Cone Photoreceptor Structure and Function in Adolescents and Young Adults with Type 1 Diabetes

by

Laura Elizabeth Finkelberg

A thesis submitted in conformity with the requirements for the degree of Master of Science

Institute of Medical Science
University of Toronto

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Abstract

This cross-sectional study assessed properties of cone photoreceptors in patients with type 1 diabetes, as potential biomarkers of sub-clinical retinopathy. Patients with no vascular signs of retinopathy (ages 12-25) exhibited abnormal neuroretinal structure and function of the cone photoreceptor layer as compared to age-similar controls. White flash electroretinogram a-waves, demonstrating L- and M-cone function, were significantly less steep in patients than in controls (by 1.3 mV/ms, 95% CI: -0.3, -2.3, p=0.01). Red flash electroretinograms did not differ between groups. Adaptive optics retinal imaging was used to visualize L- and M-cones in vivo. At 7.1° eccentricity along the 45° meridians, cone density was lower in patients than in controls. This difference was significant in the nasal hemiretina (168 cones/degree², 95% CI: 62, 273, p=0.002), but not in the temporal hemiretina. To definitively assess the ability of a-wave slope and cone density to predict subsequent development of retinopathy, longitudinal analyses are needed.
Acknowledgments

Just as it takes a village to raise a child, so it takes an entire academic team, clinical department, and support system to guide a Master’s student to her destination. This culmination of my studies was made possible by innumerable hours of collaborative effort from many individuals.

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## Contributions

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<th>Description</th>
</tr>
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<tbody>
<tr>
<td>AO</td>
<td>Adaptive optics</td>
</tr>
<tr>
<td>AOSLO</td>
<td>Adaptive optics scanning laser ophthalmoscopy</td>
</tr>
<tr>
<td>CDA</td>
<td>Canadian Diabetes Association</td>
</tr>
<tr>
<td>DME</td>
<td>Diabetic macular edema</td>
</tr>
<tr>
<td>DR</td>
<td>Diabetic retinopathy</td>
</tr>
<tr>
<td>ERG</td>
<td>Electroretinogram or electroretinography</td>
</tr>
<tr>
<td>mfERG</td>
<td>Multifocal electroretinogram</td>
</tr>
<tr>
<td>A1C</td>
<td>Glycosylated hemoglobin A1c</td>
</tr>
<tr>
<td>HTT</td>
<td>Huntingtin (gene or protein)</td>
</tr>
<tr>
<td>IN</td>
<td>Inferior nasal</td>
</tr>
<tr>
<td>IT</td>
<td>Inferior temporal</td>
</tr>
<tr>
<td>NPDR</td>
<td>Non-proliferative diabetic retinopathy</td>
</tr>
<tr>
<td>PDR</td>
<td>Proliferative diabetic retinopathy</td>
</tr>
<tr>
<td>Phot</td>
<td>Photopic</td>
</tr>
<tr>
<td>SLO</td>
<td>Scanning laser ophthalmoscopy</td>
</tr>
<tr>
<td>SCE</td>
<td>Stiles-Crawford effect</td>
</tr>
<tr>
<td>Scot</td>
<td>Scotopic</td>
</tr>
<tr>
<td>SN</td>
<td>Superior nasal</td>
</tr>
<tr>
<td>ST</td>
<td>Superior temporal</td>
</tr>
<tr>
<td>T1D</td>
<td>Type 1 diabetes</td>
</tr>
<tr>
<td>T2D</td>
<td>Type 2 diabetes</td>
</tr>
<tr>
<td>Td</td>
<td>Troland</td>
</tr>
<tr>
<td>Td-s</td>
<td>Troland-seconds</td>
</tr>
<tr>
<td>TUNEL</td>
<td>Terminal transferase dUTP nick end labeling</td>
</tr>
<tr>
<td>VTDR</td>
<td>Vision threatening diabetic retinopathy</td>
</tr>
<tr>
<td>WESDR</td>
<td>Wisconsin Epidemiological Study of Diabetic Retinopathy</td>
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A1 Patient Sample

A2 Predicting Impact of Red-Green Colour Vision Deficiencies on AO Results
1 Introduction

1.1 Overview

The purpose of this thesis is to determine the potential impact of type 1 diabetes on cone photoreceptor function, density, and distribution in adolescents and young adults. The first part of this introduction orients the reader to the retina, and the clinical tools used to investigate its structure and function. An overview of type 1 diabetes is given, and diabetic retinopathy, the most common eye complication of diabetes, is explored. Experimental research on the impact of diabetes on the retina, and specifically its impact on cone photoreceptors, is addressed. Finally, this introduction examines demonstrations of nasal-temporal retinal asymmetry in individuals with diabetes and in healthy controls.

1.2 Function and Structure of the Eye

1.2.1 Gross Anatomy of the Eye

The human eye is the organ that is the direct point of contact of the visual system with the external world (Remington, 2012). It is a fluid-filled sphere enclosed by three layers of tissue (Purves et al., 2008): an outer fibrous layer of connective tissue, a middle vascular layer, and an inner neural layer (Remington, 2012). The inner neural layer, the retina, is comprised of multiple cell types forming a complex and organized network that ultimately relays signals to the brain.

1.2.2 Optics of the Eye

The refractive power of the cornea and lens, the anterior structures of the eye, focus light onto the retina. The lens has an anterior and posterior node. Light converging from the environment onto the anterior node at a given angle is refracted at the same angle from the posterior node onto the retina (Tan, 2012).

An emmetropic eye is an optical system where the lens is at the appropriate power to focus light directly onto the retina. Eyes with a longer vitreous chamber or a more powerful lens will focus light in front of the retina. This condition is known as myopia, and is associated with “minus” refractive errors. Eyes with a shorter vitreous chamber or a less powerful lens will focus light
behind the retina. This condition is known as hyperopia, and is associated with “plus” refractive errors (Purves et al., 2008).

1.3 Function and Structure of the Retina

1.3.1 Representations of Retinal Space

Spatial coordinates on the retina or “eccentricities” are normally described with reference to the fovea, the central darkened region of the retina permitting the highest visual acuity. The literature often refers to the four primary meridians: superior (up), inferior (down), nasal (medial/towards the midline), and temporal (lateral/toward the temple). These meridians divide the eye into four areas called quadrants: superior temporal, superior nasal, inferior temporal, and inferior nasal. The oblique meridians refer to the meridians bisecting these quadrants at 45 degrees.

A length of the retinal surface is normally expressed in mm (as if the retina were flat) or in degrees (the angle subtended by that length). The conversion from mm to degrees is not consistent throughout the retina, but depends upon eccentricity from the fovea, and varies between 3 degrees to 4 degrees per mm.

1.3.2 Overview of the Neural Retina

Photoreceptors are the outermost layer of neural cells and the first to fire in response to light stimulation. In the most basic path of neural signal transmission, photoreceptors transmit signals to bipolar cells, which in turn transmit signals to retinal ganglion cells. Retinal ganglion cells are the source of all retinal output, and project their axons towards the brain. Additional cells project laterally and modify the responses of these three cell types. Horizontal cells receive input from photoreceptors and modify the responses of photoreceptors and bipolars. Amacrine cells receive input from bipolars and modify the responses of bipolar cells, ganglion cells, and other amacrine cells (Bear, 2007; Remington, 2012).

1.3.3 Stratified Retinal Blood Supply and Metabolism

The choroidal capillary bed provides nutrients and oxygen exclusively to the outer nuclear layer (photoreceptors). Metabolites from the choroid must diffuse through the retinal pigment epithelium to reach the outer nuclear layer. The central retinal artery, which enters the eye at the optic disc, provides nutrients to the rest of the neural retina. The central retinal artery gives rise
to the deep capillary network and the superficial capillary network. The deep capillary network supplies the inner nuclear layer, while the superficial capillary network supplies the retinal ganglion cell layer (Remington, 2012).

Glucose is the main energy source of the retina. The retinal pigment epithelium has glucose transporters on its apical and basal membranes that move glucose from the blood into the neural retina via facilitated diffusion. The retina is capable of both aerobic and anaerobic cellular respiration. In darkness, when photoreceptors are continuously depolarized, there is very little oxygen in the outer nuclear layer and so photoreceptors depend on anaerobic respiration (Remington, 2012). It has been demonstrated that the outer segments of photoreceptors are capable of regenerating ATP through non-mitochondrial biochemical pathways that use glycolytic energy (Pugh & Lamb, 1993).

1.4 Characteristics of Rod and Cone Photoreceptors

1.4.1 Spectral Sensitivity of Rods and Cones

Photoreceptors respond to electromagnetic radiation in the visible spectrum (with wavelengths between 400 and 700 nm) with a change in membrane potential. In other words, they convert light energy into a neural signal. This process is called phototransduction (Remington, 2012; Tessier-Lavigne, 2000). There are two principal types of photoreceptors, rods and cones. Rods are optimized for vision in dim or dark (scotopic) conditions, whereas cones are responsible for vision in bright (photopic) conditions and colour vision (Remington, 2012).

1.4.2 Spectral Sensitivity of Specific Cone Classes

There are three classes of cones: long-wavelength (L), medium-wavelength (M), and short-wavelength (S) cones (Neitz, Mancuso, Kuchenbecker, & Neitz, 2011). Each type of cone has a peak wavelength of light to which it is maximally sensitive, and a specific absorption spectrum around that peak wavelength. L-cones are maximally sensitive to wavelengths near 560 nm, and after post-synaptic processing give rise to the perception of red. M-cones are maximally sensitive to wavelengths near 530 nm, and after post-synaptic processing give rise to the perception of green. S-cones are maximally sensitive to wavelengths near 415 nm, and after post-synaptic processing give rise to the perception of blue. Specific combinations of activity among these cone classes give rise to perceptions of intermediate colours (Neitz et al., 2011).
1.5 Cellular Structure of Rods and Cones

Rods and cones are linear, elongated cells that are oriented along the visual axis (meaning that they point towards the pupil). Each cell is named for the shape that their posterior tips often take: in the case of rods, thin and cylindrical, and in the case of cones, conical (although some cones are shaped more like rods). Both types of photoreceptor are comprised of contiguous subcellular compartments. From posterior to anterior, these are: outer segment, cilium, inner segment, outer fiber, cell body, inner fibre, and synaptic terminal (Remington, 2012).

1.5.1 Structure of the Photoreceptor Outer Segment

The rod outer segment in primates is approximately 25 um in length and 2 um in diameter. The proportions of the cone outer segment in primates vary with retinal location (from retinal centre to periphery). The shorter, thicker cone outer segments at the periphery of the retina are approximately 13 um in length, 3 um in diameter at the base, and 1 um in diameter at the posterior tip (Pugh & Lamb, 2000).

Each outer segment is made up of a long stack of about 400 to 900 flat, membrane-bound sacs (discs), spaced uniformly at intervals of about 28 nm (Pugh & Lamb, 2000). Interspersed throughout the disc membranes are all the proteins necessary to initiate phototransduction. In rods, the disc membranes have been pinched off from the plasma membrane that encases the entire cell, and are free-floating within the outer segment. In cones, the disc membranes are formed by incompletely-pinched off invaginations that are continuous with the cell’s plasma membrane (Mustafi, Engel, & Palczewski, 2009; Tessier-Lavigne, 2000).

1.5.2 Outer Segment Renewal

Like other neurons, photoreceptors cannot be renewed. However, photoreceptors constantly regenerate their outer segments by shedding old discs and forming new ones. Old discs are discarded at the posterior tip of the outer segment, and are phagocytosed by the cells of the retinal pigment epithelium, while new discs are generated at the outer segment's anterior base (Tessier-Lavigne, 2000; Young, 1967).
1.6 Cellular Mechanism of Phototransduction

1.6.1 Membrane Properties of Photoreceptors and Equilibrium Potential

Unlike most other neurons, the equilibrium potential of photoreceptors in the absence of light stimulation is approximately -40mV. In dark conditions, photoreceptors are continuously depolarizing. This uncommon potential is the result of membrane channels that have a differential distribution across the inner and outer segment. The channels that dominate the photoreceptor equilibrium potential (cGMP-gated Na+ channels, non-gated K+ channels, and Na+/K+ ATPases) are outlined in Table 1.1 (adapted from Tessier-Lavigne, 2000).

1.6.2 Photoreceptor Dark Current

The synaptic terminals of both rods and cones house vesicles containing glutamate, their only neurotransmitter. Rods and cones continuously depolarize in darkness, releasing glutamate. Upon exposure to light, they undergo graded hyperpolarization (Remington, 2012).

Continuous depolarization in dark (scotopic) conditions is created by a circuit of self-sustaining ion flow termed the dark current. The dark current is created by the combination of (a) the intracellular Na+ flow from the outer segment to the inner segment and (b) extracellular K+ flow from the inner segment to the outer segment. These currents create a continuous circuit of positive ions seeking areas of negative potential. (Remington, 2012; Tessier-Lavigne, 2000).

The distinctive properties of membrane channels that contribute to the equilibrium potential are summarized in Table 1.1.
<table>
<thead>
<tr>
<th>Name of Protein</th>
<th>Density of Protein in Outer Segment Membrane</th>
<th>Density of Protein in Inner Segment Membrane</th>
<th>Action</th>
<th>Intracellular Effect</th>
<th>Extracellular Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>cGMP-gated Na(^+) Channel</td>
<td>High</td>
<td>Absent</td>
<td>Allows Na(^+) influx into outer segment only, when cGMP present (as in scotopic conditions); Na(^+) also diffuses through cilium to inner segment</td>
<td>Photoreceptor depolarized to -40mV in darkness</td>
<td>Low [Na(^+)] locally; relatively negative extracellular potential as compared to extracellular membrane of inner segment</td>
</tr>
<tr>
<td>K+ Channel (non-gated)</td>
<td>Absent</td>
<td>High</td>
<td>Allows continuous efflux of K(^+) from inner segment only</td>
<td>Low [K(^+)] in inner segment; some K(^+) migrates from outer segment to inner segment through cilium</td>
<td>High [K(^+)] locally; relatively positive extracellular potential as compared to extracellular membrane of outer segment; K(^+) flows extracellularly toward outer segment membrane</td>
</tr>
<tr>
<td>Na(^+)/K(^+) ATPase (antiporter)</td>
<td>Low</td>
<td>High</td>
<td>Na(^+) extruded from inner segment and K(^+) imported</td>
<td>Maintains steady intracellular concentrations of Na(^+) and K(^+) in spite of high rates of diffusion through ion-specific channels</td>
<td>Maintains higher [Na(^+)] and lower [K(^+)] extracellularly; establishes the Na(^+) and K(^+) gradients upon which the ion channels can act</td>
</tr>
</tbody>
</table>

**Table 1.1:** Properties of membrane channels that dominate the equilibrium potential in photoreceptors. Distributions throughout the cell membrane, types of activity, and intracellular and extracellular effects are described. Adapted from Tessier-Lavigne, 2000.
1.6.3 The Phototransduction Cascade

Phototransduction is initiated when rhodopsin molecules, which span the membranes of outer segment discs, are activated by light energy. Once rhodopsin is activated, phototransduction is carried out by a G-protein cascade. This cascade utilizes signal amplification to close cGMP-gated channels and to ultimately induce graded hyperpolarization of the photoreceptor. The specific substrates involved in phototransduction and their respective functions are outlined in Table 1.2 and Table 1.3.

<table>
<thead>
<tr>
<th>Inactive Form</th>
<th>Mechanism of Activation</th>
<th>Active Form</th>
<th>Post-Activation Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Rhodopsin/Photopsin</td>
<td>Photoisomerization</td>
<td>Rh*/Ph*</td>
<td>Activates G-protein</td>
</tr>
<tr>
<td>2 G-protein (transducin)</td>
<td>Contact with Rh*/Ph*</td>
<td>G*</td>
<td>Activates PDE</td>
</tr>
<tr>
<td>3 Phosphodiesterase (PDE)</td>
<td>Binding by G*</td>
<td>PDE*</td>
<td>Hydrolyzes cGMP</td>
</tr>
</tbody>
</table>

**Table 1.2:** Summary of the first phase of phototransduction, which generates active phosphodiesterase molecules (adapted from Breton, Schueller, Lamb, & Pugh, 1994).

<table>
<thead>
<tr>
<th>Active Form</th>
<th>Pre-Deactivation Function</th>
<th>Mechanism of Deactivation</th>
<th>Effect of deactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 Cyclic guanosine monophosphate (cGMP)</td>
<td>Holds open cation channels in plasma membrane</td>
<td>Hydrolysis by PDE*</td>
<td>Reduced binding to ligand-gated cation channels</td>
</tr>
<tr>
<td>5 Cation channels (cGMP-gated)</td>
<td>Permit Na+ and Ca2+ influx (dark current)</td>
<td>Reduced ligand binding by cGMP</td>
<td>Less Na+/Ca2+ influx $\rightarrow$ hyperpolarization of cytoplasm</td>
</tr>
<tr>
<td>6 Synaptic vesicles containing glutamate</td>
<td>Release glutamate into synaptic cleft in response to Ca2+ (dark current)</td>
<td>Cytoplasmic hyperpolarization/ reduced intracellular Ca2+</td>
<td>Reduced glutamate release</td>
</tr>
</tbody>
</table>

**Table 1.3:** Summary of the second phase of phototransduction, which results in a graded hyperpolarization of the cell and reduced release of glutamate (adapted from Breton et al., 1994).
1.7 Measuring Photoreceptor Function with Full Field Electroretinography (ERG)

1.7.1 Advantages and Disadvantages of ERG

The full-field electroretinogram (ERG) is a well-established clinical electrophysiology technique used to assess global retinal function in many types of retinal disease. Unlike multifocal ERG, the full-field ERG cannot isolate function or dysfunction to specific retinal areas. However, the full-field ERG has several advantages, including a high signal-to-noise ratio in its collected data. Moreover, a greater variety of stimuli can be employed in full-field ERGs, such that specific cone classes can be isolated. In our study, for instance, the ERG was used to selectively investigate the function of L- and M-cones, but not S-cones.

1.7.2 Cellular Contributions to ERG Recordings (First 40 Milliseconds)

The human ERG response is depicted as a waveform, comprised of a predictable series of valleys (hyperpolarizations) and peaks (depolarizations). The first valley is referred to as the a-wave. The leading edge of the a-wave is created by the collective activity of hyperpolarizing photoreceptors. This particular contribution to the scotopic ERG is termed P3 by Granit. After approximately 10ms, bipolar cells begin to depolarize. This contribution is Granit’s P2 (Granit, 1933). The negative photoreceptor response (P3) is summed with positive bipolar response (P2), gradually truncating the leading edge of the a-wave. The a-wave peak (local minimum, with slope = 0), is the point at which the photoreceptor response (P3) and competing bipolar response (P2) are of equal magnitude. The subsequent upswing created by dominance of bipolar cell activity (P2) is the beginning of the b-wave (Figure 1.1).
Clinically, the amplitude and timing (“implicit time”) of the a-wave peak are used to make inferences regarding photoreceptor activity. However, the value of these inferences is limited by the sizeable contribution of bipolar cell activity to the a-wave peak. Therefore, extrapolation of photoreceptor (P3)-driven activity requires examining properties of the a-wave that occur prior to the a-wave peak. (Hood & Birch, 1997)

1.7.3 Isolating Photoreceptor (P3) Activity by Calculating the Maximum Rate of Rise of the ERG a-wave

The maximum rate of rise of the a-wave (Breton et al., 1994) is an important property of photoreceptor function, and could potentially be used to study health and disease states of photoreceptors. However, maximum rate of rise as a standalone measure is not generally applied to ERG analysis in clinical settings. By definition, the maximum rate of rise is the maximum negative slope achieved prior to the a-wave peak. Because the maximum slope occurs prior to the bipolar (P2)-driven shallowing of the a-wave, the maximum rate of rise is an ideal proxy for the isolated photoreceptor (P3) response.
1.8 Assessing Structure of the Photoreceptor Mosaic with Adaptive Optics (AO) Retinal Imaging

1.8.1 Scope of AO Imaging

Adaptive optics (AO) retinal imaging is an emerging research and clinical tool that captures high resolution images of the retina at the microvascular and cellular level, including the cone photoreceptor mosaic. Historically, measuring the spatial density of cone photoreceptors has only possible via post-mortem histological study. In recent years, investigators have used AO as an in vivo technique in humans to investigate cone spatial density. This has been done both in healthy individuals, and in individuals with retinal degenerative diseases (as determined by genetic testing, ophthalmic examination, and/or visual electrophysiological tests).

1.8.2 Using AO to Assess Cone Density in Retinal Disease: Stargardt’s Macular Dystrophy and Retinitis Pigmentosa

AO has been able to detect markedly lower cone densities in individuals with retinal degenerative diseases as compared to healthy controls. In one such study, Xue and colleagues (Xue, Choi, Doble, & Werner, 2007) imaged five participants: one individual with Stargardt’s macular dystrophy, age 29; and one individual with retinitis pigmentosa, age 12; and three healthy controls, ages 20, 22, and 32. Histologically-acquired cone density data (Curcio et al., 1990) were used as the gold standard. In controls, cone densities were assessed along the horizontal meridian at 2 degrees and 4 degrees temporal, and along an oblique meridian at 4 degrees temporal/4 degrees superior. Cone densities in controls were consistently in 99% agreement with histological data. In the individual with Stargardt’s, an image was taken along the vertical meridian at 4 degrees inferior. In this individual, cone density was 21% of the value predicted by histology. The individual with retinitis pigmentosa was imaged along oblique meridians, at 4 degrees temporal/4 degrees inferior, and at 2 degrees temporal/4 degrees superior. In comparison to cone densities predicted by histology, cone densities in these regions were at 27% and 15%, respectively.
1.8.3 AO Imaging of Cone Photoreceptors in Other Retinal Disease Case Studies

Other investigators using AO retinal imaging have confirmed the lower cone densities seen in Stargardt’s and in retinitis pigmentosa (Chen et al., 2011; Duncan et al., 2007; Talcott et al., 2011). AO retinal imaging has also demonstrated disruption of the normal cone photoreceptor mosaic in additional types of retinal and macular dystrophies (Bessho et al., 2008; Choi et al., 2006; Duncan et al., 2007; Wolfing, Chung, Carroll, Roorda, & Williams, 2006), colour vision deficiency (Baraas et al., 2007; Carroll, Neitz, Hofer, Neitz, & Williams, 2004), rod monochromacy (Carroll, Choi, & Williams, 2008), mitochondrial DNA T8993C mutation (Yoon et al., 2009), central serous chorioretinopathy (Ooto et al., 2010), Usher syndrome (Ratnam, Västinsalo, Roorda, Sankila, & Duncan, 2013; Talcott et al., 2011), acute zonal occult outer retinopathy (Mkrtchyan et al., 2012), enhanced S-cone syndrome (S. P. Park, Hong, et al., 2013), idiopathic macular telangiectasia (Ooto et al., 2013), and surgically closed macular holes (Ooto et al., 2012).

1.8.4 Factors Mediating Cone Density and Distribution in Healthy Controls

There is a great amount of inter-individual variation in cone density and distribution (Elsner, Chui, Feng, & Burns, 2012; Lombardo, Lomoriello, Ducoli, Stirpe, & Serrao, 2013). Therefore, it is critical to disentangle what biological factors mediate these differences and to what degree.

Several investigators have predicted that axial length is associated with cone density. Variations in axial length are usually created by a change in shape of the vitreal chamber and not the cornea or lens (Chui, Song, & Burns, 2008a; Li, Tiruvedhula, & Roorda, 2010). These changes are inextricably linked with refractive error, as they cause light rays to converge in front of or behind the retina (creating myopia or hyperopia, respectively). Myopia may be associated with retinal stretching, whereby a constant number of photoreceptors are distributed over a larger area, thus reducing photoreceptor density relative to other eyes (Atchison, Schmid, & Pritchard, 2006; Coletta & Watson, 2006; Rossi, Tarrant, & Roorda, 2007).

Chui and colleagues (Chui et al., 2008a) found cone density in cones/mm² and cone density in cones/degree² to have differing relationships with axial length. At 1mm, 1.5mm, and 2mm eccentricity along the superior meridian, cone density in cones/mm² was significantly negatively
correlated with axial length (longer eyes exhibited lower densities). Conversely, at 3, 5, and 7 degrees eccentricity, cone density in cones/degree$^2$ showed no relationship with axial length. The authors suggested expressing densities in cones/degree$^2$ minimizes inter-individual variation.

Li and colleagues predicted a different relationship between cone density and axial length at the fovea (Li et al., 2010). They noted that marmosets with experimentally induced myopia had higher cone densities relative to emmetropic eyes (Troilo, 1998), which conflicts with the parafoveal data of Chui and colleagues (Chui et al., 2008a). The authors found cone density in cones/mm$^2$ showed no relationship with axial length at 0.1mm and 0.2mm eccentricity, but did show a negative correlation with axial length beginning at 0.3mm. Additionally, at 0.1mm, 0.2mm, and 0.3mm eccentricity, cone densities in cones/degree$^2$ were significantly positively correlated with axial length (longer eyes exhibited higher cone densities). At 0.33 degrees, 0.67 degrees, and 1.00 degrees eccentricity, cones/mm$^2$ were negatively correlated with axial length, Cones/degree$^2$ exhibited no relationship with axial length at the same eccentricities. These results suggest that at the foveal centre, cone density is actually higher in myopic eyes. However, these relationships are sensitive to how eccentricity is reported, particularly at the fovea, where conversions from mm to degrees are affected strongly by axial length (Li et al., 2010).

Therefore, although cones/degree$^2$ is an ideal method of expressing cone density at parafoveal eccentricities, there may not be a single systematic way to assess cone density at the foveal centre. There is evidence that uniformly-distributed retinal stretching in myopia is an oversimplification of the biological underpinnings of inter-individual variation in cone photoreceptor density and distribution (Chui et al., 2008a; Li et al., 2010).

Song and colleagues (Song, Chui, Zhong, Elsner, & Burns, 2011) found that within the central fovea, up to eccentricities of 0.45 mm, cone densities in older adults (50 to 65 years old) were significantly lower than cone densities in younger adults (22 to 35 years old). This effect was not observed at more peripheral retinal locations.

Park and colleagues (S. P. Park, Chung, Greenstein, Tsang, & Chang, 2013) measured cone densities at 0.5mm, 1.0mm, and 1.5mm eccentricity and found a weakly negative (not significant) correlation between cone density and age). Gender, ocular dominance, and ethnicity had no impact on cone density.
1.8.5 Description of AO Technology

Adaptive optics (AO) is the practice of correcting optical distortions to dramatically improve image quality (Max, 2012). These optical distortions are termed wavefront aberrations. When light rays that were once parallel (forming a plane wave) encounter disturbances in the media they are travelling through, and they become refracted at slightly different angles with respect to one another, that wavefront is said to have incurred aberrations (Max, 2012).

These aberrations are low-order (described by simple geometrical properties) or high-order (described by complex geometrical properties; Roorda, 2012). One way of expressing or quantifying the shapes of aberrations geometrically and mathematically is with Zernike polynomials, which denote specific three-dimensional perturbations of the unit circle (Noll, 1976). Linear combinations of one or more Zernike polynomials describe virtually any kind of aberration at a given time point of a wavefront passing through a circular pupil (Max, 2012). This is highly applicable to retinal imaging.

Wavefront aberrations have not only static components (whose properties are stable over time), but also dynamic components (whose properties change with time; Roorda, 2012). While an un-pliable, rigid lens of the appropriate shape might be capable of compensating for a certain type of static aberration, it would not cope with the dynamic components of optical aberrations.

Obtaining in vivo images of the retina requires shining light onto the retina via the pupil, and receiving backscattered light (light reflected off the retina) with the recording device of interest. Light entering the eye must pass through the cornea and lens, which contain microscopic irregularities, and so the wavefront of backscattered light is highly prone to aberrations (Roorda, 2012; Roorda et al., 2002). Ocular imaging systems with AO correction are able to overcome much, but not all, of these aberrations.

The essential components of an AO retinal imaging system are the Hartmann-Shack wavefront sensor and the deformable mirror. The wavefront sensor detects and quantifies aberrations to the wavefront and communicates these data to the deformable mirror. In turn, the deformable mirror adjusts its shape to accommodate for and correct these aberrations in real time (Liang, Williams, & Miller, 1997).
The mechanism of action of the Hartmann-Shack wavefront sensor is based on the Hartmann test, which quantifies the local slopes of a wavefront (Liang, Grimm, Goelz, & Bille, 1994). An ideal plane wave has equal slopes throughout, whereas a deformed wave will have differing slopes. The sensor is comprised of a sheet of many tiny apertures (a lenslet array), each of which brings light into focus on a focal plane (a detector). Each lenslet in the array forms a small dot on the detector called a “focus spot”. An ideal plane wave will bring light into focus directly along the optical axes of the lenslets, such that the focus spots form sharp dots in an evenly spaced grid. This is the reference pattern. An aberrated wavefront will produce focus spots that deviate from the reference pattern in shape or position. The detector assesses these deviations to calculate the local slopes of the wavefront (Liang, Grimm, Goelz, & Bille, 1994).

The detector inputs these local slopes to the deformable mirror, which reflects the incoming wavefront. There are several types of deformable mirrors, but the most conventional types adhere to the following construction. Behind the reflecting surface of the deformable mirror are many pistons, called actuators. There are at least as many as the number of focus spots. Each actuator is attached either to a continuous, thin, pliable face sheet mirror or to individual segments of rigid mirror (Kubby, 2012). The actuators push and pull the mirror sheet or segments as commanded by the detector, in order to achieve a shape that will best correct the wavefront. In the case of segmented mirrors, actuators are sometimes capable also of tipping and tilting individual mirror segments in addition to pushing and pulling (Kubby, 2012).

In sum, successful AO retinal imaging requires the coordination of the wavefront sensor, the deformable mirror, and a skilled operator to enable the capture of cone photoreceptor images from which density and distribution can be assessed.
1.9 Type 1 Diabetes (T1D)

1.9.1 Definition and Pathophysiology of T1D

Type 1 diabetes (T1D) is a chronic deficit in insulin. T1D is characterized by sudden onset, and is normally the result of autoimmune-driven destruction of the beta cells of the islets of Langerhans of the pancreas. Beta cells are the sole producers of endogenous insulin in the human body (Campbell & Reece, 2002). Insulin is a peptide hormone that fuels cellular metabolism. It is chiefly responsible for transporting glucose from the bloodstream into hepatic tissue, adipose tissue, and skeletal muscle. Neurons are capable of glucose uptake in the absence of insulin. Without insulin, blood glucose remains high, whereas intracellular glucose remains low. Therefore, the physiological effects of T1D are mediated by two types of dysglycemia: hyperglycemia and hypoglycemia (Campbell & Reece, 2002).

Hyperglycemia can cause excess glucose to be excreted in the urine. The altered osmolarity of the urine causes excess fluid to be excreted along with it, creating the common symptoms of increased urination (polyuria) and increased thirst (polydipsia; Campbell & Reece, 2002). Intracellular hypoglycemia causes cellular starvation, and over time, marked weight loss. Ultimately, fat becomes the primary substrate for cellular metabolism. The ketones released during fat breakdown accumulate in the blood, and can cause the blood to become dangerously acidic. This is a critical condition known as diabetic ketoacidosis (Campbell & Reece, 2002). Without treatment, the prolonged dysglycemia caused by T1D can be fatal. Even with exemplary treatment by qualified healthcare professionals, T1D can still compromise the health of many organs, notably the heart, kidneys, nerves, blood vessels, and eyes (Canadian Diabetes Association, CDA, 2008).

1.9.2 Diagnosis of T1D

Diabetes is diagnosed clinically based on one or more of the following criteria: fasting plasma glucose, an oral glucose tolerance test, or abnormal casual plasma glucose in the presence of classic symptoms of diabetes (CDA, 2008). Specific criteria are outlined in Table 1.4.
### Table 1.4: Diagnostic criteria for diabetes, adapted from the Canadian Diabetes Association Clinical Practice Guidelines (CDA, 2008).

Prolonged hyperglycemia causes increased binding of glucose to hemoglobin in red blood cells, known as glycosylation of hemoglobin. Glycosylated hemoglobin (A1C) levels can be useful in determining long-term glucose control in individuals with T1D. Still, A1C is not currently used as a diagnostic criterion for T1D, due to a lack of standardization in measurement (CDA, 2008).

#### 1.9.3 T1D Treatment Strategies

The most common treatment for T1D is insulin replacement therapy. The goal is to restore and stabilize plasma glucose levels within a healthy range, known as euglycemia. Insulin is delivered via syringe, pen, or continuous subcutaneous insulin infusion (CSII or “pump”). Most types of insulin used in treatment are produced by recombinant DNA technology. This process generates two categories of insulin. The first is insulin that is structurally identical to the endogenous human form. The second is comprised of insulin analogues, which have slight modifications to their structure that favourably alter their pharmacokinetics (CDA, 2008).

The current standard for treatment is a basal-prandial insulin regimen. This involves the administration of a short-acting insulin or a rapid-acting insulin analogue prior to meals (“prandial” insulin), on top of an intermediate-acting insulin or a long-acting insulin analogue (“basal” insulin) administered once or twice daily. The basal-prandial regimen mimics the timing
of endogenous insulin release in normoglycemic individuals. With respect to efficacy of insulin sub-types, the more rapid the onset of a given prandial insulin, the better it performs at glycemic control. Users experience less postprandial hyperglycemia, fewer hypoglycemic episodes, and lower A1C levels. The same is true of longer-acting basal insulins (CDA, 2008).

The recommended glycemic targets for each age group are outlined in Table 1.5. These targets represent compromise that balances (a) minimization of hyperglycemia, which can trigger complications in the long-term with (b) minimization of hypoglycemic episodes, which pose acute health risks. Severe hypoglycemia is especially dangerous in younger children, as it is associated with cognitive impairment later in life. Therefore, glycemic targets are less stringent for younger individuals (CDA, 2008).

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>A1C</th>
<th>Fasting/Preprandial Plasma Glucose</th>
<th>2-hour Postprandial Plasma Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 6</td>
<td>&lt;8.5%</td>
<td>6.0 – 12.0 mmol/L</td>
<td>--</td>
</tr>
<tr>
<td>6 – 12</td>
<td>&lt;8.0%</td>
<td>4.0 – 10.0 mmol/L</td>
<td>--</td>
</tr>
<tr>
<td>13 – 18</td>
<td>≤7.0%</td>
<td>4.0 – 7.0 mmol/L</td>
<td>5.0 – 10.0 mmol/L</td>
</tr>
<tr>
<td>18 +</td>
<td>≤7.0%</td>
<td>4.0 – 7.0 mmol/L (5.0 – 8.0 mmol/L if A1C targets not being met)</td>
<td>5.0 – 10.0 mmol/L</td>
</tr>
</tbody>
</table>

Table 1.5: Glycemic targets (long-term, A1C; and short-term, fasting glucose and postprandial glucose) for children and adolescents with T1D, stratified by age-group (CDA, 2008).

1.10 Definition and Classification of Diabetic Retinopathy (DR)

1.10.1 Definition of DR

Diabetic retinopathy (DR) is the primary eye complication of diabetes, caused by the cumulative effects of hyperglycemia and insulin deficiency. It is a progressive disease of the vascular and neural retina that gradually impairs vision. Clinically, DR is diagnosed based on vascular abnormalities. Vascular abnormalities are classified into two broad categories, nonproliferative
(mild) and proliferative (severe). Nonproliferative changes include microvascular abnormalities, such as microaneurysms, hard exudates, and dot hemorrhages. Nonproliferative vascular abnormalities may appear, disappear, and reappear over time. Proliferative changes are generally macroscopic, propagating, and are permanent without treatment. Proliferative changes include neovascularization and proliferation of fibroblasts.

Non-proliferative changes that are risk factors for progression to proliferative changes include: macular edema, cotton-wool spots, intraretinal microvascular abnormalities, and venous beading (Antonetti, Klein, & Gardner, 2012).

### 1.10.2 Classification of DR

DR as a disease is similarly classified into two broad categories, nonproliferative DR (NPDR) and proliferative DR (PDR; Wilkinson, 2010). The Early Treatment Diabetic Retinopathy Study (ETDRS) grading scale is the gold standard for evaluation of DR severity in clinical trials (Early Treatment Diabetic Retinopathy Study Research Group, 1981, 1991). The ETDRS grading scale is based upon the modified Airlie House classification of DR, and divides proliferative and nonproliferative DR each into several sub-classifications with categorical labels (mild to severe) and numerical labels (10 to 85). Ultimately, the severity of DR is classified based on the presentation and quantity of each type of lesion. Seven standard 30-degree-field photographs are captured of the patient’s fundus (en face images of the retinal surface). These photographs are compared with a standard set of photographs documenting prototypical lesions (Wilkinson, 2010). An abbreviated summary of the ETDRS grading scale is presented in Table 1.6 (Wilkinson, 2010).

Diabetic macular edema (DME) severity is classified separately with its own scale, the DME Disease Severity Scale (Wilkinson et al., 2003). DME is defined as retinal thickening from accumulation of fluid within one disc diameter of the macula (Ferris & Patz, 1984; Patz, Schatz, Berkow, Gittelsohn, & Ticho, 1977; in Guess & Dubovy, 2010) DME is the primary contributor to vision loss in NPDR (Guess & Dubovy, 2010).
<table>
<thead>
<tr>
<th>Level</th>
<th>Severity</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>No retinopathy</td>
<td>DR absent</td>
</tr>
<tr>
<td>20</td>
<td>Very mild NPDR</td>
<td>Microaneurysms only</td>
</tr>
<tr>
<td>35</td>
<td>Mild NPDR</td>
<td>Hard exudates, soft exudates, and/or mild retinal hemorrhages</td>
</tr>
<tr>
<td>43A</td>
<td>Moderate NPDR</td>
<td>Moderate retinal hemorrhages in four quadrants, or severe retinal hemorrhage in one quadrant</td>
</tr>
<tr>
<td>43B</td>
<td>Moderate NPDR</td>
<td>Mild intraretinal microvascular abnormalities in one to three quadrants</td>
</tr>
<tr>
<td>47A</td>
<td>Moderate NPDR</td>
<td>Both level 43 characteristics</td>
</tr>
<tr>
<td>47B</td>
<td>Moderate NPDR</td>
<td>Mild intraretinal microvascular abnormalities in four quadrants</td>
</tr>
<tr>
<td>47C</td>
<td>Moderate NPDR</td>
<td>Severe retinal hemorrhages in two to three quadrants</td>
</tr>
<tr>
<td>47D</td>
<td>Moderate NPDR</td>
<td>Venous beading in one quadrant</td>
</tr>
<tr>
<td>53A</td>
<td>Severe NPDR</td>
<td>Two or more level 47 characteristics</td>
</tr>
<tr>
<td>53B</td>
<td>Severe NPDR</td>
<td>Severe retinal hemorrhages in four quadrants</td>
</tr>
<tr>
<td>53C</td>
<td>Severe NPDR</td>
<td>Moderate to severe intraretinal microvascular abnormalities in at least one quadrant</td>
</tr>
<tr>
<td>53D</td>
<td>Severe NPDR</td>
<td>Venous beading in at least two quadrants</td>
</tr>
<tr>
<td>53E</td>
<td>Very severe NPDR</td>
<td>Two or more level 53 characteristics</td>
</tr>
<tr>
<td>61</td>
<td>Mild PDR</td>
<td>New vessels remote from optic disc, smaller in size than half the disc area, in one or more quadrants</td>
</tr>
<tr>
<td>65A</td>
<td>Moderate PDR</td>
<td>New vessels remote from optic disc, equal to or larger than half the disc diameter, in one or more quadrants</td>
</tr>
<tr>
<td>65B</td>
<td>Moderate PDR</td>
<td>New vessels less than 1 disc diameter from the optic disc, size is less than a quarter to a third of the disc area</td>
</tr>
<tr>
<td>71,75</td>
<td>High risk PDR</td>
<td>New vessels less than 1 disc diameter from the optic disc, size is equal to or greater than a quarter to a third of the disc area, OR new vessels remote from optic disc, equal to or larger than half the disc diameter, plus vitreous hemorrhage or preretinal hemorrhage, OR vitreous hemorrhages or preretinal hemorrhage obscuring an area of retina equal to or greater than the optic disc area</td>
</tr>
<tr>
<td>81,85</td>
<td>Advanced PDR</td>
<td>Fundus partially or completely obscured by vitreous hemorrhages, new vessels ungradeable in at least one field, or retina detached at the center of the macula</td>
</tr>
</tbody>
</table>

*Table 1.6:* Abbreviated summary of ETDRS grading scale (Early Treatment Diabetic Retinopathy Study Research Group, 1981; in Guess & Dubovy, 2010).
1.11 Risk Factors for DR: Evidence from Epidemiological Studies

1.11.1 Risk of DR in T1D Versus Type 2 Diabetes (T2D)

A recent meta-analysis by Yau and colleagues (Yau et al., 2012) has shed light on risk factors for and prevalence rates of DR in T1D and type 2 diabetes (T2D). The authors combined individual patient data from epidemiological studies worldwide, spanning 1980 to 2008, for patients ages 20 to 79. They reported the age-standardized prevalence of DR, PDR, DME, and vision-threatening DR (VTDR; includes all with PDR or DME or PDR+DME). The authors confirmed that type of diabetes (T1D vs. T2D), duration of diabetes, long-term glucose control (as measured by A1C), blood pressure, and ethnicity impact one’s likelihood of developing DR (Yau et al., 2012). The prevalence of DR in individuals with T1D versus those with T2D is outlined in Table 1.7 (adapted from Yau et al., 2012).

<table>
<thead>
<tr>
<th>Type</th>
<th>DR</th>
<th>PDR</th>
<th>DME</th>
<th>VTDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1D</td>
<td>77.31% (76.34 - 78.28)</td>
<td>32.39% (31.76 - 33.01)</td>
<td>14.25% (13.86 - 14.64)</td>
<td>38.48% (37.80 - 39.16)</td>
</tr>
<tr>
<td>T2D</td>
<td>25.16% (24.96 - 25.36)</td>
<td>2.97% (2.91 - 3.02)</td>
<td>5.57% (5.48 - 5.66)</td>
<td>6.92% (6.83 - 7.02)</td>
</tr>
</tbody>
</table>

Table 1.7: Age-standardized prevalence rates of DR, PDR, DME, and VTDR, in T1D vs. T2D. 95% CI in brackets. Taken from worldwide meta-analysis spanning 1980-2008 (adapted from Yau et al., 2012).

The prevalence of DR, PDR, DME, and VTDR in T1D is greater than analogous prevalence rates in T2D. For instance, the prevalence of PDR in T1D is ten times the prevalence of PDR in T2D. Therefore, T1D is a major risk factor for the development of DR. It should be noted that T2D sample here appears to be comprised of a large proportion of individuals who have had T2D for less than ten years, as the prevalence of DR in T2D jumps from 18% in the 0-10 year duration stratum to 51% in the 10-20 year duration stratum.
1.11.2 Duration of T1D and Risk of DR

The prevalence of DR stratified by duration of T1D is outlined in Table 1.8 (adapted from Yau et al., 2012). Among those with T1D, the prevalence of DR increases dramatically with increased time since diagnosis. Most patients (86%) who have had T1D for 20 years or more will have clinical levels of DR.

<table>
<thead>
<tr>
<th>Duration of T1D</th>
<th>DR</th>
<th>PDR</th>
<th>DME</th>
<th>VTDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10 years</td>
<td>20.53%</td>
<td>0.37%</td>
<td>0.55%</td>
<td>0.74%</td>
</tr>
<tr>
<td>(Total n = 456)</td>
<td>(18.73 - 22.34)</td>
<td>(0.31 - 0.43)</td>
<td>(0.48 - 0.63)</td>
<td>(0.65 - 0.82)</td>
</tr>
<tr>
<td>10 to &lt; 20 years</td>
<td>55.55%</td>
<td>19.46%</td>
<td>12.27%</td>
<td>14.29%</td>
</tr>
<tr>
<td>20 + years</td>
<td>86.22%</td>
<td>40.36%</td>
<td>17.31%</td>
<td>47.2%</td>
</tr>
<tr>
<td>(Total n = 1,026)</td>
<td>(85.07 - 87.37)</td>
<td>(39.60 - 41.12)</td>
<td>(16.83 - 17.8)</td>
<td>(46.38 - 48.03)</td>
</tr>
</tbody>
</table>

Table 1.8: Age-standardized prevalence rates of DR, PDR, DME, and VTDR, in individuals with T1D only, sub-classified by duration of diabetes. 95% CI in brackets. Taken from worldwide meta-analysis spanning 1980-2008 (adapted from Yau et al., 2012).

1.11.3 A1C and Risk of DR

Patients following the recommended guidelines for glycemic control (for ages 13+; A1C ≤ 7.0%) exhibit the lowest prevalence of DR, with prevalence rates increasing in each category of increased A1C level. However, the prevalence is still 20% for those who are meeting glycemic targets, suggesting that excellent glycemic control alone at the time of measurement is not sufficient to prevent DR. Table 1.9 summarizes prevalence rates of DR, in patients with T1D or T2D, that are stratified by A1C level (adapted from Yau et al., 2012).
### Table 1.9: Age-standardized prevalence rates of DR, PDR, DME, and VTDR, in individuals with T1D and T2D combined, sub-classified by A1C level. 95% CI in brackets. Taken from worldwide meta-analysis spanning 1980-2008 (adapted from Yau et al., 2012).

<table>
<thead>
<tr>
<th>A1C</th>
<th>DR</th>
<th>PDR</th>
<th>DME</th>
<th>VTDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 7.0%</td>
<td>17.99%</td>
<td>3.1%</td>
<td>3.59%</td>
<td>5.40%</td>
</tr>
<tr>
<td>(Total n = 3,290)</td>
<td>(17.64 - 18.33)</td>
<td>(2.93-3.26)</td>
<td>(3.42-3.76)</td>
<td>(5.19-5.60)</td>
</tr>
<tr>
<td>7.1 - 8.0%</td>
<td>33.13%</td>
<td>6.87%</td>
<td>6.30%</td>
<td>10.82%</td>
</tr>
<tr>
<td>(Total n = 2,344)</td>
<td>(32.64 - 33.62)</td>
<td>(6.63-7.10)</td>
<td>(6.06-6.54)</td>
<td>(10.53-11.10)</td>
</tr>
<tr>
<td>8.1-9.0%</td>
<td>43.1%</td>
<td>9.64%</td>
<td>7.69%</td>
<td>13.64%</td>
</tr>
<tr>
<td>(Total n = 1,843)</td>
<td>(42.53-43.66)</td>
<td>(9.37-9.90)</td>
<td>(7.46-7.93)</td>
<td>(13.33-13.95)</td>
</tr>
<tr>
<td>&gt;9.0%</td>
<td>51.2%</td>
<td>10.93%</td>
<td>12.49%</td>
<td>18.35%</td>
</tr>
<tr>
<td>(Total n = 4,346)</td>
<td>(50.8-51.6)</td>
<td>(10.76-11.11)</td>
<td>(12.31-12.67)</td>
<td>(18.13-18.58)</td>
</tr>
</tbody>
</table>

### 1.11.4 Hypertension and Risk of DR

Individuals who have blood pressure greater than 140/90 mmHg or who are currently being treated for hypertension have a greater prevalence of DR than those with normal blood pressure. The disparity in prevalence between those with and without hypertension is particularly pronounced in severe forms of DR. **Table 1.10** describes the prevalence rates of DR in individuals with T1D or T2D who have normal blood pressure, versus those who are hypertensive (adapted from Yau et al., 2012).

### Table 1.10: Age-standardized prevalence rates of DR, PDR, DME, and VTDR, in individuals with T1D and T2D combined, sub-classified by blood pressure. 95% CI in brackets. Taken from worldwide meta-analysis spanning 1980-2008 (adapted from Yau et al., 2012). *Hypertension is defined as blood pressure greater than 140/90 mmHg, or and/or self-report of treatment for hypertension.

<table>
<thead>
<tr>
<th>Blood Pressure</th>
<th>DR</th>
<th>PDR</th>
<th>DME</th>
<th>VTDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>30.84%</td>
<td>4.16%</td>
<td>5.45%</td>
<td>7.60%</td>
</tr>
<tr>
<td>(Total n = 6,516)</td>
<td>(30.59 - 31.09)</td>
<td>(4.07 - 4.25)</td>
<td>(5.35 - 5.55)</td>
<td>(7.48 - 7.72)</td>
</tr>
<tr>
<td>Hypertensive*</td>
<td>39.55%</td>
<td>12.32%</td>
<td>10.59%</td>
<td>17.63%</td>
</tr>
<tr>
<td>(n = 7,900)</td>
<td>(39.19 - 39.91)</td>
<td>(12.08 - 12.57)</td>
<td>(10.37 - 10.81)</td>
<td>(17.36 - 17.9)</td>
</tr>
</tbody>
</table>
1.11.5 Ethnicity and Risk of DR

Diabetic retinopathy is most prevalent in Caucasian and African American populations, followed by the Hispanic population, while Asian and South Asian populations have the lowest prevalence rates. The relative contributions of environmental versus genetic factors to these disparities is unknown (Liew, Klein, & Wong, 2009; Yau et al., 2012). Nonetheless, ethnicity modifies the risk of developing DR in either T1D or T2D, as seen in Table 1.11 (adapted from Yau et al., 2012).

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Age-Standardized Prevalence (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DR</td>
</tr>
<tr>
<td>Caucasian (Total n = 6,021)</td>
<td>45.76% (45.44 - 46.07)</td>
</tr>
<tr>
<td>Chinese (Total n = 1,025)</td>
<td>25.08% (24.45 - 25.91)</td>
</tr>
<tr>
<td>South Asian (Total n = 5,220)</td>
<td>19.12% (18.88 - 19.35)</td>
</tr>
<tr>
<td>African American (Total n = 678)</td>
<td>49.56% (48.59 - 50.52)</td>
</tr>
<tr>
<td>Hispanic (Total n = 2,830)</td>
<td>34.56% (33.24 - 25.87)</td>
</tr>
</tbody>
</table>

Table 1.11: Age-standardized prevalence rates of DR, PDR, DME, and VTDR, in individuals with T1D and T2D combined, sub-classified by ethnicity. 95% CI in brackets. Taken from worldwide meta-analysis spanning 1980-2008 (adapted from Yau et al., 2012).

Yau and colleagues did not stratify prevalence rates of DR in Scandinavian or in First Nations populations, which have especially high prevalence rates of T1D and T2D respectively. Two recent examples will be mentioned here to supplement their data. The Linköping study in Sweden (Heintz, Wiréhn, Peebo, Rosenqvist, & Levin, 2010) found the prevalence of DR to be 41.8% (95% CI: 38.9-44.6) in T1D, and 27.9% (95% CI: 27.1-28.7) in T2D. In Linköping, VTDR had a prevalence of 12.1% (95% CI: 10.2-14.0) among those with T1D and 5.0% (95% CI: 4.6-5.4) among those with T2D. The Sandy Lake Diabetes Complication Study (update by Hanley et al., 2005), which examined complications of T2D in a First Nations Community in Northern Ontario, estimated the prevalence of DR to be 23% (95% CI N/A), and the prevalence of PDR to be 1.5% (95% CI: 0.3-6.1%).

When studies are pooled by era (pre-2000 vs. post-2000; Table 1.12, adapted from Yau et al., 2012), it is evident that the prevalence rates of DR, PDR, DME, and VTDR have been reduced by approximately 50% from the 1980’s and 1990’s to the 2000’s.

<table>
<thead>
<tr>
<th>Era</th>
<th>Age-Standardized Prevalence (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-2000</td>
<td>DR 49.57% (49.21 - 49.93) PDR 10.58% (10.43 - 10.73) DME 9.28% (9.14 - 9.43) VTDR 15.62% (15.43 - 15.81)</td>
</tr>
<tr>
<td>(Total n = 6,162)</td>
<td></td>
</tr>
<tr>
<td>Post-2000</td>
<td>DR 24.79% (24.57 - 25.00) PDR 3.47% (3.40 - 3.55) DME 5.46% (5.35 - 5.56) VTDR 7.86% (7.74 - 7.98)</td>
</tr>
<tr>
<td>(Total n = 9,415)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1.12: Age-standardized prevalence rates of DR, PDR, DME, and VTDR, in individuals with T1D and T2D combined, sub-classified by era. 95% CI in brackets. Taken from worldwide meta-analysis spanning 1980-2008 (adapted from Yau et al., 2012).

Ronald and Barbara Klein (Klein & Klein, 2010) have observed these reductions in prevalence independently, and have reviewed the potential reasons for this positive development. They cite as major catalysts for this shift: an improvement glucose monitoring, more frequent regimens of insulin replacement therapy, better control of blood pressure, and a consensus among scientists and clinicians on the impact of intensive treatment on prevention of diabetic complications (Klein & Klein, 2010). The Kleins have conducted their own epidemiologic study, the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR; data included in Yau et al.’s 2012 meta-analysis), which followed a group of individuals with T1D from 1980 to 2007. The authors have tabulated data on how these factors (glucose monitoring, insulin management, blood pressure management) have changed over time in their sample (Table 1.13). The Kleins have also documented a substantial drop in the incidence of proliferative retinopathy and an even more substantial drop in the rate of progression of retinopathy from 1980 to 2007 (Klein & Klein, 2010).
Table 1.13: Changes in monitoring and management of glucose, insulin, and blood pressure in individuals with T1D enrolled in the WESDR, 1980-2007 (Klein & Klein, 2010).

### 1.12 Cause of Vision Loss in DR

The clinical impression of DR is that it is primarily a vascular disease. Therefore, most clinicians view vision loss as an event triggered by vascular abnormalities and their downstream effects, in particular, DME and ischemia resulting from PDR. These events occlude vision and affect the health of the neural retina, and in turn cause vision loss (Gardner and Aiello, 2010). However, investigators have produced an extensive body of evidence that has shown diabetes to affect the neural retina independently of the vasculature, often even before vascular abnormalities occur (many studies, reviewed by: Adams & Bearse Jr, 2012; Antonetti et al., 2006, 2012; Bresnick,
1986; Gardner, Antonetti, Barber, LaNoue, & Levison, 2002; Ghirlanda, Di Leo, Caputo, Cercone, & Greco, 1997; Lieth et al., 2000; Lorenzi & Gerhardinger, 2001; Parisi & Uccioli, 2001; Tzekov, Arden, Cotlier, & Weinreb, 1999). Therefore, it is critical to investigate the components of the neural retina as potential early biomarkers of sub-clinical retinopathy.

1.13 Structural Deterioration of the Neural Retina in Humans with T1D

1.13.1 Investigations of Human Post-Mortem Retinas: 1960s

Early investigations of human neuroretinal degeneration in DR—as an event that is isolated temporally and physically from DR vascular lesions—were carried out in the 1960s by Wolter and Bloodworth. From post-mortem retinae of humans with diabetes, Wolter discovered degeneration of the inner nuclear layer and atrophy of ganglion cells in retinae that did not yet show vascular signs of DR (Wolter, 1961). Bloodworth arrived at similar findings in his assessment of nearly 300 post-mortem diabetic human retinae, citing degeneration of the inner plexiform layer and ganglion cell layer. He also observed in retinal ganglion cells what are now recognized as features of apoptosis or necrosis: pyknosis (irreversible condensation of chromatin) and fragmentation of nuclei (Bloodworth, 1962).

1.13.2 Investigations of Human Post-Mortem Retinas: 1990s

Since these early studies, Barber and colleagues, in collaboration with the Penn State Retina Research Group, sought to confirm the possibility of increased apoptosis in human diabetic retinae as suggested by Bloodworth’s findings (Barber et al., 1998).

Three control retinae from non-diabetic eyes and two case retinae from individuals with T1D were studied. One case retina came from an individual who had had diabetes for 6 years, and had no signs of retinopathy when retinal vasculature was viewed with microscopy. The other case retina came from an individual who had had diabetes for 30 years duration, and had many microaneurysms characteristic of non-proliferative diabetic retinopathy (NPDR). Terminal transferase dUTP nick end labeling (TUNEL) was performed on all retinae. This technique detects fragmented DNA, a nuclear feature that is specific to apoptotic cell death. The density of TUNEL-positive cells per square centimetre in each retina was observed with light microscopy (Barber et al., 1998).
The diabetic eyes had a markedly greater density of TUNEL-positive cells than the non-diabetic eyes (case mean = 145/cm², SD = 21; control mean = 71/cm², SD = 22). This difference was even more pronounced when the non-diabetic eye with retinal branch vein occlusion was omitted from the analysis (control mean = 58/cm², SD = 15). Moreover, TUNEL-positive cells in the retina with NPDR were remote from microaneurysms, suggesting that neuroretinal apoptosis in diabetes has pathophysiological origins that are distinct from those of vascular lesions (Barber et al., 1998).

Although the authors distinguished among different retinal layers in the animal portion of their study, they did not do so for their human study, so no comment can be made about the specific impact of human diabetes on photoreceptors. Still, these limited data suggest that structural differences in the neural retina warrant further investigation as a potential diagnostic tool for subclinical DR.

1.14 Descriptions of Cone Photoreceptors in Animal Models of T1D

1.14.1 Rodent Model of T1D: Cones Unaffected (Barber et al., 1998)

The investigation of the specific impact of diabetic retinopathy on the photoreceptor layer in animal models has produced inconsistent results. Some authors report a relatively low impact on the outer nuclear layer as compared to other retinal layers, others report substantial impact on photoreceptors.

Barber and colleagues’ human study was an adjunct to a larger rodent study, and it was the first such study to investigate the putative role of cone photoreceptors in the pathogenesis of early DR (Barber et al., 1998).

A model of T1D was induced in male Sprague-Dawley rats by administration of streptozotocin, a compound that destroys the beta cells of the pancreas and renders rodents unable to produce insulin. Diabetes was verified 3 days post-administration by blood glucose levels in excess of 250 mg/dl. After the second month of diabetes, rats received a weekly injection of 2 units of insulin (Humulin) to stabilize weight loss. Throughout the study, rats had access to food and water ad libitum.
After 7.5 months duration of diabetes, rodents were sacrificed and the posterior portion of their retinae dissected to study the impact of diabetes on retinal layer thickness. Diabetic retinae were compared to retinae from age-matched control rats. Sections taken from the midpoint of each retina were stained for neural cell bodies with thionin (a Nissl stain) and viewed under 40x magnification. Although the inner plexiform layer and inner nuclear layers showed reduced thickness in diabetes, the outer nuclear and outer plexiform layers did not (Barber et al., 1998). If these results are to be applicable to human diabetes, they suggest that bipolar cells, horizontal cells, and/or amacrine cells undergo destruction whereas photoreceptors do not.

1.14.2 Rodent Model of T1D: Cones Selectively Affected (Park et al., 2003)

Park and colleagues investigated outer nuclear layer thickness in male Sprague-Dawley with streptozotocin-induced diabetes (S.-H. Park et al., 2003). Rodents with fasting glucose levels in excess of 300 mg/dl two days post-streptozotocin injection were selected for the study. Food and water access was ad libitum, but rats were not treated with insulin and were therefore maintained in a truly hyperglycemic state. Rodents were sacrificed after 1, 4, 8, 12, or 24 weeks duration of diabetes; controls were sacrificed at 8 weeks and 24 weeks. The central portion of the superior nasal quadrant was dissected, and 1μm thick sections were cut and cells were stained with 1% toluidine blue. These sections were viewed under light microscopy with a 400x magnification. The outer nuclear layer underwent a dramatic reduction in thickness in the diabetes condition, particularly between weeks 12 and 24 (Figure 1.2). The outer nuclear layer after 24 weeks duration of diabetes had only 3 to 7 layers of photoreceptor nuclei, whereas age-matched retinae had 8 to 12 such layers. Reductions in the thickness of the inner nuclear layer, inner plexiform layer, and ganglion cell layer were less pronounced.
Figure 1.2: Adapted from Park et al., 2003. Light microphotographs of 1-um thick sections of superior nasal retina from control and diabetic rats (S.-H. Park et al., 2003). The outer nuclear layer (ONL; photoreceptor nuclei), which decreases in thickness dramatically especially from 12 to 24 weeks of diabetes, is highlighted in yellow.

In Park and colleagues’ study, ultra-thin 70-nm sections were cut from the 1-μm superior nasal retinal sections. The ultra-thin sections were double stained with 1% uranyl acetate and lead citrate, and were viewed with electron microscopy. Some photoreceptor terminals contained degenerating mitochondria as well as electron-dense cytoplasm, features of cell death. These characteristics were observed as early as 8 weeks post-streptozotocin, but became more frequent starting at 12 weeks. Whole rat retinas (all quadrants) were also preserved, for TUNEL staining, to determine whether cell death was apoptotic in nature. Using light microscopy, TUNEL was able to identify photoreceptor apoptosis as early as 4 weeks post-streptozotocin; photoreceptor apoptosis became visibly more pervasive at 12 and 24 weeks. In contrast to Barber and colleagues’ results, these data show that photoreceptors are uniquely impacted by T1D, and that the outer nuclear layer is affected more severely than other retinal layers in the streptozotocin model.

The results of these studies may conflict due to the differing levels of insulin delivered, or the differing levels of hyperglycemia achieved. Still, Park and colleagues’ results (S.-H. Park et al.,
2003) give merit to the investigation of cone photoreceptors in human diabetes, which other groups have since undertaken.

1.14.3 Zebrafish Model of T1D: Double Cones Selectively Affected (Alvarez et al., 2010)

In 2010, Alvarez and colleagues studied hyperglycemic zebrafish, a relatively new animal model of diabetes (Alvarez et al., 2010). Zebrafish are particularly useful in modelling the effects of diabetes on cone photoreceptors, since their retinae are more cone-rich than are rod-dominated (nocturnal) rodent retinae (Alvarez et al., 2010). In this study, zebrafish were placed in one of three conditions: (1) fresh water for 30 days; (2) 15 days in 2% glucose solution alternating with 15 days of freshwater; or (3) 15 days of 2% mannitol solution alternating with 15 days of freshwater. Rods were relatively well-preserved in all three groups, whereas cones were not. Of the glucose-treated zebrafish, 60% demonstrated abnormal cone histology, and 30% showed necrotic degeneration of cones. Freshwater and mannitol-treated zebrafish did not display these cone abnormalities. Additionally, in the glucose-treated zebrafish, double cones—which express both red and green photopigments, and are therefore analogous to L- and M- cones—were more affected than other photoreceptors (blue cones, UV cones, and rods). Affected double cones were characterized by discontinuous labelling and irregular, stubby shaped morphology, not observed in the other types of photoreceptors.

1.15 Descriptions of Cone Photoreceptors in Human Diabetes

1.15.1 Abnormally Low Directional Reflectance of Cones in Adults with Diabetes (Zagers, Pot, and Van Norren, 2005)

Cones have specific waveguide properties, and depending on their position and orientation, exhibit differing directional and spectral reflectance. This is referred to as the optical Stiles-Crawford Effect (SCE; Stiles & Crawford, 1933). It has previously been shown that assessing the optical SCE using a Foveal Reflection Analyzer is a sensitive method for evaluating cone photoreceptor disturbances (DeLint, Berendschot, & Van Norren, 1998).

In 2005, Zagers and colleagues at the University Medical Center Utrecht in the Netherlands used a Foveal Reflection Analyzer to assess cone photoreceptor integrity in diabetic adults and age-similar controls (Zagers, Pot, & Van Norren, 2005). Patients were aged 23 to 61 years, had T1D...
or T2D for several years (range: 6-28), and had varying levels of macular edema and/or vascular signs of DR. The authors found that diabetic eyes exhibited smaller amplitude of directional reflectance at the central 1.9° of the retina as compared to controls (p < 0.001).

This result can arise from altered photoreceptor orientation, altered refractive indices on the inside or outside of the outer segment, or altered proportions of the inner to outer segment diameter (DeLint et al., 1998). The authors speculated that cone misalignment and cone damage were responsible for the abnormally low reflectance in diabetes (Zagers et al., 2005). However, the applicability of this study to sub-clinical DR is limited, as the effects of macular edema—which afflicted many of the study participants—cannot be disentangled from the isolated effects of diabetes on the eye.

1.15.2 Abnormal Cone Functional Properties in Adults with Diabetes (Holopigian et al., 1997)

Holopigian and colleagues (Holopigian, Greenstein, Seiple, Hood, & Carr, 1997) used ERGs to investigate sensitivity and maximal response of L- and M- cone photoreceptors in twelve adults with T1D or T2D for 5 years or more, with varying levels of DR (ranging from none to PDR), and nine age-similar controls (mean age = 46 in both groups). The use of very bright flashes, and a red gel filter that removes light energy of lower wavelengths (i.e. towards the blue end of the spectrum), suppresses rods and S-cones while stimulating L- and M- cones in a 10:1 ratio. A 95% confidence interval was established for the control participants in terms of both cone sensitivity and cone maximal response. Only one individual with diabetes fell below this confidence interval in terms of maximal response. However, nine of the twelve individuals with diabetes demonstrated lower cone sensitivity that fell below the 95% confidence limit for controls. Moreover, one individual with T2D and abnormal sensitivity had no signs of retinopathy on fundus, indicating that sensitivity could be an early biomarker of DR.

1.15.3 Possibility of Lower Cone Densities in Young Adults with T1D (Parravano et al., 2012; Tan, 2012)

In 2012, Marco Lombardo and colleagues presented pilot data from an AO study investigating cone photoreceptor density in T1D (Parravano et al., 2012). A flood illuminated AO imaging system was used. Participants were twelve young adults, five of whom had no signs of retinopathy, and seven of whom had mild NPDR. Cone density was measured at increasing
eccentricities along the horizontal meridian, at approximately 0.8 degrees, 1.2 degrees, and 1.6 degrees from the fovea. The authors were not able to obtain images from two individuals who had macular edema. Among the remaining participants, the mean densities observed ranged from 50,573 cones/mm\(^2\) at 0.8 degrees eccentricity to 38,664 cones/mm\(^2\) at 1.6 degrees eccentricity. These results were reportedly at the lower end of the limits for non-diabetic controls in that age group based on other AO studies. Unfortunately, due to several factors—the small sample size, the combining of results from participants with and without retinopathy, and the lack of a control group—these results are difficult to interpret.

Concurrently, the SickKids pilot study (Tan, 2012), which was the precursor to this project, produced similar results. In controls and patients with T1D, in vivo images of cone photoreceptors were collected via an AO-corrected scanning laser ophthalmoscope. Images were taken at seven degrees eccentricity along the four oblique meridians. Densities in each retinal quadrant (superior nasal, inferior nasal, superior temporal, and inferior temporal) were compared between groups. No comparisons were significant, but the superior nasal quadrant showed a trend (p = 0.09) of being less cone-dense in the T1D group as compared to the control group.


A series of recent pilot studies by Schaal and colleagues in Louisville, KY, presented at three consecutive annual meetings of the Association of Research in Vision and Ophthalmology (ARVO), has elegantly demonstrated the distinction between the impact of diabetes on the inner retina and on the outer retina.

1.16.1 Apoptotic Death of Cells in Outer Nuclear Layer, in Humans with NPDR (Schaal et al., 2011)

The introductory study (Schaal, Tang, Zeng, Kaplan, & Tezel, 2011) investigated protein expression in the inner and outer nuclear layers of diabetic retinae and control retinae. Stratified proteomic analyses were performed on three pairs of diabetic post-mortem retinae, with signs of NPDR visible on microscopy, and three pairs of age-matched control retinae. Proteome analysis—which identifies all proteins present in a tissue sample—was performed separately on the inner retina of one eye and the outer retina of the fellow eye. Four-fold differences in protein
expression between the inner and outer retina were identified. The related pathways of identified proteins were studied using Ingenuity Pathway Analysis Software. Within diabetic eyes, there were several differences between the outer and inner retina, indicating that neuronal cell death in each of these layers has very different origins (apoptotic and non-apoptotic, respectively). Findings are summarized in Table 1.14.

<table>
<thead>
<tr>
<th><strong>Diabetic Outer Retina</strong></th>
<th><strong>Diabetic Inner Retina</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Compound/Pathway</strong></td>
<td><strong>Associated with</strong></td>
</tr>
<tr>
<td>VWF/PLG (coagulation pathway), IL-6 (inflammatory marker), and TNF-signalling activation</td>
<td>See compounds/pathways below that are altered in outer retina</td>
</tr>
<tr>
<td>Upregulation of amyloid precursor protein (APP) and downregulation of carbonic anhydrase</td>
<td>Photoreceptor injury Mueller cell dysfunction</td>
</tr>
<tr>
<td>MAP3K5 (pro-apoptotic) pathway activation</td>
<td>Photoreceptor apoptosis</td>
</tr>
</tbody>
</table>

**Table 1.14:** Main findings by Schaal et al., 2011, in stratified proteomic analysis of diabetic outer and inner retinas

These data indicate that human diabetes affects the inner and outer retina via distinct mechanisms. Based on these findings, the authors argue that DR should be sub-classified as inner and outer DR. Moreover, these results imply that optimal pharmacological or surgical treatments for DR lesions in the outer retina could differ from optimal treatments for lesions in the inner retina.

Additionally, the authors discovered increased expression of the huntingtin protein (HTT) in both the inner and outer retina of diabetic eyes as compared to control eyes (Schaal et al., 2011). They note that this is indicative of increased vesicle trafficking and endocytosis, but do not speculate what compounds might be undergoing increased transport.
1.16.2 Intravitreal and Serum Huntingtin (HTT) Protein Concentrations are Positively Correlated with DR Severity in Humans (Schaal et al., 2012)

Following up on the observation of increased HTT expression in diabetic retinae, the authors’ next study (Schaal, Darabad, Zeng, Daxin, & Tezel, 2012) investigated whether HTT in the serum is associated with the presence or severity of DR. Human participants were 31 individuals with diabetes (6 with T1D, and 25 with T2D), and 6 non-diabetic controls. Presence and severity of DR were thoroughly assessed by clinical exam, fundus photography, and fluorescein angiography. In the diabetes group, four patients had no signs of DR; 14 had NPDR; and 13 had PDR. The mean levels of serum HTT in each group were as follows (Table 1.15).

<table>
<thead>
<tr>
<th></th>
<th>Serum HTT (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Control</td>
<td>0.00</td>
</tr>
<tr>
<td>Diabetes, no DR</td>
<td>0.12</td>
</tr>
<tr>
<td>Diabetes, NPDR</td>
<td>0.14</td>
</tr>
<tr>
<td>Diabetes, PDR</td>
<td>0.66</td>
</tr>
</tbody>
</table>

**Table 1.15**: Mean concentration of huntingtin protein in the serum of individuals with diabetes and varying grades of DR, and controls (Schaal et al., 2012).

HTT level was highly correlated with severity/grade of DR ($r = 0.88$). In individuals with PDR, the intravitreal level of HTT was also measured; HTT was the highest here (mean = 0.94 μg/mL, SD = 0.23). These results indicate that retinal/intravitreal HTT may sequestered in the blood (though other organs have not been ruled out as potential sources of serum HTT). If this is the case, serum HTT may be an non-invasive biomarker of sub-clinical DR (Schaal et al., 2012).

1.16.3 In Mouse Model of T1D, Retinal HTT Concentration Associated with Severity of Hyperglycemia (Schaal et al., 2012)

As part of the same study, HTT levels were measured in the serum and retinae of diabetic mice (heterozygous C57BL/6-Ins2Akita/J). Female diabetic mice of this strain develop mild hyperglycemia, while male mice develop severe hyperglycemia. Control mice (C57BL/6J) were
also tested. While there was no HTT found in the serum in any group, it was detected in the retina. The level of hyperglycemia attained was strongly correlated with retinal HTT expression \((r = 0.99)\). Taken together, these results (Schaal et al., 2012, 2011) suggest that hyperglycemia drives HTT expression in the retina, and that HTT expression is either a cause or result of the neurodegenerative changes comprising DR.

1.16.4 In Photoreceptors of Humans with Diabetes, Physical Coincidence of HTT with Apoptotic Markers (Schaal et al., 2013)

Schaal and colleagues’ most recent study (Schaal, Zeng, & Tezel, 2013) sought to determine whether there is any physical coincidence between HTT presence in the neural retina (Schaal et al., 2012, 2011), and outer nuclear layer apoptosis (Schaal et al., 2011) in diabetes. In the human portion of the study, post-mortem retinae from three individuals with diabetes and NPDR, and three non-diabetic controls, were tested for the presence of HTT. These retinae were also tested for polyglutamine aggregates of HTT, which are associated with neurodegeneration that is characteristic of Huntington’s disease (Zhang et al., 2005). Finally, retinae underwent TUNEL staining to identify apoptotic cells.

HTT was primarily expressed in the inner retina in control eyes. Conversely, HTT showed increased expression in the photoreceptors of diabetic eyes, and was highly concentrated in the outer segments. Perinuclear polyglutamine aggregates of HTT were seen only occasionally in the ganglion cells of control eyes. In diabetic eyes, these aggregates were found in greater amounts in both photoreceptors and ganglion cells. On average, 4.4% of photoreceptors (SD = 2.7%) stained positive for TUNEL in diabetic eyes, whereas none of the control retinae stained positive for TUNEL. Importantly, this apoptotic signal was “almost always” coincident with perinuclear polyglutamine aggregates of HTT in the photoreceptors of diabetic retinae (Schaal et al., 2013). Quantitative analysis of the coincidence was not reported.

1.16.5 In Photoreceptors of Mice with T1D, Physical Coincidence of HTT with Apoptotic Markers (Schaal et al., 2013)

The animal portion of the study employed diabetic and non-diabetic mice of the same strains as in the previous study (Schaal et al., 2012; severe diabetes, male heterozygous C57BL/6-Ins2Akita/J; mild diabetes, female heterozygous C57BL/6-Ins2Akita/J; control, C57BL/6J). As in humans, HTT was upregulated in the photoreceptors of hyperglycemic mice as compared to
control mice, and was also concentrated in the outer segments. On average, 1.9% (SD = 1%) of photoreceptors in diabetic mice stained TUNEL positive, whereas no photoreceptors were TUNEL positive in control mice. Again, HTT expression and photoreceptor apoptosis were highly coincident, but a quantitative report was not given by the authors. No vascular lesions were identified in the diabetic mice (Schaal et al., 2013).

1.16.6 Summary of Results (Schaal et al., 2011, 2012, and 2013)

Taken together, the results of these pilot studies suggest that HTT upregulation in the outer segments of photoreceptors occurs prior to DR-related vasculopathy. Moreover, serum HTT could have tremendous efficacy as an early biomarker of photoreceptor neurodegeneration in sub-clinical DR (Schaal et al., 2012, 2011, 2013). Forthcoming publications from this group expanding on these data will undoubtedly shed more light on the complex pathogenesis of clinical and sub-clinical DR in the outer nuclear layer.

1.17 Possible Asymmetrical Impact of NPDR on the Neural Retina: Evidence of Nasal-Temporal Differences (Holm and Lövestam Adrian, 2012)

In 2011, Holm and Lövestam Adrian of University Hospital in Lund, Sweden, were the first to investigate whether the neural retina might be impacted by asymmetrically by DR. They administered OCT and multifocal ERG (mfERG) to a group of middle-aged to older adults, 27 of whom had diabetes (mean age = 58 years, SD = 14) and 18 age-similar controls (mean age = 57 years, SD = 11 years).

Diabetes was classified as T1D (5/27 participants) or T2D (22/27 participants) based on age of onset of diabetes and insulin treatment. Individuals diagnosed prior to age 30 or treated with insulin within 2 years of diagnosis, were classified as T1D. Average duration of diabetes was 12.5 years (SD = 9.1 years), and average A1C level was 7.10 (SD = 1.8). All participants with diabetes had NPDR, defined as microaneurysms and hemorrhages, with or without hard exudates and/or central macular thickening >300µm (22% of participants; ETDRS, 1985). In the diabetes group, individual eyes with prior or current cataract, argon laser treatment, anti-VEGF therapy, intravitreal injections, or photocoagulation treatment in the macular area were excluded.
Ultimately, thirty-six eyes were tested in the diabetes group, and eighteen in the control group (Holm & Lövestam Adrian, 2012).

1.17.1 In NPDR, Possible Loss of Nasal-Temporal Asymmetry of Macular Thickness (Holm and Lövestam Adrian, 2012)

Holm and Lövestam Adrian assessed macular thickness in participants using ocular coherence tomography (OCT), a high-resolution, cross-sectional imaging technique that operates on the same basic principles as ultrasound imaging (Fujimoto, Pitriss, Boppart, & Brezinski, 2000). Using an OCT2 system, nasal and temporal macular thickness were measured in three 6mm cross-sections intersecting at the fovea: one along the horizontal meridian, one rotated 30 degrees clockwise, and one rotated 30 degrees counter-clockwise. OCT results were only reported for the diabetes group, who displayed no nasal-temporal asymmetry in macular thickness (Holm & Lövestam Adrian, 2012).

This result stands in contrast to a different OCT study which showed the temporal macula to be thinner than the nasal macula in healthy adults (Chan, Duker, Ko, Fujimoto, & Schuman, 2006). On speculation, let us imagine that in Holm and Lövestam Adrian’s NPDR group (Holm & Lövestam Adrian, 2012), the nasal macula had been thicker than the temporal macula prior to the onset of DR. If this were the case, then it might be concluded that DR has a selective nasal effect on macular thinning. Rigorous longitudinal studies would be required to investigate this very preliminary hypothesis.

1.17.2 In NPDR, Nasal Retina Exhibits Poorer Function than Temporal Retina on mfERG (Holm and Lövestam Adrian, 2012)

For the functional arm of their study, Holm and Lövestam Adrian employed mfERG. The mfERG tests neuroretinal function at many different retinal locations (usually 61 or 103) using a stimulus comprised of black and white flashing hexagons, alternating in a particular sequence. The temporal characteristics of the sequence allow the extraction of spatial data from the recordings. Like other forms of ERG, mfERGs collect summed electrical potentials from the retina using a lens placed on the cornea (Hood et al., 2012).

The mfERG stimulus Holm and Lövestam Adrian used for this study contained 103 hexagons. The N1-P1 amplitude and the implicit time (time to the P1 peak) were measured. The N1-P1
amplitude and implicit time represent the activity bipolar cells and photoreceptors (Donald C Hood, Frishman, Saszik, & Viswanathan, 2002).

The nasal and temporal regions, as in other studies, were divided into foveola, inner sectors, and outer sectors. Though precise coordinates were not specified, the inner sectors appear to span 1-2mm eccentricity, and the outer sectors appear to span 2-3mm eccentricity (Holm & Lövestam Adrian, 2012).

In comparing mfERG results between groups, it was found that the diabetes group had significantly smaller amplitudes and longer implicit times compared with the control group, in both the nasal and temporal macula.

With respect to within-groups comparisons of mfERG responses arising from the nasal versus temporal perifoveal area, the control group displayed no overall nasal-temporal asymmetry in terms of amplitude or implicit time.

Meanwhile, participants with diabetes displayed significantly smaller N1-P1 amplitudes and significant longer implicit times in the nasal retina as compared to the temporal retina (p<0.001 and p<0.005 respectively). When nasal-temporal comparisons in the diabetes group were broken down into inner sector and outer sector, it was found that both inner and outer sectors had nasal amplitudes that were significantly less then temporal. The outer sector, but not the inner sector, exhibited nasal implicit times were significantly longer than temporal implicit times (Holm & Lövestam Adrian, 2012).

The nasal-temporal asymmetry (with nasal function poorer than temporal) seen on mfERG in older adults with diabetes stands in contrast to retinal function in healthy older adults, who exhibit no asymmetry on mfERG (Holm & Lövestam Adrian, 2012). It is impossible to draw conclusions about causality because this study is cross-sectional. However, let us speculate that future longitudinal studies will show that adults with diabetes start out with symmetrical function, and gradually develop functional asymmetry (nasal function < temporal function) as they develop NPDR. This finding would suggest that nasal function is selectively impacted by NPDR, and that the nasal retina is uniquely susceptible to the long-term sequelae of diabetes.
It is also interesting to compare functional asymmetry seen on mfERG in healthy younger adults, in whom nasal function is superior to temporal function (Silva et al., 2010), to the absence of asymmetry in healthy older adults on mfERG (Holm & Lövestam Adrian, 2012). It is possible that the nasal-temporal functional asymmetry (nasal advantage) seen in young individuals is gradually attenuated with age. Again, longitudinal work will be required to confirm this hypothesis.

Based on this limited evidence, one could guess that the nasal retina, which initially has a functional advantage, is preferentially affected both by age-related processes and by clinical or sub-clinical DR. When both ageing and DR are at play, the potentially nasally-selective effects of both processes might be compounded to ultimately create a nasal functional disadvantage. This very preliminary hypothesis merits rigorous investigation. Still, this line of thought was formative in designing the hypotheses for this study.

1.17.3 Need for Investigation of Nasal-Temporal Asymmetry in Related Populations, and in Specific Cell Types

It remains to be seen if this potential nasal susceptibility, should it be a real phenomenon, would be observed in a larger sample of individuals who have sub-clinical DR or NPDR as a consequence of T1D, as opposed to T2D. Furthermore, it would be interesting to investigate whether this asymmetrical pattern could be observed in pre-retinopathic adolescents and young adults with T1D. Finally, it is unclear whether nasal susceptibility, should it exist, affects the density or distribution of cone photoreceptors in particular, which have been shown to be affected in DR. It is our hope that our study will begin to answer these questions.

In order to understand the results of our study, it is important to understand the existing body of work on nasal-temporal asymmetry in healthy controls, including nasal-temporal asymmetry of cone density and distribution. The remainder of the introduction will explore this literature.
1.18 Evidence of Nasal-Temporal Asymmetry in Retinae of Healthy Adults

1.18.1 Nasal Macula Thicker than Temporal Macula in Healthy Adults (Chan et al., 2006)

Chan and colleagues (Chan, Duiker, Ko, Fujimoto, & Schuman, 2006) assessed macular thickness in different quadrants in healthy adults, using OCT3. Thirty-seven individuals (26 women, 11 men), aged 22 to 71 years (median = 43) participated in the study. These individuals had no retinal pathology, no diabetes, no family history of glaucoma, intraocular pressure ≤ 21 mmHg, and normal visual fields and acuity. Six macular scans were acquired in each participant at equally spaced angular orientations (30°), each 6mm in length and centred on the fovea.

Retinal thickness was defined as the distance from the vitreoretinal surface to the anterior surface of the retinal pigment epithelium (therefore, including all neuroretinal layers). Retinal thickness was mapped in the foveola (central circle with 0.5mm radius), and in two concentric annuli, the inner spanning 0.5 to 1.5 mm, and the outer spanning 1.5 to 3.0 mm (as defined by the Early Treatment Diabetic Retinopathy Study; ETDRS, 1991).

As expected, the foveola was the thinnest sector, the inner annulus was the thickest, and the outer annulus diminished in thickness towards its periphery. The annuli were also each segmented into quadrants: superior, inferior, nasal, and temporal (8 sectors total). In 29 participants (78% of the total), the thickest sector was the inner nasal sector. In assessing the mean thickness across the group, it was discovered that the inner nasal sector was 16 μm thicker (approximately 1SD) than the inner temporal sector, and the outer nasal sector was 36 μm thicker (more than 2.5SD) than the outer temporal sector. Additionally, the outer superior sector was thicker than the outer inferior sector (by 19 μm, more than 1.5SD), but this relationship did not hold in the inner ring. Unfortunately, the authors did not report the statistical significance of these results, as this was not a primary aim of their study (Chan et al., 2006).

1.18.2 Nasal Macula Thicker than Temporal Macula in Healthy Adults (Silva et al., 2010)

Silva and colleagues (Silva et al., 2010) repeated this study using a Stratus OCT3, using the same sectioning protocol, except that retinal thickness was defined as the distance between the vitreoretinal interface and the inner-outer segment junction of photoreceptors. Thus, in this
study, the photoreceptor cell bodies were included in the calculations, but not the outer segments. Eighty eyes from 40 healthy adults (13 male, 27 female; mean age = 43 years, SD = 16 years) were tested. Participants had no eye or retinal disease, no cataracts or optic nerve pathology, low refractive error, and normal visual acuity. Mirroring Chan and colleagues’ study, the group means revealed that the inner nasal retina was markedly thicker than the inner temporal retina (by 15 μm, p<0.0001) and the outer nasal retina differed even further from the outer temporal retina (by 38 μm, p<0.0001). They additionally found the superior retinal sectors to be thicker on average than the inferior retinal sectors (inner superior vs. inner inferior, 7 μm, p<0.0001; and outer superior vs. outer inferior, 7 μm, p<0.0001). Though significant, the superior-inferior differences were not nearly as sizeable as the nasal-temporal differences.

Because both studies (Chan et al., 2006; Silva et al., 2010) examined the thickness of the entire neuroretina, the nasal-temporal (and superior-inferior) asymmetries that were found cannot necessarily be attributed to differences in the photoreceptor layer. The differences may arise from the inner retina, or the outer retina, or a combination of both. Isolating the thickness of the outer nuclear layer, inner nuclear layer, and ganglion cell layer in future OCT studies employing similarly executed scans will be critical to disentangling the contributions of each layer to these asymmetries.

1.18.3 Nasal Function Exceeds Temporal Function in Central 13 Degrees of Retina (Silva et al., 2010)

Silva and colleagues extended their OCT results by administering binocular multifocal electroretinograms (mfERGs) in the same participants (Silva et al., 2010). This particular protocol employed 61 hexagons, alternating at a rate of 60 Hz. The amplitude from the N1 trough to the P1 peak of the response was analyzed (Silva et al., 2010), representing the activity bipolar cells and photoreceptors (Donald C Hood et al., 2002).

Responses from each hexagon were grouped into concentric rings, the first between 2.2 and 6.8 degrees eccentricity from the fovea, the second between 6.8 and 12.9 degrees, the third between 12.9 and 20.4 degrees, and the fourth between 20.4 and 29.4 degrees. As in the OCT portion of the study, each ring was divided into nasal, temporal, superior, and inferior quadrants for hemiretinal comparison. There were no differences in amplitude between any superior and inferior ring section. The nasal retina had a greater N1-P1 amplitude than the temporal retina in
rings 1 and 2 (p = 0.001 and p = 0.002, respectively), but not in rings 3 or 4 (Silva et al., 2010). The mfERG portion of this study demonstrates a functional nasal-temporal asymmetry in healthy adults in the central 13 degrees (with a nasal advantage), that corroborates prior structural findings.

Again, because the mfERG N1-P1 response does not isolate cone photoreceptors, it is impossible to draw conclusions from this study about the unique role of cones in producing the nasal-temporal symmetry that was observed.

The following sections will address studies that have assessed nasal-temporal asymmetry in the density and distribution of cone photoreceptors. These studies employed histology, optical density techniques, and AO retinal imaging, and were performed on healthy (or in the case of post-mortem histology, previously healthy) individuals.

1.19 Nasal-Temporal Asymmetry in Human Cone Photoreceptor Density and Distribution Patterns, Observed on Histology

The first comprehensive report of cone photoreceptor density and distribution in the human retina was by Osterberg (Osterberg, 1935). The specimen studied was a single post-mortem eye from a 16-year-old male. The author estimated a total number of cones that fell somewhere between 6.3 million and 6.8 million (Osterberg, 1935, in Jonas, Schneider, & Naumann, 1992). The peak density, 147,000 cones/mm², was at the fovea, and density decreased precipitously with increasing eccentricities from that point (Osterberg, 1935, in Curcio, Sloan, Packer, Hendrickson, & Kalina, 1987) (Osterberg in Curcio 1987). A nasal-temporal asymmetry was observed, consisting of greater cone density in the nasal retina as compared with the temporal retina. Cone densities were also slightly higher in the superior retina (Osterberg, 1935, in Curcio, Sloan, Packer, Hendrickson, & Kalina, 1987). In 1941, Polyak published results of peak foveal cone density that were consistent with these findings (Osterberg, 1935, in Jonas, Schneider, & Naumann, 1992).

While there were other publications in the decades that followed (Hartridge, 1950; O'Brien, 1951; Miller, 1979; Yuodelis & Hendrickson, 1986; Farber et al., 1985), the most widely-cited series of studies on cone density and distribution were from Christine Curcio and colleagues
Curcio and colleagues examined four post-mortem retinae, fixed within 3 hours of death, from four human eye bank donors under 45 years old. These individuals had no history of eye disease, no ocular disease detected under the dissecting microscope, and no post-mortem retinal folds. Their refractive error and visual acuity were not known. Cross-sections of the retina were analyzed at the level of cone inner segments, proximal to the external limiting membrane, by way of computer-video-microscope system (Curcio et al., 1987). These authors developed a digital model of the retina, sampled at regular intervals (with many samples in the fovea, and progressively fewer samples in the periphery.

Cone density was highest in the foveal centre and was highly variable in young, healthy adult eyes (range = 96,000 to 281,000 cones/mm²; mean = 161,900). There was less variability observed in the density of peripheral cones. Density decreased with eccentricity, with steep reductions initially near the fovea, which graduated to shallow reductions more peripherally.

Isodensity contours (two-dimensional spatial maps of the retina encoding areas of equal density) revealed elliptical rather than circular contours. This is because cone density dropped off more steeply in the vertical direction than in the horizontal direction with increased eccentricity. This pattern was referred to by the authors as the cone streak. The cone streak was present in the peripheries of all retinae, and in the foveae of 3 of the 4 retinae (Curcio et al., 1987).

In addition to the cone streak, a nasal-temporal asymmetry was observed. Three retinae displayed 10-40% greater cone density in the nasal retina as compared to the temporal retina, and one retina displayed 40-70% greater cone density in the nasal retina as compared to the temporal retina. This horizontal asymmetry was more pronounced with increasing eccentricity. The pattern was not consistently present at eccentricities outside the optic disk (approximately 10 to 15 degrees). There were no apparent differences between the superior and inferior hemiretinae (Curcio et al., 1987).

In 1990, the Curcio group expanded on their original sample with four more donor retinae. Two were fellow eyes from the same cadaver; one, a single eye from a cadaver; and one, a single eye that was surgically enucleated. Again, all donor eyes were pre-screened with microscopy for ocular disease and post-mortem retinal folds. The seven individuals were four females and three
males who ranged from 27 to 44 years old (mean = 34.7, SD = 5.1). A small degree of areal expansion of the retinae (1-12%) occurred due to fixing and mounting of retinae. The authors did not take this into account in their calculations; however, this caveat should be considered when comparing their results to those of other studies. Videos were captured of the photoreceptor layer at the level of the inner segments. Cones were counted at 100x magnification at the fovea, and at 40x when surrounded by a ring of rods (approx. 1 mm+ eccentricity). A digital model of each eye was created, and these were averaged to generate a model eye.

These data (Curcio et al., 1990) confirmed the findings of the previous study (Curcio et al., 1987). The total number of cones in each retina ranged from 4.08 million to 5.29 million. A 3.3-fold difference in peak cone density was observed (range = 98,200 to 324,100 cones/mm²). Again, cone density decreased in all directions with increasing eccentricity, with the greatest drop near the fovea and shallowing slopes moving outward.

Both nasal-temporal asymmetry and the cone streak were observed in the model eye (Curcio et al., 1990). Nasal-temporal asymmetry was found to be evident near 1mm (approximately 3.5 degrees) eccentricity, and became more pronounced with increasing eccentricity. The ratio of nasal to temporal cone density was 1.25 near the optic disc and 1.40-1.45 at 9 mm+ eccentricity. Some individuals exhibited slightly greater cone densities in the inferior than the superior retina (with the cone streak extending slightly further inferiorly), but this was not consistent across all retinae. In the model eye, at most retinal locations outside the fovea, inferior cone densities were approximately 2% higher than superior cone densities.

These findings (Curcio et al., 1990) were compared graphically with Osterberg’s data (Osterberg, 1935). Densities were found to be relatively consistent across studies. However, there were isolated patches at 1-2mm eccentricity where Osterberg’s densities were 30% lower than Curcio’s (almost 2 SD). More peripherally (outside approximately 8 or 10mm eccentricity), Osterberg’s densities were 15-40% (1 SD or more) higher than Curcio’s. Essentially, the cones in Osterberg’s retina were distributed slightly differently, with lower foveal and higher peripheral densities. It is difficult to assess how meaningful these differences are given the small sample sizes involved. The most salient difference between the two studies was the age of the participants involved (16 years old, Osterberg, vs. 27-44 years old, Curcio). Thus, age cannot be discounted as a possible factor in the difference in patterns observed in the two studies.
Curcio and colleagues later addressed the potential impact of age on photoreceptor density and distribution (Curcio et al., 1993). They found overall cone density to be moderately correlated with age between 16 and 90 years (r=-0.4, p<0.05). Still, inter-age comparisons were not made by eccentricity, so these results are not easily applied to Osterberg’s data (Osterberg, 1935).

In 1992, Jonas and colleagues (Jonas et al., 1992) assessed a larger sample of participants, and repeated Curcio’s findings of nasal-temporal asymmetry. The sample included eleven males and ten females, aged 2 years to 90 years (mean = 46.8, SD = 22.2). They examined 21 single donor eyes, harvested 3.5 hours post-mortem at the latest. Gross examination of the retinae under light microscopy revealed no visible ocular surgery, retinal disease, or optic nerve damage. One foveal section (5mm in diameter) and 6 concentric rings (3-4mm in width) were analyzed. Nasal, temporal, superior, and inferior quarters of rings were analyzed separately. Cone densities on the nasal meridian were higher than densities along temporal, superior, and inferior meridians. Specifically, nasal densities were 10-50% greater than temporal cone densities at all locations studied. The authors did not note the presence of a horizontally elliptical cone streak.

To the best of my knowledge, the most recent histological study to comprehensively examine cone density and distribution in a healthy control population came from Ahnelt and colleagues in 1998 (Ahnelt, 1998; data first presented in Pum, Ahnelt, & Grasl, 1990). Six retinae were harvested from 5 adults (4 male, and one individual’s sex unknown), ages 18 (2 fellow retinae contributed), 47, 54, 72, and one individual’s age unknown. Retinae had preserved foveal architecture and intact cone mosaic topography. As in Curcio’s and Osterberg’s studies (Curcio et al., 1990, 1987; Osterberg, 1935), the authors identified a cone streak, marked by horizontally elliptical isodensity contours, which indicated a steeper decline in density along the vertical meridians (Pum et al., 1990).

These authors also noted nasal-temporal asymmetry, with 40% lower cone densities in the temporal retina than in the nasal retina (Ahnelt, 1998). In this study, the nasal-temporal asymmetry was not restricted to the periphery; it continued through to the fovea (Pum et al., 1990). The nasal advantage in cone density was attributable entirely to L-/M-cone distribution, and not to S-cones (Ahnelt, 1998).
1.20 Nasal-Temporal Asymmetry in Optical Density of Human Cone Photopigments

Kilbride and colleagues (Kilbride, Read, Fishman, & Fishman, 1983) investigated the optical density of cone photopigments (mainly of L- and M-cones) in the fovea, up to six degrees eccentricity. Six participants (age, sex not reported) had their pupils dilated and accommodation paralyzed, and were dark-adapted for 15 minutes. They were then exposed to illumination that maximally stimulated L- and M- cones for less than one second (spectral peak at 560 nm, 10nm bandpass); a digital photograph of the fundus was taken. This was followed by a very bright red light delivered for 30 seconds (6.12 log phot Td, spectral peak at 605 nm, 20nm bandpass), which bleached more than 97% of L- and M-cones, virtually no S-cones, and about 1/3 of rods. A second digital photograph was taken immediately after the bleaching light, and the first photograph was subtracted from the second to obtain a measure of the cone pigment density difference between the two time points.

The foveola (0-2 degrees) and two concentric annular regions (2-4 degrees and 4-6 degrees) were analyzed. As the authors expected, there was a decrease in cone pigment density from the foveola to the first annulus, and the first to the second annulus. Additionally, the horizontal and vertical meridians were analyzed continuously from 3.5 degrees temporal to 3.5 degrees nasal, and 3.5 degrees superior to 3.5 degrees inferior. All six participants had a greater cumulative L- and M- cone photopigment density between 0 and 3.5 degrees on the nasal side as compared to the temporal side, and the mean nasotemporal difference was statistically significant (p < 0.05, Wilcoxon test). This asymmetry was not observed in the superior-inferior direction (Kilbride et al., 1983).

There are a number of potential reasons for this observed asymmetry. Firstly, cones in the nasal fovea may have been either longer or greater in diameter than cones elsewhere. These structural parameters may have allowed nasal cones to contain more discs per outer segment (if longer), or to have a greater surface area of discs (if wider). At a constant concentration of photopigment per unit surface area of disc, both of these scenarios would have allowed a greater number of photopigments per cone. Secondly, the concentration of photopigment per unit surface area of disc could have been higher in the nasal cones, which would have created a greater optical density of photopigment without a concurrent change in cell morphology. Finally, the number of
photopigments per cone could have been constant across the entire fovea, while the nasal side simply had a greater number of cones.

Curcio and colleagues have commented on each of these possibilities with regard to Kilbride et al.’s results (Curcio et al., 1987). Based on their own results—that did not consistently show a cumulative nasotemporal asymmetry this close to the foveola—they argued that nasal-temporal differences in the number of cones present were a less likely scenario than nasal-temporal differences in cone morphology or cone photopigment concentration.

However, given the small sample sizes in both studies, the possibility of a differing number of cones cannot be entirely ruled out. Kilbride and colleagues (Kilbride et al., 1983) were themselves not able to speculate as to what the root cause of the observed nasal-temporal asymmetry might have been, as the most complete set of cone distribution data available at that time came from Osterberg’s single retina study (Osterberg, 1935), which did not report on the nasal fovea.

1.21 Human Cone Photoreceptor Density and Distribution Patterns, Observed on AO Retinal Imaging: Lack of Evidence for Nasal-Temporal Asymmetry

Summarized here are the most important and comprehensive findings to date on cone density in AO studies. For the most part, these studies corroborate histological data (Ahnelt, 1998; Curcio et al., 1990, 1987; Jonas et al., 1992; Osterberg, 1935). Generally, AO retinal imaging captures images of L- and M- cone photoreceptors only. S-cones are smaller and are more morphologically similar to rods. Though recent developments have allowed investigators to image rods with AO retinal imaging, S-cones and rods were not captured in the studies presented here.

Chui and colleagues studied cone photoreceptor distribution using AO extensively (Chui, Song, & Burns, 2008b). They reported single eyes in a group of four healthy adult controls (three males, one female; ages 24 to 54 years, mean 37), with minimal refractive error (0D to +0.5D) and no retinal pathology or systemic diseases. The central 10 to 12 degrees of the retina in each participant was imaged. Along the temporal meridian, the authors found cone densities of 30,000 cones/mm² at 0.5mm eccentricity (approximately 1.5-2 degrees), and 15,000 cones/mm² at
1.5mm eccentricity (approximately 5-6 degrees). In Curcio’s study (Curcio et al., 1990),
densities of 37,000 cones/mm² and 15,000 cones/mm² were found in these respective locations.
Along the superior meridian, they found cone densities of 25,000 cones/mm² at 0.5mm
eccentricity (approximately 1.5-2 degrees), and 10,000 cones/mm² at 1.5mm eccentricity
(approximately 5-6 degrees). Curcio (Curcio et al., 1990) reported 28,000 cones/mm² and
13,000 cones/mm² in these locations. Evidence of a steeper decline of cone photoreceptor
density along the vertical meridian than the horizontal meridian demonstrated the cone streak;
however, no nasal-temporal asymmetry in cone density was observed within the central 5mm
(Chui et al., 2008b).

Later, these authors (Chui et al., 2008a) examined AOSLO-obtained cone density in single eyes,
in a group of individuals with variable refractive errors. Participants were eleven healthy young
adults (five men and six women; ages 21-31 years, mean = 26.6), with no retinal or systemic
diseases. The data were from emmetropic eyes (five participants; refractive error, 0D to +0.5D).
At 0.9-1.0mm eccentricity (approximately 3-4 degrees), cone densities along the meridians were:
superior (16,868 cones/mm²) > temporal (16,497 cones/mm²) > inferior (14,007 cones/mm²) >
nasal (13,994 cones/mm²). At 1.8-2.0mm eccentricity (approximately 7-8 degrees), cone
densities along the meridians were: temporal (11,372 cones/mm²) > nasal (10,212 cones/mm²) >
superior (9,942 cones/mm²) > inferior (8,952 cones/mm²). While the authors observed the
expected pattern of decreasing density with increasing eccentricity, the existence of the cone
streak was less convincing (particularly at 1.0mm). Moreover, if there was any nasal-temporal
asymmetry, there appeared in this study to be a temporal advantage in cone density.

Song and colleagues (Song et al., 2011) reported cone density variations with retinal eccentricity
and meridian. Participants were divided into two groups: young adults (ten participants, ages 22-
35 years) and older adults (ten participants, ages 50-65 years). A steeper decline in cone density
with increasing eccentricity was observed along the vertical meridian compared to the horizontal
(cone streak). Nasal-temporal asymmetry was not reported. Cone densities from the older adults
showed a more consistent trend towards nasal-temporal asymmetry than those from the younger
adults (Table 1.16 and Table 1.17).
### Table 1.16: Mean cone densities (cones/mm²) along each meridian, stratified by eccentricity, found on AO retinal imaging in younger adults (ages 22 to 35 years); adapted from Song et al., 2011.

<table>
<thead>
<tr>
<th>Meridian</th>
<th>0.18</th>
<th>0.27</th>
<th>0.36</th>
<th>0.45</th>
<th>0.54</th>
<th>0.72</th>
<th>0.90</th>
<th>1.08</th>
<th>1.35</th>
<th>1.62</th>
<th>1.89</th>
<th>2.16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superior</td>
<td>63,500</td>
<td>52,700</td>
<td>42,400</td>
<td>34,000</td>
<td>29,600</td>
<td>22,100</td>
<td>18,500</td>
<td>15,700</td>
<td>13,100</td>
<td>11,700</td>
<td>10,000</td>
<td>8,700</td>
</tr>
<tr>
<td>Superior</td>
<td>62,800</td>
<td>55,300</td>
<td>44,600</td>
<td>36,300</td>
<td>31,400</td>
<td>23,900</td>
<td>19,400</td>
<td>16,600</td>
<td>12,800</td>
<td>11,500</td>
<td>10,200</td>
<td>8,100</td>
</tr>
<tr>
<td>Nasal</td>
<td>68,200</td>
<td>59,700</td>
<td>50,000</td>
<td>43,700</td>
<td>37,800</td>
<td>29,100</td>
<td>24,200</td>
<td>19,100</td>
<td>16,800</td>
<td>14,500</td>
<td>11,900</td>
<td>10,400</td>
</tr>
<tr>
<td>Temporal</td>
<td>75,200</td>
<td>59,200</td>
<td>50,500</td>
<td>41,200</td>
<td>37,300</td>
<td>28,100</td>
<td>24,100</td>
<td>19,900</td>
<td>16,300</td>
<td>13,200</td>
<td>11,500</td>
<td>9,700</td>
</tr>
</tbody>
</table>

### Table 1.17: Mean cone densities (cones/mm²) along each meridian, stratified by eccentricity, found on AO retinal imaging in older adults (ages 50 to 65 years); adapted from Song et al., 2011.

<table>
<thead>
<tr>
<th>Meridian</th>
<th>0.18</th>
<th>0.27</th>
<th>0.36</th>
<th>0.45</th>
<th>0.54</th>
<th>0.72</th>
<th>0.90</th>
<th>1.08</th>
<th>1.35</th>
<th>1.62</th>
<th>1.89</th>
<th>2.16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superior</td>
<td>50,200</td>
<td>46,500</td>
<td>39,900</td>
<td>33,700</td>
<td>29,400</td>
<td>23,200</td>
<td>19,000</td>
<td>16,400</td>
<td>12,800</td>
<td>11,100</td>
<td>10,000</td>
<td>9,100</td>
</tr>
<tr>
<td>Superior</td>
<td>50,100</td>
<td>43,600</td>
<td>39,500</td>
<td>31,200</td>
<td>28,800</td>
<td>22,800</td>
<td>18,400</td>
<td>15,200</td>
<td>11,300</td>
<td>11,100</td>
<td>8,800</td>
<td>8,300</td>
</tr>
<tr>
<td>Nasal</td>
<td>52,600</td>
<td>48,300</td>
<td>43,000</td>
<td>35,600</td>
<td>33,100</td>
<td>27,500</td>
<td>22,500</td>
<td>20,900</td>
<td>17,600</td>
<td>15,400</td>
<td>12,400</td>
<td>13,000</td>
</tr>
<tr>
<td>Temporal</td>
<td>46,600</td>
<td>40,700</td>
<td>39,000</td>
<td>35,600</td>
<td>33,600</td>
<td>25,800</td>
<td>22,000</td>
<td>18,300</td>
<td>14,900</td>
<td>13,300</td>
<td>11,000</td>
<td>9,000</td>
</tr>
</tbody>
</table>

**Blue:** Temporal > Nasal (approximately 1 SD or more difference)

**Purple:** Nasal > Temporal (approximately 1 SD or more difference)
Lombardo and colleagues (Lombardo et al., 2013) investigated whether nasal and temporal cone densities along the horizontal meridian were comparable in fellow eyes. For this purpose, they used a flood illuminated AO imaging system. Participants were 20 young adults (15 females, 5 males; ages 24-36 years), with refractive errors ranging from 0D to -5.5D. Images were obtained from both eyes, at eccentricities from the fovea of: 0.25mm (approximately 0.5 degrees), 0.42mm (approximately 1.5 degrees), 0.76mm (approximately 3 degrees), and 1.3mm (approximately 6 degrees; Table 1.18). Cone density was symmetrical among fellow eyes and there was no evidence of nasal-temporal asymmetry (Table 1.18).

<table>
<thead>
<tr>
<th>Meridian and Eye</th>
<th>Mean Cone Density (cones/mm²) in Lombardo et al., 2013</th>
<th>Retinal Eccentricity (From Fovea)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.25 mm</td>
</tr>
<tr>
<td>Nasal RE</td>
<td>50,986</td>
<td>39,567</td>
</tr>
<tr>
<td>Temporal RE</td>
<td>51,339</td>
<td>39,724</td>
</tr>
<tr>
<td>Nasal LE</td>
<td>49,928</td>
<td>39,872</td>
</tr>
<tr>
<td>Temporal LE</td>
<td>49,847</td>
<td>39,688</td>
</tr>
</tbody>
</table>

Table 1.18: Mean cone densities (cones/mm²) reported in Lombardo et al., 2013, at increasing eccentricities along the nasal and temporal meridians in both right and left eyes.

In the largest and most varied demographic sample to date, Park and colleagues (S. P. Park, Chung, et al., 2013) assessed cone density in single eyes of 192 individuals. Participants were 88 females and 104 males with no ocular pathology, 10-69 years of age (mean = 33.6, SD = 13.2). Refractive errors ranged from -9.8D to +2.5D, and axial lengths from 22.0 to 28.4mm (mean = 24.4, SD = 1.41). The sample was comprised of individuals of varied ethnic backgrounds: Asian (25.5%), African (11.5%), Caucasian (35.4%), and Hispanic (27.6%). The results of Park et al., 2013 are summarized in Table 1.19.
Table 1.19: Mean cone densities (cones/mm²) reported in Park et al., 2013, at increasing eccentricities along the superior, inferior, nasal, and temporal meridians in single eyes.

<table>
<thead>
<tr>
<th>Meridian</th>
<th>Retinal Eccentricity (From Fovea)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 mm mean</td>
</tr>
<tr>
<td>Superior</td>
<td>32,047</td>
</tr>
<tr>
<td>Inferior</td>
<td>32,105</td>
</tr>
<tr>
<td>Nasal</td>
<td>32,456</td>
</tr>
<tr>
<td>Temporal</td>
<td>32,187</td>
</tr>
<tr>
<td>Significant differences between meridians (One-way ANOVA)</td>
<td>No</td>
</tr>
</tbody>
</table>

The only eccentricity which revealed significant differences among meridians was 1.0 mm. Post-hoc testing revealed that the nasal and temporal meridians at 1.0mm had significantly higher cone densities than the superior and inferior meridians at 1.0mm. No superior-inferior or nasal-temporal asymmetries were reported by the authors (S. P. Park, Chung, et al., 2013). The horizontal cone streak and the nasal-temporal asymmetry that have been observed in several histological samples (Ahnelt, 1998; Curcio et al., 1990, 1987; Jonas et al., 1992; Osterberg, 1935) were not present in the largest participant sample having undergone AO retinal imaging to date (S. P. Park, Chung, et al., 2013).

1.22 Summary of Cone Densities at Measured at Eccentricities Assessed in Our Study

Since cone density varies dramatically with eccentricity, it is useful to focus on the results of studies which have sampled eccentricities that are similar to those sampled in our study. With a conversion rate of 0.27 mm per degree (Song et al., 2011), our retinal eccentricity of 7.07 degrees from the fovea translates to approximately 1.9mm eccentricity from the fovea. Table 1.20 summarizes histological and AO retinal imaging studies that have reported cone densities at or close to 1.9 mm eccentricity.
<table>
<thead>
<tr>
<th>Author, Year</th>
<th>Type</th>
<th>Mean Age (Range)</th>
<th>Eccentricity (mm)</th>
<th>Superior</th>
<th>Inferior</th>
<th>Nasal</th>
<th>Temporal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osterberg, 1935 (in Curcio, 1990)</td>
<td>Histology (n=1)</td>
<td>16 years</td>
<td>2.0</td>
<td></td>
<td></td>
<td></td>
<td>10,750</td>
</tr>
<tr>
<td>Curcio et al, 1990</td>
<td>Histology (n=6)</td>
<td>35 years (27-44)</td>
<td>2.0</td>
<td>10,000</td>
<td>10,500</td>
<td>12,500</td>
<td>11,700</td>
</tr>
<tr>
<td>Jonas et al., 1992</td>
<td>Histology (n=21)</td>
<td>47 years (2-90)</td>
<td>1.95</td>
<td></td>
<td></td>
<td>7,500</td>
<td></td>
</tr>
<tr>
<td>Chui et al, 2008a</td>
<td>AOSLO (n=5)</td>
<td>26.6 years (21-31)</td>
<td>1.8 -2.0</td>
<td>9,942</td>
<td>8,952</td>
<td>10,212</td>
<td>11,372</td>
</tr>
<tr>
<td>Song et al., 2011</td>
<td>AOSLO (n=10)</td>
<td>(22-35 years)</td>
<td>1.89</td>
<td>10,000</td>
<td>10,200</td>
<td>11,900</td>
<td>11,500</td>
</tr>
</tbody>
</table>

**Table 1.20:** Mean cone densities (cones/mm²) observed along each horizontal and vertical meridian, found at approximately 1.9 mm eccentricity from the fovea in histological and AOSLO studies.
2 Research Aims and Hypotheses

2.1 Research Aims

1. To investigate L- and M-cone photoreceptor function, density, and distribution in adolescents and young adults with T1D prior to the potential future onset of DR.

2. To determine which of these properties, if any, is a candidate for longitudinal analysis in this cohort as a possible biomarker of sub-clinical DR.

2.2 Hypotheses Regarding Cone Density and Distribution, by Retinal Quadrant

1. The superior nasal retinal quadrant will have significantly lower cone densities in adolescents and young adults with T1D (patients) as compared to age-similar, typically developing individuals (controls).

2. The inferior nasal quadrant will have significantly lower cone densities in patients than in controls.

3. The superior temporal quadrant will not differ significantly in cone density between patients and controls.

4. The inferior temporal quadrant will not differ significantly in cone density between patients and controls.

5. In controls, the superior nasal quadrant will be significantly more cone dense than the superior temporal quadrant.

6. In controls, the inferior nasal quadrant will be significantly more cone dense than the inferior temporal quadrant.

7. In patients, the superior nasal quadrant will not differ significantly in cone density from the superior temporal quadrant.

8. In patients, the inferior nasal quadrant will not differ significantly in cone density from the inferior temporal quadrant.
2.3 Hypotheses Regarding Cone Density and Distribution, by Hemiretina

1. The nasal hemiretina will be significantly less cone dense in patients than in controls.
2. Cone density in the temporal hemiretina will not differ significantly between patients and controls.
3. Controls will exhibit a nasal-temporal asymmetry in cone density, such that cone densities in the nasal hemiretina are significantly higher than those in the temporal hemiretina.
4. Patients will have cone densities that do not differ significantly between the nasal and temporal hemiretina.
5. The degree of nasal-temporal asymmetry in cone density will be significantly greater in controls than in patients.

2.4 Hypotheses Regarding Cone Function

1. The maximum rate of rise of the a-wave of the red flash ERG will be less steep in adolescents and young adults with T1D than in age-similar typically developing controls.
2. The maximum rate of rise of the a-wave of the white flash ERG will be less steep in adolescents and young adults with T1D than in age-similar typically developing controls.

2.5 Hypotheses Regarding Associations of Outcomes with Duration of T1D

1. Cone properties (function, density, or distribution) exhibiting differences between patients and controls will be demonstrate an association with duration of T1D in the patient group.
3 Methods

3.1 Study Design and Recruitment

This cross-sectional study addressed retinal structure and function in adolescents and young adults with T1D (patients), and in age-similar typically-developing individuals without T1D (controls). Only one eye (right or left) was tested in each participant, and was selected randomly.

Patients were 12 to 25 years of age and were recruited from the Endocrinology Clinic at The Hospital for Sick Children in Toronto, Canada. Individuals with T1D meeting eligibility criteria were identified by a designated research coordinator, and were mailed an information package describing the study, as well as an opt-out slip to be mailed back to the coordinator. After three weeks, the coordinator forwarded to our team a list of individuals that had not opted out. We contacted these remaining individuals by telephone and invited them to participate in our study. Siblings and friends without T1D were also invited to participate as controls.

Recruitment of both patients and controls also took place online, via The Hospital for Sick Children’s Research4Kids Clinical Studies Recruitment Database. In addition, controls were recruited via posters mounted at the University Toronto Campus.

All testing took place in the clinical and research spaces belonging to the Visual Electrophysiology Unit, as well as the Westall Retinal Imaging Laboratory, at The Hospital for Sick Children.

3.2 Research Ethics Board Approval and Consent

This study was approved by the Research Ethics Board at the Hospital for Sick Children, and was carried out in compliance with the TCPS2 (Tri-Council Policy Statement 2: Ethical Conduct for Research Involving Humans) guidelines set out by the Government of Canada’s Interagency Advisory Panel on Research Ethics (TCPS2, 2010).

Prior to participation, a research coordinator or graduate student described the elements of the study and its potential risks and benefits. The voluntary nature of participation was emphasized. Participants were given an opportunity to ask questions and address concerns before informed consent was obtained. All participants were compensated at a rate of ten dollars per hour.
3.3 Inclusion and Exclusion Criteria

3.3.1 Criteria Applying to All Participants

Table 3.1 outlines the inclusion and exclusion criteria pertaining to all participants in the control and patient groups.

<table>
<thead>
<tr>
<th>Inclusion</th>
<th>Exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ages 12-25 years</td>
<td>Prior refractive surgery</td>
</tr>
<tr>
<td>History of normal visual development</td>
<td>Nystagmus, strabismus, or amblyopia</td>
</tr>
<tr>
<td>Good visual acuity (&lt; 0.3 LogMAR)</td>
<td>Disorders affecting retinal or visual function</td>
</tr>
<tr>
<td>Normal colour vision</td>
<td>History of epilepsy or repeated seizures</td>
</tr>
<tr>
<td>Normal contrast sensitivity</td>
<td>Other neurological disorders and diseases (e.g., MS)</td>
</tr>
<tr>
<td>No or low refractive error (≤ + or - 6D)</td>
<td>Presently taking medications with neurological effects</td>
</tr>
</tbody>
</table>

Table 3.1: Inclusion and exclusion criteria for all study participants.

3.3.2 Criteria Specific to Patients

All patients had acquired a diagnosis of T1D, which was ongoing for 5 years or longer. Patients with a prior diagnosis of diabetic retinopathy (ETDRS level 20 or greater) were excluded from the study.

Fundus photos were taken on the day of testing, and were screened at a later date for signs of retinopathy by a retinal specialist or fellow. (Details of photography and retinopathy screening are outlined in Section 3.7) Data from patients who had signs of retinopathy on the fundus at the time of testing were excluded from our analyses.

3.3.3 Criteria Specific to Controls

Controls were screened for euglycemia. A glucometer (OneTouch Ultra 2 Meter, Lifescan; Zurich, Switzerland) and disposable lancets were used to obtain a random (non-fasting) glucose sample. All controls fell within the acceptable ranges for individuals without diabetes (Table 3.2), based on self-reports of how recently they last ate.
Table 3.2: Normal blood glucose concentrations in non-diabetic individuals. Adapted from the Canadian Diabetes Association Clinical Practice Guidelines, 2008 (CDA, 2008).

<table>
<thead>
<tr>
<th>Normal range for individuals without diabetes</th>
<th>Target range for most individuals with diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Glucose: Fasting (mmol/L)</td>
<td>Blood Glucose: 0-2 Hours After Meal (mmol/L)</td>
</tr>
<tr>
<td>4.0 to 6.0</td>
<td>5.0 to 8.0</td>
</tr>
<tr>
<td>4.0 to 7.0</td>
<td>5.0 to 10.0</td>
</tr>
</tbody>
</table>

3.4 Glucose Management in Patients

Glucose management was supervised by a R.N. on staff at The Hospital for Sick Children. Blood glucose was monitored in all patients at regular intervals during testing, approximately once per hour. Vision screening and electroretinography were only performed when blood glucose concentration fell between 4.0 and 10.0 mmol/L, as the results of these tests can be impacted by ambient glucose levels. Patients who remained above or below this range for a prolonged period were asked to return for testing at a future date.

3.5 Vision Screening

3.5.1 Visual Acuity

Visual acuity was assessed using one of two standard self-illuminated ETDRS (Early Treatment of Diabetic Retinopathy Study) charts at 4 metres distance. The test involves reading several lines of text, each containing five individual letters. With successive lines, the letters decrease in size by 0.1 log units, and finer acuity is required to resolve them. A line is scored as correct if the participant is able to read 3 or more of the 5 letters. The test is scored from 1.0 LogMAR (poorest acuity) to -0.3 LogMAR (best acuity). Where possible, both uncorrected (no visual aid) and corrected (with lenses, or pinhole) visual acuity scores were obtained.

3.5.2 Contrast Sensitivity

Contrast sensitivity was assessed in light conditions, using one of two Pelli-Robson charts at 1 metre distance. The lowest possible score is 0.00, and the highest 2.25. Normal scores for this age group fall between 1.65 and 1.95 (Mantyjarvi & Laitinen, 2001).
3.5.3 Colour Vision

Trichromacy was assessed in daylight, or under daylight-simulating illumination, using two separate tests: the Mollon-Reffin Minimalist Test (MRM) and the Hardy-Rand-Rittler (HRR) Pseudoisochromatic Plates.

The MRM involves identification of a coloured chip among several grey chips of various shades. The coloured chips fall into three categories of potential colour vision deficiency—protan (P), deutan (D), and tritan (T)—each testing the function of a different class of cones, L-, M-, and S- respectively (Neitz et al., 2011). As the test goes on, the chips become progressively less saturated, making it increasingly difficult to distinguish the coloured chip from the grey chips. The least saturated chips are designated with a score of “1”, so that a perfect score representing normal trichromacy is P1, D1, T1. (In an older version of the test employed for part of the study, T0.5 also existed; however, a score of T1 was still considered normal). Any score greater than 1 is indicative of a deficiency in colour vision, with a higher score indicating a stronger deficiency along that axis.

The HRR Pseudoisochromatic Plates are images containing many small evenly-spaced circles. Most of the circles are in various shades of grey, but a select number of circles are subtly coloured and form a pattern (such as a circle, X, or triangle) in one or more corners of the plate. A trichromat is able to see all of the patterns present on the 6 test plates. As with the MMR, each plate tests colour vision along a specific axis: protan, deutan, or tritan. Individuals making errors on a given plate are provided with supplemental plates, where they are asked to identify patterns comprised of more saturated colours along the same axis. After supplemental plates are administered, the protan, deutan, or tritan colour vision deficiency is classified as mild, medium, or strong.

3.6 Mydriasis, Cycloplegia, and Anesthesia

One drop of each of the ophthalmic solutions listed in Table 3.3 was administered after vision screening, and at least 20 minutes prior to the following tests: fundus photography, refraction, retinal imaging, and electrophysiological testing. Participants’ pupils were dilated to approximately 8 mm for the tests that followed. Pupil size was measured and recorded. Pupil diameter between 7 and 8 mm ensured consistency of retinal illumination during
electroretinography. For participant comfort, corneal anesthetic was re-applied as needed prior to electroretinography and axial length measurement.

<table>
<thead>
<tr>
<th>Commercial Name</th>
<th>Compound</th>
<th>Concentration</th>
<th>Potential Uses</th>
<th>Purpose in Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mydfrin</td>
<td>Phenylephrine hydrochloride</td>
<td>2.5%</td>
<td>Vasoconstrictor, mydriatic</td>
<td>Pupil dilation</td>
</tr>
<tr>
<td>Mydriacyl</td>
<td>Tropicamide</td>
<td>0.5%</td>
<td>Cycloplegic, mydriatic</td>
<td>Paralysis of ciliary muscles controlling accommodation; pupil dilation</td>
</tr>
<tr>
<td>Alcaine</td>
<td>Proparacaine hydrochloride</td>
<td>0.5%</td>
<td>Surface or deep anesthesia</td>
<td>Topical anesthesia of cornea</td>
</tr>
</tbody>
</table>

Table 3.3: Description of medicated eye drops administered to each participant after vision screening tests. All drops were manufactured by Alcon Canada, in Mississauga, Ontario, Canada.

3.7 Fundus Photography

A Zeiss Digital FF 450 Fundus Camera or Zeiss Visucam 200 Fundus Camera (Carl Zeiss Canada Ltd., Toronto, Canada) enabled the acquisition of stereoscopic colour fundus photographs from all participants. The pattern of areas photographed, and the grading scheme used to identify and classify signs of retinopathy, followed the guidelines set out by the Early Treatment Diabetic Retinopathy Study Research Group (Early Treatment Diabetic Retinopathy Study Research Group, 1991). These guidelines are an extension of the modified Airlie House classification of diabetic retinopathy. Seven semi-overlapping fields were photographed. Photos were graded for signs of retinopathy by at least one retinal specialist or fellow.

3.8 Refraction

After pupil dilation, a qualified orthoptist, optometrist, or student of optometry measured the refractive error of each participant. Polar notations of refractive error (three dimensions: sphere, cylinder, and axis) were converted to spherical equivalents (one dimension), which are expressed in units of + or – Diopters (D). Diopters refer to the optical power of the cornea and lens, with
respect to an ideal optical system that focuses light directly on the retina (an emmetropic eye; 0 D). Eyes that focus light in front of the retina (usually eyes with longer axial lengths) are termed myopic, and have “minus” refractive errors in Diopters. Eyes that focus light behind the retina (usually eyes with shorter axial lengths) are termed hyperopic, and have “plus” refractive errors in Diopters.

3.9 Axial Length Measurement

After re-application of corneal anesthetic, the length of the eye from the surface of the cornea to the front of the retina was measured with an I³ System A/B Scan Diagnostic Ultrasound (Innovating Imaging Inc.; Sacramento, CA). Three axial length measurements were recorded, and the mean of these measurements was calculated.

3.10 Ocular Health and Safety

At the conclusion of testing, a fluorescein stain was applied to the participant’s eye, and the corneal surface was carefully observed under an ultraviolet lamp. Any participant with a possible or suspected corneal abrasion was referred to a resident or fellow in the Department of Ophthalmology at The Hospital for Sick Children for follow-up care.

3.11 AO Retinal Imaging

3.11.1 AO Scanning Laser Ophthalmoscopy

Several types of adaptive optics (AO) retinal imaging have allowed the resolution of individual cells in the human eye in vivo. We have used a scanning laser ophthalmoscope (SLO) with AO correction to capture en-face videos of the outer nuclear layer, where cone photoreceptors can be resolved. The classes of cone photoreceptors imaged are L- and M- cones. S-cones are smaller and are more morphologically similar to rods. Neither S-cones nor rods are captured by our AO system. Videos were obtained in several retinal areas in all participants. Each video was processed to create a single image. Using these images, cone photoreceptor densities were measured.
3.11.2 Description of Westall Lab AO System and Components

The SLO is part of a larger Multimodal Adaptive Optics Retinal Imaging System (Physical Sciences Incorporated, Andover, MA, USA). The advantage of using an SLO with AO correction over a conventional SLO is the ability to acquire images of much higher resolution and clarity, as depicted in Figure 3.1.

![Figure 3.1: Image from a patient taken with our AO system, at approximately 7 degrees eccentricity from the centre of fixation. Cone photoreceptors and retinal vasculature are visible.](image)

In ocular imaging systems, the wavefront of light reflected back ("backscattered") from the eye to the camera that forms the final image is disorganized by aberrations created by the optics of the cornea and lens (Roorda et al., 2002). In an AO system, a wavefront sensor quantifies these aberrations (the local slopes of the incoming wavefront) using feedback from a lenslet array, depicted in Figure 3.2. The wavefront sensor communicates the local wavefront slopes obtained by the lenslet array to a deformable mirror, which then corrects the wavefront in real time. Our system uses a Hartmann-Shack wavefront sensor (Adaptive Optics Associates Inc., Cambridge, MA, USA/UNIQ Vision Inc., Santa Clara, CA, USA) and a continuous surface deformable mirror (ALPAO, Gières, France) with 97 semi-independently movable components called actuators. These actuators adapt to the precise pattern of aberrations communicated by the wavefront sensor at any given moment.
3.11.3 Preparation of Participants for Imaging

In preparation for imaging, participants had their pupils fully dilated and were seated in front of the AO system. His or her head was comfortably secured in place using a tripod comprised of a chinrest and a brace at each temple. Participants were carefully aligned with the imaging beam along all three spatial dimensions. Ideal alignment was achieved when the participant was able to clearly see the fixation target and imaging beam, and the AO operator was able to see clear and consistent lenslet array feedback. Minor adjustments were continuously made throughout the testing session to keep the imaging beam focused on the outer nuclear layer.

3.11.4 AO System Operator Protocol

SLO videos with AO correction were recorded at a rate of 24 frames per second, in each of the four retinal quadrants: superior temporal, inferior temporal, superior nasal, and inferior nasal. Specifically, videos were obtained along the principal oblique (45° and 135°) meridians, at approximately 7° eccentricity (5° horizontal and 5° vertical) with respect to the centre of fixation (Figure 3.3). It should be noted that the centre of fixation did not always correspond exactly with the centre of the fovea. Three to four videos were obtained in each retinal quadrant.
Figure 3.3: Schematic of AO imaging protocol in all participants. SN = superior nasal, IN = superior temporal, ST = superior temporal, IT = inferior temporal

Each video captured a 1.8° x 1.8° area of the retina, and was stored as 1000 pixels in width and as 1024 pixels in height, at a resolution of 72 dots per inch (28.346 pixels per cm). The magnification of each video in the horizontal and vertical directions is detailed below for an emmetropic eye with 24 mm axial length (Table 3.4). For an eye of these proportions imaged with our AOSLO system, 1 degree of retina is equivalent to 291 μm of retina (or 1.8 degrees = 523.8μm).

<table>
<thead>
<tr>
<th></th>
<th>Image Size (cm)</th>
<th>Image Size (mm)</th>
<th>Retinal Area (μm)</th>
<th>Retinal Area (mm)</th>
<th>Magnification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Horizontal</strong></td>
<td>35.28</td>
<td>352.8</td>
<td>523.8</td>
<td>0.5238</td>
<td>673.5X</td>
</tr>
<tr>
<td><strong>Vertical</strong></td>
<td>36.12</td>
<td>361.2</td>
<td>523.8</td>
<td>0.5238</td>
<td>689.6X</td>
</tr>
</tbody>
</table>

Table 3.4: Magnification factors in the horizontal and vertical directions for an eye of 24mm axial length.
3.11.5 Extraction of Images from Videos

Video pre-processing required accurate interlacing and registration of all images, to form a final averaged image where cones were clearly visible. Odd lines and even lines of pixels were recorded separately, such that columns of pixels were often dispersed horizontally in the image. Interlacing a single frame involved translating alternating horizontal lines of pixels with respect to one another to ensure that single columns of pixels lined up vertically.

Registration involved translating and/or rotating two or more separate images in a video with respect to one another, so that the elements in each image lined up precisely across the duration of the video. This adjusted for the effects of involuntary microsaccades and any other source of small movements across video capture. Wherever possible, interlacing and registration were performed automatically in Matlab using Fourier domain analysis of pixel brightness. Some poorly registered videos were processed manually with TurboReg in ImageJ.

The quality of videos varied, such that some videos did not permit extraction of images where cone photoreceptors were visible. Only those images with a visible cone mosaic were used in cell counting, as evaluated collectively by one or more members of the video processing and cell counting teams. The specific participants and the respective quadrants that produced viable images are detailed in the Results section.

3.11.6 Selection of Images

When participants were assessed at more than one time point, images from only one testing session were used. The testing session with the greater number of viable images was selected, or if more than one session produced an equal number of images, the most recent session was selected.

3.11.7 Cone Photoreceptor Cell Counting

Image quality and visibility of cone photoreceptors varied both between images, and throughout individual images. Patches of images where cones were likely present were blurred or occluded by vessels, which ran through the image longitudinally and cross-sectionally. A single, optimally visible rectangular section of each image, with a minimal surface area of 56 μm x 56 μm (recommended by Lombardo, 2012 and Hirsch and Miller, 1987), was selected by one of
three observers (WT, YGS, and LEF). This surface area was converted to square degrees for the shortest axial length included in our study. These sections were counted manually in triplicate, once each by three observers (WT, YGS, and DR). The mean of these counts was computed.

3.11.8 Calculations of Surface Area and Cone Density

Cone density was calculated in two different ways: cones per square degree (cones/degree²), and cones per square millimetre (cones/mm²).

The surface areas of the rectangular counted sections were calculated in square degrees for each image as follows:

\[
\text{section width (deg)} = \frac{1.8^\circ}{1000 \text{ pix}} \times \text{section width (pix)}
\]

\[
\text{section height (deg)} = \frac{1.8^\circ}{1024 \text{ pix}} \times \text{section height (pix)}
\]

\[
\text{section } SA (deg^2) = \text{section width (deg)} \times \text{section height (deg)}
\]

The surface areas of the rectangular counted sections were calculated in square millimetres for each image as described by Tan, 2012, as follows.

Axial length is the distance from the surface of the cornea to the front of the retina. The lens of the human eye has an anterior node and a posterior node. Light converging from the environment onto the anterior node at a given angle is refracted at the same angle from the posterior node onto the retina. The distance from the anterior node to the front of the cornea is the anterior nodal distance (AND). The distance from the posterior node to the retina is the posterior nodal distance (PND). The distance between the two nodal points is negligible, such that:
\[\text{AND (mm)} + \text{PND (mm)} \cong \text{Axial Length (mm)}\]

It is assumed that the proportion of AND to PND holds for eyes of differing lengths (and therefore, also the proportion of AND to axial length, and that of PND to axial length), such that:

\[
\frac{PND_2}{Axial\ Length_2} = \frac{PND_1}{Axial\ Length_1}
\]

and

\[
PND_2 = Axial\ Length_2 \times \frac{PND_1}{Axial\ Length_1}
\]

For a 24.00 mm emmetropic eye, the PND is approximately 17.67 mm. We used this standard to calculate the PND in other eyes for which axial length was known. In eyes for which axial length was not known, the average axial length across our sample was used to estimate PND.

\[
PND (\text{mm}) = Axial\ Length (\text{mm}) \times \frac{17.67\text{mm}}{24.00\text{mm}}
\]

Knowing the angle of refraction (or the angle subtended on the retina by the imaging beam, 1.8°) and the estimated PND allows calculation of the width and height of the original video in mm, and therefore the surface area of the original image.

\[
\tan 1.8^\circ = \frac{\text{Image Height (mm)}}{PND (\text{mm})}
\]
\[
\tan 1.8^\circ = \frac{Image \, Width \, (mm)}{PND \, (mm)}
\]

\[Image \, SA \, (mm^2) = Image \, Width \, (mm) \times Image \, Height \, (mm)\]

All raw images have the following surface area in square pixels:

\[1000 \, pix \times 1024 \, pix = 1.024 \times 10^4 \, pix^2\]

And so the ratio of square millimeters to square pixels for a specific individual is:

\[Ratio \left( \frac{mm^2}{pix^2} \right) = \frac{Image \, SA \, (mm^2)}{1.024 \times 10^4 \, pix^2}\]

Given this ratio, and the surface area in square pixels of the counted section,

\[Section \, SA \, (pix^2) = section \, width \, (pix) \times section \, height \, (pix)\]

The surface area of the counted section was extrapolated in square millimetres.

\[Section \, SA \, (mm^2) = Ratio \left( \frac{mm^2}{pix^2} \right) \times Section \, SA \, (pix^2)\]
3.12 Full-Field Electroretinography (ERG)

3.12.1 Red Flash and White Flash ERGs

The function of cone photoreceptors was studied using the leading edge of the a-wave of the full-field ERG. Two separate ERGs were performed: the first comprised of red flashes on a white rod-suppressing background, and the second comprised of white flashes on a white rod-suppressing background. Red flashes isolate L- and M- cone responses in approximately a 10:1 ratio, while suppressing S-cones. White flashes activate all cone classes, though L- and M- cones are still the dominant players in the a-wave because S-cones only comprise 10% of the cone population.

3.12.2 ERG Equipment and Testing Protocol

The electrical potentials arising from the retina in response to light stimuli were measured on the surface of the cornea using a Burian-Allen contact lens electrode (Hansen Ophthalmic Development Laboratory; Iowa City, IA, USA).

This type of lens is a bipolar electrode, containing an active electrode and a reference electrode. The active electrode lies directly on the corneal surface. The active electrode detects local electrical activity with respect to the reference electrode, which is flush with the inside of the eyelid. A separate ground electrode is secured to the forehead, and picks up signals arising from the environment. The presence of the ground electrode allows the subtraction of background noise.

Data from the ground electrode and bipolar electrode were recorded by Espion V6 hardware and software (Diagnosys LLC; Lowell, MA, USA). The electrical potential on the corneal surface (nV) was measured from 20ms prior to the onset of each light stimulus and until 300ms post-stimulus, and was sampled at a rate of 1000Hz (one reading per ms).

Prior to testing, corneal anesthetic was applied to produce surface anesthesia. Care was taken to ensure that the participant felt as relaxed as possible to allow easy insertion of the lens, and it was reiterated that the potential minor discomfort involved in the test was not harmful. The eye not tested was occluded with a patch.
The size of the dilated pupil (diameter \( \geq 7 \text{mm} \)) was entered into the system to standardize the level of retinal illuminance, in Trolands, delivered by the system. Trolands are a unit of measure that depend upon both luminance (candelas per square metre; cd/m\(^2\)) and the area of the pupil \( \pi r^2 \), as follows:

\[
1 \text{Td} = 1 \text{ cd/m}^2 \times \pi r^2
\]

Where \( r \) = the radius of the pupil, in mm.

Light stimuli were delivered by a ColorDome Ganzfeld Stimulator (Diagnosys LLC; Lowell, MA, USA) in a photopic environment (normal overhead indoor lighting). Before data collection, the participant was light adapted for five minutes to a constant, white, rod-suppressing background at a luminance of 30 cd/m\(^2\). Because the white flash protocol was performed immediately following the red flash protocol, light adaptation was not repeated prior to the white flash ERG. For both the red flash and the white flash protocols, the system generated white flashes at six gradually increasing photopic intensities, over the background stimulus. Each flash was very brief, lasting approximately 4 ms or less. Red flashes were achieved by filtering white flashes through a Wratten 25 red optical gel filter (Kodak; Rochester, NY, USA). The specific parameters of the red flash and white flash protocols can be found in Table 3.5 and Table 3.6, respectively.

3.12.3 Processing of Raw ERG Recordings

Raw data were pre-processed manually in Espion by one observer (LEF). All five waveform responses collected at each step were examined subjectively for internal consistency. Waveforms that were inconsistent with the modal response(s) were discarded. One example of a typical rejection criterion was a pre-stimulus and post-stimulus slope that differed greatly from that of the other waves. A second example of a rejection criterion was an a-wave peak that was irregular in its amplitude, shape, or timing. The third most common reason for rejecting a wave was a low signal-to-noise ratio. A low signal-to-noise ratio was marked by waves of a different temporal frequency obscuring and/or shifting the regular leading edge of the a-wave. At each flash intensity, the most consistent traces (normally two to four of the original five responses) were
<table>
<thead>
<tr>
<th>Step</th>
<th>Stimulus Description</th>
<th>Target Flash Intensity</th>
<th>Measured Flash Intensity</th>
<th>Stimulus Duration</th>
<th>Number of Repetitions</th>
<th>Inter-flash Interval</th>
<th>Background</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light Adaptation</td>
<td>Bulb: Xenon Colour: White (6500K) Wratten 25 (Red Filter)</td>
<td>2.20 log scot Td (158 scot Td; 3.15 scot cd)</td>
<td>0.94 log phot Td (8.77 phot Td; 0.174 phot cd)</td>
<td>≤ 4 ms</td>
<td>5</td>
<td>2 sec</td>
<td>30 scot cd/m² Bulb: Xenon Colour: White (6500K)</td>
</tr>
<tr>
<td>Red Flash 1</td>
<td>Bulb: Xenon Colour: White (6500K) Wratten 25 (Red Filter)</td>
<td>2.60 log scot Td (398 scot Td; 7.92 scot cd)</td>
<td>1.30 log phot Td (19.9 phot Td; 0.396 phot cd)</td>
<td>≤ 4 ms</td>
<td>5</td>
<td>2 sec</td>
<td>30 scot cd/m² Bulb: Xenon Colour: White (6500K)</td>
</tr>
<tr>
<td>Red Flash 2</td>
<td>Bulb: Xenon Colour: White (6500K) Wratten 25 (Red Filter)</td>
<td>2.97 log scot Td (926 scot Td; 18.4 scot cd)</td>
<td>1.65 log phot Td (44.7 phot Td; 0.889 phot cd)</td>
<td>≤ 4 ms</td>
<td>5</td>
<td>2 sec</td>
<td>30 scot cd/m² Bulb: Xenon Colour: White (6500K)</td>
</tr>
<tr>
<td>Red Flash 3</td>
<td>Bulb: Xenon Colour: White (6500K) Wratten 25 (Red Filter)</td>
<td>3.40 log scot Td (2512 scot Td; 50.0 scot cd)</td>
<td>2.10 log phot Td (126 phot Td; 2.51 phot cd)</td>
<td>≤ 4 ms</td>
<td>5</td>
<td>5 sec</td>
<td>30 scot cd/m² Bulb: Xenon Colour: White (6500K)</td>
</tr>
<tr>
<td>Red Flash 4</td>
<td>Bulb: Xenon Colour: White (6500K) Wratten 25 (Red Filter)</td>
<td>3.80 log scot Td (6310 scot Td; 126 scot cd)</td>
<td>2.49 log phot Td (310 phot Td; 6.16 phot cd)</td>
<td>≤ 4 ms</td>
<td>5</td>
<td>5 sec</td>
<td>30 scot cd/m² Bulb: Xenon Colour: White (6500K)</td>
</tr>
<tr>
<td>Red Flash 5</td>
<td>Bulb: Xenon Colour: White (6500K) Wratten 25 (Red Filter)</td>
<td>4.20 log scot Td (15849 scot Td; 315 scot cd)</td>
<td>2.91 log phot Td (805 phot Td; 16.0 phot cd)</td>
<td>≤ 4 ms</td>
<td>5</td>
<td>5 sec</td>
<td>30 scot cd/m² Bulb: XenonColour: White (6500K)</td>
</tr>
</tbody>
</table>

Table 3.5: Parameters of Red Flash ERG Testing Protocol. Red flashes on white background. Scot = scotopic, phot = photopic, Td = Troland, x log Td = 10^x Td; cd = candela. Target and measured flash intensities in candelas are calculated for an 8mm diameter pupil.
<table>
<thead>
<tr>
<th>Step</th>
<th>Stimulus Description</th>
<th>Target Flash Intensity</th>
<th>Measured Flash Intensity</th>
<th>Stimulus Duration</th>
<th>Number of Repetitions</th>
<th>Inter-flash Interval</th>
<th>Background</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Flash 1</td>
<td>Bulb: Xenon Colour: White (6500K) No Optical Gel Filter</td>
<td>2.20 log scot Td (158 scot Td; 3.15 scot cd)</td>
<td>1.79 log phot Td (62.0 phot Td; 1.234 phot cd)</td>
<td>≤ 4 ms</td>
<td>5</td>
<td>2 sec</td>
<td>30 scot cd/m² Bulb: Xenon Colour: White (6500K)</td>
</tr>
<tr>
<td>White Flash 2</td>
<td>Bulb: Xenon Colour: White (6500K) No Optical Gel Filter</td>
<td>2.60 log scot Td (398 scot Td; 7.92 scot cd)</td>
<td>2.18 log phot Td (152 phot Td; 3.03 phot cd)</td>
<td>≤ 4 ms</td>
<td>5</td>
<td>2 sec</td>
<td>30 scot cd/m² Bulb: Xenon Colour: White (6500K)</td>
</tr>
<tr>
<td>White Flash 3</td>
<td>Bulb: Xenon Colour: White (6500K) No Optical Gel Filter</td>
<td>2.97 log scot Td (926 scot Td; 18.4 scot cd)</td>
<td>2.54 log phot Td (347 phot Td; 6.90 phot cd)</td>
<td>≤ 4 ms</td>
<td>5</td>
<td>2 sec</td>
<td>30 scot cd/m² Bulb: Xenon Colour: White (6500K)</td>
</tr>
<tr>
<td>White Flash 4</td>
<td>Bulb: Xenon Colour: White (6500K) No Optical Gel Filter</td>
<td>3.40 log scot Td (2512 scot Td; 50.0 scot cd)</td>
<td>2.99 log phot Td (985 phot Td; 19.6 phot cd)</td>
<td>≤ 4 ms</td>
<td>5</td>
<td>5 sec</td>
<td>30 scot cd/m² Bulb: Xenon Colour: White (6500K)</td>
</tr>
<tr>
<td>White Flash 5</td>
<td>Bulb: Xenon Colour: White (6500K) No Optical Gel Filter</td>
<td>3.80 log scot Td (6310 scot Td; 126 scot cd)</td>
<td>3.38 log phot Td (2420 phot Td; 48.2 phot cd)</td>
<td>≤ 4 ms</td>
<td>5</td>
<td>5 sec</td>
<td>30 scot cd/m² Bulb: Xenon Colour: White (6500K)</td>
</tr>
<tr>
<td>White Flash 6</td>
<td>Bulb: Xenon Colour: White (6500K) No Optical Gel Filter</td>
<td>4.20 log scot Td (15849 scot Td; 315 scot cd)</td>
<td>3.80 log phot Td (6250 phot Td; 124 phot cd)</td>
<td>≤ 4 ms</td>
<td>5</td>
<td>5 sec</td>
<td>30 scot cd/m² Bulb: Xenon Colour: White (6500K)</td>
</tr>
</tbody>
</table>

Table 3.6: Parameters of White Flash ERG Testing Protocol. White flashes on white background. Scot = scotopic, phot = photopic, Td = Troland, x log Td = 10x Td; cd = candela. Target and measured flash intensities in candelas are calculated for an 8mm diameter pupil.
averaged to yield the mean corneal surface potential (nV) from 20ms pre-stimulus to 300ms post-stimulus.

3.12.4 Maximum Rate of Rise Calculations

Each averaged waveform was truncated several milliseconds after the a-wave peak (occurring at approximately 20ms at the lowest intensity, and slightly sooner with each increment in intensity). The rate of rise was calculated at all time points from 0 ms (stimulus onset) onward. The rate of rise (slope, \( \frac{dR}{dt} \)) at each time point was calculated as follows:

\[
\frac{dR}{dt} = \frac{A_t - A_{t-1}}{t - (t - 1)}
\]

Or more simply,

\[
\frac{dR}{dt} = A_t - A_{t-1}
\]

Where \( t = \) time (ms), and \( A_t = \) amplitude of response (millivolts, mV) at time \( t \).

The maximum rate of rise (maximum absolute value of the slope, \( \frac{dR}{dt} \)\( \mid_{max} \)) of each waveform was retrieved in Microsoft Excel (Microsoft Office 2010; Mississauga, Ontario) using the “=MAX(…)” function. The mean \( \frac{dR}{dt} \)\( \mid_{max} \) at each intensity was calculated in the control group and in the patient group.
3.13 Statistical Analyses

3.13.1 Description of Mixed Model Analysis

In the analysis of both the AO data and the ERG data, a mixed linear model was used. Mixed linear models account for some of the potentially complex relationships inherent in our data (for example, relationships between cone densities within individual participants). Such complexities would not be captured by simple pairwise tests. Moreover, the mixed model permits the inclusion of both fixed variables and random variables in the analysis.

Whether a variable should be employed in the model as either fixed or random depends on the type of inferences that are to be made from the results of experiment. For instance, in an experiment measuring the performance of three different hospitals in Toronto, performance could be entered into the model either as a fixed or as a random variable. Performance would be used a fixed variable in the model if inferences about performance are to be applied only to those same three hospitals. Performance would instead be used as a random variable in the model if inferences are to be made about performance in other Toronto hospitals (Ferguson & Takane, 2005).

The Kenward-Roger degrees of freedom approximation was used in all mixed model analyses. This minimized the effect of missing data points on the calculations. This was crucial for the cone density analysis in particular, as most participants did not have data for all four quadrants.

3.13.2 Selection of Covariance Matrices

Computation of the results of a mixed model requires the creation of a covariance matrix. A covariance matrix can take on many types of structures, but always retains several basic properties. First, the covariance matrix quantifies the interrelationships between all conditions in the experiment (e.g., all diabetes status x retinal quadrant subdivisions). Second, variances lie on the diagonal of the matrix, and covariances lie on either side of the diagonal (Ferguson & Takane, 2005).

There were three types of covariance structures considered in our analyses: unstructured, heterogeneous compound symmetry, and compound symmetry.
An unstructured covariance matrix assumes that no patterns underlie the structure of the matrix. It therefore requires estimating all possible variance and covariance parameters from the data. A covariance matrix with compound symmetry employs a simple estimate of covariance, whereby the variance values along the diagonal are equal to one another, and the covariance values are also equal to one another. Thus, a covariance matrix with compound symmetry generates only two parameters. Finally, a covariance matrix with heterogeneous compound symmetry allows unequal variances at the diagonal elements, but requires symmetry in the covariance values on either side of the diagonal.

Using an unstructured covariance matrix allows the most flexibility in the values comprising the matrix, but consumes the most degrees of freedom. Meanwhile, a covariance matrix with compound symmetry allows the least flexibility in the values comprising the matrix, but consumes the least degrees of freedom. Heterogeneous compound symmetry is the intermediate choice in both regards.

In each analysis (quadrants, hemiretinae, red flash, and white flash), the optimal covariance structure was selected among these three choices using Akaike’s Information Criterion (AIC).

### 3.13.3 Cone Density Mixed Model, by Quadrants

In the quadrant-based cone density analysis, diabetes status (control, patient) and retinal quadrant (superior nasal, inferior nasal, superior temporal, inferior temporal) were included in the model as fixed variables. Participant identity was included in the model as a random variable, since the participants comprised only a sample of the population to which we wished to extrapolate.

The inclusion of participant identity as its own variable allowed the modelling of within-subject cone density correlations between retinal quadrants. Therefore, the mixed model was employed in such a way so as to account for multiple measurements from a single individual.

The mixed model was used to assess the main effect of diabetes status on cone density, and the main effect of quadrant (regardless of diabetes status) on cone density. The interaction between diabetes status and quadrant was also assessed. In each of these tests, an alpha level of $\alpha = 0.05$ was used.
In addition, the model generated least squares means estimates of cone density in each retinal quadrant, for both patients and controls. It also generated differences in least squares means estimates, which quantified the differences between each Quadrant x Diabetes Status estimate.

The differences in least squares means were used to assess a total of eight a priori hypotheses. Differences in cone density between controls and patients were evaluated in identical retinal locations (four comparisons). The differences in least squares means were also used to identify within-group cone density patterns across the vertical meridian: superior nasal vs. superior temporal, and inferior nasal vs. inferior temporal (two comparisons per group x two groups). The alpha level was adjusted with a Bonferroni correction of $\alpha / 8$ to account for multiple comparisons ($\alpha = 0.00625$). This approach was somewhat conservative, because these eight comparisons were not completely independent.

Finally, duration of T1D was included as a random variable in the model to determine whether this factor was correlated with cone density in the patient sample.

3.13.4 Cone Density Mixed Model, by Hemiretinae

The hemiretina-based cone density analysis was similar to the quadrants-based analysis, except that superior nasal and inferior nasal observations were pooled into a single group (nasal), and superior temporal and inferior temporal observations were pooled into a single group (temporal). Superior and inferior data points from the same hemiretina in the same participant were treated as separate observations, rather than averaged.

Diabetes status (control, patient) and hemiretina (nasal, temporal) were included in the model as fixed variables. Participant identity was again included as a random variable.

The main effect of diabetes status on cone density was assessed, as was the main effect of hemiretina. The interaction between diabetes status and hemiretina was also assessed. In each of these tests, an alpha level of $\alpha = 0.05$ was used.

In addition, the model generated least squares means estimates of cone density in each hemiretina, for both patients and controls. It also generated differences in least squares means estimates, which quantified the differences between each Hemiretina x Diabetes Status estimate.
The differences in least squares means were used to assess a total of four a priori hypotheses. Differences in cone density between controls and patients were evaluated separately for the nasal hemiretina and for the temporal hemiretina (two comparisons). The differences in least squares means were also used to identify nasal-temporal asymmetry in cone densities within each group (two comparisons). The alpha level was adjusted with a Bonferroni correction of $\alpha / 4$ to account for multiple comparisons ($\alpha = 0.0125$). Again, this approach was somewhat conservative given that the comparisons were not completely independent.

Finally, duration of T1D was included as a random variable in the model to determine whether this factor was correlated with cone density in the patient group.

3.13.5 Maximum Rate of Rise Mixed Model, for Red Flash and White Flash ERGs

The format of the mixed model was identical for the red flash and the white flash ERG data. Diabetes status (control, patient) and flash intensity (6 levels) were included in the model as fixed variables. Participant identity was included as a random variable.

The main effect of diabetes status on maximum rate of rise was assessed. The interaction between diabetes status and flash intensity was also assessed. For both of these tests, an alpha level of $\alpha = 0.05$ was used.

In addition, the model generated least squares means estimates of maximum rates of rise at each flash intensity for both patients and controls. It also generated differences in least squares means estimates, which included between-groups differences in maximal rates of rise at each flash intensity.

Finally, duration of T1D was included as a random variable in the model to determine whether this factor was correlated with maximum rate of rise in the patient group.

3.13.6 Software Used in Statistical Analysis

To generate the mixed models, the “PROC MIXED” procedure was used SAS version 9.3 (released July 2011, Statistical Analysis Software Institute Inc., Cary, North Carolina, USA).
4 Results

4.1 Exclusion of Participants in AO and ERG Studies

4.1.1 Decisions to Include or Exclude Participants with Colour Vision Abnormalities in Both Studies

Across both studies, all controls and of 22 of 23 patients completed the Mollon-Reffin Minimalist Test (Minimalist). In addition, 35 of 36 controls and 21 of 23 patients completed the Hardy-Rand-Rittler Pseudoisochromatic Plates (HRR).

Nearly all participants across both AO and ERG studies had normal colour vision according to the Minimalist (scores of P1D1T1 or P1D1T0.5), and also had normal colour vision on the HRR. Only red-green (R-G) colour deficiencies were found, in two controls and two patients (Table 4.1). All participants with abnormal colour vision were male.

Congenital R-G colour deficiencies that are not associated with other retinal pathologies are caused by specific genetic mutations that affect opsin protein expression, and therefore alter the spectral sensitivity of photopigments within a given cone class (Neitz et al., 2011). While altered spectral sensitivity could alter the results of the ERG, the overall number of cones present should remain unchanged (see A.2). Therefore, those with R-G colour deficiencies on one or both tests were included in the AO study but excluded from the ERG study.

<table>
<thead>
<tr>
<th>Participant ID</th>
<th>Test ID</th>
<th>Gender</th>
<th>Group</th>
<th>HRR Colour Deficiency Classification</th>
<th>Minimalist Test Score</th>
<th>Decision: AO Data</th>
<th>Decision: ERG Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>486</td>
<td>237</td>
<td>M</td>
<td>Control</td>
<td>Strong R-G</td>
<td>P2D1T0.5</td>
<td>No usable data</td>
<td>Excluded</td>
</tr>
<tr>
<td>495</td>
<td>246</td>
<td>M</td>
<td>Control</td>
<td>Medium R-G</td>
<td>P2D4T0.5</td>
<td>No usable data</td>
<td>Excluded</td>
</tr>
<tr>
<td>399</td>
<td>213</td>
<td>M</td>
<td>T1D</td>
<td>Mild R-G</td>
<td>P1D1T0.5</td>
<td>Included</td>
<td>No data</td>
</tr>
<tr>
<td>451</td>
<td>266</td>
<td>M</td>
<td>T1D</td>
<td>No deficiency detected</td>
<td>P1D2T0.5</td>
<td>Included</td>
<td>Excluded</td>
</tr>
</tbody>
</table>

Table 4.1: Red-green colour deficiencies in the control and T1D groups, according to Hardy Rand Rittler Pseudoisochromatic Plates (HRR) and Mollon-Reffin Minimalist Test (Minimalist). Subsequent decisions for inclusion or exclusion in AO and ERG studies are listed.
4.1.2 Exclusion of Patients with Signs of Retinopathy on Fundus

Patients with DR lesions on fundus, from a current visit or from a previous visit, had their ERG and AO data excluded from the analysis. Clinical assessments of fundus photos, and excluded testing sessions, are described in Table 4.2.

<table>
<thead>
<tr>
<th>Participant ID</th>
<th>Excluded Test(s) ID</th>
<th>Test ID at Photo</th>
<th>Photo Date (Y-M-D)</th>
<th>Clinical Assessment: Lee</th>
<th>Clinical Assessment: Lam</th>
</tr>
</thead>
<tbody>
<tr>
<td>346</td>
<td>218</td>
<td>218</td>
<td>2012-08-10</td>
<td>F4: solitary flame shaped heme, just outside of superior temporal arcade</td>
<td>F1: Right small flame shaped hemorrhage (superior temporal), mild proliferative diabetic retinopathy, F6: small preretinal fibrosis vs cotton wool spots</td>
</tr>
<tr>
<td>350</td>
<td>256</td>
<td>76</td>
<td>2007-11-05</td>
<td></td>
<td>Small microaneurysm vs dot hemorrhage - consistent with mild NPDR</td>
</tr>
<tr>
<td>446</td>
<td>222 and 273</td>
<td>222</td>
<td>2011-08-06</td>
<td>F1: moderately dilated and tortuous veins, prominent nerve fibre layer superiorly and inferiorly; F2: flame heme and microaneurysm inside superior temporal arcade; F6: arterio-venous notching</td>
<td>Small hemorrhage along the white centre, mild proliferative DR</td>
</tr>
<tr>
<td>419</td>
<td>207</td>
<td>162</td>
<td>2009-08-17</td>
<td>No DR; F1: cup/disc ratio 0.45-0.5; F6: small nevus superior nasal, flat, no associated fluid or overlying orange pigment</td>
<td>Dot hemorrhage in posterior pole</td>
</tr>
<tr>
<td>429</td>
<td>214</td>
<td>189</td>
<td>2010-11-20</td>
<td></td>
<td>Small patch of exudate, inferior temporal from disc; prominent nerve fibre layer along superior temporal arcade</td>
</tr>
</tbody>
</table>

Table 4.2: Patients whose AO and/or ERG data were excluded, on the basis of DR lesions found on fundus photos. Excluded Test(s) ID specifies the testing session for which data was excluded. Test ID at Photo specifies the testing session during which the photo indicative of DR was taken. Photo Date is given in year-month-day format. Clinical assessments are transcribed from reports by Dr. Jacob Lee, MD, a surgical retina fellow, and Dr. Wai Ching Lam, MD, a practicing ophthalmologist and retinal specialist.
4.1.3 Other Causes for Exclusion of Participants

One control had been diagnosed with strabismus (Participant ID: 482; Test ID: 232). Two controls did not attend testing and did not contact the research team to cancel their appointments (Participant IDs: 475, 476; Test IDs not assigned).

One patient had been diagnosed with multiple sclerosis (Participant ID: 342; no Test ID assigned). One patient (ID: 513; Test ID 282) could not complete testing due to feeling unwell.

4.2 Characteristics of Participants in Both Studies

After decisions for inclusion and exclusion were finalized, total of 36 controls and 23 patients participated across the two studies.

4.2.1 Age

The mean age of controls was 17.6 years (SD = 3.2; range = 12.4 to 24.3). The mean age of patients was 18.9 years (SD = 2.4; range = 15.5 to 23.6).

4.2.2 Gender

Across both studies, 37 females (22 controls, 15 patients) and 22 males (14 controls, 8 patients) participated.

4.2.3 Other Characteristics

Other descriptive statistics pertaining to the participant sample, including the remainder of ophthalmic exam results, will be described for each study individually. Age and gender will also be re-examined for each study individually.

4.3 Characteristics of Patients in Both Studies

4.3.1 Duration of T1D

The patient participants were a cross-section of adolescents and young adults with T1D from a larger longitudinal study (see A.1 for specific Participant IDs and Test IDs). Across both studies, their mean duration of T1D was 11.2 years (SD = 4.2, range = 5.4 to 19.5).
4.4 Characteristics of Participants in AO Study

Twenty-three controls and nineteen patients, who attended testing sessions between December 2010 and May 2013, participated in the AO study.

4.4.1 Age

The mean age of controls was 17.4 years (SD = 3.1, range = 12.6 to 24.3). The mean age of patients was 18.9 years (SD = 2.4, range = 15.6 to 23.6).

4.4.2 Gender

There were 14 females and 9 males comprising the control group. There were 11 females and 8 males in the patient group.

4.4.3 Eye Tested

In the control group, 12 left eyes and 11 right eyes were tested. In the patient group, 9 left eyes and 10 right eyes were tested. The optic disc and retinal vasculature from (a) fundus photos and (b) AO images were used as landmarks to identify retinal locations on AO images (nasal versus temporal, and superior versus inferior). Therefore, no further left-to-right translation was required for AO images.

4.5 Ophthalmic Exam Results for Participants in AO Study

4.5.1 Visual Acuity

Visual acuity was measured for 22 of 23 controls and for 18 of 19 patients. The best demonstration of visual resolution was taken as the visual acuity score for each participant, whether vision was corrected or uncorrected. In both groups, best visual acuity ranged from -0.2 LogMAR (finest resolution) to 0.2 LogMAR (coarsest resolution). In both the control and the patient groups, the median best visual acuity was 0.0 LogMAR.

4.5.2 Contrast Sensitivity

Contrast sensitivity was obtained for all controls and for 18 of 19 patients. The modal Pelli-Robson score for contrast sensitivity was 1.65 log units in both the control group and the patient group. Two controls and three patients exhibited contrast sensitivity below this level (control
scores: 1.5, 1.35; patient scores: 1.55, 1.5, 1.4). Three controls and three patients exhibited contrast sensitivity above this level (control scores: 1.8, 1.8, 1.95; patient scores: 1.8, 1.8, 1.95).

4.5.3 Refractive Error

Refractive errors were measured in 22 of 23 controls, and in 12 of 19 patients. The median refractive error in controls was -0.25 Diopters (range = -4.0 to +1.25). The median refractive error in patients was -0.75 Diopters (range = -2.5 to 0.0).

4.5.4 Axial Length

An axial length was obtained for 16 of 23 controls and for 9 of 19 patients. The mean axial length among controls that were measured was 23.57 mm (SD = 0.95). The mean axial length among patients that were measured was 23.18 mm (SD = 0.61).

4.6 Characteristics of Patients in AO Study

4.6.1 Duration of T1D

Of 19 patients, 18 had a known duration of T1D. The mean duration of T1D among patients in the AO study group was 11.4 years (SD = 3.8, range = 5.4 to 19.5).

4.7 Available AO Data, and Retinal Surface Area Assessed

The maximum surface area available for cone counting, free of blur and vascular intrusion, varied between images. Table 4.3 lists the surface areas of the counting grids in each 1.8° x 1.8° image, for retinal quadrants that yielded usable images.
<table>
<thead>
<tr>
<th>GROUP ID</th>
<th>SURFACE AREA OF GRID (degrees²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SN</td>
</tr>
<tr>
<td>Control</td>
<td>420</td>
</tr>
<tr>
<td>Control</td>
<td>427</td>
</tr>
<tr>
<td>Control</td>
<td>428</td>
</tr>
<tr>
<td>Control</td>
<td>443</td>
</tr>
<tr>
<td>Control</td>
<td>470</td>
</tr>
<tr>
<td>Control</td>
<td>471</td>
</tr>
<tr>
<td>Control</td>
<td>472</td>
</tr>
<tr>
<td>Control</td>
<td>473</td>
</tr>
<tr>
<td>Control</td>
<td>474</td>
</tr>
<tr>
<td>Control</td>
<td>480</td>
</tr>
<tr>
<td>Control</td>
<td>483</td>
</tr>
<tr>
<td>Control</td>
<td>485</td>
</tr>
<tr>
<td>Control</td>
<td>490</td>
</tr>
<tr>
<td>Control</td>
<td>491</td>
</tr>
<tr>
<td>Control</td>
<td>494</td>
</tr>
<tr>
<td>Control</td>
<td>496</td>
</tr>
<tr>
<td>Control</td>
<td>498</td>
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<tr>
<td>Control</td>
<td>500</td>
</tr>
<tr>
<td>Control</td>
<td>501</td>
</tr>
<tr>
<td>Control</td>
<td>503</td>
</tr>
<tr>
<td>Control</td>
<td>504</td>
</tr>
<tr>
<td>Control</td>
<td>505</td>
</tr>
<tr>
<td>Control</td>
<td>506</td>
</tr>
<tr>
<td>T1D</td>
<td>204</td>
</tr>
<tr>
<td>T1D</td>
<td>213</td>
</tr>
<tr>
<td>T1D</td>
<td>310</td>
</tr>
<tr>
<td>T1D</td>
<td>316</td>
</tr>
<tr>
<td>T1D</td>
<td>317</td>
</tr>
<tr>
<td>T1D</td>
<td>331</td>
</tr>
<tr>
<td>T1D</td>
<td>347</td>
</tr>
<tr>
<td>T1D</td>
<td>370</td>
</tr>
<tr>
<td>T1D</td>
<td>371</td>
</tr>
<tr>
<td>T1D</td>
<td>384</td>
</tr>
<tr>
<td>T1D</td>
<td>385</td>
</tr>
<tr>
<td>T1D</td>
<td>399</td>
</tr>
<tr>
<td>T1D</td>
<td>401</td>
</tr>
<tr>
<td>T1D</td>
<td>423</td>
</tr>
<tr>
<td>T1D</td>
<td>451</td>
</tr>
<tr>
<td>T1D</td>
<td>461</td>
</tr>
<tr>
<td>T1D</td>
<td>481</td>
</tr>
<tr>
<td>T1D</td>
<td>484</td>
</tr>
<tr>
<td>T1D</td>
<td>487</td>
</tr>
</tbody>
</table>

**Table 4.3**: Surface areas of counting grids (in square degrees) in each 1.8 degree x 1.8 degree image. SN, superior nasal; IN, inferior nasal; ST, superior temporal; IT, inferior temporal. Purple: data collected after publication of WT thesis (Tan, 2012). Turquoise: a different AO testing session from the same participant was included in WT thesis (Tan, 2012). Empty cell indicates no usable images for that retinal location, from that testing session.
4.8 Units of Surface Area in Cone Density Calculations: Selection of Square Degrees over Square Millimetres

The superior temporal quadrant had the greatest number of observations available that were associated with known axial lengths (Controls: n = 11; Patients: n = 6). Thus, in the superior temporal quadrant, linear regressions were performed between cone density and axial length. Regressions were performed separately for controls and patients. For the first set of regressions, density was calculated in cones per square degree. For the second set of regressions, density was calculated in cones per square millimetre. The cones per square millimetre calculation required the use of an individual’s axial length, while the cones per square degree calculation did not.

Regressions generated a squared correlation coefficient, $r^2$ (0 < $r$ < 1), representing the degree of linear dependence (the strength of the association) between axial length and surface area. A value of 0 indicates no correlation, and a value of 1 indicates a total positive correlation.

When cone density in the superior temporal quadrant was measured in cones per square millimetre and regressed with axial length, the $r^2$ value for controls was 0.1, and the $r^2$ value for patients was 0.09.

When cone density in the superior temporal quadrant was measured in cones per square degree and was regressed with axial length, the $r^2$ value for controls was 0.03, and the $r^2$ value for patients was 0.01.

For both controls and patients, $r^2$ values were greater in the case of cones per square millimetre. This suggests that measurements in cones per square millimetre tend to vary more with axial length than do measurements in cones per square degree.

For the AO study, we want to maximize the ability of the mixed model to detect differences between groups, and therefore, we want to limit the amount of inter-individual variation in cone density that is due to axial length. The squared correlation coefficients found here indicate that measurements in cones per square degree minimize such inter-individual variation, as has been suggested by other investigators looking at cone density with AO imaging (Chui et al., 2008a). Measurements in cones per square degree are thus appear to be the better choice for our study.
This conclusion may seem counterintuitive, since factoring axial length into cone density calculations should theoretically standardize measurement and thus reduce inter-individual variation. However, in the parafoveal area, eyes with shorter axial lengths tend to be more cone-dense, and eyes with longer axial lengths tend to be less cone-dense (Chui et al., 2008a). Measuring cone density in cones per square degree may compensate for these differences by applying a slightly smaller surface area to shorter eyes, and a slightly larger surface area to longer eyes.

This idea is supported by the slope ($m$) of the equations generated by our linear regressions. When cone densities were measured in cones per square millimetre and were regressed with axial length, the values of $m$ in controls and patients were -615 and -559 respectively. When cone densities were measured in cones per square degree and were regressed with axial length, the values of $m$ in controls and patients were 26 and 19 respectively.

In the case of the regression of cones per square millimetre with axial length, the slope is negative and relatively steep. The direction and the magnitude of this result suggest that this unit of measure is sensitive to the cone denseness of shorter eyes and the cone sparseness of longer eyes. In the case of the regression of cones per square degree with axial length, the slope is positive and relatively shallow. This result suggests that this unit of measure is relatively insensitive to axial length-related variations in cone density.

Overall, when both correlation coefficients ($r^2$) and the slopes ($m$) of the linear regressions were considered, we ultimately decided to use cones per square degree as our unit of measure for the AO study.
4.9 Cone Density Raw Data, by Quadrant

A total of 91 usable observations across controls and patients were available from all retinal locations combined. To facilitate the Retinal Quadrant x Diabetes Status mixed model analysis, each data point was illustrated individually in scatterplot form in Figure 4.1.

Figure 4.1: Scatterplot of all 91 cone densities, in cones/degree², grouped primarily by retinal quadrant (SN: superior nasal; IN: inferior nasal; ST: superior temporal; IT: inferior temporal), and secondarily by diabetes status (control values: blue diamonds; patient values: red squares).
4.9.1 Outliers in Cone Density Data, when Grouped by Quadrant and Diabetes Status

Participant 385 (patient) had a relatively low inferior nasal cone density as compared to inferior nasal cone densities of other patients. In exploring potential reasons for this discrepancy, it was found that the portion of the photograph from where cones were counted had a relatively large surface area. Moreover, there was a degree of inter-observer disagreement about the number of cones present, but all observers counted the area reasonably. Data entry was accurate.

Conversely, Participant 347 (patient) had a relatively high superior temporal cone density as compared to superior temporal cone densities of other patients. Upon further study, it was found that the portion of the photograph from where cones were counted had a relatively small surface area. Again, there was a degree of inter-observer disagreement about the number of cones present, but all observers counted the area reasonably. Data entry was accurate.

Both observations were included in the quadrants dataset, but the strength of their contributions to the results of the mixed model was later tested with a sensitivity analysis.

Descriptions of the outliers are summarized in Table 4.4.

<table>
<thead>
<tr>
<th>Participant ID</th>
<th>Diabetes Status</th>
<th>Retinal Quadrant</th>
<th>Cone Density (cones/deg$^2$)</th>
<th>Lower Limit: Q1 - 1.5(IQR)</th>
<th>Upper Limit: Q3 + 1.5(IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>385</td>
<td>Patient</td>
<td>IN</td>
<td>331</td>
<td>523</td>
<td></td>
</tr>
<tr>
<td>347</td>
<td>Patient</td>
<td>ST</td>
<td>1065</td>
<td></td>
<td>1045</td>
</tr>
</tbody>
</table>

*Table 4.4*: Outliers in the quadrants dataset (bolded). Lower limits and upper limits were calculated for each Retinal Quadrant x Diabetes Status subdivision. The lower limit is the first quartile (Q1), less 1.5 times the interquartile range (IQR = Q3 – Q1). The upper limit is the third quartile, plus 1.5 times the interquartile range (IQR = Q3 – Q1). Outliers in the quadrants dataset were defined as densities falling below the lower limit, or above the upper limit, of the Retinal Quadrant x Diabetes Status subdivision to which they belonged.
4.10 Mixed Model Analysis of AO Data, by Quadrant

The mixed model was first applied to the dataset where cone densities were grouped by quadrant. A covariance matrix with compound symmetry structure was favoured as per Akaike’s Information Criterion (AIC).

4.10.1 F-Test: Effect of Diabetes Status x Retinal Quadrant on Cone Density

The interaction between diabetes status and quadrant did not have a significant effect on cone density ($p = 0.2$). This result indicates that the intra-retinal distribution of cones according to quadrant does not differ between controls and patients.

4.10.2 F-Test: Main Effect of Diabetes Status on Cone Density

When the interaction term was removed from the mixed model, there was a significant main effect of diabetes status on cone density ($p = 0.008$). The least squares mean estimate of cone density in controls was 925 cones/degree$^2$ (95% CI: 871, 980). The least squares mean estimate of cone density in patients was 815 cones/degree$^2$ (95% CI: 756, 874). The confidence intervals of these estimates overlap only very slightly (upper limit of 874 cones/degree$^2$ in patients, versus lower limit of 871 cones/degree$^2$ in controls). The difference in cone density between the two groups estimated by the model, regardless of quadrant, was 110 cones/degree$^2$ (controls > patients; 95% CI: 30, 190). This result demonstrates that overall, cones were less densely packed in adolescents with T1D as compared to controls.

4.10.3 F-Test: Main Effect of Retinal Quadrant on Cone Density

There was no main effect of quadrant on cone density when the control and patient groups were pooled ($p = 0.7$). Therefore, there was no intra-retinal quadrant-based pattern of cone density that was consistent across controls and patients.

4.10.4 Additional Pairwise Comparisons Between Quadrants

Although the interaction term (diabetes status x quadrant) was not significant, specific a priori hypotheses permitted returning to the model containing the interaction term to address a limited number of pairwise comparisons. First, each group was examined in isolation, and cone density differences were assessed between adjacent quadrants across the vertical meridian (four
comparisons). Second, cone densities in identical retinal locations were compared between
groups (an additional four comparisons; total, eight comparisons). A Bonferroni correction of
0.05/8 was used to protect against chance findings from multiple comparisons. Therefore, the
threshold for significance became \( \alpha = 0.00625 \). This approach was somewhat conservative,
because the pairwise comparisons were not completely independent.

### 4.10.5 Intra-Retinal Quadrant-Based Cone Distribution in Controls

In the control group, the nasal-temporal differences in cone density were approximately twice as
large in magnitude as were the superior-inferior differences. However, the nasal-temporal
differences were not unequivocally in the nasal > temporal direction, because in both cases (for
superior quadrants and inferior quadrants) the lower 95% confidence limit fell far below zero. In
addition, the nasal-temporal differences did not approach significance (\( p = 0.2 \) for both superior
and inferior cases). In sum, there were no quadrant-based patterns in the intra-retinal distribution
of cones in controls. The least squares mean estimates of cone density for each quadrant in
controls, and the estimated differences across meridians, are summarized in **Table 4.5**.

<table>
<thead>
<tr>
<th>CONTROLS ONLY</th>
<th>NASAL</th>
<th>TEMPORAL</th>
<th>DIFFERENCE across vertical meridian</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUPERIOR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 10</td>
<td>LSM = 989 (887, 1091)</td>
<td>LSM = 899 (816, 982)</td>
<td>Superior Quadrants: Nasal &gt; Temporal ( \text{DLSM} = 89 (-37, 215) ) ( p = 0.2 )</td>
</tr>
<tr>
<td>n = 15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INFERIOR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 13</td>
<td>LSM = 947 (858, 1037)</td>
<td>LSM = 867 (769, 964)</td>
<td>Inferior Quadrants: Nasal &gt; Temporal ( \text{DLSM} = 81 (-43, 204) ) ( p = 0.2 )</td>
</tr>
<tr>
<td>n = 11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIFFERENCE across horizontal meridian (do not expect difference)</td>
<td>Nasal quadrants: Superior &gt; Inferior ( \text{DLSM} = 41 (-88, 170) ) ( p = 0.5 )</td>
<td>Temporal quadrants: Superior &gt; Inferior ( \text{DLSM} = 33 (-92, 157) ) ( p = 0.6 )</td>
<td></td>
</tr>
</tbody>
</table>

**Table 4.5**: Least Mean Squares (LSM) estimates (**bolded**) of quadrant-specific cone densities
in the control group, generated by the mixed model with compound symmetry covariance
structure. Differences of Least Mean Squares (DLSM; **underlined**) express the difference
between LSMs of adjacent quadrants across the vertical meridian (nasal-temporal). DLSMs of
adjacent quadrants across the horizontal meridian (superior-inferior) are provided for reference.
All LSM and DLSM values are expressed in cones per square degree, with 95% CI (lower,
upper).
4.10.6 Intra-Retinal Quadrant-Based Cone Distribution in Patients

In the superior retina of patients, there was no difference between nasal and temporal cone densities. The magnitude of the difference was less than that of both superior-inferior quadrant comparisons. In the inferior retina of patients, the nasal-temporal relationship was the opposite of that seen in controls: the temporal side was more cone-dense than the nasal side. However, this difference was not unequivocal, as the lower 95% confidence limit fell far below zero. Moreover, the difference did not approach significance (p = 0.2). In sum, there were no quadrant-based patterns in the intra-retinal distribution of cones in patients. The least squares mean estimates of cone density for each quadrant in patients, and the estimated differences across meridians, are summarized in Table 4.6.

<table>
<thead>
<tr>
<th>PATIENTS ONLY</th>
<th>NASAL</th>
<th>TEMPORAL</th>
<th>DIFFERENCE across vertical meridian</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUPERIOR</td>
<td>SUPERIOR NASAL n = 11 LSM = <strong>819</strong> (721, 916)</td>
<td>SUPERIOR TEMPORAL n = 13 LSM = <strong>800</strong> (711, 890)</td>
<td>Superior Quadrants: Nasal &gt; Temporal DLSM = <strong>18</strong> (-108, 145) p = 0.8</td>
</tr>
<tr>
<td>INFERIOR</td>
<td>INFERIOR NASAL n = 8 LSM = <strong>770</strong> (656, 884)</td>
<td>INFERIOR TEMPORAL n = 10 LSM = <strong>862</strong> (760, 964)</td>
<td>Inferior Quadrants: Temporal &gt; Nasal DLSM = <strong>93</strong> (-54, 239) p = 0.2</td>
</tr>
</tbody>
</table>

Table 4.6: Least Mean Squares (LSM) estimates (bolded) of quadrant-specific cone densities in the patient group, generated by the mixed model with compound symmetry covariance structure. Differences of Least Mean Squares (DLSM; underlined) express the difference between LSMs of adjacent quadrants across the vertical meridian (nasal-temporal). DLSMs of adjacent quadrants across the horizontal meridian (superior-inferior) are provided for reference. All LSM and DLSM values are expressed in cones per square degree, with 95% CI (lower, upper).
4.10.7  Comparisons of Identical Quadrants Between Groups

Cone density in the superior temporal and inferior temporal quadrants did not differ between groups. In superior nasal and inferior nasal quadrants, there was a borderline significant difference between groups, where controls had greater cone densities than patients. However, given the conservative Bonferroni correction ($p = 0.00625$), the inter-group difference was not significant in either case ($p = 0.02$ for both). Still, the relatively large magnitude of the inter-group differences (170 cones/degree$^2$ for SN, and 178 cones/degree$^2$ IN), and the fact that the lower 95% confidence limits fell far above zero, suggests that a greater sample size might achieve significance. In sum, controls had greater cone densities than patients in the superior nasal and inferior nasal quadrants, but these differences were only borderline significant. It is possible that this pattern represents a between-groups difference that is selectively nasal, which could be captured by the hemiretinae mixed model. The differences of least squares means which compare identical retinal locations between groups are summarized in Table 4.7.

<table>
<thead>
<tr>
<th></th>
<th>CONTROLS</th>
<th>PATIENTS</th>
<th>DIFFERENCE between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUPERIOR NASAL</td>
<td>n = 10</td>
<td>n = 11</td>
<td>Control &gt; T1D</td>
</tr>
<tr>
<td></td>
<td>LSM = 989 (887, 1091)</td>
<td>LSM = 819 (721, 916)</td>
<td>DLSM = 170 (29, 310)</td>
</tr>
<tr>
<td>INFERIOR NASAL</td>
<td>n = 13</td>
<td>n = 8</td>
<td>Control &gt; T1D</td>
</tr>
<tr>
<td></td>
<td>LSM = 947 (858, 1037)</td>
<td>LSM = 770 (656, 884)</td>
<td>DLSM = 178 (33, 323)</td>
</tr>
<tr>
<td>SUPERIOR TEMPORAL</td>
<td>n = 15</td>
<td>n = 13</td>
<td>Control &gt; T1D</td>
</tr>
<tr>
<td></td>
<td>LSM = 899 (816, 982)</td>
<td>LSM = 800 (711, 890)</td>
<td>DLSM = 99 (-23, 221)</td>
</tr>
<tr>
<td>INFERIOR TEMPORAL</td>
<td>n = 11</td>
<td>n = 10</td>
<td>Control &gt; T1D</td>
</tr>
<tr>
<td></td>
<td>LSM = 867 (769, 964)</td>
<td>LSM = 862 (760, 964)</td>
<td>DLSM = 4 (-136, 145)</td>
</tr>
</tbody>
</table>

**Table 4.7:** Re-iteration of Least Mean Squares (LSM) estimates of quadrant-specific cone densities, generated by the mixed model with compound symmetry covariance structure, in the control group and in the patient group. Differences of Least Mean Squares (DLSM; underlined) express the between-groups differences in LSMS at identical retinal locations. All LSM and DLSM values are expressed in cones per square degree, with 95% CI (lower, upper).
4.11 Sensitivity Analyses for Quadrants Model

The impact of outliers and of influential observations on the outcome of the mixed model were tested with sensitivity analyses. In these analyses, the data were removed from the dataset and the model was re-run.

4.11.1 Sensitivity Analysis, Testing the Impact of Outliers on Mixed Model Results

In the first sensitivity analysis, two outliers were removed from the dataset (see Table 4.4) and the model was run anew, to ensure that the original outcome of the model was not reliant on those observations.

When these two outliers were removed, the effect of the interaction term (diabetes status x retinal quadrant) on cone density remained not significant.

When the interaction term was removed from the model, the main effect of diabetes status on cone density remained significant. The results were essentially equivalent (Originally: Control > T1D; DLSM = 110 cones/degree²; 95% CI: 30, 190; p = 0.008. With no outliers: Control > T1D; DLSM = 108 cones/degree²; 95% CI: 32, 183; p = 0.007).

The main effect of quadrant on cone density remained not significant.

We returned to the pairwise comparisons employing the model with the interaction term. As before, an alpha level of α = 0.00625 was set for pairwise comparisons. All patterns of intra-retinal distribution remained not significant, both within controls and within patients.

However, there was some flux in the between-groups comparisons of identical retinal locations.

The inferior nasal quadrant, which had a borderline significant result in the original model (Control > Patient; DLSM = 178 cones/degree²; 95% CI: 33, 323; p = 0.02), became less significant (Control > Patient; DLSM = 122 cones/degree²; 95% CI: -21, 266; p = 0.09). The magnitude of the between-groups difference in the inferior nasal quadrant was reduced, and the lower limit of the confidence interval now crossed zero.

The superior temporal quadrant, which had a not significant result in the original model (Control > T1D; DLSM = 99 cones/degree²; 95% CI: -23, 221; p = 0.1) became borderline significant.
(Control > T1D; DLSM = 125 cones/degree²; 95% CI: 7, 244; p = 0.03). The between-groups difference increased in magnitude, and the lower limit of the confidence interval no longer crossed zero.

The superior nasal quadrant had been borderline significant (Control > T1D; DLSM = 170 cones/degree²; 95% CI: 29, 310; p = 0.02) and remained borderline significant (Control > T1D; DLSM = 171 cones/degree²; 95% CI: 38, 305; p = 0.01). The inferior temporal quadrant remained not significant.

In sum, the two superior quadrants (superior nasal and superior temporal) exhibited more robust between-groups differences when the outliers were removed, as compared to the original model where it was the two nasal quadrants (superior nasal and inferior nasal) that exhibited larger differences.

4.11.2 Identifying Influential Observations in the Quadrants Mixed Model

In the mixed model where flash intensity was modeled as categorical and where the covariance matrix was unstructured, two observations were identified as being substantially more influential than the other observations as per restricted likelihood distances. These two most influential observations are described in Table 4.8.

The observation from Participant 385 was also an outlier, and has been described in Section 4.9.1. The observation from Participant 461 (which had not been identified as an outlier) was a relatively high superior temporal cone density, as compared to superior temporal cone densities of other patients. Upon further study, it was found that the portion of the photograph from where cones were counted had a relatively small surface area. There was a degree of inter-observer disagreement about the number of cones present, but all observers counted the area reasonably. Data entry was accurate.

These two observations were included in a second sensitivity analysis.
Table 4.8: Most influential observations in the Quadrants dataset (bolded). The value predicted by the model is the least squares mean estimate for the Diabetes Status x Quadrant subdivision to which the observation belongs. ID, Participant ID. RLD, restricted likelihood distance. Int, model with interaction term. Main, model without interaction term (main effects only). Highest RLD accepted was the largest RLD that was not substantially larger than the rest of the RLDs.

<table>
<thead>
<tr>
<th>ID</th>
<th>T1D Status</th>
<th>Quad</th>
<th>Value Predicted by Model (cones/deg²)</th>
<th>RLD</th>
<th>Highest RLD Accepted</th>
<th>Clinical Assessment</th>
<th>Data Entry</th>
<th>Decision</th>
</tr>
</thead>
<tbody>
<tr>
<td>385</td>
<td>Patient</td>
<td>IN</td>
<td>331</td>
<td>Int: 770</td>
<td>Int: 1.5</td>
<td>Large SA counted, 0</td>
<td>Accurate</td>
<td>Sensitivity Analysis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Main: 813</td>
<td>Main: 0.97</td>
<td>rater disagreement,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>but counted</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>reasonably</td>
<td></td>
<td></td>
</tr>
<tr>
<td>461</td>
<td>Patient</td>
<td>IT</td>
<td>1368</td>
<td>Int: 863</td>
<td>Int: 2.1</td>
<td>Small SA counted,</td>
<td>Accurate</td>
<td>Sensitivity Analysis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Main: 806</td>
<td>Main: 2.0</td>
<td>some inter-rater</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>disagreement, but</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>counted reasonably</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.11.3 Sensitivity Analysis, Testing the Impact of Influential Observations on Mixed Model Results

A second sensitivity analysis involved removing the two most influential observations from the dataset (see Table 4.8) and re-running the model to ensure that the original outcome of the model was not reliant on those observations.

When the two most influential observations were removed, the effect of the interaction term (diabetes status x retinal quadrant) on cone density remained not significant.

When the interaction term was removed from the model, the main effect of diabetes status on cone density remained significant. The results were essentially equivalent (Originally: Control > T1D; DLSM = 110 cones/degree²; 95% CI: 30, 190; p = 0.008. Without the most influential observations: Control > T1D; DLSM = 113 cones/degree²; 95% CI: 45, 183; p = 0.002).

The main effect of quadrant on cone density remained not significant.

We returned to the pairwise comparisons employing the model with the interaction term. As before, an alpha level of $\alpha = 0.00625$ was set for pairwise comparisons. All patterns of intra-retinal distribution remained not significant, both within controls and within patients.
However, as with the model where outliers were removed, there was some flux in the between-groups comparisons of identical retinal locations when the two most influential observations were removed. The direction of the flux was the same, and was likely driven by the data point shared by the two sensitivity analyses (Participant 385, IN).

The inferior nasal quadrant, which had a borderline significant result in the original model (Control > Patient; DLSM = 178 cones/degree\(^2\); 95% CI: 33, 323; p = 0.02), became less significant (Control > T1D; DLSM = 124 cones/degree\(^2\); 95% CI = -12, 260; p = 0.07). The magnitude of the between-groups difference in the inferior nasal quadrant was reduced, and the lower limit of the confidence interval now crossed zero.

The superior temporal quadrant, which had a not significant result in the original model (Control > T1D; DLSM = 99 cones/degree\(^2\); 95% CI: -23, 221; p = 0.1) became borderline significant (Control > T1D; DLSM = 107 cones/degree\(^2\); 95% CI = -1, 216; p = 0.05). The lower limit of the confidence interval approached zero.

The superior nasal quadrant had been borderline significant (Control > T1D; DLSM = 170 cones/degree\(^2\); 95% CI: 29, 310; p = 0.02) and remained borderline significant (Control > T1D; DLSM = 166 cones/degree\(^2\); 95% CI: 40, 292; p = 0.01). The inferior temporal quadrant remained not significant.

In sum, the two superior quadrants (superior nasal and superior temporal) exhibited more robust between-groups differences when the outliers were removed, as compared to the original model where it was the two nasal quadrants (superior nasal and inferior nasal) that exhibited larger differences.

As with the sensitivity analysis omitting outliers, the sensitivity analysis omitting the two most influential observations created a set of results in which the two superior quadrants (superior nasal and superior temporal) exhibited more robust between-groups differences. This does not totally agree with the original model. Where the two nasal quadrants (superior nasal and inferior nasal) exhibited the most robust differences.
4.11.4 Summary of Sensitivity Analyses

In sum, the majority of the results did not change when the two outliers were removed, or when the two most influential observations were removed. The only noteworthy change, in both cases, was that the between-groups differences became more robust in the superior temporal quadrant and less robust in the inferior nasal quadrant. Given the conservative Bonferroni correction, however, ($\alpha = 0.00625$), no result that was not significant became significant, or vice versa.

4.12 Association of Cone Density with Duration of T1D

Within the patient group, there was no association of cone density with duration of T1D ($p = 0.3$). There was also no association of cone density with the interaction of quadrant x duration of T1D ($p = 0.6$); this result indicates that the intra-retinal distribution of cones does not vary with increased duration of T1D.

4.13 Summary of Quadrants Mixed Model Results

Retinas of adolescents and young adults with T1D were, overall, significantly less cone-dense than retinas of age-similar controls.

There were no distinct quadrant-based patterns of intra-retinal distribution of cones in either patients or controls.

When identical retinal locations were compared between groups using a conservative alpha level ($\alpha = 0.00625$), no individual quadrant was significantly less cone-dense in T1D. However, the between-groups difference in the superior nasal quadrant approached significance. In addition, the between-groups difference approached significance in the inferior nasal quadrant, but this result was not resistant to sensitivity analyses that excluded outliers and influential observations from the dataset.
4.14 Cone Density Raw Data, by Hemiretina

The same 91 observations that were used in the quadrants dataset were used in the hemiretinae dataset. The one exception was that superior nasal and inferior nasal observations were pooled into a nasal group, and superior temporal and inferior temporal observations were pooled into a temporal group. To facilitate the Hemiretina x Diabetes Status mixed model analysis, each data point is illustrated individually in scatterplot form in Figure 4.2.

Figure 4.2: Scatterplot of all 91 cone densities, in cones/degree$^2$, grouped primarily by hemiretina (nasal or temporal), and secondarily by diabetes status (control values: blue diamonds; patient values: red squares).
4.14.1 Outliers in Cone Density Data, when Grouped by Hemiretina and Diabetes Status

Participant 385 had a relatively low nasal cone density as compared to other nasal cone densities for patients. The portion of the photograph from where cones were counted had a relatively large surface area. There was a degree of inter-observer disagreement about the number of cones present, but all observers counted the area reasonably. Data entry was accurate.

Conversely, Participant 461 had a relatively high temporal cone density as compared to other temporal cone densities for patients. The portion of the photograph where cones were counted had a relatively small surface area. Again, there was a degree of inter-observer disagreement about the number of cones present, but all observers counted the area reasonably. Data entry was accurate.

Both observations were included in the hemiretinae dataset, but the strength of their contributions to the results of the mixed model was later tested with a sensitivity analysis.

Descriptions of the two outliers are summarized in Table 4.9.

<table>
<thead>
<tr>
<th>Participant ID</th>
<th>Diabetes Status</th>
<th>Retinal Quadrant</th>
<th>Cone Density (cones/deg²)</th>
<th>Lower Limit: Q1 - 1.5(IQR)</th>
<th>Upper Limit: Q3 + 1.5(IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>385</td>
<td>Patient</td>
<td>Nasal</td>
<td>331</td>
<td>495</td>
<td></td>
</tr>
<tr>
<td>461</td>
<td>Patient</td>
<td>Temporal</td>
<td>1368</td>
<td>1107</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.9: Outliers in the hemiretinae dataset (bolded). Lower limits and upper limits were calculated for each Hemiretina x Diabetes Status subdivision. The lower limit is the first quartile (Q1), less 1.5 times the interquartile range (IQR = Q3 – Q1). The upper limit is the third quartile, plus 1.5 times the interquartile range (IQR = Q3 – Q1). Outliers in the hemiretinae dataset were defined as densities falling below the lower limit, or above the upper limit, of the Hemiretina x Diabetes Status subdivision to which they belonged.
4.15 Mixed Model Analysis of AO Data, by Hemiretina

The mixed model was then applied to the dataset where cone densities were grouped by hemiretina. A covariance matrix with compound symmetry structure was favoured as per Akaike’s Information Criterion (AIC).

4.15.1 F-Test: Effect of Diabetes Status x Hemiretina on Cone Density

The results of the model revealed a borderline significance of the interaction between diabetes status and hemiretina on cone density (p = 0.09). This result indicates that the intra-retinal distribution of cones according to hemiretina may differ between controls and patients. This result will be discussed in further detail in Section 4.15.5.

Because the interaction term was not clearly significant, it was still permissible to examine the main effects of diabetes status and hemiretina on cone density.

4.15.2 F-Test: Main Effect of Diabetes Status on Cone Density

When the interaction term was removed from the mixed model, there was a significant main effect of diabetes status on cone density (p = 0.01). The least squares mean estimate of cone density in controls was 924 cones/degree² (95% CI: 870, 978). The least squares mean estimate of cone density in patients was 816 cones/degree² (95% CI: 758, 875). The confidence intervals of these estimates overlapped only very slightly (upper limit of 875 cones/degree² in patients, versus lower limit of 870 cones/degree² in controls). The difference in cone density between the two groups estimated by the model, regardless of hemiretina, was 107 cones/degree² (controls > patients; 95% CI: 29, 187). This result demonstrates that overall, cones were less densely packed in adolescents with T1D as compared to controls. The finding mirrors the main effect of diabetes status found in the quadrant model.

4.15.3 F-Test: Main Effect of Hemiretina on Cone Density

There was no main effect of hemiretina on cone density when the control and patient groups were pooled (p = 0.3). Therefore, there was no intra-retinal nasal-temporal pattern of cone density that was consistent across controls and patients.
4.15.4 Additional Pairwise Comparisons Between Hemiretinae

A total of four a priori hypotheses relating to the hemiretinae mixed model were tested by returning to the model with the interaction term. First, cone densities in identical hemiretinae were compared between controls and patients (nasal versus nasal, and temporal versus temporal). Second, each group was examined in isolation, and the magnitude of nasal-temporal asymmetry within each group was assessed. A Bonferroni correction of 0.05/4 was used to protect against chance findings from multiple comparisons. Therefore, the threshold for significance became $\alpha = 0.0125$. This approach was somewhat conservative, because the pairwise comparisons were not completely independent.

Table 4.10 lists nasal and temporal cone densities in each group, and summarizes the results of the four a priori tests.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>T1D</th>
<th>DIFFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NASAL</td>
<td>n = 23</td>
<td>n = 19</td>
<td>Between Groups Comparison, Nasal: Control &gt; T1D DLSM = 168 (62, 273)\n</td>
</tr>
<tr>
<td>TEMPORAL</td>
<td>n = 26</td>
<td>n = 23</td>
<td>Between Groups Comparison, Temporal: Control &gt; T1D DLSM = 58 (-38, 155)\n</td>
</tr>
<tr>
<td>DIFFERENCE</td>
<td>Retinal Asymmetry, Control: Nasal &gt; Temporal DLSM = 81 (-4, 166) $p = 0.06$</td>
<td>Retinal Asymmetry, T1D: Temporal &gt; Nasal DLSM = 29 (-66, 124) $p = 0.5$</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.10: Least Mean Squares (LSM) estimates (bolded) of nasal and temporal cone densities in the control group and in the patient group, generated by the mixed model with compound symmetry covariance structure. Differences of Least Mean Squares (DLSM; underlined) along the right-hand column express the between-groups differences in LSMs at identical retinal locations. DLSMs along the bottom row express the difference between nasal and temporal LSMs within each group. All LSM and DLSM values are expressed in cones per square degree, with 95% CI (lower, upper).
4.15.5 Comparisons of Identical Hemiretinae Between Groups

The most striking result in the hemiretinae mixed model was seen in the comparison of nasal hemiretinae between controls and patients. The magnitude of the relative deficit in nasal cone density in the T1D group was estimated to be 168 cones/degree² (95% CI: 62, 273). The certainty of the difference was quite marked (p = 0.002), and reflected that the lower limit of the confidence interval fell far above zero.

In the temporal retina, the relative deficit in cone density in the T1D group as compared to the control group was lesser in magnitude, with a lower confidence limit below zero (DLSM = 58 cones/degree²; 95% CI: -38,155). Moreover, the between-groups difference in temporal cone densities was not significant (p = 0.2).

Therefore, in the nasal hemiretina of adolescents and young adults with T1D, cones were less densely packed than in controls. This was not true of the temporal hemiretina in adolescents and young adults with T1D.

4.15.6 Intra-Retinal Nasal-Temporal Cone Distribution in Controls

A pattern of nasal-temporal asymmetry in cone density was detected in the control group, with a nasal advantage of 81 cones/degree² (-4,166). However, the lower confidence limit falls just below zero, and the certainty of p = 0.06 does not meet the threshold of the conservative Bonferroni correction (α = 0.0125). Therefore, the nasal-temporal symmetry in cone density seen in controls can only be considered borderline significant.

4.15.7 Intra-Retinal Nasal-Temporal Cone Distribution in Patients

Nasal-temporal asymmetry in cone density (where nasal > temporal) was entirely absent in the T1D group (temporal > nasal, and DLSM = 29 cones/degree², 95% CI: -66, 124; p = 0.5).

4.15.8 Interaction of Diabetes Status x Hemiretina: Differential Nasal-Temporal Symmetry Between Groups

Though the nasal-temporal asymmetry in cone density was only borderline significant in controls, the vastly different result seen in the T1D group suggests a difference between groups in the degree of nasal-temporal asymmetry. This difference is reflected in the borderline significance of the interaction term (diabetes status x hemiretina; p = 0.09) when it is included in
the mixed model. The model estimates the degree of difference in nasal-temporal asymmetry between controls and patients to be 110 cones/degree² (95% CI: -18, 237). While the magnitude of the difference is sizeable, the inter-individual variation within each group has created a large confidence interval, of which the lower limit crosses zero. In sum, there is a borderline significant difference between groups in the degree of nasal-temporal asymmetry (nasal > temporal); this asymmetry is present in controls and is absent in patients.

4.16 Sensitivity Analyses for Hemiretinae Model

The impact of outliers and of influential observations on the outcome of the mixed model were tested with sensitivity analyses. In these analyses, the data were removed from the dataset and the model was re-run.

4.16.1 Sensitivity Analysis, Testing the Impact of Outliers on Mixed Model Results

A sensitivity analysis was performed by excluding two values identified as outliers (see Table 4.9), to ensure that the original results of the mixed model were not skewed by these data.

When the outliers were removed, the interaction term (diabetes status x hemiretina) was no longer borderline significant (Originally: p = 0.09. With no outliers: p = 0.3). This result implies that the degree nasal-temporal asymmetry no longer differed between groups.

When the interaction term was removed from the model, the main effect of diabetes status on cone density remained significant (Originally: Control > T1D; DLSM = 107 cones/degree²; 95% CI: 29, 187; p = 0.01. With no outliers: Control > T1D; DLSM = 113 cones/degree²; 95% CI: 44, 181; p = 0.002).

Unlike the original model where the main effect of hemiretina on cone density was not significant (Nasal > temporal; DLSM = 32 cones/degree²; 95% CI: -32, 96), the main effect of hemiretina now approached significance (Nasal > temporal; DLSM = 55 cones/degree²; 95% CI: -3, 114; p = 0.06). Nasal-temporal asymmetry was larger in magnitude, and the lower confidence limit approached zero. This result suggested that nasal-temporal asymmetry was present in both controls and in patients. This result was in keeping with the loss of significance of the interaction
term: if nasal-temporal asymmetry is present in both controls and patients, then the pattern of asymmetry should not differ between groups.

Removing the outliers created some flux in the results of the pairwise comparisons, which employed the model containing the interaction term, and for which the alpha level was $\alpha = 0.0125$.

The between-groups difference in the nasal hemiretina remained significant (Originally: Control > T1D; DLSM = 168 cones/deg$^2$; 95% CI: 62, 273; $p = 0.002$. With no outliers: Control > T1D; DLSM = 143 cones/deg; 95% CI: 50, 236; $p = 0.003$).

The between-groups difference in the temporal hemiretina went from not significant to borderline significant (Originally: Control > T1D; DLSM = 58 cones/deg$^2$; 95% CI: -38, 155; $p = 0.2$. With no outliers: Control > T1D; DLSM = 87 cones/deg$^2$; 95% CI: 2, 173; $p = 0.05$).

This result reflects a global deficit in cone density in T1D, which includes the temporal retina. However, the nasal cone deficit in T1D is still much larger in magnitude and is associated with greater certainty.

The nasal-temporal asymmetry seen in controls remained borderline significant (Originally: Nasal > Temporal; DLSM = 81 cones/deg$^2$; 95% CI: -4, 166; $p = 0.06$. With no outliers: Nasal > Temporal; DLSM = 80 cones/deg$^2$; 95% CI: 2, 158; $p = 0.04$).

The hemiretina that was more cone-dense in T1D changed from temporal to nasal once the outliers were removed (Originally: Temporal > Nasal; DLSM = 29 cones/deg$^2$; 95% CI: -66, 124; $p = 0.5$. With no outliers: Nasal > Temporal; DLSM = 24 cones/deg$^2$; 95% CI: -65, 112; $p = 0.6$). This result, by reducing the between-groups difference in nasal-temporal asymmetry, was likely to have been the cause of the loss of borderline significance of the interaction term. It was also likely to have increased the main effect of hemiretina on cone density. However, with or without the outliers, nasal and temporal cone densities in T1D should be treated as equal: in both cases, the magnitude of the asymmetry was quite small, the lower ends of the confidence limits fell far below zero, and the $p$ values were not nearly borderline significant.

In sum, regardless of the presence or absence of outliers, there was a borderline significant nasal-temporal asymmetry in controls (Nasal > Temporal) that was not seen in patients. However,
since the interaction term varied between borderline significance and non-significance, it is not possible to state that there was an unequivocal difference in nasal-temporal asymmetry between the two groups.

### 4.16.2 Identifying Influential Observations in the Hemiretinae Mixed Model

Two data points (Table 4.11) were identified as being more influential than the rest, as per their restricted likelihood distances. These two observations were identical to the data that were identified as outliers. Therefore, an additional sensitivity analysis was not required.

<table>
<thead>
<tr>
<th>ID</th>
<th>T1D Status</th>
<th>Hemi</th>
<th>Cone Density (cones/deg²)</th>
<th>Value Predicted by Model (cones/deg²)</th>
<th>RLD</th>
<th>Highest RLD Accepted</th>
<th>Clinical Assessment</th>
<th>Data Entry</th>
<th>Decision</th>
</tr>
</thead>
<tbody>
<tr>
<td>385</td>
<td>Patient</td>
<td>N</td>
<td>331</td>
<td>Int: 799</td>
<td>Int: 0.77 Main: 0.73</td>
<td></td>
<td></td>
<td>Large SA counted, some inter-rater disagreement, but counted reasonably</td>
<td>Accurate</td>
</tr>
<tr>
<td>461</td>
<td>Patient</td>
<td>T</td>
<td>1368</td>
<td>Int: 829</td>
<td>Int: 1.6 Main: 1.5</td>
<td></td>
<td></td>
<td>Small SA counted, some inter-rater disagreement, but counted reasonably</td>
<td>Accurate</td>
</tr>
</tbody>
</table>

**Table 4.11**: Most influential observations in the Hemiretinae dataset (bolded). The value predicted by the model is the least squares mean estimate for the Diabetes Status x Hemiretina subdivision to which the observation belongs. ID, Participant ID. RLD, restricted likelihood distance. Int, model with interaction term. Main, model without interaction term (main effects only). Highest RLD accepted was the largest RLD that was not substantially larger than the rest of the RLDs.

### 4.17 Association of Cone Density with Duration of T1D

A final mixed model examined only patients for which duration of T1D was known, to identify a potential association of cone density with duration of T1D. The interaction of duration of T1D x hemiretina had no impact on cone density (p = 0.3). When the interaction term was removed from the model, there was no main effect of duration on cone density (p = 0.1).
4.18 Summary of Hemiretinae Mixed Model Results

As was seen in the quadrants model, the hemiretinae model revealed that overall, the retinae of adolescents and young adults with T1D were significantly less cone-dense than retinae of age-similar controls.

The nasal hemiretinae of patients were less densely packed than the nasal hemiretinae of controls. This difference between groups was not demonstrated in the temporal hemiretina.

Controls demonstrated a borderline significant nasal-temporal asymmetry in cone density (Nasal > Temporal). Such nasal-temporal asymmetry was not present in patients.

When the degree of nasal-temporal asymmetry was compared between groups, the difference between patients and controls was borderline significant. However, this result did not hold when outliers/influential observations are removed from the dataset.
4.19 Characteristics of Participants in Red Flash ERG Study

Twenty-eight controls and twelve patients, who attended testing sessions between January 2012 and May 2013, participated in the red flash ERG study.

4.19.1 Age

The mean age of controls was 17.8 years (SD = 3.2, range = 12.4 to 24.3). The mean age of patients was 19.2 years (SD = 2.4, range = 15.5 to 23.6).

4.19.2 Gender

The control group was comprised of 18 females and 10 males. The patient group had 9 females and 3 males.

4.19.3 Eye Tested

In the control group, 14 left eyes and 14 right eyes were tested. In the TID group, 4 left eyes and 8 right eyes were tested. No left-to-right translation was required for ERG recordings.

4.20 Ophthalmic Exam Results for Participants in Red Flash ERG Study

4.20.1 Visual Acuity

Visual acuity was measured for all controls and for 11 of 12 patients. The best demonstration of visual resolution was taken as the visual acuity score for each participant, whether vision was corrected or uncorrected. In the control group, best visual acuity ranged from -0.2 LogMAR (finest resolution) to 0.3 LogMAR (coarsest resolution). The median best visual acuity among controls was -0.1 LogMAR. In the patient group, best visual acuity ranged from -0.2 LogMAR (finest resolution) to 0.1 LogMAR (coarsest resolution). The median best visual acuity among patients was 0.0 LogMAR.

4.20.2 Contrast Sensitivity

Contrast sensitivity was obtained for all controls and for 11 of 12 patients. The modal Pelli-Robson score for contrast sensitivity was 1.65 log units in both the control group and the patient group. One control and two patients exhibited contrast sensitivity below this level (control score:
1.5; patient scores: 1.5, 1.4). Eight controls and two patients exhibited contrast sensitivity above this level (control scores: 1.75, 1.8 x 4, 1.95 x 3; patient scores: 1.8, 1.8).

4.20.3 Refractive Error

Refractive errors were measured in 26 of 28 controls, and in 8 of 12 patients. The median refractive error among controls that were measured 0.0 Diopters (range = -4.5 to +1.25). The median refractive error among patients that were measured was -0.75 Diopters (range = -6.0 to +1.0).

4.20.4 Axial Length

An axial length was obtained for 26 of 28 controls and for 9 of 12 patients. The mean axial length among controls that were measured was 23.77 mm (SD = 0.96). The mean axial length among patients that were measured was 23.39 mm (SD = 1.42).

4.21 Characteristics of Patients in Red Flash ERG Study

4.21.1 Duration of T1D

Of 12 patients, 11 had a known duration of T1D. The mean duration of T1D among patients in the red flash ERG study group was 10.6 years (SD = 5.1, range = 5.4 to 19.5).
4.22 Red Flash ERG Raw Data

Across controls and patients, a total of 240 observations of maximal rate of rise were available from all flash intensities combined. To facilitate the Red Flash ERG mixed model analysis, each data point is illustrated individually in scatterplot form in Figure 4.3.

**Figure 4.3:** Scatterplot of all 240 maximal rates of rise, in mV/ms, grouped primarily by flash intensity (from dimmest to brightest, in log10 phot Trolands), and secondarily by diabetes status (control values: blue diamonds; patient values: red squares).
4.22.1 Outliers in Red Flash Maximal Rate of Rise Data, when Grouped by Flash Intensity and Diabetes Status

Outliers in the Red Flash dataset are detailed in Table 4.12. Six maximal rates of rise from controls and two maximal rates of rise from patients qualified as outliers, each falling above the upper limit (Q3 + 1.5xIQR) of the Flash Intensity x Diabetes Status subdivision to which they belonged. Data entry was reviewed, and was accurate in all cases. When the tracings for each of the original waveforms were revisited, it was found that the recordings were clinically untenable. Individual recordings were either out of keeping with recordings at other intensities, or contained excessive noise, or both. Specific descriptions of waveforms can be found in the Clinical Assessment column in Table 4.12. These eight observations were deemed untenable, and were excluded from the mixed model analysis. Thus, a total of 232 observations remained.
### Table 4.12: Outliers in the Red Flash dataset (bolded). Lower limits and upper limits were calculated for each Flash Intensity x Diabetes Status subdivision. The lower limit is the first quartile (Q1), less 1.5 times the interquartile range (IQR = Q3 – Q1). The upper limit is the third quartile, plus 1.5 times the interquartile range (IQR = Q3 – Q1). Outliers in the Red Flash dataset were defined as densities falling below the lower limit, or above the upper limit, of the Flash Intensity x Diabetes Status subdivision to which they belonged.

<table>
<thead>
<tr>
<th>ID</th>
<th>T1D Status</th>
<th>Flash Intensity (log10 phot Td)</th>
<th>Max dR/dt (mV/ms)</th>
<th>Upper Limit: Q3 + 1.5(IQR)</th>
<th>Clinical Assessment</th>
<th>Data Entry</th>
<th>Decision</th>
</tr>
</thead>
<tbody>
<tr>
<td>489</td>
<td>Control</td>
<td>0.9</td>
<td>-8.6</td>
<td>-7.4</td>
<td>Extremely noisy/too steep</td>
<td>Accurate</td>
<td>Exclude</td>
</tr>
<tr>
<td>489</td>
<td>Control</td>
<td>1.3</td>
<td>-7.9</td>
<td>-6.9</td>
<td>Extremely noisy/too steep</td>
<td>Accurate</td>
<td>Exclude</td>
</tr>
<tr>
<td>489</td>
<td>Control</td>
<td>1.7</td>
<td>-13.5</td>
<td>-7.5</td>
<td>Extremely noisy/too steep</td>
<td>Accurate</td>
<td>Exclude</td>
</tr>
<tr>
<td>493</td>
<td>Control</td>
<td>0.9</td>
<td>-8.2</td>
<td>-7.4</td>
<td>Extremely noisy/too steep</td>
<td>Accurate</td>
<td>Exclude</td>
</tr>
<tr>
<td>504</td>
<td>Control</td>
<td>0.9</td>
<td>-7.8</td>
<td>-7.4</td>
<td>Noisy/too steep</td>
<td>Accurate</td>
<td>Exclude</td>
</tr>
<tr>
<td>506</td>
<td>Control</td>
<td>1.3</td>
<td>-7.8</td>
<td>-6.9</td>
<td>Could be clinically appropriate if not nearly overlapping with R3 (R3 in keeping with R1)</td>
<td>Accurate</td>
<td>Exclude</td>
</tr>
<tr>
<td>8</td>
<td>Patient</td>
<td>2.9</td>
<td>-28.3</td>
<td>-22.7</td>
<td>Too steep/out of keeping with own R1-R5</td>
<td>Accurate</td>
<td>Exclude</td>
</tr>
<tr>
<td>423</td>
<td>Patient</td>
<td>1.3</td>
<td>-7.1</td>
<td>-5.7</td>
<td>Noisy/too steep</td>
<td>Accurate</td>
<td>Exclude</td>
</tr>
</tbody>
</table>

4.23 Mixed Model Analysis of Red Flash Data, where Flash Intensity is Treated as a Categorical Variable

For the Red Flash maximal rate of rise dataset, an unstructured covariance matrix was favoured as per Akaike’s Information Criterion (AIC). In this first mixed model analysis, flash intensity was first treated as a categorical variable.
4.23.1 F-Test: Effect of Diabetes Status x Flash Intensity on Maximal Rate of Rise

The interaction between diabetes status and flash intensity did not have a significant effect on the maximal rate of rise in response to red flashes (p = 0.5). This result indicated that the variation of maximal rate of rise with flash intensity did not differ between groups.

Moreover, no a priori hypotheses suggested that such between-group differences in maximal rate of rise patterns would occur. Therefore, further pairwise comparisons employing the model containing the interaction term were not performed. (This would have included, for example, comparisons of maximal rate of rise between groups at individual flash intensities, but was not warranted in this case). **Table 4.13** enumerates the least square means of the maximal rates of rise, for each group, at each flash intensity.

<table>
<thead>
<tr>
<th>RED Flash Intensity (log10 Phot Td)</th>
<th>CONTROL</th>
<th>T1D</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9</td>
<td>n = 25</td>
<td>n = 12</td>
</tr>
<tr>
<td></td>
<td>LSM = -3.3 (-2.9, -3.7)</td>
<td>LSM = -3.6 (-3.1, -4.2)</td>
</tr>
<tr>
<td>1.3</td>
<td>n = 26</td>
<td>n = 11</td>
</tr>
<tr>
<td></td>
<td>LSM = -4.0 (-3.6, -4.3)</td>
<td>LSM = -4.0 (-3.5, -4.5)</td>
</tr>
<tr>
<td>1.7</td>
<td>n = 27</td>
<td>n = 12</td>
</tr>
<tr>
<td></td>
<td>LSM = -5.1 (-4.7, -5.5)</td>
<td>LSM = -4.5 (-3.9, -5.2)</td>
</tr>
<tr>
<td>2.1</td>
<td>n = 28</td>
<td>n = 12</td>
</tr>
<tr>
<td></td>
<td>LSM = -7.4 (-6.8, -8.1)</td>
<td>LSM = -7.0 (-6.0, -7.9)</td>
</tr>
<tr>
<td>2.5</td>
<td>n = 28</td>
<td>n = 12</td>
</tr>
<tr>
<td></td>
<td>LSM = -9.9 (-9.1, -10.6)</td>
<td>LSM = -9.0 (-7.9, -10.3)</td>
</tr>
<tr>
<td>2.9</td>
<td>n = 28</td>
<td>n = 11</td>
</tr>
<tr>
<td></td>
<td>LSM = -12.1 (-11.0, -12.1)</td>
<td>LSM = -11.8 (-10.2, -13.4)</td>
</tr>
</tbody>
</table>

**Table 4.13**: Mixed model estimates of Least Square Means (LSM) of the maximal rates of rise of ERG a-waves in response to red flashes (in mV/ms). 95% CI are in brackets (lower, upper). LSMs at each flash intensity (where flash intensity is expressed in log10 Phot Trolands) are listed separately for controls and patients. The mixed model has an unstructured covariance matrix, and flash intensity has been treated as a categorical variable.
4.23.2 F-Test: Main Effect of Diabetes Status on Maximal Rate of Rise

When the interaction term was removed from the model, and flash intensity remained a categorical variable, there was no main effect of diabetes status on maximal rate of rise (p = 0.9). The least squares mean of the maximal rate of rise in controls, regardless of flash intensity, was -6.9 mV/ms (95% CI: -6.5, -7.3). The least squares mean of the maximal rate of rise in patients, regardless of flash intensity, was very similar: -6.8 mV/ms (95% CI: -6.4, -7.3). There was no detectable difference between groups: the difference in least squares means comparing patients and controls was 0.03 mV/ms (Control steeper than T1D; 95% CI: -0.38, 0.45). This result demonstrates that there was no overall difference between controls and patients in the maximal rate of rise of the ERG a-wave in response to red flashes.

4.24 Mixed Model Analysis of Red Flash Data, where Flash Intensity is Treated as a Continuous Variable

Treating flash intensity as a continuous variable, did not alter the findings. When the interaction term (diabetes status x flash intensity) was included in the model, the interaction still had no effect on maximal rate of rise (p = 0.3).

When the interaction term was removed from the model, and flash intensity remained a continuous variable, diabetes status did not have a main effect on maximal rate of rise (p = 0.7). The least square means of maximal rates of rise for each group, regardless of flash intensity, were essentially the same as in the categorical case: in controls, -6.9 mV/ms (95% CI: -6.5, -7.3), and in patients, -7.0 mV/ms (95% CI: -6.5, -7.5). Again, there was no detectable difference between groups: the difference in least square means was 0.07 (T1D steeper than Control; 95% CI: -0.5, 0.3; p = 0.7).

4.24.1 Applying a Linear Transformation to Maximal Rate of Rise

Finally, the maximal rate of rise data in response to red flashes were transformed by taking the base-10 log of the slopes, in an attempt to make the data more linear. Flash intensity was kept as a continuous variable. Still, neither the interaction term nor the main effect of diabetes status achieved significance (p = 0.1 and p = 1, respectively).
In sum, the models where flash intensity was treated as a continuous variable were rejected in favour of the original model, where flash intensity was treated as a categorical variable.

4.25 Sensitivity Analysis for Red Flash Mixed Model, where Flash Intensity Treated as a Categorical Variable

Since modelling flash intensity as a continuous variable and applying linear transformations conferred no advantage, we returned to the mixed model where flash intensity was treated as a categorical variable. After the eight outliers were removed, no observations stood out as being particularly influential relative to other observations, as per restricted likelihood distance values. Therefore, a sensitivity analysis was not performed on the Red Flash dataset.

4.26 Correlation of Red Flash Maximal Rate of Rise with Duration of T1D

A correlation between duration of T1D and red flash maximal rate of rise in the patient group was not performed, since patients’ electrophysiological responses did not differ from those of controls.

4.27 Summary of Red Flash Mixed Model Results

There was no main effect of diabetes status on maximal rate of rise of the ERG a-wave, in response to red flashes. Thus, adolescents and young adults with T1D do not exhibit decreased maximal rates of rise in response to red flashes, when compared with controls. There was also no effect of the interaction between diabetes status and flash intensity on maximal rate of rise. Thus, the pattern of variation of maximal rate of rise with flash intensity does not differ between groups.
4.28 Characteristics of Participants in White Flash ERG Study

Twenty-six controls and eleven patients, who attended testing sessions between January 2012 and May 2013, participated in the white flash ERG study. The white flash ERGs were performed on the same participants as those in the red flash ERG study, less two controls (IDs: 490, 509) and less one patient (ID: 331).

4.28.1 Age

The mean age of controls was 18.1 years (SD = 3.2, range = 12.4 to 24.3). The mean age of patients was 18.8 years (SD = 2.1, range = 15.5 to 22.7).

4.28.2 Gender

The control group was comprised of 17 females and 9 males. The patient group had 8 females and 3 males.

4.28.3 Eye Tested

In the control group, 13 left eyes and 13 right eyes were tested. In the T1D group, 3 left eyes and 8 right eyes were tested. No left-to-right translation was required for ERG recordings.

4.29 Ophthalmic Exam Results for Participants in White Flash ERG Study

4.29.1 Visual Acuity

Visual acuity was measured for all controls and for 10 of 11 patients. The best demonstration of visual resolution was taken as the visual acuity score for each participant, whether vision was corrected or uncorrected. In the control group, best visual acuity ranged from -0.2 LogMAR (finest resolution) to 0.3 LogMAR (coarsest resolution). The median best visual acuity among controls was -0.1 LogMAR. In the patient group, best visual acuity ranged from -0.2 LogMAR (finest resolution) to 0.1 LogMAR (coarsest resolution). The median best visual acuity among patients was 0.0 LogMAR.
4.29.2 Contrast Sensitivity

Contrast sensitivity was obtained for all controls and for 10 of 11 patients. The modal Pelli-Robson score for contrast sensitivity was 1.65 log units in both the control group and the patient group. One control and two patients exhibited contrast sensitivity below this level (control score: 1.5; patient scores: 1.5, 1.4). Seven controls and one patient exhibited contrast sensitivity above this level (control scores: 1.75, 1.8 x 4, 1.95 x 2; patient score: 1.8).

4.29.3 Refractive Error

Refractive errors were measured in 24 of 26 controls, and in 7 of 11 patients. The median refractive error among controls that were measured -0.13 Diopters (range = -4.5 to +1.25). The median refractive error among patients that were measured was -1.13 Diopters (range = -6.0 to +1.0).

4.29.4 Axial Length

An axial length was obtained for 24 of 26 controls and for 8 of 11 patients. The mean axial length among controls that were measured was 23.79 mm (SD = 0.97). The mean axial length among patients that were measured was 23.31 mm (SD = 1.49).

4.30 Characteristics of Patients in White Flash ERG Study

4.30.1 Duration of T1D

Of 11 patients, 10 had a known duration of T1D. The mean duration of T1D among patients in the red flash ERG study group was 9.7 years (SD = 4.4, range = 5.4 to 18.0).
4.31 White Flash ERG Raw Data

Across controls and patients, a total of 222 observations of maximal rate of rise were available from all flash intensities combined. To facilitate the White Flash ERG mixed model analysis, each data point is illustrated individually in scatterplot form in Figure 4.4.

**Figure 4.4**: Scatterplot of all 222 maximal rates of rise, in mV/ms, grouped primarily by flash intensity (from dimmest to brightest, in log10 phot Trolands), and secondarily by diabetes status (control values: blue diamonds; patient values: red squares).
4.31.1 Outliers in White Flash Maximal Rate of Rise Data, when Grouped by Flash Intensity and Diabetes Status

Outliers in the White Flash dataset are detailed in Table 4.14. Two maximal rates of rise from one control participant qualified as outliers, each falling below the lower limit (Q1 - 1.5xIQR) of the Flash Intensity x Diabetes Status subdivision to which they belonged. Data entry was reviewed, and was accurate in both cases. When the tracings for each of the original waveforms were revisited, it was found that the recordings were clinically plausible, and were in keeping with waveforms from the same participant at other intensities. Both observations were included in the White Flash dataset, but the strength of their contributions to the results of the mixed model was later tested with a sensitivity analysis.

<table>
<thead>
<tr>
<th>ID</th>
<th>T1D Status</th>
<th>Flash Intensity (log10 phot Td)</th>
<th>Max dR/dt (mV/ms)</th>
<th>Lower Limit: Q1 - 1.5(IQR)</th>
<th>Clinical Assessment</th>
<th>Data Entry</th>
<th>Decision</th>
</tr>
</thead>
<tbody>
<tr>
<td>507</td>
<td>Control</td>
<td>1.8</td>
<td>-3.2</td>
<td>-3.3</td>
<td>Clinically plausible</td>
<td>Accurate</td>
<td>Sensitivity Analysis</td>
</tr>
<tr>
<td>507</td>
<td>Control</td>
<td>3.8</td>
<td>-14.1</td>
<td>-14.2</td>
<td>Clinically plausible</td>
<td>Accurate</td>
<td>Sensitivity Analysis</td>
</tr>
</tbody>
</table>

Table 4.14: Outliers in the White Flash dataset (bolded). Lower limits and upper limits were calculated for each Flash Intensity x Diabetes Status subdivision. The lower limit is the first quartile (Q1), less 1.5 times the interquartile range (IQR = Q3 – Q1). The upper limit is the third quartile, plus 1.5 times the interquartile range (IQR = Q3 – Q1). Outliers in the White Flash dataset were defined as densities falling below the lower limit, or above the upper limit, of the Flash Intensity x Diabetes Status subdivision to which they belonged.

4.32 Mixed Model Analysis of White Flash Data, where Flash Intensity is Treated as a Categorical Variable

For the White Flash maximal rate of rise dataset, an unstructured covariance matrix was favoured as per Akaike’s Information Criterion (AIC). In this first mixed model analysis, flash intensity was first treated as a categorical variable.
4.32.1 F Test: Effect of Diabetes Status x Flash Intensity on Maximal Rate of Rise

The interaction between diabetes status and flash intensity did not have a significant effect on the maximal rate of rise in response to white flashes (p = 0.9). This result indicated that the variation of maximal rate of rise with flash intensity did not differ between groups.

Moreover, no a priori hypotheses suggested that such between-group differences in maximal rate of rise patterns would occur. Therefore, further pairwise comparisons employing the model containing the interaction term were not performed. Table 4.15 enumerates the least square means of the maximal rates of rise, for each group, at each flash intensity.

<table>
<thead>
<tr>
<th>WHITE Flash Intensity (log10 Phot Td)</th>
<th>CONTROL</th>
<th>T1D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.8</td>
<td>n = 26</td>
<td>n = 11</td>
</tr>
<tr>
<td></td>
<td>LSM = -7.0 (-6.4, -7.6)</td>
<td>LSM = -5.9 (-5.0, -6.7)</td>
</tr>
<tr>
<td>2.2</td>
<td>n = 26</td>
<td>n = 11</td>
</tr>
<tr>
<td></td>
<td>LSM = -9.4 (-8.6, -10.2)</td>
<td>LSM = -8.1 (-6.8, -9.4)</td>
</tr>
<tr>
<td>2.5</td>
<td>n = 26</td>
<td>n = 11</td>
</tr>
<tr>
<td></td>
<td>LSM = -11.9 (-10.9, -12.9)</td>
<td>LSM = -11.1 (-9.6, -12.7)</td>
</tr>
<tr>
<td>3.0</td>
<td>n = 26</td>
<td>n = 11</td>
</tr>
<tr>
<td></td>
<td>LSM = -16.0 (-14.7, -17.2)</td>
<td>LSM = -14.9 (-13.0, -16.8)</td>
</tr>
<tr>
<td>3.4</td>
<td>n = 26</td>
<td>n = 11</td>
</tr>
<tr>
<td></td>
<td>LSM = -19.4 (-17.8, -21.0)</td>
<td>LSM = -18.9 (-16.4, -21.3)</td>
</tr>
<tr>
<td>3.8</td>
<td>n = 26</td>
<td>n = 11</td>
</tr>
<tr>
<td></td>
<td>LSM = -23.9 (-22.1, -25.7)</td>
<td>LSM = -22.2 (-19.4, -25.0)</td>
</tr>
</tbody>
</table>

Table 4.15: Mixed model estimates of Least Square Means (LSM) of the maximal rates of rise of ERG a-waves in response to white flashes (in mV/ms). 95% CI are in brackets (lower, upper). LSMs at each flash intensity (where flash intensity is expressed in log10 Phot Trolands) are listed separately for controls and patients. The mixed model has an unstructured covariance matrix, and flash intensity has been treated as a categorical variable.
4.32.2 F-Test: Main Effect of Diabetes Status on Maximal Rate of Rise

When the interaction term was excluded from the model, and flash intensity remained a categorical variable, there was a significant main effect of diabetes status on maximal rate of rise. The least squares mean of the maximal rate of rise in controls, regardless of flash intensity, was \(-14.7 \text{ mV/ms} \) (95% CI: \(-13.7, -15.6\)). The least squares mean of the maximal rate of rise in patients, regardless of flash intensity, was \(-13.4 \text{ mV/ms} \) (95% CI: \(-12.3, -14.5\)). There was some overlap between the confidence intervals of each group. Still, the between-groups difference in least squares means was significant \((p = 0.01)\), and was estimated by the model to be \(-1.3 \text{ mV/ms} \) (Control steeper than T1D; 95% CI: \(-0.3, -2.3\)). Though the difference was not large in magnitude, the direction of the difference was unequivocal, as the lower limit of its confidence interval did not reach zero. This result demonstrates that adolescents and young adults with T1D have shallower a-wave maximal rates of rise during white flash ERGs, as compared to controls.

4.33 Mixed Model Analysis of White Flash Data, where Flash Intensity is Treated as a Continuous Variable

Modelling flash intensity as a continuous variable did not afford any advantage to the model containing the interaction term. The interaction of diabetes status with flash intensity remained non-significant \((p = 1)\).

Modelling intensity as a continuous variable and removing the interaction term generated borderline significance for the between-group difference of least square means of maximal rates of rise \((p = 0.06)\). This difference was not as robust as when intensity was modelled as categorical (see 4.19.2; \(p = 0.01\)). Since the data appeared relatively linear, one might expect that modelling intensity as a continuous variable would yield a more significant result than in the categorical case. Normally, the additional degrees of freedom made available by modelling flash intensity as continuous would afford the model greater power. However, it is possible that there were non-linearities in the data that prevented this from occurring.

4.33.1 Applying a Linear Transformation to Maximal Rate of Rise

In an attempt to improve the model where flash intensity was modelled as continuous, the white flash maximal rate of rise data were transformed by taking the base-10 log of the slopes, with the aim of making the data more linear. After the transformation, the interaction term became
borderline significant (p = 0.055). This result suggests that the variation in maximal rate of rise with flash intensity differs between groups. However, when reviewing the raw data, there is no obvious difference in maximal rate of rise patterns between the two groups.

A significant or borderline-significant interaction obscures the interpretation of significant main effects, should they exist. Since the interaction was just borderline, however, the main effect of diabetes status on maximal rate of rise was still assessed. The main effect of diabetes status was not significant (p = 0.2).

Although there was a borderline significant interaction of diabetes status and flash intensity, this did not appear to translate into consistent differences in patterns of maximal rates of rise between the two groups. Therefore, this model was not more successful than the categorical model.

In sum, the models where flash intensity was treated as a continuous variable were rejected in favour of the original model, where flash intensity was treated as a categorical variable.

**4.34 Sensitivity Analyses for White Flash Mixed Model, where Flash Intensity is Treated as a Categorical Variable**

Since modelling flash intensity as a continuous variable and applying linear transformations conferred no advantage, we returned to the mixed model where flash intensity was treated as a categorical variable.

**4.34.1 Sensitivity Analysis, Testing Impact of Outliers on Mixed Model Results**

In the first sensitivity analysis, two outliers were removed from the dataset (see Table 4.14) and the model was run anew, to ensure that the original outcome of the model was not reliant on those observations. When these two outliers were removed, the interaction term remained not significant (p = 0.8). Moreover, the main effect of diabetes status on maximal rate of rise remained significant (p = 0.004). The between-groups difference in least square means regardless of flash intensity was similar in magnitude, at -1.4 mV/ms (95% CI: -0.5, -2.4). As in the original model, the lower confidence limit did not cross zero.
4.34.2 Identifying Influential Observations in Mixed Model

In the mixed model where flash intensity was modeled as categorical and where the covariance matrix was unstructured, two observations were identified as being substantially more influential than the other observations as per restricted likelihood distances. These two most influential observations are described in Table 4.16.

<table>
<thead>
<tr>
<th>ID</th>
<th>T1D Status</th>
<th>Flash Intensity (log10 phot Td)</th>
<th>Max dR/dt (mV/ms)</th>
<th>Value Predicted by Model (mV/ms)</th>
<th>RLD</th>
<th>Highest RLD Accepted</th>
<th>Clinical Assessment</th>
<th>Data Entry</th>
<th>Decision</th>
</tr>
</thead>
<tbody>
<tr>
<td>489</td>
<td>Control</td>
<td>3.4</td>
<td>-13.1</td>
<td>-19.4</td>
<td>Int: 13.1 Main: 13.4</td>
<td>Int: 5.6 Main: 5.6</td>
<td>Clinically plausible</td>
<td>Accurate</td>
<td>Sensitivity Analysis</td>
</tr>
<tr>
<td>489</td>
<td>Control</td>
<td>3.8</td>
<td>-29.3</td>
<td>-23.9</td>
<td>Int: 11.3 Main: 11.5</td>
<td>Int: 5.6 Main: 5.6</td>
<td>Clinically plausible</td>
<td>Accurate</td>
<td>Sensitivity Analysis</td>
</tr>
</tbody>
</table>

Table 4.16: Most influential observations in the White Flash dataset (bolded). The value predicted by the model is the least squares mean estimate for the Diabetes Status x Flash Intensity subdivision to which the observation belongs. RLD, restricted likelihood distance. Int, model with interaction term. Main, model without interaction term (main effects only). Highest RLD accepted was the largest RLD that was not substantially larger than the rest of the RLDs.

4.34.3 Sensitivity Analysis, Testing Impact of Influential Observations on Mixed Model Results

A second sensitivity analysis involved removing the two most influential observations from the dataset (see Table 4.16) and re-running the model to ensure that the original outcome of the model was not reliant on those observations. When the two most influential observations were removed, the interaction term remained not significant (p = 0.9). In addition, the main effect of diabetes status on maximal rate of rise remained significant (p = 0.01). The between-groups difference of least squares means remained the same, at -1.3 mV/ms (Control steeper than T1D; 95% CI: -0.3, -2.3) and its lower confidence limit still did not cross zero.

4.34.4 Summary of Sensitivity Analyses

In sum, the results of the model did not change when the two outliers were removed, nor did they change when the two most influential observations were removed.
4.35 Association of Maximal Rate of Rise with Duration of T1D

The patient sample with known durations of T1D (n = 10) were assessed alone. Duration of T1D, as well as the interaction term duration of T1D x flash intensity (still as a categorical variable) were included in the model. It was found that the interaction of duration of T1D x flash intensity had a significant effect on maximal rate of rise in response to white flashes (p = 0.03). This result implies that as duration of T1D increases, the relationship between maximal rate of rise and flash intensity changes. The maximal rates of rise at each flash intensity were plotted individually for each patient in Figure 4.5 so that this pattern could be examined.

Figure 4.5: Maximal rates of rise in response to white flashes, versus flash intensity, for patients only (n = 10). Patients are colour coded according to their duration of T1D.
The patient group exhibited a unimodal distribution of maximal rates of rise in response to dim white flashes, such that all patients were in the same range. This distribution gradually became a bimodal distribution in response to brighter flashes, such that part of the patient group had higher maximal rates of rise, while the rest of group exhibited lower maximal rates of rise. The bimodal distribution demonstrated no clear pattern with respect to duration of T1D. While we would expect that the lower maximal rates of rise would belong to patients with longer durations of T1D, they instead belonged to patients intermediate durations of T1D. It is possible that these patients differ in other respects, such as age, or level of glucose control. This possibility warrants further study.

4.36 Summary of White Flash Mixed Model Results

The a-waves of white flash ERGs have shallower maximal rates of rise in adolescents and young adults with T1D as compared to age similar controls. In addition, there is an effect of the interaction of duration of T1D with flash intensity on the maximal rate of rise in patients. This interaction creates a pattern whereby individuals with T1D exhibit a unimodal distribution of maximal rates of rise at dimmer flash intensities, and a bimodal distribution at brighter flash intensities. It is unclear what factor in the disease process might be responsible for such an interaction effect, but it is unlikely to be duration itself.
5 Discussion

5.1 Summary of Results

5.1.1 Cone Density, Analyzed by Quadrants

The quadrants mixed model analysis revealed a main effect of diabetes status on cone density: retinal densities of patients were less cone-dense than those of age-similar controls. There was no main effect of quadrant on cone density. There was no effect of the interaction between diabetes status and quadrant on cone density.

In controls, cone density in the superior nasal quadrant did not differ significantly from that of the superior temporal quadrant. There was also no significant difference in cone densities between inferior nasal and inferior temporal quadrants in controls.

In patients, cone density in the superior nasal quadrant did not differ significantly from that of the superior temporal quadrant. There was also no significant difference in cone densities between inferior nasal and inferior temporal quadrants in patients.

When identical retinal locations were compared between groups, cone densities in the superior nasal quadrant demonstrated a borderline significant difference between groups, with patients exhibiting lower cone densities than controls.

The inferior nasal quadrant also had a lower cone density in patients that was borderline significant. However, this result did not hold when outliers and influential observations were removed from the dataset. When these data were removed, the superior temporal quadrant exhibited a borderline significant difference, with patients having less cone density in this region than controls.

Cone density in the inferior temporal quadrant did not differ between patients and controls.

There was no association between the duration of T1D and cone density in patients. The interaction of duration of T1D x quadrant also demonstrated no association with cone density.
5.1.2 Cone Density, Analyzed by Hemiretinae

The hemiretinae mixed model revealed a main effect of diabetes status on cone density. Just as in the quadrants model, it was found that the retinas of patients were less cone-dense than those of age-similar controls. There was no main effect of hemiretina on cone density, prior to sensitivity analyses.

In the nasal hemiretina, patients had significantly lower cone densities than controls. Cone densities did not differ between groups in the temporal hemiretina.

With respect to nasal-temporal cone distribution within groups, there was a borderline significant asymmetry in cone density in controls (nasal > temporal). Nasal-temporal asymmetry was not observed in patients.

The interaction between diabetes status and hemiretina had a borderline significant effect on cone density. This reflected a between-groups difference in the degree of nasal-temporal asymmetry in cone density: the nasal > temporal asymmetry was (almost significantly) stronger in controls than in patients.

When outliers and influential observations were removed, the interaction term (diabetes status x hemiretina) was no longer significant. Meanwhile, the main effect of hemiretina became borderline significant. The between-groups difference in temporal cone densities also became borderline significant (control > T1D).

Even when outliers and influential observations were removed, however, the borderline significant nasal-temporal asymmetry (nasal > temporal) seen in controls remained. The lack of asymmetry in patients also remained.

There was no association observed between duration of T1D and cone density. Additionally, there was no association observed between the interaction of duration of T1D x hemiretina on cone density.
5.1.3 Maximum Rate of Rise of Red Flash ERG a-waves

There was no main effect of diabetes status on maximal rate of rise of the ERG a-wave, in response to red flashes. Thus, there was no overall difference between controls and patients in terms of maximum rate of rise.

There was also no effect of the interaction of diabetes status x flash intensity on maximal rate of rise. This indicates that the way in which the maximum rate of rise changed with increasing intensities was not different between patients and controls.

Due to a lack of between-groups differences, maximum rate of rise was not correlated with duration of T1D.

5.1.4 Maximum Rate of Rise of White Flash ERG a-waves

There was a main effect of diabetes status on maximum rate of rise in response to white flashes. The maximum rate of rise of ERG a-waves was significantly less steep in patients than in controls.

There was no effect of the interaction of diabetes status x flash intensity on maximum rate of rise. This implies that the change in maximum rate of rise with increasing flash intensity did not differ between patients and controls.

The removal of outliers and influential observations did not change these results.

Among patients, there was no main effect of duration of T1D on maximum rate of rise. There was however, a significant effect of the interaction of duration of T1D x flash intensity on maximum rate of rise in patients.

When the maximal rates of rise of patients were plotted per individual patient, a pattern emerged. The patients all exhibited similar maximum rates of rise at dimmer flash intensities, creating a unimodal distribution, and then diverged at brighter intensities into a bimodal distribution of maximum rates of rise. The group with the lower maximum rates of rise at brighter intensities, however, was comprised of patients with intermediate durations of T1D (rather than relatively long, or relatively short, for this sample). The interaction effect might be related to a factor that was not included in the model.
5.2 Coincidence of Structural and Functional Findings

The structural and functional techniques used in this study are complementary, as their scope is limited exclusively (in AO) or mostly (in white flash ERG) to L- and M- cone photoreceptors. Unfortunately, the sample size shared between the two studies was not large enough to support direct correlation between structural and functional findings. Still, the significantly lower overall cone densities observed in T1D corroborate the shallower a-wave maximum rates of rise observed in T1D in response to white flashes. These results suggest that L- and M- cones are compromised in individuals with T1D, both structurally and functionally.

5.3 Interpretations of Results and Alternative Explanations

5.3.1 Lower Cone Densities Overall in T1D

One interpretation the observation of lower cone densities seen in T1D is that there were fewer L- and M- cones in patients than in controls. However, there are alternative explanations for this result which merit exploration.

An alternative interpretation is that, rather than missing cones, some cones are misaligned and pointing in sub-optimal directions (away from the pupil), thereby affecting waveguide properties and visibility on AO retinal imaging (Stiles Crawford Effect, or SCE; Stiles & Crawford, 1933). Properties inherent to the cones themselves could cause them to change orientation. An additional potential mechanism for misalignment could be the deterioration of the interphotoreceptor matrix (IPM). The IPM lies between the retinal pigment epithelium (RPE) and the photoreceptor layer, and contains specialized domains that ensheath cone photoreceptors in vertebrates, including humans. These domains have a putative role in maintaining the orientation of cone photoreceptors (Johnson, Hageman, & Blanks, 1986). Changes to the RPE could also contribute to alterations in cone photoreceptor orientation. In support of this notion, histologists have documented altered protein expression in the RPE of humans with T1D or T2D and no vascular signs of DR (Decanini et al., 2008).

A final interpretation is that true cone densities in patients were masked by sub-clinical signs of DR. In support of this hypothesis a recent study by Burns and colleagues, involving AO retinal imaging of adults with NPDR, demonstrated that the photoreceptor layer in these individuals was comprised of “regions of bright cones and dark regions”. Specifically, “dark [cone] regions
typically matched areas of overlying vascular remodelling… overlying retinal edematous changes…[or] relatively small [overlying] changes such as a haziness of the inner retinal layer” (Burns et al., 2014).

These potential artefacts are important to take into account in interpreting the results of our study. While our sample of patients had no signs of retinopathy on fundus photos, it is possible that they had sub-clinical lesions, sub-clinical edema, or slight inner retinal changes that could have affected the cone counts.

However, in assessing the images produced by Burns and colleagues (Burns et al., 2014), the “dark regions” appear most analogous to areas that our team would have deemed uncountable. If sub-clinical signs of DR were present in the patients in our study, these might have instead led to missing data, thereby skewing which photographs were selected from the outset. This is an inherent limitation of our study that will be discussed in greater detail in Section 5.4.6.

5.3.2 Borderline Significant Lower Densities in the Superior Nasal Quadrant in T1D

Results from our group’s pilot AO study (Tan, 2012) revealed lower cone densities in the superior nasal quadrant in T1D, which achieved borderline significance (p = 0.09, α = 0.05). This result was replicated in the current study (p = 0.02, α = 0.00625). The superior nasal quadrant was the only quadrant that retained a borderline significant difference between groups both before and after sensitivity analyses.

Again, this lower cone density could reflect a real lack of L- and M- cones in T1D relative to controls, a misalignment of cones, or overlying sub-clinical retinopathic lesions.

It is difficult to speculate what pathophysiological process, if any, would selectively affect L- and M- cones of the superior nasal retina. However, other investigators have identified the superior nasal quadrant as an area of the retina that is uniquely impacted by the progression of DR. One OCT study examined retinal thickness in controls, and in individuals who had diabetes with an absence of clinically significant macular edema. The diabetes group was split into those with no DR, and those with NPDR. Differences in retinal thickness between controls and those with no DR, and between individuals with DR and those with NPDR, were significant and were most pronounced in the superior nasal quadrant (Schaudig, Glaefke, Scholz, & Richard, 2000).
If future longitudinal work reveals the superior nasal quadrant to be selectively affected by the effects of T1D, it will be interesting to see whether this finding translates to animal models of T1D. In particular, a selective superior nasal pattern of cone photoreceptor destruction could help to retrospectively explain the discrepant results found in past studies of streptozotocin rodent models of T1D. For instance, Barber and colleagues reported that the outer nuclear layer of the retina was preserved in streptozotocin treated rats, whereas Park and colleagues reported a rapid and severe ablation of photoreceptors in streptozotocin treated rats (Barber et al., 1998; S.-H. Park et al., 2003). This dramatic difference in findings could owe to the fact that Park et al. examined only the superior nasal retina, while Barber and colleagues examined a more central portion of the retina that was distributed equally among all quadrants.

5.3.3 **Significantly Lower Nasal, but Not Temporal, Cone Densities in T1D**

The nasal hemiretina had significantly lower cone densities in T1D, while the temporal hemiretina did not. This result suggests that the nasal hemiretina could be uniquely susceptible to the effects of T1D or to the effects of sub-clinical DR. For this hypothesis to be evaluated rigorously, a longitudinal study in our sample which compares patients who go on to develop NPDR versus those who do not will be crucial.

The pathophysiological basis of this result not known. In future, it will be important for investigators to relate sub-clinical neuroretinal changes to sub-clinical vascular changes, as sub-clinical vascular changes have the potential to be asymmetrical across the vertical meridian in diabetes (Hudson et al., 2005).

5.3.4 **Borderline Significant Nasal-Temporal Asymmetry Present in Controls**

The borderline-significant finding of nasal-temporal asymmetry in control cone densities (nasal > temporal) is particularly interesting, as this phenomenon has been repeatedly cited in histological studies (Ahnelt, 1998; Curcio et al., 1990, 1987; Jonas et al., 1992; Osterberg, 1935) but has rarely been observed on AO retinal imaging (trend seen at select eccentricities in Song et al., 2011).
It would be important to see whether the borderline significant nasal-temporal asymmetry seen in controls would reach significance with a larger sample. Still, nasal-temporal asymmetry in controls would have to meet additional requirements in order to have clinical utility as a baseline for retinal disease and dysfunction. First, the asymmetry would need to stand up to measures of test-retest reliability. Second, the asymmetry would need to be replicable on other AO retinal imaging systems, in many other healthy individuals from this age group. If these two goals were to be accomplished, then longitudinal analysis of asymmetry in the same healthy controls, or cross-sectional assessments of other healthy controls that are younger and older, would be needed to set a baseline for other age categories.

5.3.5 Absence of Nasal-Temporal Asymmetry in Cone Densities in T1D

Relative to controls, adolescents and young adults with T1D exhibited a lack of asymmetry. In addition, the between-groups difference in the degree of asymmetry was borderline significant, though this result did not hold when outliers and influential observations were removed from the dataset. It would be interesting to see whether the interaction effect would reach the significance threshold in a larger sample.

If the presence of nasal-temporal asymmetry proves to be a reliable baseline in controls, then its absence in T1D could be meaningful. It will be important to investigate the capacity of a lack of asymmetry in T1D to predict the development of sub-clinical or clinical DR, or to predict the rate of progression of DR. This aim will require longitudinal analysis of our sample, until a subset of patients develops signs of retinopathy.

5.3.6 Lack of Association Between Cone Density and Duration of T1D

There was no association observed between cone density and duration of T1D. There was also no impact of the interaction between duration of T1D and retinal location on cone density, in either the quadrants or the hemiretinae model.

It is possible that factors that have not been included in the model could explain at least some of the variation in cone density in patients. Several factors that might explain some of the variation in cone density include age at testing, age at onset of T1D, cumulative long-term A1C levels, and sex. More broadly, some of the variation in cone density in patients could be explained by
ethnicity, genetic and epigenetic factors, or concentration of serum biomarkers that are predictive of DR progression.

It is also a possibility that the differences in cone density observed in our patients were present prior to the onset of T1D. For instance, it is possible that a common genetic factor first caused differences in retinal development in utero, and then went on to cause the auto-immune destruction of beta cells. Studying individuals at risk for T1D early in life, prior to the onset of T1D, and following those individuals in a longitudinal AO retinal imaging study, would provide invaluable data.

5.3.7 Lack of Difference Between Groups in Maximum Rates of Rise on Red Flash ERG

Since L- and M- cones showed between groups differences on AO and on white flash ERGs, it is interesting that the red flash ERG did not elicit a similar difference in responses between groups. Importantly, the intensity of the red flashes was considerably less than that of the white flashes. The use of a gel filter in the red flash protocol restricted the wavelengths of light so as to minimize the activity elicited from S-cones, but in doing so, also restricted the total amount of light allowed to pass through. Thus, the red flashes were likely not bright enough to elicit a response that differed between patients and controls.

One alternative explanation is that the S-cone response, which was not totally eliminated from the white flash responses, was entirely responsible for the difference in maximum rate of rise seen between groups. Studies performed in our lab and by other investigators have shown S-cones to be implicated in T1D (Cho, Poulsen, Ver Hoeve, & Nork, 2000; McFarlane, Wright, Stephens, Nilsson, & Westall, 2012; McFarlane, 2010; Wright, Nilsson, McFarlane, & Westall, 2009). Still, this scenario is unlikely, as S-cones only comprise 10% of the cone population, and proportionally contribute the least of all cone classes to the white flash ERG response. Generally, specialized ERGs which eliminate intermediate and high frequency wavelengths (towards the red end) on the visible spectrum are required to isolate the S-cone response.
5.3.8 Lower Maximum Rates of Rise of White Flash ERG a-waves in T1D

On its own, a lower density of L- and M-cones in individuals with T1D could create abnormal white flash ERG responses. Let us assume that the differences between groups seen on AO retinal imaging reflect an actual lower number of L- and M-cones. A large number of L- and M-cones missing from a retinal population is known to cause the amplitude of the a-wave peak to be smaller than normal (Donald C Hood & Birch, 1993, 1995, 2006). An a-wave peak that has smaller amplitude and normal (or longer than normal) implicit time would have to be generated by a maximal rate of rise that is less steep. Therefore, lower cone density alone could be sufficient to explain the shallower a-wave maximum rate of rise seen in patient white flash ERGs.

It is also possible that the number of missing cones was not large enough to affect the maximum rate of rise of the a-wave. (Or, that the differences in cone density observed were not real, but were instead artefacts of misalignment or of overlying sub-clinical retinopathic lesions). In this case, the differences seen on ERG were most likely attributable to a population or sub-population of remaining, physically intact cones that were either non-functional or dysfunctional. One could speculate that non-functional cones would have created a shallower maximum rate of rise and smaller amplitude, similar to missing cones.

On the other hand, a large number of dysfunctional cones would have also created a shallower maximal rate of rise, but would have done so via a different mechanism from that of missing cones. An example of a type of cone dysfunction that might have created this result is abnormally low sensitivity. Lower sensitivity of L- and M-cones has been observed in adults with T1D and T2D, with and without DR (Holopigian et al., 1997). We used similar ERG protocols to those of Holopigian and colleagues, but their study employed substantially higher flash intensities and more sophisticated mathematical modelling. Given the parameters of our protocol, it is impossible to determine whether changes in sensitivity were responsible for shallower maximal rates of rise in our study, but it is certainly a possibility.
5.3.9 Effect of Duration of T1D x White Flash Intensity on Maximum Rate of Rise in Patients

Among patients, there was no main effect of duration of T1D on maximum rate of rise of the a-wave in response to white flash ERGs. There was however, a significant effect of the interaction of duration of T1D x flash intensity on maximum rate of rise in patients. At dimmer flashes, patients had a unimodal distribution of maximum rates of rise. As flash became brighter, the patient group gradually diverged into a bimodal distribution of maximal rates of rise: some steeper, and some more shallow. We would expect the shallow group to be comprised of individuals who had longer durations of T1D. Instead, they had intermediate durations of T1D; the steeper group was comprised of the longest and shortest durations.

In terms of biological plausibility, it does not make sense for intermediate durations of T1D to cause maximum rates of rise to be shallower, and longer or shorter durations of T1D to create steeper maximum rates of rise. It is far more likely that one or more other factors not included in the mixed model are responsible for the divergence in maximum rates of rise at higher intensities. If this is true, then it could be shown that the significance of the interaction term (duration T1D x flash intensity) is artefact of these other factors. It would be worthwhile to pursue what such factors might be. As previously mentioned, one could include any of the following factors in the model: age at testing, age at onset of T1D, cumulative A1C, sex, ethnicity, genetic or epigenetic markers, or serum biomarkers.

5.4 Limitations in Study Design, and Potential Impact on Results

5.4.1 Cross-Sectional Nature of Study

The cross-sectional nature of our study creates an inherent limitation in the interpretation of our results. By comparing patients to controls at a single time point, we can only make educated guesses about how patient outcomes change over time. A longitudinal study would have the ability to address changes over time in cone function, density, and distribution in T1D. A longitudinal study would allow us to determine the temporal relationships between variables of interest and patient outcomes. Thus, we could identify potential early biomarkers of sub-clinical DR.
A rigorous and well-constructed longitudinal study would allow us to begin to make inferences about causal relationships within our data. For example, our study could be repeated as a prospective cohort study, involving young children at risk for T1D. The baseline assessment would occur prior to the onset of T1D, and participants would be reassessed on a yearly basis. As subsets of the cohort went on to develop T1D, sub-clinical DR, and clinical DR, it would be possible to test hypotheses about factors that may have contributed to those outcomes.

5.4.2 Power of Study

Some controls were relatives of patients, often siblings. This may have introduced an overmatching bias, whereby the chance of controls and patients having similar outcomes is increased. Thus, the enrolment of patient relatives in the control group may have reduced the power of the study.

The high inter-individual variability in cone density that exists in healthy controls also may have reduced the power of our study. Expressing our cone density data in cones/degree\(^2\) removed some of the variation in our data attributable to axial length (Chui et al., 2008a; Li et al., 2010). Even after this correction, however, a great deal of inter-individual variability in cone density remained.

There were no test-retest reliability measures collected from the control group for either AO or ERG studies. This would have helped to determine the effect size and required power for the study at the outset.

The number of participants that overlapped between the two studies was simply not large enough to correlate overall cone densities with maximum rates of rise. Such correlations would have been very underpowered.

Similarly, we did not have access to a large enough sample of patients with clinical DR to include them as another stratum in the analysis. At the outset of the study, our knowledge of the larger patient population at SickKids suggested that this analysis would be underpowered. Thus, patients with DR were excluded outright.
5.4.3 Participant Characteristics

There was no pairwise or group matching of patients to controls for age, sex, and ethnicity. This may have introduced confounding bias into the results. For instance, a difference between groups may have arisen due to a baseline characteristic that was not balanced between groups, rather than the presence or absence of T1D.

Participants with colour vision abnormalities were excluded from ERG, but not from AO retinal imaging. Since performing the statistical analysis, we have discovered literature that contradicts the notion that cone photopigments are merely dysfunctional in red-green colour deficiency. Depending on the genetic mutation that leads to the colour deficiency, there may be a reduction in the overall number of cone photoreceptors (Carroll et al., 2004). This may have also introduced confounding bias.

5.4.4 AO Retinal Imaging Experimental Protocol

The AO retinal imaging protocol, whereby sampled retinal locations fall along the oblique meridians, was predetermined before the beginning of the M.Sc. project. The rationale behind the original AO retinal imaging protocol was based on different studies (e.g., Kern & Engerman, 1995) from those used to develop the rationale for the M.Sc. project.

In contrast to the Westall Lab, many other AO investigators have chosen to image participants along the horizontal and vertical meridians. Due to the potential presence of the cone streak (horizontal elliptical isodensity contours found on histological and AO retinal imaging studies: Ahnelt, 1998; Chui et al., 2008b; Curcio et al., 1993, 1990, 1987; S. P. Park et al., 2013; Pum et al., 1990; Song et al., 2011), comparing our cone density data to that of others groups is not a straightforward process. Even when our results lie at the same eccentricity, they lie at different angular locations and are thus not comparable. Taking the arithmetic means of cone densities at retinal locations which lie on either side of a target location for comparison would still not be sufficient, as this assumes a linear change in density with an angular change in location. Ideally, we would have instead sampled retinal locations along the horizontal and vertical meridians.

It would have also been ideal to sample cone density at multiple eccentricities. One study assessing cone density via AO retinal imaging in healthy controls (Elsner et al., 2012) has shown that it is not possible to use cone density at one eccentricity to estimate cone density at a different
eccentricity in the same individual. This is because it is impossible to generate a mathematical function that is consistent between individuals which is able to describe changes in cone density with eccentricity along a given meridian. Elsner frames this problem eloquently, and speculates what the underlying cause is:

“The lack of a constant ratio between more central and more peripheral cone densities indicates that there is not a normogram for cone density vs. eccentricity that scales across individuals. Instead, cones from the outside of the fovea are likely lower in numbers when a larger proportion have migrated [during development] to provide foveal specialization.”

(Elsner et al., 2012)

Because the distribution of cones with respect to retinal eccentricity is so apparently variable among controls, we might have gained more useful information about cone distribution within groups or cone density differences between groups by assessing multiple eccentricities in both controls and patients.

5.4.5 ERG Experimental Protocol

Unfortunately, the flash intensities employed in the ERG study were not as bright as was originally intended. Had brighter intensities been achieved, a more sophisticated analysis of cone photoreceptor function (a-wave modelling; Hood & Birch, 1993, 1995, 2006) would have been possible. The results of a-wave modelling indicate whether cone photoreceptors have abnormal sensitivity, an abnormal maximal response, or both.

Deriving the sensitivity and maximal response of cones, rather than maximum rate of rise, would have provided additional insights into cone function or dysfunction in T1D. Because a-wave modelling quantitatively reflects the biology of the phototransduction cascade, it is possible to use the results to make guided inferences about the biological mechanisms underlying cone dysfunction. For instance, lower sensitivity often arises from abnormalities in one or more steps of the phototransduction cascade, or slowing of one or more steps. Furthermore, lower sensitivity may frequently be a feature that is secondary to retinal hypoxia. Meanwhile, a lower maximal
response is often the product of having a fewer number of functional outer segment discs than normal, either due to missing or shortened photoreceptors (Donald C Hood & Birch, 2006).

While the ERG has the advantage of being able to isolate cone photoreceptor function, and has a lower signal-to-noise ratio than mfERG, ERG entirely lacks spatial resolution. An inherent limitation to using ERG in our study, rather than mfERG, is that ERGs are unable to detect spatial patterns such as nasal-temporal functional asymmetry. This is one factor that limited our ability to directly compare our cone density and distribution results with our cone function results. Using mfERG instead of ERG would not necessarily have been the ideal solution. While the mfERG isolates cone pathways, it is unable to isolate the cone photoreceptor response. However, given that mfERG data was collected in most participants, it would have been an excellent adjunct to our analysis insofar as providing spatial maps of retinal function.

5.4.6 Sampling Bias

Missing data is usually not problematic they occur at random. However, there are several reasons to believe that data in our study, particularly for AO retinal imaging, may not have been missing at random.

The “dark regions” identified by Burns and colleagues in a recent study exploring sub-clinical DR on AO (Burns et al., 2014) resemble images that our group has deemed uncountable. Dark regions have been associated with overlying sub-clinical signs of DR and sub-clinical macular edema. By excluding images with dark regions, we could be excluding a large proportion of patients with sub-clinical DR and thereby skewing our patient sample towards those who do not have sub-clinical signs. This type of error is classified as sampling bias.

Currently, our AO study does not investigate sub-clinical vasculopathy or edema, or its spatial relationship to cone density or image quality. Incorporating these items into the AO protocol would help to minimize sampling bias, if it did have an effect on our study.

There is also a chance that missing data occurred due to high blood glucose, which may have created unfavourable behavioural characteristics for imaging (inability to sit still), or osmotic shifts that altered the refractive power of the lens. High blood glucose may have introduced sampling bias into our results, and should be controlled in future during AO retinal imaging.
5.4.7 Unexamined Factors that Could Account for Variation in Outcomes Demonstrating Between-Groups Differences

That duration of T1D was the only variable of interest to be correlated with outcomes demonstrating differences between patients and controls was an important limitation of our study. Expanding our repertoire of variables of interest would have been beneficial in two ways.

First, factors that are applicable to both patients and controls, such as age or sex, could have been included in the model, and could have accounted for some of the variance in outcomes. This might have given the model more power to detect differences between groups.

Second, correlating additional patient-specific variables (such as cumulative A1C values or route of insulin administration) with patient outcomes could have revealed important associations that were overlooked by our analyses.

A notable barrier to obtaining data on certain additional variables was accessibility. For example, our access to repeated A1C measurements in our patient sample almost always abruptly ended when patients were transferred from SickKids to an adult hospital at age 18.

Ideally, additional factors that would be addressed by our study include cumulative A1C, age, sex, ethnicity, family history of T1D, age at diagnosis, route of insulin administration, genetic and epigenetic markers, and serum biomarkers.

Using AO retinal imaging to detect sub-clinical signs of DR, the characteristics of which have been established by other investigators (Burns et al., 2014; Prager et al., 2012), would have had great utility. Detecting spatial associations between sub-clinical signs of DR on AO, and either (a) lower cone densities, or (b) reduced imaging quality of the cone photoreceptor layer, would have been invaluable to the interpretation of the results of this study.

5.4.8 Statistical Analyses

There was no step in the statistical analysis that accounted or adjusted for unmatched participant characteristics such as age, sex, and ethnicity. In addition, the statistical analysis was not designed to account for familial relationships among patients and controls.
Cone counting of AO retinal images was performed manually in this study, and was naturally subject to human error. There were attempts to employ a computer program that would count cones automatically, but its accuracy was not adequate. Even though we had to rely on manual counting, it would have been possible to measure the inter-rater reliability of cone counts, and to force agreement on images for which vastly different counts were generated. This step may have increased the accuracy of our dataset, and may have possibly prevented some outliers from being generated.

Applying a Bonferroni correction to $\alpha = 0.05$ to address certain a priori hypotheses was an approach that could be considered overly conservative, because the pairwise comparisons we performed were not completely independent of one another. Though this protects against chance findings from multiple comparisons, it made commission of type II errors more likely.

5.5 Exploration of Potential Pathophysiological Mechanisms of Cone Photoreceptor Loss in T1D

Recent histological investigation has shown outer nuclear layer apoptosis in diabetic post-mortem retinas exhibiting signs of NPDR (Schaal et al., 2011). The work of Schaal and colleagues (Schaal et al., 2013) suggests that the possible cone loss observed in the current study is an apoptotic process that is related to aberrant expression of the huntingtin protein.

Huntingtin and its related proteins have not only been implicated in the pathogenesis of DR, but also in the pathogenesis of T1D. In one study, the prevalence of diabetes among individuals with Huntington’s disease (HD) was found to be more than four times greater than the prevalence of diabetes in the general population (Farrer, 1985). Moreover, this study demonstrated that relatives of HD probands have differential susceptibility to diabetes based on their HD status. Specifically, HD-affected relatives of an HD proband are seven times more likely to have diabetes than relatives of the proband who do not have HD (Farrer, 1985).

R6/2 transgenic mice modelling human HD—that is, expressing a portion of the human huntingtin gene alongside 140 CAG repeats—demonstrate drastically reduced insulin production by pancreatic $\beta$-cells, 15% of that of control mice (Hurlbert et al., 1999). While this study did not find a reduced mass of pancreatic $\beta$-cells, a later study found that R6/2 mice have only 35% of the pancreatic $\beta$-cell mass of control mice (Björkqvist et al., 2005). Björkqvist and colleagues...
also discovered an absence of exocytosis in β-cells (but not α-cells), which accounted for a 96% reduction in the number of insulin-secreting secretory vesicles (Björkqvist et al., 2005).

A recent human genome-wide linkage study that aimed to identify disease-causing genes in T1D (Berchtold et al., 2011) identified huntingtin interacting protein 14 (HIP14) as one of the top 3 candidate genes, among a total of 11 identified genes, implicated in the pathogenesis of T1D. Follow up studies revealed that HIP14 is almost exclusively expressed in insulin-positive cells in the Islets of Langerhans. Moreover, HIP14 is anti-apoptotic, and is critical to β-cell survival and to glucose-stimulated insulin secretion. The investigators demonstrated that pro-inflammatory cytokines that mediate β-cell dysfunction in T1D down-regulate the expression of HIP14 in isolated human islets. In addition, overexpression of HIP14 is protective against cytokine-mediated apoptosis (Berchtold et al., 2011).

The role of huntingtin protein, HIP14, and related proteins in the pathogenesis of T1D could be related to the putative action of huntingtin protein in global cone loss in T1D. Additionally, the presence of abnormalities in the huntingtin protein network could also explain the elevated risk of developing DR in T1D relative to T2D. Finally, the activity of this class of proteins could also explain the weak relationship between long-term glucose control and development of DR in T1D. In other words, huntingtin and related proteins may comprise at least part of the genetic component that mitigates development of DR in individuals with excellent glucose control, or absence of DR in those with poor glucose control.
6 Conclusions

At the outset of the study, we hypothesized that the density, distribution, and function of L- and M- cone photoreceptors would demonstrate differences in adolescents and young adults with T1D as compared to age-similar typically developing controls.

Overall, cone densities were lower in patients than in controls.

We predicted that the superior nasal quadrant and inferior nasal quadrants would have significantly lower cone densities in patients. However, only the superior nasal quadrant had lower cone density in patients, and this result was borderline significant. As predicted, cone densities in the superior temporal and inferior temporal quadrants did not differ between groups. Though we expected the nasal quadrants to be more cone dense than the temporal quadrants in controls, this was not the case. As expected, there were no significant patterns of intra-retinal distribution by quadrant within patients.

As hypothesized, the nasal hemiretina was significantly less cone dense in patients than in controls, while the temporal hemiretina did not differ between groups. We predicted that controls would exhibit a nasal-temporal asymmetry in cone density (nasal > temporal), and we found this result with borderline significance. As predicted, there was a lack of nasal-temporal asymmetry in patients. We also predicted that patients would have a significantly greater degree of nasal-temporal asymmetry than controls. This result was borderline significant, but did not hold when influential observations were removed from the dataset.

We predicted that the maximum rate of rise of the ERG a-wave would be significantly less steep in patients than in controls. This was true of white flash, but not red flash, ERGs.

Finally, we predicted that outcomes exhibiting differences between groups would demonstrate associations with duration of T1D in the patient group. Neither cone density, nor white flash ERG a-wave maximum rate of rise, were correlated with duration of T1D. However, the interaction of duration of T1D with flash intensity was significantly correlated with white ERG a-wave maximum rate of rise. This manifested as a unimodal distribution of maximum rates of rise within the patient group at dimmer flash intensities, and a bimodal distribution at brighter intensities (some high, and some low). Patients that had lower maximum rates of rise at high
flash intensities had intermediate durations of T1D, rather than long or short durations of T1D. Thus, a different variable of interest (such as cumulative A1C) is more likely to be associated with the bimodal distribution than duration of T1D itself.

The results of our study demonstrate that L- and M- cone photoreceptor density, distribution, and function are abnormal in adolescents and young adults with T1D. Moreover, these results raise the possibility that the nasal retina is uniquely susceptible to the effects of T1D. Rigorous longitudinal analysis is warranted to support or refute this hypothesis. Lower overall cone density, selectively low nasal cone density, and shallow maximum rate of rise of the white flash ERG all merit longitudinal investigation as potential early biomarkers of sub-clinical DR.
7 Future Directions

7.1 Goals and Recommendations

Specific goals are identified for the continuation of the current study, and recommendations are made for future studies, based on the limitations outlined in Section 5.4.

7.1.1 Implementation of a Longitudinal Study

A prospective cohort study, enrolling young children at risk for T1D, would be an optimal study design. It would have the capacity to investigate whether the abnormal cone structural and functional properties observed in this study occur prior to or after the onset of T1D. Assessment would ideally take place on an annual basis to assess changes in outcomes over time. Strata of the study would develop, as subsets of patients eventually develop sub-clinical and clinical signs of DR. This design would permit identification of early biomarkers of DR.

TrialNet has an existing registry of families with members at risk for T1D. This would be an ideal source of recruitment for such a longitudinal study.

7.1.2 Increasing Sample Size for Structural-Functional Overlap

In future, a greater number of participants will be recruited to overlap between the structural and functional arms of the study. This will allow us to perform important structural-functional correlations among our data.

7.1.3 Matching of Participants on Baseline Characteristics

Ideally, in a new study, patients and controls would be pairwise matched for age, sex, and ethnicity.

7.1.4 Adapting AO Retinal Imaging Experimental Protocol

Blood glucose will be kept within the 4-10 mmol/L range for AO retinal imaging, to avoid sampling bias due to restless patient behaviour and osmotic shifts in the lens. Ideally, AO retinal imaging would sample retinal locations along the horizontal and vertical meridians, at multiple eccentricities. If possible, we would also assess sub-clinical signs of DR, and their spatial associations with cone density and photoreceptor layer image quality.
7.1.5 Adapting ERG Experimental Protocol and Analyzing Existing mfERG Data

In future work, ERG flash intensities could be calibrated to higher intensities such that the sensitivity and maximal response of cones could be measured via a-wave modelling.

In addition, existing mfERG data could be analyzed in much the same way as AO data. N1-P1 amplitudes and implicit times should be pooled into nasal observations and temporal observations, and compared between groups. Moreover, the degree of nasal-temporal symmetry of N1-P1 amplitudes and implicit times should be assessed in both controls and patients, and should be compared between groups.

7.1.6 Recording Detailed Accounts of Missing Observations

In order to avoid selection bias, it will be critical to document the specific reasons for missing observations on AO retinal imaging. Categories should include, but not be limited to: system dependent factors, system operator factors, patient behaviours, and signs of sub-clinical DR. We will also need to adopt a more systematic approach to acceptance or rejection of AO images for counting.

7.1.7 Adding Unexamined Variables of Interest to Analysis and Data Collection

Age, sex, family history of T1D, and age at diagnosis will be added to the mixed model analysis, as factors that could explain variation in patient outcomes.

We will work with our research coordinator to find a way to obtain better access to A1C values for participants that have left SickKids. Ideally, we would also collect data on ethnicity and route of insulin administration.

Finally, if possible, we would use recent data generated by the Type 1 Diabetes Genetics Consortium to identify genes and proteins of interest in our participant sample.
7.1.8 Refining Statistical Analyses

The statistical analyses will be re-designed to account for familial relationships among patients and controls. In addition, cone counts will be subject to tests of inter-rater reliability, and we will force agreement on images with significant inter-rater counting discrepancies.
References


McFarlane, M. (2010). *Poor Glycemic Control is Associated with Neuroretinal Dysfunction in Short-Wavelength Cone Pathways of Adolescents with Type 1 Diabetes by Poor Glycemic Control is Associated with Neuroretinal Dysfunction in Short-Wavelength Cone Pathways of Adolescents w.*


Introduction


Tan, W. (2012). Localizing Structural and Functional Damage in the Neural Retina of Adolescents with Type 1 Diabetes by Localizing Structural and Functional Damage in the Neural Retina of Adolescents with Type 1 Diabetes.


Appendices

A1 Patient Sample

The patient participants in these studies were a cross-section of adolescents and young adults with T1D from a larger longitudinal study. For reference to past and future studies from our group, Table A.1 provides specific information for patient.

<table>
<thead>
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<th>Participant ID</th>
<th>Test ID</th>
<th>Sex</th>
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<th>Duration T1D (Years)</th>
<th>Test Performed</th>
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<td>18.0</td>
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<td>12.8</td>
<td>AO</td>
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<td>F</td>
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<td>AO</td>
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</table>

Table A.1: Description of each patient, including Participant ID and Test ID, across both AO and ERG studies. Age at testing date (years) and duration of T1D at testing date (years) are listed. Tests performed are described, and include at least one of: Adaptive Optics (AO), Red Flash ERG (ERG-R), Red Flash and White Flash ERG (ERG-R,W).
A2 Predicting Impact of Red-Green Colour Vision Deficiencies on AO Results

During the writing of this manuscript, the views of the author have changed with respect to the potential impact of red-green colour vision deficiencies cone density. In the past, the “replacement model” of cone photopigments has been favoured, whereby dysfunctional or non-functional opsins occupy otherwise intact photoreceptors. This theory has been supported by directional reflectance studies, psychophysical experiments, and ERG findings (Carroll et al., 2004). However, an important AO finding reveals that red-green colour deficiencies may cause either replacement or loss of an entire class of photoreceptors, depending on the genetic mutation has caused the deficiency (Carroll et al., 2004) This finding will be considered in any future publication of data.