POOR GLYCEMIC CONTROL PREDICTS INCREASED NEURO-RETINAL DYSFUNCTION IN ADOLESCENTS WITH TYPE 1 DIABETES

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
Graduate Department of Institute of Medical Science
University of Toronto

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Poor Glycemic Control Predicts Increased Neuro-Retinal Dysfunction in Adolescents with Type 1 Diabetes

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Master of Science

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Abstract

Studies demonstrate localized neuro-retinal dysfunction in patients with diabetes and no visible diabetic retinopathy (DR). Poor glycemic control is a strong risk factor for DR. We hypothesized that poor glycemic control predicts increased areas of localized neuro-retinal dysfunction in patients with diabetes.

Forty-eight adolescents with diabetes and 45 controls were tested using the standard (103 hexagons) multifocal electroretinogram (mfERG). Negative binomial regression analysis was conducted with number of abnormal hexagons (delayed responses) as the dependent variable and glycated hemoglobin (HbA$_{1c}$), disease duration, age and sex as covariates.

Results indicate that a one-unit increase in HbA$_{1c}$ predicts an 80% ($p = 0.002$) increase in the number of abnormal hexagons when controlling for age. Increased areas of neuro-retinal dysfunction are predicted by worsening glycemic control in patients with no visible DR. Standard mfERG may be useful in monitoring patients with diabetes and identifying those who may be at risk of developing DR.
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<tbody>
<tr>
<td>BRB</td>
<td>Blood retinal barrier</td>
</tr>
<tr>
<td>CDA</td>
<td>Canadian Diabetes Association</td>
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<tr>
<td>CSME</td>
<td>Clinically significant macular edema</td>
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<tr>
<td>DCCT</td>
<td>Diabetes Control and Complications Trial</td>
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<tr>
<td>DR</td>
<td>Diabetic Retinopathy</td>
</tr>
<tr>
<td>DRS</td>
<td>Diabetic Retinopathy Study</td>
</tr>
<tr>
<td>ERG</td>
<td>Electroretinogram</td>
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<tr>
<td>ETDRS</td>
<td>Early Treatment Diabetic Retinopathy Study</td>
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<tr>
<td>HbA1c</td>
<td>Glycated hemoglobin</td>
</tr>
<tr>
<td>HRR</td>
<td>Hardy Rand &amp; Rittler</td>
</tr>
<tr>
<td>iBRB</td>
<td>Inner blood retinal barrier</td>
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<tr>
<td>IRMAs</td>
<td>Intraretinal microvascular abnormalities</td>
</tr>
<tr>
<td>ISCEV</td>
<td>International Society for Clinical Electrophysiology of Vision</td>
</tr>
<tr>
<td>mfERG</td>
<td>Multifocal electroretinogram</td>
</tr>
<tr>
<td>mfOPs</td>
<td>Multifocal oscillatory potentials</td>
</tr>
<tr>
<td>NPDR</td>
<td>Nonproliferative diabetic retinopathy</td>
</tr>
<tr>
<td>oBRB</td>
<td>Outer blood retinal barrier</td>
</tr>
<tr>
<td>OPs</td>
<td>Oscillatory potentials</td>
</tr>
<tr>
<td>PDR</td>
<td>Proliferative diabetic retinopathy</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>PRP</td>
<td>Panretinal photocoagulation</td>
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<tr>
<td>sf-mfERG</td>
<td>Slow-flash multifocal electroretinogram</td>
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<tr>
<td>T1D</td>
<td>Type 1 diabetes</td>
</tr>
<tr>
<td>T2D</td>
<td>Type 2 diabetes</td>
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<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
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<tr>
<td>WESDR</td>
<td>Wisconsin Epidemiologic Study of Diabetic Retinopathy</td>
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Introduction

Diabetic retinopathy is a chronic and progressive vision-threatening complication of diabetes mellitus. It is a serious public health concern as it is the leading cause of blindness in working-age individuals in the developed world (B. E. Klein, 2007) and is anticipated to be on the rise due to the burgeoning population of individuals with diabetes worldwide (2004; B. E. Klein, 2007; Wild, Roglic, Green, Sicree, & King, 2004). The current means of diagnosing the disease reflects the common assumption that diabetic retinopathy is primarily a vascular disease as it is diagnosed based on vascular lesions that are visible upon clinical examination. These vascular lesions however manifest when the disease has progressed to its later stages and are often vision-threatening. Therefore, to prevent vision loss in patients with diabetes, it is essential to establish reliable clinical tools to monitor retinal integrity and most importantly, to identify patients at risk of developing these vascular lesions.

Localized neuro-retinal function, measured using visual electrophysiological tools, could be a potential biomarker of diabetic eye disease. The multifocal electroretinogram (mfERG) can record localized responses simultaneously from over 100 small retinal areas in the central 50 degrees of the retina. The standard paradigm primarily reflects the function of neurons in the middle retinal layers, predominantly, bipolar cells, and the slow-flash paradigm reflects the function of neurons in inner retinal layers such as amacrine cells. Several electrophysiological studies using the standard and slow-flash multifocal electroretinographic paradigms have demonstrated delayed responses (Fortune, Schneck, & Adams, 1999; Han, Barse et al., 2004a) and reduced amplitudes of
the responses (Palmowski, Sutter, Bearse, & Fung, 1997) in patients with diabetes without any clinically visible signs of retinopathy. These indices of neuro-retinal function have been shown to worsen in patients with increasing severity of retinopathy (Fortune et al., 1999). Moreover, localized retinal regions with delayed standard mfERG responses have been shown to be predictive of new vascular lesions in patients with nonproliferative diabetic retinopathy (Han, Bearse et al., 2004b; Ng, Bearse, Schneck, Barez, & Adams, 2008). Thus there is strong evidence that diabetic retinopathy is a neurodegenerative disease rather than solely a vascular disease and that neuro-retinal dysfunction is an important component.

The Diabetes Complications and Control Trial (DCCT, 1993, 1994a) and other population-based studies have implicated poor glycemic control, measured using glycated hemoglobin (HbA1c) levels, as a strong risk factor for incidence and progression of diabetic retinopathy. The DCCT study demonstrated that intensive glycemic control significantly reduced the development and progression of retinopathy (DCCT, 1993). Histological studies have also demonstrated that administering insulin in vitro significantly reduces the number of retinal cells undergoing apoptosis (Barber et al., 1998). If measurement of neuro-retinal function is to be considered as a biomarker for monitoring retinal integrity in patients with diabetes and for identifying populations at risk of developing vascular signs of diabetic retinopathy, it is important to consider whether neuro-retinal function changes with variation in glycemic control. Therefore this study examines whether neuro-retinal function in patients with diabetes worsens with poor glycemic control or increasing HbA1c levels.
The subsequent sections of this chapter provide an overview of topics relevant to the underlying objective of this study; namely, diabetes, diabetic retinopathy, and electrophysiology.

1. Background

1.1 Diabetes

Diabetes mellitus is a metabolic disorder caused by reduced sensitivity to insulin, a lack of insulin production by pancreatic beta cells, or both (Kaufman, 1997). Hyperglycemia, the state of having abnormally high blood glucose levels, is the primary feature of diabetes (Kaufman, 1997). The two major types of diabetes are type 2 diabetes (T2D) which is commonly diagnosed in adults and is a result of reduced sensitivity to insulin, and type 1 diabetes (T1D) which is a result of decreased or a lack of insulin production.

Diabetes mellitus and its often devastating complications are a major source of morbidity and mortality worldwide. According to the International Diabetes Federation, two individuals are diagnosed with diabetes in the world every ten seconds (IDF, 2007). The numbers continue to rise in part due to aging, population growth, a rise in obesity and physical inactivity, and urbanization (Wild et al., 2004). The number of people with diabetes worldwide is projected to rise to 336 million in the year 2030 from a total of 171 million in the year 2000 (Wild et al., 2004). Regarding prevalence of diagnosed diabetes in Canada, the numbers are expected to rise from 1.8 million adults in 2005 (IDF, 2007) to 2.4 million by the year 2016 (Ohinmaa, 2004). The current rising trend in the prevalence of diabetes continues to incur significant costs to the Canadian health care
system and contributes to prolonged wait times for treatment in emergency rooms and for surgeries (CDA, 2008).

Typical clinical symptoms of diabetes include excess urination (polyuria), excessive thirst (polydipsia), and hunger (polyphagia) (Kaufman, 1997). Primary diagnostic criteria (based on venous samples) include a fasting plasma blood glucose level greater \( \geq 7 \) mmol/L or a plasma glucose level \( \geq 11.1 \) mmol/L at any time of the day, plus symptoms of diabetes (including those noted above) or a two-hour plasma glucose level in an oral glucose tolerance test of \( \geq 11.1 \) mmol/L (ADA, 2008).

Since this study involves patients with T1D, the following paragraphs describe the disease and provide an overview of its epidemiology, management practices, and complications.

Type 1 diabetes is characterized by deficient insulin production due to the destruction of pancreatic beta cells. This is frequently the result of a complex autoimmune process mediated by T lymphocytes which leads to a progressive decline in the secretion of insulin (Srikanta et al., 1983). The autoimmune disease can be attributed to genetic susceptibility or environmental factors such as exposure to certain viruses or toxins (Kaufman, 1997). Evidence of the autoimmune process includes the presence of antibodies in the blood against islet antigens (Zipris, 2009) such as insulin (Naik et al., 2004), before disease onset. Suggested pathogenic mechanisms, based on evidence from human and animal studies involve both humoral and cell-mediated immunity (Zipris, 2009). Following antigen presentation, destruction of islets is mediated by helper (CD4) T-cells, B cells, macrophages (Atkinson & Eisenbarth, 2001; Gianani & Eisenbarth,
2005; Imagawa et al., 1999; Kaufman, 1997; Moriwaki et al., 1999; Somoza et al., 1994),
and the production of cytokines (Kaufman, 1997). In addition to the autoimmune process,
the classification of T1D also encompasses the less common idiopathic diabetes in which
the etiology of beta cell destruction is unknown (CDA, 2008; Kaufman, 1997).

1.1.1 Epidemiology

Type 1 Diabetes most commonly affects children and adolescents; the peak age of
diagnosis being 12 years (Kaufman, 1997). Nearly 80% of individuals are diagnosed
before the age of 30 (Kaufman, 1997). Although less common, a second peak of
prevalence of T1D can occur in the elderly between the ages of 60 and 70 years (Gill,
Lucas, & Kent, 1996; Kilvert, FitzGerald, Wright, & Nattrass, 1984).

Epidemiological data indicate that the incidence of T1D is rising. For instance, the
International Diabetes Federation estimates 70 000 new cases of T1D occurring each year
worldwide (IDF, 2007). The number of children diagnosed with T1D between the ages of
0 to 14 years in 2006 was estimated to be 440 000 worldwide (IDF, 2007). According to
the Canadian Diabetes Association, over two million Canadians currently have diabetes,
of whom 10% have T1D (CDA, 2008).
1.1.2 Management

The primary goal of managing diabetes is to achieve normal or near-normal levels of glycemic control. Targets for glycemic control include glycated haemoglobin (HbA$_{1c}$) levels of $\leq 7.0\%$, fasting or preprandial plasma glucose levels of 4 to 7 mmol/L and three-hour post prandial plasma glucose levels of 5 to 10 mmol/L (CDA, 2008). Achieving these targets decreases the risk of developing diabetes-associated complications. The targets are generally recommended but may not be achieved safely in some individuals (CDA, 2008). Clinicians tailor treatment goals to each patient based on individual factors such as age, duration of disease, the presence of comorbidities, and the risk of hypoglycaemia (abnormally low blood glucose levels) (CDA, 2008). Children with T1D are particularly at risk of developing hypoglycaemia (CDA, 2008) which depending on its severity can cause seizures, loss of consciousness and death (Kaufman, 1997).

Diabetes can be well-managed with education and support provided by an interdisciplinary team of diabetes health care professionals (CDA, 2008). Management protocol includes taking multiple blood glucose readings, two or more insulin injections daily (or the use of an insulin pump), an appropriate meal plan and exercise regime, and HbA$_{1c}$ measurements every 3 months (CDA, 2008; Kaufman, 1997).
1.1.3 Complications

Short-term complications in addition to hyperglycemia include hypoglycaemia and diabetic ketoacidosis, which are significant sources of morbidity and in some cases, mortality in patients with both T1D and T2D. Hypoglycemia develops when the blood glucose level (typically < 4.0 mmol/L) (CDA, 2008) is abnormally low, which occurs when the rate of appearance of glucose in blood is greater than the rate of its disappearance (Cryer, 2003). It often hinders patients with T1D from achieving normal blood glucose levels (CDA, 2008). In the DCCT (1993) study, patientssss in the intensive treatment group had a three-fold increase in risk of developing hypoglycaemia in comparison with the patients in the conventional treatment group. Hypoglycemia manifests in neurogenic (autonomic) symptoms such as seating, trembling, anxiety and nausea, as well as neuroglycopenic symptoms such as confusion, dizziness and difficulty in concentrating and speaking (CDA, 2008; Towler, Havlin, Craft, & Cryer, 1993). When severe, it can result in seizures, coma or even death (CDA, 2008; Kaufman, 1997). Risk factors include HbA\textsubscript{1c} less than 6% (Davis, Keating, Byrne, Russell, & Jones, 1997; DCCT, 1991, 1997; Egger, Davey Smith, Stettler, & Diem, 1997), adolescence (DCCT, 1994b), previous hypoglycaemic episodes (DCCT, 1995; Muhlhauser, Overmann, Bender, Bott, & Berger, 1998), and longer duration of diabetes (Davis et al., 1997; Mokan et al., 1994). Treatment options depending on the severity of hypoglycaemia include oral ingestion of some carbohydrate such as glucose or sucrose tablets, or the administration of glucagon subcutaneously (CDA, 2008).

Diabetic ketoacidosis, which is present in 15 to 67% of children with T1D at the time of diagnosis (Levy-Marchal, Patterson, & Green, 2001), is a medical emergency and can
lead to death (Dahlquist & Kallen, 2005). In patients with diagnosed diabetes, it is commonly caused by inappropriate management or purposely omitting insulin (Morris et al., 1997). The consequent insulin deficiency leads to hyperglycemia, an increase in counterregulatory hormones (those that cause an increase in blood glucose) such as epinephrine, glucagon or cortisol, and the accumulation of ketoacids (Kaufman, 1997). Symptoms, in addition to polyuria, polydipsia and weight loss, include shortness of breath, vomiting, dehydration, abdominal pain and tiredness (Wolfsdorf et al., 2007). Patients can go into a coma in advanced stages (CDA, 2008). Treatment includes correcting the metabolic disturbances and lowering the blood glucose level by administering insulin in a way that would allow regulation of its absorption (CDA, 2008).

Hyperglycemia in the can result in a variety of debilitating complications associated with diabetes due to damage to various organs including the heart, the kidneys and the eyes. Studies have also shown that diabetes is associated with an increased incidence of psychiatric disorders such as depression (Anderson, Freedland, Clouse, & Lustman, 2001; Dantzer, Swendsen, Maurice-Tison, & Salamon, 2003; Goldney, Phillips, Fisher, & Wilson, 2004), anxiety disorders such as phobias (Mollema, Snoek, Ader, Heine, & van der Ploeg, 2001; Popkin, Callies, Lentz, Colon, & Sutherland, 1988) and generalized anxiety disorder (Kovacs, Goldston, Obrosky, & Bonar, 1997), as well as eating disorders in young women and teenaged girls (Daneman, Olmsted, Rydall, Maharaj, & Rodin, 1998; Jones, Lawson, Daneman, Olmsted, & Rodin, 2000).

Other systemic complications include nephropathy, neuropathy, hypertension, and diabetic retinopathy (Brink, 2001; CDA, 2008). Although microalbuminuria, a marker of nephropathy, is rare in prepubertal children (Donaghue et al., 2005), studies have shown
that 30 to 40% of patients with T1D develop renal failure over time (Andersen, Christiansen, Andersen, Kreiner, & Deckert, 1983). Annual screening for the presence of protein in urine is recommended beginning at puberty or five years post-diagnosis (Brink, 2001). Hyperglycemia can result in diabetic neuropathy which consists of a direct and a vascular insult to autonomic and peripheral nerves (Clarke, 1998; Greene, Lattimer, & Sima, 1987; Young et al., 1986). Mostly, neuropathy is subclinical in children and adolescents (Karavanaki & Baum, 2003) however it can result in significant morbidity (Clarke, 1998). Approximately 16% of adolescents with T1D develop hypertension (Eppens et al., 2006). The Canadian Diabetes Association recommends screening at least twice a year (CDA, 2008). Although the incidence of diabetic retinopathy is rare in prepubertal children, nearly all patients with T1D develop some degree of retinopathy 15 to 20 years post-diagnosis (R. Klein, Klein, Moss, Davis, & DeMets, 1984a). The subsequent sections provide an in-depth overview of diabetic retinopathy, which is an essential component of this study.

1.2 Diabetic Retinopathy

1.2.1 Epidemiology

Diabetic retinopathy (DR) is a chronic, progressive and sight-threatening complication of diabetes. According to the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR), some degree of DR is prevalent in nearly all patients with T1D (97.5%) for 15 or more years (R. Klein et al., 1984a). In fact, DR is the leading cause of blindness among adults of working age in Canada ("Canadian National Institute for the Blind
National Office. CNIB Client Database.," 2002) and the rest of the developed world (B.
E. Klein, 2007). Approximately 10% of individuals with diabetes develop severe vision
loss after having diabetes for 15 years and about 2% become legally blind (Brassard,
Robinson, & Dumont, 1993). About 40% of Canadians are estimated to have some
degree of clinically visible DR (Ross, McKenna, Mozejko, & Fick, 2007). In the United
States, approximately 86% of people with T1D have some degree of DR (Varma, 2008).

There is some evidence suggesting that since the WESDR study, which was undertaken
over 30 years ago, the prevalence of DR has declined and that current rates of prevalence
may be overestimated (Roy et al., 2004). This has been thought to be attributed to greater
awareness, better glycemic control and more intensive diabetes management protocols.

For instance, the twenty-five year follow-up study in the WESDR group of younger-onset
patients showed a decrease in the annual rates of progression of DR and incidence of
proliferative DR over a period of 15 years prior to the current study interval (R. Klein,
Knudtson, Lee, Gangnon, & Klein, 2008). Le Caire et al. (2006) examined the prevalence
of DR in patients with duration of 4 to 14 years of T1D from the same region of
Wisconsin as the WESDR cohort (R. Klein et al., 1984a). In the Le Caire et al. study, the
prevalence of DR in patients with nine to ten years of duration of diabetes was 47%
compared to a prevalence of 74% in the WESDR study. In patients with diabetes duration
of 13 to 14 years, a prevalence of 73% was found in the Le Caire et al. study compared to
the 95% prevalence reported in the WESDR study. In addition, by duration of 14 years,
the Le Caire et al. study only found a 10% prevalence of moderate-severe
nonproliferative DR (NPDR) compared to 35% in the WESDR study, and only one
individual with proliferative DR (PDR) compared to the 25% prevalence found in the
The WESDR study. The decline in the prevalence of DR was attributed to a greater percentage of patients monitoring their blood glucose levels daily (66%) and taking ≥ 3 insulin injections daily (45%). Mean HbA$_1c$ in the Le Caire et al. group was 9%. In comparison, most patients in the WESDR cohort administered 1-2 insulin injections per day, only 12% took blood glucose readings three or more times per day, and the mean HbA$_1c$ was 10.1%. A decrease in prevalence of DR has also been observed in population studies in Sweden and Finland (Flack, Kaar, & Laatikainen, 1996; Henricsson et al., 2003; Kernell et al., 1997). Patients in the Henricsson et al. study (2003) followed an intense regimen including multiple insulin injections and taking frequent blood glucose readings per day. It has been suggested that the decline in prevalence in the Swedish study may be due to lower HbA$_1c$ levels as a result of the intensive treatment regimen (LeCaire et al., 2006).

The WESDR study (R. Klein et al., 1984a) which was conducted in the early 1980s was the last major population-based study on the incidence and progression of DR (Wong & Hyman, 2008). A key question that remains unanswered is whether recent advances in medical therapies, better diabetes management procedures, more DR screening programs and greater awareness of DR risk factors have affected the prevalence and incidence of DR (B. E. Klein, 2007; Wong & Hyman, 2008). Although there have been a few studies conducted recently, it has been difficult to draw definitive conclusions from them due to differences in characteristics of the study population and study design (Kempen et al., 2004). Even if current estimates of the prevalence and incidence of DR may be relatively lower than they were three decades ago, DR is still likely to remain a major public health
issue due to the aging population and consequently, the projected rise in the number of persons diagnosed with diabetes (Kempen et al., 2004; B. E. Klein, 2007).

1.2.2 Clinical Features

Diabetic retinopathy is commonly described as a progressive disease of the retinal vasculature. The clinical features of DR manifest from increased vascular permeability, capillary closure, and ultimately retinal edema and ischemia, which is caused by damage to the retinal vasculature (Aiello et al., 1998; Hudson, 2008).

The gold standard for the classification of DR was established by the Early Treatment Diabetic Retinopathy Study (ETDRS; 1991a). Diabetic retinopathy is typically classified into two broad categories: nonproliferative and proliferative DR. Nonproliferative DR (NPDR) encompasses clinically-detectable microvascular abnormalities before the onset of neovascularisation (ETDRS, 1991a). The stage at which the proliferation of retinal blood vessels is observed is referred to as proliferative DR (PDR) (ETDRS 1991b; (DRS, 1981). It is important to note that because DR is a progressive disease, several microvascular abnormalities can coexist simultaneously.

Nonproliferative Diabetic Retinopathy

The broad category of NPDR is further divided into levels of severity based on the extent of the various microvascular abnormalities. These subdivisions, in order of increasing severity include mild to moderate, moderate to severe, severe, and very severe NPDR (ETDRS, 1991b).
Mild to moderate

This stage consist of the presence of microaneurysms, as well as one or more of: intraretinal hemorrhages, cotton wool spots, hard exudates and intraretinal microvascular abnormalities (IRMAs). Most of these abnormalities are shown in Figure 1.1.

Microaneurysms are saccular outpouchings of capillary walls (Watkins, 2003) that are typically located in the inner nuclear layer (Negi & Vernon, 2003). Upon clinical examination, they appear as small, round red dots typically temporal to the macula (Watkins, 2003) with smooth margins and a central light reflex (ETDRS, 1991c; Watkins, 2003). They range from 12 to 100 µm in diameter (Negi & Vernon, 2003). They often tend to appear and disappear over time (Meyerle, 2009) however an increase is number is indicative of disease progression and is accompanied by the appearance of additional microvascular abnormalities (Donnelly, 2002). Possible mechanisms of microaneurysm formation include the proliferation of capillary endothelial cells, bulging of the capillary wall due to the loss of pericytes, and abnormalities of the adjacent retinal areas (Cogan, Toussaint, & Kuwabara, 1961; Wise, 1956).

The rupture of a microaneurysm, capillary or venule can result in intraretinal hemorrhages which may appear as dots or blots, or flame-shaped (Negi & Vernon, 2003; Watkins, 2003). The hemorrhages most often originate in the outer plexiform and inner nuclear layers (Negi & Vernon, 2003). Dot hemorrhages appear dot-shaped with distinct margins (Donnelly, 2002) and can be difficult to distinguish from microaneurysms by ophthalmoscopy or fundus photography. Blot hemorrhages on the contrary do not have distinct margins and are relatively larger than dot hemorrhages (Negi & Vernon, 2003).
Flame hemorrhages are found in the superficial nerve fiber layer and are often associated with hypertension (Negi & Vernon, 2003; Watkins, 2003). These hemorrhages in addition to microaneurysms increase with increasing severity of DR (Donnelly, 2002).

Hard exudates are glistening yellowish-white spots with well-defined margins that are often found in the outer plexiform layer (ETDRS, 1991c; Negi & Vernon, 2003; Watkins, 2003). They are lipid deposits formed due to leakage of serous fluid from the highly permeable blood vessels (Watkins, 2003). They may be seen as individual spots or form a circinate ring around leaking microaneurysms or areas of edema (ETDRS, 1991c; Watkins, 2003).

Moderate to severe

As vascular leakage and closure increases and the tissue becomes more ischemic, IRMAs appear, as well as cotton wool spots and their often associated dark blot hemorrhages, and venous beading. Cotton wool spots appear a pale, yellowish-white or greyish-white colour with feathery edges and often, as striations parallel to the nerve fiber layer (ETDRS, 1991c; Negi & Vernon, 2003; Watkins, 2003). They represent swelling of ganglion cell axons as a result of obstruction of axoplasmic flow (McLeod, 1981).

IRMAs are irregular, tortuous blood vessels adjacent to nonperfused retinal areas (ETDRS, 1991c; Meyerle, 2009) that may be dilated pre-existing capillaries or the result of intraretinal neovascularisation (Hudson, 2008; Meyerle, 2009). Venous beading appears as a string of beads along a venous segment and is the result of venous dilation near areas of capillary nonperfusion (ETDRS, 1991c).
Severe and Very severe

Characteristics of the previously-mentioned subdivisions are exacerbated at these stages due to increased ischemia, capillary closure and leakage of vessels (Hudson, 2008; Meyerle, 2009).

Clinically significant macular edema

Diabetic macular edema occurs as a result of a breakdown of the endothelial cell tight junctions that comprise the inner blood-retinal barrier (Hudson, 2008; Meyerle, 2009; Watkins, 2003). It is characterized by thickening of the retina as a consequence of leakage of fluid and plasma constituents from the vasculature (Hudson, 2008; Meyerle, 2009). Diabetic macular edema is referred to as clinically significant macular edema (CSME) when it threatens the center of the macula, which can lead to serious visual impairment (ETDRS, 1991c; Watkins, 2003). The ETDRS defines CSME as one of the following:

I. Retinal edema or thickening at or within 500 µm of the fovea

II. Hard exudates at or within 500 µm of the fovea if it is associated with thickening of the adjacent retinal area

III. Thickening of the retina that is 1 disc diameter (1500 µm) (Watkins, 2003) or larger with part of the it within 1 disc diameter of the fovea

Clinically significant macular edema can occur at any stage of DR however it is the most common cause of vision loss in patients with NPDR (Meyerle, 2009).
Figure 1.1. Dilated fundus photograph of the right eye of a patient with NPDR. Dot and blot hemorrhages and microaneurysms are scattered throughout. Hard exudates are clearly visible inferotemporal to the optic disc and just below the macula. Cotton wool spots are also visible temporal and superior to the macula.

Source: http://www.neec.com/Pages/Services/Vitreoretinal/Diabetic_Retinopathy.php

**Proliferative Diabetic Retinopathy (PDR)**

In addition to the clinical features of NPDR, PDR is characterized by neovascularisation or the growth of new blood vessels forward into the vitreous in the optic disc area or elsewhere in the retina (Hudson, 2008; Watkins, 2003). An example of this can be seen in Figure 1.2. The fragile new vessels can proliferative and grow into the vitreous (Hudson, 2008; Watkins, 2003). Movement of the vitreous as a result may give rise to
haemorrhage, which may reduce vision (Gibson, 2001). Another complication of neovascularization is tractional retinal detachment, which occurs due to the contraction of fibrovascular tissue that grows between the new vessels and the posterior-hyaloid face (Gibson, 2001). New vessels fragile / rip easily

![Figure 1.2: Dilated fundus photograph of patient with PDR. Neovascularization and thin-walled, tortuous blood vessels are visible not just around the optic disc but elsewhere in the retina. In addition, hard exudates, hemorrhages and microaneurysms can be seen nasal to the optic disc.](image)

Source: Courtesy of Dr. Wai-Ching Lam
1.2.3 Primary Causes of Vision Loss

The major causes of visual impairment in patients with DR are CSME and PDR (Hudson, 2008; Watkins, 2003). Clinically significant macular edema, the primary cause of visual impairment in patients with T2D and NPDR, leads to slow and progressive vision loss. It can lead to serious vision loss in 20% of patients in two years if left untreated as opposed to 8% if treated in a timely manner (Watkins, 2003). On the contrary, PDR, the predominant cause of vision loss in patients with T1D, can result in sudden and severe vision loss due to tractional retinal detachment (Hudson, 2008).

1.2.4 DR Detection and Management

Irrespective of the duration of diabetes, DR rarely occurs in prepubertal children with T1D who are below ten years old. There is however a dramatic rise in prevalence in post-pubertal children after five years of disease duration (R. Klein et al., 1984a). Accordingly, the CDA recommends that screening for DR be initiated five years after the onset of T1D in all patients over the age of 15 and that screening be performed annually if DR is not present (CDA, 2008).

Methods for screening of DR include a dilated ophthalmic exam, indirect slit-lamp fundoscopy or seven-fields $30^\circ$ colour stereoscopic fundus photography, which is the gold standard (CDA, 2008).

Treatment includes retinal laser photocoagulation and vitreoretinal surgery. Laser photocoagulation is only recommended for patients with high-risk characteristics such as
disc neovascularization or any neovascularization with vitreous hemorrhage (DRS, 1981), which is typically seen in patients with severe NPDR, high-risk PDR or clinically significant macular edema (Aiello et al., 1998). Different modes of laser photocoagulation are applied for different pathologies. Panretinal or scatter photocoagulation (PRP) is used to treat PDR and involves the application of several burns of specific sizes to the peripheral retina which slows down the development of new vessels and facilitates their regression (Chalam, 2005). The DRS study (1981), which investigated the effectiveness of PRP in reducing the risk of vision loss due to PDR, showed that the surgery has significant benefits, with severe vision loss seen in 4% of treated eyes as opposed to 15.9% of untreated eyes. The greatest benefits were observed among patients with high risk characteristics (DRS, 1981).

While the DRS and ETDRS studies have demonstrated the effectiveness of laser photocoagulation in decreasing further vision loss, it is important to note that laser photocoagulation does not typically restore vision that is already lost (Fong et al., 2003). In addition, laser surgery is accompanied by serious complications including reduced peripheral and parafoveal vision (Hudson, 2008), reduced night vision and in some cases, increased macular edema (Aylward, 2000).

Vitrectomy surgery is performed in patients with nonclearing vitreous hemorrhage, tractional retinal detachments and very severe PDR (Ali, 2006). The surgery, which involves removing the vitreous humor has been shown to be highly beneficial in patients when performed early (Pollack, Leiba, Bukelman, Abrahami, & Oliver, 1992). The surgery however is also not without complications which may include eye pain, vision
loss, retinal tears and acceleration of cataract development if the lens is touched during the procedure (Aylward, 2000; Chalam, 2005).

Diabetic retinopathy is often asymptomatic until the later stages of the disease when the risk of vision loss is high. Given that surgical intervention cannot reverse pre-existing vision loss, the best strategy for preventing or slowing the progression of DR includes regular screening and tight control of blood pressure, blood glucose and cholesterol levels (Hudson, 2008).

1.2.5 Risk Factors

Age

The WESDR study demonstrated that an increase in age was significantly associated with an increase in the prevalence and severity of DR in patients with T1D (R. Klein et al., 1984a). This finding is consistent with results from other population-based studies (Ballard et al., 1986; Burger, Hovener, Dusterhus, Hartmann, & Weber, 1986; Moss, Klein, & Klein, 1998). The WESDR study also reported that DR was rare in patients below the age of 13 regardless of duration of diabetes (R. Klein et al., 1984a).

Age at diagnosis

After controlling for duration of diabetes, the WESDR found no significant association between age at diagnosis and the four-year incidence or progression of DR in either the younger or older onset groups (R. Klein, Klein, Moss, Davis, & DeMets, 1989a, 1989b).
Duration of Diabetes

Studies have shown duration of diabetes to be significantly associated with increased incidence and severity of DR (Burger et al., 1986; R. Klein et al., 1984a). The WESDR study reported that after 20 or more years of diabetes, 98% of individuals in the younger onset group (< 30 years old at diagnosis) developed some degree of DR (R. Klein et al., 1984a). In addition, the four-year incidence of PDR was reported to increase from 0% during the first three years post-diagnosis to 28% after 13 to 14 years of diabetes (R. Klein et al., 1984a).

Sex

Several studies have indicated a greater prevalence of DR in males (Bodansky et al., 1982; Dornan et al., 1982; R. Klein et al., 1984a; R. Klein et al., 2008; Malone, Grizzard, Espinoza, Achenbach, & Van Cader, 1984). In the 25-year incidence WESDR study, males had a 33% increased risk of DR progression independent of other risk factors (R. Klein et al., 2008). Studies involving adolescent populations however have indicated a higher prevalence of DR among females due to an earlier onset of puberty (Malone et al., 1984), greater insulin resistance (Moran et al., 1999) and a greater requirement for insulin during puberty (Komulainen, Akerblom, Lounamaa, & Knip, 1998; Mortensen, Villumsen, Volund, Petersen, & Nerup, 1992) compared to males.

Puberty

Puberty often worsens metabolic control in adolescents with T1D (Daneman, Wolfson, Becker, & Drash, 1981; DCCT, 1994b; Mortensen et al., 1992) as a result of a
Combination of psychosocial and physiological factors. The psychosocial aspect includes the transition from dependence on the immediate family for care to a more independent lifestyle (Daneman & Frank, 1996). Physiologically, several major hormonal changes take place including an increase in insulin-like growth factor I, increased insulin resistance and activation of the hypothalamic-pituitary-gonadal axis, that lead to the development of secondary sexual characteristics and a growth spurt (Hamilton & Daneman, 2002).

Studies have demonstrated poor glycemic control in adolescents. In a study that included approximately 3000 children and adolescents with T1D from 18 countries, adolescents had significantly higher HbA1c values in comparison with younger children (Mortensen & Hougaard, 1997). In the Diabetes Control and Complications Trial (DCCT), adolescents were found to have HbA1c values 1% higher on average than those found in adults in both intensive and conventional treatment groups (DCCT, 1994b).

In the WESDR study cohort, post-pubertal individuals were 3.2 times more likely to have DR than pre-pubertal individuals in the younger-onset group after controlling for diastolic blood pressure and duration of diabetes (B. E. Klein, Moss, & Klein, 1990). The WESDR study as well as others have also found that duration of diabetes after puberty significantly increase the risk of DR compared with duration of diabetes before puberty (Frank et al., 1982; Jackson, Ide, Guthrie, & James, 1982; B. E. Klein et al., 1990; R. Klein et al., 1984a; R. Klein, Klein, Moss, Davis, & DeMets, 1985; Knowles, Guest, Lampe, Kessler, & Skillman, 1965; Malone et al., 1984; Murphy et al., 1990).
Glycemic control

Hemoglobin A\textsubscript{1c} forms from the glycosylation of haemoglobin A, a process which occurs throughout the life span of an erythrocyte (approximately 120 days) (Bunn, Haney, Gabbay, & Gallop, 1975; Bunn, Haney, Kamin, Gabbay, & Gallop, 1976). Therefore, measuring the HbA\textsubscript{1c}\% value gives an indication of the amount of glucose in the systemic circulation for the preceding three months or so. It is thus used as a measure of glycemic control in patients with diabetes.

Several epidemiological studies have shown that poor glycemic control is associated strongly with an increased incidence and progression of DR. A key study to establish this relationship is the DCCT (1993). The study examined the effect of intensive glycemic control on the incidence and progression of DR. Participants, including T1D patients with no DR and patients with mild to moderate DR at baseline, were randomly assigned to an intensive or conventional treatment group. Results demonstrated that intensive glycemic control significantly reduced the mean risk of developing DR by 27\%. It also resulted in a 23\% decrease in macular edema in the DR patients, a 56\% reduction in the number of patients requiring panretinal photocoagulation, and a 78\% decline in the progression of DR in the patients with no DR at baseline. The data reflects a mean follow-up period of 6.5 years. In addition, the WESDR study found that a higher HbA\textsubscript{1c} at baseline was significantly predictive of greater incidence and progression of DR, as well as and the incidence of PDR and macular edema (R. Klein, Klein, Moss, & Cruickshanks, 1994; R. Klein, Klein, Moss, Davis, & DeMets, 1988; R. Klein et al., 2008; R. Klein, Moss, Klein, Davis, & DeMets, 1989). These studies demonstrate how crucial it is that patients monitor their HbA\textsubscript{1c} levels and strive to maintain them close to the normal range.
1.2.6 Pathogenesis and Histopathology of DR

Structure and Physiology of a Healthy Retina

The retina is primarily composed of neural tissue which is transparent and thus not visible during clinical examination. Normal vision is dependent on proper functioning and communication among the major retinal cell types including neurons, the vascular cells, glia and microglia (Antonetti et al., 2006; T. W. Gardner, Antonetti, Barber, LaNoue, & Levison, 2002).

Normally, an intact blood retinal barrier (BRB) helps maintain retinal structure and function (Kaur, Foulds, & Ling, 2008). The BRB separates the systemic circulation from the neural tissue and is selectively permeable (Antonetti et al., 2006; Kaur et al., 2008). It has two major components, namely, the inner (iBRB) and outer (oBRB) components (Kaur et al., 2008). The iBRB is comprised of tight junctions between adjacent capillary endothelial cells which are nonfenestrated (Shakib & Cunha-Vaz, 1966). Other cell-types that contribute to the integrity and proper functioning of the iBRB include pericytes that are separated from the endothelial cells by a basal lamina (Kaur et al., 2008). Pericytes are cells that locally regulate blood flow downstream of the arterioles which are primarily responsible for blood flow regulation (personal communication Chris). In addition, Muller cells and astrocytes, which wrap their foot processes around the capillaries, maintain the BRB and help regulate neuronal activity (T. W. Gardner et al., 2002; Kaur et al., 2008). Muller cells make contact with blood vessels and neurons in the nerve fiber and inner plexiform layers whereas astrocytes are limited to the nerve fiber and ganglion cell layers (Antonetti et al., 2006). Astrocytes make contact with blood vessels and
amacrine cells (Antonetti et al., 2006). Both glial cells provide nutrition and regulate neuronal activity (Antonetti et al., 2006). The oBRB, also selectively permeable, is composed of tight junctions between retinal pigment epithelial cells and separates the outer neural retina from the fenestrated capillaries of the chorio-capillaris (Cunha-Vaz, 1976). The main functions of the BRBs include regulating the delivery of nutrients and removal of waste products between the retina and blood vessels, protecting the neural retina from inflammatory cells found in systemic circulation and their toxic products (T. W. Gardner et al., 2002; Kaur et al., 2008).

**Microvascular Pathology in DR**

The exact mechanisms and fundamental cause(s) of DR are yet to be determined however several histological and biochemical changes have been observed.

While the role of the oBRB in DR is unclear, the breakdown of the iBRB, induced by tissue hypoxia, plays an important role (Kaur et al., 2007). Breakdown of the iBRB in DR has been associated with several changes that may be attributed to hyperglycemia, (Joussen et al., 2003), including the loss of pericytes (Cogan et al., 1961; Kuwabara & Cogan, 1963), degeneration of endothelial cells (Engerman, 1989) and thickening of the capillary basement membrane (Engerman, 1989; Kaur et al., 2008). Damage to the blood vessels leads to capillary closure and hypoxia, which in turn lead to the up-regulation of vascular permeability factors (Kaur et al., 2008) such as the vascular endothelial growth factor (VEGF). The vascular endothelial growth factor, which is reportedly expressed in Muller cells, vascular endothelial cells, and astrocytes (Kaur et al., 2008), induces increased vascular permeability (Mayhan, 1999) which ultimately leads to increased
ischemia and edema and later on, the growth of new blood vessels. These new blood vessels lack the structural integrity of the mature retinal vessels (Lawrenson, 2000). They lack proper tight junctions, show fenestrations and have a reduced number of pericytes (Lawrenson, 2000). These features make them fragile and therefore susceptible to hemorrhage.

**Neuronal Abnormalities**

Diabetic retinopathy is commonly considered to be solely a microvascular disease as its clinical features, microvascular lesions, are clearly detectable via ophthalmoscopy. There is a significant amount of evidence however, in the form of morphological and functional retinal changes, which supports the view of DR as both a vascular and a neurodegenerative disease.

As early as 40 years ago, Bloodworth (Bloodworth, 1962) and Wolter (Wolter, 1961) observed neuronal cell loss in human diabetic eyes. Since then, several animal and human studies have demonstrated cell loss and morphological abnormalities in various retinal cell types due to diabetes.

Studies have shown impairment of retinal glial cells which integrate vascular and neuronal activity, during diabetes. For instance, astrocytes significantly decrease the expression of the intermediate filament called the glial fibrillary acidic protein (GFAP), which correlates with astrocyte function (T. W. Gardner et al., 2002). The decrease in GFAP is therefore indicative of astrocyte dysfunction (T. W. Gardner et al., 2002). There is also evidence that Muller cells which are known to proliferate during PDR (Hiscott et al., 1984), are altered before the onset of any overt vasculopathy. Studies have
demonstrated that Muller cells display a decrease in the activity of glutamate transporters (Li & Puro, 2002) and a decrease in ability to convert glutamate to glutamine (Lieth, LaNoue, Antonetti, & Ratz, 2000; Mizutani, Gerhardinger, & Lorenzi, 1998). This in turn results in an increase in extracellular glutamate, and glutamate excitotoxicity has been implicated in neuronal loss in DR (Barber, 2003). Gardner et al. (2003) suggest that glial cell dysfunction in diabetes may impair their ability to maintain and induce the BRB.

Microglia also increase in size and become more active during diabetes, particularly in the ganglion cell and inner nuclear layers (Rungger-Brandle, Dosso, & Leuenberger, 2000; Zeng, Ng, & Ling, 2000). When activated, they release various cytokines and chemokines that further increase retinal vascular permeability and ultimately induce inflammation in the diabetic retina (T. W. Gardner et al., 2002).

Diabetes also induces apoptosis of other retinal neurons. Studies in rats in which diabetes has been induced, have shown loss of ganglion cells early in the course of diabetes (Barber et al., 2005; Barber et al., 1998; Martin, Roon, Van Ells, Ganapathy, & Smith, 2004) as well as the corresponding decrease in thickness of the nerve fiber layer (Sugimoto et al., 2005) as a result of loss of inner retinal neurons. Bipolar and amacrine cells may also undergo apoptosis (Barber et al., 1998). Anatomical evidence of thinning of the outer nuclear layer, apoptosis and shortening of photoreceptor outer segments have also been observed in diabetic rats (Aizu, Oyanagi, Hu, & Nakagawa, 2002; Park et al., 2003). Short wavelength cones (S cones) have been shown to be particularly vulnerable to the effects of diabetes. Histological studies have shown a significant loss of S cones in humans with DR (Mortlock, Chiti, Drasdo, Owens, & North, 2005). Studies have reported a tritan or blue-like defect in patients with DR (Barton, Fong, & Knatterud, 2003).
that increases with the progression of the disease (Mortlock et al., 2005).

Functional changes in retinal neurons and the visual pathways have been demonstrated by psychophysical and electrophysiological studies in patients with diabetes. For instance, colour vision and contrast sensitivity have been reported to be impaired (Simonsen, 1980). Several studies using visual electrophysiological techniques have demonstrated neuronal dysfunction at the level of the retina in patients with diabetes before the onset of clinically visible DR, and increasing with disease progression.

1.2.7 Visual Electrophysiology

Visual electrophysiology involves the measurement of functional changes in the retina and visual pathways in response to visual stimuli. The electroretinogram is a visual electrophysiological technique that measures the functional integrity of the retina. Modifications to the stimulus can enable examiners to assess aggregate responses from cells in the entire retina, or localized responses from specific retinal locations and specific layers of the retina. Studies using the electroretinogram and its various modifications have demonstrated neuro-retinal dysfunction in patients with diabetes and no vascular lesions, as well as in patients with DR.
The Full-Field Electroretinogram

The full-field electroretinogram (ERG) is a noninvasive tool for studying retinal function in vivo. It can be recorded using a corneal contact lens electrode that picks up electrical activity from the retina following a flash of light. The electrical activity is generated by radial currents in the extracellular space from retina neurons or from glial cells as a result of changes in the extracellular potassium ion concentration (Frishman, 2006).

Full-field ERGs are recorded under dark (scotopic) or light-adapted (photopic) conditions. Under photopic conditions, flashes of light are presented against a rod-suppressing light background and the responses are cone-driven (Frishman, 2006).

The responses, constituting voltage changes that vary over time, are plotted as waveforms. The resulting waveform is a global response that reflects the activity of all classes of retina cells (Frishman, 2006). Major components of the waveform include the “a wave”, the “b wave” and oscillatory potentials (OPs; see Fig. 1.3). The “a wave” is the initial negative deflection on the waveform that mainly results from the hyperpolarization of photoreceptors in response to light (Frishman, 2006; Leat, 1999). The “b wave” is the peak that follows the “a wave” and is the largest component of the ERG response. It is primarily attributed to the depolarization of ON bipolar cells (Gurevich & Slaughter, 1993; Knapp & Schiller, 1984; Robson & Frishman, 1995; Stockton & Slaughter, 1989). There is some experimental evidence suggesting a contribution from Muller cells however there is strong evidence that their contribution is smaller than previously thought in comparison to the contribution from bipolar cells (Karwoski & Xu, 1999; Robson & Frishman, 1995; Robson, Maeda, Saszik, & Frishman, 2004; Xu & Karwoski, 1994).
Oscillatory potentials are a series of high frequency, small amplitude wavelets climbing on the ascending limb of the “b wave” (Frishman, 2006; Leat, 1999). They may contain contributions from both rod and cone systems and are present under light and dark-adapted conditions (Peachey, Alexander, & Fishman, 1987). Most studies indicate that they originate near the inner plexiform layer (Heynen, Wachtmeister, & van Norren, 1985; Ogden, 1973) and may be generated by amacrine or interplexiform cells (Fishman, 1990). Whether ganglion cells contribute to the OPs is controversial. It has been suggested that their contribution may be more prominent under fully photopic conditions (E. Sutter, Bearse, M. A., Jr., 1999) as opposed to conditions also involving rod activity (Frishman et al., 1996; Ogden, 1973).

Figure 1.3. a) Cartoon of cross-section of the retina indicating the cells and locations where major components of the ERG waveform originate. b) Normal dark-adapted ERG waveform in response to a bright flash, indicating the “a wave”, “b wave” and OPs (arrow) on the ascending limb of the “b wave”.

Source: http://webvision.med.utah.edu/
Full-field ERG abnormalities have been identified in individuals with diabetes before the development of clinically visible DR, which worsens abnormalities in patients with DR. Oscillatory potentials particularly have been shown to be altered. Since the early 1960s, a number of studies have reported significantly delayed timing and reduced OP amplitudes in individuals with diabetes (Bresnick, Korth, Groo, & Palta, 1984; Bresnick & Palta, 1987a, 1987b; Frost-Larsen, Christiansen, & Parving, 1983; Hancock & Kraft, 2004; Juen & Kieselbach, 1990; Lovasik & Kergoat, 1993; Phipps, Fletcher, & Vingrys, 2004; Shirao & Kawasaki, 1998; Simonsen, 1980) indicating amacrine cell and inner retinal dysfunction. Reduced OP amplitudes and delayed times have also been found in patients with early stages of DR (Coupland, 1987; Juen & Kieselbach, 1990; Yonemura & Kawasaki, 1979). It has been suggested that reduced OP amplitudes in patients with diabetes conferred a ten-fold risk of progression to more severe stages of DR compared to normal OPs (Bresnick et al., 1984). Moreover, abnormal OPs have been thought to predict the onset of PDR better than vascular lesions seen on fundus photography or capillary nonperfusion observed on fluorescein angiograms (Bresnick & Palta, 1987b). In addition to altered OPs, animal and human studies have found reduced “b wave” amplitudes (Li, Zemel, Miller, & Perlman, 2002; Papakostopoulos, Hart, Corrall, & Harney, 1996).

Multifocal Electroretinogram

The multifocal electroretinogram (mfERG) is a noninvasive technique that unlike the full-field ERG enables the examiner to simultaneously assess localized neuro-retinal function by assessing focal responses from many locations across the retina. This localized nature of the mfERG makes it ideal for assessing neuro-retinal function in the
diabetic eye for a number of reasons. Firstly, the vascular lesions and edema associated with DR tend to be localized therefore it is reasonable to expect that the functional changes induced by diabetes will also be localized (Bearse et al., 2006). The full-field ERG response on the other hand is an average response constituting responses from normal and abnormal areas and is therefore not sensitive enough to detect localized abnormalities. Secondly, the spatial extent of various areas of dysfunction can be indicative of the probability that a particular localized retinal area will subsequently develop vascular abnormalities (Bearse et al., 2006). Finally, given that the various components of the mfERG response arise from different retinal layers (Hood, 2000; E. Sutter, 2000; E. Sutter, Bearse, M. A., Jr., 1999), examining retinal function measured using the mfERG has the potential to provide insight into the cellular mechanisms underlying the retinal changes induced by diabetes (Bearse et al., 2006).

The mfERG, developed by Sutter and colleagues (E. Sutter, 1991; E. Sutter & D. Tran, 1992) measures focal cone-driven responses from over 100 locations across the retina simultaneously in about eight minutes. The stimulus consists of an array of hexagons subtended over the central retina (see Fig. 1.4). The sizes of the hexagons, which increase with eccentricity, reflect the change in the cone density gradient (decreases away from the fovea). Hence responses of approximately equal amplitude across the array of hexagons are produced in controls (E. Sutter & D. Tran, 1992). The central hexagon contains a cross on which the subject fixates during the recording session. During stimulation, the hexagons alternate between black and white presentations according to a pseudo-random m-sequence (Hood, 2000). Each hexagon goes through the same m-
sequence but each one starts at a different point along the sequence (E. Sutter, 1991).

Each hexagon has a 50% probability of being black or white per frame (Hood, 2000).

Figure 1.4. Standard mfERG stimulus array of 103 hexagons subtended over the central retina.

Source: www.veris-edi.com; courtesy of Dr. Mike Fendick

After the recording, mfERG responses are derived from the cross-correlation between the continuous recording and stimulation sequence (Hood, 2006). The typical standard mfERG response (first order kernel) is a biphasic waveform (see Fig 1.5) with the main components including an initial negative peak (N1) followed by a positive peak (P1) and thereafter, maybe another negative peak (N2) (Hood, 2000). N1 is analogous to the “a wave” of the full-field ERG response as it is comprised of the same components whereas P1 is comprised of the same components of the full-field ERG “b wave” as well as OPs.
(Hood, Seiple, Holopigian, & Greenstein, 1997). The response however is dominated by bipolar cell activity with smaller contributions from photoreceptors and inner retinal cells (Hood, Frishman, Saszik, & Viswanathan, 2002). Therefore the response predominantly originates in the middle retinal layers.

**Figure 1.5.** Standard mfERG waveform and its major components


A few studies using the standard mfERG have demonstrated functional changes in patients with diabetes and no detectable signs of DR and also in patients with DR. Studies have found delayed implicit times in patients with diabetes and no DR compared to controls (Fortune et al., 1999; Han, Bearse et al., 2004a). These delays were found to be exacerbated in patients with NPDR (Fortune et al., 1999). Implicit time has also been shown to correlate with severity of DR and be spatially associated with vascular abnormalities (Fortune et al., 1999). Second-order responses, which some have suggested
arise from the inner retina, were also shown to have reduced amplitudes in patients with diabetes (Palmowski et al., 1997).

**Slow-flash Multifocal Electroretinogram**

The slow-flash mfERG paradigm (sf-mfERG), unlike the standard mfERG paradigm discussed in the preceding section, provides focal responses (see Fig. 1.6) from inner retinal neurons (these responses are termed multifocal oscillatory potentials). The interval of time between focal flashes in the standard paradigm is short such that the retinal response to the preceding flash does not fully develop before the subsequent flash is presented (Bearse, Han, Schneck, & Adams, 2004). Thus the higher-order effects are superimposed on the latter part of the standard mfERG first-order kernel response (Cai & Boulton, 2002; Frank, 2002; Hood, 2000, 2004; Keating, Parks, Smith, & Evans, 2002; Kondo, Miyake, Horiguchi, Suzuki, & Tanikawa, 1995; Palmowski et al., 1997; E. E. Sutter, 2001; E. E. Sutter & D. Tran, 1992) making them difficult to measure (Bearse, Han, Schneck, & Adams, 2004). The sf-mfERG paradigm allows for the isolation of the inner retinal contribution or oscillatory content of the standard mfERG response by separating focal flashes by a number of dark frames (Bearse, Shimada, & Sutter, 2000; Fortune et al., 2003; Hood et al., 1997; Rangaswamy, Hood, & Frishman, 2003; Wu & Sutter, 1995). In the following sections, the term multifocal oscillatory potential (mfOP) will be used in lieu of sf-mfERG.
Since previous full-field ERG studies have demonstrated inner retinal dysfunction (abnormal OPs) in individuals with diabetes and no DR, it is of interest to examine localized inner retinal function in these patients. The few studies examining mfOPs have reported decreased amplitudes (E. Sutter & D. Tran, 1992) and delayed implicit times in subjects with diabetes and no DR (Bearse, Han, Schneck, & Adams, 2004; Bearse, Han, Schneck, Barez et al., 2004; Kurtenbach, Langrova, & Zrenner, 2000) as well as in subjects with NPDR (Bearse, Han, Schneck, & Adams, 2004; Bearse, Han, Schneck, Barez et al., 2004; Onozu & Yamamoto, 2003), suggesting localized inner retinal dysfunction before overt DR lesions are clinically detected.

Figure 1.6. Sample mfOP waveform.
2. Purpose and Rationale

The purpose of this study is to determine whether poor glycemic control, measured using HbA$_{1c}$ levels, is predictive of increased localized neuro-retinal dysfunction in an adolescent population with type 1 diabetes before DR is clinically detectable.

Poor glycemic control is a strong risk factor for the progression and incidence of DR. Most notably, the DCCT study (1994) demonstrated that adolescents in an intensive treatment group had significantly lower HbA$_{1c}$ levels, and decreased incidence and progression of DR compared to those in the conventional treatment group. As discussed previously (see section 1.2.7), electrophysiological studies have shown abnormalities in neuro-retinal function in patients with diabetes and no clinically detectable vascular lesions. These abnormalities worsen with increasing severity of DR. Recently, after the initiation of the current project, one group found a correlation between HbA$_{1c}$ and standard mfERG implicit times ($R^2 = 0.22$) in 30 adolescents with T1D (Bronson-Castain et al., 2008a, 2008b). The study design of the current thesis project accounted for specific known confounders of the association between electrophysiological markers and HbA$_{1c}$.

Firstly, to the best of our knowledge, our sample size is the largest yet amongst other studies of localized neuro-retinal function in patients with diabetes. Secondly, the patients were recruited on site allowing more accurate assessment of diabetes history. Thirdly, blood glucose levels were monitored and maintained within 4 to 10 mmol/L throughout the testing session, which is important as acute changes in blood glucose levels have been shown to affect ERG responses (Klemp, Larsen et al. 2004; Klemp, Sander et al. 2005). Finally, the relationship between HbA$_{1c}$ and localized neuro-retinal function was tested, while taking into account other covariates such as duration of disease, age at testing and the sex of the patient.

It is of interest to examine the relationship between changes in glycemic control and localized neuro-retinal function. This knowledge is essential as it would support the application of tools used to evaluate localized neuro-retinal function as a practical means for assessing and monitoring patients who are at risk of later developing clinically visible DR. This idea is supported by Fletcher and colleagues (2007). In addition, as poor
glycemic control is a strong risk factor for DR, patients and clinicians can strive to achieve stricter HbA1c targets by altering management protocols if that patient is identified as having poor neuro-retinal function.

Importantly, this study was conducted in an adolescent population. This provides greater accuracy for identifying age at onset of diabetes, and results in the absence of potentially confounding factors such as other coexisting diseases and the effects of decades of insulin resistance (Goran, Bergman, Cruz, & Watanabe, 2002). Finally, considering that DR is often diagnosed when the disease is already in its later stages, research on early detection is essential to prevent vision loss. If tools assessing neuro-retinal function can identify patients at risk of developing early vascular lesions, these patients can be monitored and appropriate measures can be taken to prevent vision loss. Moreover, these patients may also be good candidates for clinical trials. Therefore given that the ultimate goal is to identify patients at risk of developing DR, adolescents are the ideal population to study as the effects of diabetes are still in their early stages.
3. Hypothesis

We hypothesize that poor glycemic control, measured using HbA$_1c$ levels, predicts increased areas of localized neuro-retinal dysfunction in patients with diabetes before DR is clinically visible.
4. Methods

4.1 Study Design

This is a cross sectional, controlled, and prospective study.

4.2 Recruitment

Participants with type 1 diabetes mellitus (T1D)

Adolescent patients with type 1 diabetes were recruited at The Hospital for Sick Children’s (SickKids) Diabetes Clinic in collaboration with Dr. Denis Daneman, an endocrinologist and Chief of Pediatrics at the hospital. A brochure explaining the nature of the study and its relevance, as well as the electrophysiological tests conducted during the study was provided to eligible participants.

Control participants

Age-similar participants who did not have diabetes were recruited using the following modes of advertising:

a. Siblings and friends of participants with type 1 diabetes were invited to take part in the study
b. Posters were displayed in the Visual Electrophysiology Unit at SickKids, as well as in community areas around the hospital
c. An advertisement was placed in the SickKids newsletter, Two Weeks, formerly known as This Week.
4.3 Inclusion Criteria

Participants with type 1 diabetes
  a. Duration of disease $\geq$ 5 years
  b. Aged 12 to 19 years, inclusive
  c. Normal visual development before diagnosis of diabetes

Control Participants
  a. Aged 12 to 29 years
  b. Normal visual development

4.4 Exclusion Criteria

Participants with type 1 diabetes
  a. Any severity of diabetic retinopathy
  b. Participants with hemoglobinopathy (i.e. those in whom we cannot obtain reliable HbA1c readings)

All participants
  a. Distance refractive error worse than $\pm$ 5 diopters
  b. LogMAR visual acuity worse than 0.3
  c. Any other eye disease (inherited, inflammatory, infectious etc.) including lenticular opacity greater than NC2, NO2, C2 and/or P1 according to the Lens Opacities Classification System II
  d. Psychiatric or neurological disorders known to affect retinal/visual function
  e. Systemic medication with known central nervous system effects

4.5 Patient Information

The SickKids database (KidCare) was used to obtain information such as glycated haemoglobin (HbA$_{1c}$) levels, dates of diagnosis of diabetes, whether the patient had any
psychiatric or neurological disorders, and systemic medications that the patient may be taking. Patient charts were also accessed if necessary.

4.6 SickKids Research Ethics Board Approval

This study underwent a scientific peer review by members of the SickKids Ophthalmology and Vision Sciences Department. The study was approved the SickKids. See Appendix A for the letter of approval.

4.7 Consent

This research conforms to the tenets of the Declaration of Helsinki.

All participants had the capacity to give consent. Consent forms for patients and controls included the names and contact information for all individuals involved in the study, as well as their respective roles in it. The forms also consisted of a statement of purpose of the research, a description of the protocol, plus the potential harms and benefits of participating in the study. Further details on the content of the consent forms can be seen in Appendix B.

All participants had sufficient time to read the consent forms. In addition to reading the form, the study was explained verbally to each participant, and any questions or concerns were addressed fully by research staff before the consent form was signed.

4.8 Study Protocol

All participants were tested once in the Visual Electrophysiological Unit. A coin was tossed to assign one eye randomly for the study. All clinical, psychophysical, and electrophysiological testing was therefore monocular, and the eye not selected for testing was occluded.
4.8.1 Patient Intake

The Research Co-ordinator performed an intake examination for control and patient participants. This included medical and ophthalmic history, visual acuity, colour vision and contrast sensitivity testing. The participant’s medical history and any drugs the participant was taking at the time were documented.

4.8.2 Blood Glucose Level Monitoring

A Registered Nurse from the SickKids Endocrinology Department was available to monitor patients’ blood glucose levels periodically during their visit to ensure patient safety. As figure 4.1 illustrates, blood glucose readings were taken before, during and after tests.

<table>
<thead>
<tr>
<th>Consent obtained</th>
<th>Intake and psychophysical</th>
<th>Clinical exam</th>
<th>mfERG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blood glucose</td>
<td>Blood glucose</td>
<td>Blood glucose</td>
</tr>
</tbody>
</table>

**Figure 4.1.** Timeline of blood glucose readings taken periodically during participant’s visit

To prevent variability in blood glucose readings due to the various glucose meters used by patients, readings from all patients were obtained using a calibrated One Touch Ultra Blood Glucose Monitoring System (LIFESCAN, a Johnson & Johnson Company). We also provided One Touch UltraSoft™ Lancets and One Touch Ultra glucose test strips (LIFESCAN, a Johnson & Johnson Company).

Blood glucose levels were required to be within the range of 4 to 10 mmol/L as short-term changes in blood glucose levels can affect retinal function. In the event that patients’ blood glucose readings were over 10 mmol/L during the initial reading, the nurse suggested moderate exercise such as taking a short walk or an appropriate dose of insulin.
In the event that the blood glucose reading was too low for the patient’s safety, the nurse provided fruit juice or crackers until the patient’s blood glucose levels returned within a safe range.

4.8.3 Clinical Examination and Psychophysical Testing

Please refer to Appendix D for refractive errors, visual acuity and contrast sensitivity scores.

Visual Acuity

Visual acuity refers to the smallest detail that an individual can perceive (Leat, Shute, Westall 1999). It measures one’s ability to discriminate two objects separated at a specific distance at optimal levels of illumination and high contrast relative to a background (Kniestedt 2003).

Visual acuity was determined using ETDRS Charts and was measured using logMAR units. The logarithm of the minimum angle of resolution (logMAR), refers to the logarithm of the smallest angle separating adjacent letters (Leat, 1999).

Participants were asked to read the letters on the chart while sitting at a distance of 4 meters. Each letter that was correctly identified has a score of 0.02 logMAR.

Contrast Sensitivity

Contrast sensitivity measures the threshold or the lowest contrast that can be detected (Leat, 1999). It is a measure of one’s ability to distinguish objects of low contrast from their background.

The Pelli-Robson Contrast Sensitivity Chart (Pelli et al 1988) was used to score contrast sensitivity in patient and control participants. The chart consists of letters of shades of grey of decreasing intensity. The letters are arranged in groups of three, with two groups
appearing per line. Letters in each triplet have the same level of contrast. The level of contrast decreases with each subsequent group but the size of the letters remains constant.

The participant was seated one meter away from the chart and was asked to read letters starting from those at highest contrast and continuing down the chart to those of the lowest contrast until the last letter visible to the participant was reached. Each letter that was correctly identified was given a score of 0.05 log units.

4.8.4 Colour Vision

H.R.R. (Hardy Rand & Rittler) Pseudoisochromatic Plates

The HRR is a clinical test that can be used for the screening and diagnosis of acquired and congenital colour vision defects (protan, deutan and tritan) (Leat, Shute & Westall 1999). The test was administered under standard illumination (C.I.E. source C).

The participant was presented first with the demonstration plates (plates 1 to 4), which were not scored, to demonstrate how the test worked. The examiner explained that each plate may include one or two coloured symbols (‘X’, ‘O’ or ‘Δ’) against a background of circles of various sizes and of various shades of grey. The participant was asked to identify the symbol(s) on each plate.

Thereafter, the participant was presented with the screening plates (plates 5 to 10), which were scored. Plates 5 and 6 screen for blue-yellow colour defects and plates 7 to 10 screen for red-green defects. The test was scored using a scoring sheet with a check mark placed near each correctly identified symbol. If a participant made errors on plates 5 or 6, which indicates a blue-yellow defect, the examiner proceeded to show diagnostic plates 21 to 24. If a participant made errors on plates 7 to 10, indicative of a red-green defect, the examiner proceeded to diagnostic plates 11 to 20. Identified defects are classified as mild, moderate or severe.
Mollon-Reffin Minimalist

This is a clinical test that primarily serves to screen for acquired colour vision defects (Leat, Shute & Westall 1991). The test consists of coloured chips: 3 sets coinciding with the protan (green), deutan (red) and tritan (blue-yellow) axes, one set of grey chips, and one orange test chip. The protan set consists of a series of 5 chips, the deutan and tritan sets of 6 chips. All sets are arranged from most (# 6) to the least saturated (# 1). The set of grey chips are of various saturations.

Initially, the task was demonstrated to the participant. The examiner added the orange chip to a group of 5 grey chips, mixed the chips randomly and asked the participant to identify the “odd-one-out”. The grey chips serve as “distractor chips” and are used to ensure that the task is not solved on the basis of perceived lightness (Leat, Shute & Westall 1999).

The examiner then proceeded with presenting chips from each of the three colour series, one at a time using the “odd-one-out” procedure explained above. The test was scored by noting the number (saturation) of the least saturated chip that was correctly identified for each of the three colour series.

4.8.5 Cycloplegic Refraction and Fundus Exam

Each patient had a cycloplegic refraction and a dilated fundus exam performed by qualified personnel. This was done to ensure that participants did not have any pre-existing eye diseases and that refractive errors were within the range specified in the inclusion criteria for the study.

To perform the refraction and fundus exam, a topical corneal anesthetic (0.5% proparacaine) and mydriatic eye drops (2.5% phenylephrine and 1% tropicamide) were used. Pupil size post-dilation was 8 to 9 mm.

A retinoscope was used for an objective assessment of refractive error and an ophthalmoscope was used to examine the posterior pole (fundus) and ocular media.
4.8.6 Multifocal Electroretinogram

Recordings were performed in accordance with the International Society for the Clinical Electrophysiology of Vision (ISCEV) guidelines (Hood et al., 2008).

A gold-plated cup electrode was placed on each participant’s forehead to serve as ground. A drop of 0.5% carboxymethylcellulose sodium solution was placed onto the surface of a bipolar Burian-Allen (Hansen Ophthalmic Development Laboratory, Iowa City, IA, USA) contact lens electrode to protect the cornea. The contact lens electrode, which provides high quality recordings of localized electrical signals generated from retinal neurons, was placed onto the cornea. The Burian-Allen lens is composed of a speculum, which holds the eye lids apart to prevent the participant from blinking and contains the reference electrode. The Burian-Allen also consists of a clear lens that makes contact with the corneal surface, and a circular wire that surrounds the lens and acts as the active electrode. Some of the lenses contained a built-in infrared light source whereas others did not; in which case, an external infrared light source was placed at the outer canthus of the eye. The infrared source and an infrared fundus camera enabled the examiner to view and monitor the fundus image throughout the recording to ensure that the participant’s fixation did not wander away from the stimulus. The eye that was not being tested was occluded with an eye patch to prevent blinking and the possible introduction of electrical artifacts. Before the recordings began, the participant was seated with his/her head stabilized using a chin rest. The participant could view the stimulus when looking into the fundus camera, which was positioned in front of the tested eye. Before testing began, the examiner adjusted the stimulus unit to bring the stimulus into focus for the participant.

Recordings were performed using the VERIS™ Science System 5.1.12 (Electro-Diagnostic Imaging, Inc., Redwood City, CA, USA). Total recording time for each mfERG paradigm was divided into 16 segments so that participants could rest at the end of a segment if necessary and so that if a segment resulted in a poor recording, it could be discarded and rerun. During the recordings, raw as well as processed mfERG traces, could be viewed on the computer monitor. In addition to the fundus image, these tracings
were also monitored to ensure the quality of the recording for each segment. Segments with poor quality recordings were repeated.

Raw signals were amplified using an AC amplifier (GRASS ICP511 [Grass-Telefactor, Astro-Med Inc., West Warwick, RI, USA]) for both the standard and slow-flash mfERG recordings.

**Standard mfERG**

Before testing, another drop of topical corneal anesthetic was administered. The stimulus (Fig. 4.2), which was comprised of an array of 103 hexagonal elements, subtended the central 45 degrees of the retina. The hexagons were scaled with eccentricity by a stretch factor of 10.46 to produce responses of approximately equal amplitudes in individuals with normal retinal function. A cross, on which each participant would fixate throughout the recording, is superimposed on the central hexagon, which is the smallest of the 103. The size of the cross was adjusted by the examiner if necessary, so the participant could see it. Each hexagon alternated in a pseudo-random fashion between white and black according to a predetermined m sequence \(2^{15} - 1\) with a base period (inter-stimulus interval) of 13.3 ms. Luminance of each of the white and black hexagons was 200 cd/m\(^2\) and 0 cd/m\(^2\) respectively. Each hexagon per video frame had approximately a 50% probability of being white or black. As a result, the overall mean luminance of the stimulus display was maintained at approximately 100 cd/m\(^2\). Signals were amplified by 50,000 times and were filtered using an analog bandpass filter of 10 to 300 Hz. Total recording time was 8 minutes.
Figure 4.2. The standard mfERG stimulus array of 103 hexagons with the central fixation cross. The size of the hexagons increases with eccentricity.

Primary responses (first order kernel responses) were extracted for each hexagon using a cross-correlation technique implemented by the VERIS software, which involves correlating the continuously recorded ERG signal with the stimulation sequence (Hood, 2000). An example of the resulting array of traces is illustrated in Fig. 4.3.
Figure 4.3. An example of a standard mfERG trace array from the right eye of a healthy individual. Each waveform is associated with each of the 103 hexagons.

MfOPs

Before recording mfOPs, another drop of 1% methylcellulose solution was applied to the contact lens to reduce any slight discomfort the participant might experience.

The set-up and stimulus for the recording was similar to that used for the standard mfERG with a few exceptions. The stimulus, although also an array of hexagons, was comprised of 61 as opposed to 103 hexagons. The 61 hexagons were scaled with eccentricity by a factor of 12.86. The m sequence was of length $2^{12} - 1$ with each step consisting of 5 dark frames. That is, the first video frame would have a 50% chance of being white while the subsequent 5 frames would be dark. Therefore the base period was 80 ms. Total recording time was approximately 8 minutes.
Signals went through two stages of filtering. During the first stage, signals were filtered with an analog filter of 10 to 300 Hz implemented by the amplifier. During the second stage, signals went through a high pass digital filter of 75 to 300 Hz, isolating only high frequency waveform components over 75 Hz.

### 4.8.7 Stereoscopic Fundus Photography

Patients had stereoscopic colour fundus photographs taken with a Zeiss Digital FF 450plus Fundus Camera by a retinal imaging specialist at SickKids to identify areas of retinal vascular changes. Seven fields of the fundus were photographed including Field 1 (centred on the optic disk) and Field 2 (centered on the macula) of the modified Airlie House classification (ETDRS 1991). The photographs were graded based on the Airlie House classification of DR (ETDRS 1991) and analyzed for the presence or absence of any severity of DR by retinal specialists Drs. Shelley Boyd or Wai Ching Lam.

### 4.8.8 Statistical Analysis

Descriptive statistics were performed on SPSS version 15.0 and regression modeling was performed on R version 2.8.1. Between group comparisons were conducted using the Mann-Whitney test where $p \leq 0.05$ was considered to be statistically significant.

**Sample Size Calculation**

Sample size was estimated using the general rule-of-thumb for regression modeling. The rule of thumb for logistic regression is that ten events are required for every covariate included in the model, while the rule for multiple linear regression is that ten to fifteen observations are required for every covariate included in the model (Harrell, Lee, Matchar, & Reichert, 1985). Since we judged that the Poisson and negative binomial regression models were closest in form to the multiple linear regression model, we chose to use the rule of thumb for multiple linear regression modeling. Also, based on a previous diabetes study involving adolescents that was conducted in the lab, we assumed
that 15% of the recruited patients may be excluded from the study for various reasons. These include not showing up for testing sessions or having blood glucose levels that are too high to be titrated within a reasonable amount of time. Taking this into consideration, we estimated that 47 to 71 participants would need to be recruited and of these, 40 to 60 participants would be required for the study.

Standard mfERG

Timing and amplitude of each waveform was measured using Hood and Li’s template stretching method (Hood & Li, 1997), which is illustrated in Fig. 4.4. This method involves generating a mean waveform from control data called the template waveform. Focal responses from each participant were compared with the template wave, which was multiplicatively scaled along the amplitude and time dimensions to obtain the least squares best fit estimate of amplitude and timing (Hood & Li, 1997). Z-scores for timing and amplitude parameters of the response waveforms were calculated for each hexagon per participant. A hexagon was defined as abnormal for the timing parameter if its associated z-score was \( \geq 1.96 \), and for the amplitude parameter if its associated z-score was \( \leq -1.96 \). Total number of abnormal hexagons for timing and amplitude per participant was calculated.
Figure 4.4. Demonstration of stretch fitting a template wave to a target waveform. The solid line represents a target waveform, the dashed line represents the template waveform stretched by a factor of 2 and the dotted line represents the same template waveform stretched by a factor of 0.5.


MfOPs

The amplitude and timing per waveform were derived by taking the mean timing and summing the amplitudes of the three most prominent peaks of the waveform. This is illustrated in Figure 4.5.
Figure 4.5. A sample multifocal OP waveform with its three most prominent peaks indicated by the three arrows. Amplitude and timing measurements of the three peaks were averaged.

Similar to the standard mfERG, z-scores for amplitude and timing parameters were determined for each hexagon’s associated waveform per participant. A hexagon was defined as abnormal for the timing parameter if its associated z-score was $\geq 1.96$, and for the amplitude parameter if its associated z-score was $\leq -1.96$. Total number of abnormal hexagons for timing and amplitude per participant was calculated.

Regression Modeling

Regression modeling was conducted using data from the patient group. Independent variables considered for inclusion in the regression model comprised of HbA$_{1c}$ levels, time since T1D diagnosis, age at diagnosis of T1D, sex, and age at testing. The dependent variable of interest was the number of abnormal hexagons based on the timing of mfERG responses rather than amplitude. There are a number of reasons for not conducting the
regression modeling based on the amplitude of the responses. Studies have demonstrated that measurement of implicit time of standard first-order kernel mfERG responses is more sensitive in detecting neuro-retinal dysfunction in eyes with diabetes (no DR) and early NPDR (Fortune et al., 1999; Han, Bearse et al., 2004a; Han, Bearse et al., 2004b). Also, amplitude has been shown to have poor spatial correspondence with localized areas of retinal damage (Schneck et al., 2004). This is attributed to significantly greater inter-subject variability in amplitude measurements in comparison with implicit time measurements among healthy individuals (Fortune et al., 1999; Han, Bearse et al., 2004b). Bearse and colleagues (2006) also suggest a possible physiological explanation; namely, a delay in signal transduction through the retinal circuitry due to abnormal functioning of post-photoreceptor components including bipolar cells. It is expected that the amplitude of responses will not be significantly reduced (Hood 2002, 1999, Hare 2002) because the retinal tissue is likely not necrotic in diabetes or early NPDR and that the middle-inner rather than outer retina is primarily affected (Bearse et al., 2006).

**Poisson Regression**

Classical linear regression such as multiple regression is used to model data that is continuous, normally-distributed, and is a linear function of a set of independent variables. The independent variables, unlike the dependent variable, do not have to be continuous and can have a wide variety of distributions. The general equation for a linear regression model can be written as

\[ y = \beta_0 + \beta_1 X_{i1} + \beta_2 X_{i2} + \ldots + \beta_j X_{ij} + \epsilon \]

where \( y \) is the dependent variable, \( \beta_0 \) is the intercept, \( \epsilon \) is a normally-distributed error term, and the \( \beta \)s are parameter estimates which are an index of the amount of variation in the dependent variable explained by the associated independent variable (Dunteman, 2006).

Discrete outcomes (e.g. normal / abnormal) which are often encountered in the clinical sciences are not normally distributed. As this sort of data does not meet the assumption of normality, it cannot be modelled using classical linear regression techniques. Generalized linear models are an appropriate alternative (Lindsey & Jones, 1998). The poisson regression model is one example of a generalized linear model. The Poisson model is used to model discrete, non-negative count data, and assumes that the mean and variance...
are equal (Byers, Allore, Gill, & Peduzzi, 2003; Dunteman, 2006). This relationship between the mean and the variance must be met as it is incorporated into the model and the estimation of the $\beta$ values (Dunteman, 2006). The Poisson model also assumes that the Poisson-distributed DV is not a linear function of the $\beta$s. The function however can be linearized by conducting a non-linear transformation. This can be done by taking the natural logarithm of both sides of the general equation yielding the following equation:

$$\log(\mu_i) = \beta_0 + \sum_{j=1}^{p} \beta_j X_{ij},$$

where $\mu_i$ is the estimated mean of the Poisson-distributed dependent variable (Dunteman, 2006).

The residual deviance can be used to assess the goodness of fit (Dunteman, 2006; Kianifard & Gallo, 1995) of a model. When the observed variance of the dependent variable is significantly larger than the variance expected for a Poisson distribution, we encounter overdispersion in the model. This is indicated when the ratio of the residual deviance and degrees of freedom (sample size minus number of parameters in the model) is greater than one (Byers et al., 2003; Dunteman, 2006; Kianifard & Gallo, 1995). Overdispersion results in an inaccurate model as a consequence of the Poisson distribution assumption that the mean equals the variance (Byers et al., 2003; Dunteman, 2006; Kianifard & Gallo, 1995). The assumption results in the standard error estimates associated with each of the $\beta$s being much smaller than the true standard errors (Dunteman, 2006; W. Gardner, Mulvey, & Shaw, 1995). As a result, the t or z tests for the $\beta$ values are inflated and the associated p values are lower than they would actually be (W. Gardner et al., 1995). This may lead one to draw misleading conclusions from the regression.

The issue of overdispersion can be resolved by fitting a negative binomial regression model to the data (Allison, 1999; W. Gardner et al., 1995).
Negative Binomial Regression Modeling

The negative binomial regression model is an extension of the Poisson regression model. It is the model of choice for count data with a large number of zeroes and overdispersion. It takes this into account by incorporating an error term in the general equation, which is an extension of the Poisson regression equation (Agresti, 1990; Allison, 1999). Incorporation of this error term, also known as the dispersion parameter, results in a more accurate estimate of the standard errors, $\beta$ values, and associated p values. This results in a valid model that provides a better fit to the data (Byers et al., 2003).

Model Selection and Interpretation

A backward selection procedure was used to arrive at the final model. The procedure starts with the full model, which includes all the predictor variables. The aim is to cut the full model down to one that only includes the independent or predictor variables needed to fit the data well.

An alpha level of 0.157, which corresponds to using Akaike’s information criterion (discussed below) for selecting variables (Steyerberg, Eijkemans, & Habbema, 1999), was chosen as the criterion for removing a predictor variable from a model rather than 0.05. This was to ensure that variables that may make important contributions to the model are not excluded. Next, the full model including all the predictor variables was formulated. R automatically conducts a z test on each $\beta$ value associated with each predictor variable, and the p value is displayed. Variables with a p value > 0.157 are removed as they do not significantly explain the variability in the dependent variable. This resulted in the reduced model.

The fit of each model was assessed using the likelihood ratio test. The likelihood ratio statistic, which is equal to the difference in deviances of the two models being compared, has a chi-square distribution with the degrees of freedom equal to the difference in the number of parameters included in the two models (Dunteman, 2006). The likelihood ratio test was used to compare the reduced model with the full model to determine whether the variable removed from the full model made a significant contribution to the model. A p
value greater than 0.157 would indicate that the variable did not make a significant contribution. The likelihood ratio test was also used to determine whether the reduced model that was suspected to be the final model (p < 0.157 for all ßs) was significantly better than the null model (all ßs = 0). In other words, it was used to test the null hypothesis that none of the predictor variables in the suspected final model could explain the variability in the dependent variable.

In addition to conducting the likelihood ratio test, the p values associated with the ßs as well as the sign of each ß value was noted to determine whether it was logical based on theoretical knowledge of the research topic. Akaike’s Information Criterion (Akaike, 1973), which is provided in the R output and is another tool to assess the fit of the model, was also noted. The Akaike Information Criterion penalizes for overfitting a model to one’s particular data set by restricting the number of variables that can be added to the model (Lindsey & Jones, 1998). Therefore, it leads to the selection of the model that fits the data best and has the fewest number of parameters (Lindsey & Jones, 1998). A lower Akaike Information Criterion value is indicative of a better model (Lindsey & Jones, 1998).

The model with p values associated with the ßs less than 0.157, the lowest Akaike Information Criterion value, and appropriate LRT results was selected as the final model.

Regression Model with One-Year HbA1c Averages

The average HbA1c of each patient over a period of one year from the date of testing was obtained. Average values were obtained for 39 of the 48 patients as only one HbA1c value was available for the remaining patients. A regression model was generated by replacing the HbA1c values obtained closest to the date of testing with the one-year averages to further explore the relationship between glycemic control and neuro-retinal function.
5. Results

5.1 Participants

Eighty-five patients and 53 control subjects were recruited and scheduled for testing between March 2006 and November 2008. Of these 85 patients, 29 did not show up for their scheduled testing sessions. Two patients were excluded from the study – one due to high refractive error and the other because she was on medication with visual system and CNS side effects. A third patient could not undergo testing as his blood glucose reading was deemed too high by the nurse to be titrated within a reasonable amount of time. In addition, following analysis of their stereoscopic fundus photographs by retinal specialists, four patients were found to have vascular lesions. The fundus photograph of a fifth patient also showed a vascular lesion that was most likely not associated with DR. The vascular lesions are further described in Table 5.1. Due to the manifestation of vascular lesions on the fundus photographs of these five patients, their data were also excluded from analysis. Of the 53 control subjects, two were excluded from the study as they were on medications with visual system and CNS side effects, one was not tested due to equipment malfunction, one control subject’s visual acuity was worse than 0.3 logMAR, and data for the first four controls tested in 2006 is not available. Furthermore, mfOPs were not recorded in two control subjects due to experiencing discomfort from wearing the contact lens electrode, and data for one control are not available. Therefore, for these three control subjects, only the standard mfERG recording was available. Figure 5.1 illustrates the proportion of patients and controls who were tested after initial contact was made.
Figure 5.1. Summary of the proportion of patients (a) and controls (b) who were tested and the proportion of those who were not tested for various reasons.
Table 5.1. Fundus photography results for patients with DR.

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>305</td>
<td>F1, F2 - microaneurysms, cotton wool spots</td>
</tr>
<tr>
<td>307*</td>
<td>F1, F2 - large nerve fiber layer hemorrhage at disc margin</td>
</tr>
<tr>
<td>346</td>
<td>F6 - peripheral retinal hemorrhage</td>
</tr>
<tr>
<td>350</td>
<td>F1 - small microaneurysm vs dilated vessel below optic disc</td>
</tr>
<tr>
<td>380</td>
<td>F7 - small retinal hemorrhage in periphery</td>
</tr>
</tbody>
</table>

Overall, data for 48 patients and 45 control subjects were analyzed for the standard mfERG, and data for 48 patients and 42 controls were analyzed for the sf-mfERG. Their demographic information is presented in Table 5.2 (see Appendix C for details). On average, control subjects were older than patients at the time of testing by about two years; however, this difference was not found to be statistically significant (Mann-Whitney test, p = 0.15). Figure 5.2 illustrates the distribution of ages for participants in the patient (Fig. 5.2a) and control (Fig. 5.2b) groups. Half the patients have had diabetes for less than or equal to ten years and all have had diabetes for less than 15 years (see Fig. 5.3). Also, most of the patients (37 out of the 48) were diagnosed with T1D as young children and before the age of 10 (Fig. 5.4). Nearly all patients had HbA1c levels greater than the Canadian Diabetes Association’s recommended target of 7% with the highest being 12% (Fig. 5.5).

* As the retinal specialists did not find any other vascular abnormalities consistent with DR, this patient was thought to have some other condition.
Table 5.2. Demographic information for patient and control groups. Data is presented as mean ± standard deviation and the range is included in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age at testing (years)</strong></td>
<td>15.66 ± 1.76 (11.95 to 18.25)</td>
<td>17.55 ± 4.22 (12.15 to 27.05)</td>
</tr>
<tr>
<td>**Sex (Males/Females)</td>
<td>23/25</td>
<td>16/29</td>
</tr>
<tr>
<td>**Age at diagnosis (years)</td>
<td>6.24 ± 3.54 (1.41 to 13.12)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Time since diagnosis (years)</strong></td>
<td>9.40 ± 3.17 (4.90 to 14.2)</td>
<td>-</td>
</tr>
<tr>
<td><strong>HbA1c (%)</strong></td>
<td>8.71 ± 1.24 (6.4 to 12)</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 5.2. Distribution of ages at the time of testing of patients (a) and controls (b)
Figure 5.3. Distribution of time since diagnosis of T1D
**Figure 5.4.** Distribution of the ages at which patients were diagnosed with T1D

**Figure 5.5.** Distribution of patient HbA₁c levels closest to the day of testing
5.2 Clinical Examination and Psychophysical Testing

Visual acuity scores were available for 45 patients, contrast sensitivity scores were available for 43 patients, and refractive errors were available for 36 patients and for 21 control subjects. There was no significant difference in visual acuity or contrast sensitivity scores between patients and control subjects included in the analysis (see Table 5.3).

Table 5.3. Visual acuity and contrast sensitivity results presented as mean ± standard deviation

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual acuity (logMAR)</td>
<td>0.00 ± 0.10</td>
<td>-0.03 ± 0.12</td>
</tr>
<tr>
<td>Contrast sensitivity</td>
<td>1.71 ± 0.10</td>
<td>1.74 ± 0.13</td>
</tr>
</tbody>
</table>

HRR scores were available for 43 patients and all controls whereas minimalist scores were available for 43 patients and 44 control subjects. All available HRR scores were normal in patients and control subjects. Two patients had a slight tritan defect based on their Minimalist scores (P1D1T1, normal is P1D1T0.5); however both these patients had normal HRR scores.

All available refractive errors were within ± 5 diopters. Data for one patient, who had a refractive error of -5.75 diopters were excluded. No abnormalities were found on ophthalmoscopic fundus exams for participants with the exception of one patient (ID 307) whose exam revealed a large hemorrhage near the optic disc in the left eye. This was confirmed in this patient’s fundus photographs (see Table 5.1).

5.3 MfERGs: Between Group Comparisons

The number of abnormal hexagons was determined for both timing and amplitude of standard mfERG responses and mfOPs. Descriptive statistics for the abnormal hexagon counts are shown in Table 5.4 for the standard mfERG and Table 5.5 for mfOPs. Data for the number of abnormal hexagons for timing of patient standard mfERG responses had a large amount of variability and a
wider distribution than the data for timing of patient mfOPs. Figures 5.6 and 5.7 illustrate the distributions for the number of abnormal hexagons for the timing and amplitude of standard mfERG responses respectively for patients and control subjects. The distribution of the number of abnormal hexagons for timing of mfOPs for both groups is shown in Figure 5.8. Data for the number of abnormal hexagons for the amplitude of mfOPs had a tight distribution with zero or one abnormal hexagons for patients and control subjects. Ninety-two percent of patients and 88% of control subjects had no abnormal hexagons.

**Table 5.4.** Descriptive statistics for standard mfERG abnormal hexagon counts

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 48)</th>
<th>Controls (n = 45)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Timing</strong></td>
<td><strong>Amplitude</strong></td>
<td><strong>Timing</strong></td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>0 to 19</td>
<td>0 to 28</td>
</tr>
<tr>
<td><strong>Mean ± SD</strong></td>
<td>3 ± 4.76</td>
<td>1.27 ± 4.32</td>
</tr>
<tr>
<td><strong>Variance</strong></td>
<td>22.64</td>
<td>18.67</td>
</tr>
</tbody>
</table>

**Table 5.5.** Descriptive statistics for sf-mfERG abnormal hexagon counts

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 48)</th>
<th>Controls (n = 42)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Timing</strong></td>
<td><strong>Amplitude</strong></td>
<td><strong>Timing</strong></td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>0 to 8</td>
<td>0 to 1</td>
</tr>
<tr>
<td><strong>Mean ± SD</strong></td>
<td>1.94 ± 1.90</td>
<td>0.08 ± 0.28</td>
</tr>
<tr>
<td><strong>Variance</strong></td>
<td>3.59</td>
<td>0.08</td>
</tr>
</tbody>
</table>
Figure 5.6. Standard mfERG: distribution of the number of abnormal hexagons for timing of responses for: a) patients and b) controls
Figure 5.7. Standard mfERG: distribution of the number of abnormal hexagons for amplitude of responses for: a) patients and b) controls
Figure 5.8. Sf-mfERG: distribution of the number of abnormal hexagons for timing of responses for: a) patients and b) controls
The mean number of abnormal hexagons for timing of standard mfERG responses was greater for the patient group (3 ± 4.76) in comparison with control subjects (1.38 ± 2.38), however the difference was not statistically significant (Mann-Whitney test, p = 0.11; see Fig. 5.9a). There was also no significant difference in the mean number of abnormal hexagons for the amplitude of the responses between patients (1.27 ± 4.32) and control subjects (1.93 ± 4.08; Mann-Whitney test, p = 0.15; see Fig. 5.9b).

![Figure 5.9](image1.png)

**Figure 5.9.** Standard mfERG: Percentage of abnormal hexagons for a) timing and b) amplitude

On average, patients (1.94 ± 1.90) had significantly more abnormal hexagons for timing of mfOPs compared to controls (0.93 ± 1.09; Mann-Whitney test, p = 0.005; see Fig. 5.10a) however no significant difference was found in the mean number of abnormal hexagons for amplitude between the two groups (Mann-Whitney test; p = 0.29; see Fig. 5.10b).
5.4 Regression Modeling

Regression modeling was conducted for the patient population for both standard and sf-mfERG data to determine whether neuro-retinal function was associated with poor glycemic control. Predictor variables considered for the modeling included the age of the patient at the time of testing, time since diagnosis of T1D, the sex of the patient, the age at which the patient was diagnosed with T1D, and the HbA1c closest to the time of testing. The dependent variable was the number of abnormal hexagons for timing of the response waveforms.

Before generating models, correlations were conducted among the covariates to determine whether there was any evidence of multicollinearity. There was a significant negative correlation between age at diagnosis and time since diagnosis (Spearman’s rho = -0.86, p < 0.01). A moderate but significant correlation was found between age at testing and age at diagnosis (Spearman’s rho = 0.48, p < 0.01). Thus to avoid multicollinearity, age at diagnosis was excluded from the regression modeling.

Poisson regression modeling was attempted. The resulting models were not significant due to excessive variability in the data. This variability was greater than would be expected for a
Poisson distribution (see Regression Modeling section, p. 57), and thus resulted in overdispersion. Subsequently, the data were modeled using negative binomial regression which compensates for the excessive variability in the data.

Negative binomial regression modeling with patient sf-mfERG data did not yield significant results for any of the covariates. Modeling with standard mfERG data however did result in significance.

The backward selection procedure was used to build the negative binomial regression model. In accordance with this procedure, the full model which included all the predictor variables was first formulated (see Table 5.6). With the highest $\beta$ value and lowest p value, HbA$_{1c}$ was found to be significantly predictive of the number of abnormal hexagons, followed by age at testing with a negative parameter estimate value. Sex and time since diagnosis were not significantly predictive of the number of abnormal hexagons as the p values associated with their $\beta$ values were greater than 0.157, which made these variables eligible for removal from the model in the subsequent steps. The Akaike Information Criterion value for this model was 198.66.
**Table 5.6.** Description and results for the full model

<table>
<thead>
<tr>
<th>Model 1: # Abnormal hexagons = $\beta_0 + \text{HbA}_{1c} + \text{time since diagnosis} + \text{age} + \text{sex}</th>
<th>\beta</th>
<th>e^\beta</th>
<th>\text{Standard error}</th>
<th>\text{Confidence interval (95%)}</th>
<th>p \text{ value}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept ($\beta_0$)</td>
<td>-1.64</td>
<td>0.19</td>
<td>2.69</td>
<td>0.00 – 37.73</td>
<td>0.54</td>
</tr>
<tr>
<td>$\text{HbA}_{1c}$</td>
<td>0.67</td>
<td>1.96</td>
<td>0.19</td>
<td>1.34 – 2.87</td>
<td>0.0005*</td>
</tr>
<tr>
<td>Time since diagnosis</td>
<td>0.09</td>
<td>1.10</td>
<td>0.08</td>
<td>0.93 – 1.29</td>
<td>0.26</td>
</tr>
<tr>
<td>Age</td>
<td>-0.28</td>
<td>0.75</td>
<td>0.14</td>
<td>0.57 – 0.99</td>
<td>0.04*</td>
</tr>
<tr>
<td>Sex</td>
<td>0.10</td>
<td>1.10</td>
<td>0.50</td>
<td>0.41 – 2.96</td>
<td>0.85</td>
</tr>
<tr>
<td>Residual deviance</td>
<td>46.3 on 43 degrees of freedom</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Akaike Information Criterion</td>
<td>198.66</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The next step involved removing the predictor variable with the highest p value and one that is greater than 0.157. Therefore, the predictor sex was removed from this model. $\text{HbA}_{1c}$ was still the strongest predictor of the number of abnormal hexagons in this model, followed by age at testing. Further details on Model 2 are elaborated in Table 5.8.

* $p < 0.157$
Table 5.7. Description and results for Model 2 excluding sex as a predictor

| Model 2: # Abnormal hexagons = β₀ + HbA₁c + time since diagnosis + age |
|--------------------------|-------------------|-------------------|--------------------------|
|                          | β     | e^β             | Standard error | Confidence interval (95%) | p value |
| Intercept (β₀)           | -1.40 | 0.25           | 2.60          | 0.00 – 40.34             | 0.59    |
| HbA₁c                   | 0.67  | 1.95           | 0.19          | 1.33 – 2.84              | 0.0005* |
| Time since diagnosis     | 0.09  | 1.09           | 0.08          | 0.94 – 1.26              | 0.26    |
| Age                     | -0.29 | 0.75           | 0.14          | 0.57 – 0.98              | 0.04*   |
| Residual deviance        | 46.3  | on 44 degrees of freedom |
| Akaike Information Criterion | 196.69 |

The likelihood ratio test comparing Model 2 with Model 1 (see Table 5.9) was not significant (p = 0.86), which indicated that removing sex from Model 1 did not make a difference. The Akaike Information Criterion for this model however was 196.69, which is slightly lower than that of the full model, indicating that this second model fit the data better.

*p < 0.157
Table 5.8. Likelihood ratio test results comparing Model 2 with Model 1 (full model)

<table>
<thead>
<tr>
<th>Model</th>
<th>Log likelihood</th>
<th>Degrees of freedom</th>
<th>Chi-square</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-93.328</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-93.345</td>
<td>-1</td>
<td>0.03</td>
<td>0.86</td>
</tr>
</tbody>
</table>

As the p value associated with the $\beta$ for time since diagnosis was still greater than 0.157, for the next model, this variable was removed. The resulting model (see Table 5.10) indicated that HbA$_{1c}$ was still strongly predictive of the number of abnormal hexagons, followed by age at testing. Since all the variables in this model have a p value of less than 0.157, the backward selection process was stopped.
Table 5.9. Description and results for Model 3 excluding time since diagnosis as a predictor

<table>
<thead>
<tr>
<th></th>
<th>$\beta$</th>
<th>$e^{\beta}$</th>
<th>Standard error</th>
<th>Confidence interval (95%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept ($\beta_0$)</td>
<td>-0.36</td>
<td>0.70</td>
<td>2.53</td>
<td>0.00 – 98.9</td>
<td>0.89</td>
</tr>
<tr>
<td>HbA$_{1c}$</td>
<td>0.59</td>
<td>1.80</td>
<td>0.19</td>
<td>1.25 – 2.62</td>
<td>0.002*</td>
</tr>
<tr>
<td>Age</td>
<td>-0.26</td>
<td>0.77</td>
<td>0.14</td>
<td>0.59 – 1.00</td>
<td>0.054*</td>
</tr>
<tr>
<td>Residual deviance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>46.7 on 45 degrees of freedom</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Akaike Information Criterion</td>
<td>195.98</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

As seen in Table 5.11, a likelihood ratio test comparing Model 3 to the null model was significant which indicated that Model 3 fit the data better than the null model. The test comparing Model 3 with Model 2 was not significant which indicated that removing time since diagnosis as a predictor did not make a difference. The Akaike Information Criterion for Model 3 however was 195.98, which is lower than that for Models 1 and 2. Together, these factors make Model 3 the final model.

* $p < 0.157$
Table 5.10. Likelihood ratio test results comparing Model 3 to the null model and to Model 2

<table>
<thead>
<tr>
<th>Model</th>
<th>Log likelihood</th>
<th>Degrees of freedom</th>
<th>Chi-square</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Null</td>
<td>-99.65</td>
<td>-2</td>
<td>11.32</td>
<td>0.003*</td>
</tr>
<tr>
<td>Model 3</td>
<td>-93.99</td>
<td></td>
<td>0.003*</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-93.99</td>
<td>-1</td>
<td>1.29</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Based on the results in Table 5.10, the final model can be interpreted as follows: a one unit increase in HbA1c would result in an increase in the number of abnormal hexagons for the timing of standard mfERG responses by a factor of 1.80 or by 80% when age is held constant. Also, a one unit increase in age would result in a decrease in the number of abnormal hexagons by a factor of 0.77 or by 23% when HbA1c is held constant.

A scatterplot of the univariate correlation between the number of abnormal hexagons for implicit time of standard mfERG responses and HbA1c (see Fig. 5.11) yielded a significant Spearman’s rho of 0.423 (p = 0.001).
Figure 5.11. Univariate correlation between HbA$_{1c}$ and the number of abnormal hexagons for implicit time of standard mfERG responses.

Modeling using the one-year HbA$_{1c}$ averages in lieu of the single HbA$_{1c}$ values obtained closest to the date of testing did not yield any significance (see Table 5.11). The one-year HbA$_{1c}$ average did not significantly predict the number of abnormal hexagons for implicit time of standard mfERG responses ($p = 0.25$). Similar to the final model including single HbA$_{1c}$ values, age at testing however was found to predict a decrease in the number of abnormal hexagons by a factor of 0.72 or 28%.
Table 5.11. Description and results for modeling including one-year HbA$_1c$ average

| Model: # Abnormal hexagons = $\beta_0 + $HbA$_1c$ + age at testing |
|---|---|---|---|---|
| Intercept ($\beta_0$) | 3.08 | 21.76 | 3.45 | 0.025 – 188807.29 | 0.37 |
| HbA$_1c$ | 0.35 | 1.42 | 0.30 | 0.78 – 2.57 | 0.25 |
| Age at testing | -0.33 | 0.72 | 0.17 | 0.51 – 1.01 | 0.056* |

Residual deviance 35.6 on 36 degrees of freedom

* $p < 0.157$
6. Discussion and Conclusions

The DCCT study (1993) brought to the forefront the importance of good glycemic control in reducing the development and progression of DR in patients with T1D. A subsequent study conducted by the same group (DCCT, 1994b) involving the adolescent population from the previous study, found that intensive insulin therapy significantly reduced the incidence and progression of DR in comparison with conventional therapy. The study showed that the incidence of DR had reduced by 53% in adolescent patients with no DR at baseline, and that the progression of DR was reduced by 70% in adolescents with NPDR at baseline. Hence, poor glycemic control, indicated by high HbA1c levels, has been correlated strongly with the development and progression of DR. The objective of the current study was to determine whether poor glycemic control is predictive of worsening localized neuro-retinal dysfunction. Results confirm the hypothesis and indicate that poor glycemic control is indeed predictive of worsening neuro-retinal dysfunction in patients with diabetes before DR is clinically visible. This result represents an important step towards the larger goal of identifying accurate and sensitive biomarkers for monitoring retinal integrity in patients with diabetes, and identifying those at risk of developing DR.

There are a number of unique characteristics about the current study that must be addressed. While many other electrophysiological studies have demonstrated localized and global neuro-retinal dysfunction in patients with diabetes, there is firstly a deficiency in mfERG studies conducted specifically on adolescents with T1D. Most of the studies to date have involved patients with T2D or a mix of both T1D and T2D. It follows that most of the subjects participating in these studies are adults. While one group recently found a moderate correlation between HbA1c and mfERG implicit times in adolescents with T1D (Bronson-Castain et al., 2008a, 2008b), there are some characteristics that distinguish our study. To the best of our knowledge, the sample size of this study is the largest in comparison with the others. Also, patient blood glucose levels were monitored and maintained within 4 to 10 mmol/L throughout the testing session. This is important as full-field ERG (Dawson, Hazariwala, & Karges, 2000; Hirsch-Hoffman, 1993; Macaluso, Onoe, & Niemeyer, 1992) and mfERG studies (Klemp 2004) have demonstrated significant changes in implicit times of the responses due to changes in ambient blood glucose levels. The 4 to 10 mmol/L range was suggested by Dr. Denis Daneman, the Chief of Pediatrics/endocrinologist at SickKids. It is a broad enough range to ensure the
safety of the patients and allows the blood glucose levels to be adjusted within a reasonable amount of time. While it is likely that blood glucose levels may change slightly during the electrophysiological testing, the glucose levels were adjusted in consultation with the nurse such that any changes would be minor and still within the safe range of 4 to 10 mmol/L. The blood glucose levels were monitored frequently throughout the testing session to ensure that they remained within the specified range so as to avoid any major fluctuations that may have influenced results. Finally, we have developed a predictive model demonstrating a strong relationship between HbA$_{1c}$ and areas of localized neuro-retinal dysfunction after taking into account other covariates including age, sex and duration of diabetes.

To achieve the aim of the study, a negative binomial regression model using patient data was generated with the number of abnormal hexagons for timing of mfERG responses as the dependent variable. Predictor variables included the sex of the patient, time since diagnosis of T1D, the HbA$_{1c}$ level, and age at testing.

Before generating the regression model however, neuro-retinal function was measured using both the standard and sf-mfERG paradigms. Timing and amplitude of the responses obtained from both paradigms were measured, and based on z-scores, the number of abnormal hexagons was determined. No significant difference was found between the patient and control groups in the mean number of abnormal hexagons for the amplitude of neither the standard nor sf-mfERG responses. As for the mean number of abnormal hexagons for timing of standard mfERG responses, although there was a trend with patients on average having more abnormal hexagons (3 ± 4.76) compared to controls (1.38 ± 2.38), the difference was not statistically significant. This is contrary to findings from other studies that have demonstrated delayed timing of standard mfERG responses in patients with diabetes (Fortune et al., 1999; Han, Bearse et al., 2004a). The nonsignificant result is likely due to the wide distribution of the data and its large variability. On the other hand, the mean number of abnormal hexagons for the timing of mfOP responses was significantly larger for patients (1.94 ± 1.90) compared to controls (0.93 ± 1.09). This is the case even though the difference between the means of the two groups is smaller for the sf-mfERG responses than the standard mfERG responses. A possible explanation is that the distribution of the data in the case of the mfOP results is much tighter and has lower variability in comparison with the standard mfERG data. Nevertheless, the results suggest that inner retinal neurons in particular are susceptible to functional changes associated with the effects of diabetes in patients.
before vascular lesions are visible. This finding is consistent with findings from many other electrophysiological studies. Several full-field ERG studies have demonstrated decreased amplitudes and delayed timings of OPs in patients before DR is clinically visible (Bresnick et al., 1984; Bresnick & Palta, 1987a; Frost-Larsen et al., 1983; Hancock & Kraft, 2004; Juen & Kieselbach, 1990; Lovasik & Kergoat, 1993; Shirao & Kawasaki, 1998; Simonsen, 1980). More recently, sf-mfERG studies in diabetic eyes have also demonstrated localized implicit time delays in mfOPs (Bearse, Han, Schneck, & Adams, 2004; Bearse, Han, Schneck, Barez et al., 2004; Kurtenbach et al., 2000).

Following the initial between-group analysis of results from both mfERG paradigms, a negative binomial regression model was attempted for the timing of responses (number of abnormal hexagons) for both standard and sf-mfERG patient data. Although attempted, a model could not be generated for the sf-mfERG results as there was not enough variation in the data to conduct a regression analysis. A negative binomial regression model, however, was successfully generated for standard mfERG data. The final model consisted of HbA$_{1c}$ and age at testing as predictor variables. Hemoglobin A$_{1c}$ was found to be significantly predictive of the number of abnormal hexagons. The model indicates that a one unit increase in HbA$_{1c}$ predicts an increase in the number of abnormal hexagons for timing of standard mfERG responses by a factor of 1.80 when controlling for the age of the patient. In other words, if two patients of the same age were to undergo testing using the standard mfERG, the patient with the higher HbA$_{1c}$ value would have a greater number of abnormal hexagons in terms of timing of the responses. The model also revealed that a one unit increase in age at testing would result in a decrease in the number of abnormal hexagons by a factor of 0.77 when controlling for HbA$_{1c}$. The one-year HbA$_{1c}$ model on the other hand indicated that average glycemic control over a one year period did not significantly predict the number of abnormal hexagons. This suggests that the model is more sensitive to short-term glycemic control over a period of about three months rather than more chronic glycemic control over a period of one year. Also, similar to the single HbA$_{1c}$ model, age at testing was found to predict a decrease in the number abnormal hexagons when controlling for HbA$_{1c}$ (one-year average). Future validation studies will further investigate this small effect of age at testing.

The sex of the patient and, time since diagnosis did not significantly account for the variability in the number of abnormal hexagons. In regards to the sex of the patient as a predictor variable, the
nonsignificant result was not unexpected given that the literature does not indicate that it is a strong risk factor for DR. Although studies have implicated time since diagnosis or the duration of diabetes as a strong risk factor for DR (Burger et al., 1986; R. Klein et al., 1984a; R. Klein, Klein, Moss, Davis, & DeMets, 1984b), it was not found to be significantly associated with neuro-retinal function in this study, and was excluded during the second step of the modeling process. Many studies have found that it is the number of years post-puberty that significantly affect the risk of developing DR as opposed to the years before puberty (Frank et al., 1982; Jackson et al., 1982; B. E. Klein et al., 1990; R. Klein et al., 1985; Knowles et al., 1965; Malone et al., 1984). Pubertal status was not assessed in this study and so the duration of diabetes post-puberty in the patient group is unknown. Had these data been collected and included in the regression analysis, it is unclear whether it would have emerged as a significant predictor; the reason being that it is also possible that the post-pubertal duration of diabetes may not be long enough since the patients are adolescents.

Multivariate predictive models generated by the Adams group support the nonsignificant results for the variable sex in the negative binomial regression model. An overview of these predictive models follows as they also provide support for the potential use of mfERG implicit time measurements in predicting the development of new diabetic retinopathy.

The predictive models generated by the Adams group followed patients from one to three years to examine whether standard mfERG implicit time delays at specific retinal locations preceded the appearance of new NPDR lesions (Bearse et al., 2006; Han, Schneck et al., 2004; Ng et al., 2008). The one-year study (Han, Schneck et al., 2004) examined 12 patients with mild NPDR and 16 patients with no visible DR at baseline, and one year later. Independent variables considered for the modeling procedure consisted of mfERG implicit times, duration of diabetes, blood glucose level, baseline DR status, gender, and diabetes type. To map the standard mfERG functional abnormalities, obtained from recordings done at baseline only, with the vascular lesions seen on fundus photographs taken during the follow-up visit, the standard mfERG array was divided into 35 contiguous “retinal zones”. Results demonstrated that new DR had developed in 11 of 12 NPDR eyes at follow-up. Sex, which was not found to be predictive of neuro-retinal dysfunction in our study, was not found to be predictive of new DR, whereas mfERG implicit times and duration of diabetes had significant predictive power. Sensitivity and specificity of the predictive model were found to be 86% and 84% respectively. Similarly, the
three-year predictive model (Ng et al., 2008) demonstrated that delayed standard mfERG implicit times were associated with approximately an 8-fold greater risk of developing recurring DR, that is, lesions that were present in a retinal zone during at least two if not all three annual follow-up visits. The model, which also included duration of diabetes and blood glucose levels as covariates, demonstrated that baseline standard mfERG implicit times, duration of diabetes and blood glucose concentration were significant predictors of recurring DR. Sensitivity and specificity for the model were determined to be 88% and 98% respectively. Findings from these predictive models, as noted by the Adams group (Bearse et al., 2006), however must be interpreted with caution. The one-year model involved a follow-up visit after only 12 months and so it is possible that the some of the new lesions that were observed during follow-up visits were transient in nature (Ng et al., 2008). Microaneurysms and hemorrhages for instance have been known to appear and disappear over time during the early stages of DR (Meyerle, 2009). The group also concedes that a more vigorous approach could be used to validate the models; that is, the models could be validated using a new group of subjects as opposed to those whose data were used to generate the models (Bearse et al., 2006). Overall, results from the predictive models indicate that standard mfERG implicit time is a significant predictor of the development of future DR in patients with existing DR at baseline.

It should be noted that the negative binomial regression modeling results of the current study must also be interpreted with caution because the model has yet to be validated.

Limitations of our study include missing data; particularly, refractive errors. Much of the missing data however cannot be recovered by bringing the patients in for testing as it was collected about two years ago by a former lab member during the initial stages of the study. In regards to the missing refractive errors however, given that most patients and controls had normal visual acuities and that all available visual acuities were below the 0.3 logMAR exclusion criterion, the missing refractive errors most likely did not influence the main study findings. Another limitation is the loss of participants, most of whom were patients, after initial contact had been established. Many of the participants withdrew from taking part in the study after initial contact because they were afraid of having the contact lens electrode on the eye. Others could not attend as they moved to a location that was too far from the hospital, and some simply did not show up for the testing session without explanation.
Overall, the modeling results support the study’s hypothesis and lead to the conclusion that poor-long term glycemic control is predictive of increasing neuro-retinal dysfunction in patients with diabetes before DR is clinically visible. This is the first study that has shown a direct association between glycemic control and localized neuro-retinal dysfunction. Studies examining OP amplitude and dark adaption in patients with diabetes however have demonstrated normalization of these parameters after 11 days of strict glycemic control (Frost-Larsen et al., 1983; Lauritzen, Frost-Larsen, Larsen, & Deckert, 1983). The modeling results also indicate that poor glycemic control, indicated by high HbA1c levels, may impair the function of neurons in the middle retina, particularly the bipolar cells. Other studies have also demonstrated functional changes in electrophysiological responses predominantly emerging from bipolar cells during diabetes. For instance, full field ERG studies have found a reduction in the amplitude of the “b wave”, which is thought to reflect bipolar cell function, during diabetes (Fletcher, Phipps, Ward, Puthussery, & Wilkinson-Berka, 2007). Other standard mfERG studies have also shown delayed implicit times in patients (Fortune et al., 1999; Han, Bearse et al., 2004a) as has been elucidated in the preceding paragraphs. In addition, anatomic studies have demonstrated reduced thickness of the inner nuclear layer (Barber et al., 1998; Martin et al., 2004), which consists of bipolar cell nuclei.

In summary, this study’s findings highlight the importance of maintaining good glycemic control in reducing neuro-retinal dysfunction in patients with diabetes who have yet to develop the irreversible vascular lesions associated with DR. They also indicate that for a given patient with a large number of hexagons with delayed standard mfERG responses (i.e. abnormal hexagons), as well as a high HbA1c reading, the specific retinal locations of neuronal dysfunction should be monitored for the appearance of subsequent vascular lesions. Thus the findings lead to the bigger question of whether the patients with high HbA1c values subsequently develop DR and additionally, whether the vascular lesions correlate spatially with retinal areas associated with the abnormal hexagons with delayed standard mfERG timings. These questions can be addressed in a future longitudinal study.
7. Future Directions

A number of potential studies and different analytical approaches can be undertaken to strengthen and expand on the results of the current study. An interesting alternative approach to the analysis of the standard mfERG timing data collected in this study is cluster analysis, which is commonly used to analyze static perimetry and more recently, multifocal visual evoked potential data. Cluster analysis is a method used to form and analyze clusters, a cluster being a group of two or more spatially related defects (Chauhan, Henson, & Hobley, 1988). The advantage of using this approach is that it may lower the false positive rate of results. Based on the technique used by Hood and colleagues (Hood et al., 2004) and similar to the approach used in the current study, z-scores for standard mfERG timing data will be assigned to each of the 103 hexagons per participant. Analogous to the probability density plots created in the Hood study (2004), hexagons will be colour-coded. Hexagons with z-scores ≥ 1.96 (critical p value of 0.05) will have a desaturated colour and those with z-scores ≥ 2.58 (critical p value of 0.01) will have a saturated colour. A modified cluster criterion, based on the one suggested by Goldberg et al. (Goldberg, Graham, & Klistorner, 2002) will be applied; that is, a cluster would consist of at least 3 contiguous hexagons with z-scores ≥ 1.96, one of which must have a z-score ≥ 2.58. Thereafter, the number of clusters per participant can be tallied and compared between patient and control groups to determine whether there is a difference in the number of clusters between the groups. Additionally, to investigate whether defects are more likely to be found in a particular retinal region such as one of the four quadrants, the proportion of patients with clusters in particular retinal quadrants can be determined.

In regards to expanding the current analysis, namely, the negative binomial regression modeling results, the next step is validation of the model by recruiting and testing another group of adolescents with T1D who meet the current inclusion criteria. These patients would be tested using the standard mfERG. The number of abnormal hexagons with regards to the timing of the responses will be determined. In addition, the patients’ HbA1c levels, time since diagnosis of T1D, sex and age will be recorded. Based on these variables, the model will be used to predict the number of abnormal hexagons for each patient to compare with the actual number of abnormal hexagons obtained from the standard mfERG recording. Sensitivity and specificity of the model will also be evaluated.
An interesting question that can be explored in a future study is whether the functional neuro-retinal changes observed in adolescent patients with T1D correlate spatially with areas of structural defect, visualized using high resolution adaptive optics technology. Ocular aberration, caused by structures in the eye such as the cornea and lens, hinder high resolution imaging of fine retinal structures (Roorda et al., 2006; Shahidi, Blair, Mori, & Zelkha, 2004). These aberrations can be measured and corrected using adaptive optics technology (Roorda et al., 2006). This technology, with high resolution retinal imaging instruments, can yield images of the highest possible resolution at the microscopic level in vivo such that individual cells, fine blood vessels, and nerve fibers can be clearly visible (Roorda et al., 2006). Cross-sectional and axial images can be obtained from patients using a confocal scanning laser ophthalmoscope and ultra high resolution optical coherence tomography with adaptive optics correction. Areas of neuro-retinal dysfunction identified by mfERG can specifically be targeted for imaging. These images can in turn be assessed for structural defects including the presence of microaneurysms, damage to the photoreceptor outer segments and thinning of the nerve fiber layer. The spatial correlation between the structural and functional defects can further elucidate the underlying disease process of DR.

The key question that no study has definitively answered is whether worsening neuro-retinal dysfunction is predictive of the vascular lesions that characterize DR. The conclusion of the current study is that poor glycemic control or increasing HbA₁c levels are predictive of worsening neuro-retinal dysfunction. A longitudinal study must be conducted to expand on this result and to answer the question of whether worsening neuro-retinal function is predictive of diabetic retinopathy. Of particular interest is whether the patients who have high HbA₁c levels during this study subsequently develop retinopathy and moreover, whether the vascular lesions correlate spatially with specific localized areas of delayed mfERG responses. A logistic regression model with DR/no DR as the dependent variable and standard mfERG implicit times, HbA₁c, duration of diabetes, age at testing, and the sex of the patient could be generated and validated by testing a new group of adolescents with NPDR. Results from the longitudinal study might provide greater insight and further elucidate the mechanisms underlying the pathophysiology of DR. Longitudinal analysis would help identify the contributions of neurodegenerative disease and visible vascular disease by demonstrating whether areas of future visible vascular lesions were preceded by delayed standard mfERG responses. Furthermore, it
would help identify patients who are at risk of developing DR before vascular lesions are visible. This is essential so appropriate preventative measures can be taken to prevent vision loss.
8. References


DCCT. (1994b). Effect of intensive diabetes treatment on the development and progression of long-term complications in adolescents with insulin-dependent diabetes mellitus:


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first 4-14 years of type 1 diabetes: the Wisconsin Diabetes Registry Study. *Am J Epidemiol, 164*(2), 143-150.


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Appendix A: Research Ethics Board Approval Letter

Research Ethics Board (REB)

The Research Ethics Board for The Hospital for Sick Children is organized and operates according to the principles and practices outlined in the Tri-Council Policy Statement, the ICH Harmonized Tripartite Guidelines: Good Clinical Practice, and Division 5 and the Medical Devices Regulations of the Food and Drug Act as well as the Natural Health Products Regulations of Health Canada. This signed document is in lieu of the Health Canada Research Ethics Board Attestation Form.

Approval & Terms of Agreement

Investigators: Dr. Carol Westall, E. Lakhani, S. Boyd, W. Lam, T. Wright

Study Title: Association between functional markers and structural retinal changes in patients with Type I diabetes

REB File number: 1000012654 Level of Continuing Review: H D


Consent & Assent Form Version Date(s): Research Consent Form (Controls) – July 31, 2008; Research Consent Form (Parents/Caregivers of Controls) – July 31, 2008; Assent Form (Controls) – July 31, 2008; Research Consent Form (Patients) – July 31, 2008; Research Consent Form (Parents/Caregivers of Patients) – July 31, 2008; Assent Form (Patients) – July 31, 2008.

Investigator's Brochure Version Date: N/A


I agree to carry out the proposed research involving human subjects in accordance with the above-noted guidelines and regulations (as applicable) and using only the REB-approved study protocol and consent/assent form(s). I shall notify the division/department head and the REB prior to implementing any amendments in the protocol and consent/assent forms and of any deviations or any changes in study activity. I shall also notify the REB of any unexpected adverse events as per REB guidelines. As applicable, I certify that the research contract and corresponding protocol are consistent and will inform the contract manager of any protocol amendments as required.

I agree that, in accordance with the Personal Health Information Protection Act of Ontario, I am responsible for adhering to all conditions and restrictions imposed by the REB governing the use, security, disclosure, return and disposal of the research subjects' personal health information. I am also responsible for reporting immediately any privacy breaches to the REB Chair and to Janice Campbell, the Sick Kids privacy officer. I will ensure that the personal health information is used, only as necessary, to fulfill the specific research objectives and related research questions described in this application and approved by the REB.

Signature of Principal Investigator: [Signature] DATE

I approve of this research protocol, agree to share responsibility for its proper conduct, and will ensure that the REB is notified of concerns, as appropriate:

Signature of Division/Department Head: [Signature] DATE

The REB of the Hospital for Sick Children has reviewed and approved the above-named research study.

Mr. Richard Sugarman, REB Chair
555 University Avenue, Toronto, Ontario, M5G 1X8
Tel: 416-813-6152 Fax: 416-813-5085 Email: richard.sugarman@sickkids.ca

DATE OF APPROVAL: Nov. 21, 2008 EXPIRY DATE: Nov. 2009

REB Form Version Date: 2008-02-01
Appendix B: Sample Consent Form

Department of Ophthalmology

Hospital for Sick Children (SickKids)

RESEARCH CONSENT FORM FOR PATIENTS WITH CAPACITY TO CONSENT

TITLE OF STUDY:

Association between functional markers and structural retinal changes in patients with Type 1 Diabetes.

INVESTIGATORS:

Director of Electrophysiology: Dr. Carol Westall (416) 813-6516
Responsible Individual: Dr. Carol Westall (416) 813-6516
Retinal Specialist/Scientist: Dr. Shelley Boyd (416) 864-6060 x. 3138
Endocrinologist: Dr. Denis Daneman (416) 813-4490
Endocrinologist Dr. Lawrence Leiter (416) 867-7441
Endocrinologist Dr. Amir Hanna (416) 867-3721
Retinal Specialist: Dr. Wai-Ching Lam (416) 813-6525
Biostatistician: Mr. Mohamed Abdolell MSc, P. Stat (902) 473-6102
Orthoptist/Visual Electrophysiology Technologist: (416) 813-7790
Melissa Cotesta BSc. O.C. (C.)
Orthoptist/Assistant Director Visual (416) 813-6133
Electrophysiology Unit: Carole Panton C.O., O.C. (C.)
Purpose of the Research:

The purpose of this study is to help us understand more clearly if early eye and brain changes occur in children and young persons with Type I Diabetes. Current tests for diagnosing diabetic eye disease are based on the assumption that the primary problem is damage to blood vessels of the retina. Based on this blood vessel damage, treatment is given late in the disease when there is high risk of sudden, severe visual loss. Recent evidence suggests that the nerve cells within the retina and the brain may be damaged before, or along with, blood vessels.

We will evaluate three nerve function tests together to see how well they detect diabetic damage, and how well they can follow changes over time. This has not been done before.

We will then check your vision using tests for visual acuity (ability to see fine detail), colour, and contrast sensitivity. The multifocal electroretinogram (mERG), slow-flash electroretinogram (sfERG) and short wavelength electroretinogram (s-cone ERG) will also be conducted. Lastly, we will complete the routine eye exam with refraction (whether you need glasses) and ophthalmoscopy (examine the back of the eye).

If the routine clinical examination (visual acuity, refraction, opthalmoscopy, fundus photography) show any abnormalities we will discuss these findings with you and refer you for further testing if required.

Description of the Research:

Participation in this study will require you to visit the hospital once for approximately 2 to 3 hours. The order in which the tests will be administered is as follows:
1) Introduction to study by coordinator, discussion of inclusion criteria, consent form signed, questions answered.

2) A study nurse will test your blood glucose level. The blood glucose will be stabilized if it is too high or low. If you prefer a local anesthetic (Ametop) can be used before any blood is drawn.

3) Vision: We will examine your vision by asking you to read some letters on a regular vision chart to measure what you can see. We will also test how well you can see colour and read fading letters.

[* The above listed tests should take about 20 mins.]*

**NOTE:** To do the tests listed below we will need to put drops in one of your eyes for pupil dilation which will make your pupil bigger. After testing, your vision will be blurred, especially up close for about 4-8 hours and probably until the next day.

* **Snack Break** (during pupil dilation)

4) **Multifocal Electro-retinogram (mfERG):**

While your pupil is still large we will place a small contact lens sensor (electrode) at the front of your eye after we have placed an anaesthetic drop in your eye, which will numb the front of your eye. The contact lens will pick up electrical signals from your retina. You will be asked to look into a camera at a pattern of hexagons. The test is painless and will take approximately fifteen minutes to complete.

5) **Slow-flash electroretinogram (sfERG):**

The slow-flash flash ERG will seem almost identical to the multifocal electroretinogram. The difference is that bright flashes are inter-dispersed by more dark images. The data give us information about structures deeper in your retina. The test will take approximately ten minutes to complete.

6) **Short-wavelength electroretinogram (s-cone ERG):**

The s-cone ERG will tell us how the nerve cells that are sensitive to blue light in the retina are functioning. The set-up is similar to mfERG and sfERG so you will be wearing the same contact lens. You will be asked to look at white light for about ten minutes. After ten minutes, the
background will change to amber and you will see blue flashes of light for a few seconds. This test takes approximately ten to fifteen minutes to complete.

7) **Ophthalmoscopy:**

We will look at the inside of your eye with a bright light. This is a very routine clinical test.

8) **Refraction:**

We will test if you are near sighted (able to see things better up close than far away), far sighted (able to see things better far away than close), or if you have clear vision for both close and far. We will then shine a light into your eyes and hold lenses in front of your eyes.

9) **Fundus Photography:**

We will take some photographs of the inside of your eyes. We will ask you to complete a separate consent form for these photographs.

[* Ophthalmoscopy, Refraction and Fundus Photography should take about 15 mins.*]

[* Ophthalmoscopy and Refraction should take about 10 mins.*]

If we subsequently use information about your condition a separate consent form will need to be completed. If the study involves taking photographs, videotaping or sound recordings, a separate consent form will need to be completed.

New information from this study or other studies may affect whether you want to continue to take part in the study. If this happens, we will tell you about this new information.

The entire examination will require about 3 hrs. Patient’s health records will be reviewed for purposes of this study.

**Potential Harms (Injury, Discomforts or Inconvenience):**

We know of no harm that taking part in this study could cause you. The eye drops and anaesthetic drop may cause slight discomfort, but this resolves within 10 seconds. The drops, which we use to dilate your pupil, may cause your vision to be slightly blurred for 4-8 hours and
pupils may remain dilated until the next day. Lastly, testing will require about 3 hours so you may be tired at the end of the day from doing all the tests.

**Potential Benefits:**

You will not benefit directly from participating in this study. However, if you display problems with your vision then we can give you special help if needed.

**Confidentiality:**

We will respect your privacy. No information about who you are will be given to anyone or be published without your permission, unless required by law. For example, the law could make us give information about you if a child has been abused, if you have an illness that could spread to others, if you or someone else talks about suicide (killing themselves), or if the court orders us to give them the study papers.

Sick Kids Clinical Research Monitors, employees of the funder or sponsor, or the regulator of the study may see your health record to check on the study. By signing this consent form, you agree to let these people look at your records. We will put a copy of this research consent form in your patient health record and give you a copy as well.

The data produced from this study will be stored in a secure, locked location. Only members of the research team (and maybe those individuals described above) will have access to the data. This could include external research team members. Following completion of the research study the data will be kept as long as required then destroyed as required by Sick Kids policy. Published study results will not reveal your identity.

**Reimbursement**

A reimbursement of reasonable out of pocket expenses will be provided for the visit. We will pay for all your expenses for being in this study e.g., meals, babysitters, parking and getting you to and from Sick Kids. If you stop taking part in the study, we will pay you for your expenses for taking part in the study so far.
**Participation:**

Participation in research is voluntary. It is your choice to take part in this study. You can stop at any time. The care you get at Sick Kids will not be affected in any way by whether you take part in this study.

New information that we get while we are doing this study may affect your decision to take part in this study. If this happens, we will tell you about this new information. And we will ask you again if you still want to be in the study.

During this study we may create new tests, new medicines, or other things that may be worth some money. Although we may make money from these findings, we cannot give you any of this money now or in the future because you took part in this study.

We will give you a copy of this consent form for your records.

In some situations, the study doctor or the company paying for the study may decide to stop the study. This could happen even if the medicine [or treatment] given in the study is helping you. If this happens, the study doctor will talk to you about what will happen next.

If you become ill or are harmed because you took part in this study, we will treat you for free. Your signing this consent form does not interfere with your legal rights in any way. The staff of the study, any people who gave money for the study, or the hospital are still responsible, legally and professionally, for what they do.

**Sponsorship**

The sponsors/funders of this research are the Juvenile Diabetes Research Foundation and miscellaneous sponsors/funders for Dr. Westall.

**Conflict of Interest**

Some of the people doing this study may have a conflict of interest. That means that they may benefit personally, financially, or in some other way from this study.
Dr. Westall (Principal Investigator) has received or may receive money, or other benefits (speaker's fees, travel assistance, industry-initiated research grants, investigator-initiated research grants, consultant fees, honoraria, gifts, intellectual property rights such as patents, etc.) for research related to the present study from sponsor(s) that have activities related to the present study.

**Consent**

By signing this form, I agree that:

1) You have explained this study to me. You have answered all my questions.
2) You have explained the possible harms and benefits (if any) of this study.
3) I know what I could do instead of taking part in this study. I understand that I have the right not to take part in the study and the right to stop at any time. My decision about taking part in the study will not affect my health care at Sick Kids.
4) I am free now, and in the future, to ask questions about the study.
5) I have been told that my medical records will be kept private. You will give noone information about me, unless the law requires you to.
6) I understand that no information about who I am will be given to anyone or be published without first asking my permission. 
7) I have read and understood pages 1 to 6 of this consent form. I agree, or consent, to take part in this study.

_________________________________________                _______________________________
Printed Name of Subject & Age                      Subject’s signature & date

_________________________________________                _______________________________
Printed Name of person who explained consent         Signature & date

_________________________________________                _______________________________
Printed Witness’ name (if the subject/legal guardian does not read English) Witness’ signature & date
If you have any questions about this study, please call Peter Glazer at 416-813-7654 ext. 3606.

If you have questions about your rights as a subject in a study or injuries during a study, please call the Research Ethics Manager at 416-813-5718.
## Appendix C: Patient Demographic Information

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