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To Investigate the Foveal Avascular Zone in a Young Healthy Population Using Optical Coherence Tomography Angiography

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TO INVESTIGATE THE FOVEAL AVASCULAR ZONE IN A YOUNG HEALTHY POPULATION USING OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY

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MPhil Thesis

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Abstract

Introduction/Aims: Inflammatory diseases such as diabetes, glaucoma and age-related macular degeneration (AMD) can alter the size and shape of the foveal avascular zone (FAZ). Macular pigment (MP), a powerful antioxidant, located at the macula can protect the eye from oxidative stress damage. This study aims to investigate possible factors affecting the FAZ, such as vessel perfusion and overweight/obesity, in association with MP status in a young, healthy population. Normative values for FAZ size/shape and vascularity will also be proposed.

Methods: One hundred and fifty-four subjects aged 18-35 years old were recruited. Superficial FAZ area, diameter, ganglion cell layer, central macular thickness (CMT), vascular perfusion and density were measured by Optical Coherence Tomography Angiography (OCTA). FAZ area/vascularity were assessed in relation to body mass index (BMI), trunk fat % and macular pigment optical density (MPOD).

Results: Mean FAZ area was 0.22±0.07millimetres squared (mm^2). Reduced vessel perfusion central (≤ 24%), low MPOD (≤ 0.4optical density units (OD)) and high BMI (> 25kilograms (kg)/metre (m^2)) were associated with a larger FAZ area on multivariate analysis. Age, vessel perfusion and CMT were all negative predictors of FAZ area. Trunk fat % was a positive predictor of FAZ area ($p = 0.03$) while BMI was positively correlated with FAZ area, (Pearson's r = 0.18, $p = 0.03$).

Conclusions: Optical Coherence Tomography Angiography has potential as a screening tool aiding in the earlier detection and monitoring of eye diseases associated with oxidative stress i.e., hypertensive and diabetic retinopathy (DR), glaucoma and AMD. FAZ size in association with MPOD assessment, may be useful in detecting and advising patients at risk of retinal oxidative stress damage.

Declaration

I certify that this thesis which I now submit for examination for the award of Master of Philosophy, is entirely my own work and has not been taken from the work of others, save and to the extent that such work has been cited and acknowledged within the text of my work. This thesis was prepared according to the regulations for graduate study by research of the Technological University Dublin and has not been submitted in whole or in part for another award in any other third level institution. The work reported on in this thesis conforms to the principles and requirements of the TU Dublin's guidelines for ethics in research. TU Dublin has permission to keep, lend or copy this thesis in whole or in part, on condition that any such use of the material of the thesis be duly acknowledged.

Signature  Date: 17th June 2022

Candidate: Susan O'Shea

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To my husband, Colm, I cannot thank you enough for all that you have done to support me. You are my best friend; I could not have done this without you.

Dedication

This thesis is dedicated to Thea O'Shea.

Abbreviations list

AI	Artificial Intelligence
ANOVA	Analysis of Variance
AMD	Age Related Macular Degeneration
BIA	Bioelectrical Impedance Analysis
BMI	Body Mass Index
BP	Blood Pressure
BRB	Blood Retinal Barrier
cm	Centimetre
CMT	Central Macular Thickness
c-HFP	Customised Heterochromatic Flicker Photometry
DNA	Deoxyribonucleic Acid
DR	Diabetic Retinopathy
ELM	External Limiting Membrane
ETDRS	Early Treatment of Diabetic Retinopathy Study
FA	Fluorescein Angiography
FAZ	Foveal Avascular Zone
GCL	Ganglion Cell Layer
GDPR	General Data Protection Regulation
HFP	Heterochromatic Flicker Photometry
ICGA	Indocyanine Green Angiography
ILM	Inner Limiting Membrane
INL	Inner Nuclear Layer
IOP	Intraocular Pressure
IPL	Inner Plexiform Layer
kg	kilogram
LED	Light Emitting Diode
LZ	Lutein-Zeaxanthin
m	Metre
mm	Millimetre(s)

MP	Macular Pigment
MPOD	Macular Pigment Optical Density
OCT	Optical Coherence Tomography
OCTA	Optical Coherence Tomography Angiography
OD	Optical Density units
OH	Hydroxyl
ONL	Outer Nuclear Layer
OPL	Outer Plexiform Layer
RNFL	Retinal Nerve Fibre Layer
ROS	Reactive Oxygen Species
RPE	Retinal Pigment Epithelium
SPSS	Statistical Package for Social Sciences
TU	Technological University
USB	Universal Serial Bus
UV	Ultraviolet

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1 CHAPTER ONE

INTRODUCTION

1.1 Background and Context

The foveal avascular zone (FAZ) is a region in the centre of the retina which is devoid of blood vessels to ensure clarity of vision. Photoreceptors which detect light are densely packed into this area; while capillaries are arranged in rings around its centre, an area known as the FAZ. The fovea is a region of high metabolic activity, where light is focused to form images. It thus requires a balance between adequate provision of oxygen and nutrients from the blood supply, whilst remaining avascular and transparent at the centre to allow unimpeded passage of light to photoreceptors. The size and shape of the FAZ, in addition to the surrounding vessel density and perfusion, can be altered by age, sex and some diseases associated with oxidative stress.[1–4] For example, the shape of the FAZ becomes more irregular with increasing severity of diabetes.[5] Whereas in glaucoma; the FAZ size increases and circularity and vascularity of the FAZ decreases.[6,7] Reduced FAZ vessel density and perfusion has also been found in subjects with Covid-19.[8]

The FAZ was first imaged using fluorescein angiography (FA), as early as 1961 and is still considered the gold standard in retinal vascular imaging today.[9,10] FA requires injection of fluorescent dye into the bloodstream which then fluoresces under cobalt blue light, thus identifying blood flow as well as leakage and pooling of blood.[9] It is used to identify alterations in blood flow and is particularly useful in the treatment of diabetic retinopathy (DR) and age-related macular degeneration (AMD). However, there are some drawbacks associated with this imaging modality; it is an invasive technique which can be uncomfortable for the patient and may result in nausea and/or vomiting. Such negative experiences when associated with the procedure may result in

the loss of patients to follow-up. The injection of fluorescent dye into the bloodstream carries a small risk of anaphylaxis or seizures.[11–13] While the prevalence of anaphylactic response to fluorescein injection is reported to be between 0.3-0.59%, clinics where the procedure is performed must have epinephrine available, the shelf-life of which is 90 days and suitably qualified staff must be on-hand to deal with any possible reaction or suspected reaction.[14–16] The risks and costs associated with FA can be wide-ranging and despite its clear diagnostic benefits, it may not be suitable for every patient and would not be practical for screening purposes. Optical coherence tomography angiography (OCTA) is a newer technology which facilitates superior imaging of the FAZ, its composite layers and is comparable to FA, even in the presence of certain diseases.[13] This novel imaging modality can measure the size/shape of the FAZ, the surrounding vessel density and perfusion, and has proven to be useful in the management of certain eye diseases associated with oxidative stress; such as DR, hypertension, glaucoma and AMD.[17–20]

1.1.1 Oxidative Stress and the Role of Antioxidants

Oxidative stress is a causative factor in eye conditions such as DR, hypertensive retinopathy, glaucoma and AMD.[21–24] It is caused by an imbalance between the production and accumulation of reactive oxygen species (ROS) in cells and the ability of the biological system to detoxify them.[25] ROS are by-products of normal chemical processes which, if allowed to build up without adequate protective antioxidants for counter-balance; can damage cells, proteins, lipids and deoxyribonucleic acid (DNA).[21,25] Retinal tissue is particularly sensitive to oxidative stress due to its high oxygen demand and exposure to high-energy short wavelength light.[21] In diabetes mellitus, chronic hyperglycaemia adversely affects various molecular pathways, inducing oxidative stress.[26] The imbalance of ROS ultimately results in the breakdown

of the blood-retinal barrier (BRB) and damages the cells that comprise these structures, including endothelial cells and pericytes, causing them to leak fluid which is particularly destructive when it occurs in the retina as it can threaten sight.[22] In hypertension, uncontrolled build-up of the Superoxide ion and hydrogen peroxide is responsible for damage to proteins within arterial endothelial cells leading to vasoconstriction and increased blood pressure (BP).[23,27] Glaucoma is a multifactorial disease associated with the loss of retinal ganglion cells, again, partly due to uncontrolled build-up of ROS causing mitochondrial dysfunction.[28] Levels of serum oxidative stress markers may also increase in glaucoma.[24] While the exact cause of AMD is unknown, it is accepted that oxidative stress plays a role.[29,30] Uncontrolled oxidative stress and ROS accumulation have been associated with retinal pigment epithelial cell damage.[31] More recently, oxidative stress signs have also been reported in subjects with Covid-19 such as cotton wool spots, haemorrhages and venous dilation.[32] While oxidative stress can cause damage within the body, antioxidants offer protection by restoring redox balance.[23]

Macular pigment (MP) is a powerful antioxidant and blue light filter found in high concentrations in the central fovea. MP is made up of carotenoids: lutein, zeaxanthin and *meso*-zeaxanthin.[33–35] It plays an important role in protecting the eye from oxidative stress and helps to optimise quality of vision.[36] Humans are not born with MP and therefore must absorb these carotenoids from food. A diet rich in coloured fruit and vegetables such as spinach and red/orange peppers can enhance MP levels. Higher MP levels may confer antioxidant protection in the retina, however, it can become depleted due to many factors including age, smoking and overweight/obesity.[37,38] Retinal conditions associated with oxidative stress may lead to lower MP levels, concomitantly, it is possible that MP confers protection and slows the progression of these diseases,

such as AMD and hypertensive and DR.[21,39,40] Of interest, adipose tissue competes with the macula to store carotenoids present in MP.[38,41] Therefore, individuals with higher levels of visceral fat can often have lower levels of MP present in the eye.[38]

1.2 Study Aims/Objectives

a) To establish normative data using the Cirrus 5000 HD-OCT (Cirrus 5000) (Zeiss, California) on the superficial FAZ in a young, healthy population (aged 18-35), free of ocular pathology. A larger FAZ size and reduced vascularity has been associated with pathology relating to oxidative stress/inflammation such as diabetic and hypertensive retinopathy, glaucoma and AMD.[3,20,42,43]

b) The second aim of this study was to investigate the size of the superficial FAZ, its vascular profile and central macular thickness (CMT); in relation to other health parameters including overweight/obesity, dietary intake of carotenoids lutein and zeaxanthin and MP optical density (MPOD) in a young, healthy population.

1.3 Study Rationale

FAZ parameters such as size, shape, vascular density and perfusion can differ with age, sex and between various ethnicities.[1,2,4,44] Hence, there is a need for normative data on the FAZ and its vascularity. While commercially available machines display excellent repeatability and reliability, considerable variability has been found when comparing different machines.[45] It is therefore important to establish machine-specific normative data on various ethnic groups in order to more accurately detect changes which may be occurring in the FAZ due to disease. This formed the basis for paper one published in the European Journal of Ophthalmology, which determined normative data for the superficial FAZ in a predominantly White population using the Cirrus 5000, which will be discussed in Chapter Six.[46]

Given the apparent association between FAZ parameters and retinal pathology and the putative protective effects of MP against oxidative stress, the second part of the study aimed to assess the relationship, if any, between factors that may affect the superficial FAZ (i.e., BP, vessel density/perfusion, overweight/obesity) and possible links with MP status, in a young healthy population, using the Cirrus 5000. This part of the study formed the basis for the second article and will be discussed in Chapter Seven.

1.4 Research Benefits

The emerging technology, OCTA has many benefits including quick, non-invasive image capture, offering a wealth of retinal information. The advent of new imaging technologies offers great potential for detection and management of ocular diseases. Quick, non-invasive imaging modalities are of particular importance in the context of Covid-19, as clinicians can easily gather information with minimal patient contact, which may not have been possible previously. As will be discussed in Chapter Four, there are variations between the current commercially available machines, therefore, machine-specific normative data are paramount to easily detect abnormalities for early intervention and disease prevention. Scans using OCTA can be readily performed with no patient discomfort and are completely non-invasive without the need for vital dyes for visualisation. Given that some inflammatory conditions are associated with changes to the FAZ and surrounding vasculature, this study aimed to examine factors, such as body weight and/or BP which may affect the FAZ and analyse the possible relationship with MPOD (i.e., a powerful antioxidant). These investigations aim to uncover further use for OCTA as a potential screening device for ocular conditions related to oxidative stress.

Chapter Two will examine the anatomy and physiology of the retina, including the FAZ and how it can be affected by oxidative stress.

2 CHAPTER TWO

ANATOMY AND PHYSIOLOGY OF THE RETINA

2.1 The Retina

The retina is a thin layer of light sensitive tissue which lines the inside of the eye. Its average thickness is approximately 230-360µm and varies from thickest at the optic disc to thinnest at the ora serrata.[47,48] The retina is strongly attached to the choroid at these two locations. The inner face of the retina borders the posterior vitreous body while the choroid lies posterior to the retina. The sclera is located behind the choroid, providing structural integrity to the globe. Figure 2.1 shows a horizontal section of the anatomy of the adult human eye.

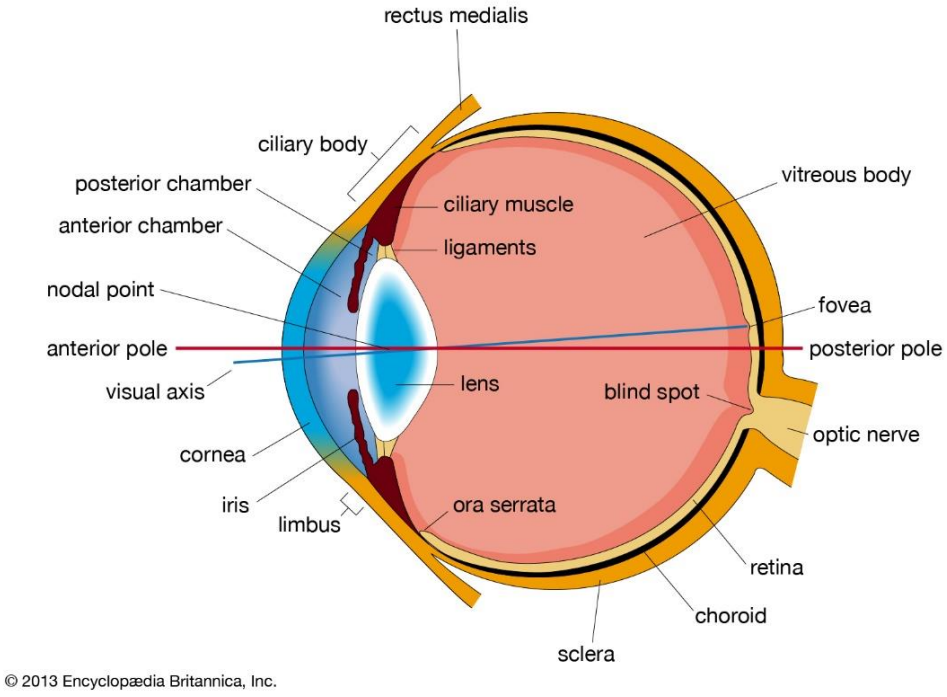

Figure 2.1. *Human eye. A horizontal section of the adult eye.*[49]

The retina comprises layers of cells and tissue specially designed to capture light. This focused light converges on the macula where it is converted into electrical signals and transported to the brain via the optic nerve, forming images.[50] The macula is a highly

specialised region of the retina where light is focused. At its centre is the fovea, which is responsible for high visual resolution and colour vision. The retina is the only part of the central nervous system which can be viewed directly and non-invasively.[51] Ten main layers comprise the retina as shown in Figure 2.2 and they include the following:

1. Inner Limiting Membrane (ILM)
2. Retinal Nerve Fibre Layer (RNFL)
3. Ganglion Cell Layer (GCL)
4. Inner Plexiform Layer (IPL)
5. Inner Nuclear Layer (INL)
6. Outer Plexiform Layer (OPL)
7. Outer Nuclear Layer (ONL)
8. External Limiting Membrane (ELM)
9. Photoreceptor layer (layer of rods and cones)
10. Retinal Pigment Epithelium (RPE)

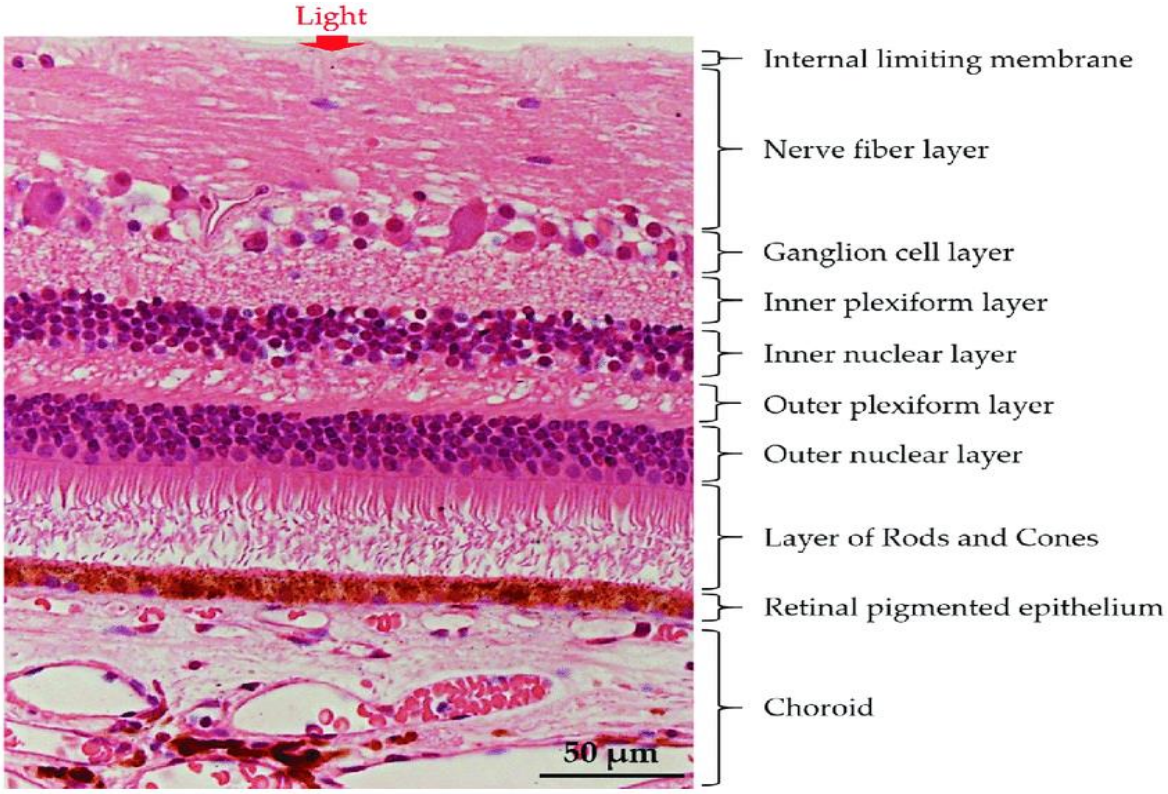

Figure 2.2. *Histology of the retina.*[52]

The layers from the ILM to the ELM make up the inner sensory retina. In order to allow light to pass through the sensory retina to the deeper pigmented retina for processing, these layers are transparent and invisible to the human eye. However, they can be seen using optical coherence tomography (OCT) which confirms what was previously only understood using histology samples. OCT exploits the principle of total internal reflection to visualise the transparent layers of the inner retina and will be discussed in greater detail in Chapter Four.[53] An example of an image formed by an OCT scan is shown in Figure 2.3.

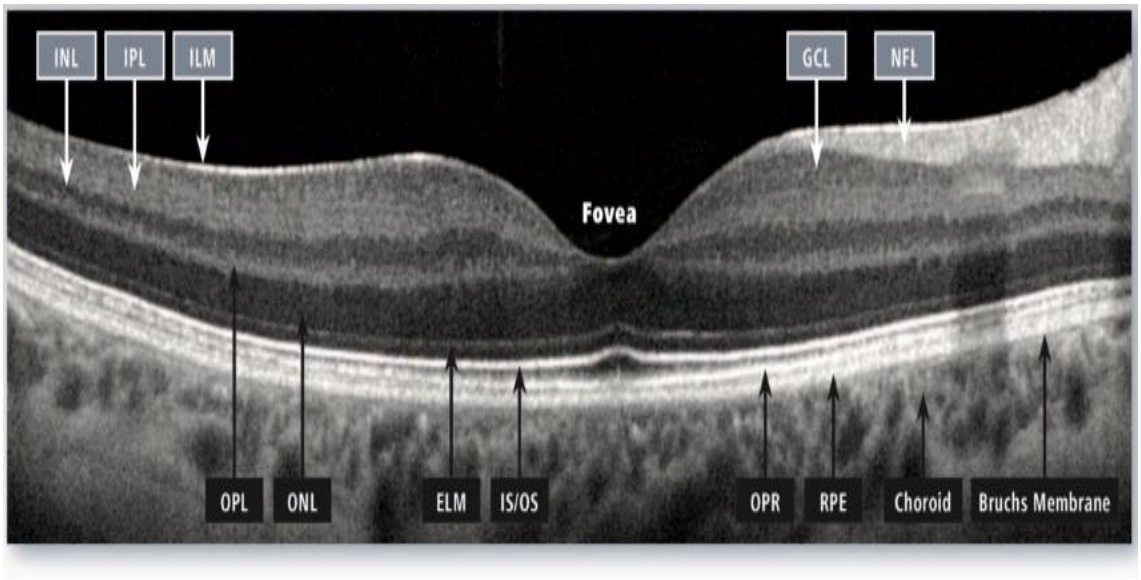

Figure 2.3. *Cirrus OCT scan showing retinal layers.*[54]

Light can pass through the inner sensory retina, due to their specialised cellular arrangement, to the RPE where it is absorbed. The functions of the retinal layers, photoreceptors and cells will be discussed in more detail later in this chapter.

2.2 The Macula

The macula is an area 4.5-6millimetres (mm) in size situated in the anatomic centre of the retina at the posterior pole.[51] Average macular thickness is approximately 170-250µm according to the UK Biobank study.[55] While it makes up a tiny proportion (approximately 4%) of the retina, the macula is almost entirely responsible for central vision due to its highly specialised architecture.[56] The macula is also known as the macula lutea which refers to its yellow appearance due to the presence of carotenoid pigments (lutein, zeaxanthin and *meso*-zeaxanthin). Collectively, these pigments are known as MP, which is thought to enhance visual quality by absorbing short wavelength blue light, thus reducing chromatic aberration caused by the light scatter of

different wavelengths of light.[57] Short wavelength light is damaging to the eye as it is more energetic than long wavelength light and promotes production of ROS.[58] MP putatively protects against the effects of harmful blue short wavelength light as the peak absorption of MP is 460 nanometres (nm). In addition, MP acts as a powerful anti-oxidant, protecting this highly metabolically active region of the retina against oxidative stress.[56] MP and oxidative stress will be discussed in greater detail in Chapter Three.

2.2.1 The Fovea

Development of the macula in the human retina occurs due to cells from the INL and GCL moving outwards, allowing cone cells to migrate towards the centre and accumulate at the macula.[59] The combined displacement of the inner retinal layers and accumulation of cone cells in this region contribute to enhancing vision quality. The resulting retinal topography begins to slope downwards at the macula, forming the foveal pit at its centre. This central area of approximately 0.35mm width is known as the foveola or fovea centralis.[51] The slope is called the clivus and it marks the border between the rod-dominated vascular fovea and the cone-dominated avascular fovea. Blood vessels are present on the clivus, but are absent in the fovea centralis, an area known as the FAZ.[51,56,60] The bottom of the foveal pit has a flat base, known as the umbo, where peak cone density is reached.[61] Figure 2.4 shows an image of the foveola in cross section.

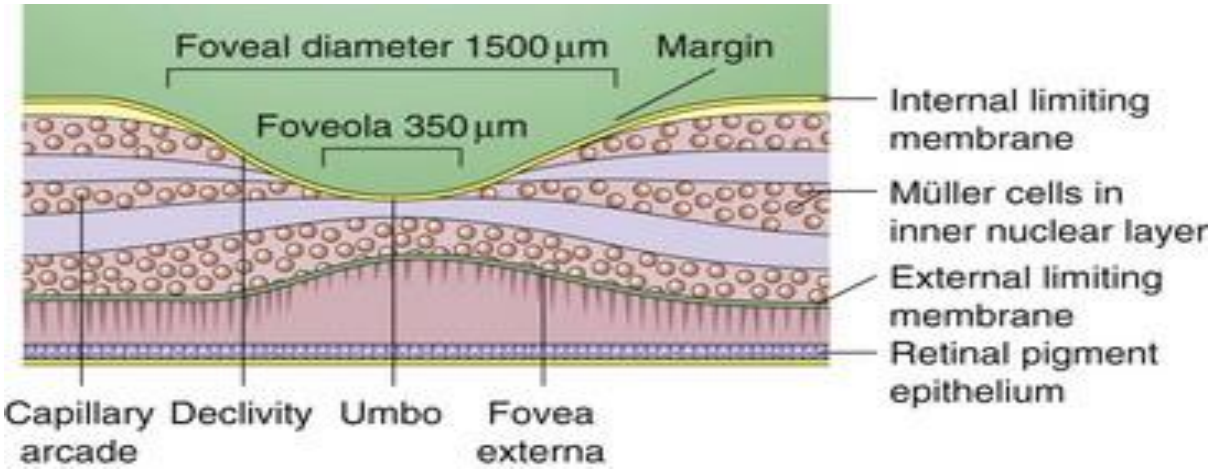

Figure 2.4. *Cross section of the retina showing the foveola.*[61]

The fovea is solely responsible for central visual acuity and colour vision. Light can pass unobstructed to the photoreceptor layer due to the absence of inner sensory retinal layers at the fovea. Cones and Müller cells are present exclusively at the foveola.[51]

2.2.2 Photoreceptors

Cone photoreceptors, responsible for central visual acuity and colour sensitivity, are found in highest density in the central 0.15mm of the foveola, which varies between individuals from 100,000 to 324,000/mm^2, while rods and ganglion cells are absent here.[62] Rods are responsible for vision in low light conditions. Ganglion cells, which transmit light signals to the brain for processing, will be discussed in detail in section 2.9. There are approximately 4.5 million cones and 100 million rods in the human eye, making the latter the second most common neuron in the human body.[47,51] The ratio of rods to cones is 20:1 in the peripheral retina, while at the macula, it is 9:1, with the central foveola remaining rod-free.[63] Both rods and cones contain a cell axon and at their termination each have individual projections, the rod's spherule and the cone's pedicle.[64] A schematic diagram of a rod and a cone cell is shown in Figure 2.5.

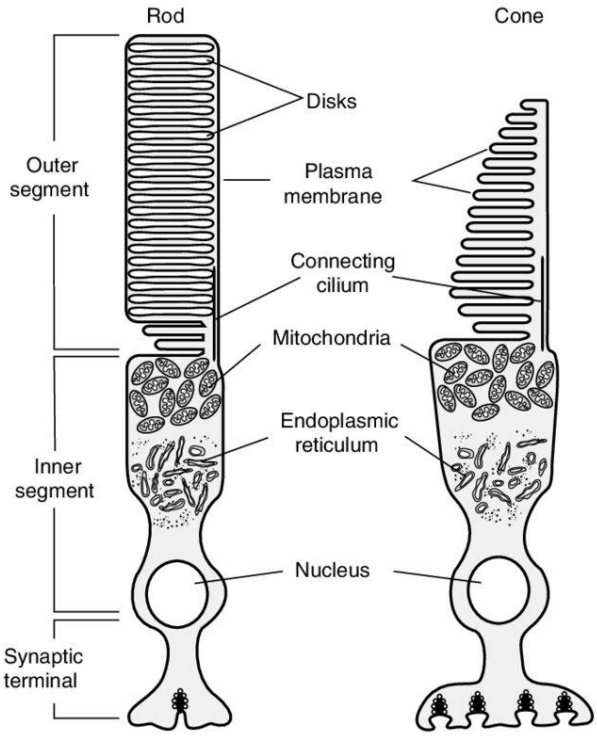

Figure 2.5. *Schematic diagram of a rod and a cone cell.*[65]

Each cone contains a specific type of light sensitive pigment called opsin.[47] They are thus sensitive to red, green or blue light, depending on the opsin-type present and function best in photopic conditions.[64] Blue light sensitive cones are absent from the central fovea.[64] Cones are connected to the brain by a three-neuron pathway.[64] In the peripheral retina, the relationship between cones and ganglion cells is convergent, meaning that multiple cones synapse with each ganglion cell. At the macula, the cone's shape is specialised, giving it a narrower, elongated and more conical appearance. Due to this altered morphology, cones can pack densely in the region of the fovea. The relationship between cones and ganglion cells is divergent here, with each cone cell connecting to three midget ganglion cells; these factors contribute to increased spatial and temporal resolution of images at this location.[64] Rods function best in scotopic conditions and are connected to ganglion cells via amacrine cells.[64] All rods contain the

same photopigment called rhodopsin.[47] They are not sensitive to colour but are extremely sensitive to light and are better motion sensors than cones.[64]

2.2.3 Müller Cells

Müller cells are non-neural glial cells which play a number of important roles within the retina. They are unique in that they extend through the whole thickness of the retina, providing structural support throughout to photoreceptors and neurons and regulating immune response to oxidative stress.[64,66,67] Müller cells connect neurons and improve the quality of the signals they send by increasing their signal to noise ratio and insulating cell axons. They also have a role in guiding light to the photoreceptors and regulate the BRB by insulating blood vessels.[17,20] The BRB will be discussed further in section 2.6. In addition, Müller cells can proliferate in response to injury.[64]

2.2.4 Regions of the Macula

The macula can be subdivided into regions for analysis with the fovea at its centre. Surrounding the fovea is the parafovea which is a ring approximately 0.5mm in size.[68] Some retinal vessels are present in this region while ganglion cell density is at its highest here.[51] Both rod and cone photoreceptors are present in the parafovea at a ratio of 4:1 in favour of rods.[51] The perifovea extending to the peripheral retina contains a high density of blood vessels.[51] While cones are still present in the perifovea, they are greatly outnumbered by rod photoreceptors here by a ratio of 33-130:1.[51] The GCL in this region is greater than one cell thick. Density of cones and ganglion cells is highest at the fovea and decreases toward the perifovea and peripheral retina. The subdivisions of the macula are shown in Figure 2.6.

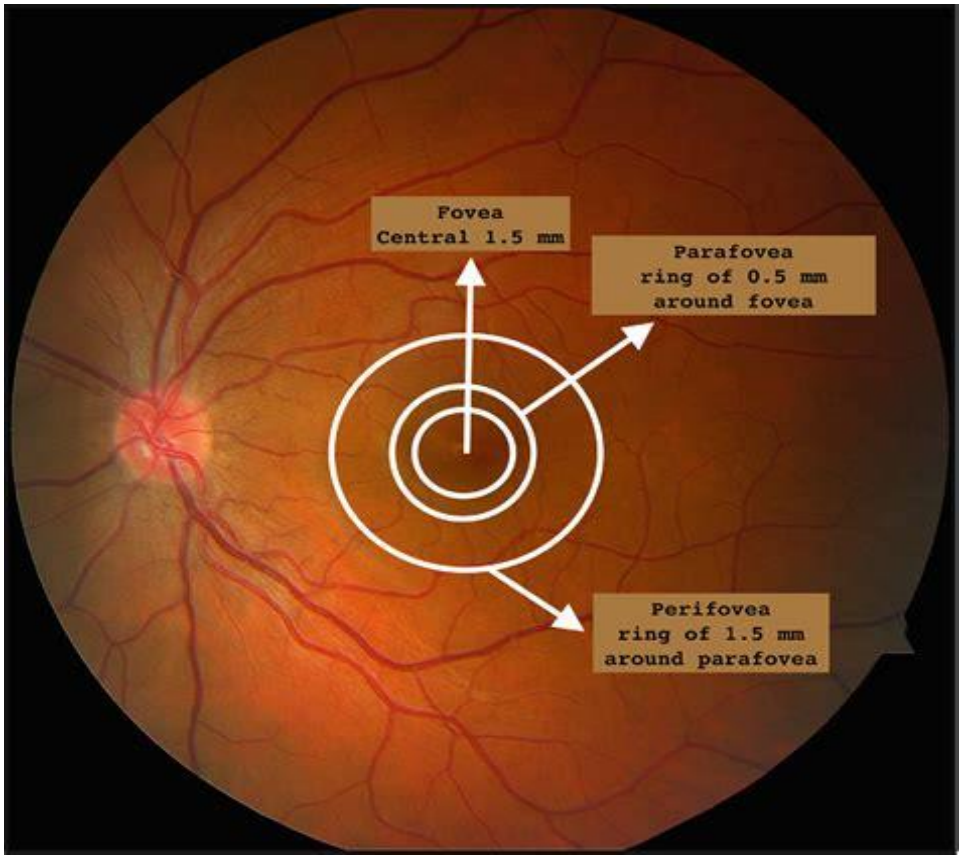

Figure 2.6. *Regions of the macula showing the fovea (central 1.5mm) and the parafoveal (outer 0.5mm diameter ring) and perifoveal regions (extreme outer 1.5mm diameter ring).*[69]

2.3 The Foveal Avascular Zone

Vessels are absent from the central 500µm of the fovea, which is known as the FAZ, and roughly corresponds with the location of the fovea centralis.[51] This region has developed in humans and some primates to provide precise central and colour vision.[53] It contains solely cone photoreceptors and Müller cells and relies on the vasculature of the choriocapillaris for energy and nutrient supply.[51] Cone photoreceptors are tightly spaced in this region and it is imperative that the overlying retina is devoid of vasculature which would obscure the light pathway to the photoreceptor layer.[53] The closely packed cones in the fovea connect to the brain via parvocellular pathways in which the cells have small cell bodies.[56] This means that each cone in the foveal region

connects to one midget bipolar cell and three midget ganglion cells. By contrast, in the peripheral retina, multiple photoreceptor cells communicate with one bipolar and ganglion cell, i.e., the magnocellular, or large cell body, pathway. Due to the organisation of the parvocellular pathways, they are arranged in parallel, meaning that they can easily be drawn apart from each other during the formation of the foveal pit without affecting their established connections, which indicates that these pathways are established prior to foveal pit formation.[56]

The tissue of the fovea is unusual in that it has a high demand for oxygen, due to its high metabolic activity, and yet it does not receive a direct blood supply. Instead, a capillary plexus forms around the foveal region.[56] Animal studies on macaques and marmosets have shown that the FAZ is present prior to formation of the foveal pit and may play a role in its formation.[70] Provis et al have also shown that the FAZ is present in human foetal retinas and remains avascular after birth.[60] The foveal pit develops due to the combined mechanical forces of intraocular pressure (IOP) and retinal stretch which occurs as the eye elongates with growth, leading to the characteristic topographic change.[53] The presence of the FAZ is assumed to provide a certain elasticity to the developing retina which aids in the formation of the foveal pit. The remaining retina is more rigid due to the presence of its vasculature. Development of the fovea begins early in foetal development but full maturation is not reached in humans until between two and four years of age.[56]

Studies on foveal morphology have shown that there is great variation in foveal diameter and depth even in normal eyes.[71,72] FAZ size is larger in females than in males and seems to increase with age.[1,2,4] Foveal morphology studies have also indicated that the FAZ is often small or absent in subjects with a history of retinopathy of prematurity.[73] FAZ area is also affected by ethnicity, and appears to be larger in Asian

eyes (approximately 0.33-0.37mm^2) when compared with Caucasians (0.22-0.28mm^2).[74,75] Studies have shown increased FAZ area in certain diseases, such as in subjects with diabetes, glaucoma and Alzheimer's Disease.[3,5,7,20,76] The associations between pathology and the FAZ will be discussed in more detail in Chapter Four.

2.4 Retinal Blood Supply

Due to its thickness in humans, the retina has a dual blood supply which does not overlap: the retinal vessels supply the inner retina and the choroid provides blood to the outer retina and the foveola.[51,56] The OPL is thought to be supplied by both. The central retinal artery enters the retina through the optic nerve, then branches superiorly and inferiorly. These branches further divide into the superior and inferior arteriolar arcades which supply each quadrant of the peripheral inner retina, spreading outward from the optic disc.[51,77] A cilioretinal artery is also present in approximately 25% of eyes which provides an additional blood supply exclusively to the macula.[77] The retinal vasculature is situated mainly in the nerve fibre layer, with the larger vessels closest to the ILM.[77] A plexus of capillaries is formed by the arterioles which is divided into a superficial layer, located at the level of the nerve fibre layer or GCL; and a deeper layer, which lies in the INL, near the OPL.[77] The retinal vessels can be visualised non-invasively using OCTA, as will be discussed in Chapter Four. Figure 2.7A shows a printout of an angiography scan using the Cirrus 5000. The vessels in the central 3mm of the macula can be visualised and analysed in the superficial layer (from the ILM to the IPL). The central 0.25mm^2 marks the FAZ which is devoid of vessels. The macular region is subdivided according to the Early Treatment of Diabetic Retinopathy Study (ETDRS) in circles of 1, 3 and 6mm diameter, which roughly correspond to the foveal anatomy as described in Section 2.2.1.[78] An example of the ETDRS grid is shown in Figure 2.7B. In the Angioplex Metrix circled in red in Figure 2.7A, the central portion corresponds with the

central 1mm (the foveola and partial inner fovea), while the inner (1mm ring) is concerned with the parafovea and partially takes in the outer portion of the fovea. The full section refers to the whole 3mm diameter of both the fovea and parafovea. The outer 1.5mm ring is used in a 6mm scan but does not apply to this 3mm scan. Vessel density (the total length of perfused blood vessels per unit area, mm/mm^2) and vessel perfusion (percentage of the total area of perfused vasculature per unit area in the same region) are analysed within these rings. Blood exits the eye through the central retinal vein.

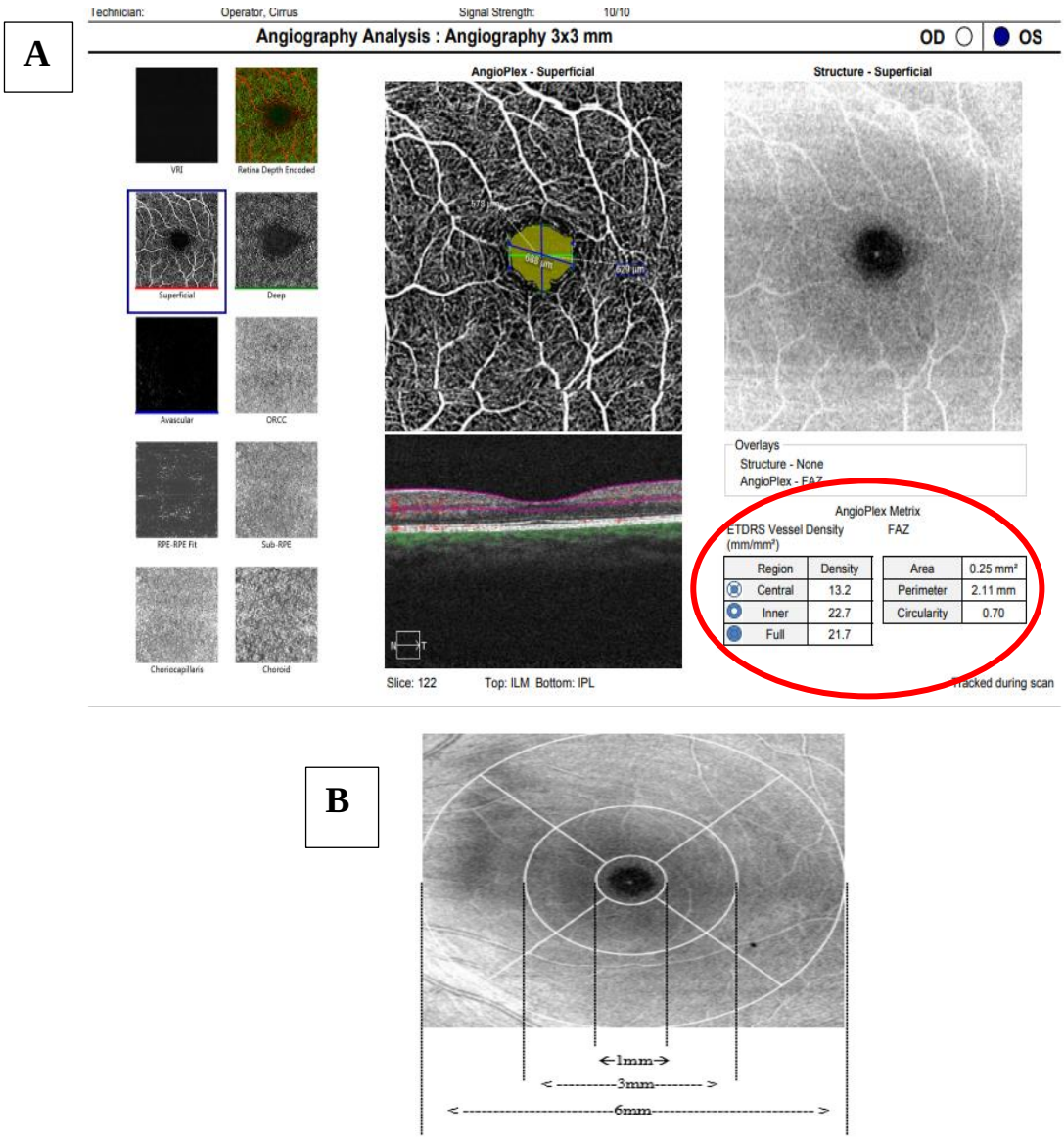

Figure 2.7. *A. Angiography analysis using a 3x3mm scan. Angioplex Metrix highlighted in red displays the vessel density at the central (fovea), inner (parafovea) and full sections of the macula according to the ETDRS. ETDRS; Early Treatment of Diabetic Retinopathy Study. B. ETDRS grid centred on the macula. [Image: Author's Own]*

2.5 The Choroid

The choroid is an extremely important vascular layer. It receives 80% of all the ocular blood supply from the ophthalmic artery, while the ciliary body receives 15% and the remaining 5% is directed to the retina. The ophthalmic artery supplies the choroidal vasculature through the short posterior ciliary arteries.[51] Choroidal thickness varies from 0.1 to 0.25mm and it is split into three layers: the layer of Haller, Sattler and the

choriocapillaris.[51] The innermost layer is the choriocapillaris, which has fenestrated, leaky capillaries and is separated from the RPE by a porous, elastic membrane, known as Bruch's membrane.[56] Choroidal blood flow is one of the fastest in the body, at 2000ml/min/100g tissue and is approximately 40 times faster than retinal blood flow which is 50ml/min/100g tissue.[64] This speed of blood flow, coupled with proximity to the photoreceptors facilitates quick diffusion into the cells.[51] At the foveola, the only blood supply is from the choriocapillaris where oxygen must diffuse through 100µm of tissue to reach the cones. At the peripheral retina, the choroidal blood supply is complemented by the retinal blood supply to the photoreceptors in addition to oxygen diffusing only 50-60µm from the choroid.[56] The foveal cones, which have a high oxygen demand, are supplied with oxygen solely from the choroidal blood vessels, with no additional supply from the retinal vessels. The fovea is particularly susceptible to damage by oxidative stress as it naturally has a high metabolic demand and a limited blood flow. So it is very vulnerable to oxidative stress conditions if any further restriction of choroidal blood flow were to occur, due to disease or other injury.[56] To maximise the inflow of oxygen to the fovea, the vessels of the choriocapillaris have adapted to increase their internal diameter while the thickness of Bruch's membrane is reduced at the region of the fovea. However, these adaptations to increase oxygen supply at the fovea create a natural location for lipids and other blood-borne material to accumulate over time in the sub-retinal space. These accumulations can contribute to reduction in choroidal blood flow and increased stress conditions in the retina, leading to AMD.[56]

2.6 The Blood-Retinal Barrier

A close relationship must exist between the blood vessels and the cells surrounding them in order to allow nutrients to flow into the cell while waste products are removed.

The BRB can be separated into two entities: the inner and outer BRB. The endothelium of intra-retinal blood vessels is considered to comprise the inner BRB. They have tight junctions and no fenestration to allow molecules such as glucose to transport across the barrier. The outer BRB consists of three entities:[77]

1) The fenestrated endothelium of the choriocapillaris, which is quite porous and provides glucose to the RPE.
2) The elastic and permeable Bruch's membrane.
3) The specialised cells of the RPE which allow vitamin A to pass to the photoreceptors.

2.7 Retinal Pigment Epithelium

The RPE is a monolayer of approximately 3.5 million epithelial cells which are bound together by junctional complexes and is one of the most metabolically active sites in the human body.[51,79] This layer is dark brown or black in colour, due to the presence of melanin.[51] RPE cells are densely packed into the region of the fovea at 5000 cells/mm^2. In the peripheral retina, there are approximately 2000 cells/mm^2.[51] This layer forms a selective BRB between the choroid which is rich in blood supply, and the inner neural retina.[80] This barrier maintains the integrity of the retina by allowing small molecules such as oxygen to pass through while blocking the entry of larger blood cells into the retina. Presence of blood in the specialised neurosensory retina would render it impossible for light to pass through leading to reduced visual acuity.[81] The entirety of the retina is not tightly attached to the choroid. A space exists between the RPE and the choroid known as the sub-retinal space. Bruch's membrane is an elastic membrane which stretches from the ora serrata to the optic disc and separates the RPE from the vascular network of the choriocapillaris.[51] The accumulation of deposits in the sub-

retinal space blocks nutrients including vitamin A from reaching the RPE which leads to degradation, seen as drusen deposits.[56]

2.8 Photoreceptor Layer

The photoreceptor layer is the only light sensitive layer in the neurosensory retina and this is where light signals are converted into electrical energy in the process of phototransduction.[50,51] This thin layer is made up of photoreceptors, known as rods and cones, as discussed in section 2.2.2.

2.9 Inner Neurosensory Retina

Specific cell types are found in the various layers of the inner neurosensory retina, as detailed below:

a) Bipolar cells: These cells synapse with photoreceptor cells, forming the next link in the pathway to the brain. There are ON and OFF types of bipolar cells, depending on whether information will be transmitted or halted.[47] Bipolar cells can be further divided into rod or cone bipolar cells, relating to which photoreceptor type they connect with.[64] Cone bipolar cells have more differentiation, with approximately 10 different types, while there is only one type of rod bipolar cell.[64]

b) Horizontal cells: Horizontal cells are mainly found in the outer portion of the INL. Their long processes with dendritic terminations reach the OPL. The dendrites synapse with cone cells while the axons communicate with rod photoreceptors.[64] Their function is to modulate the output of the photoreceptor cells depending on illumination levels, thus reducing the effect of bright objects from dazzling the retina.[47]

c) Amacrine cells: These are mainly located in the INL, but some are also found in the GCL. These axon-lacking cells modulate neurological signals in the IPL and

communicate with rods, cones, bipolar and ganglion cells.[64] There are approximately 30 types of amacrine cells and they are involved in motion and direction detection.[47]

d) Interplexiform cells: These cells manage feedback between the OPL and IPL and communicate with amacrine and bipolar cells.[64]

e) Müller cells: These were described in section 2.2.3.

2.9.1 External Limiting Membrane

This is not a true membrane, but a meshwork made up of junctions between photoreceptor cells and Müller cells.[51] The ELM can act as a protective barrier restricting the passage of large molecules through to the lower layers of the retina.

2.9.2 Outer Nuclear Layer

The cell bodies of photoreceptors (rods and cones) are located in the ONL. This layer is thickest at the fovea, where it has 10 layers of cones; compared to the peripheral retina where the layers comprise three to four rod nuclei and one layer of cone nuclei.[51]

2.9.3 Outer Plexiform Layer

This is a communication layer which contains the synapses and processes of photoreceptor, bipolar and horizontal cells.[51]

2.9.4 Inner Nuclear Layer

The nuclei of bipolar, horizontal, interplexiform and Müller cells are contained in this layer. These are known as interneurons which connect the GCL to the photoreceptor layer.[51]

2.9.5 Inner Plexiform Layer

This layer is used for communication and contains a complex network of the processes and synapses of bipolar, amacrine and ganglion cells from the other layers. Visual information is processed extensively in the IPL and synapses occur between bipolar cell axons and ganglion cell dendrites.[64] These dendrites and their synapses become damaged and die prior to ganglion cell death in glaucoma animal studies.[82,83] Tribble et al have shown that this may also occur in humans.[84] The IPL is involved in detection of colour, motion, contrast and hue and is absent at the fovea.[64]

2.9.6 Ganglion Cell Layer

The GCL is solely comprised of ganglion cells which transmit visual information from the retina to the brain.[51] There are approximately 1.2 million ganglion cells in the retina.[51] In the central retina, this layer can be 10 cells deep in thickness while in the peripheral retina the GCL is much more sparsely populated, at only one cell thick, however there are no ganglion cells present at the foveola.[51] Approximately 20 types of ganglion cells have previously been described in the literature, midget and parasol ganglion cells are the two most common types.[51] The axons of the ganglion cells eventually form the optic nerve. Ganglion cell loss is associated with visual field loss and glaucoma. Software of OCT machines group the GCL together with the IPL for analysis, as the two layers are closely aligned. Thickness of the GCL + IPL can be measured in each eye and compared to normative data to analyse glaucoma progression.[85] This will be discussed in detail in Chapter Four.

2.9.7 Retinal Nerve Fibre Layer

The RNFL contains the axons of ganglion cells and grows thicker towards the optic nerve where all the ganglion cell axons converge.[51] They are arranged radially in an organised manner and never cross the equator of the globe. This demarcation between

superior and inferior retina is known as the horizontal raphe in the retina. At the macula, the axons do not cross the fovea and instead, travel in a different arrangement, arcing around the macula towards the optic nerve, this is known as the papillomacular bundle.[51] The inferior and superior nerve fibres are thickest as all the nerve fibres from the peripheral retina are packed together, arcing around the papillomacular bundle, which is thinnest.[51] Tan et al showed that thinning of the RNFL, GCL and IPL occur in glaucoma and that this is often detectable prior to visual field loss.[86] Retinal blood vessels are located predominantly at the level of the RNFL.

2.9.8 Inner Limiting Membrane

This barrier layer demarcates the boundary between the posterior vitreous face and the retina. It is comprised of Müller cells and covered by a basement membrane.

2.10 Oxidative Stress in Retinal Disease

As shown earlier in this chapter, the macula is a highly metabolically active region of the retina which leads to the creation of an abundance of ROS.[35,51,64,80] These are molecules containing oxygen which have one or more unpaired electrons and are therefore highly reactive.[25] These molecules are created as normal by-products of chemical reactions in the body; however their unrestricted accumulation in cells can lead to an imbalance causing damage, known as oxidative stress, which can damage cells, lipids, proteins and DNA.[21,25] As discussed earlier in this chapter, photoreceptors function in hypoxic conditions which increases their vulnerability to slight changes in the balance of ROS and antioxidants within the fovea. Excessive oxidative stress can therefore lead to inflammation, compromise of the BRB leading to leaky blood vessels, and tissue damage in this highly sensitive region.[25] Oxidative stress damage can be mitigated by the presence of antioxidants which neutralise the highly reactive ROS in a cell system.[87,88] The presence of the antioxidant MP in high concentrations at the

macula thus plays a role in preventing photoreceptor cell death.[89] In Chapter Three, MP will be discussed in detail, along with its function in prevention and mitigation of certain ocular diseases.

3 CHAPTER THREE

MACULAR PIGMENT

3.1 Macular Pigment

The carotenoids lutein, zeaxanthin and *meso*-zeaxanthin are collectively known as MP. These carotenoids are found in high concentrations in the central fovea and IPL of the macula where they act as powerful antioxidants and as a blue light filter.[90] At the fovea, MP is located mainly in the fibres of Henle, while it is situated in the IPL and OPL of the parafovea.[33,91] Figure 3.1 shows where MP is located within the macula.

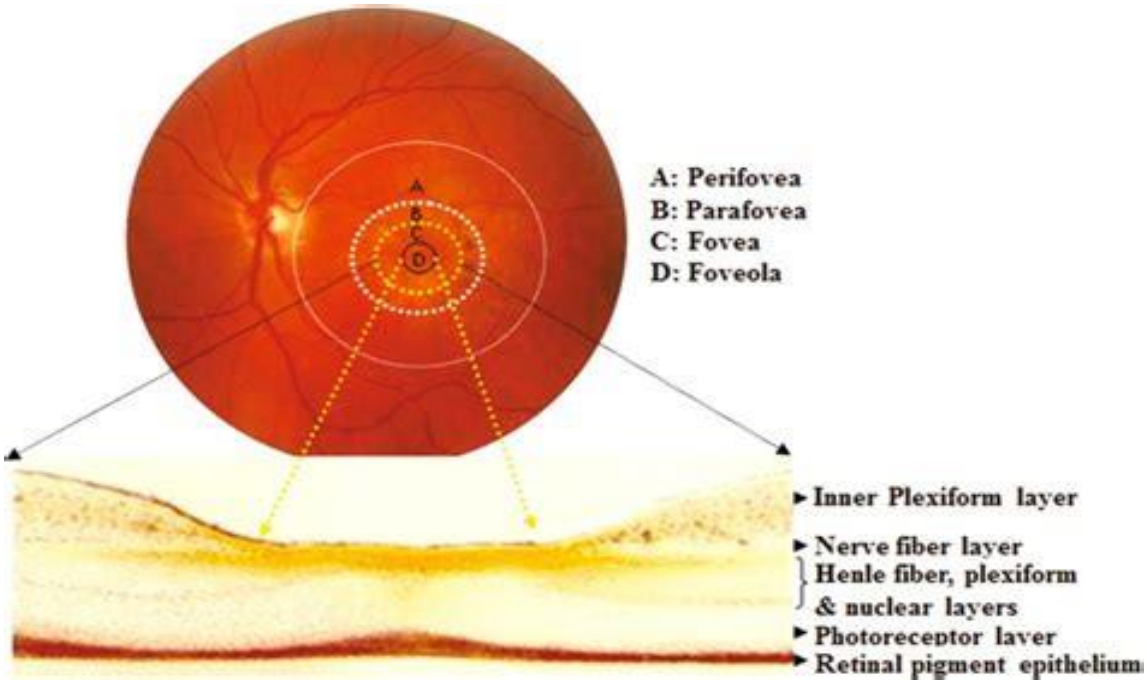

Figure 3.1. *Location of macular pigment within the macular layers.*[90]

Bernstein et al found that MP is present in almost all structures of the human eye but it is found in highest concentrations at the fovea.[92] Figure 3.2 shows the density of MP in different ocular structures. According to Bone et al the concentration of MP varies from 13ng/mm^2 at the fovea to 0.05ng/mm^2 at the peripheral retina.[93] At the foveola, the concentration of MP is approximately 1000 times greater than it is in the rest of the

body.[94] These pigments belong to the xanthophyll family of carotenoids which are found in plants.[90] Approximately 40 carotenoids have been found in human foods; of these 15 have been identified in human blood and tissues.[95] Only three carotenoids are found in the human retina: lutein, zeaxanthin and *meso*-zeaxanthin, the latter of which is thought to be formed from lutein, however, it has also been found in some foods.[96]

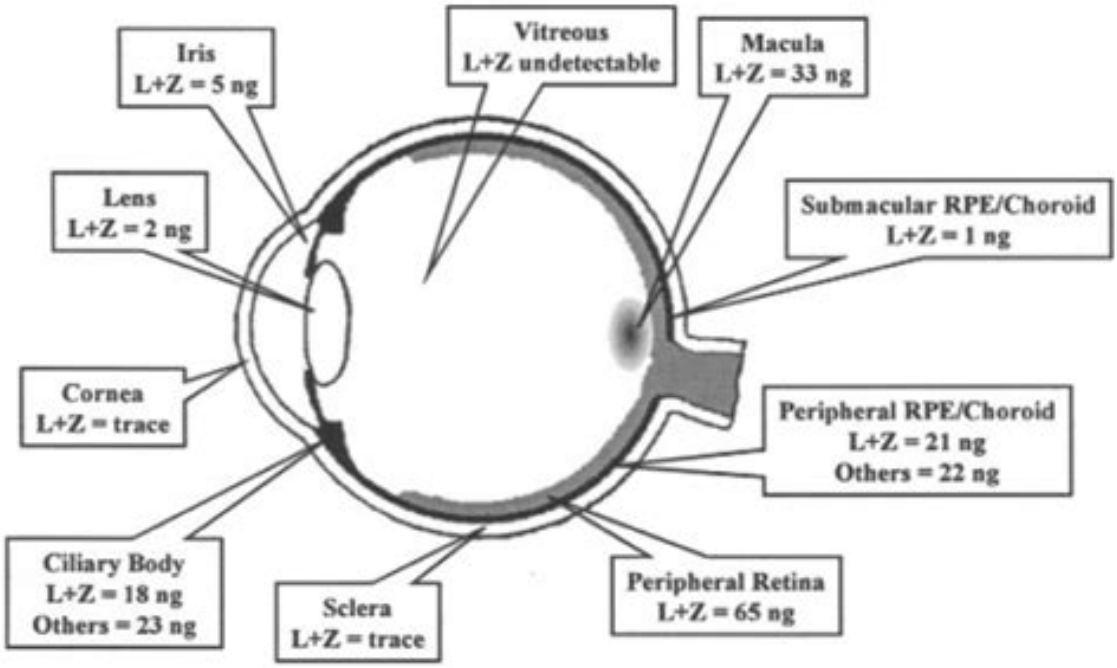

Figure 3.2. *Lutein and Zeaxanthin density in different parts of the eye*[97] *(Meso-zeaxanthin not included in this diagram).*

The yellow colour of MP gives the macula its full name, the macula lutea or yellow spot.[98] The peak absorption of MP is 460nm and its yellow colour makes it ideal for blue light absorption which protects the retina from harmful, short wavelength (450-500nm) light.[90] Figure 3.3 shows the absorption spectra of lutein (445nm) and zeaxanthin (450nm) in olive oil.[99] Blue light is more reactive, due to its short wavelength, and is involved in photochemical reactions, leading to oxidative stress which will be discussed in more detail in Section 3.3.1.

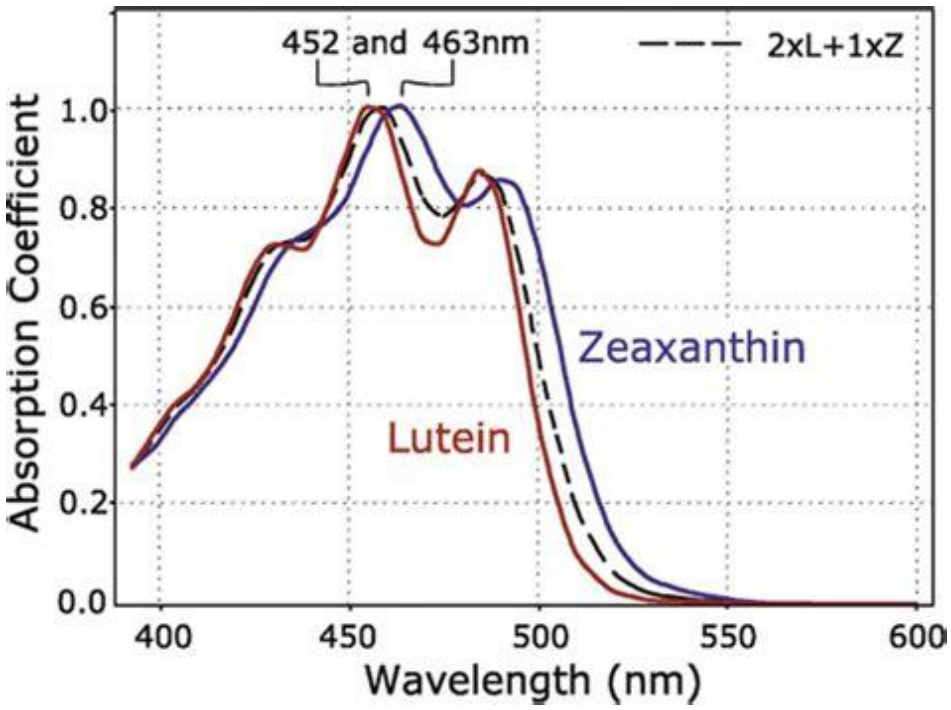

Figure 3.3. *Absorption spectra of lutein (red) and zeaxanthin (blue) in olive oil. The dashed line shows a mixture of the two.*[90]

Distribution of the three carotenoids varies throughout the macula, with *meso*-zeaxanthin found in its highest concentration at the centre of the fovea, zeaxanthin levels are highest in the parafovea and lutein is dominant in the perifovea.[33] At the fovea, zeaxanthin out numbers lutein by a ratio of 2:1 but in the peripheral retina, lutein is present in higher quantities, and this ratio reverses, 1:2 in favour of lutein.[92] The pattern of accumulation of MP, particularly the specificity at the foveola, has lead scientists to believe it plays a significant role in the macula.[93]

De novo synthesis of MP is not possible in humans meaning that it must be acquired through diet.[90] Xanthophylls are pigments found in brightly coloured plants. Lutein is found in most fruits and dark green leafy vegetables, such as spinach and broccoli; while zeaxanthin concentrations are highest in red and orange vegetables and fruits such as corn, nectarines or squash.[92,100] Egg yolk and maize also have high concentrations of

both lutein and zeaxanthin.[101] The human body does not easily access carotenoids from green leafy vegetables but eggs have proven to be an easily bioavailable source of these nutrients.[102] *Meso*-zeaxanthin is not found in many dietary sources and is thought to be formed from lutein in the retina. Nolan et al, however, have recently confirmed its presence in the skin of salmon, sardines and trout.[103] Studies have shown that MP levels can rise if dietary lutein and zeaxanthin are increased.[100,104]

3.1.1 Stereochemistry of Macular Pigment

Chemically, lutein, zeaxanthin and *meso*-zeaxanthin are stereoisomers, each with at least nine conjugated double bonds and two hydroxyl functional groups attached.[99] The conjugated bonds allow lutein and zeaxanthin to absorb blue light and neutralise ROS from oxidative reactions, while the hydroxyl groups provide the ability to cross blood-ocular and blood-brain barriers.[105] Figure 3.4 shows the chemical structures of MP with conjugated bonds which alternate between single and double bonds.[105]

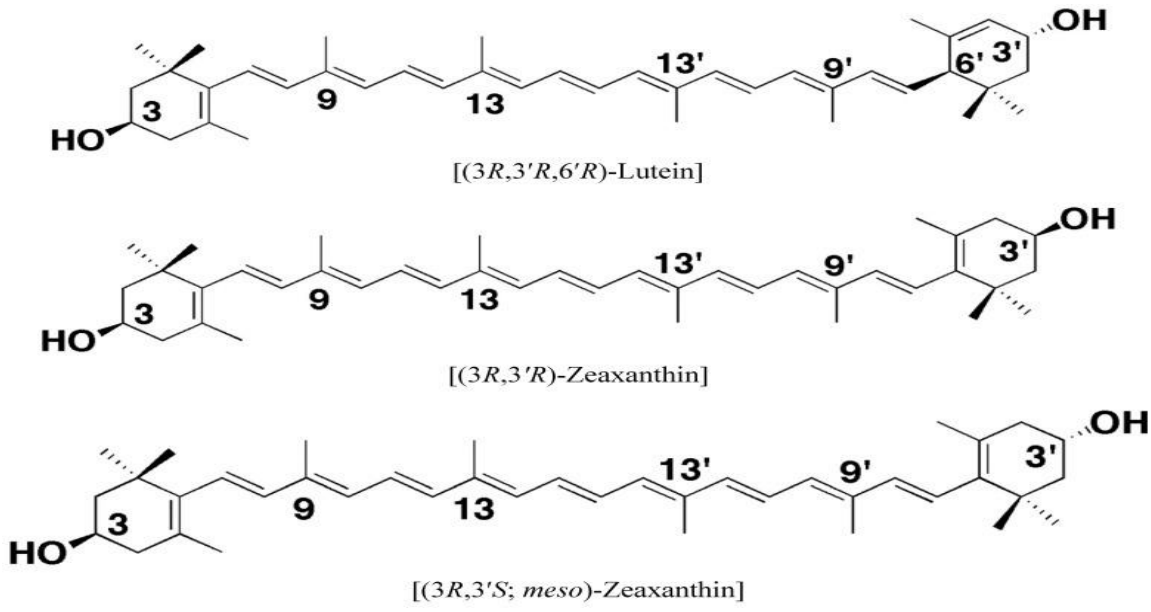

Figure 3.4. *The chemical structures of lutein, zeaxanthin and meso-zeaxanthin.*[106]

While zeaxanthin, with eleven conjugated double bonds, is highly efficient at quenching singlet oxygen, *meso*-zeaxanthin is the most efficient antioxidant of the three MP

carotenoids for this purpose, of course all three acting together is preferable.[105] In lipid membranes, MP has a regular orientation which enhances the rigidity of the membrane and may confer additional protection against oxidative damage.

3.1.2 Bioavailability of Carotenoids

Bioavailability refers to the amount of carotenoids available for use in bodily functions or storage and depends on the amount, source and type of carotenoid ingested through the diet.[90] Carotenoids are hydrophobic which means they are poorly bioavailable. Studies have shown that the presence of lipid in the diet improves carotenoid bioavailability, while increased dietary fibre inhibits MP absorption.[107,108] Sources of human dietary fats that would aid bioavailability include vegetable oil, avocados, eggs and oily fish such as salmon. Several stages are involved in the absorption of carotenoids by the intestine, once absorbed, they are transported by the lymphatic system to the liver and delivered to their final destination tissue by the circulatory system.[90] Lutein and zeaxanthin are transported on lipoproteins to the eye, with high density lipoprotein playing an important role in this transport system.[109] This highlights the importance of a diet rich in coloured fruit and vegetables, olive oil and nuts and lower in sugars and refined carbohydrates to promote high density lipoprotein availability and thus, maximise MP absorption in the retina.[110]

Adipose tissue, in particular visceral fat, is another important location for the accumulation of the same carotenoids which constitute MP.[111,112] Johnson et al found that adipose tissue and specifically visceral fat competes with the retina for the accumulation of carotenoids, lutein in particular.[113] This study found that subjects with a higher body fat percentage (%) had less available lutein in blood serum and this difference was more pronounced in women than in men. Hammond et al found similar results with inverse relationships becoming apparent between MPOD and both BMI

greater than 29 kilograms (kg)/metre (m)^2 and body fat percentage over 27%.[38] They also reported that the effect was found regardless of sex.

3.2 Role of Macular Pigment

It is thought that MP has a dual role: as an antioxidant protecting against oxidative stress damage and also as a blue light filter.[92] Snodderly et al suggest that in a healthy eye, the organisation of MP molecules takes a non-random orientation in order to maximise the absorption of light.[114,115] Improved ocular and visual health has been found to have an association with increased levels of MP.[21]

3.2.1 Macular Pigment as a Blue Light Filter

Visible blue/violet and invisible ultraviolet (UV) light are examples of short wavelength light. The shorter the wavelength of light, the higher the energy, short wavelength light is, therefore, more excitable and reactive in ocular tissue, leading to involvement in oxidative stress reactions.[105,116] Shorter wavelength light also has a higher refractive index, the difference in refraction of various coloured lights can lead to colour fringes around images known as chromatic aberration. The ability of MP to absorb blue light means it provides enhanced contrast sensitivity and visual acuity by reducing the effects of chromatic aberration and glare.[36,117] Both the cornea and lens are capable of filtering out most incident UV light on the eye.[105] Lutein and zeaxanthin are also found in the crystalline lens where they likely play a significant role in absorbing short wavelength light before it reaches the retina.[97] MP also has a protective effect at the macula in absorbing residual harmful blue and other short wavelength light.[21,33] Blue light has been found to interact particularly with lipofuscin, which is a by-product of Vitamin A, resulting in increased production of the free-radicals involved in oxidative stress.[118] Blue light from light emitting diode (LED) screens such as laptops and smartphones has been associated with increased myopia, asthenopia, disrupted sleep patterns and macular

degeneration.[119] LED screens are omnipresent in modern life to the extent that blue-light filtering spectacles are increasingly prescribed to young people, regardless of refractive status, to combat the damaging effects of blue light. It is possible that as the current teenage to working-age population ages, the effects of long-term exposure to blue light may present as premature ocular damage.

3.2.2 Macular Pigment as an Antioxidant

Oxidative stress, which was introduced in Chapter Two, is a factor involved in normal ageing but is also often a causative factor in the development and aggravation of eye disease such as AMD, glaucoma, DR and even hypertensive retinopathy.[21–24,120] It is caused by an imbalance of ROS within cells. ROS are highly reactive molecules containing oxygen which have unpaired electrons. There are endogenous and exogenous sources of ROS. Endogenous sources occur due to chemical reactions within cells and lead to the formation of singlet oxygen, the superoxide ion, the hydroxyl ion and nitric oxide among others.[120] Exogenous sources are generally external factors such as tobacco smoking, alcohol consumption, fats, pollution or drugs which are metabolised into free radicals within the body.[120] Molecules known as antioxidants, which have conjugated double bonds and are capable of safely bonding with the unpaired electrons, are required to restore balance. This state of balance is known as redox balance. Due to its high oxygen demand and exposure to UV and blue light, retinal tissue is particularly susceptible to oxidative stress damage. The chemical structure of MP is ideally adapted to neutralise ROS in the retina, thus restoring redox balance and decreasing the harmful effects of oxidative stress.[114,117] All three MP carotenoids are very effective antioxidants in absorbing energy from free radicals.[105,121]

3.3 Measurement of Macular Pigment

MP levels can be measured in living humans in a variety of ways, using psychophysical (requiring input from the subject) or objective methods which require no input, however, some of these methods are complex, requiring specialised equipment and are only suitable for laboratory or research purposes. Objective techniques include fluorophotometry, fundus reflectometry, auto-fluorescence attenuation and resonance Raman spectroscopy.[90,122,123] Auto-fluorescence using OCT or fundus photography is a non-invasive and simple method to assess MPOD.[123] The waste product lipofuscin fluoresces under blue light, while the presence of MP reduces this fluorescence. Specialised instrumentation use this reduction in fluorescence to calculate the level of MPOD.[123] This is a quick and objective method of measuring MPOD which has shown good agreement with the most widely accepted subjective test, Heterochromatic Flicker Photometry (HFP).[123] However, it requires the use of expensive specialist equipment, pupil dilation and a very bright light is used as part of the test to bleach the photoreceptors, which is very uncomfortable for the subject.[123] A new method, called MP reflectometry uses a spectrometer to analyse the absorption spectra of light reflected from the macula, given that the absorption spectra of lutein and zeaxanthin are known, it is possible to calculate MPOD levels in a minimally invasive way.[124] However, this method is very new and while it has shown good agreement with HFP in small samples (n =19, n = 30) that have been investigated thus far, validation using larger sample sizes would be more reliable.[124,125] This method holds promise and has the potential to differentiate between lutein and zeaxanthin in the MPOD measurement which would be of interest in tailoring dietary supplementation specifically to the individual.[124] Another new method which is currently used clinically is the MP-eye.[126] This new method uses polarised light to create the phenomenon of Hadinger's Brushes as MP levels positively

correlate with the ability to identify this optical phenomenon.[126,127] A benefit to this technology is its speed, as the test only takes one to two minutes.[126] The gold standard in MPOD measurement is still HFP presently.[124] Psychophysical methods include HFP, customised HFP (c-HFP), threshold spectral sensitivity, motion-based photometry and colour matching.[122,123,128] The method of c-HFP was used to measure MPOD in the current study, using the Macular Metrics Densitometer (Macular Metrics, Massachusetts, USA).[129] This measures the absorption of blue light by MP and MPOD is quantified as a value between 0 and 1 optical density units (OD), indicating the amount of lutein and zeaxanthin in the macula.[58] A value of < 0.2 OD can be considered as low, while 0.2-0.5 is mid-range and > 0.5 is high.[58]

3.3.1 Heterochromatic Flicker Photometry

The methodology of the macular metrics densitometer has been described by Wooten et al.[122] It is a psychophysical test which requires the subject to fixate a target which is flickering between a wavelength that is easily absorbed by MP (460nm) and one that is not (550-570nm) and has been validated against the actual absorption spectrum of MP.[130–132] The technique is based on the assumption that the spectral sensitivities of the fovea and parafovea are the same. In brief, the subject is required to adjust the 460nm (blue) light until flicker cannot be perceived. The amount of blue light required to achieve this is directly related to the level of MPOD. The test is repeated at a parafoveal location where MP levels are negligible. The optical density of MP is equal to the log ratio of the difference in the measurements found at these two locations. The methodology followed in the current study was the customised technique which uses a predetermined optimal flicker frequency for each subject, to refine the endpoint and is outlined in Chapter Five.

Several studies have found an inverse relationship between MPOD levels and AMD risk factors such as age, smoking, family history of AMD and obesity suggesting that low levels of MPOD could be associated with greater risk of development of AMD.[133–135] The greatest risk factor for AMD is thought to be genetic variants, such as the Y402H variant on the complement factor H gene, although its exact role is unknown.[136] Presence of these genetic factors may compound existing AMD risk from the other factors named above, therefore, those with genetic factors in particular could benefit from adopting healthy lifestyle changes such as increased intake of green leafy vegetables and smoking cessation.[137]

3.3.2 Food Frequency Questionnaire and Serum Analysis

Food frequency questionnaires can be used to assess dietary intake of carotenoids in relation to MP levels. They can be used cautiously to give a general overview of dietary carotenoid intake, given that they are associated with certain limitations.[138] These questionnaires are subject to bias as responders may artificially under or over report on certain foods or may have difficulty recalling specific details.[139,140] The lutein-zeaxanthin (LZ) screener is a validated test which aims to improve upon the questionnaire format by using approximate bioavailability of four dietary carotenoid sources: eggs, broccoli, spinach and corn and categorising carotenoid intake into low, medium and high.[141] Dietary estimates found using this method correlated with serum levels of carotenoids, controlling for age and BMI.[141] The LZ screener was used in the current study.

Serum analysis of blood can also be used to measure levels of lutein and zeaxanthin.[142] Serum levels of MP can vary widely between individuals.[99,121] This is thought to relate to a number of factors, such as lipid intake, genetic predisposition, BMI and body composition.[38] As such, serum measurement of MP plays an important role to examine

whether carotenoids are absorbed efficiently.[142] Blood serum analysis, while useful, is invasive, time consuming and requires specialised equipment so it is not suitable in a clinical setting and as such, is limited to use in research only.

3.4 Macular Pigment and Retinal Disease

The young retina is subject to large amounts of incident light, most notably, UV and blue light, which promotes oxidative stress in the eye and builds up over time.[105,143] In addition, the crystalline lens is not as effective in filtering out harmful short wavelength light in young eyes, although it does begin to develop this filtration ability from approximately three years of age, through the natural formation of yellow chromophores in photooxidation reactions.[105,144] Photooxidation is a normal and useful occurrence when chromophores within the lens are exposed to low levels of UV light which cause them to release ROS and develop into a protective UV filter over time.[105] Chromophores alter due to chronic exposure to UV light which leads to an increase in ROS production as the eye ages over the course of a lifetime.[105] However, fewer protective antioxidants are produced after middle age. Overall, this leads to the lens becoming clouded i.e., cataracts, which occur as a result of an imbalance in the amount of unquenched ROS.[105] Similarly, and as part of normal ocular metabolism, there is a build-up of the waste product lipofuscin in the RPE. In low levels, lipofuscin is minimally invasive within the eye. However, as lipofuscin builds up over decades, it begins to contribute to oxidative stress reactions by releasing more ROS and further sensitising the RPE to blue light.[145] Various inflammatory diseases, such as hypertensive retinopathy, glaucoma, DR and AMD have been associated with oxidative stress.[21–24] Thus, as the eye ages. the retina naturally becomes more vulnerable to the effects of harmful oxidative stress conditions and this is compounded if an oxidative stress disorder, such as diabetes, hypertension, AMD or glaucoma develop.[105] These

oxidative stress conditions can also deplete MP levels, and studies have shown that increased levels of MP may confer protection in the retina by filtering harmful blue light, quenching ROS and reducing the production of lipofuscin.[57,145–147] The presence of high levels of MP may, therefore, be useful in protecting against oxidative stress damage from a young age.

3.4.1 Age-Related Macular Degeneration

According to a recent review by the World Health Organisation, AMD remains one of the leading causes of avoidable blindness worldwide.[145,148] While the exact causes of AMD are still unknown, inflammation and oxidative stress are thought to be involved.[149] Increased levels of oxidative stress markers; the inflammatory protein carbonyl and the products of lipid oxidation, have been found in subjects with AMD.[31] Oxidative stress has been associated with RPE cell damage contributing to AMD.[31] As discussed in Section 3.4, the normal ageing process results in the production of lipofuscin, a by-product of Vitamin A metabolism. With advancing age, and after a lifetime of build-up of lipofuscin and exposure to blue light, this natural process becomes toxic, blocking the passage of Vitamin A and other key nutrients to the retina and leading to the degradation of the macula known as AMD.[105] MP is understood to play a preventative role in the development of AMD.[90,133,150] Lutein has a protective effect in the RPE in reducing formation of damaging lipofuscin.[145] Lower MP levels are associated with known risk factors for AMD.[134] While heredity and other lifestyle factors, such as smoking and diet may also contribute to this, it is likely that the antioxidant effect of MP plays an important protective role in preventing the development of AMD.[134,151] Levels of MP decrease with advancing age which contributes to the increased vulnerability of the macula to oxidative stress damage.[134] High cholesterol, i.e., high levels of low density lipoprotein combined with low levels

of high density lipoprotein may also contribute to lower levels of MP as this would impair transport of MP to the retina, given that high density lipoprotein is a transporter of MP, as discussed in Section 3.4.[134] The risk of developing AMD appears to reduce in subjects whose diet is rich in carotenoids, particularly lutein and zeaxanthin.[152] consequently it would appear that following a careful diet including varied fruit and vegetables and healthy fats from a young age may help boost MP levels and reduce the risk of developing AMD in advanced age.[37]

3.4.2 Diabetic Retinopathy

In diabetes mellitus, chronic hyperglycaemia induces oxidative stress and the imbalance of ROS results in excess available glucose in the blood, which in turn damages blood vessels, causing them to leak fluid.[22] When this occurs in the retina, it is particularly destructive as can lead to diabetic macular oedema and proliferative DR, which threaten sight.[22] The International Diabetes Federation estimates that diabetes mellitus may affect 700 million people globally by 2045.[153] DR is the most common cause of blindness among patients with diabetes among the working population.[154] Oxidative stress is a known factors in diabetic disease process, possibly due to hyperglycaemia.[155] Studies have shown that lutein and zeaxanthin are involved in ROS detoxification leading to a reduction of hyperglycaemic effects and can reduce oxidative stress damage in the retina.[156,157] Cennamo et al found lower levels of MP in addition to reduced retinal vessel density, as measured by OCTA, in subjects with Type One diabetes.[40] Of interest, subjects without visible DR had lower MP than those with retinopathy which may have been due to oxidative stress or sub-optimal carotenoid intake. Hu et al found that subjects with DR had lower serum levels of lutein and zeaxanthin, additionally, they found that supplementation with these carotenoids improved visual acuity and contrast sensitivity while macular oedema decreased.[158] MPOD levels were found to be

lower in patients with Type Two diabetes than in those with Type One and in non-diabetic controls.[159] Abdominal obesity is a known risk factor in Type Two diabetes.[160] As discussed in section 3.2.1, adipose tissue, in particular visceral fat, competes with the macula for storage of MP carotenoids, which may explain this reduction. These findings are consistent with the hypothesis that oxidative stress is involved in DR and that retinal vessel density and MPOD have potential as diagnostic indicators in progression of this disease.[40] There is an association between Type Two diabetes and diet: an increased risk of developing Type Two diabetes is associated with consumption of high levels of sugar and fatty foods, while a diet rich in vegetables appears to lower this risk.[161] While the risks of developing complications from diabetes increase with increasing duration of diabetes, it is concerning that the incidence of Type Two diabetes is rising among the population under 40 years of age.[162] Early intervention with lifestyle changes such as increased exercise and diet alterations to include more fruit and vegetables (i.e., carotenoids) can reduce the likelihood of developing Type Two diabetes and the complications which follow.[161,162]

3.4.3 Glaucoma

In addition to being the leading cause of irreversible blindness worldwide, the global prevalence of glaucoma is also expected to increase by almost 75% to approximately 112 million people by the year 2040.[163] This neurodegenerative ocular disease is characterised by visual field loss caused by damage to the optic nerve head and inner retina leading to loss of ganglion cells.[164] While the main cause of glaucoma is poorly understood, redox imbalance is thought to play a part in increasing IOP which is the only known modifiable risk factor of primary open angle glaucoma.[164] Benoist d'Azy et al found that levels of serum oxidative stress markers may increase in glaucoma.[24] Reduced oxygen supply and inadequate blood perfusion are other causes.[165] Damage to

retinal cell axons has been linked to an increase in ROS production which then leads to ganglion cell death.[165] The antioxidant properties of MP have been discussed in Section 3.2.2. Literature on the role of MP in preventing or attenuating glaucomatous damage is limited but lutein and zeaxanthin are thought to have a neuroprotective effect in the retina in animal studies.[164] Lutein, in particular, appears to act as an antioxidant in protecting the retina and ganglion cells from ischemia and hypoxia by preventing the formation of inflammatory markers and gliosis and apoptosis of Müller cells.[166,167] In some human studies, MPOD appears to be lower in the presence of glaucoma.[168,169] In addition, one study found that GCL thickness, which was reduced in subjects with primary open angle glaucoma, also had a strong correlation with lower levels of MPOD.[170] While some interventional studies have investigated the role of dietary carotenoids in slowing the progression of glaucoma, results have been contradictory.[164] One epidemiology study found that the risk of developing primary open angle glaucoma was reduced by 20 to 30% in subjects who consumed a diet rich in green leafy vegetables, such as spinach. However, it was not clear if the association was related specifically to the carotenoid content of these nutrient-rich foods.[164,171] While further study is required to elucidate the reasons, nevertheless, there is evidence to suggest that adopting a healthy diet containing collard greens, kale and spinach from a young age may delay or protect against the incidence of glaucoma in high-risk groups.[164]

3.4.4 Hypertension

Hypertension is a key factor responsible for 10.4 million deaths worldwide per year.[172] Hua et al found that raised BP was associated with decreased retinal vessel density, and the presence of hypertensive retinopathy was associated with reduced RNFL and thickness.[5,7] There is evidence to suggest that inflammation, caused by oxidative stress, may be a causative factor in hypertensive retinopathy, which in turn leads to a reduction

in blood flow and an increase in inner retinal thinning and that earlier detection of these changes may, in fact, aid the treatment of hypertension.[3,35,76] Nolan et al found lower levels of MPOD in subjects with self-reported hypertension.[39] Indeed, Kumari et al also found that lower serum levels of lutein and zeaxanthin were associated with signs of hypertensive retinopathy such as increased vessel tortuosity, narrower arterioles and wider venules.[173] While there may be a variety of explanations for these findings, it is interesting to note that oxidative stress has also been implicated as a causative factor in hypertension.[23,174] Whether the reduction in antioxidant MP is caused by or contributing to hypertension is unclear. Dietary studies have found that increasing intake of vegetables while moderating intake of cholesterol and trans/saturated fats can have a positive effect in reducing the risk of cardiovascular disease such as hypertension.[175,176] As with other acquired diseases, earlier intervention is paramount to successful prevention.

In relation to these inflammatory diseases, OCTA facilitates quick, non-invasive examination of the retinal vasculature.[177] Studies using OCTA have associated early hypertension with changes in the retinal microvasculature, even in the absence of visible retinopathy.[178,179] Poorly controlled diabetes has been linked to reduced retinal blood flow and perfusion, resulting in capillary dropout in the foveal region.[3,77] Microvascular changes associated with DR have been revealed by OCTA which were otherwise clinically undetectable.[180] Areas of capillary non-perfusion and enlargement of the FAZ which are indicative of DR can be identified using OCTA.[181] Reduced blood flow has also been associated with the pathogenesis of glaucoma.[182] The pathophysiology of AMD is more complex but has also been linked to a reduction in retinal blood flow.[77] The diagnostic capacity of OCTA for early signs of retinal damage due to oxidative stress and investigation of retinal blood-flow will be discussed in Chapter Four.

4 CHAPTER FOUR

OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY

4.1 Introduction

There are currently various methods for imaging the eye and retina. OCT, an upgrade from retinal photography, was first developed in 1991 and uses interference reflectometry to display the retinal layers in cross section.[183] The first images of human ocular structures were successfully taken using OCT in 1993.[184] A key benefit to this technology is the ability to image the individual retinal layers in vivo, something that could previously only be done ex-vivo, using histological samples.[185] The contrast of OCT images is too low to differentiate between small blood vessels so it is not used to assess ocular vasculature in detail.[186] In order to check for irregular blood flow or leakage within the eye, fluorescent dye such as fluorescein or indocyanine green is injected into the blood stream and photographed passing through the ocular blood vessels.[11] This is FA or indocyanine green angiography (ICGA) and is considered to be the current gold standard in vascular imaging, although it is an invasive test.[11] Advancing on these technologies is OCTA which was developed in 2010 and allows parts of the retina and its vasculature to be imaged in three dimensions without the use of invasive fluorescein dye.[187] The ability to easily record blood flow is another major advantage of this new technology over OCT which lacks the depth-resolution and contrast to create these images.[188] The non-invasive nature of OCTA imaging has proven particularly useful during the recent pandemic which has required social distancing and necessitates quick, effective uncomplicated consultations.

4.1.1 Benefits of the Technology

Imaging using OCTA is a safe and fast method of examining ocular structures.[189] While FA is considered to be the gold standard in retinal vascular imaging, OCTA technology

has the potential to present higher contrast images than FA or ICGA as it is not hindered by hyper-fluorescence from the dyes used.[11,190] Until this new development, FA or ICGA were the only methods which would allow visualisation of the blood vessels in the FAZ by injecting a dye into the veins which binds to protein in the blood and fluoresces under a coloured filter.[191] These are the primary tests used to investigate retinal vasculature; however, there are some drawbacks associated with these examination methods.[3] It is necessary to inject fluorescein or indocyanine dye into the vein to perform the examination, which requires a high level of skill; and planning. Vomiting, nausea and more rarely, anaphylaxis (approximately 0.3%) have been reported in some cases.[15,192] Due to pooling and fluorescence of the dyes, it can also be difficult to view certain structures depending on the dye patterns. The images obtained from FA and ICGA are also one-dimensional and thus, the retinal layers cannot be segmented and viewed separately; while OCTA can easily provide three-dimensional images which offer improved insight into location of lesions or vessels within the tissue.[189] While it is currently limited by the field of view of the device, it may still allow for some disease detection without the need to subject patients to invasive testing.[193] The technology is also improving, with wide-field and montage imaging allowing more of the retina to be visualised with OCTA. It is also easily repeatable and not time dependent as with dye tests, as each image obtained with OCTA represents the complete vascular status of the retina.[194] The advent of OCTA allows eyes with no retinal vascular disease to be examined in more detail without the risks associated with conventional techniques.[192] Choroidal neovascular membranes, for example, can be visualised directly with this new technology.[195,196]

4.1.2 Basic Principles

Initially OCT, and then OCTA were first developed using the principle of time domain followed by spectral domain OCT.[197] The principle behind OCT is similar to ultrasound technology as it involves measuring the reflectance of light (or ultrasound) waves from a structure to produce an image.[198] In OCT, infrared light is used in place of ultrasound.[198] This allows much greater resolution of images (approximately 100 times greater than with ultrasound).[184] The light is passed through a beam-splitter creating two beams: one is sent to the biological sample under view while the other is reflected as a reference beam.[197] Spectral domain OCT uses a spectrometer to analyse the reflectance which is much faster than time domain which utilises mirrors for this purpose. The combination of these two beams creates an interference pattern which can be used to form an image. To visualise depth within tissue, the reflectance mirror or spectrometer is moved in an axial direction along the visual axis, creating what is known as an A-Scan which is a one-dimensional image of the ocular structures taken from the direction that light travels through the eye. Composite A-Scans can be formed by also moving the mirror or spectrometer transversely through the tissue, creating a two-dimensional transverse optical cross-section, known as a B-Scan.[197] Examples of A and B-Scans are shown in Figure 4.1.

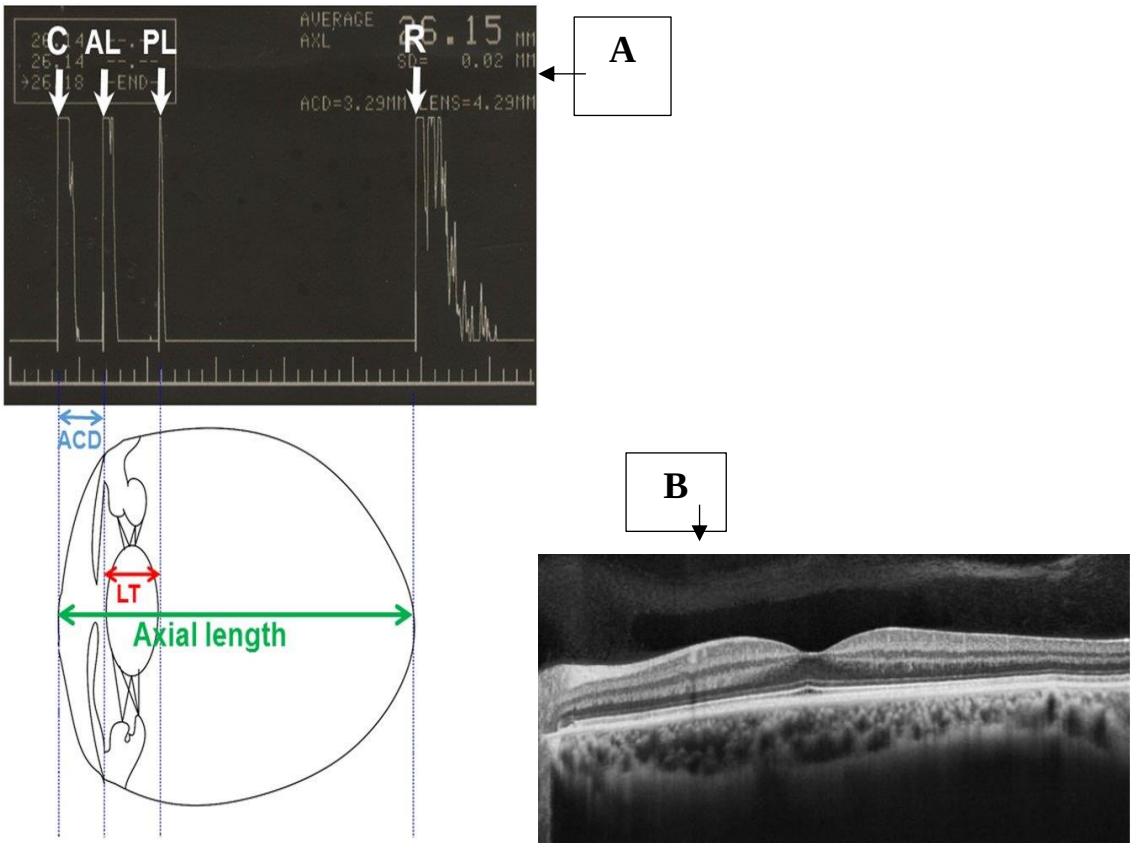

Figure 4.1 A and B. *The difference between A-scan and B-Scan. Figure 1A shows an A-scan in relation to the optical section, C; cornea, AL; axial length, PL; posterior lens surface, R; retina, ACD; anterior chamber depth, LT; lens thickness.*[199] *Figure 1B shows the more detailed B-scan made up of composite A-scans to show the retina in cross section. [Image: Author's Own]*

The Cirrus 5000, used in this study, is a spectral domain OCT device. It combines the capability of OCT and OCTA, providing structural information from cube scans and additional vascular information in angiography scans.[85] One of the main differences between OCT and OCTA is that OCTA can be used to identify movement within tissue. OCTA identifies movement of red blood cells, called erythrocytes, by scanning the same point in the eye at a rate of approximately 68,000 times per second, rates of acquisition depend on the instrument used.[187] An algorithm is used to analyse the collected data and produce angiographic images of the retina. Reducing the effect of miniscule eye movements and blinks is therefore important to ensure the images

obtained by OCTA are accurate.[190] ZEISS uses proprietary gaze tracking called FastTrac to accommodate this.[187] The Cirrus 5000 uses a proprietary algorithm called Angioplex, which has been validated, called Optical Microangiography to the power of Complex, to analyse the amplitude and phase structure of the OCT signals to form images.[74] It can therefore detect movement of blood cells as blood-flow. It must also be acknowledged that the blood vessels however, can cause a scotoma due to light scatter which may obscure some areas in the underlying tissue.[200] Software is improving all the time to identify and remove these artifacts from OCT and OCTA images.[201,202]

4.2 Scan Acquisition

Both OCT and OCTA scans can be quickly and easily acquired without the use of mydriatic drops. The routine for both is the same and is described here. A macular cube scan uses OCT to give structural information and can be followed by an OCTA scan if desired to give additional vascular information. To achieve an ideal scan, the image should be evenly illuminated, with no dark corners and in clear focus with the branching blood vessels visible. The pupil can be realigned to remove corneal reflections. If floaters present an issue, they can be moved by asking the subject to move their eyes up, down, right and left. The normative data of the Cirrus 5000 are based on 284 healthy adults aged 18-84 and refractive error between -12.00 to +8.00D.[203] Scans can still be performed on subjects outside this refractive error but caution should be used in analysing them as the instrument has no normative database for such subjects.[85] The Cirrus 5000 has two focus screens for the operator.[85] The iris should be brought into focus first with the pupil centre of the image in the upper screen. The lower screen shows the fundus, this image should be adjusted to bring the blood vessels just into focus. During the scan, the live B-scan can be seen on the acquisition screen. The B-Scan should be kept in the centre of the screen during acquisition for an optimal scan

quality, minor adjustments can be made during the scan to achieve this. After acquisition, the scan quality check screen is shown which displays the signal strength of the scan which is a value between 1 and 10.[85] Scans with signal strength below 6 are generally of poor quality and unacceptable.[85] Figure 4.2 shows the operator view.

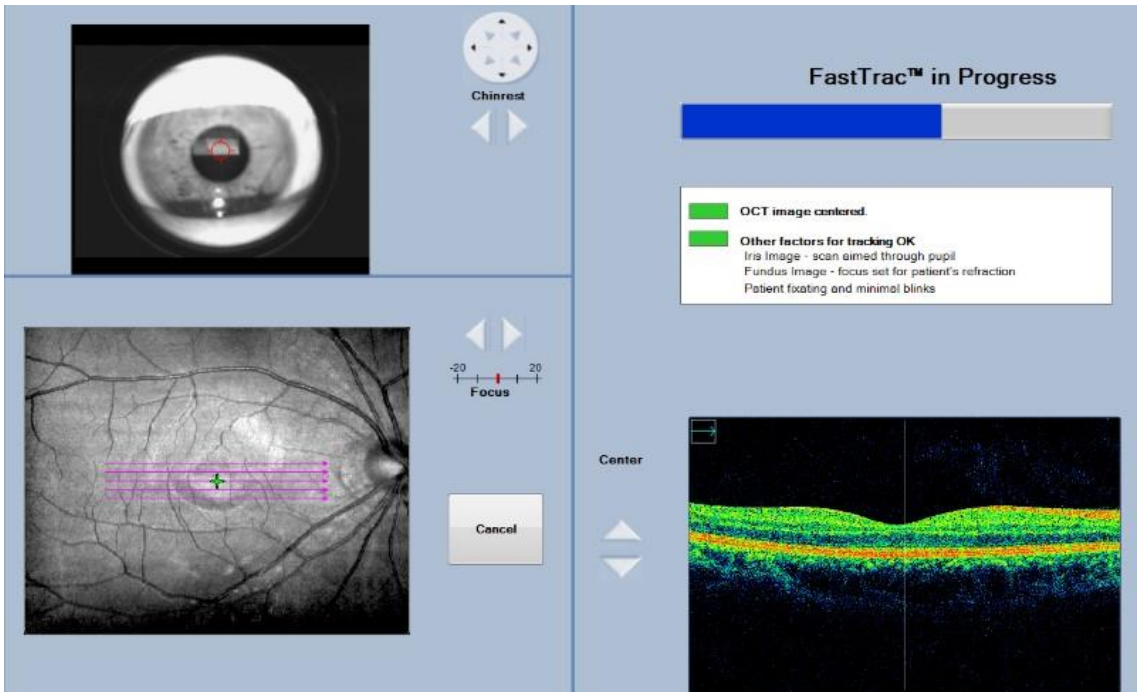

Figure 4.2. *Acquisition screen for Cirrus 5000. The images on the left side show the two focus screens while the live scan is shown on the bottom of the right side. FastTrac monitors the live scan and stops progress if alignment is lost and improves the scan by minimising errors.*[85]

4.2.1 Possible Scan Types

While anterior scans are also possible, the scans listed in this section refer to scans of the posterior segment of the human eye used in this study:

1. *Macular cube (200x200 or 512x128)*

This is a cube of data obtained through a 6mm grid using a series of horizontal line scans and one central B-scan. The difference between them is that the 200x200 uses 200

line scans composed of 200 A-scans while the 512x128 uses 128 line scans made up of 512 A-scans which gives a higher definition scan in a slightly longer acquisition time.[85]

2. Angiography Scan of the Macula (3x3mm, 6x6mm or 8x8mm)

This is very similar to the macular cube scan, but very detailed images of vasculature are possible due to an intensity-based frequency filtering technique. Each B-scan is repeated several times to detect change in contrast which is thought to be due to blood cell movement, thus detecting blood flow.[85] The size of each scan (3x3mm, 6x6mm and 8x8mm) refers to the field of view or size of the scan chosen. In relation to the ocular anatomy, the smallest scan focuses on the detail of the fovea, while the 6mm scan encompasses the perifoveal region and the 8mm scan includes some of the surrounding retina in the scan.[61] The metrics for each of these scans are not interchangeable i.e., vessel density from a 3x3mm scan will not be equivalent to a 6x6mm scan so care must be taken when assessing change that the exact same scan size is compared.[85]

3. Angiography Scan of Optic Disc (4.5x4.5mm)

This allows the vascular profile of the optic disc to be imaged in a similar way to the macular angiography scan. Repeated consecutive B-Scans are used to provide an angiographic image.[85]

4. Montage Angio Scan (6x6 or 8x8mm)

Because the field of view of the device is fixed, the montage scan allows a greater area of the retina to be imaged. The field of view can be increased to 10x14mm by using six 6x6mm montage or to 14x14mm using five 8x8mm montage scans. The montage image is created automatically by the software.[85]

4.3 Quantitative OCTA Features

Since its inception, OCTA has increasingly proven useful in detection and diagnosis of

vascular diseases. Some of the modalities it employs include measurement of vascular density and perfusion, FAZ area, circularity, diameter and perimeter.[185] There are an abundance of OCTA machines commercially available from various suppliers, each with variation in the features measured as well as the methods used. As such, comparisons cannot readily be made between different OCTA manufacturers. For the purposes of this study, the Cirrus 5000 with Angioplex will be discussed.

4.3.1 Vessel Density

This is the total length of perfused blood vessels per unit area of an OCTA image.[185] This would be equivalent to taking all the vessels in a particular area and measuring their length with a ruler, then dividing that number by the area they originally occupied.[85] The Angioplex software measures this in mm/mm^2. Other machines, such as the Triton OCTA (Topcon, Japan) represent this measurement as a percentage.[204] Decreased vessel density, perhaps due to capillary dropout, has been found in association with diseases such as DR, glaucoma, AMD and hypertension.[179,205–207]

4.3.2 Vessel Perfusion

Vessel perfusion is a percentage of the total area of perfused vasculature per unit area.[78] It is found by adding up all the pixels containing perfusion in an area and dividing the sum by the total number of pixels in that region.[85] Vessel perfusion may also be reduced in DR and Sun et al found that reduced blood flow was associated with hypertension.[42,206]

The main difference between vessel density and perfusion is that density treats all vessels equally while larger vessels will have a greater influence on perfusion. The vessel density measurement is more sensitive to capillary loss but is also more sensitive to visual noise.

4.3.3 FAZ Area

The FAZ area is defined as the area within the borders of the avascular zone in the fovea, as identified by the machine. It is possible to manually draw the FAZ outline if the automatically highlighted area is unsatisfactory. This is done using the edit function which opens a drawing tool and allows the FAZ area to be drawn using the computer mouse.[85] FAZ area is increased in certain diseases. Sun et al found the deep FAZ was larger in uncontrolled hypertension.[42] Fleissig et al found that both superficial and deep FAZ area was larger in subjects with Type Two diabetes than controls or those with Type One.[208] Superficial FAZ area can also increase in normotensive glaucoma.[207] While it is possible to image the deep FAZ, the Angioplex software provides detailed analysis for the superficial FAZ only.[85]

4.3.4 FAZ Perimeter and Diameter

The perimeter refers to the length of the boundary of the FAZ and is measured in mm. This is automatically identified by the machine. The perimeter of the FAZ can be larger in the presence of hypertension.[42] A callipers can also be used to manually measure anything shown on the B-scan. It can be placed within the FAZ to measure any diameter and is measured in μm.[85] Figure 4.3 shows the use of the callipers function to measure various diameters of the FAZ.

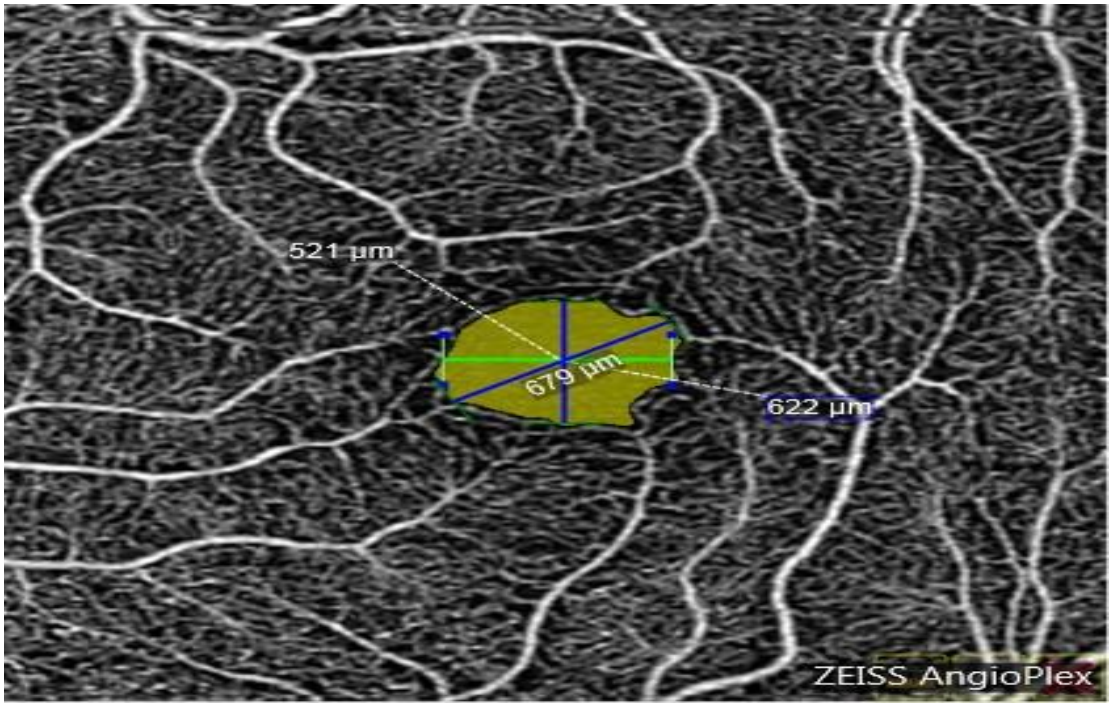

Figure 4.3. *Foveal avascular zone measured on Cirrus 5000. The FAZ is highlighted with lines drawn using callipers to measure horizontal, vertical and longest diameter in µm. [Image: Author's own]*

4.3.5 FAZ Circularity

This is a measurement of the regularity of the shape of the FAZ. It is the ratio of the measured FAZ perimeter compared to a reference circle of the same area.[185] It is shown as a number on a scale between 0 to 1, where 1 is a perfect circle.[7] Irregular circularity has an association with diabetes, for example, Krawitz et al found that circularity was reduced in eyes with DR when compared with controls, using a similar metric called acircularity index which differs from circularity in that 1 is a perfect circle and irregularity results in a value higher than 1.[3]

4.3.6 Macular Thickness

CMT measures the thickness of the macula in µm. Different OCTA machines vary in where they measure between, all use the ILM as the internal border, while the Cirrus 5000 measures to the RPE, the Spectralis (Heidelberg, Germany) reaches to Bruch's Membrane for example.[209] One study found a significant difference in CMT as

measured by the Cirrus 5000 and the Topcon 3D OCT 1000, (Topcon, Japan), possibly because the Topcon instrument measures to the border of the RPE, at a point slightly more anterior than the Cirrus 5000.[209]

4.3.7 Ganglion Cell Layer + Inner Plexiform Layer Thickness

Many OCTA machines analyse these two retinal layers as one, as the border between them appears quite weak in human eyes.[85,210] These layers were discussed in Chapter 2 and have been shown to have an intrinsic relationship, with synapses between bipolar cell axons and ganglion cell dendrites occurring in the IPL. Changes to these dendrites and damage to the ganglion cells are thought to occur early in glaucoma,[64,211] OCTA identifies this region between the RNFL and the beginning of the INL. GCL + IPL thickness reduces in glaucoma, therefore, examination of the GCL has become a useful diagnostic indicator of early glaucomatous change.[212]

4.4 OCTA and Ocular Diseases

The images created by OCTA are useful in the diagnosis and monitoring vascular diseases such as DR, AMD, hypertension and glaucoma due to its quick and non-invasive method of image capture.[17,18,20,213] The use of OCTA for analysis of retinal vasculature and perfusion has particular value in advanced glaucoma and in cases of high myopia where conventional visual field and optic nerve head analysis become less useful due to extensive nerve damage.[20] As will be discussed in the following sections, changes to FAZ area seem to occur in DR, AMD, glaucoma and hypertension while vessel density appears to decrease also.[42,214–216] In addition, diseases such as DR result in irregular FAZ shape while GCL + IPL thinning occurs in early stages of glaucoma.[3,20] The ability to quickly and non-invasively screen for these diseases is a major advantage of OCTA.

4.4.1 Value of Normative Data

More uses for OCTA in diagnosis and monitoring of ocular diseases are being found as the technology matures. As discussed in Section 4.3.1, there are subtle differences between various brands of OCTA machines. To this end, it is of great value to establish machine-specific normative parameters for measurements of FAZ area, vascular profile, macular thickness and GCL, so that deviations from these normative values can be flagged and further investigated.[202] FAZ area is considered to be larger in females than males and also tends to increase in size with advancing age.[1,2,4,46] However, Fujiwara et al found that age did not affect FAZ area before 40 years.[217] Various studies have found that age negatively affects vessel density and perfusion in healthy eyes.[1,2,206] FAZ size, shape and vascular profile appear to be altered in the presence of certain diseases, some of which will be discussed in this section.[3,20,216]

4.4.2 Diabetic Retinopathy

Progressive retinal ischemia contributes to the sight-threatening complications of DR, such as proliferative DR (growth of abnormal new vessels) and diabetic macular oedema, which is associated with uncontrolled inflammation and oxidative stress in the retina.[218] Standard tests for screening patients with diabetes for DR include fundus examination, fundus imaging and FA, if deemed necessary.[219] Classic signs of DR include microaneurysms, neovascularisation, macular oedema and intra-retinal microvascular abnormalities.[219] These signs can also be visualised using OCTA with the addition of other beneficial features such as capillary dropout and FAZ changes.[220,221] While FA is considered to be the gold standard for diagnosis of and monitoring progression of DR, studies have found that OCTA provides better discrimination of parafoveal macular capillaries, FAZ changes and capillary dropout when compared with FA images.[222–224]

The use of OCTA in the detection of preclinical diabetes has been of increasing interest to investigators in recent years.[219,225] In a small study, Thompson et al identified micro-aneurysms using OCTA in subjects with no apparent clinical signs of DR visible to the human eye, using dilated indirect fundoscopy.[221] Furthermore, OCTA can also be easily used as a screening tool in subjects with background or with no visible signs of DR where FA would not be advised due to possible complications relating to the invasive nature of the test.[5,191] This new technology also has advantages over FA in late stages of DR: intra-retinal microvascular abnormalities have also been identified more readily using OCTA when compared to fundus photography and may indicate more advanced retinopathy earlier than conventional methods can identify.[226] Neovascularisation, which occurs in more advanced stages of disease can also be identified by OCTA in higher quantities and sooner than it can be seen using fundoscopy or conventional imaging.[227]

The importance of the FAZ for optimal vision has been discussed In Chapter Two. Capillary non-perfusion in DR leads to death and drop-out of central capillaries and thus enlargement of the FAZ.[215] An association between DR and larger FAZ has been found in a number of studies.[181,228,229] It appears that FAZ area also increases as the stage of retinopathy advances.[5] The area, perimeter, diameter and circularity of the FAZ can all be quantified using OCTA which has proven useful in early detection of DR and may also be useful in monitoring progression of the disease.[219] As outlined in Section 4.3.1, FAZ size can vary among normal subjects due to a variety of reasons. It has therefore been suggested that FAZ shape or circularity may be a more useful prognostic indicator.[219,230] For example, Krawitz et al found that circularity decreased in subjects with DR in comparison with controls and appeared to decrease further with severity of the disease.[3] Durbin et al found that vessel density and perfusion were smaller, while

FAZ perimeter was larger in subjects with DR, compared with controls.[206] Cao et al found reduced vessel density in subjects with Type Two diabetes with no other clinically detectable signs of retinopathy.[231] According to Sun et al the presence of a larger FAZ area and lower vessel density in the deep capillary plexus predicted the development of DR, while lower vessel density in the superior plexus meant an increased likelihood of developing diabetic macular oedema.[232] If identified early, diabetes can be well managed or even prevented, reducing the likelihood of developing complications as associated with DR.[218] Early intervention and changes to lifestyle factors can prevent prediabetes from developing into Type Two diabetes.[233] Screening for this condition has the potential to reduce diabetes related blindness by 30-50%, thus a simple test such as OCTA, that can be used as a screening tool to detect early signs may be very useful in the management of this disease.[5,218]

4.4.3 Glaucoma

The causes of glaucoma are poorly understood. Low ocular perfusion pressure, which is the difference between IOP and mean arterial pressure is associated with this disease.[189] Glaucoma can be treated but the prognosis is improved if intervention is made early before extensive visual field loss occurs. The gold standard tests for glaucoma include perimetry, IOP measurement and examination of the optic disc. Recent studies have begun to uncover a use for OCT in glaucoma testing, given the GCL+ IPL appears to thin in early glaucoma due to the death of ganglion cells, axons and dendrites.[212,234] More recently, OCTA has also been used to examine the optic nerve head and measurement of peripapillary perfusion in addition to ganglion cell analysis.[189] One study found that macular vessel density was lower in subjects with glaucoma and minimal visual field loss and this reduced density corresponded with the area where glaucomatous field loss occurred.[235] In addition, Zivkovic et al found that FAZ area

increased and vessel density at the macula decreased in normal tension glaucoma.[207] The literature suggests that vascular change occurs prior to visual field loss in glaucoma.[207,235] This may indicate a new role for OCTA in the early diagnosis of glaucomatous change within the eye, perhaps indicating closer monitoring or intervention before irreversible visual field loss occurs.

4.4.4 Macular Degeneration

AMD is the leading cause of blindness in the older population and is associated with inflammatory response and oxidative stress.[149] While the use of OCTA in the diagnosis and management of AMD is in its infancy, some studies have reported a reduction in vessel density in eyes with early or intermediate AMD when compared with controls.[205,216] Assessment of FAZ area has proved controversial, with many studies finding an association between an increase in size and AMD, however, others have not drawn this conclusion.[205,216,236] Circularity and vascular profile appear to be more reliable markers in assessment of AMD.[216] Neovascular or wet AMD poses a significant threat to visual performance, although it is often treatable, if intervention is swift. Neovascular AMD is characterised by the growth of new vessels which are weak and easily leak fluid.[237] It causes disruption to the organisation of the retinal layers through fluid build-up.[238] Treatment involves injection with anti-vascular endothelial growth factor which halts the proliferation of these porous new vessels and thus stops fluid building up within the retina.[237] While structural OCT has become useful in identifying changes within the retinal layers which may relate to neovascular membranes, confirmation by FA is always required in order to visualise the membranes and leaking vessels.[216] There is certainly scope for increased use of OCTA in AMD management as the technology improves. This non-invasive testing method is of interest as it can be quickly and easily employed without risk of causing anaphylaxis and therefore could

potentially be used on a much wider scale than FA, testing for disease, screening and monitoring. OCTA is capable of imaging neovascular membranes which neither FA nor structural OCT can do.[239] The latter two are only capable of showing signs that indicate choroidal neovascularisation.[239] Precise segmentation of the retinal layers is paramount to the function of OCTA and by its nature, neovascular AMD makes accurate segmentation very difficult.[216,238] However, the definition and contrast provided by OCTA images are much higher when compared to FA as they are not impeded by leaking or pooling of fluorescent dye.[238] The ability to triage patients with a non-invasive method such as OCTA is of particular benefit in a pandemic situation, such as with Covid-19. The Cirrus 5000 has the added benefit of being possible to operate remotely with the use of screen sharing to another device to control the machine.[240]

4.4.5 Hypertension

The effects of hypertension can be seen in the retina on fundus examination, the eye is unique in that it is the only area in the body where these microvascular changes can be viewed directly.[42,241] Arteriovenous nicking, micro-aneurysms, retinal haemorrhages, cotton wool spots are all signs of ischemia within the eye that can be related to hypertension.[42,241] Cotton wool spots are lesions of neural axons caused by ischemia due to occlusion of the retinal arterioles.[242,243] Hypertensive retinopathy can be graded using the Keith Wagener-Barker classification from zero to four.[241] Grades one and two, in particular, are quite difficult to differentiate using normal fundoscopy or photography as they both describe mild signs which are difficult to detect and compare to normal vessel thickness. Table 4.1 shows this grading classification.

Table 4.1. *Keith Wagener-Barker classification of hypertensive retinopathy.*[241]

Grade	Feature
0	Normal
1	Mild generalised arteriolar narrowing
2	Definite focal narrowing and arteriovenous nipping
3	Signs of Grade 2 retinopathy and retinal haemorrhages, exudates and cotton wool spots
4	Severe Grade 3 retinopathy and papilloedema

If hypertension is addressed early with lifestyle changes such as adopting a healthy diet and losing weight, its effects can be mitigated.[172] A clinical test that could more easily identify and grade these early hypertensive changes could aid in timely intervention. OCTA has the potential to easily identify these early signs of hypertensive changes within the eye.[42] Hypertension studies using OCTA have found a reduction in RNFL and GCL + IPL thickness as well as reduced blood flow in subjects with hypertension.[241,244] Studies have also found that higher BP was associated with reduced number of capillaries in the deep plexus of the FAZ.[42,245,246] Donati et al also found that FAZ area appeared larger in subjects with hypertension compared with controls.[246] Given that changes to the appearance of retinal blood vessels are associated with hypertension and that it is difficult to accurately assess and grade these changes using conventional methods, measurement of capillary density could have potential as a prognostic indicator in retinal hypertension.[245–247]

4.4.6 Covid-19

The novel coronavirus, Covid-19, is another disease characterised by inflammation. It causes inflammatory reactions in the respiratory and digestive systems by binding to proteins and in particular to angiotensin-converting-enzyme 2, which leads to endothelial cell damage. The same enzyme is also found in the endothelium of ocular tissue.[248] Indeed the retina and choroid have a high metabolic demand and are highly

vascularised tissues, such that signs of inflammation including haemorrhages, cotton wool spots and venous dilation have been identified in eyes following Covid-19 infection.[32,249] Similarly, other ocular tissues have also shown signs of inflammatory events.[32] Some recent small OCTA studies, on post-Covid-19 subjects, have shown that vessel density and perfusion flow reduced and RNFL thickness decreased following infection with Covid-19.[8,32] One study even showed that macular blood flow was reduced up to 6 months after recovery from the disease.[248] While these studies are limited in size and quantity, given the novel nature of the illness, OCTA has proven to be a useful investigative test which is quick and non-invasive. It is possible that these vascular changes occur as a result of the coronavirus infection, however, more research using larger populations and longitudinal studies are required to verify the research.

4.5 Future of OCTA

FA and ICGA can be used to provide information about flow and leakage which no other similar investigative test currently does.[193] As OCTA detects movement of blood cells, it is therefore limited in its ability to detect leakage and pooling of blood that is stationary, as only flow can be detected.[194] Its limitations mean that OCTA may be a useful tool in addition to FA or ICGA in some cases but not fully replacing them at this time, however, with improvements in the technology being made, there is scope for OCTA to become even more useful.

4.5.1 Advances in Technology

As an emerging technology, OCTA has already shown great potential with its ease of use and ability to detect preclinical changes in a variety of ocular diseases, used in addition to FA or ICGA. As it continues to develop, we may see even more capabilities of OCTA emerge. Instruments, with faster acquisition times, have already been developed which improves the quality of the captured images, reducing artefacts from

minor eye movements or blinks.[201] In tandem with these developments, interest is growing in the area of artificial intelligence (AI), in all areas of life and medical technology is no exception.[201] An imaging modality such as OCTA is a perfect example of a technology that can benefit from the development of AI for the early detection and for monitoring the progression of eye diseases. It already employs the use of proprietary algorithms to analyse and interpret data. While AI is also perfectly suited to perform image capture, for example. Comprehensive normative data from all the various OCTA machines would be required to improve analysis by AI as it uses deep learning to recognise patterns and thus teach itself to identify particular signs. It can also be employed to recognise artefacts in acquired images and delete them, thus improving image quality.[201]

The current study used OCTA to examine the size, shape and vascular profile of the FAZ in young healthy subjects. These measurements were analysed along with MPOD measurements, BMI and other health markers. The detailed methodology will be discussed in Chapter Five.

5 CHAPTER FIVE

RESEARCH METHODOLOGY

This chapter describes the methodology used in this study. The testing protocol and instrumentation are discussed.

5.1 Subject Recruitment

One hundred and fifty-four participants were recruited mainly from the Technological University (TU) Dublin student cohort, staff and nearby office workers. One hundred and thirteen were white and 67.5% of all participants were female. Informed consent was obtained from all subjects to permit the use of their pseudo-anonymous data in accordance with General Data Protection Regulation (GDPR) guidelines. All subjects were assigned a letter code for research purposes (refer Appendix 1).

- Inclusion criteria: Subjects aged 18-35, free of ocular pathology (self-reported) and visual acuity ≥6/12 (LogMAR 0.3).
- Exclusion criteria: Subjects who had taken dietary supplements containing lutein or zeaxanthin in the six months prior to the study or had a refractive error > +/- 8.00DS were excluded. 5.2 Ethics, Data Management and Storage

Approval for this study was obtained from the TU Dublin ethics committee. The research adheres to the principles of the Declaration of Helsinki.

GDPR governs the protection and management of personal data. In accordance with this regulation, all soft data was wiped from individual instruments on a daily basis. Hardcopy data was anonymised and stored in a locked cabinet with restricted access within TU Dublin. Soft copies of OCTA measurements were stored on an encrypted Universal Serial Bus (USB).

5.3 Statistical Analysis

G*Power version 3.1 was used to calculate the minimum sample size required to achieve statistical significance. A priori analysis was carried out for two-tailed t-test, difference between two independent means; alpha = 0.05, power = 0.80, arbitrary effect size = 0.5; minimum sample size required = 128 subjects. Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) version 26.0 (IBM Corp, Armonk, NY, USA). The Kolmogorov-Smirnov test was used to assess the normality of the sample data. Parametric tests such as Independent samples t-test or analysis of variance (ANOVA) test was used to test for differences in normally distributed study parameters, whereas, a non-parametric test, the Kruskal-Wallis H test was used to test for differences in group medians in non-normally distributed data. Pearson's product-moment and Spearman's Rho were used to assess the relationship between FAZ area and other study variables where appropriate. Partial correlations were carried out to control for sex. All parametric data values were expressed as mean ± standard deviation throughout, while non-parametric data were expressed as median and range. A *P*-value of < 0.05 was considered significant. Graphical representations of correlations were presented using scatter plots. A multivariate linear regression was used to determine predictors of FAZ area.

5.4 Experimental Protocol and Techniques

5.4.1 Spectacle Prescription, Visual Acuity and Ocular Dominance

All measurements were taken by the author. Spectacle prescription, if worn, was measured on a manual focimeter, otherwise the non-cycloplegic refractive error was measured using an auto refractor (Dong Yang Rekto ORK-11 Auto Ref-Keratometer, Everview, Seoul, Korea). While the Cirrus 5000 is validated for refractive error up to ±12.00DS, subjects with myopia >-8.00DS were excluded to ensure good quality

scans.[85,217] Visual acuity was measured with the presenting correction in place using the LogMAR chart (Thompson Software Solutions, United Kingdom). The eye with the best acuity was used for the study, as FAZ size and area have previously shown inter-individual symmetry.[250] If acuity was equal, the dominant eye was chosen which was identified using the sighting method (Miles test), which was repeated three times for each participant.[251] Subjects with VA lower than 6/12 were excluded as it is more difficult to measure MPOD if VA is less than 6/12, as the small target must be visible to the subject in order to identify flicker.[129]

5.4.2 Demographic Information and Dietary Questionnaire

Participants answered a number of questions detailing their demographic information. The questions detailed general and ocular health, smoking history (non-smoker, current or past), age and ethnicity (refer Appendix 2). They also completed a food frequency questionnaire, known as the LZ screener (refer Appendix 2).[252] The data from this dietary questionnaire were reviewed for accuracy and completeness within the fields and later transferred to an Excel spreadsheet (Microsoft, USA Version 2201) for analysis. This questionnaire assessed lutein and zeaxanthin intake from foods such as eggs, broccoli, corn and green leafy vegetables, weighted according to frequency of intake and bioavailability of the food. Participants were categorised as low, medium or high based on their intake.[141] Based on the frequency of different foods eaten, participants were assigned a grouping from 1-3, according to the amount of lutein and zeaxanthin estimated to be in the diet; 1 being the lowest scoring category and 3 the highest scoring. An example of the questionnaire is shown in Appendix B.

5.4.3 Blood Pressure

BP was measured using an Omron M2 Digital Sphygmomanometer (Omron, Netherlands) with the subject seated upright and relaxed with feet flat on the floor. They were asked to remove any tight or thick clothing from the upper arm. The arm cuff was applied to the right arm in all cases for convenience. The blue strip was centred on the subject's arm, with the air tube running down the inside of the forearm. The bottom of the cuff was placed 1-2centimetres (cms) above the elbow, fitting snugly around the arm with no kinks in the tubing. The subject's arm rested on the arm of the chair to keep the cuff at the same level as the heart and the subject was instructed not to move or talk during the measurement and to breath normally, with fists unclenched. The cuff completely inflated, then deflated again during the measurements and the subject was asked to stay perfectly still until all measurements were finished. Two sets of readings for systolic and diastolic pressure were taken and averaged. A third reading was included if there was a large variation between the first two readings. Participants were categorised into groups according to their hypertensive status: normal (systolic BP ≤120mmHg, diastolic BP ≤ 80mmHg), pre-hypertensive (systolic BP =121-139mmHg, diastolic BP = 81-89mmHg) and hypertensive (systolic BP ≥ 140mmHg, diastolic BP ≥ 90mmHg) as described by Schwartz & Sheps, (1999).[253,254]

5.4.4 Measurement of Macular Pigment Optical Density

MPOD was measured using the macular metrics clinical densitometer and is expressed as OD. The methodology of the macular metrics densitometer has been described by Wooten et al. [122] For the current study, the customised method was used which customises the task for each subject, as described by Stringham et al.[129] The subject's initials and date of birth were entered into the machine. The initial flicker frequency and luminance was then set based on the subject's age. The purpose and procedure of the

test were explained to each subject before beginning and the subject was reminded to blink frequently during this test. Spectacle correction was worn if required and one eye (the pre-determined test eye) was examined in each case. During the test, the subject fixated an object which initially appeared to be flickering. Each subject was afforded a practice session before beginning the test proper. In the first instance, the operator controlled the rate of change of flicker and the subject was asked to report when flicker was no longer perceived. This point is known as the null zone. This was repeated until the subject was confident in recognising this null zone. For the test proper, the subject could control the rate of change. If the null zone could not be identified i.e., flicker never appeared to cease, the flicker frequency was increased until the null zone could be perceived. Conversely, the flicker frequency was reduced if the null zone appeared too large. Five readings were taken, each at 0.5 degrees eccentricity and at 7 degrees eccentricity. The test was considered reliable if the standard deviation of the measurements was less than 0.05. Figure 5.1 shows an example of the targets seen in the Densitometer.

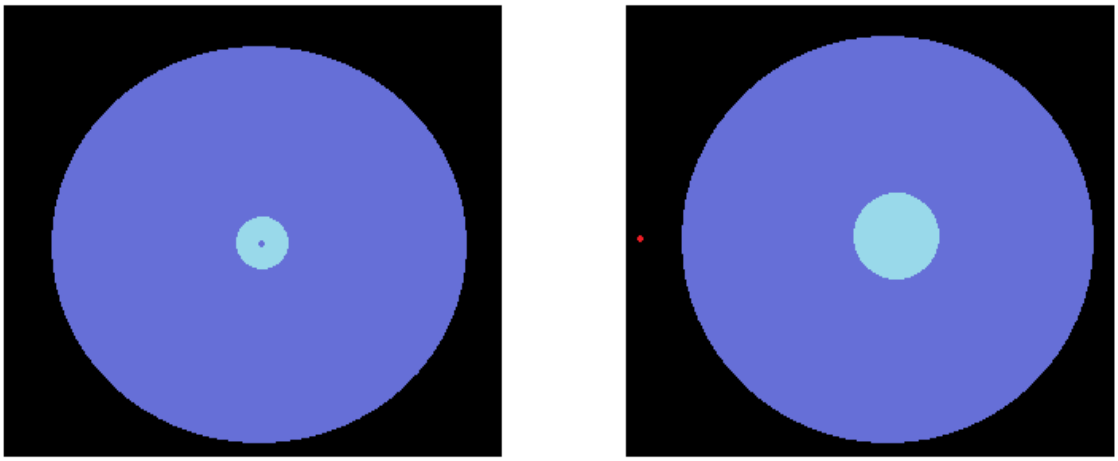

Figure 5.1. *An approximate example of the targets seen in the Densitometer. The foveal test field is displayed on the left and the parafoveal field is shown on the right [Image: Author's own, not to scale].*

For analysis purposes, MPOD values were divided around the mean into low (≤ 0.4 OD and high (> 0.4) OD.

5.4.5 Optical Biometry

Axial length was measured using the IOL Master (ZEISS, CA). Instructions for use of the IOL Master 4 are detailed on Dr Warren Hill's website.[255] The subject's details were entered into the machine's database. No details were saved to the machine. Measurements were only performed on the chosen test eye. The subject fixated a red light for axial length measurement. The IOL Master uses partial coherence interferometry with a Michelson interferometer to perform an A-scan to measure axial length. This is based on a composite signal, which means that the signal improves with every scan taken. Good readings are indicated by the signal to noise ratio on screen which must be at least greater than two. This is denoted by a green traffic light shown on screen and a representation of the peak. A minimum of five good readings are required for an adequate composite signal.

5.4.6 Anthropomorphic Measurements

These measurements were taken following the protocol outlined by The Irish Longitudinal Study on Aging.[256]

Height

Participants were required to remove all heavy outer garments e.g., coat/jacket and shoes. The subject then stood on the platform with their back to the measuring rod, knees straight with feet together. A ruler was placed on top of the head to accurately measure to the top of the head and taken to the nearest cm.

Waist

Waist was measured midway in cms between the iliac crest and the lower rib (costal margin) with a measuring tape. The subject was asked to remove all heavy outer garments but if the midway point corresponded to the waistband of trousers or skirt, the waist was measured outside the waistband. The subject was asked to breathe out gently and look straight ahead, the tape was to be kept horizontal and measurement to the nearest mm was taken at the end of expiration. In situations where the waistband was not level all around, waist was measured on the waistband where higher and off the waistband where it was lower.

Waist to Height Ratio

The ratio of waist to height measurement was calculated and participants were divided into normal (≤ 0.5) and high (> 0.5) groups for analysis.[257]

Weight

Weight was measured in kg to one decimal place using the Tanita Bioelectrical Impedance analyser (BIA) (Tanita Europe BV, Amsterdam, The Netherlands). The procedure for this machine will be described in Section 5.4.7.

Body Mass Index

BMI was calculated from measured height and weight as: weight (kg)/height (m^2). For this study, BMI was categorised as normal (≤ 25 kg/m^2) and overweight (> 25 kg/m^2), according to World Health Organisation guidelines.[258]

5.4.7 Tanita Bioelectrical Impedance Analysis

Bioelectrical impedance is a quick, non-invasive method of measuring body composition.[259] The measurement is achieved by measuring the resistance of a small safe electrical current (approximately 50 Kilohertz) as it passes through different tissues in the body.[260] Impedance is a measure of the inherent resistance to electrical current within the body. Muscle conducts an electrical current, giving a lower impedance value, while fat resists it, resulting in a higher value.[261] This simple test can determine a variety of measurements within the body including, BMI, trunk fat % and impedance (Ω). Trunk fat % is the percentage of the weight of the body's core taken up with fat (mainly visceral fat).[261] Trunk fat % was divided around the mean for males and females and categorised as normal (males = ≤ 33, females = ≤ 30) and high (males = > 33, females = > 30).[112]

Participants were asked to remove all heavy garments and shoes and socks (as before). If nylon socks were worn which could not be removed, a small drop of saline solution was placed on the weighing platform to allow an electrical current to flow.

The clothes weight was entered for each test, a default of 0.8kg was used for all participants. Age was entered next and then height in cms. The subject then stepped onto the platform with bare feet touching the electrodes and stood facing forwards in a stable position without bending the knees. They then held the two handles out from the body at either side and slightly in front to ensure the arms were not in contact with the

sides of the body. The results automatically printed out once all measurements were complete.

5.4.8 Optical Coherence Tomography Angiography

For these measurements, the subject's date of birth was input into the Cirrus 5000 machine. The machine generated an identification number for each subject to ensure anonymity. Two scans were performed on the subject's test eye which was pre-determined as either the eye with better acuity or the dominant eye. Ordinary macular cube was carried out first, followed by OCTA 3x3mm angiography scan. The procedure was the same for both scans.

The subject placed their chin on the left chin rest to measure the right eye and on the right chin rest to measure the left eye. Sensors within the chin and forehead rest identify where the subject rests their chin and will default to measuring that eye. The chin and forehead rests are designed to optimise the positioning of the eye as shown in Figure 5.2. The coloured lines on the forehead rest indicate left (blue) and right (white). When the subject rests on the left-hand side, the right eye is optimally placed for scanning, and vice versa.

Figure 5.2. *Adapted chin and forehead rest.*[85]

There were two focus screens for the operator. The iris was brought into focus first with the pupil centre of the image in the upper screen. The lower screen showed the fundus, this image was adjusted to bring the blood vessels just into focus. The optimise button was used to improve scan quality. During the acquisition, which takes a few seconds, the subject was instructed to fixate the centre of a green star superimposed on a black background (see Figure 5.3) and to blink often before the test begins but not to blink or follow the red line during the scan.

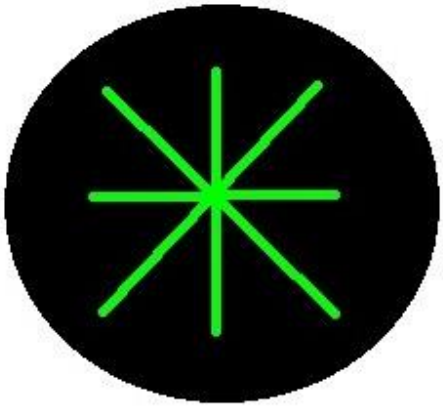

Figure 5.3. *An approximate example of the fixation star used in the Cirrus 5000. [Image: author's own, not to scale]*

The live B-scan could be seen by the operator on the acquisition screen. The B-scan was kept in the centre of the screen using the mouse wheel during acquisition for an optimal scan quality, minor adjustments could be made during the scan to achieve this. Once the scan was finished, the operator checked the scan for quality. It was graded in terms of acceptability, which was noted on the printout. The FAZ area was highlighted by clicking the FAZ button. This area could be adjusted if the researcher deemed it necessary, for example if the software had not picked up the FAZ. An inbuilt callipers

was then opened and used to measure the horizontal, vertical and longest diameters. Vessel density and perfusion were also assessed numerically. A greyscale photograph of the macular region was examined to confirm the relevant ocular health of all eligible participants. All required parameters were saved to an encrypted USB in PDF format. Images were also saved anonymously.

Chapters Six & Seven will detail the procedures and results of Study One: Normative Data on the Foveal Avascular Zone in a Young Healthy Irish Population using Optical Coherence Tomography Angiography and Study Two: Investigation of Factors Associated with Retinal Oxidative Stress and Inflammation that affect the Foveal Avascular Zone in Healthy Eyes: An Optical Coherence Tomography Angiography Study.

EXPERIMENTAL WORK, RESULTS AND ANALYSIS

6 CHAPTER SIX

NORMATIVE DATA ON THE FOVEAL AVASCULAR ZONE IN A YOUNG HEALTHY IRISH POPULATION USING OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY

6.1 ABSTRACT

Purpose

To establish normative data on the size, shape and vascular profile of the FAZ in a young, healthy, Irish population, using the Cirrus 5000. Certain diseases may alter FAZ appearance. Normative databases provide normal baseline values for comparison, thus improving diagnostic ability.

Methods

One hundred and fifty-four participants aged 18-35 years old were recruited, 67.5% were female. Superficial FAZ area, diameter, circularity, GCL, CMT, vascular perfusion and density were measured using the Cirrus 5000. Axial length was measured with the IOL Master and BP was measured using the Omron sphygmomanometer.

Results

Mean FAZ area was 0.22±0.07mm^2, mean CMT was 263.08±18.73µm. Both were larger in females than males (p <0.05, p < 0.01). Mean vessel density and perfusion central were 14.11±2.77mm/mm^2 and 24.70±4.96% respectively. Both were lower in females (p < 0.05 for both). Vessel density and perfusion inner correlated positively with minimum GCL+ IPL thickness (p < 0.05 for both). CMT correlated positively with vessel density and perfusion central (p < 0.01 for both) and negatively with FAZ area (p < 0.01).

Conclusions

This study provides normative data for FAZ appearance and vascularity for the first time in a young, healthy, Irish population, using the Cirrus 5000. Establishing machine and population specific normative data, particularly in relation to vessel density and perfusion, is paramount to the early identification of ocular disease using OCTA.

6.2 INTRODUCTION

The FAZ is a region in the fovea devoid of blood vessels. Absence of vessels in this area reduces light scatter, allowing light to pass to photoreceptors unimpeded, thereby, providing precise vision from this region of the macula.[81] While the FAZ is surrounded by a capillary ring, nutrients are provided to this region by the choriocapillaris.[262] The FAZ can be visualised using FA, however, with OCTA, this area can now be examined in greater detail, non-invasively.[187]

The current study used the Cirrus 5000, which identifies the movement of red blood cells by scanning the retina approximately 68,000 times per second.[187] An algorithm provides analysis, detecting movement as blood flow and producing three-dimensional angiographic images.[187] The software is the Angioplex metrix which measures FAZ area, circularity, perimeter, diameter, vessel density and vessel perfusion. Circularity index is a scale from 0 to 1 of how circular an object is, 1 being a perfect circle.[7] Angioplex measures vessel density as the total length of perfused blood vessels per unit area, represented as mm/mm^2. Vessel perfusion is a percentage of the total area of perfused vasculature per unit area in the same region.[78] As there is no standardised method of representing this information, comparisons cannot be made between different OCTA machines. Therefore, normative data are required for each commercially available instrument. Vessel density and perfusion are subdivided into sections according to the ETDRS in circles of 1mm, 3mm and 6mm diameter.[5] An example is shown in Figure 6.1A. Vessel density and perfusion density are measured within central, inner and full zones which are shown in Figure 6.1B.

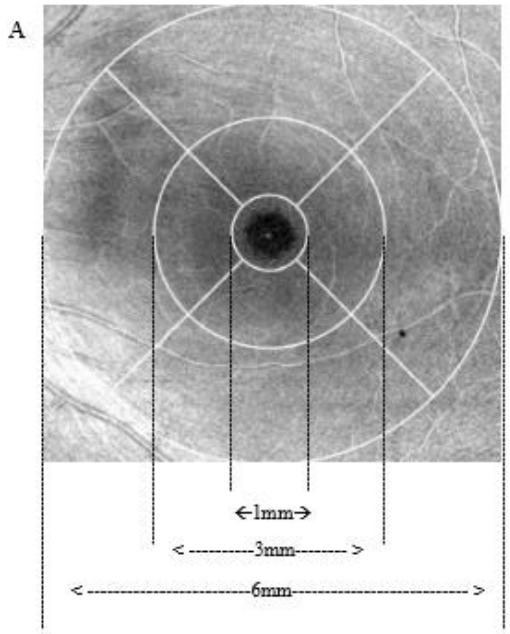

Figure 6.1. *A. ETDRS grid centred on the macula. B. Cirrus Metrix showing central, inner and full ETDRS measurement zones. The central zone corresponds to a circle of 1mm diameter, the inner zone is the outer 1mm radius ring and the full zone is a disc of 6mm diameter as shown in Figure 1A. [Image: Author's Own].*

FAZ size appears to increase with age while vessel density decreases.[1,2] FAZ size is generally larger in females than in males.[1,4] CMT has been found to be smaller in females.[4] Larger FAZ area has been associated with decreased CMT, suggesting that a thinner retina requires less blood supply and, therefore, will have a larger FAZ.[4,217] Additionally, longer axial length has been associated with larger FAZ area and decreased vessel density.[230]

Normative data for FAZ parameters are valuable in understanding the possible relationship they may have with disease process. This is particularly relevant to diseases characterised by blood flow and perfusion such as diabetes, multiple sclerosis and glaucoma.[3,20,263] Increased FAZ size and irregular shape may have diagnostic importance in diabetes.[3] Di et al found greater variability in FAZ

appearance between participants without DR compared to those with it, indicating that FAZ changes could possibly predict disease.[229] The invasive nature of FA means, however, that it is not routinely used on participants with mild DR or prediabetes.[229] Therefore, with OCTA, FAZ changes in these participants can now be investigated.[229]

The purpose of the current study is to establish normative data relating to FAZ size, shape and vascular profile in a young, healthy Irish population. There is a need for machine-specific normative data to make valid comparisons when using OCTA.[264]

6.3 METHODS

Approval was obtained from the TU Dublin ethics committee. Participants were recruited mainly from the TU Dublin student cohort in accordance with the principles of the Declaration of Helsinki.

Participants

Healthy participants aged 18-35 years old with visual acuity ≥ 6/12 were included. Exclusion criteria was refractive error ≥ +/-8.00DS spherical equivalent. Because inter-ocular symmetry of the FAZ area has been demonstrated, one eye of each participant was examined.[265]

Ocular and Clinical Examinations

Spectacle prescription, if worn, was measured on a manual focimeter, otherwise the dry refractive error was measured using an autorefractor. Visual acuity was measured with appropriate correction in place and the eye with the best acuity was used. In case of equal acuity, the dominant eye was selected, using the Miles test.[251] Axial length was

measured using the IOL Master. BP was measured using an M3 Digital Sphygmomanometer.

Optical Coherence Tomography Angiography

The OCTA scans were performed using the Cirrus 5000. A 200 x 200 mm macular cube was imaged by a single skilled examiner, followed by a 3 x 3 mm OCTA scan. Scans with a signal strength of 8 or higher were accepted. Macular health was confirmed by viewing a photograph from the macular cube scan and by self-reported medical history. CMT and ganglion cell thickness were obtained from the macular cube. The Angioplex metrix identified FAZ area, perimeter, circularity, vessel density and perfusion in the superficial FAZ, which Angioplex defines as the layer from the ILM to the IPL.[85] In cases where the FAZ was incorrectly or not identified, this was performed manually. The machine's internal callipers measured the horizontal, vertical and longest diameters of the FAZ.

Statistical Analysis

Statistical analysis was carried out using SPSS version 26.0 (IBM Corp, Armonk, NY, USA). Normality of the data was assessed using the Kolmogorov-Smirnov test. An independent samples t-test was used to test for differences in normally distributed study parameters, whereas the Kruskal-Wallis H test was used for non-normally distributed data. Pearson's product-moment and Spearman's Rho were used to assess the relationship between FAZ area and other study variables where appropriate. Partial correlations were carried out to control for sex. All values were expressed as mean ± standard deviation throughout. A *P*-value of < 0.05 was considered significant.

6.4 RESULTS

Demographic Data

Of the 154 participants recruited, 12 were excluded due to underlying systemic health conditions. Ninety-seven of the remaining participants were female. The mean age for the total group of 142 was 24.17 ± 4.92 (range 18-34). Demographic data are shown in Table 6.1.

Table 6.1. *Demographic characteristics of the study group (n=142)*

Characteristic	**n**	**%**
Sex		
Male	45	31.7
Female	97	68.3
Ethnicity		
White	115	81
Mixed	7	4.9
South Asian	8	5.6
Pakistani	5	3.5
Black	6	4.2
Hispanic	1	0.7
Blood Pressure		
Non-hypertensive	133	93.7
Hypertensive	9	12.6

Hypertension: Systolic blood pressure > 140mmHg and/or diastolic blood pressure >90mmHg.

Analysis

Data were analysed for both the full group (n=142) and whites only (n=115). While there were no significant differences in *P* values for the main findings between the two groups, the subsequent analysis is reported for the whites only group (n = 115).

FAZ characteristics

FAZ characteristics and differences between males and females are shown in Table 6.2. Mean and standard deviation for the FAZ area were 0.22 ± 0.07mm^2. Females had

significantly larger FAZ area (0.23 ± 0.07mm^2) than males (0.19 ± 0.08mm^2), (p < 0.01). FAZ perimeter and diameters were all significantly larger in females (p < 0.01 for all). Median FAZ circularity was 0.71 with no significant difference between the sexes (p > 0.05).

Vessel Density and Perfusion

Normative data for vessel density and perfusion in males and females are shown in Table 6.2. The mean and standard deviation for vessel density and perfusion central were 14.11 ± 2.77mm/mm^2 and 24.70 ± 4.96% respectively. Both were significantly smaller in females (13.66 ± 2.55mm/mm^2 and 23.96 ± 4.92%) than in males (15.06 ± 3.02mm/mm^2 and 26.26±5.35%) (p < 0.05 for all). BP was also lower in females than in males (p < 0.05).

Structural Parameters

The mean axial length for the whole group was 23.88 ± 1.16mm (range 20.6 - 27.16). There was no statistically significant difference in axial length based on sex (p > 0.05), as shown in Table 6.2. The mean CMT was 263.08 ± 18.73µm and this was lower in females than in males (p < 0.05).

Table 6.2. *Demographic and structural characteristics by sex (n=115, 67.8% female). Independent samples t- test. †Independent samples Kruskal-Wallis test of medians.*

	Total	Male	Female	*p* value
Age	22.17 (18-34.33)	26.50 (18.33-34.33)	21.45 (18-33.17)	**0.05*†**
FAZ area (mm^2)	0.22 ± 0.07	0.19 ± 0.08	0.23 ± 0.07	**0.02***
Circularity	0.71 (0.48-0.86)	0.69 (0.48-0.82)	0.72 (0.49-0.86)	0.18†
Perimeter (mm)	1.94 ± 0.07	1.84 ± 0.38	1.99 ± 0.32	**0.03***
Horizontal diameter(µm)	565 (265-829)	522 (272-829)	586 (256-750)	**0.03***†
Vertical diameter (µm)	508.26 ± 102.68	471.86 ± 113.57	525.53 ± 92.95	**0.00***
Longest Diameter (µm)	614.71 ± 103.88	579.11 ± 111.50	631.60 ± 96.26	**0.01***
Vessel Density central (mm/mm^2)	14.11 ± 2.77	15.06 ± 3.02	13.66 ± 2.55	**0.01***
Vessel Density Inner (mm/mm^2)	23.22 ± 1.01	23.25 ± 0.86	23.21 ± 0.98	0.81
Vessel Density Full (mm/mm^2)	22.20 ± 1.01	22.32 ± 0.99	22.14 ± 1.03	0.37
Vessel Perfusion central (%)	24.70 ± 4.96	26.26 ± 5.35	23.96 ± 4.92	**0.02***
Vessel Perfusion inner	41.55 ± 1.47	41.59 ± 1.35	41.53 ± 1.52	0.82
Vessel Perfusion full	39.65 ± 1.65	39.87 ± 1.56	39.54 ± 1.70	0.33
CMT (µm)	263.08 ± 18.73	274.70 ± 19.56	257.56 ± 15.65	**0.00***
Average GCL+IPL thickness (µm)	83.19 ± 5.35	83.93 ± 6.04	82.85 ± 4.99	0.32
Minimum GCL +IPL thickness (µm)	81.93 ± 5.49	82.81 ± 6.32	81.51 ± 5.03	0.24
Axial length	23.88 ± 1.16	23.91 ± 1.25	23.87 ± 1.12	0.89
Systolic BP	118.89 ± 11.80	126.97 ± 10.19	115.06 ± 10.54	**0.00***
Diastolic BP	78.31 ± 8.09	81.40 ± 6.43	76.84 ± 8.41	**0.00***

**Difference is significant at the 0.05 level.*

Abbreviations: FAZ; foveal avascular zone, CMT; central macular thickness, Avg GCL + IPL thickness; average ganglion cell layer and inner plexiform layer thickness, Min GCL+ IPL thickness; minimum ganglion cell layer and inner plexiform layer thickness.

FAZ Measurements and Vessel Density and Perfusion

Correlations between FAZ size, vessel density and perfusion are shown in Table 6.3. As expected, vessel density central and full correlated negatively with FAZ area (Pearson's r = -0.707, -0.350, $p < 0.01$) and perimeter (Pearson's r = -0.664, -0.326, $p < 0.01$). Vessel perfusion central and full correlated negatively with FAZ area (Pearson's r = -0.684, -0.323, $p < 0.01$) respectively and perimeter (Pearson's r= -0.636, -0.305, $p < 0.01$) respectively.

Table 6.3. *Partial correlations between FAZ measurements and vessel density and perfusion and blood pressure controlling for sex (n= 115).*

Pearson Correlation test⍊; Spearman's Rho test ǂ; Correlation is significant at 0.05

	FAZ Area	Circularity	Perimeter	Horizontal Diameter	Vertical Diameter	Longest Diameter
Vessel Density Central	**-0.707⍊****	-0.173ǂ	**-0.664⍊****	**-0.694ǂ****	**-0.612⍊****	**-0.692⍊****
Vessel Density Inner	-0.160⍊	-0.044ǂ	-0.148⍊	-0.127ǂ	-0.176⍊	-0.125⍊
Vessel Density Full	**-0.350⍊****	-0.098ǂ	**-0.326⍊****	**-0.306ǂ****	**-0.333⍊****	**-0.314⍊****
Vessel Perfusion Central	**-0.684⍊****	**-0.186ǂ***	**-0.636⍊****	**-0.672ǂ****	**-0.588⍊****	**-0.663⍊****
Vessel Perfusion Inner	-0.122⍊	-0.039ǂ	-0.118⍊	-0.088ǂ	-0.130⍊	-0.103⍊
Vessel Perfusion Full	**-0.323⍊****	-0.089ǂ	**-0.305⍊****	**-0.275ǂ****	**-0.301⍊****	**-0.300⍊****
Mean Systolic BP	**0.252⍊****	0.016ǂ	**0.241⍊****	**0.192ǂ***	**0.206⍊***	**0.227⍊***
Mean Diastolic BP	0.163⍊	-0.052ǂ	0.173⍊	**0.210ǂ***	0.170⍊	0.117⍊

level; Correlation is significant at 0.01 level**. Significant correlations are shown in bold.*

Abbreviations: FAZ; foveal avascular zone, BP; blood pressure.

CMT, Vessel Density, Perfusion, and GCL + IPL thickness

CMT correlated negatively with FAZ area, (Pearson's r = -0.594, $p < 0.01$), perimeter (Pearson's r = -0.566, $p < 0.01$), and diameters as shown in Figure 6.2 (A), (B), (C) and (D). CMT showed significant positive correlations with vessel density and perfusion central and full (Pearson's r = 0.644, 0.320, 0.637, and 0.342, $p < 0.01$ for all). No significant correlation was found between CMT and axial length ($p > 0.05$). Average GCL+ IPL thickness showed a positive correlation with vessel density inner, (Pearson's r = 0.292, $p < 0.01$). Minimum GCL + IPL thickness showed positive correlations with both vessel density inner and full (Pearson's r = 0.309, $p < 0.01$, 0.235, $p < 0.05$) and vessel perfusion inner, Pearson's r = 0.220, $p < 0.05$). These correlations are shown in Table 6.4.

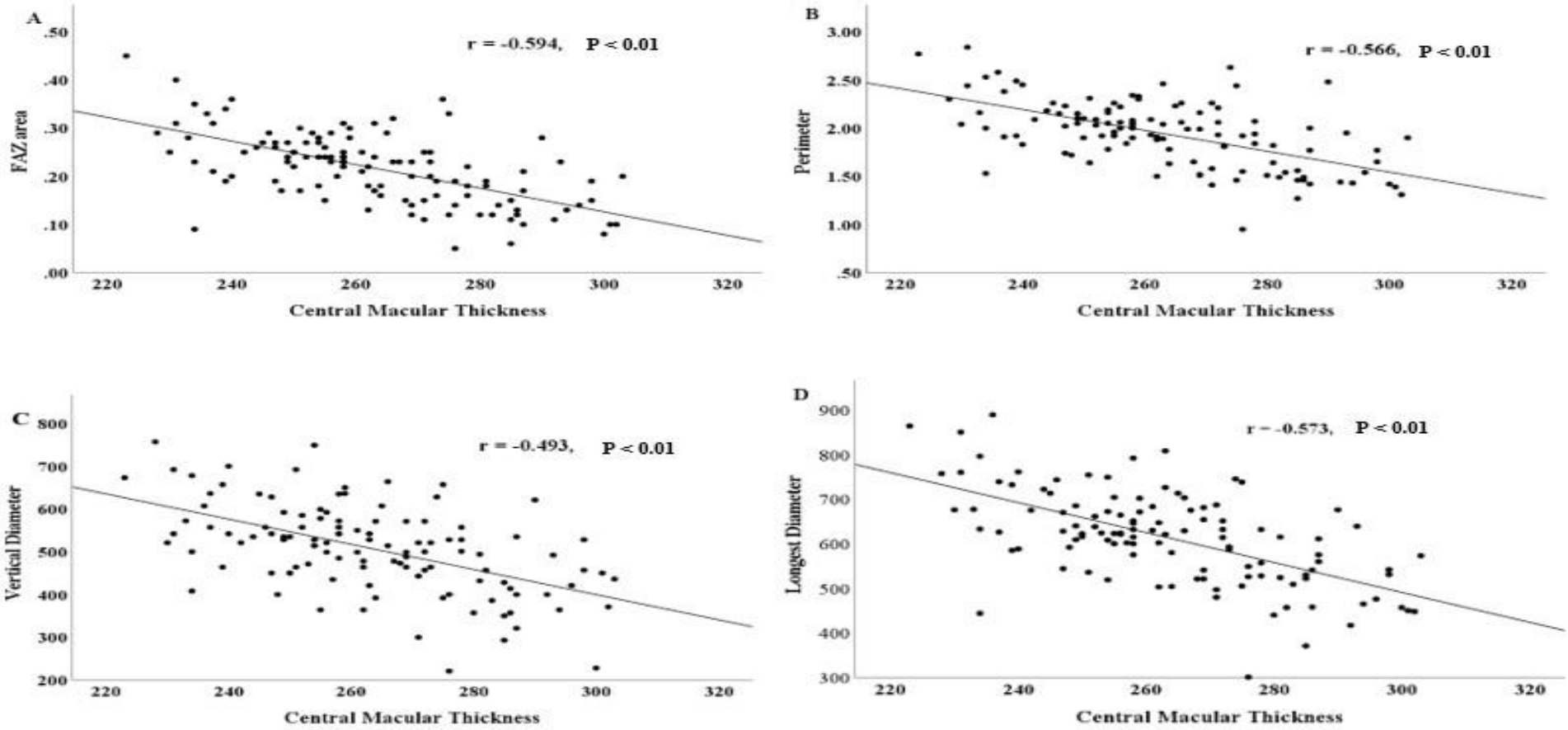

Figure 6.2. *Significant negative partial correlations between CMT and (A) FAZ area; (B) perimeter; (C) vertical diameter and (D) longest diameter. These findings indicate that a larger FAZ area is associated with lower CMT.*

Abbreviations: CMT, central macular thickness; FAZ, foveal avascular zone.

Table 6.4. *Partial Correlations of vessel density and perfusion and structural parameters controlling for ~~sex~~, (n=115).*

	Vessel Density Central	Vessel Density Inner	Vessel Density Full	Vessel Perfusion Central	Vessel Perfusion Inner	Vessel Perfusion Full
Axial Length	0.154	0.091	0.124	0.074	-0.081	-0.040
Avg GCL+IPL Thickness	-0.052	**0.292****	0.221	-0.034	0.244	0.182
Min GCL +IPL Thickness	-0.056	**0.309****	**0.235***	-0.051	**0.220***	0.160
CMT	**0.644****	0.145	**0.320****	**0.637****	0.153	**0.342****

Pearson Correlation test; Correlation is significant at 0.05 level; Correlation is significant at 0.01 level **. Significant correlations are shown in bold.*

Abbreviations: CMT; central macular thickness, Avg GCL + IPL thickness; average ganglion cell layer and inner plexiform layer thickness, Min GCL+ IPL thickness; minimum ganglion cell layer and inner plexiform layer thickness.

6.5 DISCUSSION

Imaging using OCTA represents a non-invasive, simple test with great diagnostic potential, given that FAZ area and vascular changes have been associated with various diseases.[3,20,229,263] While several studies have examined FAZ area and vascular profile in various ethnicities, relatively few studies report on a predominantly white, young, healthy population. A considerable amount of normative data exists for the AngioVue machine, (Optovue, USA) and the Topcon DRI-OCT.[1,2,4,75,217,266–268] While some normative data exists for the Cirrus 5000, there is currently limited data available on whites, on vascular profile, and to the author's knowledge, none on an Irish population.[44,74,269] Establishing normative data is paramount if FAZ changes associated with systemic or ocular disease are to be detected and monitored.

FAZ Area, Perimeter and Diameter

Differences in FAZ area and size have been found, possibly due to the various ethnicities, age and sexes being studied, along with different types of commercial instruments used.[2,4,44,269] In the current study, FAZ area was significantly larger in females (0.23mm^2) compared with males (0.19mm^2), ($p < 0.01$) and this is supported by the literature.[2,4] The mean FAZ area for the current population was 0.22±0.07mm^2, which was smaller than that found in an Asian population (0.37±0.11mm^2), which also used the Cirrus 5000.[74] A recent study found that FAZ area was significantly larger in a Chinese population (0.33 ± 0.012mm^2) compared with Polish participants (0.28 ± 0.014mm^2), also using this machine.[44] The mean age of this study group was 68.23 ± 1.42 and 65.31 ± 1.10 years respectively, and participants under the age of 54 years were excluded. Coscas et al found the mean superficial FAZ area was 0.28mm^2, when using the AngioVue OCTA (Optovue Inc, USA), in a white population, aged 20-79 years old, and noted that vessel density decreased with age.[75] Fujiwara et al found that age only became a factor affecting FAZ area in those aged 40 years and over, in a large group of Japanese participants.[217] Of interest, Dai et al found that the Cirrus 5000 consistently measured smaller FAZ area and vessel density when compared with the AngioVue machine.[270] While the current study found that the average FAZ area was smaller in whites compared with other populations, due to limited data (white n = 115 versus other ethnicities n = 27), it was not possible to compare with other ethnicities. Furthermore, no association was found between FAZ area and age in this group of young adults, aged 18-35 years old, mean age 24.4 ± 4.99 years. The variability in measurements between different groups highlight the importance of taking ethnicity, age and sex into account when making comparisons. These findings further highlight the need for machine and population-specific normative data on

FAZ size and area in a young, healthy, white population, for disease detection and monitoring using OCTA.

Vessel Density and Perfusion

Vessel density and perfusion central were found to be significantly lower in females than males ($p < 0.01$, $p < 0.05$ respectively). This may be because females have a smaller number of vessels spread throughout a larger area of the choriocapillaris plexus, as females are known to have a larger FAZ area than males. Mean vessel density central, inner and full were 14.11 ± 2.77mm/mm^2, 23.22 ± 1.01mm/mm^2 and 22.20 ± 1.01mm/mm^2, on the Cirrus 5000, while mean vessel perfusion central, inner and full were, 24.70 ± 4.96%, 41.55 ± 1.47% and 39.65 ± 1.65% respectively in this young Irish population. Findings in the literature relating to vessel density and sex, are, however, mixed. Yilmaz et al found no significant relationship, while the findings of Wang et al support the current study.[266,271] A recent study on an older population using the Cirrus 5000 found that vessel density full was significantly higher in Chinese (17.05 ± 0.24mm/mm^2) when compared with Polish participants (16.08 ± 0.43mm/mm^2).[44] The smaller vessel density full found in Polish participants compared to the whites in the current study may be due to different EDTRS segmentation used by the software of each machine. Differences in software between the various instruments mean that it is not possible to make direct comparisons on vessel density and perfusion, even on the same machine which further highlights the importance of establishing normative data for each instrument, depending on the software used. The current study found that BP was statistically significantly lower in females compared with males ($p < 0.01$), which is not surprising, as BP tends to be lower in premenopausal women than age-matched males.[272] There was, however, no significant correlation between BP and vessel density or perfusion, while controlling for sex, in the current study. While this finding was unexpected, the small number of hypertensive participants may explain this observation. A 2018 study suggested,

however, that changes in the choriocapillaris vasculature could be indicative of hypertension, indicating that hypertension may affect the microvasculature of the eye.[273] Further research on participants with hypertension versus normotension is warranted to establish cut-off points where hypertension can show an effect on vessel density and perfusion within the retina.

Central Macular Thickness, GCL + IPL Thickness

CMT was found to be lower in females than in males in the current study, which aligns with previous reported findings using various machines.[4,217,274] The mean CMT for the white group was 263.08 ± 18.73µm. CMT correlated negatively with FAZ area, a finding which has appeared elsewhere, Samara et al infer that a thicker retina may have a smaller FAZ due to increased metabolic requirements.[2,4,267] The current study supports these findings, as CMT was positively and FAZ area was negatively correlated with vessel density and perfusion, central and full. This suggests that a greater macular thickness requires a larger amount of blood vessels and perfusion and therefore, a smaller avascular zone. While the results are not directly comparable; a recent study also found a positive correlation between vessel density and foveal thickness using the Triton OCT (Topcon, Japan).[268] Although no correlation was found between CMT and BP in the current study in this young, healthy, population; Lee et al found that CMT was thinnest in a group of participants with hypertensive retinopathy or previous retinopathy when compared with healthy controls.[241] Future research on participants with hypertension could further investigate these findings.

Vessel density inner showed positive correlations with both average and minimum GCL+IPL thickness in the current study, while vessel density full and perfusion inner also correlated positively with minimum GCL + IPL thickness. This association may indicate that thicker GCL + IPL require more vascular perfusion to function normally, as would be expected. The average and minimum GCL + IPL thicknesses were found to be 83.19 ± 5.35µm and 81.93 ±

5.49µm in the current study. Again, similar normative data on the Cirrus 5000 for comparison is lacking. To the author's knowledge, to date, one small study (n = 10) reports similar normative data on average (82.1 ± 3.6µm) and minimum (79.6 ± 4.1µm) GCL + IPL thickness in a Spanish population.[78] Reduction in GCL thickness has been associated with glaucoma diagnosis.[275] Further study of correlations with these thickness values in a larger population sample would be interesting to investigate the potential use of minimum GCL + IPL thickness as a prognostic indicator for possible glaucomatous change in otherwise healthy participants with glaucoma risk factors, such as family history or raised IOP.

Differences Between Manufacturers

The Angioplex software represents vessel density as mm/mm^2 while the XR Avanti uses a percentage. Shiihara et al found that the measurement of FAZ area was comparable between the DRI-OCT Triton, the RS-3000 Advance (Nidek, Japan) and the Cirrus 5000, however, these authors used only manual measurements for the Cirrus machine as the study predated the Angioplex automatic software.[269] The variability in how FAZ data have been presented in the literature makes it difficult to draw comparisons, even using the same machine.

Study Strengths and Limitations

To the author's knowledge, this study is the first to establish normative data on FAZ area and vascular profile, in a young, healthy, Irish, white population for the Cirrus 5000. The large study size was an important strength, which represented a young healthy population. There were, however, some limitations which should also be acknowledged. Ocular health was based on self-reported case history and by examination of a greyscale photograph rather than by direct observation. The study population had a higher percentage of females than males which may have influenced some of our findings and, therefore, should be interpreted with caution. The current study did not include measurement of IOP which would have further

contributed to our understanding of factors affecting the FAZ. Shoji et al found that FAZ area decreased after IOP lowering surgery in participants with Primary Open Angle Glaucoma, while Zhou et al found no association between IOP and FAZ area in a young, healthy population.[4,276]

6.6 CONCLUSION

This study proposes normative data, for the first time, on FAZ size, shape and vascular profile in a young, healthy, Irish population, using the Cirrus 5000. The mean FAZ area for the current population was smaller than other ethnicities, which also used the Cirrus 5000. Vessel density and perfusion central were found to be lower in females compared with males ($p < 0.01$, $p < 0.05$ respectively). No significant correlation, however, was found between BP and vessel density or perfusion, in participants under the age of 35, while controlling for sex. Vessel density central and full correlated positively with CMT and negatively with FAZ area. These findings suggest that a greater macular thickness requires a larger amount of blood vessels and blood perfusion and, therefore, a smaller avascular zone. Vessel density inner showed a positive correlation with average and minimum GCL+IPL thickness in the current study, vessel density full and perfusion inner also correlated positively with minimum GCL + IPL thickness only, which is an interesting finding. Thinning GCL is considered to be a potential indicator for glaucoma.[275] Our findings suggest that minimum GCL + IPL thickness is a more useful predictor than using average thickness.

The technology of OCTA has potential for the investigation of the FAZ area and retinal vasculature for diagnostic purposes, however, there is a need for normative data for individual instruments. The variability of reporting on vascularity between the various machines means that no comparisons can be made between our results and the existing literature.

7 CHAPTER SEVEN

INVESTIGATION OF FACTORS ASSOCIATED WITH RETINAL OXIDATIVE STRESS THAT AFFECT THE FOVEAL AVASCULAR ZONE IN HEALTHY EYES: AN OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY STUDY

7.1 ABSTRACT

Purpose

The size and shape of the FAZ can change due to retinal diseases associated with oxidative stress; such as DR, glaucoma, hypertensive retinopathy and macular degeneration. MP, a powerful retinal antioxidant, may confer protection. This study aims to assess the relationship, if any, between factors that may affect the superficial FAZ (i.e., vessel density, perfusion, overweight/obesity), and possible links with MPOD, in young, healthy participants.

Methods

One hundred and fifty-four participants, aged 18 to 35 years old, were recruited. Fifteen were excluded, leaving 139 for analysis. The superficial FAZ area, foveal vascularity and CMT were assessed using the Cirrus 5000. Health parameters including, BMI, trunk fat % and MPOD were analysed, to determine possible associations with the FAZ.

Results

Mean FAZ area was $0.23 \pm 0.08 mm^2$. FAZ area was positively correlated with BMI (Pearson's r = 0.189, $p = 0.03$) and significantly larger in participants with lower MPOD, on bivariate analysis ($p = 0.04$). Significant correlates of FAZ area in the multivariate model included age, sex, vessel perfusion central, CMT and trunk fat %, which collectively contributed 65.2% of the overall variability.

Conclusions

The study findings suggest that reduced vessel perfusion, thinner CMT, higher trunk fat % and low MPOD are plausible predictors of a larger FAZ area, in healthy eyes. Non-invasive OCTA testing, in association with these predictors, may aid in the early detection and monitoring of retinal diseases associated with oxidative stress.

7.2 INTRODUCTION

The FAZ is a highly specialised region at the centre of the fovea in the retina. The fovea is tightly packed with photoreceptors to detect light, with capillaries arranged in rings around its centre, an avascular area called the FAZ. It was first imaged using FA, which is still the gold standard in retinal vascular imaging.[13] However, FA requires injection of fluorescent dye into the blood which can cause anaphylaxis, thus restricting its frequent clinical usage.[13] It also cannot segment the retinal layers or measure retinal thickness.[13] The newer technology of OCTA permits superior imaging of the FAZ area, its layers and retinal thickness and is comparable to FA, even in the presence of certain ocular diseases.[13]

Age, female sex and some retinal diseases can affect the size and shape of the FAZ.[1–4,44,229,277] With its quick, non-invasive imaging, OCTA can be useful in the management of eye diseases including DR, glaucoma, hypertensive retinopathy and AMD.[3,18,20,42] More recently, OCTA has detected microvascular changes such as reduced vessel density and perfusion in participants with Covid-19.[8] Increased FAZ size and irregular shape has diagnostic relevance in diabetes.[3,6] Changes can result from capillary dropout as smaller blood vessels die due to decreased perfusion.[3] FAZ shape may also become more irregular with increasing severity of diabetes.[6] In eyes with primary open angle glaucoma, circularity and vascular density decreased, while FAZ perimeter was statistically larger.[7] This may relate to reduced blood perfusion, as altered perfusion has been proposed as a pathogenic factor in glaucoma, and may indicate another diagnostic use for FAZ assessment using OCTA.[182] Studies have found reduced vessel density in eyes with intermediate and advanced AMD.[43] Vascular density was lower in eyes with exudative compared with non-exudative AMD, while circularity was reduced in the presence of advanced AMD, when compared with early stages of the degeneration.[43] Lee et al found that eyes with AMD had lower vascular density

than those without, although there was no difference in FAZ area between the two groups.[43] The authors suggested that alterations in FAZ area may not occur until more advanced stages of AMD.

Oxidative stress plays a prominent role in the development and acceleration of eye diseases such as DR, glaucoma, AMD and hypertensive retinopathy.[21–24] It is caused by an imbalance between the production and accumulation of ROS in cells and the ability of the biological system to detoxify them.[21] While ROS are by-products of normal chemical processes within the body, their build-up without adequate protective antioxidants for counterbalance can damage cells, proteins, lipids and DNA. Retinal tissue is particularly sensitive to oxidative stress, due to its high oxygen demand and exposure to high-energy short wavelength light.[21] Healthy retinal cells can easily inhibit pro-oxidant factors and maintain homeostasis, however, advancing age and/or disease causes decline in the efficiency of homeostatic mechanisms.[21] Excessive oxidative stress and associated inflammation may lead to blood-retinal barrier compromise and tissue damage.[21] While there is limited normative data on vessel density, perfusion and the GCL in the FAZ region, recent studies have demonstrated changes in retinal microvasculature in the presence of early hypertension, even without visible retinopathy.[179,278] Hua et al found an association between hypertension and decreased retinal vessel density.[179] Hypertensive retinopathy was also coincident with reduced RNFL and GCL thickness.[279] Evidence suggests that inflammation caused by oxidative stress is a causative factor in hypertensive retinopathy, leading to lower blood flow and increased inner retinal thinning.[23,278] Earlier detection of these changes could aid in the treatment of hypertension.[278] The novel Covid-19 virus is another inflammatory disease which can affect the eye.[8] Inflammatory signs such as retinal haemorrhages, cotton-wool spots and venous

dilation have been identified in participants with Covid-19, along with reduced retinal vessel density and perfusion.[280]

Oxidative stress is associated with retinal pathology while antioxidants play a protective role by restoring redox balance.[23] The carotenoids lutein, zeaxanthin and *meso*-zeaxanthin, collectively known as MP, are found in high concentrations in the central fovea.[34] These plant pigments protect the eyes from damage; acting as powerful antioxidants and anti-inflammatory agents.[21] This led to the hypothesis that increased levels of MP may confer protection against AMD and other ocular disorders.[37,146] Cennamo et al examined the relationship between MP and microvascular density using OCTA in a cohort of Type 1 diabetes participants.[40] Both MP and vessel density were lower in -those with diabetes. MP was also lower in participants without visible DR, indicating that these developments can precede diabetic changes in the eye, suggesting that vessel density and MPOD may be used as prognostic indicators of disease progression.[40] Recent studies have shown that MP is also lower in participants with glaucoma and high blood pressure.[39,168] MP levels, however, are variable and can become depleted by a number of factors including age, poor diet, overweight/obesity, oxidative stress and inflammation.[38,281] To the author's knowledge, only one study has examined the distribution of MP in relation to the size of the FAZ and found that eyes with a larger FAZ area were more likely to have a secondary peak in their MPOD profile, i.e., a ring of higher MPOD density 0.5 to 1 degree from the centre of the fovea.[282] Further investigation is warranted to examine the relationship, if any, between total MPOD levels and size/area of the FAZ.

Given the apparent association between FAZ parameters and retinal pathology and the putative protective effects of MP against oxidative stress, this study aims to assess the relationship, if any, between factors which may affect the superficial FAZ (i.e., vessel

density, perfusion, overweight/obesity), and possible links with MP status, in a young healthy population, using the Cirrus 5000. An understanding of these factors under normal conditions may assist in the earlier detection and monitoring progression of diseases associated with oxidative stress and, as alterations to the FAZ can be detected using OCTA.

7.3 METHODS

Ethics and Consent

Approval was obtained from the TU Dublin ethics committee in accordance with the principles of the Declaration of Helsinki.

Participants

One-hundred and fifty-four healthy participants aged 18-35 years were recruited to this prospective, cross-sectional study. Participants with visual acuity ≥ 6/12 were included. Those with refractive error > +/-8.00DS spherical equivalent and participants who had taken dietary supplements containing lutein or zeaxanthin during the six months prior to the study were excluded.

Demographics and Lifestyle Questionnaire

Participants completed a brief questionnaire on demographic information including age, general and ocular health, and a validated food frequency questionnaire known as the LZ screener as shown in Table 7.1.[252] This questionnaire assessed lutein and zeaxanthin intake from foods such as eggs, broccoli, corn and green leafy vegetables, weighted according to frequency of ingestion and bioavailability. Participants were categorised as low, medium or high intake.

Ocular and Clinical Examinations

Visual acuity was measured with presenting correction in place and the eye with the best acuity was used.

Body Mass Index

Height was measured using a measuring rod to the nearest cm. Weight was measured in kg using the Tanita BIA. Measured height and weight as were used to calculate BMI as: weight/height (kg/m^2) and categorised as healthy (≤ 25 kg/m^2) and overweight (>25 kg/m^2), according to World Health Organization guidelines.[258] Waist to height ratio was calculated and participants were divided into healthy (≤ 0.5) and high (> 0.5) groups.[257] The BIA also calculated trunk fat percentage (%) which is the % of weight of the body's core taken up with fat (mainly visceral fat).[261] Trunk fat % was divided around the mean for males and females and categorised as healthy (males = ≤ 33, females = ≤ 30) and high (males = > 33, females = > 30).[112]

Optical Coherence Tomography Angiography

Scans using OCTA were performed using the Cirrus 5000. [85] A 200 x 200mm macular cube was obtained, followed by a 3 x 3 mm OCTA scan. The Angioplex metrix identified FAZ area, perimeter, circularity, vessel density and perfusion in the superficial FAZ, which was defined from the ILM to the IPL.[85] The software also measured vessel density and perfusion in the region of the FAZ. For analysis, the macula was divided into rings as per the ETDRS. As shown in Figure 7.1, the central 6mm of the macula is subdivided into central, inner, outer and full regions, these correspond approximately with the anatomy of the macula. The central portion coincides with the central 1mm (the foveola and partial inner fovea), while the inner (1mm ring) is concerned with the parafovea and partially takes in the outer portion of the fovea. The 3x3mm scans used in the current study did not include the outer region.

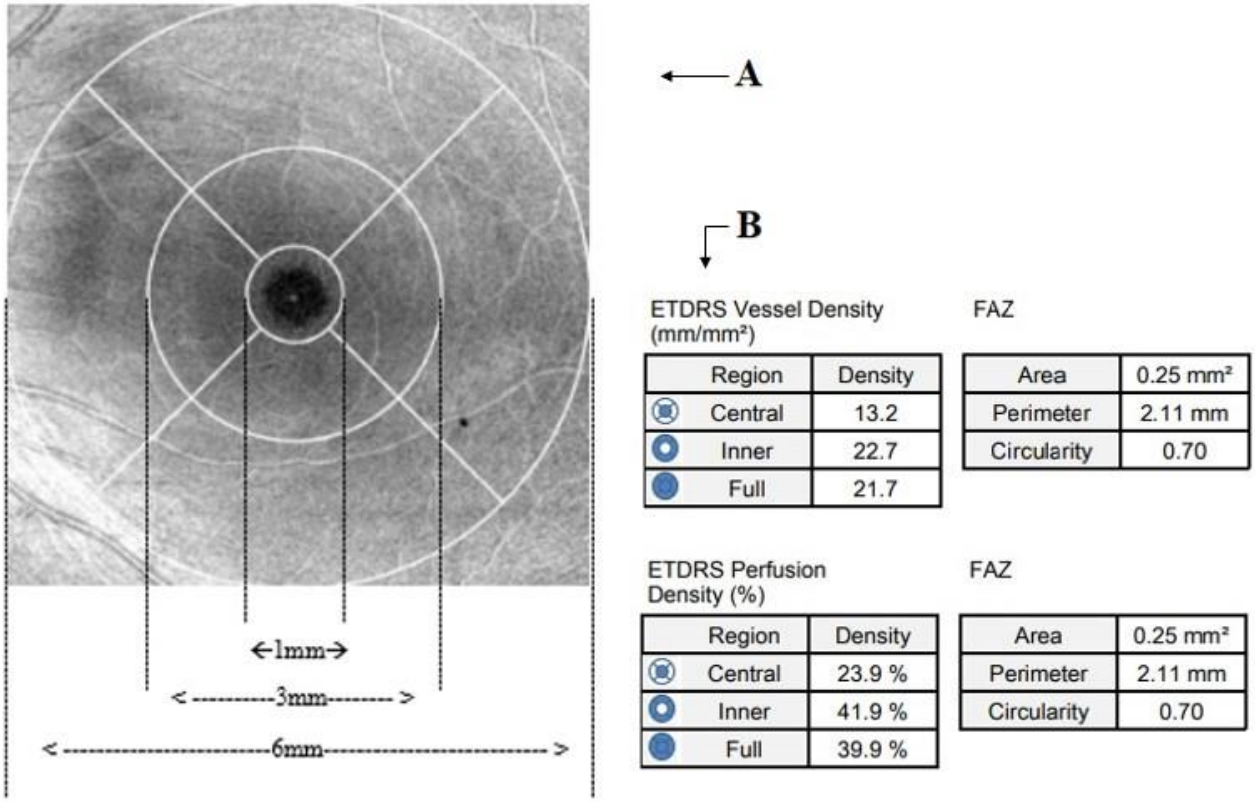

Figure 7.1. *A. ETDRS grid centred on the macula. B. Angioplex Metrix displays the vessel density and perfusion at the central (fovea), inner (parafovea) and full sections of the macula according to the ETDRS. ETDRS; Early Treatment Diabetic Retinopathy Study. [Image: Author's Own]*

Values for all vessel density and perfusion measurements were split into low and high groups around the median for analysis, informed by Lim et al.[244] The cut-off values chosen for vessel density central, inner and full were 14mm/mm^2, 22mm/mm^2 and 23mm/mm^2 and for vessel perfusion central, inner and full were 24%, 41% and 39%, for each group respectively.[244] The macular cube scan provided CMT and ganglion cell thickness measures. Cut-off values were chosen based on the mean CMT from the UK Biobank study.[55] A value of $\leq 264.5\mu m$ was considered normal and $> 264.5\mu m$ was high. GCL + IPL thicknesses were similarly divided into groups; cut-off values for GCL + IPL were chosen as follows: mean GCL + IPL of $\leq 82.1\mu m$ was low and $> 82.1\mu m$ was high, while minimum GCL + IPL $\leq 80.4\mu m$ was considered low and $> 80.4\mu m$ was high, derived from Mwanza et al.[234]

Macular Pigment

MPOD was measured using the Macular Metrics densitometer, which is based on the principal of HFP and has been described in detail elsewhere.[122] In brief, a test stimulus was presented which flickers between a luminance of 460nm (peak absorption of MP), and a luminance of 540nm, (minimum absorption by MP). The difference in luminance noted by the subject is directly proportional to the amount of MP present in the eye. Five readings were taken both centrally (at 0.5 degrees eccentricity) and parafoveally (at 7 degrees eccentricity). The ratio of blue light required in the fovea to that required in the parafovea indicates the amount of pigment present. The logarithm of this ratio is the OD of MP. A standard deviation of < 0.05 was accepted.[283] For analysis, the group was divided around the mean into low MP (≤ 0.4OD) and high MP (> 0.4OD) as shown in Table 7.2.

Statistical Analysis

The Kolmogorov-Smirnov test was used to assess normality of data. As FAZ area data were not normally distributed, a square root transformation of these data was performed. The derived data were normally distributed and used as the dependent variable for subsequent statistical analysis of the FAZ. Mean and standard deviation of FAZ data is presented as the non-transformed original measure for ease of interpretation. An independent samples t-test and/or one-way ANOVA was used to test for differences in normally distributed data. The Kruskal-Wallis H test was used to test for differences in group medians in non-normally distributed data (age, vessel perfusion inner and full and mean GCL + IPL thickness). Pearson's product-moment correlation was used to assess the relationship between normalised FAZ area and other study variables where appropriate. All values were expressed as mean ± standard deviation throughout. A *p*-value of < 0.05 was considered significant.

7.4 RESULTS

Demographic, Health and Lifestyle factors

Fifteen participants were excluded due to underlying health conditions. Demographic information for the remaining 139 is shown in Table 7.1.

Table 7.1. *Demographic characteristics of the study group (n=139)*

Characteristic	**n**	**%**
Age (years)		
18-26	96	69.1
27-34	43	30.9
Sex		
Male	44	31.7
Female	95	68.3
Ethnicity		
White	113	81.3
Mixed	6	4.3
South Asian	5	3.6
Black	6	4.3
Hispanic	1	0.7
BMI		
Healthy (≤25)	95	68.3
Overweight (>25)	44	31.7
Waist to Height Ratio		
Healthy (≤0.5)	89	64.1
High (>0.5)	50	35.9
Trunk Fat %		
Healthy (≤ 33% (men), ≤30% (women))	60	43.2
High (>33% (men), >30% (women))	79	56.8
Smoking Status		
Non-smoker	123	88.5
Current or past smoker	16	11.6
MPOD Category		
Low	75	54
High	64	46
LZ Category		
Low	39	28.1
Medium	46	33.1

High	54	38.8

BMI; Body Mass index: Healthy = ≤25 Overweight = >25, Waist to height ratio: ≤ 0.5 = healthy, >0.5 = high, Trunk fat %: healthy = ≤ 33% (men), ≤30% (women), high = >33% (men), >30% (women); MPOD category: low = ≤ 0.40, high = > 0.40, LZ; Lutein-Zeaxanthin category.

FAZ area by demographic, health and ocular variables are shown in Table 7.2. The mean age of the full group was 24.22 ± 4.92 years and ranged from 18 to 34 years; mean age of males was (26.1 ± 5.2 years); females (23.34 ± 4.6 years), and this difference was significant ($p <$ 0.001). Participants were divided into two age groups: 18 to 26 years (mean: 21.3 ± 2.38) and 27 to 34 years (mean: 30.72 ± 2.01). Age was not significantly associated with FAZ area in the bivariate model (p =0.461), (see Table 7.2) and there was no significant correlation between age and FAZ area (Spearman's rho = -0.104, p =0.222).

Sex

Mean FAZ area for the study group was 0.23 ± 0.08mm^2. Females had a larger FAZ area (0.25 ± 0.08mm^2) compared with males (0.20 ± 0.08mm^2), and this difference was significant, ($p < 0.001$); (see Table 7.2).

Body Mass Index, Waist to Height Ratio and Trunk Fat Percentage

Mean BMI was 23.63 ± 3.37 and ranged from 17.22 to 31.62. FAZ area was not significantly different between the "healthy" and "overweight" groups, (p = 0.074); (see, Table 7.2). There was, however, a small significant positive correlation, between FAZ area and BMI (Pearson's r = 0.189, p = 0.026), as shown in Figure 2. FAZ area was not significantly different between both "healthy" and "high" groups for waist to height ratio, (p = 0.079). Mean trunk fat % was 33.15 ± 14.69% in males and 30.29 ± 6.13% in females. There was no significant difference in FAZ area in the "healthy" or "high" group for either males or females, (p = 0.451, 0.323 respectively). There was no significant correlation between trunk fat % and FAZ area, (Spearman's rho = 0.031, p = 0.713).

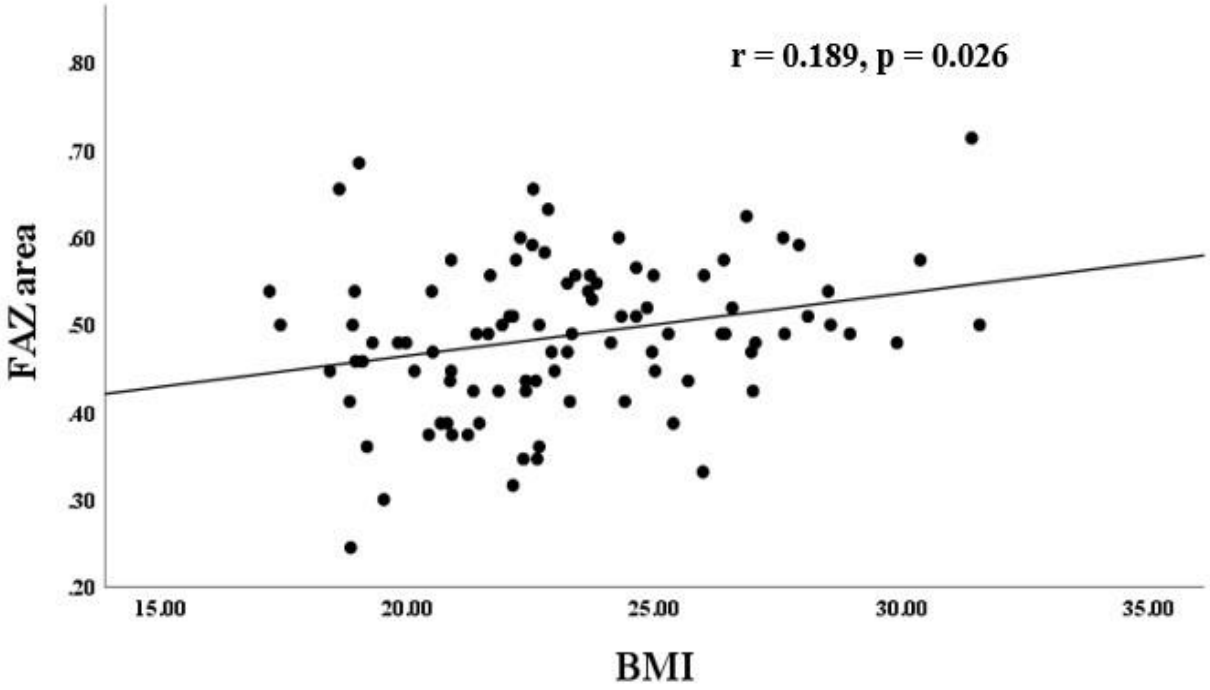

Figure 7.2. *Scatterplot showing a significant positive relationship between normalised FAZ area and BMI.*
Abbreviations: FAZ; foveal avascular zone (mm^2), BMI; body mass index (kg/m^2).

Vessel Density and Vessel Perfusion

Mean values for vessel density central, inner and full were 13.66 ± 2.93mm/mm^2, 23.18 ± 0.92mm/mm^2 and 22.11 ± 1.01mm/mm^2, respectively. The mean FAZ area was significantly larger in participants with lower vessel density central and full (p <0.001 for both); (see Table 7.2). FAZ area was negatively correlated with vessel density central and full (Pearson's r = -0.793, -0.400 respectively, p < 0.001 for both), see Figure 7.3. Vessel density inner was not, however, associated with FAZ area on bivariate analysis (p = 0.278), and there was no correlation between vessel density inner and FAZ area, (Pearson's r = -0.166, p = 0.050). Mean values for vessel perfusion central, inner and full were 23.87 ± 5.26%, 41.44 ± 1.48% and 39.46 ± 1.68%, respectively. A significantly larger FAZ area was found in participants with lower vessel perfusion central and full (p < 0.001 for both). While participants with lower vessel perfusion inner had a larger FAZ area (0.25mm^2) than those with higher

perfusion inner (0.22mm^2), this difference was not significant, (p = 0.053). FAZ area correlated negatively with both vessel perfusion central and full (Pearson's r = -0.777, -0.391 respectively, p < 0.001 for both), see Figure 7.3, however, there was no correlation with vessel perfusion inner, (Spearman's rho = -0.140, p = 0.100).

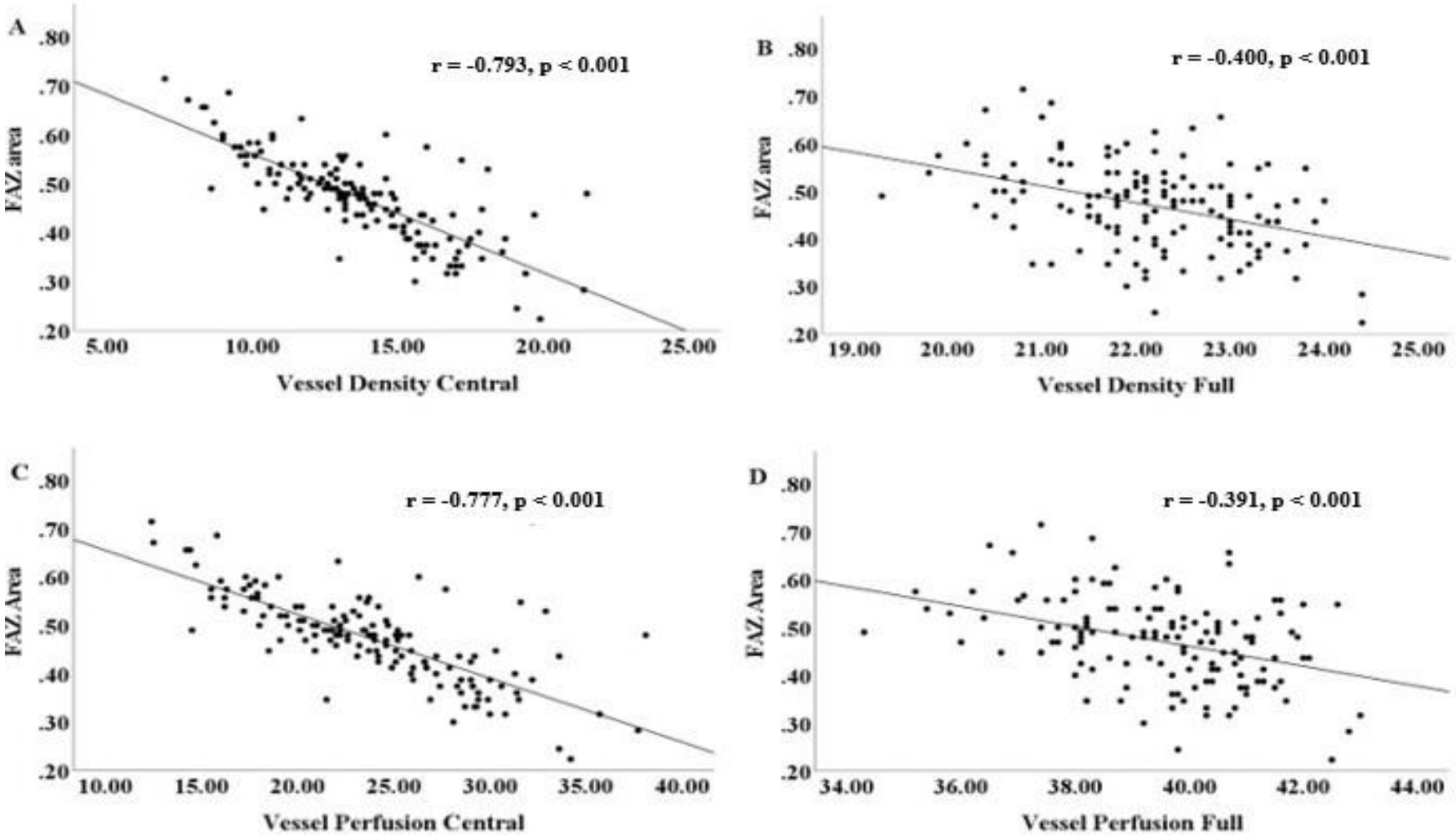

Figure 7.3. *Scatter plots showing negative correlations between normalised FAZ area (mm^2) and vessel density (mm/mm^2) and perfusion (%) central and full.*

Central Macular Thickness, Ganglion Cell + Inner Plexiform Layer Thickness

Mean CMT for the group was 258.96 ± 20.82µm. FAZ area was significantly larger in the group with thinner CMT and this finding was significant ($p < 0.001$); (see Table 7.2). There was also a significantly strong negative correlation between FAZ area and CMT (Pearson's r = -0.679, $p < 0.001$) as shown in Figure 7.4.

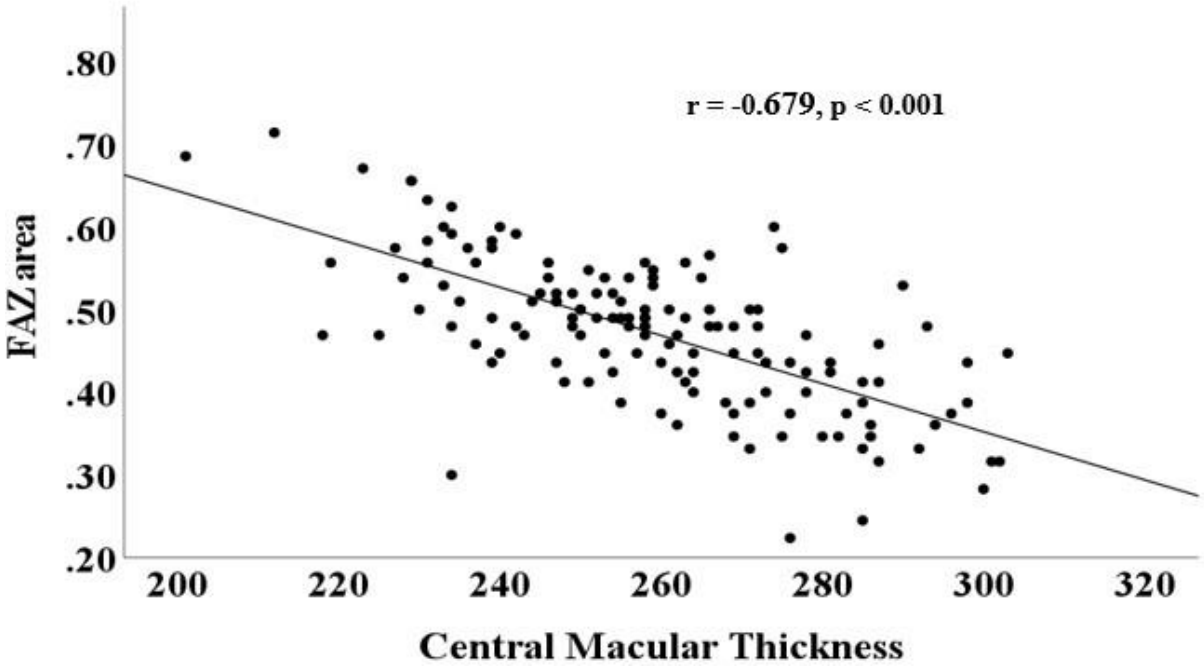

Figure 7.4. *Scatterplot showing a significant negative relationship between normalised FAZ area and CMT.*

Abbreviations: FAZ; Foveal Avascular Zone (mm^2), CMT; Central Macular Thickness (µm).

Mean values for average and minimum GCL + IPL thicknesses were 83.22 ± 5.23µm and 81.89 ± 5.31µm, respectively. There was no significant difference in FAZ area between the low and high groups for average and minimum GCL + IPL ($p > 0.05$ for both); (see Table 7.2). There was no correlation between FAZ area and either average (Spearman's rho = 0.136, $p = 0.110$) or minimum GCL + IPL thickness (Pearson's r = 0.095, $p = 0.268$).

Macular Pigment Optical Density and Lutein-Zeaxanthin Category

Mean MPOD was 0.41 ± 0.18 and it ranged from 0.33 to 1.03 OD. FAZ area was significantly larger in participants with lower MPOD compared with those with higher MPOD (p = 0.038); (see Table 7.2). However, no correlation was found between FAZ area and MPOD (Pearson's r = -0.153, p = 0.073). While there was no correlation between MPOD and BMI (r = -0.043, p = 0.617) and no difference in MPOD between the waist to height groups, (p = 0.883), there was a negative correlation between MPOD and trunk fat % (Spearman's rho = -0.181, p = 0.033). A one-way ANOVA showed no significant difference in FAZ area between low, medium and high LZ categories, although this was a borderline finding.

Table 7.2. *FAZ area by demographic, health and ocular variables.*

	N	Mean Percentile	SD	25th	50th	75th	sig
Age							
18-26	96	0.24	0.09	0.17	0.23	0.29	0.461†
27-34	43	0.22	0.08	0.17	0.24	0.27	
Sex							
Male	44	0.20	0.08	0.14	0.20	0.25	**<0.001**‡
Female	95	0.25	0.08	0.19	0.24	0.30	
BMI (kg/m^2)							
Normal	95	0.22	0.08	0.16	0.22	0.27	0.074‡
Overweight	44	0.25	0.09	0.19	0.24	0.29	
Waist to Height Ratio							
Normal	89	0.22	0.09	0.16	0.22	0.27	0.079‡
High	50	0.25	0.08	0.19	0.25	0.29	
Vessel Density Central (mm/mm^2)							
≤14.00	81	0.28	0.07	0.23	0.26	0.31	**<0.001**‡
>14.00	58	0.17	0.06	0.13	0.16	0.20	
Vessel Density Full (mm/mm^2)							
≤22.00	64	0.26	0.09	0.20	0.25	0.31	**<0.001**‡
>22.00	75	0.21	0.08	0.15	0.20	0.25	
Vessel Perfusion Central (%)							
≤24.00	74	0.28	0.07	0.23	0.26	0.31	**<0.001**‡
>24.00	65	0.17	0.06	0.13	0.17	0.20	
Vessel Perfusion Full (%)							
≤39.00	52	0.27	0.09	0.21	0.25	0.32	**<0.001**†
>39.00	87	0.21	0.08	0.15	0.21	0.26	
CMT (µm)							

≤264.5	87	0.26	0.08	0.22	0.25	0.31	**<0.001**⸸
>264.5	52	0.18	0.07	0.12	0.17	0.23	
Mean GCIPL (µm)							
≤82.1	69	0.22	0.08	0.18	0.23	0.27	0.345†
>82.1	70	0.24	0.09	0.17	0.23	0.29	
Min GCIPL (µm)							
≤80.4	57	0.21	0.08	0.15	0.22	0.25	0.062⸸
>80.4	82	0.24	0.08	0.19	0.23	0.29	
MPOD (OD)							
≤0.4	75	0.24	0.09	0.19	0.23	0.29	**0.038**⸸
>0.4	64	0.21	0.08	0.14	0.22	0.26	
LZ category							
Low	39	0.23	0.08	0.17	0.22	0.16	**0.047**‡
Medium	46	0.21	0.08	0.14	0.23	0.23	
High	54	0.25	0.09	0.19	0.25	0.25	

⸸Independent Samples t-test; †Kruskal-Wallis test of Medians; ‡One-Way ANOVA. Significant differences highlighted in bold. Abbreviations: SD; standard deviation, BMI; body mass index, CMT; central macular thickness, GCIPL; ganglion cell layer plus inner plexiform layer thickness, MPOD; macular pigment optical density, LZ; Lutein-Zeaxanthin category.

Multivariate Model

Significant correlates of FAZ area in the multivariate regression model included age, vessel perfusion central, CMT and trunk fat %, which collectively contributed 64.3% of the overall variability. Age, vessel perfusion central and CMT were negative predictors of FAZ area, after adjusting for all other covariates, (p = 0.022; < 0.001 and = 0.028 respectively). Larger FAZ area was associated with female sex on both univariate (p < 0.001) but not on multivariate analysis (p = 0.620). While FAZ area was not associated with trunk fat % on univariate analysis (see Table 7.2), trunk fat % was a positive predictor of FAZ area in the multivariate model, (p =0.023). While lower MPOD was associated with larger FAZ area on

univariate analysis, (p = 0.038), no association was found in the multivariate model, (see Table 7.3).

Table 7.3. *Multivariate regression analysis between demographic, health and ocular variables and FAZ area in a young, healthy population (n = 139).*

Independent variable	Unstandardised β coefficient	Standard error	t	*p*
Constant	0.942	0.081	11.64	<0.001
Age	-0.002	0.001	-2.082	**0.039**
Sex* (female)	0.005	0.011	0.497	0.620
MPOD	-0.016	0.028	-0.572	0.568
Vessel Perfusion Central	-0.011	0.001	-8.621	**<0.001**
CMT	-0.001	0.000	-2.041	**0.043**
Trunk Fat %	0.001	0.000	2.261	**0.025**

Adjusted r^2 = 0.643; F = 42.48; p = <0.001. Dependent variable = FAZ area square root (normalised FAZ area); Std. Error, Standard Error. *Male = control group.

Abbreviations: *MPOD; macular pigment optical density, CMT; central macular thickness.*

7.5 DISCUSSION

In this study, reduced vessel perfusion central (≤ 24%), low MPOD (≤ 0.4OD) and high BMI (> 25kg/m^2) were associated with larger FAZ area, which, to the author's knowledge, are novel findings. FAZ area was significantly larger in participants with lower vascular density/perfusion, findings which concur with previous analysis.[1] Age, vessel perfusion central and CMT were negative predictors of FAZ area in the multivariate model, while trunk fat % remained a positive predictor, after adjusting for all other covariates, collectively explaining 64.3% of the variability.

Vessel Density and Vessel Perfusion

The current findings suggest that reduced retinal vessel density and perfusion are associated

with larger FAZ area, in this young healthy group. Studies have found reduced vascular density (full) in hypertensive participants aged 60-70 years compared with non-hypertensive controls, which was particularly marked in uncontrolled hypertension.[278,284] Donati et al found that FAZ area was significantly larger among hypertensive participants, particularly in the deep FAZ.[246] Sun et al found a significant increase in the size and perimeter of the deep FAZ of eyes of participants with hypertension, but not in the superficial plexus, suggesting that the superficial FAZ is affected later in the disease process, if at all.[42] Macular blood flow and thickness were also reduced in hypertensive eyes.[42]

Recently, OCTA has found reduced vessel density and perfusion, particularly in the deep plexus, of participants who have recovered from Covid-19, while another study found that macular vascular flow was further reduced at six-month follow up.[8,32] Early identification of Covid-19 changes within the eye, using a low-contact test such as OCTA, may lead to more efficient treatment. Covid-19 causes an inflammatory response by binding to angiotensin converting enzyme 2 which damages endothelial cells in all areas of the body, including the eye.[248] Retinal vascular changes consistent with inflammation and oxidative stress such as cotton wool spots, haemorrhages and venous dilation have been reported in participants with Covid-19.[32] Longitudinal studies, however, are necessary to fully elucidate these findings. The non-invasive nature of OCTA, as an investigative test over conventional FA, is a major advantage of this technology, given that the complications and costs associated with FA make it unsuitable for use as a simple screening test.[15]

Body Mass Index, Waist to Height Ratio and Trunk Fat Percentage

The current study findings that BMI correlated positively with FAZ area ($r = 0.189$, $p = 0.026$) and that trunk fat % was a positive predictor of FAZ area in the multivariate model, ($p = 0.035$) are important given that overweight/obesity is a significant risk factor for many

health conditions, including diabetes, high cholesterol, hypertension and AMD. These findings are significant, as only 32% of our participants were overweight, with an average BMI of 24 kg/m^2. Furthermore, mean trunk fat % was within the normal range for both males (≈33%) and females (≈30%), as this was a young, healthy group. Overweight/obesity is prevalent among European adults.[285] To date there has been very little research on FAZ area and overweight/obesity. However, one small study, (n = 65) found a larger FAZ area in obeseparticipants.[286] Obesity is associated with oxidative stress, which in turn; is linked with capillary dropout and damage to the retinal vasculature.[287] Overweight/obesity is also considered an important factor in the aetiology of Type Two diabetes.[288] Higher body fat, visceral fat in particular, is associated with increased insulin resistance and inflammation.[160,289] In one study, FAZ area was larger in participants with Type Two diabetes compared with Type One diabetes and controls, possibly due to differences in glycaemic control and/or duration of disease.[208] While measurement of BMI can identify overweight/obesity, it does not isolate where adipose tissue is accumulating in the body, nor can it differentiate between fat or muscle mass.[257] The use of specific analysis, such as BIA, may provide more in-depth evaluation of body fat distribution, considering trunk fat % (i.e., visceral fat) was a positive predictor of FAZ area in the current study.

MPOD and LZ Category

The current study found a larger FAZ area in subjects with lower MPOD, to the author's knowledge a novel finding. MP is a powerful antioxidant and anti-inflammatory agent which putatively protects the macula against oxidative stress. MP, however, can become depleted for many reasons, including advancing age or poor diet.[36,37] Higher MPOD levels from an early age may offer greater retinal protection over one's lifespan. Mean MPOD was 0.41 ± 0.18OD in the current study. This is similar to Liew et al's findings that mean MPOD in a

group of healthy adults was 0.44OD (range 0.06 – 1.25), albeit this group included subjects aged 17-50 years.[290] While higher levels of MPOD is preferable, there is no consensus on what constitutes a normal amount, given that many studies include a wide age-range and employ different measurement methods.[290–292] While no correlation was found between FAZ area and MPOD and no association between FAZ area and dietary intake of LZ in the current study, there was a significant negative correlation between MPOD and trunk fat %, a finding supported by the literature.[112] Adipose tissue, visceral fat in particular, competes with the retina to store MP carotenoids.[112] Hammond et al found lower MPOD in subjects with BMI >29kg/m^2.[38] In the current study, FAZ area was larger in subjects with lower MPOD (i.e., ≤ 0.40OD) and in those with higher BMI (i.e., > 25kg/m^2) and trunk fat % (i.e., females > 30%, males > 33%). Additionally, MPOD was lower in subjects with higher trunk fat %. While MPOD is not routinely measured in clinical practice, the finding that a larger FAZ area is associated with higher trunk fat %, in participants free of ocular pathology, may indicate a lower antioxidant status in these subjects. Such individuals may benefit from dietary and lifestyle advice. There is value in counselling ophthalmic patients from a young age on the importance of a healthy diet rich in fruit and vegetables, i.e., carotenoids lutein and zeaxanthin, to help maintain MPOD levels into older age, in addition to regularising BMI and reducing visceral/trunk fat % levels for their protective properties against diseases such as AMD and diabetes.[37]

Central Macular Thickness, Ganglion Cell + Inner Plexiform Layer Thickness

In the current study, CMT was a strong negative predictor of FAZ area (p = 0.028). CMT also significantly negatively correlated with FAZ area, a finding supported by the literature.[4,217] Reduced CMT has been found in individuals with genetic risk factors for AMD.[293] Therefore, subjects with a thinner CMT may be more likely to have larger FAZ area

and thereby be more vulnerable to the effects of oxidative stress.

While there was no association between FAZ area and GCL + IPL thickness in the current study on young, healthy subjects, there was a positive correlation between average GCL + IPL thickness and vessel density and perfusion full, and a positive correlation was found between minimum GCL + IPL and vessel density full. Enlarged FAZ area has shown an association with glaucoma in disease studies.[7,207] Assessment of the FAZ area and foveal vascularity may aid in early identification of glaucomatous changes within the eye, which can prove difficult to assess visually.[241] Current best practice involves a combination of monitoring optic disc and ganglion cell changes, visual fields, IOP and the presence of risk factors such as family history. Choi et al found that reduced vessel density, decreased FAZ circularity and increased FAZ perimeter were all significant in distinguishing POAG from normal controls, however, segmentation of the FAZ regions differed as additional software was used.[20] Zivkovic et al found larger FAZ area and reduced vessel density in normal tension glaucoma subjects.[207] The authors posited that with further study of the FAZ, it may be possible to identify a value for FAZ area, which can potentially detect a glaucomatous suspect.[207] The participants in the current study were free of pathology. Furthermore, IOP was not measured which is a limitation as the relationship between IOP and FAZ parameters could not be analysed.[294] Future studies incorporating IOP measurements, in subjects with glaucoma versus controls, would be useful to further investigate if OCTA biomarkers for the early detection of glaucoma, such as vessel density/perfusion full, could be identified.

Study Strengths and Limitations

There were many strengths to the current analysis. For example, to the author's knowledge, this is the first study of its kind to investigate the association between MPOD and visceral fat, and FAZ area/vascularity using the Cirrus. In particular, the link between FAZ area and MP

has rarely been examined in a healthy population, although it has been investigated in relation to disease.[40,282] The results from the current study provide a reference from a normal, healthy perspective for comparison with disease studies. Specifically, this study has shown that OCTA analysis ought to concentrate on vascular density and perfusion central/full, as opposed to inner values which do not include the FAZ.

There were some important limitations in the current study which should be acknowledged. The current study contained more females than males and males were statistically significantly older.[1] This may have impacted these data; we found that age was a negative predictor of FAZ area, a finding which should be interpreted cautiously.[217] Regarding the examination of GCL + IPL thickness, the findings of positive correlations between GCL + IPL and vessel density and perfusion are inconclusive without IOP measurement.

7.6 CONCLUSION

The current study findings suggest that lower vessel density and perfusion (central and full), thinner CMT and higher trunk fat % are plausible predictors of a larger FAZ area in healthy eyes. Suggested normal values for young healthy subjects, from the current study are 14.00mm/mm^2 for vessel density central, 24% for perfusion central, 264.5µm for CMT and MPOD ≥ 0.4OD. Reduced vessel density and perfusion, lower CMT and high BMI/ trunk fat % are all associated with inflammatory conditions and with a larger FAZ area.[179,205–207,286] Inflammation is associated with oxidative stress, which can cause retinal damage over time. Furthermore, in the current study, larger FAZ area was associated with lower MP status and, therefore, antioxidant status in a young, healthy population. The presence of MP within the retina has a protective effect against damage from oxidative stress. Use of OCTA can non-invasively monitor vascular density and perfusion, CMT and FAZ area for deviations from the proposed normal values. The current study findings suggest that OCTA, in association

with knowledge of these predictors, may have prognostic potential in the early detection of and monitoring progression of retinal diseases associated with oxidative stress such as DR, glaucoma, hypertension, AMD and Covid-19. Further study is needed to evaluate the current study findings.

8 CHAPTER EIGHT

CONCLUSION AND FURTHER STUDY

8.1 Summary and Conclusions

The research presented in this thesis provides normative data for the Cirrus 5000 on the superficial FAZ. In addition, factors relating to oxidative stress, which can affect the FAZ, have also been identified. Machine and population-specific normative data are paramount to the early detection of disease. While a wide variety of OCTA instruments are available for clinical use, unfortunately their measurements are not interchangeable and comparisons cannot be made between them.[85,268] Therefore, there is a need for machine-specific data.

Research has shown that the size and shape of the FAZ may change in retinal diseases associated with oxidative stress, including DR, hypertension, glaucoma and AMD.[6,20,42,43] MP, a powerful antioxidant, is selectively located at the macula and may confer protection against these retinal diseases. This study explored factors which may affect the superficial FAZ (i.e., vessel density and perfusion, overweight/obesity) and possible links with MPOD, in a young, healthy population. The detection of predictors that affect the FAZ in terms of size/shape and vascularity may allow early identification of subjects at risk of retinal diseases associated with oxidative stress and low antioxidant status. An understanding of these factors under normal conditions may allow better detection and management of disease progression, given that OCTA is increasingly being employed and MPOD is not routinely measured in clinical practice. Findings and conclusions drawn from this analysis will now be discussed.

8.1.1 Normative Data on the Foveal Avascular Zone

The current study found a mean superficial FAZ area of 0.23±0.08mm^2 in young, healthy adults in Ireland using the Cirrus 5000. Consistent with previous studies, the FAZ area was larger in females compared with males.[2,4] The mean FAZ area found in this group of young, predominantly white subjects, was smaller than in other studies using the same machine in older subjects of different ethnicities.[44,74] This highlights the importance of accounting for ethnicity when analysing data relating to the FAZ. The FAZ area did not increase with age in subjects under 35 years in this dataset. Important findings from the current study related to normative data for vessel density and perfusion, as there is currently scant literature available for the Cirrus 5000 machine. Moreover, the differences in machine software make direct comparisons impossible.[85,268] Mean vessel density central and full were 13.63±2.91mm/mm^2, and 22.12±1.00mm/mm^2, while mean vessel perfusion central and full were 23.83 ± 5.23%, and 39.46 ± 1.68% respectively. These findings contribute to the limited normative data available for this machine. To the author's knowledge, this was the first study of its size and kind, using the Cirrus 5000 and in young healthy Irish subjects. Vessel density and perfusion central were found to be lower in females compared with males ($p < 0.01$), which aligns with previous findings that FAZ area is larger in females than in males.[2,4] Current literature on average and minimum GCL + IPL thickness for the Cirrus 5000 is also lacking. Here, mean values of 83.19 ± 5.35µm and 81.93 ± 5.49µm for average and minimum thicknesses respectively are reported, which contributes to the limited known data.[78] Thinning of the GCL is considered to be a potential indicator for glaucoma.[275] However, it is difficult to further contextualise this information without measuring IOP. Normative data can help clinicians to easily identify deviations from normal, which could potentially indicate retinal disease. Normative data for the Cirrus 5000 from the current study can be used for such comparison by clinicians using a similar device. In addition, given that variation in

measurement and expression of data exist between commercially available machines; instrument-specific data, such as has been provided here, is required.

8.1.2 Investigation of Factors Associated with Oxidative Stress that affect the Foveal Avascular Zone.

A novel aspect of the current study was investigation of the relationship between the FAZ size and vascularity and MPOD levels in healthy eyes, where very little information exists in the literature.[282] In the current study, reduced vessel perfusion central (≤ 24%), low MPOD (≤ 0.4OD) and high BMI (> 25Kg/m^2) were associated with a larger FAZ area, which, to the author's knowledge, are novel findings. Age, vessel perfusion central and CMT were negative predictors of FAZ area, while trunk fat % remained a positive predictor of FAZ size. Changes in the FAZ, which can be visualised and studied using OCTA, have been linked to some diseases relating to oxidative stress.[3,8,42] Hence, it is useful to understand the association, if any, between certain factors affecting the FAZ (i.e., weight, MPOD/antioxidant levels) which may be used as diagnostic indicators and potentially also to monitor the progression of retinal diseases. Testing using OCTA, in association with these predictors, may aid in the early detection and monitoring of retinal diseases associated with oxidative stress as early intervention in at-risk patients could prevent progression of inflammatory ocular conditions. For example, presence of a large or increasing FAZ area in association with other risk factors for disease, such as family history of glaucoma/AMD or overweight/obesity may warrant closer monitoring of the patient's ocular health.

The current study has presented normative data for the FAZ area and vasculature using the Cirrus 5000, in addition to identifying certain factors, such as lower vessel density and perfusion (central and full), thinner CMT and higher trunk fat % as plausible predictors of a larger FAZ area in healthy eyes, factors which are also associated with oxidative stress.

Moreover, larger FAZ area was associated with lower less MPOD, antioxidants which confer protection within the retina against oxidative stress damage. These findings suggest that the use of OCTA, in conjunction with knowledge of these predictors, may have diagnostic potential in the early identification and monitoring of diseases associated with oxidative stress; such as DR, hypertension, glaucoma and AMD.

8.2 Strengths and Limitations of the Current Research

To the author's knowledge, this is an original study using the Cirrus 5000 to measure FAZ area and vascular profile and to examine the relationship between FAZ area and trunk fat/MPOD levels. The gold standard method of c-HFP was used to measure MPOD, which was a strength of the current study, in addition to using BIA to measure trunk fat %, which is a more specific measure of body fat than BMI.[257] Limited information exists in the current literature on the relationship between MPOD and FAZ area in healthy eyes in particular, which is a strength of the current study. To the author's knowledge, only one study has examined the distribution of MP at the fovea in relation to FAZ size in normal eyes.[282] Cennamo et al also found reduced vessel density and MPOD in subjects with Type Onediabetes.[40] Having an understanding of the associations in healthy eyes can help clinicians to identify suspicious changes. In addition, the current study proposes normative values for FAZ size/shape and vascularity in adults aged 18-35 in Ireland using the Cirrus 5000. Scans using OCTA can provide detail quickly and easily in an uncomplicated manner for patients. This has proven particularly useful since the advent of Covid-19, which has necessitated patient encounters to be brief. Reduced retinal vessel density and perfusion have been found in subjects with Covid-19 using OCTA, while inflammatory signs such as retinal haemorrhages, cotton-wool spots and venous dilation have also been identified.[280]

There were, however; limitations which should also be acknowledged. There were more female participants than males in the study population and females are known to have larger

FAZ area.[2,4] The males were significantly older, which may have impacted these data, as some studies found that FAZ area can also increase in size with age.[2,295] However, the impact on the FAZ area is controversial, with other studies finding no association between FAZ area and age.[267,274] Fujiwara found that under 40 years, age did not affect FAZ area.[217] Additionally, while the current study found that average FAZ area was smaller in Whites compared with other populations, due to limited data (white n = 115 versus other ethnicities n = 27), it was not possible to compare with other ethnicities in the current study. A sample with equal numbers of males and females of similar age, with more equal representation of various ethnicities would have facilitated improved data analysis. Another limitation is that IOP was not measured in the current study. Therefore, findings relating to FAZ area/vascular profile and GCL + IPL thickness in this healthy population are inconclusive. Finally, while FAZ area was larger in subjects with lower MPOD, FAZ area was not associated with LZ intake, which was surprising. In the current study, LZ categories were established using a self-assessed dietary questionnaire which is limited by the accuracy of information provided by subjects.[252] Serum analysis would provide more accurate measure of bioavailable carotenoids, however, this would have required blood-testing which was beyond the scope of the current study.

8.3 The Role of OCTA in Clinical Practice

As a developing technology, OCTA has great potential in clinical practice as a routine diagnostic and monitoring test. It is quick and simple to use, minimally invasive for patients and provides excellent images of the internal ocular structures which could not be visualised so readily with other imaging modalities. Its non-invasive nature is a major benefit to OCTA as it can be easily performed without risk of serious adverse effects on the patient. It is likely that as understanding of the benefits of this technology increases, along with improvements to the technology itself, OCTA will become more widely used clinically. As outlined in Section

8.1.2, OCTA can be used to identify possible indicators of oxidative stress, such as reduced vessel density in an otherwise healthy eye. Eye-care practitioners are well-positioned in the community to help motivate patients to make dietary and lifestyle changes to improve their ocular as well as general health and could use OCTA findings to highlight the importance of maintaining ocular health. MPOD is not currently routinely measured in practice, however, the MP-Eye, (Azul Optics, UK) is a novel device which can measure MP in approximately 1 minute, which could easily be adopted as a screening device to measure MP in association with use of OCTA.[126]

8.4 Future Study

The current study involved young healthy subjects in order to establish normative data for the variables under investigation. Based on data from the current study, it would be interesting to repeat the study using subjects with disease, such as hypertension for example, and including measurement of blood pressure. Grading of stage one and two hypertensive retinopathy is difficult using fundoscopy, as discussed in Chapter Seven.[241] Changes in the FAZ have been observed in subjects with hypertension; thus, a study comparing controls with hypertensive subjects could investigate these changes further.[42] This study would examine the theory that changes to the FAZ do occur in the presence of hypertension and may begin to elucidate cut-off values for FAZ size/vascularity, which may indicate early onset of disease. In addition, further studies which include measurement of IOP and pachymetry, and comparing controls with subjects with glaucoma would be beneficial to investigate whether IOP has an effect on FAZ area or the vessels surrounding it. Reduced macular vessel density corresponding with visual field defects has been found in glaucomatous eyes.[235] The same study found that macular vessel density was reduced in glaucoma even where no significant visual field defect was found and concluded that this may indicate that vascular changes may be detectable prior

to visual field loss.[235] Further investigation including IOP would increase the understanding of factors at play here.

FAZ area is segmented and analysed differently by the various commercially available machines. The current study used analysis from an in-built proprietary algorithm called Angioplex, other commercially available machines each have their own algorithm.[45] Many early OCTA studies used either public domain software called Image J or image processing algorithm written in MATLAB (The MathWorks Inc, USA) to analyse images and measure FAZ area or vessel density independently of machine-specific software.[7,296] One such parameter which can be assessed in this way is fractal dimension. The pattern of the retinal blood vessels is described as a fractal, meaning that it constitutes a geometric pattern in which each section, or fractal, resembles the pattern of the whole.[297] Fractal dimension can be calculated using image processing software, such as Image J (Open Source) where OCTA images can be uploaded, converted to greyscale and superimposed with a grid pattern, which segments the image into sections, each containing at least one pixel from the image.[298,299] Fractal dimension is a real number which accounts for changes in detail due to magnification and allows a one-dimensional image to be analysed in two dimensions.[298] A further study using software such as Image J to analyse the images could therefore allow comparison with competitor machines as data can be presented in a variety of ways using this third-party software. The Cirrus 5000 can image the deep FAZ but the software does not provide analysis. Image processing could also be used to analyse the deep FAZ from images produced by the Cirrus 5000. Use of fractal dimension as a metric is also of interest in disease studies, Kostic et al for example, found lower fractal dimension in subjects with Type Two diabetes compared with normal controls.[299]

Machine-learning and AI could play an important role in improving OCT and OCTA imaging in future as it can enhance image quality cheaply and easily.[300] Improvements to OCTA are

already occurring, such as increasing the field of view of machines and the use of machine-learning and new algorithms to enhance image quality and reduce artifacts.[300] These advancements contribute to increased reliability and therefore greater clinical usage of OCTA.

8.5 Conclusion

There is great potential for wider use of OCTA as a routine diagnostic clinical test and as a monitoring tool as it is quick, easy to use and non-invasive. The normative data which the current study provides for a young, healthy Irish population using the Cirrus 5000 can contribute to baseline measurements for this machine. In addition, lower vessel density and perfusion (central and full), thinner CMT and higher trunk fat % were found to be plausible predictors of a larger FAZ area in healthy eyes. This study proposes that OCTA, through the measurement of FAZ area and vascular profile, may have prognostic potential in the diagnosis and monitoring of diseases relating to oxidative stress. While a larger FAZ area is suggestive of lower MPOD/antioxidant status and given that high levels of MP may be beneficial in the retina, OCTA, in association with a simple measurement of MPOD, such as the new MP-eye could be very useful in detecting and advising patients who are at risk of retinal oxidative stress damage. Further studies would be required to evaluate these findings.

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List of Publications

O'Shea SM, O'Dwyer VM, Scanlon G. Normative data on the foveal avascular zone in a young healthy Irish population using optical coherence tomography angiography. *Eur J Ophthalmol*. 2022. doi: 10.1177/11206721211073446. Epub ahead of print. PMID: 35001682.

O'Shea SM, O'Dwyer VM, Scanlon G. Normative Data on the Foveal Avascular Zone in a Young Healthy Irish Population, Nov 2020. [Poster]

O'Shea SM, O'Dwyer VM, Scanlon G. Factors Affecting the Foveal Avascular Zone in a Young, Healthy Population. Women in Vision Conference, Sep 2021. [Poster]

O'Shea SM, O'Dwyer VM, Scanlon G. Factors Affecting the Foveal Avascular Zone. Retina Conference, Nov 2021. [Poster]

Article submitted to Current Eye Research, June 2022:

O'Shea SM, O'Dwyer VM, Scanlon G. Investigation of Factors Associated with Retinal Oxidative Stress and Inflammation that affect the Foveal Avascular Zone in Healthy Eyes: An Optical Coherence Tomography Angiography Study.

List of Employability Skills & Discipline Specific Skills Training

There is a requirement by TU Dublin to undertake a number of postgraduate modules (30 in total, categorised by employability skills (15 ECTS credits), and discipline specific (15 ECTS credits)). The following is a list of the modules completed.

Discipline-specific postgraduate modules:

- *Research Integrity.* (compulsory) 5 ECTS
- *Going Dark: Law enforcement in cyber environment.* 5 ECTS
- *Innovation and knowledge management.* 5 ECTS

Total discipline-specific credits **15 ECTS**

Employability skills postgraduate modules:

- *Introduction to Statistics.* 5 ECTS
- *Refractive surgery for optometry.* 10 ECTS

Total employability skills credits **15 ECTS**

Appendix A

OLLSCOIL TEICNEOLAÍOCHTA BHAILE ÁTHA CLIATH
DUBLIN
TECHNOLOGICAL UNIVERSITY DUBLIN

CONSENT FORM

Researcher's Name: SUSAN O'SHEA (Use block capitals)	**Title:** MS.
Faculty/School/Department: Sciences & Health/Physics/Optometry	
Title of Study: **To investigate the association between the foveal avascular zone and macular pigment optical density.**	
To be completed by the: subject/patient/volunteer/informant/interviewee/parent/guardian *(delete as necessary)*	

3.1 Have you been fully informed/read the information sheet about this study? YES/NO

3.2 Have you had an opportunity to ask questions and discuss this study? YES/NO

3.3 Have you received satisfactory answers to all your questions? YES/NO

3.4 Have you received enough information about this study and any associated health and safety implications if applicable? YES/NO

3.5 Do you understand that you are free to withdraw from this study?

- at any time
- without giving a reason for withdrawing
- without affecting your future relationship with the Institute YES/NO

3.6 Do you agree to take part in this study the results of which are likely to be published? YES/NO

3.7 Have you been informed that this consent form shall be kept in the confidence of the researcher? YES/NO

Signed_____ Date _____

Name in Block Letters _____

Signature of Researcher _____ Date _____

1 copy for subject, 1 copy for researcher to be kept in locked cabinet in DIT

Appendix B

OCTA Macular Health Research Project

Information Sheet and Informed Consent given and signed (tick) □

Name _____ DOB_____

Phone number_____ Email_____

Male/Female (circle)

Ethnicity (please tick)

Caucasian	□	Asian	□	African American	□
Hispanic	□	Indian	□	Mixed Ethnicity	□

Medical History

Have you any of the following medical conditions?	Yes	No
Diabetes	□	□
High blood pressure	□	□
Cholesterol	□	□

Other (please specify) _____

Do you take/Have you taken any dietary supplements containing lutein or zeaxanthin in the past 6 months? (Circle one) Yes/No

If yes, what? _____

L/Z Questionnaire

Lutein/Zeaxanthin Screener*						
About how many servings do you have of the following foods?						
tick for each food. Each row should only have no more than one tick						
			SERVING			
	<1x/week	**1/week**	**2-3x/week**	**4-6x/week**	**1x/day**	**>1x/day**
Eggs						
Broccoli						
Corn						
dark green leafy vegetables						

Smoking

Have you ever smoked cigarettes, daily for a period of at least one year? (circle one) Yes/No

If yes, how many cigarettes do/did you smoke on average per day? _____

For how many years do/did you smoke altogether?_____

Ocular History

Have you ever been diagnosed with any eye conditions, e.g., cataract, glaucoma, macular degeneration, squint/lazy eye or other?

If yes, give details_____

Vision Case History

Do you currently wear spectacles and/or contact lenses? Yes/No_____

Focimeter Spectacle Rx (if any)	

Vision

Aided/Unaided (circle) (Thompson Chart) R _____ L _____LogMar

Study Eye: Right □ **Left** □

Indicate which eye will be used for the current study (eye with best VA)

Note: The study eye is the eye with best corrected visual acuity. If corrected visual acuity is the same in both eyes the study eye will be randomly selected.

Blood pressure (mmHg)

Systolic 1	
Diastolic 1	
Systolic 2	
Diastolic 2	
Mean Systolic	
Mean Diastolic	

Refractive Error (Auto Refractor)

Record the subject's refractive error for both eyes (also staple printout from auto refractor result):
*note exclusion criteria

RE	
LE	

Measurement of Macular Pigment Optical Density: The Clinical Densitometer

Critical Flicker Frequency

MPOD @0.5° eccentricity		**MPOD &SD @ 7° eccentricity**	
MPOD		**SD**	

IOL Master measurements:

Axial length measurements (mm)

1	2	3	Average

Corneal Ks

Dioptres	K1=	Axis=	K2=	Axis=
Radius	r1=	Axis=	r2=	Axis=

ACD (mm)	

Height (cm)	

Bioimpedence *(Staple results sheet here.)*

Body Type	
Gender	
Age	
Height cm	
Weight kg	
BMI	
BMR kJ	
BMR kcal	
Fat %	
Impedance Ω	
Fat Mass kg	
FFM kg	
TBW kg	
Trunk Fat %	

AVERAGE

Waist circumference (cm)				
Hip circumference (cm)				
Waist: Hip ratio (WHpR)				
Waist to Height Ratio (WHtR)				

OCTA measurements:

FAZ Area mm^2	
Superficial	
Circularity	
Perimeter mm	
Horizontal diameter um	
Vertical diameter um	
Maximum diameter um	
Vessel density central	
Vessel density inner	
Vessel density full	
Perfusion density central	
Perfusion inner	
Perfusion full	
Central Macular Thickness	
Cube Volume	

Avg ganglion cell	
Min ganglion cell	

Appendix C

Macular Health Study

Information Leaflet

Invitation to participate.

You are invited to participate in a research study designed to examine the part of your eye responsible for central vision in a novel way. This area, called the macula is rich in macular pigment (MP) which is thought to protect the eye from harmful UV light. At its centre, is the Foveal Avascular Zone (FAZ) which is responsible for clear vision as there are no blood vessels here to obscure passage of light. This study will allow us to identify any relationship(s), which may exist between this area, your macular pigment and general health. This study has been approved by the Research Ethics Committee of Dublin Institute of Technology.

Background Information

- The FAZ is a region at the back of the eye which can now be studied in greater detail since the advent of a new device using Optical Coherence Tomography Angiography (OCTA). This technology allows us to visualise blood flow in blood vessels at the back of the eye completely non-invasively.
- The aim of this study is to measure the dimensions of the FAZ in healthy eyes in order to establish normative data and investigate any possible link between the FAZ, macular pigment levels, blood pressure and other health indicators.
- Macular pigment (MP) is a naturally occurring pigment which is concentrated at the macula at the back of the eye and is made up of three dietary compounds, Lutein, Zeaxanthin and Meso-zeaxanthin. These are found in a diet rich in fruit and vegetables.

- It is believed that these antioxidant plant compounds help protect the eye by absorbing UV and harmful blue light and neutralising free radicals. MP is important for maintaining eye health.
- We wish to investigate parameters of the FAZ and correlate findings with MP density in healthy subjects.

Visit Expectations

The following tests will take place during a study visit:

Participants will have a detailed assessment of their vision and eye health examination. The assessments will be identical to what is currently standard practice with the addition of a number of tests which are non-invasive.

The additional tests are as follows:

- You will be asked to sign an informed consent document which states that you are happy to participate in the study and that all aspects of the study have been explained to you by the study investigator.
- A scan of your eye will be performed which gives a rough indication of your refractive prescription (if any) and your level of vision will be measured using an eye chart.
- You will be asked to fill out a demographics questionnaire and a brief dietary questionnaire which will determine the amount of MP in your diet.
- Your MP levels will be measured using a specialised optical device. Feedback will be given regarding your macular pigment levels.
- A photo of the retina will be taken to ensure that the eye is healthy.
- A three-dimensional scan will be taken of your eye using a specialised instrument to examine the macula in more detail.
- Your height, weight and waist circumference will be measured which must be taken in your bare feet.
- Body fat analysis using bio-electric impedance analysis will be performed.
- Blood pressure will be taken.

How long will it take?

Each visit related to the study will take approximately 45 to 60 minutes.

Subject Payment

This study is entirely voluntary. You will not be paid for your participation in this study. If you decide to take part you are still free to withdraw at any time

and without giving a reason. This will not affect the standard of care you receive.

Risks

We foresee no risks to the subjects participating in this research. The tests are all completely non-invasive and simply involve the participant identifying presented targets and completing a brief questionnaire.

Exclusion criteria

There are some conditions which may preclude you from partaking in the study and these include:
Subjects must be between ages 18-35, not having taken dietary supplements containing lutein, zeaxanthin over the six months period prior to the study. Subjects are excluded if they have eye conditions such as: glaucoma, cataract, age- related macular degeneration or diabetic retinopathy.

Subjects with glasses prescription > +/- 8 or visual acuity less than 6/12(0.3 logMAR) will also be excluded from this study.

Benefits

You will gain knowledge of your macular pigment level. It has been suggested that a person's macular pigment level is a good indicator of overall eye health. You will also gain knowledge of your body fat analysis and blood pressure.

Difference of Research Study to Clinical Practice

Your involvement in this study is for research purposes only. This is not a medical examination for your benefit.

Confidentiality

All data collected in this study will be treated as strictly confidential and will be obtained and processed in accordance with General Data Protection Regulation (GDPR). All data will be analysed collectively as a group and coded by data link to ensure participants confidentiality. The anonymous results of this study may be published in the medical literature.

Contact

For any queries or concerns contact the study investigator, Ms. Susan O'Shea on 087-9588281.

If you decide that you would like to participate, please remember to bring your glasses (distance, reading, bifocals etc.).

We hope that this information has answered most of your questions. Should you have further questions or do not fully understand the information given, please feel free to ask.

The researchers at Technological University Dublin, who are carrying out this research, would like to thank you for taking the time to read this information.

Appendix D

Macular Health MPhil

Are *you* aged 18-35?

Interested in finding out more about your eye health with brand new technology?

Got approx. 1 hour to spare?

To volunteer for a new eye study investigating the link between diet, health and the macula in the eye using brand new technology for the first time in optometry practice in Ireland.

If interested, please contact Susan O'Shea 0879588281/0872108477

Email oshea.sue@gmail.com