed photoreceptor membranes and elimination of lipids could account for this. As diabetic oedema is improved by 670nm one feature of the RPE cells that improves must be osmotic pumps that are expensive in terms of ATP consumption. However, due to the small sample size, we cannot rule out that these changed could also signify the variation in study population.

In conclusion, this is the first study looking at the effect of photobiomodulation in iAMD segregated into those with and without SDD and comparing the findings to a control arm. The results are at odds with available literature on light therapy in AMD with respect of improvement of BCVA although this could be due to different light wavelengths and length of exposure protocols. The present findings suggest possible improvement in RIT and delay in progression of AMD disease as measured by the RPE-BM complex volume. As dark adaptometry measures the integrity of photoreceptor and RPE function dynamics, the decrease is RIT and parallel decrease in RPE-BM complex volume is promising.

However, these findings need to be interpreted with cautious optimism due to the small sample size. Therefore, larger randomised-control confirmatory studies would be required to validate these findings.

Chapter 8. Discussion

8.1. General discussion

The overall aim of the work presented in this thesis was to utilise data from a preliminary phase I/IIa proof of concept study that I conducted to evaluate the discriminatory power of current and potential biomarkers to stratify eyes with intermediate AMD into those with and without SDD lesions, and place SDD within the AMD disease continuum. My hypothesis was that based on the scientific rationale of new interventions, this new classification of AMD disease severity will allow targeted interventions to specific severity levels of AMD. I also evaluated the magnitude of treatment effect from 670nm red light exposure based on this classification. As 670nm rejuvenates the mitochondria in the photoreceptors and the retinal pigment epithelium, I completed multiple functional tests to evaluate the impact of 670nm on photoreceptors and post-receptor pathway. These were then correlated to the structural changes over 12 months of 670nm therapy.

In order to include SDD in the classification, I first conducted a cross-sectional study (Chapter 4) to compare visual function and structure between healthy retinae and those that have already progressed to various stages of AMD including non-foveal atrophy. The Beckman Initiative Classification on colour fundus photograph was used to categorise participants into varying AMD severity and then I used multimodal imaging including infrared reflectance and SD-OCT to segregate the intermediate AMD group into those with and without SDD. The diagnostic capacity of these potential biomarkers to discriminate between each AMD severity level including SDD was investigated. The biomarkers consisted of functional tests that included both rod and cone mediated tests with the exception of LLQ, which accounts for a patient's own perception of their visual function status aimed to capture deficits in low luminance scenarios. Additionally, structural biomarkers, including ONL and RPE-BM complex/RPEDC were also analysed in all disease severity groups.

Histopathological work from Curcio et al. (1993) in donor eyes (ranging from 27 to 90 years of age) with macroscopically normal maculae showed selective predilection of rod photoreceptors loss in aging where an estimated of 30% of parafoveal rods are lost within 3.5° to 10° from either side of fixation. However, cone density remained stable (Curcio et al. 1993; Gao and Hollyfield 1992). The same trend appears to persist in eyes with AMD (Curcio, Medeiros, and Millican 1996). This is not to say that despite the longevity of cones exceeding that of rods, there is no decline in cone function.

In Chapter 3, static (or absolute) thresholds were measured with the novel dark-adapted chromatic (DAC) perimeter which incorporates two-color dark-adapted principle designed to measure the response from rods without the intrusion of the cone system. Healthy aged eyes had similar magnitude of scotopic sensitivity throughout all retinal locations which is in accordance with previous literature (Jackson *et al.*, 1998; Jackson and Owsley, 2000). Structurally, although rods are lost with aging, the photoreceptor mosaics are maintained and are similar to that found in younger retinae as the remaining rod inner segments enlarge to fill the void left by the degenerating rods (Curcio et al. 1993). Therefore, the quantum capacity for photon capture within the outer segments for rod-mediated phototransduction does not change with aging and the there is little evidence of a decrease in the available quantity of rhodopsin throughout adulthood (Plantner, Barbourl and Kean, 1988; Van Kuijk *et al.*, 1991). Consequently, a more diffuse decrease in scotopic sensitivity is observed as opposed focal loss.

Of note, participants with good retinal health and those with iAMD without SDD were indistinguishable functionally, suggesting that presence of macular drusen itself does not cause loss of rod system sensitivity. The fact that the scotopic sensitivity was not affected in the presence of drusen despite structural alterations of RPE implies that photoreceptor integrity is maintained in eyes with iAMD without SDD. This is in spite of photoreceptor shortening and outer segment deflection overlying drusen (Johnson *et al.*, 2003). In contrast, eyes with SDD had profound decline in scotopic thresholds of almost 10dB in the inferior retina in the vertical meridian and 4° nasally (decline at 8° and 12 ° locations was present but not statistically significant) in the horizontal meridian. This is an important finding as SDDs have been reported to be more

abundant in the superior perifovea (Curcio et al. 2013), it is unclear why scotopic sensitivity would be disproportionately reduced inferiorly.

An even greater decline in scotopic sensitivity across all loci in eyes with non-foveal atrophy compared to healthy aged eyes and those with iAMD without SDD was found in this thesis. However, this decrease was not statistically different from eyes with SDD. This indicates that scotopic sensitivity alterations do not mirror the anatomic evidence of GA. The functional impairment is far greater than structural demise. Parafoveal atrophy, by definition, indicates localised photoreceptor loss. SDDs which are located apically and internal to the RPE have been associated with photoreceptor abnormalities including outer segment shortening and loss of wave-guiding inner segments (Curcio et al. 2013). Eventually, this leads to outer retinal layer gaps over large deposits. These abnormalities are also commonly observed in histology and imaging around and ahead of GA progression (Spaide, 2013; Bird, Phillips and Hageman, 2014). Specifically, in eyes with GA, shortening of outer segments and photoreceptor loss are seen distant from the GA (Spaide, 2013). Therefore, extensive changes away from actual atrophy could explain broad decline in rod function. Nonetheless, eyes with SDD have generalised and comparable decline in rod function seen in eyes that have developed non-foveal atrophy suggesting that SDD is a marker of advanced disease despite no noticeable atrophic changes on multimodal imaging. Thus, the presence of SDD can be considered a structural sign of rod dysfunction which is independent of SDD location. Importantly, these results indicate that the overall loss of rod function is not directly correlated with drusen load, SDD location or extent of GA. In all groups, scotopic sensitivities tended to improve with increasing eccentricities which is in concordance with literature (Owsley *et al.*, 2000). This could be due to the fact that rod photoreceptor density is preserved in peripheral retina (Curcio et al. 1993).

Although the similarities of retinal sensitivities between healthy eyes and those with iAMD with drusen-only appear to be at odds with earlier literature, it is important to note that AMD was previously categorised as “early” and “late” AMD. The “early” AMD encompassed all retinal abnormalities associated with the disease with the exception of overt GA or nAMD. The SDD phenotype was not classified separately as a distinct group until more recently.

Therefore, comparable analysis with prior studies is difficult. For example, Owsley et al. (2000) measured dark-adapted sensitivity at 51 loci within the central 38° of visual field and found that the mean sensitivity was 6.7dB lower in AMD eyes when compared to healthy eyes. However, 42 of a total of 80 participants were found to have normal scotopic sensitivity whilst 38 individuals had significantly reduced function (Owsley *et al.*, 2000). It is unclear if any of the participants had SDD although 9 participants had confirmed nAMD or GA which could have yielded the overall reduced mean scotopic thresholds between these two groups. It is also quite possible that the iAMD without SDD participants recruited in the FUSCHIA study simply had normal scotopic function. Nonetheless, a study by Tan and colleagues (2018) with the same instrument support my findings of a particularly more significant visual deficit in SDD eyes compared to the other two groups. These authors also reported that scotopic sensitivities were indistinguishable following dark adaptation of 30 minutes between healthy aging and eyes AMD without SDD without preceding photobleach which is in concordance with our results. Fundamentally, this suggests that eyes exposed to a flash prior to measuring scotopic sensitivity are still dark-adapting due to residual retinal bleaching, hence absolute thresholds are not being measured. Literature comparing static thresholds in non-foveal atrophic AMD eyes to healthy eyes and iAMD with and without SDD is lacking. As the presence of SDDs have been reported to confer increased risk to progression of GA it is important that these groups are functionally and structurally compared. Although eyes with extra-foveal atrophy had reduced retinal sensitivity across all test points compared to healthy eyes and those with drusen-only, none of them differed significantly from eyes with SDD. Functionally, this is an important finding, as it highlights profound visual function deficit which could be indicative of eyes with SDD may already be on track to irreversible disease progression.

Similar decline was also reflected in delayed dark adaptation in eyes with SDD and non-foveal atrophy compared to healthy aged eyes and those with iAMD without SDD shown in chapter 4. In contrast to scotopic sensitivity, dark adaptation has been shown to be particularly affected in AMD with SDD (Owsley *et al.*, 2016; Flynn, Cukras, and Jeffrey 2018; Luu *et al.*, 2018).

The recovery of visual sensitivity following a bright flash in the dark is inherently dependant on the regeneration of visual pigment via the retinoid cycle (Lamb and Pugh, 2004). Thus, dark adaptation allows to psychophysically measure the integrity of the photoreceptor outer segments and RPE, a system supported intrinsically by the choroid and Bruch's membrane. Severe delays in RIT in eyes with SDD suggest that it is unlikely that delays in RIT are caused specifically by mechanical disturbance of RPE due to drusen morphology. Rather, it is possible that deficits may be due to RPE dysfunction related to the accumulation of SDD material internal to the RPE, although mechanical disturbances to the inner-outer segment junction and ELM may also play a role. Eyes with SDD have also been associated with thinner choroids and reduced choroidal vessel volume which could impede metabolic exchange from the choroid to the RPE and photoreceptors vis Bruch's membrane, necessary to the retinoid cycle. This can lead to defective transport of all-trans retinol and metabolites required for regeneration of visual pigment. It is important to note, that although the dark adaptation is impaired, rod sensitivity does eventually return in eyes with SDD, indicating that photoreceptors are present but dysfunctional (Luu *et al.*, 2018). Also, participants with SDD in this thesis were exclusively characterized as having at least 5 SDDs with typical conical shape breaking through the ELM, classified as stage 3 (Zweifel *et al.*, 2010). This was to ensure no ambiguity in the presence of SDD. It is not known if subjects classified with SDD at stage 1 or 2 exhibit similar rod dysfunction and warrants further investigation.

The pathophysiology of SDD is not fully understood. Similar to drusen, SDDs can grow, regress and disappear. However, the outer retinal atrophy observed subsequently to regression appears to be different between the two deposits. Specifically, Spaide (2013) reported undefined ellipsoid zone, decreased photoreceptor length and thinner underlying choroid with unaltered RPE following regression of SDD, distinct from end stage atrophy where the RPE cells die. It has been suggested that RPE from eyes with SDD may survive during outer retinal atrophy due to continued metabolic exchange with choriocapillaris whereas photoreceptor loss occurs due to increased distance from choroidal vessels leading to hypoxia (Chen *et al.*, 2020).

As the RPE remains intact, it has not been established if the production of 11-cis retinal in the regeneration of the visual pigment is compromised or there is a protein trafficking malfunction between the RPE and photoreceptor outer segments.
No formal analysis on SDD growth or regression was performed as part of this thesis nor can SDD regression in eyes with non-foveal atrophy can be ruled out. However, this does not detract from the findings in this thesis.

In chapter 4, correlations between functional and structural biomarkers were also analysed. Scotopic thresholds were well correlated with ONL volume, ONL thickness and CT whilst RIT did not show any association with these. Therefore, loss of retinal sensitivity is associated with photoreceptor degeneration. This is supported by a recent study which reported that ONL thinning was associated with marked loss of retinal sensitivity (Pfau *et al.*, 2020).
Photopic function was not particularly affected compared with scotopic function. In this work, a modest difference was found in BCVA in eyes with SDD lesions compared to healthy aged eyes (4 ETDRS letters) and those with iAMD without SDD (2 ETDRS letters) which could be representative of intersession variability (Rosser *et al.*, 2003; Patel *et al.*, 2008). Cone function was undoubtedly reduced in eyes with atrophy. However, photopic flicker ERGs were not affected in AMD. This result is not surprising as any possible deficit in the macular response is undoubtfully diluted with functional peripheral cones. It is possible that none of the tests in this thesis evaluating photopic function were able to quantify meaningful visual function deficit as they were not highly sensitive to detect small changes. Cone function has been shown to be compromised in AMD as measured by steady-state 14-Hz flicker thresholds and photostress recovery (Dimitrov *et al.*, 2011). It is also important to note that the vast majority of the participants recruited for the studies covered in this thesis had good visual acuities even though the inclusion criteria specified a minimum of 50 ETDRS letters.

In chapter 5, the magnitude of the effect of 670nm red light therapy was investigated. In aging retina, mitochondrial function declines which impairs respiration and results in an exacerbation in the generation of ROS (Eells, 2019). This leads to increased

oxidative stress impacting mitochondrial DNA and accumulation of mtDNA mutations (Karunadharma et al. 2010; Ferrington et al. 2016; Terluk et al. 2015; Nordgaard et al. 2008). Photoreceptors, specifically their inner segments, are packed with mitochondria in the ellipsoid section and constitute the most metabolically active layer in the retina (Wong-Riley, 2010).

Most of this energy produced is consumed during dark adaptation to fuel ion pumps required to maintain electrochemical gradients across the outer segment membrane (Wangsa-Wirawan, 2003; Wong-Riley, 2010). Conversely, retinal oxygen consumption declines by 40 to 60% in photopic conditions where these ion channels close and pumping activity terminates (Okawa *et al.*, 2008; Hardarson *et al.*, 2009). Mitochondrial function can be modulated with 670nm light. The mechanism of photobiomodulation is discussed in detail in the introduction. Although there are diverse views regarding the mechanism behind photobiomodulation it is widely accepted that 670nm exposure increases the oxidation of cytochrome c, boosting the transfer of electrons, increasing oxygen consumption and mitochondrial membrane potential, highly correlated with peaks in COX catalytic activity and ATP content in vivo (Karu, 1999; Karu *et al.*, 2005; Hamblin and Demidova, 2006; Karu, 2008). In addition, it has also been reported that 670 nm light increases ATP production by decreasing the viscosity of nanoscopic water bound to intramitochondrial surface around ATP rotor pumps (Sommer, Haddad and Fecht, 2015). Although the photochemistry of cytochrome c oxidase following red light exposure has been previously accepted as the main , this concept, has recently been challenged where it was shown that the proliferative effect of 660nm light was independent of COX unit in a mouse knockout for COX 10 gene and in human cell line with mtDNA mutation lacking three critical COX subunits (Lima, et al., 2019). In both models lacking COX, they were able to increase ATP levels and citrate synthase activity levels (Lima, et al., 2019). Another theory proposed that dietary chlorophyll metabolites, pyropheophorbides-a (P-a), can capture photonic energy (range 660 – 675nm) and increase ATP (Xu, *et al.*, 2014, Zhang, *et al.,* 2016). These studies, however, have not been replicated by others, but do suggest that PBM may alter cellular metabolism through alternative molecular targets not dependant on COX.

As mitochondrial dysfunction is implicated in AMD, it was hypothesized that photobiomodulation could improve mitochondrial function in the photoreceptors and RPE and prevent AMD progression. The significant improvement of rod-mediated function in the healthy aging group, but not in AMD disease suggests that there may be a window of treatment effect beyond which rod function may not be redeemed. The relatively modest effect observed in this study is likely due normal variation of human retinae compared to animal models. As the disease was already extensively established in iAMD groups, there may have been poor scope to improve retinal function. Perhaps treatment at earlier stages may have proven beneficial given conservative improvement in the healthy cohort. Crucially, improvement in photoreceptor function does not necessarily translate to halting of disease progression. It is not known if improving or slowing of dark adaptation has any beneficial impact on the retina. However, previous studies have shown that the visual cycle can be modulated. For example, improvement in dark adaptation was observed following short course of preformed Vitamin A in older adults with normal retina or early AMD (Owsley, McGwin, *et al.*, 2006). In contrast, inhibition of isomerase activity of RPE65 with a molecule called emixusat, resulted in decreased accumulation of lipofuscin and bisretinoids in a murine model of Stargardt's disease, leading to slowing down of the visual cycle (Bavik *et al.*, 2015). However, this treatment was not successful for GA secondary to AMD in a clinical trial (Rosenfeld *et al.*, 2018). Although no clinical benefit was observed in functional outcomes, photobiomodulation did show delay in disease progression as measured by RPE-BM complex volume over 12 months when compared to a control arm (chapter 7).

In diabetic animal models, photobiomodulation has shown to reduce the production of superoxide, inflammatory biomarker expression and cell death, but did not find these changes to be mediated by COX activity (Tang *et al.*, 2013) A case report of 4 patients with non-centre involving macular oedema has been shown to improve with 670nm light therapy (Tang, Herda and Kern, 2014). Although the exact mechanism is not fully understood, an improvement in the efficiency of osmotic pumps within the RPE that drive water posteriorly may be one of mechanisms beneficially impacted.

In chapter 6, longitudinal assessment of outcomes measures revealed only RIT was able to detect progression of iAMD without SDD at 12-month visit even though it was not able to distinguish between the latter and healthy aged eyes in the FUSCHIA cohort cross-sectionally. This distinction is representative of stable retinae in health aged eyes and subtle indication of disease progression in AMD without SDD. However, due to the limitation of the dark adaptation test automatically terminating at 20 minutes, the mean change in eyes with SDD and non-foveal atrophy could not be determined. Additionally, ONL thickness and volume as well as CT all decreased at annual follow up visit in eyes with SDD and those with non-foveal atrophy whilst eyes with iAMD without SDD remained stable. This distinction is important as it suggested accelerated deterioration or disease progression in eyes with SDD compared to those with drusen only. Also, given these structural alterations, it is likely that functional delays would also occur in these groups.

The overall findings of this thesis indicate that different phenotypes exhibit different patterns of visual loss suggesting inherently different pathophysiology. It is however likely that various mechanisms may be involved, and one may not be mutually exclusive especially when multiple deposits are present in the retina. The functional disparities may lie in the composition of these deposits. Although both share similar components including neutral lipids, apolipoprotein E, complement factor H, and vitronectin, SDD lacks immunoreactivity for photoreceptor, Müller cell, and RPE marker proteins (Curcio et al. 2013). It has been hypothesized that the lipid-rich drusen produce more peroxidizable and proinflammatory lipids than SDD thus recruiting immune cells (Chen *et al.*, 2020). Although previous study has reported microglia or macrophages adhering to advanced SDD (Greferath *et al.*, 2016), recent histological findings refuted any presence of immune cells within or next to SDD (Chen *et al.*, 2020). Additionally, Chen et al. (2020) have shown that the subretinal space appears to be devoid immune cells. Emergence of SDD has been associated with choroidal perfusion abnormalities and consistently with thinner choroids implicating reduced blood flow and volume (Arnold *et al.*, 1995; Alten *et al.*, 2013; Grewal *et al.*, 2014).

It is important to remember that even in healthy retinae, choroidal circulation just about meets the metabolic demands to sustain the dark current and therefore any deficiency in the haemodynamic profile of the choroidal circulation can lead to hypoxia at the apical proximity of photoreceptor inner segment (Wangsa-Wirawan, 2003). Outer retinal hypoxia can also be caused by the accumulation of deposits resulting from increased distance between choriocapillaris and oxygen consuming photoreceptors. All these factors may be involved in SDD eyes, delaying dark adaptation. In addition to hypoxia, mechanical or functional inhibition to protein trafficking necessary for rod recovery may be involved in rod recovery specific to SDD lesions.

The comparable scotopic function and quantitative OCT parameters between healthy aged eyes and iAMD without SDD suggest that eyes are probably well perfused. The fact the eyes with drusen take many years to progress to advanced stages whilst retaining good scotopic function makes hypoxia less unlikely as the principal mechanism of pathogenesis in eyes with typical drusen. In contrast, eyes with SDD lesions have been associated to confer increased risk to progress to advanced disease. Whether eyes with SDD suffer predominantly from hypoxia and eyes with drusen are more susceptible to inflammation has not been established.

Similarly, scotopic sensitivity revealed depressed function centrally and inferiorly but appeared to improve in the periphery in eyes with SDD and non-foveal atrophic AMD. In contrast to literature, scotopic thresholds did not correlate with anatomical locations or extent of deposits or non-foveal atrophy. Even though the size of the stimuli used in this thesis was large (Goldmann size V = 1.73°, equating ~ 577µm), it does not explain the lack of anatomical correspondence as rod function deficits were identified elsewhere.

The question remains as to whether there are ways of measuring functional and structural changes which can 1) precede the appearance of clinical signs of AMD, 2) predict disease progression and 3) identify those at higher risk of progression.

8.2. Conclusion

The rationale for conducting these exploratory studies was to evaluate potential clinical biomarkers and their diagnostic and prognostic capacity in varying severity of AMD including eyes with SDD. Only rod mediated tests, static and dynamic, were able to distinguish eyes with iAMD without SDD and iAMD with SDD. In a 12-month period, psychophysically, only RIT could detect disease progression in iAMD without SDD whereas structurally, ONL volume and thickness along with choroidal thicknesses were able to detect disease progression in eyes with SDD and those with established non-foveal atrophic AMD. However, eyes with drusen only remained structurally stable. Similarities between eyes with SDD and those with non-foveal atrophic AMD changes highlight that these eyes have accelerated structural alterations compared to those with drusen only. Additionally, eyes with SDD serves as a marker of advanced disease even though no structural changes of atrophy are visible on clinical examination or imaging. Given this, eyes with SDD may already be on the pathway to irreversible disease progression, and so clinical trials on prevention or treatment of progression of geographic atrophy should exclude them or undertake a sub analysis of this group.

In conclusion, AMD is a heterogenous disease and distinct phenotypes have different visual outcomes. Improved clinical classification which incorporates SDD as a distinct feature may allow more effective assessment of individuals which are more likely to respond to a potential treatment and their prognostic visual outcome.

8.3. Future work

Rod-mediated tests are particularly sensitive to SDD lesions. However, rod function has not been explored with SDD severity as classified by Zweifel *et al.* (2010). Specific retinal layers are affected at stage 3 compared to stage 1 and 2. Studies from macular hole and retinal detachment repairs have highlighted that the presence of intact ELM was critical to photoreceptor restoration and predicting visual outcome (Wakabayashi *et al.*, 2009; Landa *et al.*, 2012).

The authors proposed that restoration of the outer segments can take place as long as photoreceptor cell bodies have not been compromised (Wakabayashi *et al.*, 2009). Whether disruptions to the ELM (Stage 3) is prognostic of rod dysfunction has not been thoroughly investigated.

If indeed deposits breaking the ELM can cause irreversible damage and outer segments cannot be structurally or functionally redeemed, this would undoubtedly have implications for future novel therapeutic trials in AMD.

Furthermore, SDDs can be present in individuals without any pathology, even though they are commonly found secondary to AMD. However, they are not exclusive to AMD and have also been reported in Sorsby's fundus dystrophy, pseudoxanthoma elasticum, acquired vitelliform lesions and angioid streaks. Analysis to determine whether rod dysfunction and morphological features on OCT differ between these conditions would be of value as it could help achieve a better understanding of SDD pathophysiology.

Lastly, as AMD progresses slowly and the results in this thesis show accelerated structural deterioration in eyes with SDD, longitudinal follow-up (beyond 12 months) of the retinal status and photoreceptor function would help validate current findings.

Publications

Publications arising during the period of study:

Grewal, Manjot K., Shruti Chandra, Sarega Gurudas, Alan Bird, Glen Jeffery, and Sobha Sivaprasad. 2020. "Exploratory Study on Visual Acuity and Patient-Perceived Visual Function in Patients with Subretinal Drusenoid Deposits." *Journal of Clinical Medicine* 9 (9): 2832. https://doi.org/10.3390/jcm9092832.

Grewal, Manjot K, Chrishne Sivapathasuntharam, Shruti Chandra, Sarega Gurudas, Victor Chong, Alan Bird, Glen Jeffery, and Sohba Sivaprasad. 2020. "A Pilot Study Evaluating the Effects of 670 Nm Photobiomodulation in Healthy Ageing and Age-Related Macular Degeneration." *Journal of Clinical Medicine* 9: 1–13. https://doi.org/10.3390/jcm9041001.

Manuscripts submitted for publication:

Functional clinical endpoints and their correlations in eyes with AMD with and without subretinal drusenoid deposits – a pilot study. *Submitted to EYE*

Scotopic thresholds on dark-adapted chromatic perimetry in healthy aging and age-related macular degeneration. *Submitted to Scientific Reports*

Conference presentation:

Deep phenotyping of intermediate age-related macular degeneration (AMD) using rod function tests *ARVO, Poster presentation*

References

Age Related Eye Disease Study Group (AREDS) (2000) 'Risk factors associated with age-related macular degeneration: AREDS study report number 3', *Ophthalmology*, 107(12), pp. 2224–2232.

Age-Related Eye Disease Study Group 2 (AREDS2) (2013) 'Lutein, zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial', *Journal of the American Medical Association* 309, pp. 2005–2015.

Alten, F. *et al.* (2013) 'Localized reticular pseudodrusen and their topographic relation to choroidal watershed zones and changes in choroidal volumes', *Investigative Ophthalmology and Visual Science*, 54(5), pp. 3250–3257. doi: 10.1167/iovs.13-11923.

Anderson, D. H., Fisher, S. K. and Steinberg, R. H. (1978) 'Mammalian cones: disc shedding phagocytosis, and renewal', *Investigative Ophthalmology and Visual Science*, 17(2), pp. 117–133.

Ando, T. *et al.* (2011) 'Comparison of therapeutic effects between pulsed and continuous wave 810-nm wavelength laser irradiation for traumatic brain injury in mice', *PLoS ONE*, 6(10). doi: 10.1371/journal.pone.0026212.

Arden, G. B. *et al.* (2011) 'Regression of early diabetic macular oedema is associated with prevention of dark adaptation', *Eye*. Nature Publishing Group, 25(12), pp. 1546–1554. doi: 10.1038/eye.2011.264.

AREDS (2001) 'AREDS Report No. 8', *Arch Ophthalmol*, 119, pp. 1417–1436.

Arjamaa, O. *et al.* (2009) 'Regulatory role of HIF-1α in the pathogenesis of age-related macular degeneration (AMD)', *Ageing Research Reviews*, 8(4), pp. 349–358. doi: 10.1016/j.arr.2009.06.002.

Arnold, J. J. *et al.* (1995) 'Reticular pseudodrusen: A Risk Factor in Age-Related Maculopathy', *Retina*, 15, pp. 183–191.

Augustin, A. J. *et al.* (2013) 'Alprostadil infusion in patients with dry age related macular degeneration: A randomized controlled clinical trial', *Expert Opinion on Investigational Drugs*, 22(7), pp. 803–812. doi: 10.1517/13543784.2013.794782.

Barbosa, D. T. Q. *et al.* (2014) 'Age-related macular degeneration and protective effect of HMG Co-A reductase inhibitors (statins): Results from the National Health and Nutrition Examination Survey 2005-2008', *Eye (Basingstoke)*, 28(4), pp. 472–480. doi: 10.1038/eye.2014.8.

Barron, M. J. *et al.* (2001) 'Mitochondrial abnormalities in ageing macular photoreceptors', *Investigative Ophthalmology and Visual Science*, 42(12), pp. 3016–3022.

Bavik, C. *et al.* (2015) 'Visual cycle modulation as an approach toward preservation of retinal integrity', *PLoS ONE*, 10(5), pp. 1–16. doi: 10.1371/journal.pone.0124940.

Beatty, S. *et al.* (2000) 'The role of oxidative stress in the pathogenesis of age-related macular degeneration', *Survey of Ophthalmology*, 45(2), pp. 115–134. doi: 10.1016/S0039-6257(00)00140-5.

Begum, R. *et al.* (2013) 'Treatment with 670 nm Light Up Regulates Cytochrome C Oxidase Expression and Reduces Inflammation in an Age-Related Macular Degeneration Model', *PLoS ONE*, 8(2), pp. 1–11. doi: 10.1371/journal.pone.0057828.

Beirne, R. O. *et al.* (2006) 'Severity staging by early features of age-related maculopathy exhibits weak relationships with functional deficits on SWS grating acuity', *Investigative Ophthalmology & Visual Science*, 47(10), pp. 4624–4631. doi: 10.1167/iovs.05-1227.

Bennett, L. D. *et al.* (2017) 'Dark-Adapted Chromatic Perimetry for Measuring Rod Visual Fields in Patients with Retinitis Pigmentosa', *Translational Vision Science & Technology* 6(4). doi: 10.1167/tvst.6.4.15.

Bennett, L. D. *et al.* (2019) 'Regional Variations and Intra- / Intersession Repeatability for Scotopic Sensitivity in Normal Controls and Patients With Inherited Retinal Degenerations', *Investigative Ophthalmology & Visual Science,* 60(4), pp. 1122-1131. doi: 10.1167/iovs.18-25473.

Berrow, E. J. *et al.* (2010) 'The electroretinogram: A useful tool for evaluating age-related macular disease?', *Documenta Ophthalmologica*, 121(1), pp. 51–62. doi: 10.1007/s10633-010-9226-1.

Bhutto, I. and Lutty, G. (2012) 'Understanding age-related macular degeneration (AMD): Relationships between the photoreceptor/retinal pigment epithelium/Bruch's membrane/choriocapillaris complex', *Molecular Aspects of Medicine*. Elsevier Ltd, 33(4), pp. 295–317. doi: 10.1016/j.mam.2012.04.005.

Birch, D. G. and Anderson, J. L. (1992) 'Standardized full-field electroretinography. Normal values and their variation with age', *Arch Ophthalmol*, 110, pp. 1571–1575.

Bird, A. C. *et al.* (1995) 'An international classification and grading system for age-related maculopathy and age-related macular degeneration', *Survey of Ophthalmology*, 39(5), pp. 367–374. doi: 10.1016/S0039-6257(05)80092-X.

Bird, A. C., Phillips, R. L. and Hageman, G. S. (2014) 'Geographic atrophy: A histopathological assessment', *JAMA Ophthalmology*, 132(3), pp. 338–345. doi: 10.1001/jamaophthalmol.2013.5799.

Boddu, S. *et al.* (2014) 'Risk factors associated with reticular pseudodrusen versus large soft drusen', *American Journal of Ophthalmology*. Elsevier Ltd, 157(5), pp. 985-993.e2. doi: 10.1016/j.ajo.2014.01.023.

Bonilha, V. (2008) 'Age and disease-related structural changes in the retinal pigment epithelium', *Clinical Ophthalmology*, 2(2), p. 413. doi: 10.2147/opth.s2151.

Bonilha, V. L. *et al.* (2006) 'The retinal pigment epithelium apical microvilli and retinal function', *Advances in Experimental Medicine and Biology*, 572, pp. 519–524. doi: 10.1007/0-387-32442-9_72.

Booij, J. C. *et al.* (2010) 'The dynamic nature of Bruch's membrane', *Progress in Retinal and Eye Research*. Elsevier Ltd, 29(1), pp. 1–18. doi: 10.1016/j.preteyeres.2009.08.003.

Boon, C. J. F. *et al.* (2013) 'Cuticular drusen: Stars in the sky', *Progress in Retinal and Eye Research*. Elsevier Ltd, 37, pp. 90–113. doi: 10.1016/j.preteyeres.2013.08.003.

Boulton, M. *et al.* (1989) 'The formation of autofluorescent granules in cultured human RPE', *Investigative Ophthalmology and Visual Science*, 30(1), pp. 82–89.

Boulton, M. and Dayhaw-Barker, P. (2001) 'The role of the retinal pigment epithelium: topographical variation and ageing changes', *Nature, Eye*, 15, pp. 384–389.

Bowmaker, J. K. and Dartnall, H. J. (1980) 'Visual pigments of rods and cones in a human retina.', *The Journal of Physiology*, 298(1), pp. 501–511. doi: 10.1113/jphysiol.1980.sp013097.

Bressler, N. M. *et al.* (1994) 'Clinicopathologic correlation of drusen and retinal pigment epithelial abnormalities in age-related macular degeneration', *Retina*, 14(2), pp. 130–142. doi: 10.1097/00006982-200507001-00016.

Bressler, S. B. *et al.* (2008) 'Racial differences in the prevalence of age-related macular degeneration: The Baltimore Eye Survey', *Archives of Ophthalmology*, 126(2), pp. 241–245.

Brys, K., Vanfleteren, J. R. and Braeckman, B. P. (2007) 'Testing the rate-of-living/oxidative damage theory of aging in the nematode model Caenorhabditis elegans', *Experimental Gerontology*, 42(9), pp. 845–851. doi: 10.1016/j.exger.2007.02.004.

Buch, H. *et al.* (2005) '14-Year incidence, progression, and visual morbidity of age-related maculopathy: The Copenhagen City Eye Study', *Ophthalmology*, 112(5), pp. 787–798. doi: 10.1016/j.ophtha.2004.11.040.

Bunce, C. *et al.* (2015) 'Certifications for sight impairment due to age related macular degeneration in England', *Public Health*. Elsevier Ltd, 129(2), pp. 138–142. doi: 10.1016/j.puhe.2014.12.018.

Calaza, K. C. *et al.* (2015) 'Mitochondrial decline precedes phenotype development in the complement factor H mouse model of retinal degeneration but can be corrected by near infrared light', *Neurobiology of Aging*. Elsevier Inc, 36(10), pp. 2869–2876. doi: 10.1016/j.neurobiolaging.2015.06.010.

Campochiaro, P. A. *et al.* (1999) 'The pathogenesis of choroidal neovascularization in patients with age-related macular degeneration.', *Molecular vision*, 5(May), p. 34.

Chakravarthy, U. *et al.* (2010) 'Clinical risk factors for age-related macular degeneration: A systematic review and meta-analysis', *BMC Ophthalmology*, 10(1). doi: 10.1186/1471-2415-10-31.

Chang, M. A. *et al.* (2008) 'Racial differences and other risk factors for incidence and progression of age-related macular degeneration: Salisbury Eye Evaluation (SEE) Project', *Investigative Ophthalmology and Visual Science*, 49(6), pp. 2395–2402. doi: 10.1167/iovs.07-1584.

Chen, K. G. *et al.* (2019) 'Longitudinal Study of Dark Adaptation as a Functional Outcome Measure for Age-Related Macular Degeneration', *Ophthalmology*. Elsevier Inc, 126(6), pp. 856–865. doi: 10.1016/j.ophtha.2018.09.039.

Chen, L. *et al.* (2020) 'SUBRETINAL DRUSENOID DEPOSIT IN AGE-RELATED MACULAR DEGENERATION Histologic Insights Into Initiation, Progression to Atrophy, and Imaging', *Retina*, 40, pp. 618–631.

Cheng, Y. *et al.* (2018) 'Photobiomodulation inhibits long-term structural and functional lesions of diabetic retinopathy', *Diabetes*, 67(2), pp. 291–298. doi: 10.2337/db17-0803.

Chew, E. Y. *et al.* (2014) 'Ten-year follow-up of age-related macular degeneration in the age-related eye disease study: AREDS report No. 36', *JAMA Ophthalmology*, 132(3), pp. 272–277. doi: 10.1001/jamaophthalmol.2013.6636.

Christen, W. G. *et al.* (1996) 'A prospective study of cigarette smoking and risk of age-related macular degeneration in men', *Journal of the American Medical Association*, 276(14), pp. 1147–1151. doi: 10.1001/jama.276.14.1147.

Chung, H. *et al.* (2012) 'The nuts and bolts of low-level laser (Light) therapy', *Annals of Biomedical Engineering*, 40(2), pp. 516–533. doi: 10.1007/s10439-011-0454-7.

Ciulla, T. A. *et al.* (2002) 'Choroidal perfusion perturbations in non-neovascular age related macular degeneration', *British Journal of Ophthalmology*, 86(2), pp. 209–213. doi: 10.1136/bjo.86.2.209.

Cocce, K. J. *et al.* (2018) 'Visual Function Metrics in Early and Intermediate Dry Age-related Macular Degeneration for Use as Clinical Trial Endpoints', *American Journal of Ophthalmology*. Elsevier Inc., 189, pp. 127–138. doi: 10.1016/j.ajo.2018.02.012.

Connolly, D. M. and Barbur, J. . (2009) 'Low Contrast Acuity at Photopic and Mesopic Luminance Under Mild Hypoxia , Normoxia , and Hyperoxia', *Aviation, Space, and Environmental Medicine*, 80(11), pp. 933–940. doi: 10.3357/ASEM.2535.2009.

Costa, R. A. *et al.* (2006) 'Retinal assessment using optical coherence tomography', *Progress in Retinal and Eye Research*, 25(3), pp. 325–353. doi: 10.1016/j.preteyeres.2006.03.001.

Crabb, J. W. *et al.* (2002) 'Drusen proteome analysis: An approach to the etiology of age-related macular degeneration', *Proceedings of the National Academy of Sciences of the United States of America*, 99(23), pp. 14682–14687. doi: 10.1073/pnas.222551899.

Crossland, M. D. *et al.* (2011) 'Retinal specific measurement of dark-adapted visual function : validation of a modified microperimeter Retinal specific measurement of dark-adapted visual function : validation of a modified microperimeter', *BMC Ophthalmology*. BioMed Central Ltd, 11(1), p. 5. doi: 10.1186/1471-2415-11-5.

Cruickshanks, K. J., Klein, R. and Klein, B. E. K. (1993) 'Sunlight and Age-Related Macular Degeneration: The Beaver Dam Eye Study', *Archives of Ophthalmology*, 111(4), pp. 514–518. doi: 10.1001/archopht.1993.01090040106042.

Da Cruz, L. *et al.* (2018) 'Phase 1 clinical study of an embryonic stem cell-derived retinal pigment epithelium patch in age-related macular degeneration', *Nature Biotechnology*. Nature Publishing Group, 36(4), pp. 1–10. doi: 10.1038/nbt.4114.

Cunha-Vaz, J., Bernardes, R. and Lobo, C. (2011) 'Blood-retinal barrier', *European Journal of Ophthalmology*, 21(SUPPL.6), pp. 3–9. doi: 10.5301/EJO.2010.6049.

Curcio, C. A. *et al.* (1990) 'Human photoreceptor topography', *Journal of Comparative Neurology*, 292(4), pp. 497–523. doi: 10.1002/cne.902920402.

Curcio, C. A. *et al.* (1993) 'Aging of the Human Photoreceptor Mosaic : Evidence for Selective Vulnerability of Rods in Central Retina', *Investigative Ophthalmology and Visual Science*, 34(12), pp. 3278–3296.

Curcio, C. A. *et al.* (2011) 'The oil spill in ageing Bruch membrane', *British Journal of Ophthalmology*, 95(12), pp. 1638–1645. doi: 10.1136/bjophthalmol-2011-300344.

Curcio, C. A. *et al.* (2013) 'Subretinal drusenoid deposits in non-neovascular age-related macular degeneration: morphology, prevalence, topography, and biogenesis model', *Retina*, 33(2), doi: 10.1097/IAE.0b013e31827e25e0.

Curcio, C. A. (2018) ‘Soft drusen in age-related macular degeneration: Biology and targeting via the oil spill strategies’, *Investigative Ophthalmology and Visual Science*, 59(4), pp. AMD160–AMD181. doi: 10.1167/iovs.18-24882.

Curcio, C. A., Medeiros, N. E. and Millican, C. L. (1996) ‘Photoreceptor loss in age-related macular degeneration’, *Investigative Ophthalmology and Visual Science*, 37(7), pp. 1236–1249.

Datta, S. *et al.* (2017) ‘The impact of oxidative stress and inflammation on RPE degeneration in non-neovascular AMD’, *Progress in Retinal and Eye Research*. Elsevier Ltd, 60, pp. 201–218. doi: 10.1016/j.preteyeres.2017.03.002.

De Freitas, L. F. and Hamblin, M. R. (2016) 'Proposed Mechanisms of Photobiomodulation or Low-Level Light Therapy' *IEEE journal of selected topics in quantum electronics : a publication of the IEEE Lasers and Electro-optics Society*, *22*(3), 7000417. https://doi.org/10.1109/JSTQE.2016.2561201

Delaey, C. and Van De Voorde, J. (2000) ‘Regulatory mechanisms in the retinal and choroidal circulation’, *Ophthalmic Research*, 32(6), pp. 249–256. doi: 10.1159/000055622.

Desmet, K. D. *et al.* (2006) ‘Clinical and experimental applications of NIR-LED photobiomodulation’, *Photomedicine and Laser Surgery*, 24(2), pp. 121–128. doi: 10.1089/pho.2006.24.121.

Dimitrov, P. N. *et al.* (2008) ‘Measuring rod and cone dynamics in age-related maculopathy’, *Investigative Ophthalmology and Visual Science*, 49(1), pp. 55–65. doi: 10.1167/iovs.06-1048.

Dimitrov, P. N. *et al.* (2011) ‘Visual Function Tests as Potential Biomarkers in Age-Related Macular Degeneration’, 52(13). doi: 10.1167/iovs.10-7043.

Dimitrov, P. N. *et al.* (2012) ‘Relationship between Clinical Macular Changes and Retinal Function in Age-Related Macular Degeneration’, *Investigative Ophthalmology & Visual Science*, 53(9), pp. 5213–5220. doi: 10.1167/iovs.11-8958.

Domalpally, A. *et al.* (2019) ‘Prevalence, Risk, and Genetic Association of Reticular Pseudodrusen in Age-related Macular Degeneration: Age-Related Eye Disease Study 2 Report 21’, *Ophthalmology*. American Academy of Ophthalmology, 126(12), pp. 1659–1666. doi: 10.1016/j.ophtha.2019.07.022.

Dorey, C. K. *et al.* (1989) ‘Cell Loss in the Aging Retina Relationship to Lipofuscin Accumulation and Macular Degeneration’, *Investigative Ophthalmology & Visual Science*, 30(8), pp. 1691–1699.

Eckmiller, M. S. (2004) 'Defective cone photoreceptor cytoskeleton , alignment , feedback , and energetics can lead to energy depletion in macular degeneration', *Progress in Retinal and Eye Research*, 23, pp. 495–522. doi: 10.1016/j.preteyeres.2004.04.005.

Edwards, A. O. *et al.* (2005) 'Complement Factor H Polymorphism and Age-Related Macular Degeneration', 3(April), pp. 421–425.

Eells, J. T. (2019) 'Mitochondrial dysfunction in the aging retina', *Biology*, 8(2). doi: 10.3390/biology8020031.

Eisner, A. *et al.* (1987) 'Sensitivities in Older Eyes With Good Acuity : Eyes Whose Fellow Eye Has Exudative AMD', pp. 1832–1837.

Evans, J. R. (2001) 'Risk Factors for age-related macular degeneration', *Progress in Retinal and Eye Research*, 20(2), pp. 227–253. doi: 10.1016/S1350-9462(00)00023-9.

Fanjul-Moles, M. L. and López-Riquelme, G. O. (2016) 'Relationship between oxidative stress, circadian rhythms, and AMD', *Oxidative Medicine and Cellular Longevity*, 2016. doi: 10.1155/2016/7420637.

Farsiu, S. *et al.* (2014) 'Quantitative classification of eyes with and without intermediate age-related macular degeneration using optical coherence tomography', *Ophthalmology*. American Academy of Ophthalmology, 121(1), pp. 162–172. doi: 10.1016/j.ophtha.2013.07.013.

Feeney-Burns, L., Hilderbrand, E. S. and Eldridge, S. (1984) 'Aging human RPE: Morphometric analysis of macular, equatorial, and peripheral cells', *Investigative Ophthalmology and Visual Science*, 25(2), pp. 195–200.

Feigl, B. (2009) 'Progress in Retinal and Eye Research Age-related maculopathy – Linking aetiology and pathophysiological changes to the ischaemia hypothesis', *Progress in Retinal and Eye Research*. Elsevier Ltd, 28(1), pp. 63–86. doi: 10.1016/j.preteyeres.2008.11.004.

Fernandez, A. B. *et al.* (2018) 'Age-related macular degeneration and progression of coronary artery calcium: The multi-ethnic study of atherosclerosis', *PLoS ONE*, 13(7), pp. 1–10. doi: 10.1371/journal.pone.0201000.

Ferrington, D. A. *et al.* (2016) 'Increased retinal mtDNA damage in the CFH variant associated with age-related macular degeneration', *Experimental Eye Research*. Elsevier Ltd, 145, pp. 269–277. doi: 10.1016/j.exer.2016.01.018.

Ferrington, D. A., Sinha, D. and Kaarniranta, K. (2016) 'Defects in retinal pigment epithelial cell proteolysis and the pathology associated with age-related macular degeneration', *Progress in Retinal and Eye Research*. Elsevier Ltd, 51, pp. 69–89. doi: 10.1016/j.preteyeres.2015.09.002.

Ferris, F. L. *et al.* (2013) 'Clinical classification of age-related macular degeneration', *Ophthalmology,* 120(4), pp. 844–851. doi: 10.1016/j.ophtha.2012.10.036.

Finger, R. P. *et al.* (2013) 'Visual Impairment as a Function of Visual Acuity in Both Eyes and Its Impact on Patient Reported Preferences', PLoS ONE, 8(12), pp. 8–12. doi: 10.1371/journal.pone.0081042.

Finger, R. P. *et al.* (2014) 'Reticular Pseudodrusen A Risk Factor for Geographic Atrophy in Fellow Eyes of Individuals with Unilateral Choroidal Neovascularization', *Ophthalmology*. Elsevier Inc, 121(6), pp. 1252–1256. doi: 10.1016/j.ophtha.2013.12.034.

Finger, R. P. *et al.* (2016) 'Reticular pseudodrusen and their association with age-related macular degeneration the melbourne collaborative cohort study', *Ophthalmology*. American Academy of Ophthalmology, 123(3), pp. 599–608. doi: 10.1016/j.ophtha.2015.10.029.

Flamendorf, J. *et al.* (2015) 'Impairments in dark adaptation are associated with age-related macular degeneration severity and reticular pseudodrusen', *Ophthalmology*. Elsevier Inc, 122(10), pp. 2053–2062. doi: 10.1016/j.ophtha.2015.06.023.

Flynn, O. J., Cukras, C. A. and Jeffrey, B. G. (2018) 'Characterization of rod function phenotypes across a range of age-related macular degeneration severities and subretinal drusenoid deposits', *Investigative Ophthalmology and Visual Science*, 59(6), pp. 2411–2421. doi: 10.1167/iovs.17-22874.

Fraser-Bell, S. *et al.* (2008) 'Cardiovascular Risk Factors and Age-related Macular Degeneration: The Los Angeles Latino Eye Study', *American Journal of Ophthalmology*, 145(2), pp. 308–316. doi: 10.1016/j.ajo.2007.10.007.

Fraser, R. G. *et al.* (2016) 'Assessment of retinotopic rod photoreceptor function using a dark-adapted chromatic perimeter in intermediate age-related macular degeneration', *Investigative Ophthalmology and Visual Science*, 57(13), pp. 5436–5442. doi: 10.1167/iovs.16-19295.

Friedman, D. S. *et al.* (1999) 'Racial differences in the prevalence of age-related macular degeneration: The Baltimore Eye Survey', *Ophthalmology*, 106(6), pp. 1049–1055. doi: 10.1016/S0161-6420(99)90267-1.

Frisén, L. and Frisén, M. (1979) 'Micropsia and visual acuity in macular edema - A study of the neuro-retinal basis of visual acuity', *Albrecht von Graefes Archiv für Klinische und Experimentelle Ophthalmologie*, 210(2), pp. 69–77. doi: 10.1007/BF00409993.

Fritsche, L. G. *et al.* (2013) 'Seven new loci associated with age-related macular degeneration', *Nature Genetics*, 45(4), pp. 433–439. doi: 10.1038/ng.2578.Seven.

Fritsche, L. G. *et al.* (2014) 'Age-Related Macular Degeneration: Genetics and Biology Coming Together', *Annual Review of Genomics and Human Genetics*, 15(1), pp. 151–171. doi: 10.1146/annurev-genom-090413-025610.

Fu, Y. (2018) Phototransduction in rods and cones. https://webvision.med.utah.edu/book/part-v-phototransduction-in-rods-and-cones/phototransduction-in-rods-and-cones/

Gao, H. and Hollyfield, J. G. (1992) 'Aging of the human retina: Differential loss of neurons and retinal pigment epithelial cells', *Investigative Ophthalmology and Visual Science*, 33(1), pp. 1–17.

Gao, W. *et al.* (2008) 'Measuring retinal contributions to the optical Stiles-Crawford effect with optical coherence tomography', *Optics Express*, 16(9), pp. 6486–6501. doi: 10.1364/OE.16.006486.Measuring.

Gensler, H. L. and Bernstein, H. (1981) 'DNA Damage as the Primary Cause of Aging', *The Quarterly Review of Biology*, 56(3), pp. 279–303.

Gkotsi, D. *et al.* (2014) 'Recharging mitochondrial batteries in old eyes. Near infra-red increases ATP', *Experimental Eye Research*. Elsevier Ltd, 122, pp. 50–53. doi: 10.1016/j.exer.2014.02.023.

Gonzalez-Lima, F. and Rojas (2011) 'Low-level light therapy of the eye and brain', *Eye and Brain*, p. 49. doi: 10.2147/eb.s21391.

Green, W. R. and Enger, C. (1993) 'Age-related Macular Degeneration Histopathologic Studies: The 1992 Lorenz E. Zimmerman Lecture', *Ophthalmology*. American Academy of Ophthalmology, Inc, 100(10), pp. 1519–1535. doi: 10.1016/S0161-6420(93)31466-1.

Greferath, U. *et al.* (2016) 'Correlation of Histologic Features with in Vivo Imaging of Reticular Pseudodrusen', *Ophthalmology*. American Academy of Ophthalmology, 123(6), pp. 1320–1331. doi: 10.1016/j.ophtha.2016.02.009.

Grewal, D. S. *et al.* (2014) 'A Pilot Quantitative Study of Topographic Correlation between Reticular Pseudodrusen and the Choroidal Vasculature Using En Face Optical Coherence Tomography', 9(3). doi: 10.1371/journal.pone.0092841.

Grunwald, J. E. *et al.* (1998) 'Foveal choroidal blood flow in age-related macular degeneration (AMD)', *Investigative Ophthalmology and Visual Science*, 38(4).

Grunwald, J. E., Hariprasad, S. M. and Dupont, J. (1998) 'Effect of aging on foveolar choroidal circulation', *Archives of Ophthalmology*, 116(2), pp. 150–154. doi: 10.1001/archopht.116.2.150.

Grzybowski, A. and Pietrzak, K. (2012) 'From patient to discoverer-Niels Ryberg Finsen (1860-1904)-the founder of phototherapy in dermatology', *Clinics in Dermatology*. Elsevier B.V., 30(4), pp. 451–455. doi: 10.1016/j.clindermatol.2011.11.019.

Guymer, R. H. *et al.* (2019) 'Subthreshold Nanosecond Laser Intervention in Age-Related Macular Degeneration The LEAD Randomized Controlled Clinical Trial', *Ophthalmology*. American Academy of Ophthalmology, 126(6), pp. 829–838. doi: 10.1016/j.ophtha.2018.09.015.

Guymer, R., Luthert, P. and Bird, A. (1999) 'Changes in Bruch's membrane and related structures with age', *Progress in Retinal and Eye Research*, 18(1), pp. 59–90. doi: 10.1016/S1350-9462(98)00012-3.

Guymer, R. and Wu, Z. (2020) 'Age-related macular degeneration (AMD): More than meets the eye. The role of multimodal imaging in today's management of AMD—A review', *Clinical and Experimental Ophthalmology*, 48(7), pp. 983–995. doi: 10.1111/ceo.13837.

Haas, P. *et al.* (2015) 'Impact of visceral fat and pro-inflammatory factors on the pathogenesis of age-related macular degeneration', *Acta Ophthalmologica*, 93(6), pp. 533–538. doi: 10.1111/aos.12670.

Hageman, G. S. *et al.* (2001) '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', *Progress in Retinal and Eye Research*, 20(6), pp. 705–732. doi: 10.1016/S1350-9462(01)00010-6.

Hagins, W. A., Penn, R. D. and Yoshikami, S. (1970) 'Dark Current and Photocurrent in Retinal Rods', *Biophysical Journal*. Elsevier, 10(5), pp. 380–412. doi: 10.1016/S0006-3495(70)86308-1.

Hamblin, M. R. and Demidova, T. N. (2006) 'Mechanisms of low level light therapy', *Mechanisms for Low-Light Therapy*, 6140, p. 614001. doi: 10.1117/12.646294.

Hamblin, M. R., Huang, Y. Y. and Heiskanen, V. (2019) 'Non-mammalian Hosts and Photobiomodulation: Do All Life-forms Respond to Light?', *Photochemistry and Photobiology*, 95(1), pp. 126–139. doi: 10.1111/php.12951.

Hammond, B. *et al.* (1998) 'Scotopic sensitivity: relation to age, dietary patterns, and smoking status', *Optometry and Vision Science*, 75(12), pp. 867–872.

Hammond, C. J. *et al.* (2002) 'Genetic influence on early age-related maculophaty', *Evidence-Based Eye Care*, 3(3), pp. 162–163. doi: 10.1097/00132578-200207000-00022.

Hardarson, S. H. *et al.* (2009) 'Oxygen saturation in human retinal vessels is higher in dark than in light', *Investigative Ophthalmology and Visual Science*, 50(5), pp. 2308–2311. doi: 10.1167/iovs.08-2576.

Harman, D. (1956) 'Aging: a theory on free radical radiation chemistry', *J. Gerontol.*, 11, pp. 298–300.

Harman, D. (1981) 'Harman 1981 PNAS', 78(11), pp. 7124–7128.

Hartmann, K. I. *et al.* (2012) 'Effect of change in drusen evolution on photoreceptor inner segment/outer segment junctione', *Retina*, 32(8), pp. 1492–1499. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3792858/pdf/nihms506186.pdf.

Hayreh, S. S. (1962) 'The Ophthalmic Artery', *British Journal of Ophthalmology*, 46(4), pp. 212–247. doi: 10.1007/978-3-319-12781-1_1.

Hayreh, S. S. (1975) 'Segmental nature of the choroidal vasculature', *British Journal of Ophthalmology*, 59(11), pp. 631–648. doi: 10.1136/bjo.59.11.631.

Hayreh, S. S. (2006) 'Orbital vascular anatomy', *Eye*, 20(10), pp. 1130–1144. doi: 10.1038/sj.eye.6702377.

Hecht, B. Y. S., Haig, C. and Wald, G. (1935) 'Dark adaptation of retinal fields of different size and location.', *The Journal of general physiology*, 1932.

Hecht, S. (1937) 'RODS, CONES, AND THE CHEMICAL BASIS OF VISION', *Physiological Reviews*, 17(2), pp. 239–290.

Hecht, S. and Shlaer, S. (1936) 'Intermittent stimulation by light: V. the relation between intensity and critical frequency for different parts of the spectrum', *Journal of General Physiology*, 19(6), pp. 965–977. doi: 10.1085/jgp.19.6.965.

Henkind, P. (1979) Ocular circulation in Physiology of the human eye and visual system, Hagerstown, Md, Harper & Row

Hoang, Q. V. *et al.* (2002) 'Photoreceptor inner segments in monkey and human retina: Mitochondrial density, optics, and regional variation', *Visual Neuroscience*, 19(4), pp. 395–407. doi: 10.1017/S0952523802194028.

Hobby, A. E. *et al.* (2018) 'Effect of varying skin surface electrode position on electroretinogram responses recorded using a handheld stimulating and recording system', *Documenta Ophthalmologica*. Springer Berlin Heidelberg, 137(2), pp. 79–86. doi: 10.1007/s10633-018-9652-z.

Hogg, R. E. and Chakravarthy, U. (2006) 'Visual function and dysfunction in early and late age-related maculopathy', *Progress in Retinal and Eye Research*, 25(3), pp. 249–276. doi: 10.1016/j.preteyeres.2005.11.002.

Holopigian, K. *et al.* (1997) 'peripheral vision is largely unaffected in early AMD', *Optometry and vision science*, 74(3), pp. 152–159.

Holz, F. G. *et al.* (1994) 'Bilateral Macular Drusen in Age-related Macular Degeneration: Prognosis and Risk Factors', *Ophthalmology*, 101(9), pp. 1522–1528. doi: 10.1016/S0161-6420(94)31139-0.

Holz, F. G. *et al.* (1999) 'Inhibition of lysosomal degradative functions in RPE Cells by a Retinoid Component of Lipofuscin', *Investigative Ophthalmology & Visual Science*, 40, pp. 737–743.

Hsu, S. T. *et al.* (2019) 'Longitudinal Study of Visual Function in Dry Age-Related Macular Degeneration at 12 Months', *Ophthalmology Retina*. American Academy of Ophthalmology, 3(8), pp. 637–648. doi: 10.1016/j.oret.2019.03.010.

Huang, D. *et al.* (1991) 'Optical coherence tomography', *Science*, 254, pp. 1178–1181.

Huang, Y. Y. *et al.* (2011) 'Biphasic dose response in low level light therapy - an update', *Dose-Response*, 9(4), pp. 602–618. doi: 10.2203/dose-response.11-009.

Ivandic, B. T. and Ivandic, T. (2008) 'Low-level laser therapy improves vision in patients with age-related macular degeneration', *Photomedicine and Laser Surgery*, 26(3), pp. 241–245. doi: 10.1089/pho.2007.2132.

Iwasaki, M. and Inomata, H. (1986) 'Relation between superficial capillaries and foveal structures in the human retina', *Investigative Ophthalmology and Visual Science*, 27(12), pp. 1698–1705.

Jackson, G. R. and Edwards J. G. (2008) "A short-duration dark adaptation protocol for assessment of age-related maculopathy." *Journal of ocular biology, diseases, and informatics*, 1 (1), pp. 7-11. doi:10.1007/s12177-008-9002-6.

Jackson, G. R. *et al.* (1998) 'Aging and scotopic sensitivity', *Vision Research,* 38, pp. 3655–3662.

Jackson, G. R. *et al.* (2005) 'Photoreceptor degeneration in aging and age-related maculopathy', *Ageing Research Reviews*, 1(3), pp. 381-396.

Jackson, G. R. *et al.* (2014) 'Twelve-month natural history of dark adaptation in patients with AMD', *Optometry and vision science*, 91(8), pp. 925–931.

Jackson, G. R. and Owsley, C. (2000) 'Scotopic sensitivity during adulthood', *Vision Research*, 40, pp. 2467–2473.

Jackson, G. R., Owsley, C. and Mcgwin, G. (1999) 'Aging and dark adaptation', *Vision Research,* 39, pp. 3975–3982.

Jacobson, S. G., Apathy, P. P., and Parel, J.-M. (1991) 'Rod and Cone Perimetry: Computerized Testing and Analysis', in *Principles and Practice of Clinical Electrophysiology of Vision*, pp. 475–482.

Jampol, L. M. and Tielsch, J. (1992) 'Race, Macular Degeneration, and the Macular Photocoagulation Study', *Archives of Ophthalmology*, 110(12), pp. 1699–1700. doi: 10.1001/archopht.1992.01080240039024.

Jin, K. (2010) 'Modern biological theories of aging', *Aging and Disease*, 1(2), pp. 72–74. doi: 10.1093/jn/119.6.952.

Johnson, P. T. *et al.* (2003) 'Drusen-associated degeneration in the retina', *Investigative Ophthalmology and Visual Science*, 44(10), pp. 4481–4488. doi: 10.1167/iovs.03-0436.

Johnstone, D. M. *et al.* (2014) 'Indirect application of near infrared light induces neuroprotection in a mouse model of parkinsonism - An abscopal neuroprotective effect', *Neuroscience*. IBRO, 274, pp. 93–101. doi: 10.1016/j.neuroscience.2014.05.023.

Kalloniatis, M. and Luu, C. (2013) Psychophysics of vision. http://webvision.med.utah.edu/book/part-viii-gabac-receptors/psychophysics-of[1]vision/

Karu, T. (1999) 'Primary and secondary mechanisms of action of visible to near-IR radiation on cells', *Journal of Photochemistry and Photobiology B: Biology*, 49(1), pp. 1–17. doi: 10.1016/S1011-1344(98)00219-X.

Karu, T. I. *et al.* (2005) 'Absorption measurements of a cell monolayer relevant to phototherapy: Reduction of cytochrome c oxidase under near IR radiation', *Journal of Photochemistry and Photobiology B: Biology*, 81(2), pp. 98–106. doi: 10.1016/j.jphotobiol.2005.07.002.

Karu, Tiina I. (2008) 'Mitochondrial signaling in mammalian cells activated by red and near-IR radiation', *Photochemistry and Photobiology*, 84(5), pp. 1091–1099. doi: 10.1111/j.1751-1097.2008.00394.x.

Karunadharma, P. P. *et al.* (2010) 'Mitochondrial DNA damage as a potential mechanism for Age-Related macular Degeneration', *Investigative Ophthalmology and Visual Science*, 51(11), pp. 5470–5479. doi: 10.1167/iovs.10-5429.

Katz, M. L., Gao, C. L. and Rice, L. M. (1996) 'Formation of lipofuscin-like fluorophores by reaction of retinal with photoreceptor outer segments and liposomes', *Mechanisms of Ageing and Development*, 92(2–3), pp. 159–174. doi: 10.1016/S0047-6374(96)01817-9.

Kawasaki, R. *et al.* (2008) 'Prevalence and Risk Factors for Age-Related Macular Degeneration in an Adult Japanese Population. The Funagata Study', *Ophthalmology*, 115(8), pp. 3–10. doi: 10.1016/j.ophtha.2007.11.015.

Kent, A. L. *et al.* (2015) 'A safety and feasibility study of the use of 670nm red light in premature neonates', *Journal of Perinatology*. Nature Publishing Group, 35(7), pp. 493–496. doi: 10.1038/jp.2015.5.

Khan, J. C. *et al.* (2006) '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', *British Journal of Ophthalmology*, 90(1), pp. 75–80. doi: 10.1136/bjo.2005.073643.

Khan, K. N. *et al.* (2016) 'Differentiating drusen: Drusen and drusen-like appearances associated with ageing, age-related macular degeneration, inherited eye disease and other pathological processes', *Progress in Retinal and Eye Research*. Elsevier Ltd, 53, pp. 70–106. doi: 10.1016/j.preteyeres.2016.04.008.

Klein, R. *et al.* (1991) 'The Wisconsin Age-related Maculopathy Grading System', *Ophthalmology*. American Academy of Ophthalmology, Inc, 98(7), pp. 1128–1134. doi: 10.1016/S0161-6420(91)32186-9.

Klein, R., Klein, B. E. K. and Linton, K. L. P. (1992) 'Prevalence of Age-related Maculopathy: The Beaver Dam Eye Study', *Ophthalmology*. American Academy of Ophthalmology, Inc, 99(6), pp. 933–943. doi: 10.1016/S0161-6420(92)31871-8.

Klein, R. *et al.* (1999) 'Prevalence of age-related maculopathy in the atherosclerosis risk in communities study', *Archives of Ophthalmology*, 117(9), pp. 1203–1210. doi: 10.1001/archopht.117.9.1203.

Klein, R. *et al.* (2002) 'Ten-year incidence and progression of age-related maculopathy: The Beaver Dam eye study', *Ophthalmology*, 109(10), pp. 1767–1779. doi: 10.1016/S0161-6420(02)01146-6.

Klein, R. *et al.* (2003) 'The association of cardiovascular disease with the long-term incidence of age-related maculopathy: the Beaver Dam Eye Study.', *Ophthalmology*, 110(6), pp. 1273–1280. doi: 10.1016/S0161-6420(03)00599-2.

Klein, R. *et al.* (2006) 'Fifteen-year cumulative incidence of age-related macular degeneration: the Beaver Dam Eye Study' *Ophthalmology,* 114, pp. 253–262.

Klein, R. (2011) 'Race/ethnicity and age-related macular degeneration', *American Journal of Ophthalmology*. Elsevier Inc., 152(2), pp. 153–154. doi: 10.1016/j.ajo.2011.02.016.

Klein, R. *et al.* (2013) 'Risk Alleles in CFH and ARMS2 and the Long-term Natural History of Age-Related Macular Degeneration' *Journal of the American Medical Association*, 131, pp. 383–392.

Klein, R. *et al.* (2014) 'Markers of inflammation, oxidative stress, and endothelial dysfunction and the 20-year cumulative incidence of early age-related macular degeneration the beaver dam eye study', *JAMA Ophthalmology*, 132(4), pp. 446–455. doi: 10.1001/jamaophthalmol.2013.7671.

Klein, R. *et al.* (2015) 'Small Drusen and Age-Related Macular Degeneration: The Beaver Dam Eye Study', *Journal of Clinical Medicine*, 4(3), pp. 425–440. doi: 10.3390/jcm4030425.

Klein, R., Klein, B. E. K. and Franke, T. (1993) 'The Relationship of Cardiovascular Disease and Its Risk Factors to Age-related Maculopathy: The Beaver Dam Eye Study', *Ophthalmology*. American Academy of Ophthalmology, Inc, 100(3), pp. 406–414. doi: 10.1016/S0161-6420(93)31634-9.

Knudtson, M. D. *et al.* (2004) 'Location of Lesions Associated with Age-Related Maculopathy Over a 10-year Period : The Beaver Dam Eye Study', 45(7), pp. 2135–2142. doi: 10.1167/iovs.03-1085.

Kokkinopoulos, I. *et al.* (2013) 'Age-related retinal inflammation is reduced by 670 nm light via increased mitochondrial membrane potential', *Neurobiology of Aging*. Elsevier Inc., 34(2), pp. 602–609. doi: 10.1016/j.neurobiolaging.2012.04.014.

Kolb, H. (2011) Simple anatomy of the retina [Online]. Salt Lake City, Utah: Moran Eye Center. 2018.

Kolb H (2013) The organisation of the retina and visual system. In: Kolb, H, Fernandez, E, and Nelson, R [eds.] Webvision, http://webvision.med.utah.edu/.

Van Kuijk, F. J. G. M. *et al.* (1991) 'Spectrophotometric quantitation of rhodopsin in the human retina', *Investigative Ophthalmology and Visual Science*, 32(7), pp. 1962–1967.

Ladewig, M. *et al.* (2003) 'Cone Dysfunction in Patients With Late-Onset Cone Dystrophy and Age-Related Macular Degeneration', *Archives of Ophthalmology*, 121(11), pp. 1557–1561. doi: 10.1001/archopht.121.11.1557.

Lamb, T. D. and Pugh, E. N. (2004) 'Dark adaptation and the retinoid cycle of vision', *Progress in Retinal and Eye Research*, 23(3), pp. 307–380. doi: 10.1016/j.preteyeres.2004.03.001.

Lamb, T. D. and Pugh, E. N. (2006) 'Phototransduction, dark adaption, and rhodopsin regeneration: The proctor lecture', *Investigative Ophthalmology and Visual Science*, 47(12), pp. 5138–5152. doi: 10.1167/iovs.06-0849.

Lambert, N. G. *et al.* (2016) 'Risk factors and biomarkers of age-related macular degeneration', *Progress in Retinal and Eye Research*. Elsevier Ltd, 54, pp. 64–102. doi: 10.1016/j.preteyeres.2016.04.003.

Lamin, A. *et al.* (2019) ‘Changes in volume of various retinal layers over time in early and intermediate age-related macular degeneration’, *Eye*. Springer US, pp. 428–434. doi: 10.1038/s41433-018-0234-9.

Landa, G. *et al.* (2012) ‘External limiting membrane and visual outcome in macular hole repair: Spectral domain OCT analysis’, *Eye*. Nature Publishing Group, 26(1), pp. 61–69. doi: 10.1038/eye.2011.237.

Lee, J. *et al.* (2019) ‘Neovascularization in Fellow Eye of Unilateral Neovascular Age-related Macular Degeneration According to Different Drusen Types’, *American Journal of Ophthalmology*. Elsevier Inc., 208(January 2013), pp. 103–110. doi: 10.1016/j.ajo.2019.07.013.

Van Leeuwen, R. *et al.* (2003) ‘Epidemiology of age-related maculopathy: A review’, *European Journal of Epidemiology*, 18(9), pp. 845–854. doi: 10.1023/A:1025643303914.

Lim, L. S. *et al.* (2012) 'Age-related macular degeneration' Lancet, 379, pp. 1728-1738. doi: 10.1016/S0140-6736(12)60282-7.

Lima, P. L. V. *et al.* (2019) ‘Photobiomodulation enhancement of cell proliferation at 660 nm does not require cytochrome c oxidase’, *Journal of Photochemistry and Photobiology B: Biology*. Elsevier, 194(February), pp. 71–75. doi: 10.1016/j.jphotobiol.2019.03.015.

Lin, H. *et al.* (2011) ‘Mitochondrial DNA damage and repair in rpe associated with aging and age-related macular degeneration’, *Investigative Ophthalmology and Visual Science*, 52(6), pp. 3521–3529. doi: 10.1167/iovs.10-6163.

Lorentzen, S. E. (1970) ‘INCIDENCE OF CILIORETINAL ARTERIES’, *Acta Ophthalmologica*, 48, pp. 518–524.

Lutty, G. A. *et al.* (2020) ‘Choriocapillaris dropout in early age-related macular degeneration’, *Experimental Eye Research*. Elsevier, 192, p. 107939. doi: 10.1016/j.exer.2020.107939.

Luu, C. D. *et al.* (2018) ‘Topographic Rod Recovery Pro fi les after a Prolonged Dark Adaptation in Subjects with Reticular Pseudodrusen’, *Ophthalmology Retina*. American Academy of Ophthalmology, pp. 1–12. doi: 10.1016/j.oret.2018.06.016.

Mares-perlman, J. A. *et al.* (1995) ‘Dietary Fat and Age-Related Maculopathy’, *Arch Ophthalmol*, 113, pp. 743–748.

Mares, J. A. *et al.* (2011) ‘Healthy lifestyles related to subsequent prevalence of age-related macular degeneration’, *Archives of Ophthalmology*, 129(4), pp. 470–480. doi: 10.1001/archophthalmol.2010.314.

Marsiglia, M. *et al.* (2013) 'Association between geographic atrophy progression and reticular pseudodrusen in eyes with dry age-related macular degeneration', *Investigative Ophthalmology and Visual Science*, 54(12), pp. 7362–7369. doi: 10.1167/iovs.12-11073.

Mata, N. L. *et al.* (2013) 'Investigation of oral fenretinide for treatment of geographic atrophy in age-related macular degeneration', *Retina*, 33(3), pp. 498–507. doi: 10.1097/IAE.0b013e318265801d.

Maugeri, A. *et al.* (2018) 'Complement system and age-related macular degeneration: Implications of gene-environment interaction for preventive and personalized medicine', *BioMed Research International*, 2018. doi: 10.1155/2018/7532507.

Mcguinness, M. B. *et al.* (2020) 'Relationship Between Rod-Mediated Sensitivity , Low-Luminance Visual Acuity , and Night Vision Questionnaire in Age-Related Macular Degeneration', *Translational Vision Science & Technology*, pp. 1–10.

McGwin, G. *et al.* (2006) '3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors and the presence of age-related macular degeneration in the cardiovascular health study', *Archives of Ophthalmology*, 124(1), pp. 33–37. doi: 10.1001/archopht.124.1.33.

Merry, G. F. *et al.* (2017) 'Photobiomodulation reduces drusen volume and improves visual acuity and contrast sensitivity in dry age-related macular degeneration', *Acta Ophthalmologica*, 95(4), pp. e270–e277. doi: 10.1111/aos.13354.

Mester, E. *et al.* (1971) 'Effect of laser rays on wound healing', *The American Journal of Surgery*, 122(4), pp. 532–535. doi: 10.1016/0002-9610(71)90482-X.

Metelitsina, T. I. *et al.* (2006) 'Effect of systemic hypertension on foveolar choroidal blood flow in age related macular degeneration', *British Journal of Ophthalmology*, 90(3), pp. 342–346. doi: 10.1136/bjo.2005.082974.

Mimoun, G., Soubrane, G., Coscas, G. (1990) 'Macular drusen' [in French] Journal Français d'Ophtalmologie, 13, pp. 511–530

Mitchell, P. *et al.* (1995) 'Prevalence of Age-related Maculopathy in Australia: The Blue Mountains Eye Study', *Ophthalmology*, 102(10), pp. 1450–1460. doi: 10.1016/S0161-6420(95)30846-9.

Mitchell, P. *et al.* (2002) 'Five-year incidence of age-related maculopathy lesions: The Blue Mountains Eye Study', *Ophthalmology*, 109(6), pp. 1092–1097. doi: 10.1016/S0161-6420(02)01055-2.

Mitchell, P., Smith, W. and Wang, J. J. (1998) 'Iris color, skin sun sensitivity, and age-related maculopathy: The Blue Mountains Eye Study', *Ophthalmology*, 105(8), pp. 1359–1363. doi: 10.1016/S0161-6420(98)98013-7.

Mitchell, P. and Wang, J. J. (1999) 'Diabetes, fasting blood glucose and age-related maculopathy: The Blue Mountains Eye Study', *Australian and New Zealand Journal of Ophthalmology*, 27(3–4), pp. 197–199. doi: 10.1046/j.1440-1606.1999.00211.x.

Miyazaki, M. *et al.* (2005) 'The 5-year incidence and risk factors for age-related maculopathy in a general Japanese population: The Hisayama study', *Investigative Ophthalmology and Visual Science*, 46(6), pp. 1907–1910. doi: 10.1167/iovs.04-0923.

Motulsky, H. J. and Brown, R. E. (2006) 'Detecting outliers when fitting data with nonlinear regression - A new method based on robust nonlinear regression and the false discovery rate', *BMC Bioinformatics*, 7, pp. 1–20. doi: 10.1186/1471-2105-7-123.

Mustafi, D., Engel, A. H. and Palczewski, K. (2009) *Structure of Cone Photoreceptors*, *Nano*. doi: 10.1016/j.preteyeres.2009.05.003.Structure.

Nebbioso, M., Barbato, A. and Pescosolido, N. (2014) 'Scotopic microperimetry in the early diagnosis of age-related macular degeneration: Preliminary study', *BioMed Research International*. Hindawi Publishing Corporation, 2014. doi: 10.1155/2014/671529.

Nguyen, C. T. *et al.* (2018) 'Longitudinal changes in retinotopic rod function in intermediate age-related macular degeneration', *Investigative Ophthalmology and Visual Science*, 59(4), pp. AMD19–AMD24. doi: 10.1167/iovs.17-23084.

Nickla, D. L. and Wallman, J. (2010) 'The multifunctional choroid', *Progress in Retinal and Eye Research*. Elsevier Ltd, 29(2), pp. 144–168. doi: 10.1016/j.preteyeres.2009.12.002.

Nordgaard, C. L. *et al.* (2008) 'Mitochondrial proteomics of the retinal pigment epithelium at progressive stages of age-related macular degeneration', *Investigative Ophthalmology and Visual Science*, 49(7), pp. 2848–2855. doi: 10.1167/iovs.07-1352.

Okawa, H. *et al.* (2008) 'ATP Consumption by Mammalian Rod Photoreceptors in Darkness and in Light', *Current Biology*. Elsevier Ltd, 18(24), pp. 1917–1921. doi: 10.1016/j.cub.2008.10.029.

Ooto, S. *et al.* (2013) 'Reduction of retinal sensitivity in eyes with reticular pseudodrusen', *American Journal of Ophthalmology*. Elsevier Inc., 156(6), p. 1184. doi: 10.1016/j.ajo.2013.06.036.

Osterberg, G. (1935) 'Topography of the layer of rods and cones in the human retina' Acta Ophthalmologica (Suppl.), 6, pp. 1-103.

Owen, C. G. *et al.* (2012) 'The estimated prevalence and incidence of late stage age related macular degeneration in the UK', *British Journal of Ophthalmology*, 96(5), pp. 752–756. doi: 10.1136/bjophthalmol-2011-301109.

Owsley, C. *et al.* (2000) 'Psychophysical evidence for rod vulnerability in age-related macular degeneration', *Investigative Ophthalmology and Visual Science*, 41(1), pp. 267–273.

Owsley, C. *et al.* (2001) 'Delays in Rod-mediated Dark Adaptation in Early Age-related Maculopathy', 6420(01), pp. 1196–1202.

Owsley, C. *et al.* (2006) 'Development of a questionnaire to assess vision problems under low luminance in age-related maculopathy', *Investigative Ophthalmology and Visual Science*, 47(2), pp. 528–535. doi: 10.1167/iovs.05-1222.

Owsley, C., McGwin, G., *et al.* (2006) 'Effect of short-term, high-dose retinol on dark adaptation in aging and early age-related maculopathy', *Investigative Ophthalmology and Visual Science*, 47(4), pp. 1310–1318. doi: 10.1167/iovs.05-1292.

Owsley, C. *et al.* (2014) 'Associations between abnormal rod-mediated dark adaptation and health and functioning in older adults with normal macular health', *Investigative Ophthalmology and Visual Science*, 55(8), pp. 4776–4789. doi: 10.1167/iovs.14-14502.

Owsley, C. *et al.* (2016) 'Delayed Rod-Mediated Dark Adaptation Is a Functional Biomarker for Incident Early Age-Related Macular Degeneration', *Ophthalmology*. Elsevier Inc, 123(2), pp. 344–351. doi: 10.1016/j.ophtha.2015.09.041.

Owsley, C. *et al.* (2017) 'Natural History of Rod-Mediated Dark Adaptation over 2 Years in Intermediate Age-Related Macular Degeneration', *Translational Vision Science & Technology*, 6(3), p. 15. doi: 10.1167/tvst.6.3.15.

Owsley, C. and McGwin, G. (2016) 'Vision-targeted health related quality of life in older adults: Patient-reported visibility problems in low luminance activities are more likely to decline than daytime activities', *BMC Ophthalmology*. BMC Ophthalmology, 16(1), pp. 4–9. doi: 10.1186/s12886-016-0274-5.

Oyster, C. (2006) The Human Eye: Structure and Function. 1st edition. Cary, NC. Sinauer Associates Inc.

Panda-Jonas, S., Jonas, J. B. and Jakobczyk-Zmija, M. (1996) 'Retinal pigment epithelial cell count, distribution, and correlations in normal human eyes', *American Journal of Ophthalmology*. Elsevier Inc., 121(2), pp. 181–189. doi: 10.1016/S0002-9394(14)70583-5.

Pascolini, D. and Mariotti, S. P. (2012) 'Global estimates of visual impairment: 2010', *British Journal of Ophthalmology*, 96(5), pp. 614–618. doi: 10.1136/bjophthalmol-2011-300539.

Patel, P. J. *et al.* (2008) 'Intersession repeatability of visual acuity scores in age-related macular degeneration', *Investigative Ophthalmology and Visual Science*, 49(10), pp. 4347–4352. doi: 10.1167/iovs.08-1935.

Pauleikhoff, D. *et al.* (1990) 'Choroidal perfusion abnormality with age-related Bruch's membrane change', *American Journal of Ophthalmology*. Elsevier Inc., 109(2), pp. 211–217. doi: 10.1016/S0002-9394(14)75989-6.

Penfold, P. L. *et al.* (2001) 'Immunological and aetiological aspects of macular degeneration', *Progress in Retinal and Eye Research*, 20(3), pp. 385–414. doi: 10.1016/S1350-9462(00)00025-2.

Peoples, C. *et al.* (2012) 'Photobiomodulation enhances nigral dopaminergic cell survival in a chronic MPTP mouse model of Parkinson's disease', *Parkinsonism and Related Disorders*. Elsevier Ltd, 18(5), pp. 469–476. doi: 10.1016/j.parkreldis.2012.01.005.

Pfau, M. *et al.* (2020) 'Progression of Photoreceptor Degeneration in Geographic Atrophy Secondary to Age-related Macular Degeneration', 5479, pp. 1–9. doi: 10.1001/jamaophthalmol.2020.2914.

Plantner, J. J., Barbourl, H. L. and Kean, E. L. (1988) 'The rhodopsin content of the human eye James', *Current Eye Research*, 7(11), pp. 1125–1129.

Powner, M. B. *et al.* (2016) 'Improving mitochondrial function protects bumblebees from neonicotinoid pesticides', *PLoS ONE*, 11(11), pp. 1–11. doi: 10.1371/journal.pone.0166531.

Puell, M. C. *et al.* (2012) 'Impaired mesopic visual acuity in eyes with early age-related macular degeneration', *Investigative Ophthalmology and Visual Science*, 53(11), pp. 7310–7314. doi: 10.1167/iovs.11-8649.

Pulos, E. (1989) 'Changes in Rod Sensitivity through Adulthood', *Investigative Ophthalmology and Visual Science*, 30(8), pp. 1738–1742.

Purves, D., Augustine G. J., Fitzpatrick D. et al. (2001) Neuroscience, Sunderland (MA): Sinauer Associates.

Querques, G. *et al.* (2014) 'IMPACT OF RETICULAR PSEUDODRUSEN ON MACULAR FUNCTION', *Retina*, 34, pp. 321–329.

Ramon, C. *et al.* (2019) 'Longitudinal changes in outer nuclear layer thickness in soft drusen and reticular pseudodrusen', *Clinical and Experimental Optometry*, 102(6), pp. 601–610. doi: 10.1111/cxo.12894.

Ramrattan, R. S. *et al.* (1994) 'Morphometric analysis of Bruch's membrane, the choriocapillaris, and the choroid in aging', *Investigative Ophthalmology and Visual Science*, 35(6), pp. 2857–2864.

Ravalico, G. *et al.* (1996) 'Age-related ocular blood flow changes', *Investigative Ophthalmology and Visual Science*, 37(13), pp. 2645–2650.

Reuter, T. (2011) 'Fifty years of dark adaptation 1961-2011', *Vision Research*. Elsevier Ltd, 51(21–22), pp. 2243–2262. doi: 10.1016/j.visres.2011.08.021.

Reynolds, R., Rosner, B. and Seddon, J. M. (2010) 'Serum lipid biomarkers and hepatic lipase gene associations with age-related macular degeneration', *Ophthalmology*. Elsevier Inc., 117(10), pp. 1989–1995. doi: 10.1016/j.ophtha.2010.07.009.

Robinson, D. G. *et al.* (2018) 'Low-Level Nighttime Light Therapy for Age-Related Macular Degeneration : A Randomized Clinical Trial'.

Rodriguez, J. D. *et al.* (2018) 'Cone photoreceptor macular function and recovery after photostress in early non-exudative age-related macular degeneration', *Clinical Ophthalmology*, 12, pp. 1325–1335. doi: 10.2147/OPTH.S165658.

Rogala, J. *et al.* (2015) 'In Vivo Quantification of Retinal Changes Associated With Drusen in Age-Related Macular Degeneration', pp. 36–38. doi: 10.1167/iovs.14-16221.

Ronan, S. *et al.* (2006) 'Senile panretinal cone dysfunction in age-related macular degeneration (amd): a report of 52 amd patients compared to age-matched controls', *Transactions of the American Ophthalmological Society*, 104, pp. 232–240.

Rosenfeld, P. J. *et al.* (2018) 'Emixustat Hydrochloride for Geographic Atrophy Secondary to Age-Related Macular Degeneration: A Randomized Clinical Trial', *Ophthalmology*. American Academy of Ophthalmology, 125(10), pp. 1556–1567. doi: 10.1016/j.ophtha.2018.03.059.

Rosser, D. A. *et al.* (2003) 'How sensitive to clinical change are ETDRS logMAR visual acuity measurements?', *Investigative Ophthalmology and Visual Science*, 44(8), pp. 3278–3281. doi: 10.1167/iovs.02-1100.

Rudnicka, A. R. *et al.* (2012) 'Age and gender variations in age-related macular degeneration prevalence in populations of European ancestry: A meta-analysis', *Ophthalmology*. Elsevier Inc., 119(3), pp. 571–580. doi: 10.1016/j.ophtha.2011.09.027.

Rudolf, M. *et al.* (2008) 'Sub-retinal drusenoid deposits in human retina: Organization and composition', *Experimental Eye Research*. Elsevier Ltd, 87(5), pp. 402–408. doi: 10.1016/j.exer.2008.07.010.

Rushton, W. A. H. (1965) 'THE FERRIER LECTURE , 1962 Visual adaptation', *Proceedings of the Royal Society of London*, 162(986), pp. 20–46.

Russell, S. R. *et al.* (2000) 'Location, substructure, and composition of basal laminar drusen compared with drusen associated with aging and age-related macular degeneration', *American Journal of Ophthalmology*, 129(2), pp. 205–214. doi: 10.1016/S0002-9394(99)00345-1.

Sahni, J. N. *et al.* (2017) 'Safety and acceptability of an organic light- emitting diode sleep mask as a potential therapy for retinal disease Safety and acceptability of an organic light- emitting diode sleep mask as a potential therapy for retinal disease', *Nature Publishing Group*. Nature Publishing Group, 31(1), pp. 97–106. doi: 10.1038/eye.2016.259.

Saliba, A. *et al.* (2015) 'Photobiomodulation mitigates diabetes-induced retinopathy by direct and indirect mechanisms: Evidence from intervention studies in pigmented mice', *PLoS ONE*, 10(10), pp. 1–14. doi: 10.1371/journal.pone.0139003.

Sarks, J. P., Sarks, S. H. and Killingsworth, M. C. (1988) 'Evolution of geographic atrophy of the retinal pigment epithelium', *Eye (Basingstoke)*, 2(5), pp. 552–577. doi: 10.1038/eye.1988.106.

Sarks, J. P., Sarks, S. H. and Killingsworth, M. C. (1994) 'Evolution of soft drusen in age-related macular degeneration', *Eye (Basingstoke)*, 8(3), pp. 269–283. doi: 10.1038/eye.1994.57.

Saßmannshausen, M. *et al.* (2020) 'Longitudinal Analysis of Retinal Thickness and Retinal Function in Eyes with Large Drusen Secondary to Intermediate Age-Related Macular Degeneration', *Ophthalmology Retina*. Elsevier Inc, pp. 1–10. doi: 10.1016/j.oret.2020.07.019.

Schaumberg, D. A. *et al.* (2001) 'Body mass index and the incidence of visually significant age-related maculopathy in men', *Archives of Ophthalmology*, 119(9), pp. 1259–1265. doi: 10.1001/archopht.119.9.1259.

Schiffer, F. *et al.* (2009) 'Psychological benefits 2 and 4 weeks after a single treatment with near infrared light to the forehead: a pilot study of 10 patients with major depression and anxiety', *Behavioral and Brain Functions*, 5(1), p. 46. doi: 10.1186/1744-9081-5-46.

Schlingemann, R. O. (2004) 'Role of growth factors and the wound healing response in age-related macular degeneration', *Graefe's Archive for Clinical and Experimental Ophthalmology*, 242(1), pp. 91–101. doi: 10.1007/s00417-003-0828-0.

Schmitz-Valckenberg, S. *et al.* (2010) 'Combined Confocal Scanning Laser Ophthalmoscopy and Spectral-Domain Optical Coherence Tomography Imaging of Reticular Drusen Associated with Age-Related Macular Degeneration', *Ophthalmology*. Elsevier Inc., 117(6), pp. 1169–1176. doi: 10.1016/j.ophtha.2009.10.044.

Schmitz-Valckenberg, S. *et al.* (2011) 'Reticular drusen associated with geographic atrophy in age-related macular degeneration', *Investigative Ophthalmology and Visual Science*, 52(9), pp. 5009–5015. doi: 10.1167/iovs.11-7235.

Schuman, J. S. (2008) 'Spectral domain optical coherence tomography for glaucoma (an AOS thesis)', *Transactions of the American Ophthalmological Society*, 106, pp. 426–458.

Seddon, J. *et al.* (1996) 'A prospective study of cigarette smoking and AMD in women', *Journal of the American Medical Association*, 276(14), pp. 1141–1146.

Seddon, J. M. *et al.* (2003) 'Progression of age-related macular degeneration', *Evidence-Based Eye Care*, 4(4), pp. 202–203. doi: 10.1097/00132578-200310000-00010.

Seddon, J. M. *et al.* (2007) 'Association of CFH Y402H and LOC387715 A69S With Progression of Age-Related Macular Degeneration', *JAMA*, 297(16), pp. 1793–1800.

Shao, L. *et al.* (2014) 'Visual Acuity and Subfoveal Choroidal Thickness: The Beijing Eye Study', *American Journal of Ophthalmology*. Elsevier Inc., 158(4), pp. 702-709.e1. doi: 10.1016/j.ajo.2014.05.023.

Shijo, T. *et al.* (2017) 'Prevalence and characteristics of pseudodrusen subtypes in advanced age-related macular degeneration', *Graefe's Archive for Clinical and Experimental Ophthalmology*. Graefe's Archive for Clinical and Experimental Ophthalmology, 255(6), pp. 1125–1131. doi: 10.1007/s00417-017-3622-0.

Sivapathasuntharam, C. *et al.* (2017a) 'Aging retinal function is improved by near infrared light (670 nm) that is associated with corrected mitochondrial decline', *Neurobiology of Aging*. Elsevier Inc, 52, pp. 66–70. doi: 10.1016/j.neurobiolaging.2017.01.001.

Sivapathasuntharam, C. *et al.* (2017b) 'Aging retinal function is improved by near infrared light (670 nm) that is associated with corrected mitochondrial decline', *Neurobiology of Aging*. Elsevier Inc, 52, pp. 66–70. doi: 10.1016/j.neurobiolaging.2017.01.001.

Sivapathasuntharam, C. *et al.* (2019) 'Improving mitochondrial function significantly reduces the rate of age related photoreceptor loss', *Experimental Eye Research*. Elsevier, 185(June), p. 107691. doi: 10.1016/j.exer.2019.107691.

Sivaprasad, S. *et al.* (2016) 'Perspectives on reticular pseudodrusen in age-related macular degeneration', *Survey of Ophthalmology*. Elsevier Inc, 61(5), pp. 521–537. doi: 10.1016/j.survophthal.2016.02.005.

Sivaprasad, S. *et al.* (2018) 'Articles Clinical efficacy and safety of a light mask for prevention of dark adaptation in treating and preventing progression of early diabetic macular oedema at 24 months (CLEOPATRA): a multicentre , phase 3 , randomised controlled trial', 8587(zone 1), pp. 1–10. doi: 10.1016/S2213-8587(18)30036-6.

Smith, W. *et al.* (1998) 'Plasma fibrinogen levels, other cardiovascular risk factors, and age-related maculopathy: The blue mountains eye study', *Archives of Ophthalmology*, 116, pp. 583–587. doi: 10.1016/s0278-2391(99)90653-5.

Smith, W., Mitchell, P. and Wang, J. J. (1997) 'Gender, oestrogen, hormone replacement and age-related macular degeneration: Results from the blue mountains eye study', *Australian and New Zealand Journal of Ophthalmology*, 25(SUPPL. 1), pp. 87–89. doi: 10.1111/j.1442-9071.1997.tb01745.x.

Snell, R.S. and Lemp, M. A. (2013) Clinical Anatomy of the Eye. Second Edition. New Jersey, United States: Blackwell Science Ltd.

Sobrin, L. *et al.* (2011) 'ARMS2/HTRA1 locus can confer differential susceptibility to the advanced subtypes of age-related macular degeneration', *American Journal of Ophthalmology*, 151(2). doi: 10.1016/j.ajo.2010.08.015.

Solberg, Y., Rosner, M. and Belkin, M. (1998) 'The Association Between Cigarette Smoking and Ocular Diseases', *Survey of Ophthalmology*, 42(6), pp. 535–547.

Sommer, A. P., Haddad, M. K. and Fecht, H. J. (2015) 'Light Effect on Water Viscosity: Implication for ATP Biosynthesis', *Scientific Reports*. Nature Publishing Group, 5, pp. 1–6. doi: 10.1038/srep12029.

Spaide, R. F. *et al.* (1999) 'Characterization of peroxidized lipids in Bruch's membrane', *Retina*, pp. 141–147. doi: 10.1097/00006982-199902000-00010.

Spaide, R. F. (2013) 'Outer retinal atrophy after regression of subretinal drusenoid deposits as a newly recognized form of late age-related macular degeneration', *Retina*, 33(9), pp. 1800–1808. doi: 10.1097/IAE.0b013e31829c3765.

Spaide, R. F. (2018) 'Improving the Age-Related Macular Degeneration Construct', *Retina*, 38(5), pp. 891–899. doi: 10.1097/iae.0000000000001732.

Spaide, R. F., Armstrong, D. and Browne, R. (2003) 'Choroidal neovascularization in age-related macular degeneration - What is the cause?', *Retina*, 23(5), pp. 595–614. doi: 10.1097/00006982-200310000-00001.

Sperduto, R. D. and Hiller, R. (1986) 'Systemic Hypertension and Age-Related Maculopathy in the Framingham Study', *Archives of Ophthalmology*, 104(2), pp. 216–219. doi: 10.1001/archopht.1986.01050140070022.

Stalmans, P. and Himpens, B. (1997) 'Effect of increasing glucose concentrations and protein phosphorylation on intercellular communications in cultured rat retinal pigment epithelial cells', *Investigative Ophthalmology and Visual Science*, 38(8), pp. 1598–1609.

Starita, C., Hussain, A. A. and Marshall, J. (1995) 'Decreasing hydraulic conductivity of Bruch's membrane: Relevance to photoreceptor survival and lipofuscinoses', *American Journal of Medical Genetics*, 57(2), pp. 235–237. doi: 10.1002/ajmg.1320570224.

Stefánsson, E., Geirsdóttir, Á. and Sigurdsson, H. (2011) 'Metabolic physiology in age related macular degeneration', *Progress in Retinal and Eye Research*. Elsevier Ltd, 30(1), pp. 72–80. doi: 10.1016/j.preteyeres.2010.09.003.

Steinberg, J. S. *et al.* (2015) 'Reticular drusen in eyes with high-risk characteristics for progression to late-stage age-related macular degeneration', *British Journal of Ophthalmology*, 99(9), pp. 1289–1294. doi: 10.1136/bjophthalmol-2014-306535.

Steinberg, J. S. *et al.* (2017) 'Evaluation of Two Systems for Fundus-Controlled Scotopic and Mesopic Perimetry in Eye with Age-Related Macular Degeneration', *Translational Vision Science & Technology* 6(4). doi: 10.1167/tvst.6.4.7.

Steinmetz, R. L. *et al.* (1993) 'Symptomatic abnormalities of dark adaptation in patients with age-related Bruch's membrane change', *British Journal of Ophthalmology*, 77(9), pp. 549–554. doi: 10.1136/bjo.77.9.549.

Stockman, A. and Sharpe, L. T. (2006) 'Into the twilight zone: The complexities of mesopic vision and luminous efficiency', *Ophthalmic and Physiological Optics*, 26(3), pp. 225–239. doi: 10.1111/j.1475-1313.2006.00325.x.

Stone, J. *et al.* (2008) 'The locations of mitochondria in mammalian photoreceptors: Relation to retinal vasculature', *Brain Research*, 1189(1), pp. 58–69. doi: 10.1016/j.brainres.2007.10.083.

Strauss, O. (2005) 'The retinal pigment epithelium in visual function', *Physiological Reviews*, 85(3), pp. 845–881. doi: 10.1152/physrev.00021.2004.

Sturr, J. F. *et al.* (1997) 'Psychophysical Evidence for Losses in Rod Sensitivity in the Aging Visual System', *Vision Research*, 37(4), pp. 475–481.

Sunness, J. S. *et al.* (1985) 'Peripheral Retinal Function in Age-Related Macular Degeneration-Reply', *Archives of Ophthalmology*, 103(11), p. 1631. doi: 10.1001/archopht.1985.01050110025007.

Sunness, J. S. *et al.* (1997) 'Visual Function Abnormalities and Prognosis in Eyes with Age-related Geographic Atrophy of the Macula and Good Visual Acuity', *Ophthalmology*, 104(10), pp. 1677–1691. doi: 10.1161/CIRCULATIONAHA.111.087940.The.

Sunness, J. S. *et al.* (2008) 'Low Luminance Visual Dysfunction as a Predictor of Subsequent Visual Acuity Loss from Geographic Atrophy in Age-Related Macular Degeneration', *Ophthalmology*, 115(9), pp. 1480–1488. doi: 10.1016/j.ophtha.2008.03.009.

Suzuki, M. *et al.* (2015) 'Refractile drusen: Clinical imaging and candidate histology', *Retina*, 35(5), pp. 859–865. doi: 10.1097/IAE.0000000000000503.

Tahir, H. J. *et al.* (2017) 'Slowed dark adaptation in older eyes ; effect of location', *Experimental Eye Research*. Elsevier Ltd, 155, pp. 47–53. doi: 10.1016/j.exer.2016.11.016.

Talks, S. J. *et al.* (2017) 'The role of new imaging methods in managing age-related macular degeneration', *Asia-Pacific Journal of Ophthalmology*, 6(6), pp. 498–507. doi: 10.22608/APO.2017305.

Tan, J. S. L. *et al.* (2007) 'Cardiovascular Risk Factors and the Long-term Incidence of Age-Related Macular Degeneration The Blue Mountains Eye Study', *Ophthalmology*, 114, pp. 1143–1150. doi: 10.1016/j.ophtha.2006.09.033.

Tan, R., Guymer, R. H. and Luu, C. D. (2018a) 'Subretinal drusenoid deposits and the loss of rod function in intermediate age-related macular degeneration', *Investigative Ophthalmology and Visual Science*, 59(10), pp. 4154–4161. doi: 10.1167/iovs.18-23970.

Tan, R. S. *et al.* (2019) 'Longitudinal assessment of rod function in intermediate age-related macular degeneration with and without reticular pseudodrusen', *Investigative Ophthalmology and Visual Science*, 60(5), pp. 1511–1518. doi: 10.1167/iovs.18-26385.

Tan, R. S., Guymer, R. H. and Luu, C. D. (2018b) 'Repeatability of retinal sensitivity measurements using a medmont dark-adapted chromatic perimeter in healthy and age-related macular degeneration cases', *Translational Vision Science and Technology*, 7(3). doi: 10.1167/tvst.7.3.3.

Tang, J. *et al.* (2013) 'Low-intensity far-red light inhibits early lesions that contribute to diabetic retinopathy: in vivo and in vitro.', *Investigative ophthalmology & visual science*, 54(5), pp. 3681–3690. doi: 10.1167/iovs.12-11018.

Tang, J., Herda, A. A. and Kern, T. S. (2014) 'Photobiomodulation in the treatment of patients with non- center-involving diabetic macular oedema', *British Journal of Ophthalmology*, 98(8), pp. 1013–1015. doi: 10.1136/bjophthalmol-2013-304477.Photobiomodulation.

Taylor, H. R. *et al.* (1990) 'Visible light and risk of age-related macular degeneration', *Transactions of the American Ophthalmological Society*, 88, pp. 163–178.

Terluk, M. R. *et al.* (2015) 'Investigating mitochondria as a target for treating age-related macular degeneration', *Journal of Neuroscience*, 35(18), pp. 7304–7311. doi: 10.1523/JNEUROSCI.0190-15.2015.

Thompson, A. C. *et al.* (2018) 'Association of low luminance questionnaire with objective functional measures in early and intermediate age-related macular degeneration', *Investigative Ophthalmology and Visual Science*, 59(1), pp. 289–297. doi: 10.1167/iovs.17-22528.

Thornton, J. *et al.* (2005) 'Smoking and age-related macular degeneration: A review of association', *Eye*, 19(9), pp. 935–944. doi: 10.1038/sj.eye.6701978.

Tipton, D. A. (1984). A review of vision physiology. *Aviation, Space, and Environmental Medicine, 55*(2), pp. 145–149.

Topouzis, F. *et al.* (2009) 'Association of diabetes with age-related macular degeneration in the EUREYE study', *British Journal of Ophthalmology*, 93(8), pp. 1037–1041. doi: 10.1136/bjo.2008.146316.

Tsang, S. H. and Sharma, T. (2018) 'Retinal histology and anatomical landmarks', *Advances in Experimental Medicine and Biology*, 1085, pp. 3–5.

Uddin, D. *et al.* (2020) 'Repeatability of Scotopic Sensitivity and Dark Adaptation Using a Medmont Dark-Adapted Chromatic Perimeter in Age-related Macular Degeneration', Translational Vision Science & Technology, 9 (7). doi: 10.1167/tvst.9.7.31.

Vanderbeek, B. L. *et al.* (2011) 'Racial differences in age-related macular degeneration rates in the United States: A longitudinal analysis of a managed care network', *American Journal of Ophthalmology*. Elsevier Inc., 152(2), pp. 273-282.e3. doi: 10.1016/j.ajo.2011.02.004.

Varma, R. *et al.* (2010) 'Four-Year Incidence and Progression of Age-Related Macular Degeneration: The Los Angeles Latino Eye Study', *American Journal of Ophthalmology*. Elsevier Inc., 149(5), pp. 741–751. doi: 10.1016/j.ajo.2010.01.009.

Vidaurri, J. S. *et al*. (1984) 'Association between drusen and some of the risk factors for coronary artery disease' *Ophthalmologica*, 188(4) pp. 243-247. doi: 10.1159/000309370.

Vingerling, J. R. *et al.* (1995) 'The Prevalence of Age-related Maculopathy in the Rotterdam Study', *Ophthalmology*. American Academy of Ophthalmology, Inc, 102(2), pp. 205–210. doi: 10.1016/S0161-6420(95)31034-2.

Wakabayashi, T. *et al.* (2009) 'Foveal Microstructure and Visual Acuity after Retinal Detachment Repair. Imaging Analysis by Fourier-Domain Optical Coherence Tomography', *Ophthalmology*. American Academy of Ophthalmology, 116(3), pp. 519–528. doi: 10.1016/j.ophtha.2008.10.001.

Wang, J. S. and Kefalov, V. J. (2011) 'The Cone-specific visual cycle', *Progress in Retinal and Eye Research*. Elsevier Ltd, 30(2), pp. 115–128. doi: 10.1016/j.preteyeres.2010.11.001.

Wang, L. *et al.* (2010) ‘Abundant lipid and protein components of drusen’, *PLoS ONE*, 5(4), pp. 1–12. doi: 10.1371/journal.pone.0010329.

Wang, Q. *et al.* (1996) 'Pattern of age-related maculopathy in the macular area. The Beaver Dam Eye Study'. Investigative Ophthalmology & Visual Science, 37, pp. 2234-2242.

Wangsa-Wirawan, N. D. (2003) ‘Retinal Oxygen’, *Archives of Ophthalmology*, 121(4), p. 547. doi: 10.1001/archopht.121.4.547.

Weale, R. A. (1961) ‘The duplicity theory of vision.’, *Annals of the Royal College of Surgeons of England*, 28(March), pp. 16–35.

Weinrich, T. W. *et al.* (2017) ‘Improving mitochondrial function significantly reduces metabolic, visual, motor and cognitive decline in aged Drosophila melanogaster’, *Neurobiology of Aging*. Elsevier Inc, 60, pp. 34–43. doi: 10.1016/j.neurobiolaging.2017.08.016.

West, S. K. *et al.* (1989) ‘Exposure to sunlight and other risk factors for age-related macular degeneration’, *Archives of Ophthalmology*, 107(6), pp. 875–879. doi: 10.1001/archopht.1989.01070010897038.

Whitmore, S. S. *et al.* (2015) ‘Complement activation and choriocapillaris loss in early AMD: Implications for pathophysiology and therapy’, *Progress in Retinal and Eye Research*. Elsevier Ltd, 45, pp. 1–29. doi: 10.1016/j.preteyeres.2014.11.005.

Wong-Riley, M. (2010) ‘Energy metabolism of the visual system’, *Eye and Brain*, pp. 99–116.

Wong, W. L. *et al.* (2014) ‘Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: A systematic review and meta-analysis’, *The Lancet Global Health*. Wong et al. Open Access article distributed under the terms of CC BY-NC-ND, 2(2), pp. e106–e116. doi: 10.1016/S2214-109X(13)70145-1.

Wong, W. T. *et al.* (2013) ‘Treatment of geographic atrophy with subconjunctival sirolimus: Results of a phase I/II clinical trial’, *Investigative Ophthalmology and Visual Science*, 54(4), pp. 2941–2950. doi: 10.1167/iovs.13-11650.

Wu, Z. *et al.* (2014) ‘Low-luminance visual acuity and microperimetry in age-related macular degeneration’, *Ophthalmology*. Elsevier Inc, 121(8), pp. 1612–1619. doi: 10.1016/j.ophtha.2014.02.005.

Wu, Z. *et al.* (2016) ‘Reticular pseudodrusen in intermediate age-related macular degeneration: Prevalence, detection, clinical, environmental, and genetic associations’, *Investigative Ophthalmology and Visual Science*, 57(3), pp. 1310–1316. doi: 10.1167/iovs.15-18682.

Wu, Z., Guymer, R. H. and Finger, R. P. (2016) 'Low luminance deficit and night vision symptoms in intermediate age-related macular degeneration', *British Journal of Ophthalmology*, 100(3), pp. 395–398. doi: 10.1136/bjophthalmol-2015-306621.

Xu, C. *et al.* (2014) 'Light-harvesting chlorophyll pigments enable mammalian mitochondria to capture photonic energy and produce ATP', *Journal of Cell Science*, pp. 388–399. doi: 10.1242/jcs.134262.

Xu, L. *et al.* (2009) 'Ocular and systemic factors associated with diabetes mellitus in the adult population in rural and urban China. The Beijing Eye Study', *Eye*, 23(3), pp. 676–682. doi: 10.1038/sj.eye.6703104.

Yazdanie, M. *et al.* (2017) 'Decreased Visual Function Scores on a Low Luminance Questionnaire Is Associated with Impaired Dark Adaptation', *Ophthalmology*. Elsevier Inc, 124(9), pp. 1332–1339. doi: 10.1016/j.ophtha.2017.05.005.

Yehoshua, Z. *et al.* (2014) 'Systemic Complement Inhibition with Eculizumab for Geographic Atrophy in Age-Related Macular Degeneration The COMPLETE Study', *Ophthalmology*. American Academy of Ophthalmology, 121(3), pp. 693–701. doi: 10.1016/j.ophtha.2013.09.044.

Yip, K. K. *et al.* (2011) 'The effect of low-energy laser irradiation on apoptotic factors following experimentally induced transient cerebral ischemia', *Neuroscience*, 190, pp. 301–306. doi: 10.1016/j.neuroscience.2011.06.022.

Young, R. W. (1967) 'The renewal of photorecept o r cell outer segments', *The Journal of Cell Biology*, 33(1), pp. 61–72. Available at: http://jcb.rupress.org/content/33/1/61.full.pdf.

Young, R. W. (1987) 'Pathophysiology of age-related macular degeneration', *Survey of Ophthalmology*, 31(5), pp. 291–306. doi: 10.1016/0039-6257(87)90115-9.

Young, R. W. and Bok, D. (1969) 'Participation of the retinal pigment epithelium in the rod outer segment renewal process.', *The Journal of cell biology*, 42(2), pp. 392–403. doi: 10.1083/jcb.42.2.392.

Zhang, D. *et al.* (2016) 'Sequestration of ubiquitous dietary derived pigments enables mitochondrial light sensing', *Scientific Reports*. Nature Publishing Group, 6, pp. 1–13. doi: 10.1038/srep34320.

Zweifel, S. A. *et al.* (2010) 'Reticular Pseudodrusen Are Subretinal Drusenoid Deposits', *Ophthalmology*. Elsevier Inc., 117(2), pp. 303-312.e1. doi: 10.1016/j.ophtha.2009.07.014.

Appendix

- Case Report Forms (CRF) FUSCHIA Study
- Case Report Forms (CRF) 670nm Trial
- Patient Information Sheet (FUSCHIA)
- Patient Information Sheet (670nm trial)

Moorfields Eye Hospital NHS
NHS Foundation Trust

CASE REPORT FORM

Structure Function Correlation in aging and age related macular degeneration (FUSCHIA STUDY)

Chief Investigator: Professor Sobha Sivaprasad
Name of site: Moorfields Eye hospital
CRF Version Number: 4.0, 11/SEP/2017

Subject No. ☐☐☐☐☐☐

CRF Completion Instructions

General

Complete the CRF using a **black ballpoint pen** and ensure that all entries are complete and legible.

Avoid the use of abbreviations and acronyms.

The CRF should be completed as soon as possible after the scheduled visit.

Do not use subject identifiers anywhere on the CRF, such as name, hospital number etc., in order to maintain the confidentiality of the subject. Ensure that the header information (i.e. subject's initials and ID number) is completed consistently throughout the CRF. Missing initials should be recorded with a dash (i.e. D-L).

Each CRF page should be signed and dated by the person completing the form.
The 'completed by' Name in the footer of each page must be legible and **CRFs should only be completed by individuals delegated to complete CRFs on the Site Delegation log (and signed by the PI).**

Ensure that all fields are completed on each page:

- If a test was Not Done record **ND** in the relevant box(es)
- Where information is Not Known write **NK** in relevant box(es)
- Where information is not applicable write **NA** in the relevant box(es)

Corrections to entries

If an error is made, draw a single line through the item, then write the correct entry on an appropriate blank space near the original data point on the CRF and initial and date the change.

Do NOT

- Obscure the original entry by scribbling it out
- Try to correct/ modify the original entry
- Use Tippex or correction fluid

Medications taken by the subject during the trial should be recorded on the "Concomitant Medications Log" using the generic name whenever possible, except combination products which will be recorded using the established trade name. All non-IMPs mentioned in the protocol should also be recorded on the "Concomitant medication Log" for the duration of the trial.

Verbatim Adverse Event terms (initial medical term) should be recorded as the final diagnosis whenever possible.

Complete all **dates** as day, month, year i.e. 13/NOV/2008. Partial dates should be recorded as NK/NOV/2008.

All **times** are to be recorded in 24 hour format without punctuation and always use 4-digits; i.e. 0200 or 2130. Midnight is recorded as 0000.

Weights should be recorded to the nearest 0.1 kg.

Source documents such as lab reports, ECG reports etc. should be filed separately from the CRF (if not in the medical notes) for each subject and be signed and dated by a delegated Investigator as proof of review of the assessment during the trial. Questionnaire should be considered as the CRF appendices (except standard approved questionnaire e.g. EQ-5D)

If a subject prematurely withdraws from the trial a single line must be drawn across each uncompleted page to correspond with the last visit of the subject as mentioned on the "Trial Completion" page.

The protocol deviation/violation/serious breach log should be used to record comments relating to each CRF visit that cannot be captured on the page itself. This includes reason for delayed or missed protocol visits or trial assessments, unscheduled visits etc.

The Chief Investigator (for lead site)/Principal Investigator is responsible for the accuracy of the data reported on the CRF. The CI/PI must sign and date the Principal Investigator's Sign Off page to certify accuracy, completeness and legibility of the data reported in the CRF.

Serious Adverse Events (SAEs)
SAEs should be faxed **within 24 hours** of the site being aware of the event using the trial specific SAE report form to [redacted] **or preferably emailed to sae@ucl.ac.uk**

Storage
CRF documents should be stored in a locked, secure area when not in use where confidentiality can be maintained. Ensure that they are stored separately to any other documents that might reveal the identity of the subject.

Subject No. | | | | | | |

VISIT 1 (BASELINE) DEMOGRAPHIC DATA

Date of Assessment: __ __ / __ __ __ / __ __ __ __

(DD / MMM / YYYY)

Informed Consent:	
Date participant/ signed written consent form: __ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)	**Date of first trial-related procedure:** __ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Name of person taking informed consent: _____	

Demographic Data:				
Date of Birth: __ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)				
Ethinicity:				
White	White British ☐	White Irish ☐	White Other ☐	
Mixed race	White & Black Caribbean ☐	White & Black African ☐	White & Asian ☐	Other mixed background ☐
Asian or Asian British	Indian ☐	Bangladeshi ☐	Pakistani ☐	Other Asian background ☐
Black or Black British	Caribbean ☐	African ☐	Black Other ☐	
Chinese or other ethnicity	Chinese ☐	Other ☐ (please specify)		
Sex: ☐ Male ☐ Female				

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

Subject No. | | | | | | |

VISIT 1 (BASELINE) INCLUSION CRITERIA

Date of Assessment: __ __ / __ __ __ / __ __ __ __

(DD / MMM / YYYY)

The following criteria MUST be answered YES for participant to be included in the trial (except where NA is appropriate):		**Yes**	**No**	**N/A**
1.	Step 1- 7 of the AREDS 9-step severity scale in at least one eye	☐	☐	☐
2.	Best corrected visual acuity in the study eye better than 50 ETDRS logMAR letters at baseline	☐	☐	☐
3.	They will require media clarity, pupillary dilation, and subject cooperation sufficient for adequate imaging and psychophysical testing	☐	☐	☐
4.	Ability to return for follow-up	☐	☐	☐
5.	Ability to fully consent to process	☐	☐	☐
6.	Manual and psychological dexterity to use the device in the correct manner	☐	☐	☐
If any of the above criteria is answered NO, the participant is NOT eligible for the trial and must not be included in the study. Please list reason(s) for ineligibility for screen failure on Participant Eligibility Review page.				

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

Subject No. | | | | | | |

VISIT 1 (BASELINE) EXCLUSION CRITERIA

Date of Assessment: __ __ / __ __ __ / __ __ __ __

(DD / MMM / YYYY)

The following criteria MUST be answered NO for the participant to be included in the trial:		**Yes**	**No**
1.	Co-existent ocular disease: Any ocular condition is present such that, in the opinion of the investigator, might affect the inflammatory status, visual acuity or cone function during the course of the study	☐	☐
2.	A substantial cataract that, in the opinion of the investigator, is likely to be decreasing visual acuity by 3 lines or more (e.g. cataract would be reducing acuity to 6/12 or worse if eye was otherwise normal)	☐	☐
3.	History of major ocular surgery (including cataract extraction, scleral buckle, any intraocular surgery, etc.) within prior 3 months or anticipated within the next 6 months following enrolment.	☐	☐
4.	Epilepsy	☐	☐
5.	Allergies to adhesives or any other component used.	☐	☐
If any of the above criteria is answered YES, the participant is NOT eligible for the trial and must not be included in the study. Please list reason(s) for ineligibility for screen failure on Participant Eligibility Review page.			

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

Subject No. | | | | | | |

VISIT 1 (BASELINE) PARTICIPANT ELIGIBILITY REVIEW

End of Visit 1/ Baseline Checklist:			
		Yes	No
1.	Does the participant satisfy the inclusion and exclusion criteria to date?	☐	☐
2.	Have all Screening Visit procedures been completed?	☐	☐
3.	Have the Medical History and Concomitant Medication pages been completed?	☐	☐
4.	Is the participant still willing to proceed in the trial?	☐	☐

Participant's eligibility Investigator Sign-Off:	
Is the participant eligible to take part in the Clinical Trial? **Investigator's Signature**: _____ **Date** : __ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY) **Investigator's Name:** _____	☐ Yes ☐ No, Please give reason for screen failure below
Reason(s) for screen failure:	
1.	
2.	
3.	

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 1 (BASELINE) MEDICAL HISTORY

Date of Assessment: __ / ___ / ____

(DD / MMM / YYYY)

Has the patient had any relevant medical history?	☐ **No** ☐ **Yes,** Complete below	
Condition / illness /surgical procedure	**Duration**	**Or tick if ongoing at Screening Visit?**
		☐
		☐
		☐
		☐
		☐
		☐
		☐
		☐
		☐
		☐
		☐
		☐

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

Subject No. | | | | | | |

VISIT 1 (BASELINE) CONCOMITANT MEDICATIONS

Is the participant taken any concomitant medications at screening						
Medication (Record Generic or trade name)	**Reason for use** (Medical History diagnosis or other reason, e.g. Prophylaxis)	Dose and units	**Frequency**	**Route**	**Duration**	**Or tick if ongoing at Screening Visit**
1.						☐
2.						☐
3.						☐
4.						☐
5.						☐
6.						☐
7.						☐
8.						☐
9.						☐
10.						☐

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

Subject No. | | | | | | |

VISIT 1 (BASELINE) SMOKING / ALCOHOL STATUS

Date of Assessment: __ __ / __ __ __ / __ __ __ __

(DD / MMM / YYYY)

Has the participant ever smoked? ☐ **No** ☐ **Yes,** Complete below

☐	**Current Smoker**	**Participant's average daily use:** - Number of cigarettes : ___ ___ - Number of cigars : ____ ___ - Number of pipes : ___ ___ **Smoked for ___ ___ months/years**
☐	**Former smoker**	**Smoked for ___ ___ months/years** **Smoking ceased** __ __ __ __ (YYYY) **When smoking, participant's average daily use:** - Number of cigarettes : ___ ___ - Number of cigars : ____ ___ - Number of pipes : ___ ___

Participant's alcohol consumption

Participant's average consumption per week:
- Number of glasses of wine : ___ ___
- Number of pints/bottle of beer : ____ ___
- Number of spirits : ___ ___

(see protocol for definition of units)

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

Subject No. | | | | | | |

VISIT 1 (BASELINE) OPHTHALMIC EXAM

Date of Assessment: __ __ / __ __ __ / __ __ __ __

Was Ophthalmic Examination performed? ☐ **No** ☐ **Yes, Complete below**				
	R	**L**	**Not done**	**LLQ QUESTIONNAIRE**
BCVA EDTRS (Letters)	Score: VA	Score: VA		SCORE:
BCVA Low Luminance	Score: VA	Score: VA:		
Slit lamp examination DILATED / UNDILATED	R			L **Anterior segment** Lids/Lashes Conjunctiva Cornea Anterior chamber Lens **Posterior segment** C:D Vessels Macula Periphery
Cataract grading				
Dilation	Time : Drug : Tropicamide 1.0% 1 drop R / L / Both BN: Exp: Drug : Phenylephrine 2.5 % 1 drop R / L / Both BN: Exp:			

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

Subject No. | | | | | | |

VISIT 1 (BASELINE) VITAL SIGNS

Were Vital Signs performed?	☐ **No (comment below)** ☐ **Yes,** Complete below Comment*: _____
Date of Vital Signs:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Time of Vital Signs:	_____:_____ **HH:MM**
Blood Pressure (sitting):	___ ___ ___ / ___ ___ ___ mmHg
Weight: ___ ___ ___ . ___ kg	**Height:** ___ . ___ ___ m

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

Subject No. | | | | | | |

VISIT 1 (BASELINE) IMAGING

Tests	Date (DD /MM / YYYY)	Completed by: Name	Signature
Photographs			
Photographs wide angle OPTOS			
OCT			
OCT- A			
Infrared Imaging			
Qualitative/Quantitative Autofluorescence			
COMMENTS:			

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

Subject No. | | | | | | |

VISIT 1 (BASELINE) PHOTORECEPTOR FUNCTION

Medmont	☐ **No (comment below)** ☐ **Yes,** Complete below **Comment *:** _____
Date of test:	__ __ / __ __ __ / __ __ __ __ **(DD / MMM / YYYY)**
Time of test	_____:_____ **HH:MM**
Right eye / Left eye / Both	

Parameter	Completed	Comment
Static thresholds		

Parameter	Completed	Comment
Dyanmic test		

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

Subject No. | | | | | | |

VISIT 1 (BASELINE) PHOTORECEPTOR FUNCTION

AdaptDx	☐ **No (comment below)** ☐ **Yes,** Complete below **Comment *:** _____
Date of test:	__ __ / __ __ __ / __ __ __ __ **(DD / MMM / YYYY)**
Time of test	_____:_____ **HH:MM**
Right eye / Left eye / Both	

Parameter	**Completed**	**Comment**
Dyanamic test		

VISIT 1 (BASELINE): MAIA MICROPERIMETRY

MAIA	☐ **No (comment below)** ☐ **Yes,** Complete below **Comment *:** _____
Date of test:	__ __ / __ __ __ / __ __ __ __ **(DD / MMM / YYYY)**
Time of test	_____:_____ **HH:MM**
Right eye / Left eye / Both	

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

Subject No. | | | | | | |

VISIT 1 (BASELINE) PHOTORECEPTOR FUNCTION

RETEval	☐ **No (comment below)** ☐ **Yes,** Complete below **Comment *:** _____
Date of test:	__ __ / __ __ __ / __ __ __ __ **(DD / MMM / YYYY)**
Time of test	_____:_____ **HH:MM**
Right eye / Left eye / Both	

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

NOTES

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

Subject No. | | | | | | |

VISIT 2 (12 MONTHS): CHECKLIST

Date of Visit:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)

Visit Checklist:

		Yes	No
1.	**Have there been any new Adverse Events?** (If yes, please record in Adverse Events page)	☐	☐
2.	**Have there been any changes in Concomitant Medications?** (If yes, please record in Concomitant Medications Log)	☐	☐
3.	**Have there been any changes in Health?**	☐	☐
4.	**Have there been any other changes?**	☐	☐

Notes	

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 2 (12 MONTHS): OPHTHALMIC EXAM

Date of Assessment: __ __ / __ __ __ / __ __ __ __

Was Ophthalmic Examination performed? ☐ **No** ☐ **Yes, Complete below**				
	R	**L**	**Not done**	**LLQ QUESTIONNAIRE**
BCVA EDTRS (Letters)	Score: VA	Score: VA		SCORE:
BCVA Low Luminance	Score: VA	Score: VA:		
Slit lamp examination DILATED / UNDILATED	R			L **Anterior segment** Lids/Lashes Conjunctiva Cornea Anterior chamber Lens **Posterior segment** C:D Vessels Macula Periphery
Cataract grading				
Dilation	Time : Drug : Tropicamide 1.0% 1 drop R / L / Both BN: Exp: Drug : Phenylephrine 2.5 % 1 drop R / L / Both BN: Exp:			

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 2 (12 MONTHS) IMAGING

Tests	Date (DD /MM / YYYY)	Completed by: Name	Signature
Photographs			
Photographs wide angle OPTOS			
OCT			
OCT- A			
Infrared Imaging			
Qualitative/Quantitative Autofluorescence			
COMMENTS:			

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

Subject No. | | | | | | |

VISIT 2 (12 MONTHS): PHOTORECEPTOR FUNCTION

Medmont	☐ **No (comment below)** ☐ **Yes,** Complete below **Comment *:** _____
Date of test:	__ __ / __ __ __ / __ __ __ __ **(DD / MMM / YYYY)**
Time of test	_____:_____ **HH:MM**
Right eye / Left eye / Both	

Parameter	Completed	Comment
Static thresholds		

Parameter	Completed	Comment
Dyanmic test		

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 2 (12 MONTHS) PHOTORECEPTOR FUNCTION

AdaptDx	☐ **No (comment below)** ☐ **Yes,** Complete below **Comment *:** _____
Date of test:	__ __ / __ __ __ / __ __ __ __ **(DD / MMM / YYYY)**
Time of test	_____:_____ **HH:MM**
Right eye / Left eye / Both	

Parameter	**Completed**	**Comment**
Dyanamic test		

VISIT 2 : MAIA MICROPERIMETRY

MAIA	☐ **No (comment below)** ☐ **Yes,** Complete below **Comment *:** _____
Date of test:	__ __ / __ __ __ / __ __ __ __ **(DD / MMM / YYYY)**
Time of test	_____:_____ **HH:MM**
Right eye / Left eye / Both	

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

Subject No. | | | | | | |

VISIT 2 (12 MONTHS) PHOTORECEPTOR FUNCTION

RETEval	☐ **No (comment below)** ☐ **Yes,** Complete below **Comment *:** _____
Date of test:	__ __ / __ __ __ / __ __ __ __ **(DD / MMM / YYYY)**
Time of test	_____:_____ **HH:MM**
Right eye / Left eye / Both	

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

Subject No. | | | | | | |

VISIT 3 (24 MONTHS): CHECKLIST

Date of Visit:	__ __/ __ __ __/ __ __ __ __ (DD / MMM / YYYY)

Visit Checklist:

		Yes	No
1.	**Have there been any new Adverse Events?** (If yes, please record in Adverse Events page)	☐	☐
2.	**Have there been any changes in Concomitant Medications?** (If yes, please record in Concomitant Medications Log)	☐	☐
3.	**Have there been any changes in Health?**	☐	☐
4.	**Have there been any other changes?**	☐	☐

Notes	

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 3 (24 MONTHS): OPHTHALMIC EXAM

Date of Assessment: __ __ / __ __ __ / __ __ __ __

Was Ophthalmic Examination performed? ☐ **No** ☐ **Yes, Complete below**				
	R	**L**	**Not done**	**LLQ QUESTIONNAIRE**
BCVA EDTRS (Letters)	Score: VA	Score: VA		SCORE:
BCVA Low Luminance	Score: VA	Score: VA:		
Slit lamp examination DILATED / UNDILATED	R			L **Anterior segment** Lids/Lashes Conjunctiva Cornea Anterior chamber Lens **Posterior segment** C:D Vessels Macula Periphery
Cataract grading				
Dilation	Time : Drug : Tropicamide 1.0% 1 drop R / L / Both BN: Exp: Drug : Phenylephrine 2.5 % 1 drop R / L / Both BN: Exp:			

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 3 (24 MONTHS): IMAGING

Tests	Date (DD /MM / YYYY)	Completed by: Name	Signature
Photographs			
Photographs wide angle OPTOS			
OCT			
OCT- A			
Infrared Imaging			
Qualitative/Quantitative Autofluorescence			
COMMENTS:			

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

Subject No. | | | | | | |

VISIT 3 (24 MONTHS): PHOTORECEPTOR FUNCTION

Medmont	☐ **No (comment below)** ☐ **Yes,** Complete below **Comment *:** _____
Date of test:	__ __ / __ __ __ / __ __ __ __ **(DD / MMM / YYYY)**
Time of test	_____:_____ **HH:MM**
Right eye / Left eye / Both	

Parameter	Completed	Comment
Static thresholds		

Parameter	Completed	Comment
Dyanmic test		

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 3 (24 MONTHS): PHOTORECEPTOR FUNCTION

AdaptDx	☐ **No (comment below)** ☐ **Yes,** Complete below **Comment *:** _____
Date of test:	__ __ / __ __ __ / __ __ __ __ **(DD / MMM / YYYY)**
Time of test	_____:_____ **HH:MM**
Right or Left eye	

Parameter	**Completed**	**Comment**
Dyanamic test		

VISIT 3 (24 MONTHS): MAIA MICROPERIMETRY

MAIA	☐ **No (comment below)** ☐ **Yes,** Complete below **Comment *:** _____
Date of test:	__ __ / __ __ __ / __ __ __ __ **(DD / MMM / YYYY)**
Time of test	_____:_____ **HH:MM**
Right eye / Left eye / Both	

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 3 (24 MONTHS): PHOTORECEPTOR FUNCTION

RETEval	☐ **No (comment below)** ☐ **Yes,** Complete below **Comment *:** _____
Date of test:	__ __ / __ __ __ / __ __ __ __ **(DD / MMM / YYYY)**
Time of test	_____:_____ **HH:MM**
Right eye / Left eye / Both	

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

Subject No. | | | | | | |

VISIT 4 (36 MONTHS): CHECKLIST

Date of Visit: __ __ / __ __ __ / __ __ __ __

(DD / MMM / YYYY)

Visit Checklist:

		Yes	No
1.	**Have there been any new Adverse Events?** (If yes, please record in Adverse Events page)	☐	☐
2.	**Have there been any changes in Concomitant Medications?** (If yes, please record in Concomitant Medications Log)	☐	☐
3.	**Have there been any changes in Health?**	☐	☐
4.	**Have there been any other changes?**	☐	☐

Notes	

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 4 (36 MONTHS): OPHTHALMIC EXAM

Date of Assessment: / /

Was Ophthalmic Examination performed? ☐ **No** ☐ **Yes, Complete below**				
	R	**L**	**Not done**	**LLQ QUESTIONNAIRE**
BCVA EDTRS (Letters)	Score: VA	Score: VA		SCORE:
BCVA Low Luminance	Score: VA	Score: VA:		
Slit lamp examination DILATED / UNDILATED	R			L **Anterior segment** Lids/Lashes Conjunctiva Cornea Anterior chamber Lens **Posterior segment** C:D Vessels Macula Periphery
Cataract grading				
Dilation	Time : Drug : Tropicamide 1.0% 1 drop R / L / Both BN: Exp: Drug : Phenylephrine 2.5 % 1 drop R / L / Both BN: Exp:			

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 4 (36 MONTHS): IMAGING

Tests	Date (DD /MM / YYYY)	Completed by: Name	Signature
Photographs			
Photographs wide angle OPTOS			
OCT			
OCT- A			
Infrared Imaging			
Qualitative/Quantitative Autofluorescence			
COMMENTS:			

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 4 (36 MONTHS): PHOTORECEPTOR FUNCTION

Medmont	☐ **No (comment below)** ☐ **Yes,** Complete below **Comment *:** _____
Date of test:	__ __ / __ __ __ / __ __ __ __ **(DD / MMM / YYYY)**
Time of test	_____:_____ **HH:MM**
Right or Left eye	

Parameter	Completed	Comment
Static thresholds		

Parameter	Completed	Comment
Dyanmic test		

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 4 (36 MONTHS): PHOTORECEPTOR FUNCTION

AdaptDx	☐ **No (comment below)** ☐ **Yes,** Complete below **Comment *:** _____
Date of test:	__ __ / __ __ __ / __ __ __ __ **(DD / MMM / YYYY)**
Time of test	_____:_____ **HH:MM**
Right or Left eye	

Parameter	**Completed**	**Comment**
Dyanamic test		

VISIT 4 (36 MONTHS): MAIA MICROPERIMETRY

MAIA	☐ **No (comment below)** ☐ **Yes,** Complete below **Comment *:** _____
Date of test:	__ __ / __ __ __ / __ __ __ __ **(DD / MMM / YYYY)**
Time of test	_____:_____ **HH:MM**
Right eye / Left eye / Both	

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 4 (36 MONTHS): PHOTORECEPTOR FUNCTION

RETEval	☐ **No (comment below)** ☐ **Yes,** Complete below **Comment *:** _____
Date of test:	__ __ / __ __ __ / __ __ __ __ **(DD / MMM / YYYY)**
Time of test	_____:_____ **HH:MM**
Right eye / Left eye / Both	

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

Subject No. | | | | | | |

TRIAL COMPLETION

Did participant complete the trial?	☐ **Yes,** Please provide **date of last visit**: __ __ / __ __ __ / 2 0 __ __ (DD / MMM / YYYY) ☐ **No,** Please provide **date of withdrawal** and complete below: __ __ / __ __ __ / 2 0 __ __ (DD / MMM / YYYY)

<u>Early Withdrawal:</u> please tick most appropriate reason for participant not completing the trial:

☐ **Adverse Events related:** please state related AE: _____ (add details to AE page)

☐ **Participant's decision, specify: _____**

☐ **Investigator's decision, specify: _____**

☐ **Sponsor's decision**

☐ **Lost to follow up**

☐ **Patient deceased**

☐ **Other, specify:** _____

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

FUSCHIA

Site: Moorfields Eye Hospital

Subject No. | | | | | | |

ADVERSE EVENTS PAGE

AE No	Event Name (Please give Diagnosis if known)	Start date (DD/MMM/YYYY)	Stop date (DD/MMM/YYYY)	Serious? If serious, please complete a JRO SAE form	Con-comitant Medication given	Severity 0 - Mild 1- Mode-rate 2 - Severe	Study Drug Action 0 - None 1 - Temporarily Interrupted 2 - permanently withdrawn	Outcome 0 - Resolved 1- Resolved with sequelea 2 - Not resolved	Relationship to Study Drug 0 - Definitely 1 - Probably 2 - Possibly 3 - Unlikely 4 - Not related 5 - Not assessable
1		___/___/___	___/___/___	☐ No ☐ Yes	☐ No ☐ Yes				
2		___/___/___	___/___/___	☐ No ☐ Yes	☐ No ☐ Yes				
3		___/___/___	___/___/___	☐ No ☐ Yes	☐ No ☐ Yes				
4		___/___/___	___/___/___	☐ No ☐ Yes	☐ No ☐ Yes				
5		___/___/___	___/___/___	☐ No ☐ Yes	☐ No ☐ Yes				
6		/ /	/ /	☐ No ☐ Yes	☐ No ☐ Yes				

I have reviewed the AEs on this page and have assessed them for seriousness, causality, severity and outcome and confirm that, to the best of my knowledge, it accurately reflects the study information obtained for this participant

PI signature _____ _____ **Date:**_____

☐ **Please check box if this is the last page used**

FUSCHIA

Site: Moorfields Eye Hospital

Subject No. | | | | | | |

PRINICIPAL INVESTIGATOR'S SIGN OFF

Principal Investigator's Signature Statement:	
I have reviewed this CRF and confirm that, to the best of my knowledge, it accurately reflects the study information obtained for this participant. All entries were made either by me or by a person under my supervision who has signed the Delegation and Signature Log.	
Principal Investigator's Signature: _____ **Principal Investigator's Name:** _____	**Date of Signature:** __ __/ __ __ __/ __ __ __ __ (DD / MMM / YYYY)

ONCE SIGNED, NO FURTHER CHANGES CAN BE MADE TO THIS CRF WITHOUT A SIGNED DATA QUERY FORM.

Moorfields Eye Hospital NHS
NHS Foundation Trust

CASE REPORT FORM

A pilot study to investigate the effect of 670 nm light on visual function in ageing and age related macular degeneration (student study)

Chief Investigator: Professor Sobha Sivaprasad
Sponsor Number: 15/0274
Name of site: Moorfields Eye hospital
CRF Version Number: 1.0, 09/Mar/2017

Subject No. ☐☐☐☐☐☐

CRF Completion Instructions

General

Complete the CRF using a **black ballpoint pen** and ensure that all entries are complete and legible.

Avoid the use of abbreviations and acronyms.

The CRF should be completed as soon as possible after the scheduled visit.

Do not use subject identifiers anywhere on the CRF, such as name, hospital number etc., in order to maintain the confidentiality of the subject. Ensure that the header information (i.e. subject's initials and ID number) is completed consistently throughout the CRF. Missing initials should be recorded with a dash (i.e. D-L).

Each CRF page should be signed and dated by the person completing the form.
The 'completed by' Name in the footer of each page must be legible and **CRFs should only be completed by individuals delegated to complete CRFs on the Site Delegation log (and signed by the PI).**

Ensure that all fields are completed on each page:

- If a test was Not Done record **ND** in the relevant box(es)
- Where information is Not Known write **NK** in relevant box(es)
- Where information is not applicable write **NA** in the relevant box(es)

Corrections to entries

If an error is made, draw a single line through the item, then write the correct entry on an appropriate blank space near the original data point on the CRF and initial and date the change.

Do NOT

- Obscure the original entry by scribbling it out
- Try to correct/ modify the original entry
- Use Tippex or correction fluid

Medications taken by the subject during the trial should be recorded on the "Concomitant Medications Log" using the generic name whenever possible, except combination products which will be recorded using the established trade name. All non-IMPs mentioned in the protocol should also be recorded on the "Concomitant medication Log" for the duration of the trial.

Verbatim Adverse Event terms (initial medical term) should be recorded as the final diagnosis whenever possible.

Complete all **dates** as day, month, year i.e. 13/NOV/2008. Partial dates should be recorded as NK/NOV/2008.

All **times** are to be recorded in 24 hour format without punctuation and always use 4-digits; i.e. 0200 or 2130. Midnight is recorded as 0000.

Weights should be recorded to the nearest 0.1 kg.

Source documents such as lab reports, ECG reports etc. should be filed separately from the CRF (if not in the medical notes) for each subject and be signed and dated by a delegated Investigator as proof of review of the assessment during the trial. Questionnaire should be considered as the CRF appendices (except standard approved questionnaire e.g. EQ-5D)

If a subject prematurely withdraws from the trial a single line must be drawn across each uncompleted page to correspond with the last visit of the subject as mentioned on the "Trial Completion" page.

The protocol deviation/violation/serious breach log should be used to record comments relating to each CRF visit that cannot be captured on the page itself. This includes reason for delayed or missed protocol visits or trial assessments, unscheduled visits etc.

The Chief Investigator (for lead site)/Principal Investigator is responsible for the accuracy of the data reported on the CRF. The CI/PI must sign and date the Principal Investigator's Sign Off page to certify accuracy, completeness and legibility of the data reported in the CRF.

Serious Adverse Events (SAEs)
SAEs should be faxed **within 24 hours** of the site being aware of the event using the trial specific SAE report form to [redacted] **or preferably emailed to sae@ucl.ac.uk**

Storage
CRF documents should be stored in a locked, secure area when not in use where confidentiality can be maintained. Ensure that they are stored separately to any other documents that might reveal the identity of the subject.

VISIT 1 (BASELINE) DEMOGRAPHIC DATA

Date of Assessment: __ __ / __ __ __ / __ __ __ __

(DD / MMM / YYYY)

Informed Consent:	
Date participant/ signed written consent form: __ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)	**Date of first trial-related procedure:** __ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Name of person taking informed consent: _____	

Demographic Data:

Date of Birth: __ __ / __ __ __ / __ __ __ __

(DD / MMM / YYYY)

Ethinicity:

White	White British ☐	White Irish ☐	White Other ☐	
Mixed race	White & Black Caribbean ☐	White & Black African ☐	White & Asian ☐	Other mixed background ☐
Asian or Asian British	Indian ☐	Bangladeshi ☐	Pakistani ☐	Other Asian background ☐
Black or Black British	Caribbean ☐	African ☐	Black Other ☐	
Chinese or other ethnicity	Chinese ☐	Other ☐ (please specify)		

Sex: ☐ Male ☐ Female

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 1 (BASELINE) INCLUSION CRITERIA

Date of Assessment: __ __ / __ __ __ / __ __ __ __

(DD / MMM / YYYY)

The following criteria MUST be answered YES for participant to be included in the trial (except where NA is appropriate):		**Yes**	**No**	**N/A**
1.	Step 1- 4 of the AREDS 9-step severity scale in at least one eye	☐	☐	☐
2.	Best corrected visual acuity in the study eye better than 50 ETDRS logMAR letters at baseline	☐	☐	☐
3.	They will require media clarity, pupillary dilation, and subject cooperation sufficient for adequate imaging and psychophysical testing	☐	☐	☐
4.	Ability to return for follow-up	☐	☐	☐
5.	Ability to fully consent to process	☐	☐	☐
6.	Manual and psychological dexterity to use the device in the correct manner	☐	☐	☐
If any of the above criteria is answered NO, the participant is NOT eligible for the trial and must not be included in the study. Please list reason(s) for ineligibility for screen failure on Participant Eligibility Review page.				

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 1 (BASELINE) EXCLUSION CRITERIA

Date of Assessment: __ __ / __ __ __ / __ __ __ __

(DD / MMM / YYYY)

The following criteria MUST be answered NO for the participant to be included in the trial:		**Yes**	**No**
1.	Co-existent ocular disease: Any ocular condition is present such that, in the opinion of the investigator, might affect the inflammatory status, visual acuity or cone function during the course of the study	☐	☐
2.	A substantial cataract that, in the opinion of the investigator, is likely to be decreasing visual acuity by 3 lines or more (e.g. cataract would be reducing acuity to 6/12 or worse if eye was otherwise normal) and early cataract that might be susceptible to growth under the influence of 670 nm light	☐	☐
3.	History of major ocular surgery (including cataract extraction, scleral buckle, any intraocular surgery, etc.) within prior 3 months or anticipated within the next 6 months following enrolment.	☐	☐
4.	Epilepsy	☐	☐
5.	Allergies to adhesives or any other component used.	☐	☐
If any of the above criteria is answered YES, the participant is NOT eligible for the trial and must not be included in the study. Please list reason(s) for ineligibility for screen failure on Participant Eligibility Review page.			

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 1 (BASELINE) PARTICIPANT ELIGIBILITY REVIEW

End of Visit 1/ Baseline Checklist:			
		Yes	No
1.	Does the participant satisfy the inclusion and exclusion criteria to date?	☐	☐
2.	Have all Screening Visit procedures been completed?	☐	☐
3.	Have the Medical History and Concomitant Medication pages been completed?	☐	☐
4.	Is the participant still willing to proceed in the trial?	☐	☐

Participant's eligibility Investigator Sign-Off:	
Is the participant eligible to take part in the Clinical Trial? **Investigator's Signature:** _____ **Date** : __ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY) **Investigator's Name:** _____	☐ Yes ☐ No, Please give reason for screen failure below
Reason(s) for screen failure:	
1.	
2.	
3.	

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 1 (BASELINE) MEDICAL HISTORY

Date of Assessment: __ __ / __ __ __ / __ __ __ __

(DD / MMM / YYYY)

Has the patient had any relevant medical history?	☐ **No** ☐ **Yes,** Complete below		
Condition / illness /surgical procedure	**Start date** (DD/MMM/YYYY)	**Stop date** (DD/MMM/YYYY)	**Or tick if ongoing at Screening Visit?**
	___/___/___	___/___/___	☐
	___/___/___	___/___/___	☐
	___/___/___	___/___/___	☐
	___/___/___	___/___/___	☐
	___/___/___	___/___/___	☐
	___/___/___	___/___/___	☐
	___/___/___	___/___/___	☐
	___/___/___	___/___/___	☐
	___/___/___	___/___/___	☐
	___/___/___	___/___/___	☐
	___/___/___	___/___/___	☐
	___/___/___	___/___/___	☐

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 1 (BASELINE) CONCOMITANT MEDICATIONS

Is the participant taken any concomitant medications at screening					☐ No ☐ Yes, Complete below		
Medication (Record Generic or trade name)	**Reason for use** (Medical History diagnosis or other reason, e.g. Prophylaxis)	Dose and units	**Freque -ncy**	**Route**	**Start Date** (DD/MMM/YYYY)	**Stop Date** (DD//MMM/YYY)	**Or tick if ongoing at Screening Visit**
1.					___/___/___	___/___/___	☐
2.					___/___/___	___/___/___	☐
3.					___/___/___	___/___/___	☐
4.					___/___/___	___/___/___	☐
5.					___/___/___	___/___/___	☐
6.					___/___/___	___/___/___	☐
7.					___/___/___	___/___/___	☐
8.					___/___/___	___/___/___	☐
9.					___/___/___	___/___/___	☐
10.					___/___/___	___/___/___	☐

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 1 (BASELINE) SMOKING / ALCOHOL STATUS

Date of Assessment: __ __ / __ __ __ / __ __ __ __

(DD / MMM / YYYY)

Has the participant ever smoked? ☐ **No** ☐ **Yes,** Complete below

☐	**Current Smoker**	**Participant's average daily use:** - Number of cigarettes : ___ ___ - Number of cigars : ____ ___ - Number of pipes : ___ ___ **Smoked for ___ ___ months/years**
☐	**Former smoker**	**Smoked for ___ ___ months/years** **Date when smoking ceased:** __ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY) **When smoking, participant's average daily use:** - Number of cigarettes : ___ ___ - Number of cigars : ____ ___ - Number of pipes : ___ ___

Participant's alcohol consumption

Participant's average consumption per week:
- Number of units of wine : ___ ___
- Number of units of beer : ____ ___
- Number of units of spirits : ___ ___

(see protocol for definition of units)

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 1 (BASELINE) OPHTHALMIC EXAM

Date of Assessment: __ __ / __ __ __ / __ __ __ __

(DD / MMM / YYYY)

Was Ophthalmic Examination performed? ☐ No ☐ Yes, Complete below				
	R	L	Not done	Comments
BCVA EDTRS (Letters)				
BCVA Low Luminance				
Slit lamp examination	R Lids/Lashes Conjunctiva Cornea Anterior chamber Lens C:D Vessels Macula Periphery			L
Cataract grading				
Dilation	Time Drug R or L			

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 1 (BASELINE) VITAL SIGNS

Were Vital Signs performed?	☐ **No (comment below)** ☐ **Yes,** Complete below Comment*: _____
Date of Vital Signs:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Time of Vital Signs:	_____:_____ **HH:MM**
Blood Pressure (sitting):	___ ___ ___ / ___ ___ ___ mmHg
Pulse:	____ ___ ___ beats/min
Weight: ___ ___ ___ . ___ kg	**Height:** ___ . ___ ___ m
Oral/ Tympanic Temperature:	___ ___ . ___ **°C**

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 1 (BASELINE) FUNDUS PHOTOGRAPHY

Fundus photography performed?	☐ **No (comment below)** ☐ **Yes,** Complete below Comment: _____
Date of photograph:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Time of photograph	_____:_____ HH:MM
Right or Left eye	

Location	**Results**
Disc	
Macula	
Periphery	
AREDS classification	
I	
II	
III	
IV	

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 1 (BASELINE) OCT

OCT performed?	☐ **No (comment below)** ☐ **Yes,** Complete below Comment: _____
Date of OCT:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Time of OCT:	_____:_____ HH:MM
Right or Left eye	

Location	Results
Disc	
Macula	
Periphery	

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

Sponsor No. 1 5 / 0 2 7 4

Subject No. | | | | | | |

Site: Moorfields Eye Hosp

VISIT 1 (BASELINE) PHOTORECEPTOR FUNCTION

Medmont	☐ **No (comment below)** ☐ **Yes,** Complete below Comment *: _____
Date of test:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Time of test	_____:_____ HH:MM
Right or Left eye	

Parameter	Absolute thresholds	Unit
Static thresholds		

Parameter	Time taken to recovery point	Unit
Dynamic test		

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

Sponsor No. 15/0274

Subject No. | | | | | | |

Site Moorfields Eye Hosp

VISIT 1 (BASELINE) PHOTORECEPTOR FUNCTION

AdaptDx	☐ **No (comment below)** ☐ **Yes,** Complete below Comment *: _____
Date of test:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Time of test	_____:_____ HH:MM
Right or Left eye	

Parameter	Time taken to recovery point	Unit
Dyanamic test		

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

Sponsor No. 1 5 / 0 2 7 4

Subject No. | | | | | | |

Site Moorfields Eye Hosp

VISIT 1 (BASELINE) PHOTORECEPTOR FUNCTION

RETEval	☐ **No (comment below)** ☐ **Yes,** Complete below Comment *: _____
Date of test:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Time of test	_____:_____ HH:MM
Right or Left eye	

Parameter	Unit	Result
Timing		
Amplitude		

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 1 (BASELINE) DEVICE ADIMINSTRATION

670 NM LIGHT DEVICE

Date of Dosing (DD/MMM/YYYY)	**Time of Dosing** (24 hr)	**Dose** (including units)	**Comment**
_____/_____/_____	_____ _____:_____ _____		

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

Sponsor No. 1 5 / 0 2 7 4

Subject No. | | | | | | |

Site Moorfields Eye Hosp

VISIT 2 (ONE MONTH): CHECKLIST

Date of Visit: __ __ / __ __ __ / __ __ __ __

(DD / MMM / YYYY)

Visit Checklist:

		Yes	No
1.	**Have there been any new Adverse Events?** (If yes, please record in Adverse Events page)	☐	☐
2.	**Have there been any changes in Concomitant Medications?** (If yes, please record in Concomitant Medications Log)	☐	☐
3.	**Have there been any changes in Health?**	☐	☐
4.	**Have there been any other changes?**	☐	☐
5.	**Compliance diary noted**	☐	☐

Notes	

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 2 (ONE MONTH): OPHTHALMIC EXAM

Date of Assessment: __ __ / __ __ __ / __ __ __ __

Was Ophthalmic Examination performed? ☐ No ☐ Yes, Complete below				
	R	L	Not done	Comments
BCVA EDTRS (Letters)				
BCVA Low Luminance				
Slit lamp examination	R Lids/Lashes Conjunctiva Cornea Anterior chamber Lens C:D Vessels Macula Periphery			L
Cataract grading				
Dilation	Time Drug R or L			

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 2 (ONE MONTH): FUNDUS PHOTOGRAPHY

Fundus photography performed?	☐ **No (comment below)** ☐ **Yes,** Complete below Comment: _____
Date of photograph:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Time of photograph	_____:_____ HH:MM
Right or Left eye	

Location	**Results**
Disc	
Macula	
Periphery	
AREDS classification	
I	
II	
III	
IV	

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 2 (ONE MONTH): OCT

OCT performed?	☐ **No (comment below)** ☐ **Yes,** Complete below Comment: _____
Date of OCT:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Time of OCT:	_____:_____ HH:MM
Right or Left eye	

Location	**Results**
Disc	
Macula	
Periphery	

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 2 (ONE MONTH): PHOTORECEPTOR FUNCTION

Medmont	☐ **No (comment below)** ☐ **Yes,** Complete below Comment *: _____
Date of test:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Time of test	_____:_____ HH:MM
Right or Left eye	

Parameter	Absolute thresholds	Unit
Static thresholds		

Parameter	Time taken to recovery point	Unit
Dynamic test		

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

Sponsor No. 1 5 / 0 2 7 4

Subject No. | | | | | | |

Site Moorfields Eye Hosp

VISIT 2 (ONE MONTH):) PHOTORECEPTOR FUNCTION

AdaptDx	☐ **No (comment below)** ☐ **Yes,** Complete below Comment *: _____
Date of test:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Time of test	_____:_____ HH:MM
Right or Left eye	

Parameter	Time taken to recovery point	Unit
Dynamic test		

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 2 (ONE MONTH): PHOTORECEPTOR FUNCTION

RETEval	☐ **No (comment below)** ☐ **Yes,** Complete below Comment *: _____
Date of test:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Time of test	_____:_____ HH:MM
Right or Left eye	

Parameter	Unit	Result
Timing		
Amplitude		

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 3 (THREE MONTH): CHECKLIST

Date of Visit: __ __ / __ __ __ / __ __ __ __

(DD / MMM / YYYY)

Visit Checklist:

		Yes	No
1.	**Have there been any new Adverse Events?** (If yes, please record in Adverse Events page)	☐	☐
2.	**Have there been any changes in Concomitant Medications?** (If yes, please record in Concomitant Medications Log)	☐	☐
3.	**Have there been any changes in Health?**	☐	☐
5.	**Compliance diary noted**	☐	☐

Notes	

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 3 (THREE MONTH): OPHTHALMIC EXAM

Date of Assessment: __ __ / __ __ __ / __ __ __ __

Was Ophthalmic Examination performed? ☐ No ☐ Yes, Complete below				
	R	L	Not done	Comments
BCVA EDTRS (Letters)				
BCVA Low Luminance				
Slit lamp examination	R Lids/Lashes Conjunctiva Cornea Anterior chamber Lens C:D Vessels Macula Periphery			L
Cataract grading				
Dilation	Time Drug R or L			

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 3 (THREE MONTH): FUNDUS PHOTOGRAPHY

Fundus photography performed?	☐ **No (comment below)** ☐ **Yes,** Complete below Comment: _____
Date of photograph:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Time of photograph	_____:_____ HH:MM
Right or Left eye	

Location	**Results**
Disc	
Macula	
Periphery	
AREDS classification	
I	
II	
III	
IV	

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 3 (THREE MONTH): OCT

OCT performed?	☐ **No (comment below)** ☐ **Yes,** Complete below Comment: _____
Date of OCT:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Time of OCT:	_____:_____ HH:MM
Right or Left eye	

Location	**Results**
Disc	
Macula	
Periphery	

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 3 (THREE MONTH): PHOTORECEPTOR FUNCTION

Medmont	☐ **No (comment below)** ☐ **Yes,** Complete below Comment *: _____
Date of test:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Time of test	_____:_____ HH:MM
Right or Left eye	

Parameter	Absolute thresholds	Unit
Static thresholds		

Parameter	Time taken to recovery point	Unit
Dyanmic test		

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 3 (THREE MONTH): PHOTORECEPTOR FUNCTION

AdaptDx	☐ **No (comment below)** ☐ **Yes,** Complete below Comment *: _____
Date of test:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Time of test	_____:_____ HH:MM
Right or Left eye	

Parameter	Time taken to recovery point	Unit
Dyanmic test		

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 3 (THREE MONTH): PHOTORECEPTOR FUNCTION

RETEval	☐ **No (comment below)** ☐ **Yes,** Complete below Comment *: _____
Date of test:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Time of test	_____:_____ HH:MM
Right or Left eye	

Parameter	Unit	Result
Timing		
Amplitude		

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

Sponsor No. 1 5 / 0 2 7 4

Subject No. | | | | | | |

Site Moorfields Eye Hosp

VISIT 4 (SIX MONTH): CHECKLIST

Date of Visit: __ __ / __ __ __ / __ __ __ __

(DD / MMM / YYYY)

Visit Checklist:

		Yes	No
1.	**Have there been any new Adverse Events?** (If yes, please record in Adverse Events page)	☐	☐
2.	**Have there been any changes in Concomitant Medications?** (If yes, please record in Concomitant Medications Log)	☐	☐
3.	**Have there been any changes in Health?**	☐	☐
5.	**Compliance diary noted**	☐	☐

Notes	

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 4 (SIX MONTH): OPHTHALMIC EXAM

Date of Assessment: __ __ / __ __ __ / __ __ __ __

Was Ophthalmic Examination performed? ☐ No ☐ Yes, Complete below				
	R	L	Not done	Comments
BCVA EDTRS (Letters)				
BCVA Low Luminance				
Slit lamp examination	R Lids/Lashes Conjunctiva Cornea Anterior chamber Lens C:D Vessels Macula Periphery			L
Cataract grading				
Dilation	Time Drug R or L			

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 4 (SIX MONTH): FUNDUS PHOTOGRAPHY

Fundus photography performed?	☐ **No (comment below)** ☐ **Yes,** Complete below Comment: _____
Date of photograph:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Time of photograph	_____:_____ HH:MM
Right or Left eye	

Location	**Results**
Disc	
Macula	
Periphery	
AREDS classification	
I	
II	
III	
IV	

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 4 (SIX MONTH): OCT

OCT performed?	☐ **No (comment below)** ☐ **Yes,** Complete below Comment: _____
Date of OCT:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Time of OCT:	_____:_____ HH:MM
Right or Left eye	

Location	**Results**
Disc	
Macula	
Periphery	

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 4 (SIX MONTH): PHOTORECEPTOR FUNCTION

Medmont	☐ **No (comment below)** ☐ **Yes,** Complete below Comment *: _____
Date of test:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Time of test	_____:_____ HH:MM
Right or Left eye	

Parameter	Absolute thresholds	Unit
Static thresholds		

Parameter	Time taken to recovery point	Unit
Dyanmic test		

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 4 (SIX MONTH): PHOTORECEPTOR FUNCTION

AdaptDx	☐ **No (comment below)** ☐ **Yes,** Complete below Comment *: _____
Date of test:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Time of test	_____:_____ HH:MM
Right or Left eye	

Parameter	Time taken to recovery point	Unit
Dyanmic test		

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 4 (SIX MONTH): PHOTORECEPTOR FUNCTION

RETEval	☐ **No (comment below)** ☐ **Yes,** Complete below Comment *: _____
Date of test:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Time of test	_____:_____ HH:MM
Right or Left eye	

Parameter	Unit	Result
Timing		
Amplitude		

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

Sponsor No. | 1 | 5 | / | 0 | 2 | 7 | 4 |

Subject No. | | | | | | | |

Site: Moorfields Eye Hosp

VISIT 5 (ONE YEAR): CHECKLIST

Date of Visit: __ __ / __ __ __ / __ __ __ __

(DD / MMM / YYYY)

Visit Checklist:

		Yes	No
1.	**Have there been any new Adverse Events?** (If yes, please record in Adverse Events page)	☐	☐
2.	**Have there been any changes in Concomitant Medications?** (If yes, please record in Concomitant Medications Log)	☐	☐
3.	**Have there been any changes in Health?**	☐	☐
5.	**Compliance diary noted**	☐	☐

Notes	

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 5 (ONE YEAR): OPHTHALMIC EXAM

Date of Assessment: __ __ / __ __ __ / __ __ __ __

Was Ophthalmic Examination performed? ☐ No ☐ Yes, Complete below				
	R	L	Not done	Comments
BCVA EDTRS (Letters)				
BCVA Low Luminance				
Slit lamp examination	R L Lids/Lashes Conjunctiva Cornea Anterior chamber Lens C:D Vessels Macula Periphery			
Cataract grading				
Dilation	Time Drug R or L			

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

Sponsor No. 1 5 / 0 2 7 4

Subject No. | | | | | | |

Site Moorfields Eye Hosp

VISIT 5 (ONE YEAR): FUNDUS PHOTOGRAPHY

Fundus photography performed?	☐ **No (comment below)** ☐ **Yes,** Complete below Comment: _____
Date of photograph:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Time of photograph	_____:_____ HH:MM
Right or Left eye	

Location	**Results**
Disc	
Macula	
Periphery	
AREDS classification	
I	
II	
III	
IV	

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

Sponsor No. 1 5 / 0 2 7 4

Subject No. | | | | | | |

Site: Moorfields Eye Hosp

VISIT 5 (ONE YEAR): OCT

OCT performed?	☐ **No (comment below)** ☐ **Yes,** Complete below Comment: _____
Date of OCT:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Time of OCT:	_____:_____ HH:MM
Right or Left eye	

Location	**Results**
Disc	
Macula	
Periphery	

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 5 (ONE YEAR): PHOTORECEPTOR FUNCTION

Medmont	☐ **No (comment below)** ☐ **Yes,** Complete below Comment *: _____
Date of test:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Time of test	_____:_____ HH:MM
Right or Left eye	

Parameter	Absolute thresholds	Unit
Static thresholds		

Parameter	Time taken to recovery point	Unit
Dyanmic test		

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

Sponsor No. 1 5 / 0 2 7 4

Subject No. | | | | | | |

Site Moorfields Eye Hosp

VISIT 5 (ONE YEAR): PHOTORECEPTOR FUNCTION

AdaptDx	☐ **No (comment below)** ☐ **Yes,** Complete below Comment *: _____
Date of test:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Time of test	_____:_____ HH:MM
Right or Left eye	

Parameter	Time taken to recovery point	Unit
Dyanmic test		

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

VISIT 5 (ONE YEAR): PHOTORECEPTOR FUNCTION

RETEval	☐ **No (comment below)** ☐ **Yes,** Complete below Comment *: _____
Date of test:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)
Time of test	_____:_____ HH:MM
Right or Left eye	

Parameter	Unit	Result
Timing		
Amplitude		

Completed by: _____
Name Signature Date

* Record the comment on the protocol deviation/violation log.

TRIAL COMPLETION

Did participant complete the trial?	☐ **Yes,** Please provide **date of last visit**: __ __ / __ __ __ / 2 0 __ __ (DD / MMM / YYYY) ☐ **No,** Please provide **date of withdrawal** and complete below: __ __ / __ __ __ / 2 0 __ __ (DD / MMM / YYYY)

Early Withdrawal: please tick most appropriate reason for participant not completing the trial:

☐ **Adverse Events related:** please state related AE: _____ (add details to AE page)

☐ **Participant's decision, specify: _____**

☐ **Investigator's decision, specify: _____**

☐ **Sponsor's decision**

☐ **Lost to follow up**

☐ **Patient deceased**

☐ **Other, specify:** _____

Completed by: _____

Name Signature Date

* Record the comment on the protocol deviation/violation log.

670 nm

Sponsor No. | 1 | 5 | / | 0 | 2 | 7 | 4 |

Subject No. | | | | | | | |

Site Moorfields Eye Hosp

ADVERSE EVENTS PAGE

AE No	Event Name (Please give Diagnosis if known)	Start date (DD/MMM/YYYY)	Stop date (DD/MMM/YYYY)	Serious? If serious, please complete a JRO SAE form	Con-comitant Medication given	Severity 0 - Mild 1- Mod e-rat e 2 - Sever e	Study Drug Action 0 - None 1 - Temporarily Interrupted 2 - permanently withdrawn	Outcome 0 - Resolved 1- Resolved with sequelea 2 - Not resolved	Relationship to Study Drug 0 - Definitely 1 - Probably 2 - Possibly 3 - Unlikely 4 - Not related 5 - Not assessable
1		___/___/___	___/___/___	☐ No ☐ Yes	☐ No ☐ Yes				
2		___/___/___	___/___/___	☐ No ☐ Yes	☐ No ☐ Yes				
3		___/___/___	___/___/___	☐ No ☐ Yes	☐ No ☐ Yes				
4		___/___/___	___/___/___	☐ No ☐ Yes	☐ No ☐ Yes				
5		___/___/___	___/___/___	☐ No ☐ Yes	☐ No ☐ Yes				
6		/ /___	/ /___	☐ No ☐ Yes	☐ No ☐ Yes				

I have reviewed the AEs on this page and have assessed them for seriousness, causality, severity and outcome and confirm that, to the best of my knowledge, it accurately reflects the study information obtained for this participant

PI signature _____ _____ **Date:** _____ ☐

Please check box if this is the last page used

670 nm

Sponsor No. 15/0274

Subject No. | | | | | | |

Site Moorfields Eye Hosp

PRINICIPAL INVESTIGATOR'S SIGN OFF

Principal Investigator's Signature Statement:	
I have reviewed this CRF and confirm that, to the best of my knowledge, it accurately reflects the study information obtained for this participant. All entries were made either by me or by a person under my supervision who has signed the Delegation and Signature Log.	
Principal Investigator's Signature: _____ **Principal Investigator's Name:** _____	**Date of Signature:** __ __/ __ __ __/ __ __ __ __ (DD / MMM / YYYY)

ONCE SIGNED, NO FURTHER CHANGES CAN BE MADE TO THIS CRF WITHOUT A SIGNED DATA QUERY FORM.

Moorfields Eye Hospital NHS
NHS Foundation Trust

Patient Information Sheet:

Structure Function Correlation in aging and age related macular degeneration

(FUSCHIA STUDY)

Version 4.0 dated 23 August 2016

Principal investigator:

Professor Sobha Sivaprasad

Address: Moorfields Eye Hospital, 162, City Road, London, EC1V 2PD

Telephone: [redacted],

Fax: [redacted]

Email: [redacted]

Introduction

We would like to invite you to take part in our research study that will observe changes in the eye in patients who are above 55 years with or without signs of Age Related Macular Degeneration (AMD)

Before you decide we would like you to understand why the research is being done and what it would involve for you. Please take the time to read the following information. Talk to your friends, family or GP about the study if you wish. One of our team will go through the information sheet with you and answer any questions you have. Ask us if there is anything that is not clear or you require any further information.

Take time to decide whether or not you wish to take part.

What is AMD?

The Macula is a small area of the retina (a layer of light sensitive nerve cells that line the back of your eye) When you look straight ahead what you see is being detected by your macula. It is used for fine detailed central vision that you use to read, watch TV and recognise faces.

Age Related Macular Degeneration or AMD occurs when the cells in the retina stop working so well. There are two types of AMD, 'wet' and 'dry'.

Dry AMD

Dry AMD develops when macular cells become damaged due to a build-up of waste material. This waste looks like yellow spots on the back of the eye called and are called 'drusen' This is the most common type and occurs in 9 out of 10 people and can take a long time for vision to be seriously affected.

Drusen can occur as part of the normal aging process. You can have very small drusen which does not affect vision. However if the drusen get bigger it can indicate early AMD.

Wet AMD

Wet AMD develops when abnormal blood vessels grow from underneath the macula and damage the cells. It can cause severe vision loss over a short period of time. This is sometimes known as late AMD

What is the purpose of this study?

We are conducting a long-term study to observe and record information about the changes that occur in the retina in ageing and in patients that have a genetic make-up or changes in the eye at risk of AMD.

We would like to find out more about how aging and genetic make-up affect the retina and how visual changes occur. To do this we want to collect medical histories, eye exam data, and genetic information that may be associated with AMD.

Why Have I been invited to take part?

You have been asked to take part this study because you are aged above 55 years or you have early signs of AMD in at least one eye or because one of your relatives have AMD. About 50 participants will be taking part.

Do I have to take part?

No, it is up to you to join the study. We will describe the study and go through this information sheet. If you agree to take part, we will ask you to sign a consent form. You can choose to leave the study at any time without giving a reason. Your future care will not be affected.

What will happen to me if I take part?

If you decide to take part you will be asked to sign a consent form. You will be in the study for 3 years and will be asked to come for tests once a year. The tests may be done over two days.

What tests are done?

At your first visit, an eye researcher will do the following test to see if you are suitable for the study and you have consented to the study:

1. You will be asked questions about your medical history and eyesight history

2. You will have your blood pressure height and weight measurements recorded.

3. Visual acuity test: This tests how clearly you can see different sized letters on a chart with each of your eyes.

4. Low lighting visual acuity: visual acuity test will be repeated holding a filter in front of each eye to determine how clearly you can see in low light conditions

5. Visual field tests in light and dark: For these tests you will put your chin on a rest and look into a large bowl. One eye will be covered with a patch, you should press a button when you see lights flashing on and off in the bowl. This test will be repeated in light and dark and may take up to 45 minutes.

   For the next tests drops will be put into your eyes to make the pupils larger. You might find that bright lights may hurt your eyes for 4-6 hours after the drops, sunglasses will help

6. Eye examination: An eye doctor will examine the back of your eyes using a bright light. This will take about 5 minutes. This will include testing your eye function during flickering light.

7. Colour photographs will be taken of the retina in each eye, you will notice a bright flash after each of the photos is taken, this will not have any long term effect on your eyes; this will take about 10 minutes. Detailed imaging of the eye will include infrared imaging, pigment distribution imaging and these tests will take a further 30 minutes. These will also use bright flash but these tests do not have any long term effects on your eyes.

8. Optical Coherence Tomography (OCT): this test is similar to an ultrasound for your eye it allows us to take several different types of pictures of the back of

your eye. The test is quick and painless, for the test you will sit in front of a machine and a light beam will scan the retina in each eye this test lasts about 10 minutes

9. Quality of life questionnaires: This is a questionnaire about your vision condition and how you feel about your vision in the light and dark.

10. A sputum sample will be taken for DNA analysis. The anonymised samples will be sent away to other labs in England for genetic analysis or stored for 5 years in UCL labs for future analysis. We would like to share the DNA data with other researchers working on the same disease area. The DNA analysis is done only for the purpose of genetic testing and the results would not be used for diagnostic purpose.

11. Optional tests: If you are willing to participate in more detailed visual function tests, you will be invited for another visit where we will do detailed tests by placing you in a dark room and then testing how your eyes will adapt to change in light and dark. This can take up to 4 hours with breaks in between where your eyes will be patched alternatively to adapt to dark.

What happens next?

Everyone who agrees to take part in the study will have be tested with tests 1-10 listed above. The pictures of your eyes are will be analysed and compared to your visual function to understand AMD progression. If you are enrolled into the study you will be asked to return in 12 months to repeat these tests.

Annual visits:

Below is a timetable detailing which tests are carried out at each visit:

	Baseline	12 months	24 months	36 months
Study visit date	x	x	x	x
Demographics BP, height, weight, smoking and	x			

medical history				
BCVA (ETDRS Letters)	x	x	x	x
BCVA (LLQ and SKILL score)	x	x	x	x
Dark adaptation AdaptDX Medmont	x	x	x	x
Microperimetry Mesopic and scotopic	x	x	x	x
AVOT tests (optional)	x/-	x/-	x/-	x/-
Cone temporal contrast sensitivity (optional)	x/-	x/-	x/-	x/-
Adaptive Optics (optional)	x/-	x/-	x/-	x/-
Sputum sample	x			
RETval 30Hz	x	x	x	x
Electrophysiology (optional)	x/-	x/-	x/-	x/-
Colour photographs, infrared and qualtitative and quantitative autofluorescence of central retina and macular pigment optical density	x	x	x	x
OCT	x	x	x	x
OCT A (optional)	x/-	x/-	x/-	x/-
Optos wide-angle photographs	x	x	x	x
LLQ questionnaire	x	x	x	x
Withdrawal dates and reasons if any	x	x	x	x

How long to visits take?

It is hard to specify how long you will be in the eye clinic for but you should allow 4 hours for all the mandated tests to be completed. You are allowed to eat and drink during the waiting time. If you opt for more tests, we will do the tests over 2 days.

Will my taking part in this study be kept confidential?

If you choose to take part, some parts of your medical records and data collected for the study will be looked at by authorised persons in the Sponsor institution. Your

data will also be checked by authorised people who check that the study is being carried out correctly. Everyone involved in this study will have a duty of confidentiality to you as a research participant and we will ensure that this is followed. The results of the study may be published, or made available to other researchers for scientific purposes, however your identity will not be revealed.

What will happen to any samples I give?

Sputum samples will analysed to study DNA. Samples may be stored for future analysis. The DNA analysis would be for the purpose of genetic testing only and the results would not be used for diagnostic purposes.

Are there any benefits to me if I participate in the study?

This study is being carried to observe changes in the eye only. The information we get from this study may help to improve future treatment of people with Age Related Macular Degeneration.

Will I receive re-imbursement?

You will not receive any monetary compensation for taking part in the study, however reasonable travel expenses may be paid, where applicable. Your willingness to take part, however, may in the future help doctors better understand and/or treat other who have your condition.

What If I choose not to take part in the study?

If you do not want to take part in the study, you will receive standard care as decided by your doctor. Your participation in this study is voluntary and you may withdraw from the study at any time without your future medical care being affected.
You will be informed of any significant information that may develop during the study that may relate to your participation as a patient.

Can I withdraw from the research study once I have enrolled?

If you no longer wish to take part in the study, for any reason, please contact the study doctor immediately. If at any time during the study you or your doctor feels that it is in your best interests to withdraw from the study, you may do so without any penalty or loss of benefits to which you are otherwise entitled to at this hospital

including present and future standard care. You will be asked to return for a final visit. The procedures performed will be the same as those scheduled for the final visit in the study.

What happens if I am harmed in any way?

In the event that something goes wrong or you are harmed during the research, you will have all the rights and protection that you normally have as an NHS patient. There are no special compensation arrangements for study participants. If you are harmed due to someone's negligence then you may have grounds for legal action, but you may have to pay for it.

If you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal NHS complaints procedures are available to you:

In the first instance please contact the Patient Advice and Liaison Service (PALS) on [redacted]

I have some questions, who can I ask?

For further general information about the study, please contact your study doctor whose contact details are placed below. For general information about research and your rights as a research participant please contact PALS on [redacted].

For further general information on the study please contact your study researcher (during office hours)

Principal Investigator (study doctor) Prof Sobha Sivaprasad Tel: [redacted]

After normal working hours you may contact the on call ophthalmologist on [redacted]

The researchers conducting the research are not being paid for recruiting the patients in the study nor for looking after them. They have no conflicts of interest.

Thank you for reading this information and considering taking part in the study

Moorfields Eye Hospital NHS
NHS Foundation Trust

Information Sheet

You will be given a copy of this information sheet

Study title

A pilot study to investigate the effect of 670nm light on visual function in aging and age related macular degeneration (student study)

Chief Investigator

Professor Sobha Sivaprasad
Address: Moorfields Eye Hospital
Telephone: [redacted]
Email: [redacted]

Introduction

Please read this patient information sheet carefully. If you are unable to see the text, or if there is anything you do not understand, please ask a member of the study team for their help.

You are being invited to take part in a research study. Before you decide if you want to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with your friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

What is the purpose of the study?

The retina is the name of a thin layer of cells that line the back of the eye which are responsible for sight. The retina is not the same “all over”. In the centre there is a special part called the macula. When you look “straight ahead”, what you are looking at is detected by the macula. The macula is the only part of the retina that is specialised for detailed vision, such as reading, watching TV or recognising faces. So if the macula is damaged, there is a very great loss of vision.

The retina changes as we age and is the site of many conditions, such as Age-Related Macular Degeneration (AMD). We know that as we grow older, the cells (photoreceptors) in the retina start to decline and this decline is likely to be related to the development of AMD. AMD is the commonest cause of blindness in the elderly in the developed world. There are two types: dry and wet. The dry type progresses to the wet type in 10 to 15% of patients. Unfortunately, there are no known treatment for the dry type, as yet.

We are conducting a pilot study to assess whether a device which shines a particular wave-length of light (670 nm, which is in the red region of vision) might improve how these cells work and reduce the inflammation associated with aging and AMD. We think that this is the case because we know that 670 nm light has done this in preliminary studies.

We hope that we will be able to use this to slow-down the aging process in the eye and therefore prevent the likelihood of developing AMD and slow down its progression.

Part of the results used in this study may used as part of a postgraduate research degree (PhD) awarded by University College London (UCL).

Why have I been invited?

You are being invited to take part in this research study because you are over the age of 50 and may have early signs of AMD in at least one eye. 40 patients will be taking part.

Is there any reason I cannot take part?

You cannot take part if you have other some eye conditions (such as diabetic retinopathy or glaucoma, have severe cataracts, have had eye surgery in the last three months or in the next 6 months, epilepsy or allergies to adhesives or other components used.

Do I have to take part?

No. Your participation in the study is voluntary. You may choose not to take part in this study or you may leave the study at any time without giving a reason. Your future treatment and care will not be affected.

What will happen to me if I take part?

If you take part, you will be asked to sign a consent form, and you will be in the study for 12 months. We are asking you to commit to holding this light source to one eye for two minutes every day for 12 months. The light is less strong than being outside on a sunny day. You will come to Moorfields Eye Hospital/ The Institute of Ophthalmology for 5 visits in total. We may need to contact you after the trial.

It is important that you are able to set aside enough time for each visit. We advise that this might take 3 hours.

You will be shown how to use the device, you will be given a device to take home and you will be given an instruction leaflet.

What types of tests are done?

At your first visit, an ophthalmologist (eye doctor) will do the following tests to see if you are suitable for the study:

1) You will be asked questions about your medical history, your eyesight history and about any medications you are taking.

2) Visual acuity test: this tests how clearly you can see different sized letters on a chart with both eyes, in normal light and the dark.

3) Eye Examination: You will be given eye drops before the test to dilate your pupils (make your pupils bigger). You may find bright lights hurt your eyes for 4-6 hours after this test but sunglasses will help. You must not drive until the effects of the eye drops have worn off. We advise you not to drive to you appointment at the hospital.

4) Colour photographs will be taken of the retina in each eye. You will notice a bright flash after each photo is taken, but this will not have any long-term effect on your eye.

5) Optical Coherence Tomography (OCT) and autofluorescence: this test is like an ultrasound for your eye. It lets us take pictures of the back of your eye. The test is quick and painless. For the test, you will sit in front of a machine and a light beam

will scan the retina in each eye.

6) Dark Adaptation: This tests how quickly your eyes recover from a flash. During this test, you will sit in a dark room and look into a machine. There will be a bright flash of light and you will be asked to press a button when you see a coloured light.

7) Scotopic thresholds: This tests how well you can see in the dark. During this test, you will sit in a dark room and look into a machine. There will be a series of lights and you will be asked to press a button when you see a light.

8) 30 Hz Flicker ERG: This is a test of the electrical activity of one type of cell in your retina called cones. These are affected in macular degeneration. An electrode is placed under your eye and you will be asked to look at a flashing light. A recording is taken.

In addition to the tests mentioned above, we are trying to do further tests that will tell us more about the effect of the light on the eye. You can take part in these tests if you want.

Optional tests for those who agree to participate:

9) Psychophysical test: This is a more detailed version of the dark adaptation test. You sit in front of the apparatus and your head will be supported. You will be asked to respond to a series of lights by pressing a button. This test takes about 45 minutes.

What happens next?

Everyone who agrees to participate in the trial will be tested with tests 1-7 listed above. you are eligible for the trial, you will be provided with a light device.

What types of tests are done at other visits?

You will then be seen at 1 month, 3 months, 6 months and 12 months.

1) You will be asked questions about how you have been since your last visit and about any changes to the medications you are taking since you were last seen, and whether you have used the light.

2) Visual acuity to check the vision in both eyes (as above).

3) Eye examination (as above).

4) Photoreceptor function (as above).

5) 30 Hz Flicker (as above).

6) OCT and fundus photograph (as above)

What other treatments are there for your current condition?

There is no known treatment for early dry AMD. A large study known as the AREDs trial revealed a beneficial effect of very high doses of antioxidants (vitamin C, beta-carotene, zinc, copper) in reducing patients relative risk of progression to advanced AMD by 25%.

What is the device being tested?

The device is a small hand held light source emitting diffuse red / near infrared light. It is designed to improve health and reduce inflammation in the retina.

Information on how to use the 670nm LED device

You will be given a pack consisting of:
The light source
Batteries
A timer
Alcohol wipes for cleaning

The Light Source
The light source consists of 1 part. It is battery operated.

One end is the button to switch the light on, the other is the light source.

The light source is labelled R (right) or L (left) for the correct eye.

When the batteries finish or if the light is dimming, please contact the study investigators who will replace them for free.

What do I have to do?

You need to hold the light up to your eye, as above, for two minutes every day, for one year.

Please keep a careful record of when you use the light in the diary card provided.

Safety/ is the treatment completely safe?

The light does not contain any harmful wavelengths of light such as ultraviolet rays and is less strong than being outside on a sunny day. No ill-effects have been reported in any animal or human studies.

Safety/ what should I be aware of and worried about?

Compliance of using this device is crucial to the success of the study. Please keep all your appointments.

We are not aware of any side effects from exposing this light for only 2 minutes every day.

If when you use the light, you find the light uncomfortable, you may close your eyes

You may notice a brief after image for a few minutes, which is to be expected and is harmless.

Please let us know if you have epilepsy, particularly photosensitive epilepsy, as you will not be able to go through the tests required in this study.

You cannot take part if you have other some eye conditions (such as diabetic retinopathy or glaucoma, have severe cataracts, have had eye surgery in the last three months or in the next 6 months, epilepsy or allergies to adhesives or other components used.

How effective may it be?

The reason we are doing this study is to find out exactly how effective the device might be. Previous small studies in AMD and a study in diabetic eye disease showed improvement of the condition.

Are there any benefits to me if I participate in this study?

We believe that this treatment may reduce the rate of progression of aging changes in the eye and may improve visual function.

That is why we want to test the device. However this cannot be guaranteed.

The information we get from this study may also help us understand aging of the retina and help us develop other new treatment options for early AMD.

What if I choose not to take part in the study?

If you do not want to take part in this study, you will receive standard care as decided by your doctor. Your participation in this study is voluntary and you may withdraw from the study at any time without your future medical care being affected. If you decide to withdraw from the study for any reason, please contact your study doctor. You will be informed of any significant information that may develop during the research study
that may relate to your willingness to continue in participation as a patient.

Can I withdraw from the trial if I enter it?

If at any time during the study either you or your doctor feels that it is in your best interest to withdraw from this study, you may do so without any penalty or loss of benefits to which you are otherwise entitled to at this hospital, including the present and future standard care. Your data will be withdrawn from the study.

What happens when the research study stops?

When the research ends, you will be followed up as usual. You may keep the device if it is effective. We will follow you up in clinic and provide treatment with the best available standard care.

Who will know my results?

If you join the study, some parts of your medical records and the data collected for the study will be looked at by authorised people from the University sponsoring the research.

Some data will need to be kept for up to 5 years, as per regulations. Everyone will have a duty of confidentiality to you as a research participant and we will do our best to meet this. Recordings will be kept separately from personal information. The results of the research may be published for scientific purposes; however, your identity will not be revealed.

Will my GP know about the study?

Your GP will be informed that you are participating in the study and s/he will be kept informed of your medical progress.

Will the study cost me anything?

You will not be asked to pay for any costs associated with the study protocol or follow-up visits.

Will I receive reimbursement?

You will not receive any monetary compensation for taking part in the study.

Your willingness to take part, however, may, in the future, help doctors better understand and/or treat others who have your condition.

Travel expenses up to £20 may be reimbursed for each visit.

What happens if I am harmed in any way?

If any harm occurs while you are taking part in this research project, you will have all the rights and protection that you normally have as an NHS patient. There are no special compensation arrangements for study participants. If you are harmed due to someone's negligence, then you may have grounds for a legal action, but you may have to pay for it.

If you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints procedures are available to you.

You can contact the patient advice and liaison service (PALS) at Moorfields Eye Hospital. They can be telephoned [redacted] emailed at pals@moorfields.nhs.uk or written to: Moorfields Eye Hospital NHS Foundation Trust 162

City Road London EC1V 2PD

Who has reviewed the study?

The study has been approved by Stanmore NHS ethics committee.

Who is organising and funding the research?

The research is sponsored University College London and the research is funded by Fight For Sight and Moorfields Eye Charity. The doctors conducting the research are not being paid for recruiting the patients in the study, nor for looking after them, and they have no conflicts of interest.

I have some questions, who can I ask?

For further general information on the study, please contact your study doctor whose contact details are at the beginning of this participant information sheet.

For general information about research or if you have any questions about your rights as a research subject, please contact the hospital's Patient Advice & Liaison Service (PALS) department. Their contact details are at the beginning of this participant information sheet

Who do I contact if there is a problem?

If you believe that you are hurt or if you get sick because of something that is done during the study, you should contact your study doctor whose contact details are at the beginning of this participant information sheet.

If you choose to participate, you will be given a consent form to sign. By signing the consent form, you have not waived any of your legal rights. You will receive a copy of this patient information and the signed consent form that will show all signatures and dates.

Thank you for reading this information and considering taking part in the study.

Contact for queries

For further general information on the study, please contact your study doctor (during office

hours).

Principal Investigator (study doctor): Dr Chrishne Siva Tel: [redacted]

During normal working hours you may contact Dr Chrishne [redacted] In an emergency please contact Moorfields Eye Hospital A&E on [redacted].

For general information about research or If you have any questions about your rights as a research subject, please contact the hospital's Patient Advice & Liaison Service (PALS) department. Their contact details are: [redacted]