Longitudinal Studies utilizing Local Neural Retinal Function, measured by Multifocal Electroretinograms, for the Prediction of Diabetic Eye Disease

By

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Abstract

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Diabetes is the number one cause of preventable blindness in working aged Americans. Diabetic macular edema is one of the most common reasons for vision loss in these patients. While treatments are available for macular edema, they are invasive and target the tissue after edema has already developed. They do not restore lost vision, but rather attempt to minimize further vision loss. Earlier and less invasive treatments for edema are needed.

The multifocal electroretinogram (mfERG), a local test of neural function, has been shown to be sensitive to changes in diabetes at all stages. This thesis investigates the relationship between the mfERG and diabetic eye disease (including retinal structure and function changes), ranging from its earliest clinical changes to sight threatening macular edema.

Five related studies are included, each as a separate chapter in this thesis.

The first study examines the reproducibility of the mfERG across instruments in patients with and without diabetes. This experiment was conceived because it was necessary to develop a method to combine data from our laboratory's two mfERG instruments for use in future experiments. Examining the reproducibly of the instruments is the first step in that process.

The second study builds on the past work of the lab. It creates a multivariate model using the mfERG implicit time to predict the onset of diabetic retinopathy in patients with no previous retinopathy. We were able to construct a model, which has 80% sensitivity and 74% specificity for the local prediction of the first signs of clinical retinopathy. This study also revealed strong differences between the mfERGs of type 1 and type 2 diabetics by identifying type of diabetes as a confounder of mfERG in this patient group.

The third study examines the relationship between retinal thickness and other diabetes health measures, such as blood pressure and blood glucose, which could alter thickness measurements. This study looked at factors that may confound our interpretation of retinal edema on an ocular coherence tomography (OCT), as increased retinal thickness accompanies edema in the diabetic retina. This correlation between retinal thickness and edema facilitates OCT to often be used as an outcome measure to

identify edema. We found that increased blood pressure is associated with increased retinal thickness, even when blood pressure is in the normal range, in patients with retinopathy. However patients without retinopathy did not display the same trend.

The fourth study presented here is a cross sectional evaluation of patients with diabetic macular edema. We examined how the mfERG correlates with retinal thickness measured by OCT, edema on a fundus photo, and visual acuity, in these patients. We found local correlations between all these factors.

The fifth, and last study, builds on the previous chapters, in a longitudinal study evaluating patients with retinopathy at risk for edema. We used the mfERG to predict the onset of diabetic edema in patients with diabetic retinopathy. We found that mfERG amplitude and implicit time can predict local edema with a 72% sensitivity and specificity. Furthermore, a multivariate model, which includes the mfERG, measures along with systolic blood pressure and sex can predict the onset of edema with 84% sensitivity and 76% specificity.

Overall, we found the mfERG to be predictive of diabetic changes in the retina at many stages of diabetic eye disease. This extends from the onset of retinopathy to vision threatening diabetic edema. Our multivariate models have good sensitivity for making these local predictions. The predictive properties of these measurements in diabetes should be useful both in clinical trials or studies aimed at better treatments for diabetic eye disease at all levels, and for doctors treating patients at risk.

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Chapter One: Background and Introduction

1.1 Diabetes

1.1.1 Impact

The focus of this thesis is diabetes and the prediction of diabetic eye disease. This work is important because diabetes, in addition to the problems raised for those with the disease, has a huge impact on the health system both in the United States and around the world. According to the most recent statistics, released in early 2011, there are over 25.8 million people in the United States with diabetes, 8.3% of the population.[1] It is the sixth leading cause of death in this country, contributing to over 230,000 deaths in 2007 alone,[1, 2] and is actually underreported on death certificates (often noted as heart disease or stroke). In 2007 the cost of diabetes in the United States alone was $174 billion dollars, with $116 billion of that in direct health costs.[3]

The overall impact, on the lives of the patients with diabetes, stems mostly from the systemic complications of the disease. The complications involve many organ systems, including cardiovascular, neural, and ocular effects. Preventing these complications requires vigilance in blood glucose control and constant follow up by the patient and a team of doctors.

1.1.2.Types

There are different types of diabetes. While they are often thought to be the same disease, there are important differences between the types. This section gives a very brief introduction to the types of diabetes to highlight the similarities and differences. Studies included later in this dissertation focus on the differences between neuronal health between type 1 and type 2 diabetes.

1.1.2.1 Type 1

Type 1 diabetes is an autoimmune disorder, which destroys pancreatic beta cells and leaves the body with an inability to produce insulin. Insulin is a protein that the body uses to process glucose from the blood for use by cells. Type 1 diabetes encompasses 5-10% of all cases of diabetes.[3] By the age of 18 years approximately 1 in 300 children in the United States have type 1 diabetes and world wide the incidence of the disease has been increasing by 2-5%.[4]

The onset of type 1 diabetes is typically sudden with symptoms such as increased urination, thirst, and fatigue. Type 1 diabetics need frequent doses of insulin by injection or pump in order to live. Failure to administer insulin in a timely fashion can lead to the cells not getting the glucose they need and they begin burning fat for energy. This fat burning produces ketones as a side product which can lead to ketoacidosis, a coma which can be life threatening.[1]

1.1.2.2 Type 2

In type 2 diabetes, the pancreas can still produce some insulin but the body is not able to use the insulin effectively (also know as insulin insensitivity). The insensitivity is generally focused on the adipose tissue and skeletal muscles because those cells have high numbers of Glut 4 receptors which are the receptors which are most effected in

diabetes.[5,6] In type 2 diabetes, both glucose and insulin build up in the blood stream over time. Furthermore, the cells also do not receive adequate glucose. The build up of the combination of glucose and insulin leads to inflammation and oxidative stress, which cause cell damage, exacerbating the problem. As the disease progresses, the pancreatic beta cells lose their effectiveness resulting in insulin dependence for many type 2 patients.

The initial symptoms of type 2 diabetes are similar to that of type 1; increased thirst, hunger, urination, and fatigue. Type 2 diabetes accounts for 90-95% of the cases of diabetes. The onset is usually more gradual and usually takes place in adults but younger onset is becoming more common.[3] Type 2 diabetes is often co-morbid with other health issues such as obesity, hyperlipidemia, and hypertension.[7] The Obesity Society, a scientific society who studies obesity and related diseases, states that 90% of type 2 patients are over weight.[8]

1.1.2.3 Other Types of Diabetes

Other types of diabetes exist but are less common, and are not the focus of this thesis. Briefly, these include gestational diabetes and genetic defects of the pancreatic beta cells. Gestational diabetes can occur in late pregnancy and occurs in to 3-8 percent of pregnant woman. It increases the risk that the woman will develop type 2 diabetes later in life by 40-60 percent, and generally results in larger birth size for the baby.

Genetic defects of the pancreatic beta cells result from mutations in a single gene and result in impaired insulin production. MODY (maturity onset diabetes of the young) the most common form of these defects. It can be caused by a defect in a number of different genes.[3] We have had patients with MODY participate in our studies as individual cases but their results are not reported within this thesis.

1.1.3 Diagnosis

Diagnosis of diabetes is usually definite in type 1 diabetes but more ambiguous in type 2 diabetes, where the symptoms appear more gradually. This is an important consideration when evaluating the duration of diabetes and its impact on the health of the patient. In the studies presented later in this thesis, we often targeted patients with longer durations of diabetes as they are at a higher risk for retinal changes. However in most cases the actual duration of diabetes is likely different and longer than the value recorded, depending on how quickly the patient was evaluated by their doctor. An eye exam with retinopathy can also often be the catalyst to a diabetes diagnosis.

Diabetes is diagnosed by a fasting blood glucose level of over 126 mg/dL, a random blood glucose reading over 200 mg/dL with diabetes symptoms, or a blood glucose reading over 200 mg/dL 2 hours after an oral glucose tolerance test (a beverage with over 75 grams of glucose dissolved in water).

Although still controversial, in the update to the standard of care in 2010, the American Diabetes Association also recommended that the HbA1c could also be used to screen for and diagnose diabetes. They set 6.5% as an abnormally high value.[9] This change came due to increased standardization of the assay, and with a recommendation that the test only be done using laboratories that subscribe to the reference standards set by the DCCT study. For HbA1c measures, pre-diabetes is the range from 5.7%- 6.4%.

1.1.4 Current Treatments

Insulin is the only treatment for all patients with type 1 diabetes and is also a treatment for some patients with type 2 diabetes. Insulin was first isolated in 1921-1922 by Banting and Best at the University of Toronto. It was prepared for human use by Macleod and Collip and was first administered on Jan 11, 1922. Banting and Macleod earned the Nobel prize for this accomplishment.[10]

Insulin can be long or short acting. Lantis insulin is the longer lasting variety. It is a hexamer of insulin, which slowly breaks down over time. Humalog and Novolog are fast acting insulins (produced by different drug manufactures), which are less complex molecules. Generally, fast acting insulins can start working in 5 minutes, peak at 1 hour and last 2-4 hours. Humalog (a synthetic insulin) is created in a laboratory using E-coli bacteria which are genetically altered to produce insulin very similar to human insulin.[11] Novolog is an insulin aspart and is made in a similar fashion using recombinant DNA, meaning that to create Novolog a substitution is made where an aspartic acid is substituted in for a proline at amino acid 28. It is also created by a yeast which keeps the insulin from forming the hexamers, which slow down the insulin action.[11]

Metformin is one of the most popular drugs used to treat type 2 diabetes. It controls blood sugar by lowering glucose production in the liver and increasing uptake in the periphery.[12] It is the drug of choice in patients with heart problems and in obese patients.[13]

Sulfonylurea drugs, which include glipizide and gliburide, act by triggering insulin release. While the mechanism of action remains uncertain, they may act by increasing the number of insulin receptors and binding to specific plasma membrane receptors.[14] These drugs are used for patients with type 2 diabetes that cannot be controlled by diet.[15]

Thiazolidinediones are peroxisome proliferator-activated receptor (PPAR) agonists, which enhance the cell's use of glucose. They are the newest drugs to become available and include Avandia (Rosiglitazone). Recent post-market testing shows safety concerns in this drug class, thus Avandia has been limited to diabetic patients who cannot control their blood sugar with other medications. These safety concerns include increased risk of heart attack or stroke.[16]

1.1.5 Complications of Diabetes Throughout the Body

Complications from diabetes spread into many organ systems. The work here concentrates on the complications in the eye, and ways to predict and prevent retinopathy and macular edema. However, all the systems are connected so it is important to give a complete picture of how diabetes affects the entire body as part of this background. For example, our work in later chapters highlights higher blood pressure, part of the cardiovascular system, as an important component in ocular health in patients with retinopathy.

Furthermore, the patients in our study often ask questions about their other symptoms as they pertain to managing their diabetes. It's important for doctors who work with diabetic patients to be informed about all the risks and complications of the disease to help the patients manage their disease.

1.1.5.1 Cardiovascular and Heart Disease

According to the American Diabetes Association, (in 2004) 68% of death certificates for patients with diabetes listed heart disease and 16% listed stroke. This represents 2-4 times the risk for heart disease compared to people without diabetes.[17] Heart disease is the most prevalent cause of death in patients with diabetes. In a recent study evaluating the association between obesity and heart disease in men and woman, diabetes and hypertension were found to be independently associated with an increased risk of heart disease.[18] Furthermore, it has also been found that even before diabetes is diagnosed (in the stage or pre-diabetes or insulin resistance), there is relationship between increased insulin resistance and decreased cardiac output in African American patients.[19]

1.1.5.2 Nephropathy

Diabetes accounts for 40% of all new cases of end stage renal disease.[20] Furthermore, 20-30% of patients with diabetes acquire nephropathy during the course of their disease. The first sign of nephropathy is microalbumnuria (urine albumin level > 30 mg/day), which can then progresses to albuminuria (300 mg/day). According to the Diabetes Control and Complications Trial (DCCT), blood glucose and blood pressure control reduces the risk of these complications.[21]

1.1.5.3 Neuropathy/Neural Degeneration

According to the American Diabetes Association, 60-70 % of people with diabetes have some nerve damage.[3] Specifically, 30 % of diabetics over the age of 40 have impaired feeling in their feet, a condition known as distal symmetric neuropathy,[3] which is diagnosed with nerve conduction studies and electromyography (EMG).[17] According to the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR), the 25 year cumulative incidence of lower extremity amputation is 10.1% in patients with type 1 diabetes, and neuropathy is one of the independent risk factors.[22]

The main symptoms of neuropathy are pain and numbness. The pain is caused by damage to the nociceptors, neurons that sense pain and temperature.[23] Neuropathy can also causes issues of impotence, cardiac arrhythmia and cardiac ischemia[24, 25] The DCCT and WESDR studies also found that better blood glucose and blood pressure control can reduce the risk of neuropathy, along with not smoking.[21 22]

1.1.5.4 Ocular

There are 12,000-24,000 new cases of blindness each year from diabetes making it the leading cause of preventable blindness in working aged adults (21-74).[17] The following subsection will address changes to all parts of the eye due to diabetes.

1.1.5.4.1 Lens Changes

Often one the earliest signs of diabetes is a myopic shift in refractive error or refractive error instability. This is so often the case that clinically eye care professionals are trained to ask questions about diabetes symptoms in cases of extreme refractive error change. This shift is caused by excess glucose being processed through the polyol pathway.

Specifically, the polyol pathway is activated by excess glucose. Its rate-limiting step controlled by aldose reductase, which turns the abundant glucose into sorbitol. Because of its ability to utilize glucose, aldose reductase is a topic of diabetes research.[26] In experiments with mice that over express aldose reductase, the mice have more oxidative stress and have more cataracts.[27] The end result of the polyol pathway in diabetes is the sorbital becomes trapped in the lens; it expands changing refractive error to a more myopic prescription.

Other changes to the lens also happen as a result of diabetes. Wiener et al.[28] found that the cortex of the lenses of type 1 diabetics were thicker than controls. This relationship strengthened as the duration of diabetes lengthened. Kline et al.[29] found that diabetic patients also have increased pathology of the lens and are diagnosed with cataracts earlier and those cataracts progress faster than patients without diabetes.

1.1.5.4.2 Ocular Neuropathy

Diabetes is responsible for changes to neural tissue in all areas of the eye. This dissertation focuses heavily on changes to retinal neurons as a result of diabetes, measured with multifocal electroretinograms (mfERG). A complete discussion on the relationship of diabetes to mfERG changes is presented in a later subsection. However other changes to neurons in the eye are also related and merit brief discussion.

In the retinal neurons in animal studies, Barber et al.[30] found a 14% reduction in the thickness of the inner plexiform layer, a 22% loss in the inner nuclear layer, and a 10% loss of ganglion cells after 7.5 months of diabetes in Streptozosin (STZ) rats. That group also found increased apoptosis in the diabetic retina. They suggest glutamate excitotoxicity or the activity of advanced glycation (or glycosylation) end products (AGE) as possible causes for these changes.[31] (Streptozosin rats are a common animal model of diabetes where the drug streptozosin is used to kill pancreatic beta cells leaving the rat with diabetes.) In this same rat model, Lopes de Faria and colleagues found that diabetes and hypertension together attenuate the proliferation of retinal cells.[32]

Human patients with diabetes also commonly have reduced sensitivity in their corneal neurons,[33] leading to caution for the use of contact lenses. They are also more likely to have cranial neuropathies in the third, fourth and sixth cranial nerves.[23]

1.1.5.4.3 Glaucoma

The relationship between diabetes and primary open angle glaucoma is complicated and continues to be investigated. It will be touched on briefly here, being mentioned as another possible aspect of neural change associated with diabetes. The Ocular Hypertension Treatment Study (OHTS) found that diabetes is protective against glaucoma.[34] However, this was based on self-reports from the participants as to whether or not they had diabetes, leading to discussion and follow up on this relationship.[35] Earlier studies and studies since OHTS have found that the two are associated, with patients with diabetes being more likely to develop glaucoma.[36, 37] Yet, other large population based studies have found no relationship between the two.[38] It is known that diabetes can cause secondary glaucoma from neovascularization in the angle of the eye.[39]

1.1.5.4.4 Retinopathy

Diabetic retinopathy is the most common complication of diabetic eye disease. It is present in 28.5% of diabetic patients aged 40 and above.[40] There are several theories as to what takes place within the retina to lead to diabetic retinopathy. It could be one or a combination of several pathways that lead to the clinical damage seen. The first pathway of interest involves direct toxic effects of hyperglycemia on the tissue such as an increase in advanced glycation end products and protein kinase C activation.[41] The second pathway that can lead to retinopathic changes is alterations in the cell signaling pathways within the retina. And third, retinopathy could be the result of changes in the sorbital pathway leading to increased free radicals and oxidative damage within the retina.[42] Most likely it is a combination of alterations to all three pathways, leading to the clinical damage observed.

Clinically, the earliest visible changes of diabetic retinopathy are microaneurysms (MA). MAs are small out-pouchings of retinal vessels caused by damage to the endothelial cells. Under normal circumstances the endothelial cells provide structure to the vessels, but their death in the condition of hyperglycemia accompanied with the increased blood flow in diabetes, causes the vessels to lose their typical structure. Other early changes in the diabetic retina include dot, blot, and flame hemorrhages (hemes). These hemorrhages are visible leaking blood trapped within the retinal layers. The clinical presentation of the heme depends on the retinal layer it is trapped within.[39] In our first longitudinal study presented in chapter 4, we created a model for the prediction of the onset of retinopathy. MA's and hemes are the changes that were observed in the patients in that study. We created a model to predict these earliest changes.

Further signs of diabetic retinopathy include cotton wool spots, or local infarcts in the retinal tissue, venous beading which is further changes to the venules caused by hyperglycemia, and intraretinal microvascular abnormalities (IRMA) which are collateral vessels formed within retinal areas that are not receiving enough oxygen.[39] Finally vascular proliferation, the growth of new vessels, is the final stage of diabetic retinopathy. At this stage there can be vision loss. In addition to proliferation (PDR), diabetic macular edema can also lead to vision loss. This will be covered extensively in the next section. Proliferation of new vessels can lead to tractional detachment and vitreous hemorrhage, which are the specific causes of vision loss. PDR is treated with pan retinal photocoagulation, which reduces the stress on the retina by laser treatment of the peripheral retina tissue. PDR is touched on only briefly here as we did not include subjects with PDR in our studies, but all the other stages of retinopathy are represented.

1.2 Diabetic Macular Edema Overview

Chapters 6 and 7 examine the relationship between the mfERG and diabetic edema. Diabetic edema is a leading cause of vision loss in diabetes. There are several pathways that can lead to edema, and there are no preventative treatments specifically for edema at this time. This section gives an in depth presentation on diabetic macular edema and how it is currently detected and treated. I also discuss the previous efforts to predict edema, retinopathy, and mortality in diabetes, as it pertains to the work later in this thesis on the prediction of diabetic eye disease. We have created a model to predict retinopathy and edema in "at risk" patients in chapters 4 and 7 of this dissertation.

1.2.1 Definition

Diabetic macular edema (DME) is the leakage of fluid from the small retinal vessels into the macular tissue. The fluid accumulates in the outer plexiform layer and the inner nuclear layer. It leads to visual blurring and vision loss. There are two kinds of edema- extracellular and intracellular. Typically, extracellular edema is caused by increased vascular permeability that leads to the leakage of serum proteins and lipids into the intraretinal space. Excessive fluid then follows the osmotic gradient into the intraretinal space.[43] Intracellular edema can be caused by swelling of the Muller cells themselves.[44]

Edema can occur anywhere in retina but edema near the fovea is vision (visual acuity) threatening. The EDTRS study set up the guidelines for clinically significant macular edema (CSME), which is edema that is more likely to cause vision loss. Edema qualifies as clinically significant if it meets any one of the following three criterion 1) If there are hard exudates within 500 microns of the center of the fovea with thickening of the adjacent retina. 2) Thickening of center of the fovea within 500 microns. 3) Retinal thickening one disc diameter in size or larger within one disc diameter of the center of the fovea.[45]

1.2.2 Epidemiology

In the United States, diabetes is the leading cause of vision loss (visual acuity) in working aged people. When examining this group, DME is one of the main reasons for vision loss.[17, 46] Half of the patients with DME will lose more than two lines of visual acuity within 2 years.[47] The WESDR study found that over a 10-year period 14% of the diabetic patients included in their study developed DME and 10% developed CSME. When dividing the patients into types of diabetes the 10 year rate of macular edema in type 1 patients was 20.1%, it was 25.4% in type 2 patients who took insulin, and 13.9% in type 2 patients who did not take insulin.[48] The more recently published Los Angeles Latino Eye Study found that in a four year period 7.2% of the diabetic patients developed clinically significant macular edema and another 5.4% developed macular edema.[49]

Many factors have been found to be associated with an increased risk of DME. These factors include a longer duration of diabetes,[49] lower socioeconomic status, elevated cholesterol, increased DR,[50] higher diastolic and systolic blood pressure, Hispanic ethnicity, and prior amputation.[51]

1.2.3 Mechanism

The mechanisms leading to DME are complex and involve many different pathways. The combination of these pathways (leading to extracellular edema) is summarized by hyperglycemia and its downstream effects (free radicals and toxins) inducing an alteration of the blood retinal barrier (BRB) and the cells that compose it. The BRB is composed by the retinal pigment epithelium (RPE) on the outer side and the retinal vasculature/capillaries on the inner side. The endothelial cells of these vessels have the responsibility of maintaining the BRB. Under normal circumstances the endothelial cell tight junctions are almost impermeable to protein transport, setting up a gradient within the retina.[52] On the other side of the barrier, the RPE balances the fluids within the retina.

With increased blood glucose, the retinal capillaries elongate, dilate, and their basement membranes thicken. This increases the sheer stress on the vessels and causes a decoupling of the endothelial cell tight junctions. The leakage of proteins into the tissue follows. Inflammatory mediators and growth and oxidative stress factors, are also released, which increases cellular hypoxia.[50] The external limiting membrane (ELM) is a barrier within the retina, which is made up of zonulae adherentes junctions between the photoreceptor and Muller cells. Some proteins cannot pass through the ELM. The fluid accumulates in this location and as the fluid builds up, the RPE becomes overwhelmed. It cannot clear the fluid fast enough and edema is the end result.[53]

Other factors, cells, and mediators also contribute to this process through other mechanisms. The pericytes which live outside the BRB, are sensitive to increased advanced glycation end products (AGE's) which are abundant in the blood stream in diabetes.[54] Pericytes help control autoregulation of the retina and so their loss leads to reduced regulation of the retinal tissue. Pericyte loss correlates with MA formation, and thus is in the stream that eventually leads to the formation of DME.

Retinal leukocytes also contribute to the process of DME formation.[55] These leukocyte cells are recruited to the areas of endothelial injury. They can generate free radicals, which cause retinal damage. Furthermore, in diabetes there is also leukostasis, which can lead to capillary non-perfusion in addition to the release of free radicals. Capillary non-perfusion leads to ischemia, which also with all the other factors described earlier, promotes the release of inflammatory mediator factors such as angiotensin II and VEGF. VEGF is an angiogenic factor protein that promotes the growth of new blood vessels. Extra VEGF in the eye promotes the process of DME via inflammation and vascular permeability increases. VEGF is 50,000 times more potent that histamine in inducing vascular leakage.[44] Anti-VEGF agents are getting attention as treatments for vascular eye disease including diabetes.

1.2.4 Detection

1.2.4.1 Fundus Examination and Photography

An eye examination with a dilated fundus examination is one of the most common ways that DME is detected. With the increased use of telemedicine, fundus photographs have become an increasingly popular method of detection. Studies have shown that fundus photography is more sensitive than ophthalmoscopy for detecting early diabetic lesions and CSME, when it occurs in eyes with early diabetic changes, even amongst eye care professionals.[56, 57] This is not true in more severe cases and the two are often used together.

1.2.4.2 Fluorescein Angiography (FA)

This method involves the intravenous injection of sodium fluorescein dye into the arm of the patient. The dye then moves through the vascular system into the eye. Under normal circumstances the dye cannot pass through the retinal capillaries due to the tight junctions. However, in DME when those junctions are damaged, the dye can leak into the retinal tissue. This method is sensitive for finding early changes in diabetes and early leakage from DME.[58, 59] It can also detect capillary non-perfusion, which is not as easy to detect with other methodologies; it can be a critical clinical measure. However, the

injection is invasive, can sometimes be difficult especially in patients with diabetes who have vascular damage, and there is the slight risk of allergic reaction to the dye.

1.2.4.3 Optical Coherence Tomography (OCT)

This method, which was first introduced in 1991, allows for the visualization of a cross section of the retinal layers. According to the original publication on the method, by Huang et al., OCT uses "low-coherence interferometry to produce a two dimensional image of optical scatter from internal tissue microstructures in a way that is analogous to ultrasonic pulse-echo imaging."[60] In short, this technology allows for in-vivo imaging of retinal tissue and is now frequently used to assess retinal disorders. The Zeiss cirrus OCT, one of the OCTs used in our study, has an axial resolution on the order of 5 microns and a transverse resolution of 15 microns.[61]

OCT is an ideal method for detecting eye disease because it is both fast and non-invasive. Recent studies have found that spectral domain OCT results are highly and systematically correlated with FA[58, 62] and may be more sensitive than FA for the detection of cystoid edema in eyes with diabetes and other diseases.[63] OCT has also been used to look at sub-clinical changes in the retina, as we have, and it is discussed in Chapter 5. OCT to detect edema will be detailed in Chapter 6.

1.2.5 Treatment of Edema

1.2.5.1 Photocoagulation

The treatment of DME depends on the specifics of both the location within the retina and extent of the retinal swelling. DME can be focal or diffuse; how defined it is dictates the treatment. Currently the gold-standard treatment for CSME is either focal or grid laser photocoagulation. Focal laser coagulation has the goal of limiting leakage by aiming laser burns at leaking MAs. Grid laser also aims to reduce leakage but with a series of burns over a larger area. The ETDRS study found that laser photocoagulation reduced the risk of vision loss from DME by 50%.[45]

1.2.5.2 Injections

1.2.5.2.1 Steroids

Steroid injections of triamcinolone (Kenalog) have been used in the treatment of diabetic macular edema. They are not routinely used in solo treatment because it has been found that focal or grid laser is more effective for most patients with DME than steroid injections alone.[64] Steroid injection is also not without side effects. They routinely cause ocular hypertension (30-40% of patients) and also increase the risk of cataract and intraocular infection.[65] Steroid injections still have a place in some combination therapies for DME.

1.2.5.2.2 Anti-VEGF

Injections of the anti-VEGF agents, ranibizumab (Lucentis) and bevacizumab (Avastin), have been successful at reducing vision loss in wet age related macular degeneration. They have recently been studied in a variety of diseases where abnormal vessel growth is at the heart of the condition, including diabetes. These drugs have been

used in attempts to reduce DME and vessel growth in diabetic patients. While they are currently still an off-label use, they have been shown to be successful in reducing macular thickness and increasing visual acuity in these diabetic patients.[66, 67] They are becoming more and more widely used for these conditions.

1.2.5.2.3 Combination Therapies

Many different combinations of the above therapies have also been examined in studies looking at the treatment of DME. While the literature remains inconclusive about the benefits of steroid injections before focal laser,[68] a very recent report suggests that combination pharmaceutical and laser treatments may be quite effective in treating macular edema in diabetes. This study, performed by the Diabetic Retinopathy Clinical Research Network, found that injection of anti-VEGF or steroid followed by laser treatment was more effective than laser treatment alone but that the steroid group had a higher complication rate.[69]

1.2.6 Prediction and prevention of DME and retinopathy, and the use of retinopathy and edema as risk factors for prediction of diabetes related mortality.

Since the treatments for DME and PDR are very invasive, work on prevention and prediction of DME and retinopathy is very important. The focus of this thesis is the prediction of diabetic retinal eye disease. Previous studies on the prediction of which patients will get DME, and the prevention of DME, have concluded that better glycemic and blood pressure control can lower the risk of this complication. Both the UKPDS and DCCT studies indicated that lowering HbA1c lowers the risk of DME.[21, 70] Furthermore the DCCT [21] found that intense insulin therapy can lower the risk of DME by 50%.

Several studies have also shown a relationship between higher blood pressure and macular edema. High systolic blood pressure has been shown to be correlated with diffuse macular edema[71] and improved blood pressure control reduces the risk of macular edema.[72] The relationship between DME, retinal thickness and blood pressure is an important component to this thesis. We also found that blood pressure is an important predictive factor for edema. More discussion on this topic will be found in Chapters 5, 6, and 7.

By comparison, very little work has looked at the prediction and prevention of the earlier stages of retinopathy, probably because unlike macular edema, earlier retinopathy does not alter visual acuity. Although the UKPDS [73] did find that one or two microaneurysms were significant for progression of retinopathy and should not be ignored. Other factors such as hypertension, and neuropathy have also been found to increase the risk of earlier retinopathy.[74]

Interestingly, many studies that have looked at the prediction of cardiovascular disease and mortality from diabetes use retinopathy, edema, and other eye signs as markers and risk factors. This reminds us that these changes in the eye are a piece of a much larger picture in diabetic health. The WESDR study found that decreased arteriole size and increased venule size are associated with all causes of mortality from diabetes but not specifically with the incidence of retinopathy, proliferation or macular edema.[75] A study in Spain[76] found that although 30% of patients with diabetes had retinopathy, their overall risk for 10-year mortality was low (5%). Another study in Japan[77] found diabetic retinopathy to be a risk factor for overall mortality after coronary bypass surgery.

Thus, in addition to being important in maintaining good vision over a lifetime,

changes in the eye may be important indicators of over all health in diabetes but more work is certainly needed to accurately predict who will, and will not, develop diabetes related eye changes. The work in this thesis is a step toward that goal.

1.3 Landmark studies on the treatment of diabetic retinopathy and their results

The standard of care for the treatment of diabetic eye disease is the result of several landmark studies, which took place in the 1980's, 1990's and early 2000's. They are the UKPDS, DCCT, ETDRS, and WESDR studies. Their patient populations, goals, and results are briefly outlined here. These studies provide the building blocks for future studies on changes in the eye induced by diabetes. They also provide the basis for much of the epidemiologic data we have for eye complications; they are heavily referenced in the later chapters presented in this thesis.

In addition to discussing important studies, this section (1.3) also discusses the limitations of studies in the eye and specific concerns about the use of endpoints and surrogate endpoints in such studies. The diabetic eye has unique concerns for research studies, which largely stem from the fact that a great deal of damage can occur before vision is lost.

1.3.1 United Kingdom Prospective Diabetes Study (UKPDS*)*

The UKPDS study was conducted in the United Kingdom between 1977 and 1997 and included data for patients followed for 53,000 person/years. It enrolled 5,102 newly diagnosed type 2 diabetic patients (mean age 53). It also included within it the Hypertension and Diabetes Study (HDS), which evaluated a subset of 1,148 hypertensive diabetic patients (39% of the total enrolled patients). More than 85 publications have resulted from the UKPDS study.

While it is not practical to summarize the results of 85 publications here, the central question of the UKPDS study was to determine if improved blood glucose control prevents diabetes complications. Different groups of patients in the study took sulphonylurea drugs, insulin, and metformin. The study revealed that that intensive glucose therapy lowered risk for any diabetes end point by 12%, microvascular endpoints by 25%, heart attack by 16%, cataract by 24%, retinopathy at 12 years by 21%, and albuminuria at twelve years by 33%. It was also found that all intensive therapy medications (metformin, insulin, sulphonylurea) improved blood glucose control over diet alone and lowered HbA1c by 2% on average. This study also reduced fears about the cardiac complications of sulphonylurea drugs and insulin therapies, which did not have an increased risk of complications. However, both therapies did increase the risk of hypoglycemia and weight gain.

This study also provided information about the health care system in the United Kingdom, which could be applied to the United States and around the world. After 6 years only half of the patients had an HbA1c under 8% and 40% of subjects were still on their initial therapy.[78] Furthermore, in UKPDS, mono-therapy with sulphonylureas, insulin or metformin all proved ineffective over time. The need for stepwise addition of therapeutic agents in the UKPDS is typical of diabetes management, increasing the relevance of this study's results to current clinical practice.

The Hypertension and Diabetes Study (HDS) was part of UKPDS. It found that lowering blood pressure with an ACE inhibitor or a beta-blocker reduced the risk of

microvascular and macrovascular disease. The average blood pressures in the study went from 154/87 mmHg to 142/82 mmHg over 8 years and the study results showed that lowering the systolic blood pressure lowered the risk of any diabetes related complication by 12%.[7, 79] The inclusion of blood pressure control medications in type 2 diabetes is now common practice.

1.3.2 The Diabetes Control and Complications Trial (DCCT)

This study was conducted from 1983 to 1993 and sponsored by NIH/NIDDK. It enrolled 1,441 patients who were 13-39 years old with type 1 diabetes. The goal of the study was to intervene in part of this group to improve blood glucose control and see the effects. The study specifically asked two questions: 1) if intensive therapy prevents the development of diabetic retinopathy in patients with no retinopathy and 2) does intensive therapy alter progression of early retinopathy. Although the study was primarily interested in retinopathy as the outcome measure, it also gathered data on other organ systems, such as renal, cardiovascular, and neurological systems. The difference between the conventional and intensive therapy groups was the number of times insulin was administered every day (twice for the conventional group, and three or more times for the intensive group.) The intensive group also monitored their blood sugar more closely and adjusted their insulin based on blood glucose levels. This group attempted to maintain a preprandial blood glucose of 70-120 mg/dl. The intensive group also had more follow up visits than then conventional group.

The study found that the intervention helped delay the onset and progression of retinopathy, neuropathy, and nephropathy. The difference in the amount of retinopathy between the two groups became apparent at 36 months when the intensive group had 50% less retinopathy. In the first year the intensive group had a higher incidence of retinopathy progression compared to the conventional group, but that risk lowered at 36 months and remained lower for the rest of the study resulting in an overall lower risk for the intensive group of 54%. [21]

The improvements in the intensive group in this study showed how important blood glucose control is for maintaining good health with type 1 diabetes. The intensive group did have significantly more episodes of hypoglycemia and so the treatment is not without concerns, but overall intensive glucose therapy seems to have many benefits according to this study.

1.3.3 The Early Treatment Diabetic Retinopathy Study (ETDRS*)*

This study was conducted beginning in 1980. The subjects in this study were followed for at least 4 years. It was sponsored by the National Eye Institute and has yielded around 30 publications. 3,711 patients with diabetes and moderate, severe, or early proliferative retinopathy and/or macular edema were enrolled. The study sought to answer three questions. These questions are explicitly listed in their first publication (and quoted directly here).[45] "1. When in the course of diabetic retinopathy is it most effective to initiate panretinal photocoagulation? 2. Is photocoagulation effective in the treatment of diabetic macular edema? 3. Is aspirin treatment effective in altering the course of diabetic retinopathy?"

Generally the patients were randomized so that one eye was photocoagulated immediately and one not until proliferation developed. The patients with edema were

randomized into two groups above and then were further randomized into two subgroups. The subgroups were formed for the eyes that had immediate photocoagulation; they were further randomized to panretinal and focal, or just focal laser.

The major results of the study identified clinically significant macular edema (CSME) and defined its criterion. This study also revealed that focal or grid laser for macular edema saves sight, and thus established it as the gold standard and standard of care. The outcome measure for this study was visual acuity for the macular edema section. The results for questions 1, found no benefit to early photocoagulation, with similar incidence of vision loss in the early and delayed group.[80] The results for question number 3 found that aspirin did not make a significant different in reducing cardiovascular events in this group of patients. While it did not appear to be harmful to the patient group, it was not recommended as a therapy from this study.[81]

1.3.4 The Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR)

This study began in 1979 with a cohort of 996 type 1 and 1370 type 2 patients who had baseline exams between 1980 and 1982. It has had 5 follow up cohorts in 1984-86, 1990-92, 1995-96, 2000-2001, and 2006-2007. The National Eye Institute funded it.

The initial purpose of the study was to find the incidence and frequency of diabetes complications, specifically retinopathy, kidney complications, and amputation. They also sought to identify risk factors that may lead to the development of these complications. The many follow up cohorts have also allowed information on the cumulative incidence of complications.

This study has also generated many publications (over 200) so a complete overview of the results of the study is not feasible here, but generally the most important findings (according to the study website) are that improved blood sugar control reduces the risk and progression of retinopathy and kidney disease. The WESDR study also gives good data about the incidence and prevalence of complications within the diabetic population over time. [82-86]

1.3.5 Limitations of studies and outcome measures

All the studies discussed above are large-scale efforts that represent collaborations between epidemiologists, physicians, and statisticians. The end points in the studies differ due to the questions they are interested in, but the presence or progression of retinopathy and a decrease in visual acuity are common endpoints for diabetes and vision related studies.

Retinopathy progression as an endpoint (as with the DCCT) takes into account earlier changes in diabetes but had the challenge of coordinating fundus photography grading or fundus examinations. There is also the issue that some locations within the eye, namely near the fovea, hold more importance when diabetic lesions are involved than others, making it difficult to standardize the results. The problem with visual acuity as an endpoint in studies involving diabetic retinopathy (as with ETDRS) is that retinopathy does not alter vision until the very late stages of the disease (ETDRS study was evaluating this stage). Thus, this issue of finding acceptable surrogate endpoints for diabetic retinopathy studies is one of concern for researchers in this area. In the studies in our laboratory, one of the long-term goals is to help establish measures of neural function as a possible surrogate endpoint for studies of diabetic retinopathy and edema.

Prentice's landmark paper in 1989 was one of the first to describe surrogate endpoints. Weir and Walley[87] summarized his work in their review of surrogate endpoints and continue with further discussion detailing that while often confused, correlates are not the same as surrogate endpoints. It is important to note that just because a factor or measure is correlated with the outcome of interest that is not a strong enough reason for it to be a surrogate endpoint. Biomarkers, on the other hand, can be used as surrogate endpoints. They are advantageous because they may reduce the time in clinical trials, thus reducing costs.

Prentice stated for hypothesis testing that surrogate endpoints "yield unambiguous information about differential treatment effects on the true endpoint" and he defines a surrogate endpoint as "a response variable for which a test of the null hypothesis of no relationship to the treatment groups under comparison is also a valid test of the corresponding null hypothesis based on the true endpoint."[87, 88] Berger went on to extrapolate on Pretince's work and gives a specific list of criteria for a surrogate endpoint. Berger says that a surrogate endpoint should (1) be integrally involved in the process of the disease so that modulation of expression correlates closely with the disease course, (2) be different in the normal and disease process pathway (3) be reproducible (4) be susceptible to the treatments being employed. [89] We believe that the mfERG could fit these criterion outlined by Berger once more studies have been completed. Identifying good surrogate end points would aid in clinical trials aimed at new treatments for diabetic eye disease by reducing the number of patients required and saving costs. More discussion on the mfERG and the mfERG in diabetes is found in the next sections and in all of the subsequent chapters.

1.4 Electrophysiology

1.4.1 Early History

The standing potential of the eye was discovered in fish in 1849 by DuBois-Reymond.[90] The first human electroretinogram (ERG) was recorded a short time later in 1877 by Dewer. Einthoven and Jolly[91] (in 1908) were the first to obtain detailed recording (in frogs) and give letter designations to the different portions of the response. The first published human ERG was recorded by Kahn and Lowenstein in 1924.[92] However, the full field electroretinogram as we know it today was not developed until the 1940's, when the advent of the contact lens electrode made clinical electrophysiology more fesible.[93 90]

Significant advances came between the years of 1933 and 1947, when a scientist named Granit used chemicals to modify the ERG and found three main components. He labeled these components PI PII and PIII. The numbers were given for the order in which they disappeared under anesthesia. Later these components were likened to the a, b, and c waves that are now common nomenclature for the ERG. PIII forms the a wave, PII the b wave, and PI the c wave.[90]

1.4.2 Electrophysiology Tests

1.4.2.1 Full Field ERG

The full field ERG results in a waveform that has three general components, the a-wave, the b-wave and the oscillatory potentials (OPs). The a-wave is the fastest of the three components. It is a negative component that is generated by photoreceptor cells. [94] The next component, the b-wave, is a large positive response. It is generated largely by bipolar cells.[95] The oscillatory potentials, which are typically four to six high frequency oscillations, are located on the ascending b-wave. The OP's are thought to be feedback from ganglion to amacrine cells or bipolar to amacrine cells. [90, 96]

Clinically the full field flash ERG is mostly used for diseases that effect a large portion of the retina. It can be measured under photopic or scotopic conditions, allowing tests and responses from cones or rods, respectively. As diabetes affects a large portion of the retina, some of the first electrophysiological studies of the diabetic retina were completed with the full field ERG. The full field ERG is sensitive to the changes in the retina in diabetes at many different stages. The full field ERG has led to the use of other electrophysiological testing in diabetes involving the full field.

1.4.2.2 Pattern ERG

The response generated by the entire retina to a pattern stimulus is the pattern electroretinogram (PERG). The pattern electroretinogram is particularly useful because when dividing the display equally between black and white stimuli, the first order (linear) response cancels out and the non-linear components are highlighted. The PERG is generated by the inner retina, which means the response comes from ganglion cells, and some amacrine cells. The major peak is the P50 which may show the input activity of ganglion cells. The major negative potential is the N95 which represents output spiking activity.[90]

The pattern ERG has not been as widely used as the full field flash ERG to test the diabetic retina. The non-linear responses it highlights are not the changes that are most prevalent in diabetic neurodegeneration. The largest changes to the retinal neurons in diabetes are in central layers, the bipolar cells, this probably because of their close proximity to the blood vessels within the retina.

However, the studies performed with the PERG in diabetes found correlations between PERG changes and diabetes. Caputo et al.[97] found macular dysfunction in patients with type 1 diabetes, with and without retinopathy, using the pattern ERG. Other studies have found pattern ERG differences in timing and amplitude in diabetic patients with and without retinopathy.[98, 99]

1.4.2.3 Multifocal Electroretinogram (mfERG)

The multifocal electroretinogram (mfERG) is a newer technology, first reported in the 1990's by Sutter and colleagues. This technique allowed ERG responses to be obtained from multiple local retinal areas. The stimulus consists of multiple hexagons (103 were used in the studies presented in this thesis) that flicker between black and white each using the same binary (2^{15} -1) pseudo-random m-sequence, each slightly delayed in time. The individual responses for each location are determined by cross

correlating the location to the sequence. In the standard recording, using the first order kernel, the waveforms produced consist of an "a" wave and "b" wave. The "a" wave is derived from cones and the "b" wave from bipolar cells (mostly ON bipolar cells). The rods contribute very little to the mfERG, as is the case for the ganglion cells, and inner retinal cells.[90]

This origin of the mfERG was found using studies that selectively removed the responses of different neuron groups by chemically blocking them. The chemicals used in this process are APB (2-amino-4-phosphonobutyric acid), which is a glutamatergic receptor agonist. It blocks the signal transmission from photoreceptors to ON –bipolar cells. The second drug is PDA (cis-2, 3 piperidinedicarboxylic acid), which is a glutamatergic receptor antagonist. It blocks the transmission to OFF bipolar cells. A combination of PDA and APB is used to isolate the responses of photoreceptor cells. A third drug, TTX (terodotoxin) was also used to block the spiking activity in the retina (ganglion and amacrine cells).[100 95]

Higher order kernels, or the effects of previous flashes can also be evaluated with mfERG recordings. The mfERG is advantageous because it is non-invasive, measures at many local retinal locations, and is reproducible.[101] The mfERG has been shown to be sensitive to many diseases affecting photoreceptors and bipolar cells such as Stargardts disease, age related macular degeneration, central serous retinopathy and diabetes.[90]

A pattern multifocal ERG is a separate but related test, like the full field PERG, the first order responses cancel out leaving the non-linear responses of ganglion cells and amacrine cells. The pattern mfERG has been shown to be sensitive to diseases affecting ganglion cells (e.g. glaucoma). [102] To my knowledge there are no studies assessing the pattern mfERG in diabetes.

There have been many studies assessing the mfERG in diabetes by our group and others. The mfERG is an ideal test to assess function in diabetes as it assesses many different local retinal locations and is very sensitive to functional changes even before retinopathy is apparent. Changes to the mfERG in diabetes is the focus of this thesis and so this theme will be followed up extensively in the remaining sections and chapters.

1.5 Electrophysiology in diabetes and rationale for current work

1.5.1 Electrophysiology in diabetes

The overall focus of this thesis is the use of multifocal electroretinograms in diabetes, diabetic retinopathy and diabetic edema. The first studies using the mfERG to look at changes in diabetes were published in the late 1990's.[103] To date, studies have evaluated changes in every stage of diabetes, and the mfERG has also been used to investigate ocular treatments such as improvements in neural function following laser or injections of Lucentis or Avastin. [104, 105] Most of the studies, including those in this thesis, focus on adults mainly with type 2 diabetes. However there is now a growing interest, in our lab and with others, in neural function in adolescents.[106]

Before the mfERG was available, full field flash ERG studies on diabetic patients set a precedent for this work. These full field flash studies have continued to this day, and have revealed a reduction in OP amplitudes and increase in OP implicit time (particularly the first OP) in diabetes. In more severe diabetes there is a loss of amplitude and increase in implicit time (IT) of the full field flash b-wave under both photopic and scotopic

conditions.[107-109] Furthermore, changes in OP's have been reported to predict the progression of diabetic retinopathy in one study.[110]

Prior work with the mfERG in diabetes has focused on identification of differences between diabetic and control patients, as well as on the use of mfERG to predict future changes in diabetic eye disease at local retinal regions. It is known from this prior work that the mfERG tends to worsen as diabetes progresses, mostly visible by changes in the implicit time.[111, 112] Implicit time delays have been shown to be correlated with ischemic areas in the retina.[113] The oscillatory potentials and amplitude have also shown change and decrease in diabetes.[114-116] Studies by Klemp et al. show that the mfERG in diabetes can be influenced by changes in blood sugar at the time of recording, indicating that health factors may confound in this disease process and should be considered.[117]

Our group first used the mfERG for evaluation of retinal changes.[111] Identifying that the mfERG worsens locally as the retina has more changes from diabetes. More recently we have focused on the use of mfERG for the prediction of retinopathy and now edema. Predictive models for patient with and without retinopathy have been created for one, two and three years into the future.[118-120] Later chapters in this thesis will detail new models. The first creating a model of the onset of retinopathy in eye with no previous retinopathy, and the second a model of the onset of edema.

1.5.2 Summary and Rationale for Current Work

This thesis contains five related studies all examining different aspects of the relationship between retinal function, retinal structure and diabetes health measurements. They build on each other and on past work in our lab; they represent the logical next steps for our prior work. Our group has previously used the mfERG for evaluation of retinal changes, and as a method of prediction of new retinopathy in patients with prior retinopathy. The work here examines patients with no retinopathy and follows them until they develop retinopathy for the very first time. This is a very clinically important transition, which needed to be examined and is, in part, a follow up to the patients that had been studied in the lab earlier but had not developed any retinopathy. For our new study, presented here (Chapter 4) we included more patients, and those patients were targeted to have longer durations of diabetes to increase the likelihood of a retinopathy outcome.

Here we also look specifically at issues related to diabetic macular edema and the ability to predict its onset. As detailed in the earlier background subsection, diabetic edema is a leading cause of vision loss in diabetic patients. It is logical that if the mfERG can predict early retinopathy changes in diabetes, we should examine it to see if it can predict these more serious changes that can lead to the loss of vision. More accurate, quantitative and timely predictions of edema, who will develop it, and where it will be located in the retina, could help to save vision in this population.

For these experiments, first we examined subclinical changes in retinal thickness in patients, with and without retinopathy, and the factors that influence retinal thickness in diabetic patients. Retinal edema is accompanied by thickening of the retina. We were interested in OCT as an outcome measure of edema and also possibly as a tool that might not only provide a method of detecting thickness changes, but also help identify those for whom edema may be on its way. In short, we were gathering information about OCT as a

measurement for edema and identifying potential factors that could influence our measures. Second, we looked at the relationship between the mfERG and retinal thickness in patients with edema to build on the literature with our data set and add new information. Finally we followed patients longitudinally to establish edema onset in high-risk patients, and develop a model for the prediction of local edema using the mfERG measures and other known health risk factors for diabetic retinopathy.

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Chapter 2: General Methods

2.1 Introduction and Designs

This chapter discusses the methods of the studies included in this dissertation and the general protocol run on the patients included in all of the studies. Parts of this information will be repeated in individual chapter's methods sections as was necessary for publication of this work in the various scientific journals.

This dissertation is composed of 5 studies. Three of these studies, chapters 3, 5 and 6 are cross sectional, looking at data at one point in time and comparing different groups of patients. The other two studies, chapters 4 and 7, are longitudinal. For these studies a group of patients was followed over time. All studies were observational and no treatments were instituted to the patients as part of any of the studies presented here. The patients with edema, chapters 6 and 7, were aided in referrals back to their ophthalmologist for evaluation and any necessary management.

2.2 Patient Groups

This thesis involves four patient groups. 1) control patients, 2) patients with diabetes and no retinopathy, 3) patients with diabetes and retinopathy, with risk of edema onset, and 4) patients with retinal edema. All patients in the studies had the same general inclusion criteria. They had no previous surgeries or injuries to their eyes, a refractive error between +4.00 D and -6.00 D, and they were between the ages of 21 and 68. They had no media opacities or diseases that affected the retina except diabetes, although patients with controlled hypertension were also included. Patients in groups 1,2, and 3 had to have a visual acuity of 20/25 or better at study entry but this was not required for group 4; however as it happens all patients in group 4 had acuity of 20/100 or better.

Study subjects were paid $25 per visit for participation in each visit and informed consent was obtained from every subject yearly. The procedures followed the tenets of the Declaration of Helsinki and the protocol was approved but the Committee for the Protection of Human Subjects at UC Berkeley. Patients were recruited from Kaiser Hospital Oakland, San Francisco Veterans Administration Hospital, The Eastmont Wellness Center, and the UC Berkeley Optometry Clinic.

Information on the patient groups are as follows:

Group 1: There were approximately 80 control study patients included in all studies. 50 were run on our older mfERG instrument (mfERG1), 52 on our newer mfERG instrument (mfERG2) and 21 run on both instruments for the purposes of the reproducibility study (Chapter 3). All control patients were confirmed to have normal blood glucose levels at the time of testing. Any control with elevated random blood glucose at the time of testing (over 200 mg/dl) was not included in our control patient group. An mfERG was obtained from the better seeing eye of each control. If both eyes had equal acuity the eye with less refractive error was chosen; in the case equal refractive error in each eye, the left eye was chosen. All controls were tested once with the exception of the 21 participants in the reproducibility study and 9 control patients who returned after one year to assess the stability of control mfERG recording over time.

Group 2: Patients with diabetes and no retinopathy were followed over time annually for a range of 1-7 years. These patients met all the criteria listed above and also had a targeted duration of diabetes over 8 years. These patients are included in the studies

in Chapters 3, 4 and 5. Several of these patients were recruited for prior studies and their earlier data was reported in Han et al.,[1] and Ng et al.[2] However most of this thesis study group was recruited during my time at UC Berkeley from 2006-2010. Forty-one of these patients were included in the model in chapter 4, and their demographic data are discussed fully there. Development of retinopathy was the follow up end point for these patients. However after retinopathy development occurred many of them agreed to continue being followed as part of group 3.

Group 3: Patients with diabetes and retinopathy were also followed over time semi-annually for a range of 6 months – 4 years. This group was an entirely new cohort of patients all recruited after December 2006 for the studies presented here. This group is included in studies in Chapters 5 and 7. These patients had nonproliferative diabetic retinopathy and were also targeted to have a duration of diabetes over 8 years or an HbA1c over 8%, many of the patients met both criterion. Development of retinal edema on a fundus photograph (central 45 degrees) was the follow up end point for these patients. If retinal edema developed the patients were asked to return for a Fluorescein Angiogram (FA) to determine the extent of the edema and then they were included as part of group 4. Patients with clinically significant macular edema (CSME) as determined by our retinal specialist Shirin Barez MD, were then referred to their ophthalmologist for evaluation and any necessary management. Patients with proliferative diabetic retinopathy were not included in any study presented here but we did offer them referral help to find an ophthalmologist for treatment and management.

Group 4: Patients with retinal edema, were seen once as part of a cross sectional study. These results are noted and discussed in chapter 6. There were 13 patients (20 eyes) with type 2 diabetes and edema, and 2 patients (3 eyes) with type 1 diabetes were also evaluated. All patients had edema in the central 6 mm (macula) of the retina. Some patients came into the study with edema (6) and some developed edema after being followed as part of group 3 (above) (9 patients). Patients with retinal edema were included regardless of visual acuity or duration of diabetes but the other inclusion criteria applied.

2.3 Protocol Components

2.3.1 Visual Acuity and Contrast Sensitivity

After signing the necessary consent forms, the first component of the study was visual acuity measures. These were done at 10 feet with an ETDRS chart. High contrast and low contrast measures were obtained for each eye with habitual correction. Additionally contrast sensitivity (CS) on a Pelli-Robson chart was also obtained at 10 feet for each eye. Log CS scores were calculated for each eye. Patients with high contrast acuities worse than 20/25 were excluded from the study unless edema was present.

2.3.2 Color Vision

Although the data for this test is not shown in this thesis, An Adams destaturated D-15 color vision (DSAT) test was preformed annually on all patients.[3] It has been shown in prior studies that color vision can be affected in diabetes,[4, 5] and we are interested in evaluating those differences. Our group is looking at correlations between color vision and other measures, but this data is not included here.[6] A color confusion

score was calculated and recorded. A higher color confusion score resulted in more errors in arrangement and farther distance traveled across color space.

2.3.3 Blood Work

Three different blood tests were preformed on each patient with diabetes. First a conventional blood glucose reading (One Touch Ultra) was made to determine the blood glucose level at the time of testing. As blood glucose at the time of testing was found in another study (Klemp et al.[7]) to be a potential confounding factor by altering mfERG measures in diabetes, this information was necessary.[7] This test was also done on control patients. Second, an HbA1c was preformed to gauge blood sugar control over time (about the last 3 months). This was made with an At-Home HbA1c (Flexsite Diagnostics Palm City FL). Third, for all patients seen after (10/2008) an in house DCA-2000 analyzer HbA1c was also collected (Bayer Diabetes Care, Terrytown NY with testing reagents by Siemens Inc, Washington DC). Preliminary evaluation of the two HbA1c methods showed the results of the two to be highly correlated but they often gave slightly different results, especially at higher percentages so we continued to run both tests on each patient. All HbA1c data presented in this thesis is from the At-Home A1c test. Blood pressure (LAS) was also tested on every patient. An automatic cuff (Omron HEM-773) was used for consistency across examiners.

2.3.4 Optical Coherence Tomography

A Stratus OCT 3 (Time domain OCT, Carl Zeiss Meditec Dublin CA) was conducted to obtain retinal thickness measures, and also to aid in the evaluation of edema as an outcome measure. This is a commercially available instrument, but a specialized 12 scan protocol was designed to give more data points than the 6 scan protocol available as part of the instrument's package.[8] The data from the 12 scans was then averaged into 37 hexagons in Matlab as reported in Neuville et al.[8] These 37 hexagons match up the central 37 hexagons of the mfERG stimulus allowing for comparisons when necessary.

In November 2008 we purchased a Cirrus HD spectral domain OCT (Carl Zeiss Meditec). (Figure 1) All patients seen after November 2008 had both OCT measures. The Cirrus OCT (Spectral Domain) is advantageous because it takes 27,000 A-scans per second rather than 400 in 1.25 seconds; it is less depended on patient fixation and has better resolution (on the level of 5-8 microns).[9] The Cirrus OCT always measures retinal thicknesses to be greater than the Stratus OCT, which is due to the Cirrus OCT detection of the outer band of the retinal pigment epithelium. The Stratus OCT measures from the inner/outer segment of the photoreceptors.[10]

Figure 1

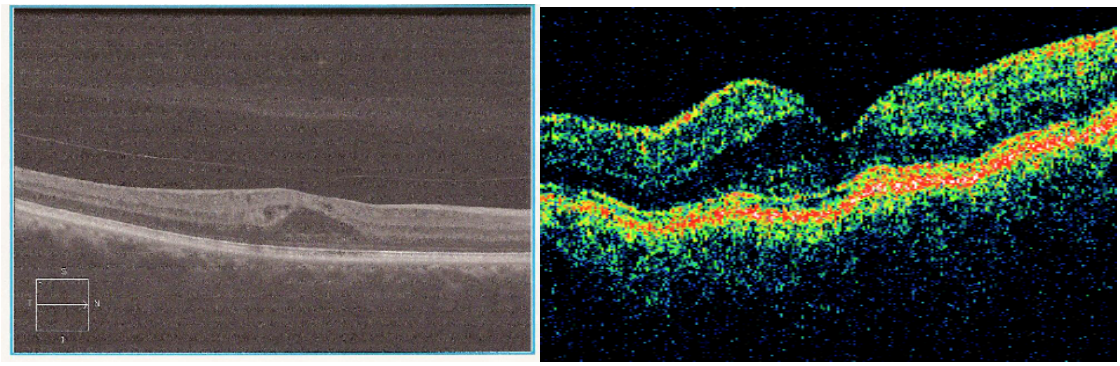

Figure 1: A Cirrus (Left) and Stratus (Right) OCT image of the same diabetic patient with clinically significant macular edema.

2.3.5 Fundus Photography

Colored fundus photographs covering the central 50 degrees were obtained for all patients. Early in the study (prior to 2009) a Topcon (Model - TRC-50X) camera was used taking 30 degree photos in 5 different fields. In January 2009, a Zeiss camera (Model- Visucam pro NM) was used. With the Zeiss, three 45 degree photos were taken. Care was taken to make sure the macula was visible in at least two images so that macular stereo pairs could be available for the edema studies for both cameras.

All photos were uploaded on to the Eyepacs web server (www.eyepacs.org). They were then accessed and graded in detailed fashion (Figure 2) for the presence and degree of retinopathy or edema by our retinal specialist, Shirin Barez MD. The multifocal electroretinogram (mfERG) hexagon locations were laid over the fundus photos so the locations of the retinal lesions could be spatially matched with the neural delays (Figure 2).

Figure 2

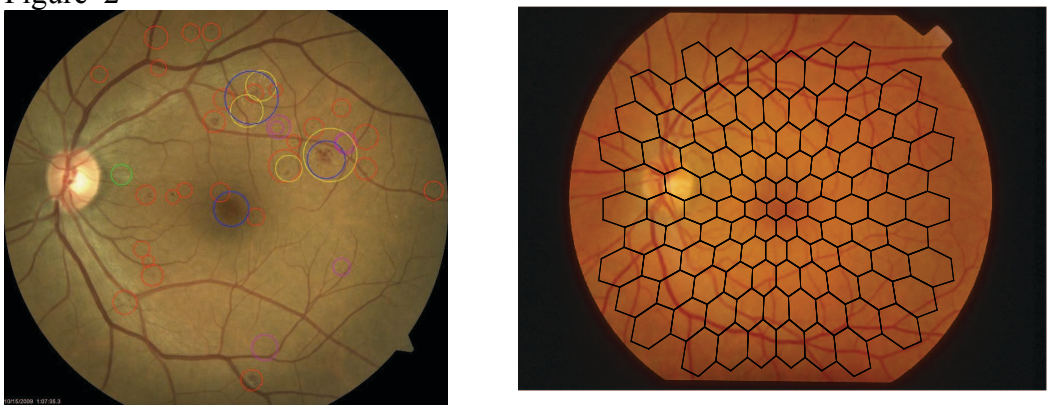

Figure 2: Left: Detailed Fundus grading of a patient with retinopathy and edema. The different colored circles highlight different retinal lesions present in diabetes using the traditional color scale. Here the red circles represent hemes, blue is edema, yellow are exudates, green are cotton wool spots, purple are IRMA. Right: The overlay of the mfERG stimulus on the fundus photo of a control patient.

2.3.6 Multifocal Electroretinography (mfERG)

2.3.6.1 Parameters

All multifocals were recorded on commercially available systems, VERIS, which are manufactured by Electro-Diagnostic Imaging Inc. We have two VERIS instruments in our laboratory space. (I have labeled them and routinely call them mfERG1 and mfERG2 for convenience.) They are very similar but mfERG1 uses the VERIS 4.3 software, mfERG2 uses a VERIS 5.2 system. Both have a 9-inch CRT display, which is a 640 x 480 pixel display. The both contain an eye-camera-refractor display. This display allows for constant retinal magnification at different refractive errors and also allows the patient to self adjust the screen to best focus. The video camera allows us to watch their fixation in real time. The stimulus is an 103 hexagon scaled display that subtended about 45 degrees. It was presented at a frame rate of 75 Hz. (13.33 milliseconds/frame) with a sampling frequency of 0.83 ms/sample (1200 Hz).

Figure 3

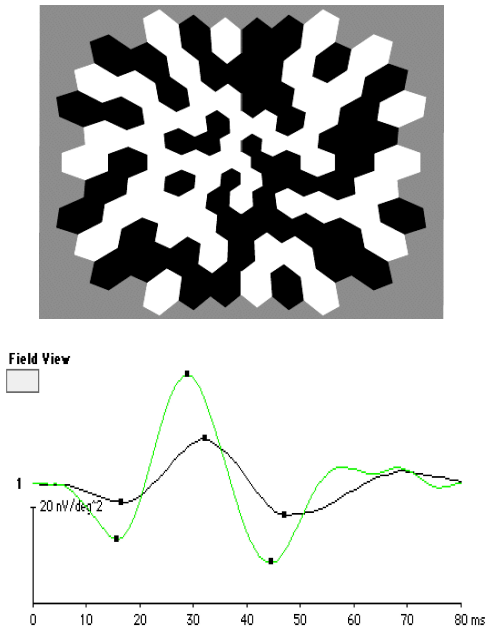

Figure 3: Top: The Veris stimulus as it was displayed to the patients in the study. Bottom: A sample waveform from a diabetic patient (black) and a control patient (green).

Subject's pupils were fully dilated with 1.0% tropicamide and 2.5% phenylephrine. The cornea was anesthetized with 0.5% proparicaine. A ground electrode filled with electrode gel (Viasys electrode gel (Madison, WI)) was clipped to an ear lobe after the ear was cleaned/exfoliated with Omni Prep skin gel (DO Weaver & Co, Aurora, CO). A Burian-Allen contact lens electrode was used for recording, filled with Refresh Celluvisc solution (Allergan, Irvine CA). The fellow eye was closed and occluded. A

grass electrode impedance meter model FEZM5 was used to check the impedance of the electrodes. This was kept under 10 kOhms.

The recording time was 7 minutes and 17 seconds. It was broken into 16 segments, set by the software. The subjects fixed on an "X" in the center of the pattern during the recordings. Each hexagon modulated between black (<2 cd/m^2) and white (200 cd/m^2) according to a 2^{15} -1 binary m-sequence. The background was set at the mean luminance of the screen. The room lights were on during all recording and the luminance of the walls behind both mfERG instruments were set to be the same (90-100 cd/m^2) with a photometer. The quality of recording was monitored in real time by the examiner. The retinal signals were amplified 100,000 times and band pass filtered at 10-100 Hz.

2.3.6.2 Analysis

mfERGs were processed with a single iteration of artifact removal. Artifact removal allows for the replacement of contaminated segments by taking into account if one segment is different than the rest, and 17% spatial averaging which was done to improve the signal to noise ratio. Spatial averaging takes the surrounding waveforms into account while processing. The 103 waveforms were then exported from VERIS. The template stretching method[11] was used to identify the P1 implicit time and amplitude. For the template stretching method, waveform templates were constructed from the mean local waveforms of the control subjects for each instrument. Each template was then fitted to the first 80 milliseconds of each subject's local response using a least squares fit. The program then reports a scaling factor for amplitude and time. The amplitude and IT are found from the product of the template data and the scaling factor. The goodness of fit for each waveform is also given by the program. It is a parameter that they label statfit. A statfit of 0 is a perfect match between the sample waveform and the template and a statfit of 1.0 means that the waveform fits as well as a flat line (mean of the data). Patients with a statfit over 0.8 had their mfERG data rejected from the study as the original publication by Hood and Li indicates that at 0.8 the signal to noise ratio is too low to be reliable.[11] Very few patients were rejected on this basis. Figure 4 shows a cartoon of how the stretching program works, more information can be found in the original publication. [11]

Figure 4

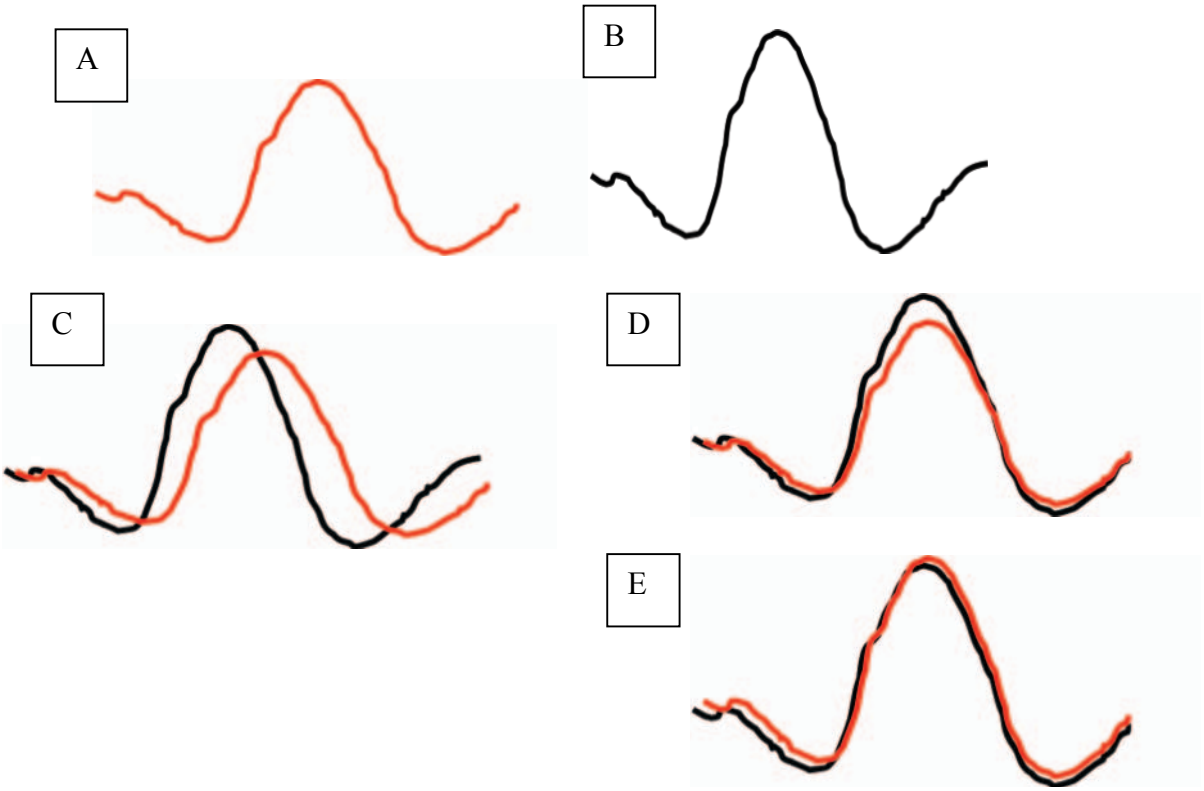

Figure 4: The red line is the template (A), the black is the subject's waveform (B). The two are offset in time and amplitude (C). The program will shift the template in time (D, in this case faster) and in amplitude (E, in this case larger) to match up to the subject's waveform. It uses the template measures and how much it has to alter it in order to calculate the implicit time and amplitude.

Implicit times and amplitudes taken from the template stretching program were imported into Microsoft excel and converted to Z-scores. Z-scores are a way to normalize data by indicating how many standard deviations a measure is from the mean of a normal population of controls subjects. A patient/subject's Z-score of 2 carries a probability of $p<0.023$ and is the criterion for abnormality for most of our studies. mfERG Z-scores are the units used in all of the mfERG related studies in this thesis. Chapter 3 highlights more about how Z-scores allow for use of data across different instruments.

For several of the studies (Chapters 4 and 7), the data was then taken from 103 hexagons and grouped into 35 zones. While this is discussed in more detail in those chapters, generally, the longest IT and the minimum Amp was assigned to the entire zone. This allows us to be more spatially conservative especially in the central retina where, because of the cone density scaling, the hexagons are smaller.

Figure 5

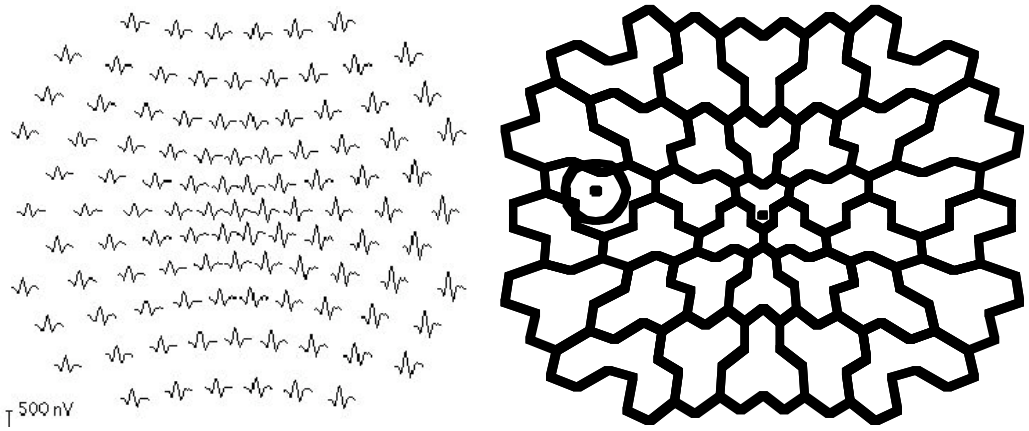

Figure 5: Left: The 103 waveforms as they are exported from the VERIS program. Right: The 35 retinal zones used in several of the studies of this thesis. Data is exported from VERIS and the converted to Z-scores, the maximum implicit time or minimum amplitude Z-score is applied to the entire zone

2.4 Statistical Treatment of Data

Statistical treatment of the data was different for the 5 different studies but generally an important component of this thesis is the use of multivariate logistic regression to create mathematical models for the prediction of diabetic eye disease. Chapters 4 and 7 are the presentation of the two multivariate models created as the largest (and arguably the most important) studies in this dissertation. Thus a complete discussion of the predictive modeling procedures used will conclude this methods section.

2.4.1 Logistic Regression

Logistic Regression[12] allows us to examine the association between risk factors and an outcome measure. The risk factors can be binary, categorical, or continuous variables but the outcome is always binary.
It follows the general equation of:

$$\text{Log } (p/1\text{-}p) = A + Bx$$

Where "p" is the probability of developing the outcome measure. A is the intercept coefficient, B is the log odds ratio associated with a 1 unit increase in x, and x is the risk factor of interest. This equation can be expanded so that many factors can be included in this model. In our studies we calculated the probability of retinopathy or edema development using risk factors measured the year before. Those risk factors included: diabetes type (binary), diabetes duration (continuous), blood glucose level (continuous), HbA1c (continuous), blood pressure (continuous), degree of retinopathy (categorical), gender (binary), age (continuous), and mfERG IT and mfERG Amp (both continuous). For the outcome, a region was given a 1 if it had the outcome of interest (retinopathy or edema) and a 0 if it did not.

2.4.1.1 General Estimating Equations

The above equation is for a simple logistic regression. However, the data used in our studies, from 35 regions within one eye and both eyes of each subject, were used in both models presented. Since, correlations may exist within the zones of the eyes of any one subject, and across eyes of the same subject, generalized estimating equations (GEEs) were used to estimate model coefficients within this thesis. GEE's are an extension of general linear models that allow for the correlation structure to be specified. GEEs were introduced in the 1980's [13, 14] and because of their many uses have become common. They are now included in the commercial statistical package used to generate our statistics, STATA (Version 10 College Station, TX).

With the GEE approach, several specifications must be made: these are that the link function must be stated, the distribution of the dependent variable must be known, and the correlation structure must be given. In our studies, the outcome variable was binary so the distribution is binomial and the link is thus a logistic regression (logit) function. The correlation structure for unordered data was used while specifying that observations on both eyes of a single subject were combined into a single group (to allow covariance between zones of the same subject) but that independence should be assumed across subjects.[14] In addition, the program allows us to specify that a robust variance be used. This is a feature of the GEE that allows us to accommodate any differences between the assumed and true covariance structures.

Thus the function typed into STATA to complete this model was:

xtgee EDEMA IT, family (b) link (logit) corr (ind) i(subject) ro

(In STATA the dependent variable comes first followed by the independent variables. The b = binary, logit = logistic regression, ind = independent, i(subject) = independence is assumed based on the variable subject, ro = robust variances will be applied.)

The outcome models can be interpreted the same way as for the results of a standard logistic regression.

2.4.2 Modeling Process

For the logistic regression model creation, we first performed a univariate analysis of all the risk factors and determined which factors were most likely to be predictive. Next, possible confounders and interaction terms were evaluated. Lastly, a stepwise forward regression was performed, where the factors which were most significant were added first ($p<0.05$ followed by those that were $p<0.20$), then all factors were evaluated one by one to see if they added to the model. Factors were retained that both improved the model and were significant at a $p<0.05$ level.

2.4.3 Validation of the models

Validation of the models in this thesis was achieved with a five-fold cross validation.[15, 16] This consists of taking all the data and dividing it into 5 groups. Then a model is created using 4/5 of the data and it is "tested" on the remaining 1/5. Cross validations are typically performed in groups of 5 or 10. Five was chosen here because of the small sample sizes used in our models. A more ideal way to validate a model is with a

new set of data, however given the length of time needed to collect the data and the already very small sample size, this was not possible; thus a cross validation provided a good means to validate the model and estimate its general accuracy.

2.4.4.Receiver Operating Characteristic Curves

Finally, data was displayed as a receiver operating characteristic curve. Receiver operating characteristic curves (or ROC curves) are commonly used in medicine today to help with decision making and evaluate the accuracy of models and new technologies.[17-20] They arose from signal detection theory as a way to visualize the charts in a summary format. For example, sample data is shown in the table below (Table 1). As for signal detection theory, the chart highlights the true positive rate, the true negative rate, the false positive rate, and the false negative rate. The choice of data points in a cell can change depending on what cut off for normality is chosen.

Table 1

Test is Positive at Given Cut off	Patient Has Outcome		
	Yes	No	Totals
Yes	30 (True Positives)	10 (False Positive)	40
No	10 (False Negative)	50 (True Negatives)	60
Totals	40	60	100

Table 1: Table showing signal detection theory outcome of a sample data set. In this data set there are 40 people with disease and 60 people without. The sample test correctly identifies 30 of the 40 people (sensitivity). It also correctly rejects 50 of the 60 who do not have disease (specificity). These numbers would be changed if the cut off of the test were different.

The ROC plot has sensitivity on the y-axis (the true positive rate) and 1-specificity on the x-axis (the false positive rate). This allows a cut off to be chosen to maximize the sensitivity and specificity of the model (or in some cases one or the other).[21] As shown in the figure below (Figure 6), a 'perfect' ROC curve would be one that follows the y-axis up the left side of the plot to the top left corner and then moves along the top of the graph. The diagonal line represents a plot that is no better than guessing. Most plots fall in between, as with our data. ROC curves of the 5 fold models are shown in Chapters 4 and 7.

Figure 6

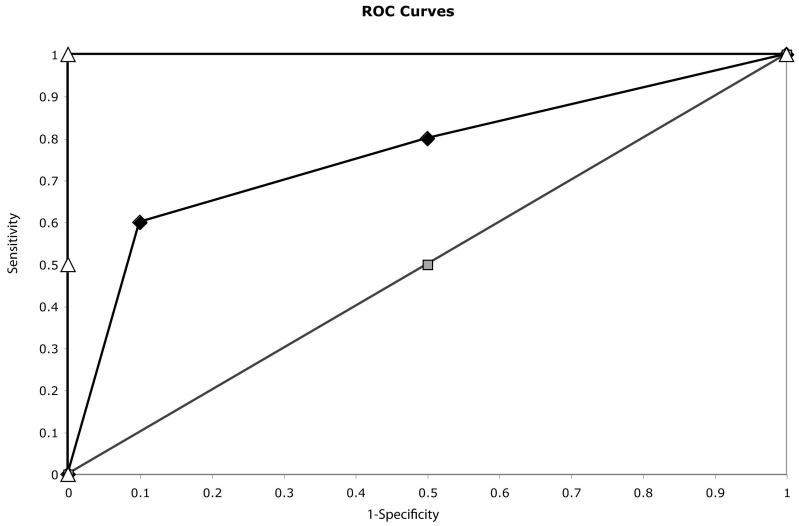

Figure 6: Graph illustrating perfect ROC curve (triangles), ROC no better than guessing (squares), and "typical" ROC curve (diamonds), which falls in the middle. The curve shown has a specificity of 90% and a sensitivity of 60%.

2.5 References

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Chapter 3: Reproducibility of the mfERG between two VERIS Instruments

3.1 Prelude

This study examining the reproducibility of the mfERG was an important first step to the other studies presented in this thesis. This investigation was originally designed to solve a specific problem in our data collection and analysis but was then expanded to examine the general reproducibility and repeatability of the mfERG.

The problem, which initiated the study, was that our lab has two VERIS mfERG instruments, which we designated for our two different longitudinal studies. One VERIS was used primarily to collect the data for the study in chapter 4 (mfERG1). This means that it was used for the patients without retinopathy. The other VERIS (mfERG2) was used primarily to collect the data presented in chapters 6 and 7, the patients with retinopathy. However, often it was unclear at the time the mfERGs were recorded which study a given subject belonged in, as most subjects are unaware of their retinopathy status. We could not check their status prior to recording as fundus photography and examination lights alter the mfERG results.

Because of this, being able to combine data from the two instruments was critical for the success of these studies. The other option was repeating the mfERG for all the patients which were mis-categorized, which is time consuming and difficult for the patients.

The literature did not provide an adequate means for us to assess the reproducibility between instruments, or a way to combine data from two VERIS instruments. We had 21 control patients repeat mfERGs on both VERIS instruments within a month's time span and compared the differences between recordings for both implicit time and amplitude.

We found the reproducibility of the mfERG to be excellent, but only after the data was normalized using Z scores. The raw implicit time data from each instrument was very different and not easily comparable. The amplitudes were similar due to the calibration of the instruments but there were also variations between the two instruments. This study also confirmed that the mfERG implicit time (IT) is very stable over short term visits in control patients but that the mfERG amplitude (Amp) is much more variable. The information in this chapter allowed us to be comfortable combining data from both mfERG instruments together in one data set using Z-scores.

This chapter has been published and is presented here exactly as it is in the literature. The publication of the chapter here was approved by both the University of California Berkeley Graduate Division, and also by Springer Publishing. Portions of this study were also presented at the 2008 ISCEV meeting.

Harrison WW Bearse MA, Ng JS, Barez S, Schneck ME, Adams AJ. Reproducibility of the mfERG between instruments. ***Documenta Opthalmologica*** 2009; June 119(1), 67-78.

3.2 Abstract

3.2.1 Purpose

First, to examine both the reproducibility of the multifocal electroretinogram (mfERG) recorded on different versions of the same instrument, and the repeatability of the mfERG recorded on a single instrument using two different amplifiers. Second, to demonstrate a means by which multicenter and longitudinal studies that use more than one recording instrument can compare and combine data effectively.

3.2.2 Methods

Three different amplifiers and two mfERG set-ups, one using VERIS™ 4.3 software (mfERG1), and one using VERIS™ Pro 5.2 software (mfERG2), were evaluated. 73 subjects with normal vision were tested in three groups. Group 1 (n=42) was recorded using two amplifiers in parallel on mfERG1. Group 2 (n=52) was recorded on mfERG2 using a single amplifier. Group 3 was a subgroup of 21 subjects from groups 1 and 2 that were tested sequentially on both instruments. A fourth group of 26 subjects with diabetes were also recorded using the two parallel amplifiers on mfERG1. P1 implicit times and N1-P1 amplitudes of the 103 local first order mfERGs were measured, and the differences between the instruments and amplifiers were evaluated as raw scores and Z-scores based on normative data. Measurements of individual responses and measurements averaged over the 103 responses were analyzed.

3.2.3 Results

Simultaneous recordings made on mfERG1 with the two different amplifiers showed differences in implicit times but similar amplitudes. There was a mean implicit time difference of 2.5 ms between the amplifiers but conversion to Z-scores improved their agreement. Recordings made on different days with the two instruments produced similar but more variable results, with amplitudes differing between them more than implicit times. For local response implicit times, the 95% confidence interval of the difference between instruments was approximately ±1 Z-score (±0.9 ms) in either direction. For local response amplitude, it was approximately ±1.6 Z-scores (±0.3 μV).

3.2.4 Conclusions

Different amplifiers can yield quite different mfERG P1 implicit times, even with identical band-pass settings. However, the reproducibility of mfERG Z-scores across recording instrumentation is relatively high. Comparison of data across systems and laboratories, necessary for multicenter or longitudinal investigations, is facilitated if raw data are converted into Z-scores based on normative data.

3.3 Introduction

The multifocal electroretinogram (mfERG) is a non-invasive objective technique that simultaneously measures retinal function at multiple retinal locations. It is used for the evaluation of retinal neuronal populations, as well as for the prediction and assessment of a wide variety of retinal diseases, including retinitis pigmentosa, diabetic retinopathy, and age-related macular degeneration [1-6]. The mfERG is also used for the evaluation of drug toxicity and surgical success [7-10], and its uses continue to expand in both the clinical and research arenas.

Although limited in number, previous studies have examined the repeatability of the mfERG and found it to be high with variations across systems [3, 11-15]. These studies have reported implicit time coefficients of variation (CV) as low as 3.1% (when achieving good repeatability was a goal of the study), and as high as 30.3% (when factors influencing variations in the mfERG were not fully controlled) [12, 15]. The CVs for amplitudes have been reported to range from 10.4% to 36% [13, 15]. Most studies have found that averaging over larger retinal areas reduces variability, and have consequently reported the CVs of rings of responses. Given all of the potential sources of variability that exist in an mfERG recording session, both intrinsic to the subjects and in the stimulus conditions and equipment, the high repeatability from these past studies is encouraging as long as the testing environment is controlled. ISCEV guidelines for clinical mfERG recording [16] are in place to help achieve uniformity in testing situations.

While the ISCEV guidelines specify that each clinic or laboratory establish their own norms, they do not address how clinics or laboratories could pool data for multicenter investigations. These may be necessary in the future to improve the statistical power of mfERG studies in the presence of relatively small samples. Additionally, malfunction or aging of the mfERG equipment being used in a clinic or laboratory can require replacing components, causing inconsistency in the data being collected. This is especially important if follow-up data are to be interpreted or in longitudinal studies over a number of years. Scientists and clinicians are faced with the dilemma of replacing aging equipment while attempting to reduce inconsistencies in the data collection and interpretation.

Reproducibility of the mfERG across instruments has not previously been examined. The purpose of this study is to evaluate the robustness and stability of the mfERG as it is recorded over both time and with different instrumentation (in the case of this study, different VERIS™ instruments and amplifiers). Our results show that the reproducibility of the mfERG across recording instrumentation is quite high and that converting raw data into Z-scores based on normative data facilitates meaningful comparison of results across recording systems and different laboratories.

3.4 Methods

3.4.1 Systems and Stimulus Characteristics

Two visual evoked response imaging systems (VERIS™) (EDI, Redwood City CA), were used to record first-order mfERGs. Both systems stimulated using luminance modulation of a 45°, 103-element hexagonal array scaled with eccentricity. The stimulus background, bright flashes, and dark elements were set to 100 cd/m^2, 200 cd/m^2, and < 2

cd/m^2 (99% contrast), respectively. In addition, the ambient room lighting was between 80-100 cd/m^2 on the wall behind each instrument. Both systems had a 75 Hz frame rate monochrome CRT monitor display and ran a standard m-sequence (2^{15}-1) that lasted approximately 8 minutes. Each recording session was broken into 16 segments, approximately 30 seconds each, and the retinal signals were band-pass filtered 10-100 Hz and sampled every 0.83ms.

However, some features were different between the two recording set-ups (Table 1). Features unique to the first system (mfERG1) include that it runs VERIS™ 4.3 software and has a stimulus screen resolution of 1024 x 768. It also has two external Grass Telefactor (Astro-Med Inc®, West Warwick, RI) amplifiers. The first amplifier ("mfERG1 New Amp"; recording channel 1; Grass model CP511) was produced in 1996. The second amplifier ("mfERG1 Old Amp"; recording channel 2; Grass model P511) was manufactured in 1983. Both amplifiers on mfERG1 were set to amplify 100,000 times. Features unique to the second system (mfERG2) include that it runs VERIS™ Pro 5.2 software, has a stimulus screen resolution of 640 x 480, and one computer-controlled Grass amplifier ("mfERG2 Amp"; Grass model 15LT) which was produced in 2006 and set to a gain of 50,000.

Table 1: Differences Between mfERG Instruments

Characteristics	**mfERG1**	**mfERG2**
Veris Software	VERIS™ 4.3	VERIS™ Pro 5.2
Amplifier Model(s)	CP511 and P511	LT15
Amplifier Setting	100,000	50,000
Monitor Display and Screen Resolution	CRT 75 Hz 1024 x 768 pixels	CRT 75 Hz 640 x 480 pixels

Comparison of the frequency response curves of the amplifiers, as specified by Grass, shows that the two newer amplifiers (mfERG1 New Amp and mfERG2 Amp) should have similar band-pass filtering characteristics but that the older amplifier (mfERG1 Old Amp) is slightly different. The difference between the amplifiers of mfERG1 was verified by inputting sine waves of varying frequencies but fixed amplitude and measuring the output amplitudes with the filters set at 10-100 Hz (Fig 1.) In addition, an artificial eye comprised of a photodiode and an R-C circuit was run on both instruments and all three amplifiers to further characterize the implicit time differences inherent between them. The peak latencies of the first order "mfERGs" recorded from the artificial eye were consistently 2.5 ms shorter for the older (mfERG1, channel 2) amplifier than for the other two amplifiers.

Figure 1

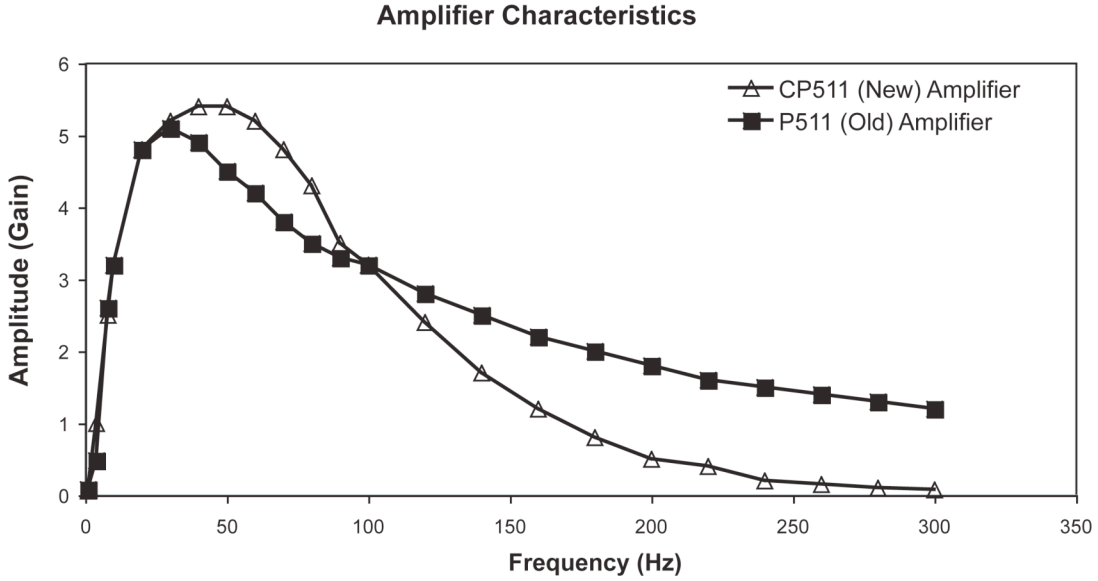

Figure 1-The measured filtering characteristics of the new (Δ) and old (■) amplifiers on mfERG1 when set at 10-100 Hz.

3.4.2 Subjects and Recordings

Seventy three subjects with normal vision and 26 subjects with diabetes were included in this study. Patient demographic information is given in Table 2. The subjects were divided into four groups. Group 1 was comprised of 42 subjects with normal vision recorded simultaneously (in parallel) on both amplifiers of mfERG1. Group 2 was comprised of 52 subjects with normal vision recorded on mfERG2. Within group 2, 9 of these subjects returned for follow-up 1 year later to examine intra-instrument repeatability over time. Group 3 was a subset of the first two groups and consisted of 21 subjects who were run on both instruments within a two month period of time (mean = 0.94 ± 0.68 months). Group 4 was composed of 26 subjects with diabetes without retinopathy, recorded on the two parallel channels of mfERG1.

MfERGs were recorded from one eye while the other eye was occluded. Pupils were fully dilated to at least 7mm with 1% tropicamide and 2.5 % phenylephrine, and 0.5% proparacaine was used to anesthetize the cornea prior to recording. A Burian-Allen bipolar contact lens electrode filled with 1.0% carboxymethlcellulose sodium solution was used for all mfERG recordings. Each instrument was used with its own dedicated contact lens electrode. A clip ground electrode was applied to the subject's earlobe and the resistance between the electrode leads was measured and kept under 10 k-Ohms. Both systems had in-line video cameras that allowed for real-time observation of the eye during testing. Recording segments contaminated by signal saturation or loss of fixation were discarded and repeated. All subjects had 20/20 (logMAR 0.0) or better visual acuity and were free of retinal disease and media opacities, as evaluated by ophthalmic

examination and masked retinal photograph grading. All subjects had refractive errors between -6D and +4D. The study adhered to the Declaration of Helsinki and was approved by the Committee for the Protection of Human Subjects at the University of California Berkeley. Written informed consent was obtained from all subjects after the study was fully explained at their first visit.

3.4.3 Waveform and Data Analysis

The first-order mfERG kernel was analyzed. A single iteration of artifact removal was used on both instruments with 17% spatial averaging. The 103 mfERGs were

Table 2: Subject Demographic Information

Subject Group	**mfERG Instrument**	**Number of Subjects**	**Age ± Std Dev**
Group 1	mfERG1: both amplifiers	42 with normal vision	45.2 ± 12.75
Group 2	mfERG2	52 with normal vision	43.7 ± 14.5
Group 3 (subgroup of groups 1 and 2)	mfERG1 and mfERG2	21 with normal vision	47.4 ± 13.6
Group 4	mfERG1	26 with diabetes	51.3 ± 11.9

exported and the Hood and Li template scaling method was applied to all waveforms to derive P1 implicit time and N1-P1 amplitude [17]. This method minimizes the least squares difference between a waveform and the local template. The template represents the mean local waveform of the subjects with normal vision and it is independently scaled in both amplitude and time to fit the individual local responses. The scaling factors are then used to derive implicit time and amplitude. The templates were created from data of all subjects with normal vision in a group and a different set of 103 local response templates was used for each of the three amplifiers. Group 4's data was analyzed using the appropriate template from group 1. Implicit times and amplitudes were evaluated for all subjects as both raw scores and Z-scores, where the mean and standard deviation were calculated from all subjects with normal vision available for that amplifier-instrument combination after determining that the normative data for each of the 103 hexagons did not differ from a normal distribution (chi-square tests; mean $p = 0.58 \pm 0.25$). Responses were analyzed as whole eye averages (103 response measures averaged together) and also as individual local mfERG measurements.

3.5 Results

3.5.1 Amplifier Comparisons on the Same mfERG Instrument

Recordings from the 2 amplifiers of mfERG1 using the two parallel channels were made from the subjects in groups 1 and 4. As the two recordings were made simultaneously, any differences between them can be attributed to the amplifiers (potential differences in gain, filtering and noise), and no other sources of variation existed. The raw measurements of the 103 local mfERGs were first examined and then they were converted to Z-scores. The 103 raw measurements were then averaged to give one value for each subject. The Z-scores were similarly averaged.

The N1-P1 amplitudes were very similar with a mean difference of 0.01 ± 0.003 μV, and a maximum difference of 0.013 μV (6.5 % of the mean value) between amplifiers for the whole eye average of any individual subject (data not shown). This was expected since the amplifiers were calibrated to provide similar overall gains. The mean amplitudes for the two channels were also similar for individual hexagons (0.21 ± 0.05 μV and 0.20 ± 0.05 μV for the first and second channels, respectively). Figure 2A shows the whole eye raw amplitude data obtained with both amplifiers from the subjects with normal vision (group1) and subjects with diabetes (group 4). Figure 2B shows the Z-scores of these same subjects. Figure 2B illustrates how the small difference between the amplifiers decreased after the conversion to Z-scores, and the data for both groups fall along a diagonal with a slope of 1.

Figure 2C shows the raw implicit time data obtained from both amplifiers. The mean implicit time difference between the two amplifiers was 2.5 ms. Figure 2D shows the Z-scores of the diabetic subjects and the subjects with normal vision with the data falling on a diagonal (slope = 1) passing through the origin. The implicit times showed a better agreement after the conversion to Z-scores.

Figure 2

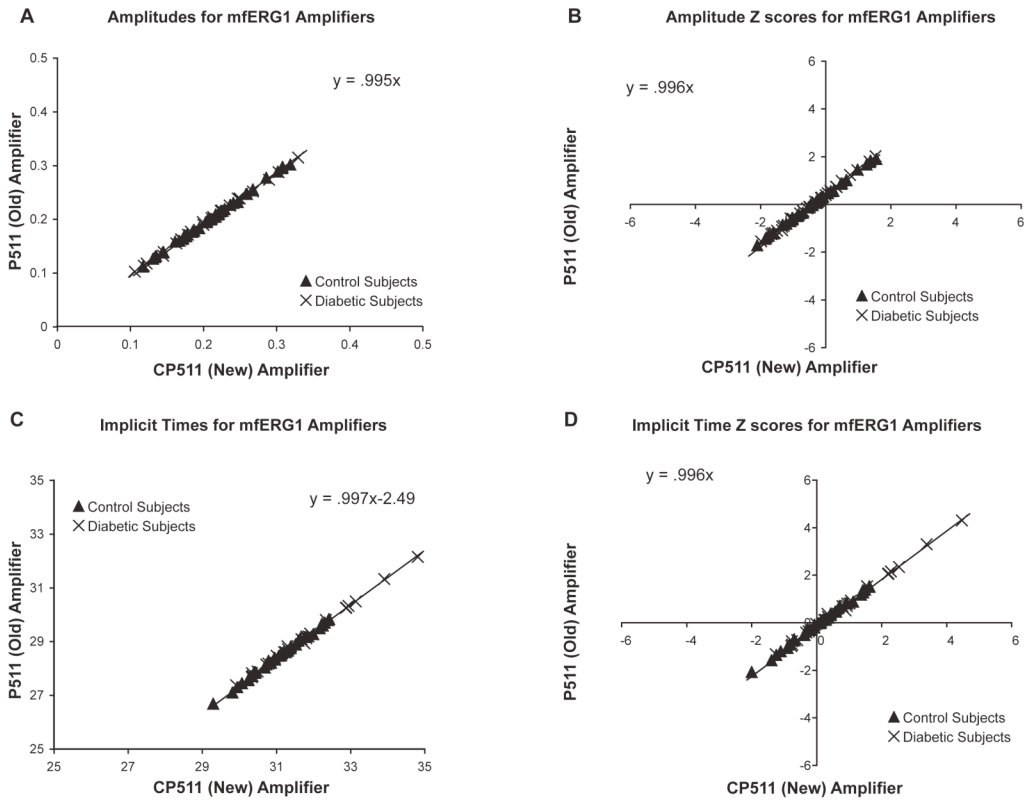

Figure 2- mfERG1 amplifier raw data and Z-score comparison for amplitude and implicit time. A) Raw amplitude data B) Amplitude Z-score data C) Raw Implicit Time data and D) Z-score Implicit Time data. Each data point indicates a whole eye average for one subject. Subjects with normal vision, Group 1 (Control) (▲) and subjects with diabetes, Group 4 (X) are plotted together.

Local response implicit time differences between the amplifiers were examined for subjects with diabetes. The 2.5 ms mean difference in implicit times between the two amplifiers also occurred locally, but with conversion to Z-scores, the amplifiers had good local agreement for all simultaneous recordings. Past studies in our lab have used implicit time Z-scores ≥ 2.0 ($p \leq 0.023$) as indications of abnormality [3, 18]. Table 3 shows that by applying this criterion to the local data from the subjects with diabetes in this study, the two amplifiers had 95.6 % agreement when classifying a local mfERG implicit time as normal or abnormal. A similar analysis was done for the subjects with normal vision using a criterion of 1.0 Z-score, also producing a high agreement of 92.5% (data not shown).

Table 3: Local Amplifier Agreement for Subjects with Diabetes (95.6%)

mfERG1 Old (P511) Amp	mfERG1 New (CP511) Amp: ≥ 2 Z-score Criterion (Abnormal)	mfERG1 New (CP511) Amp: < 2 Z-score Criterion (Normal)	Total
≥ 2 Z-score Criterion	401 (15.0%)	53 (2.0%)	454 (17%)
< 2 Z-score Criterion	63 (2.4%)	2161 (80.6%)	2224 (83%)
Total	464 (17.4%)	2214 (82.6%)	2678 (100%)

3.5.2 Reproducibility Between Different Instruments

This section presents the comparison of mfERG data collected on different days using different instruments. Figure 3 shows the results for whole-eye average comparisons between the older amplifier (Grass model P511) of the mfERG1 system and mfERG2 system (Grass model 15LT amplifier) for subject group 3. The plot of the implicit times in Figure 3A shows that, on average, there is a 2.5 ms difference between the two instruments, which is in agreement with the artificial eye. The mean implicit time of subjects on mfERG1 Old Amp was 28.80 ± 0.91 ms and the mean implicit time on mfERG2 was 31.30 ± 0.87 ms. As expected, there is a lower correlation ($R^2 = 0.81$) in the implicit time data than was observed earlier in the simultaneous recordings on a single mfERG instrument. The implicit times obtained on the two instruments are re-plotted as a Bland-Altman plot [19] in Figure 3B. The difference between the two instruments is plotted on the y axis and the mean of the instruments is plotted on the x axis for each subject. The zero slope (95% CI = -0.24 - 0.20) and the y-intercept of the least squares regression indicates that there is an implicit time offset of about 2.5 ms between them. (If the intercept and the slope of the line were both 0, the two instruments would be directly comparable. If the line had a significant slope the instruments would not be easily comparable). By converting the implicit times into Z-scores, the two instruments are now more comparable (Figure 3C). The 95% confidence interval of ±0.86 Z-scores indicates that implicit time Z-scores are highly reproducible on the two instruments. The differences between the two instruments ranged from 0.06 to 1.03 Z-scores.

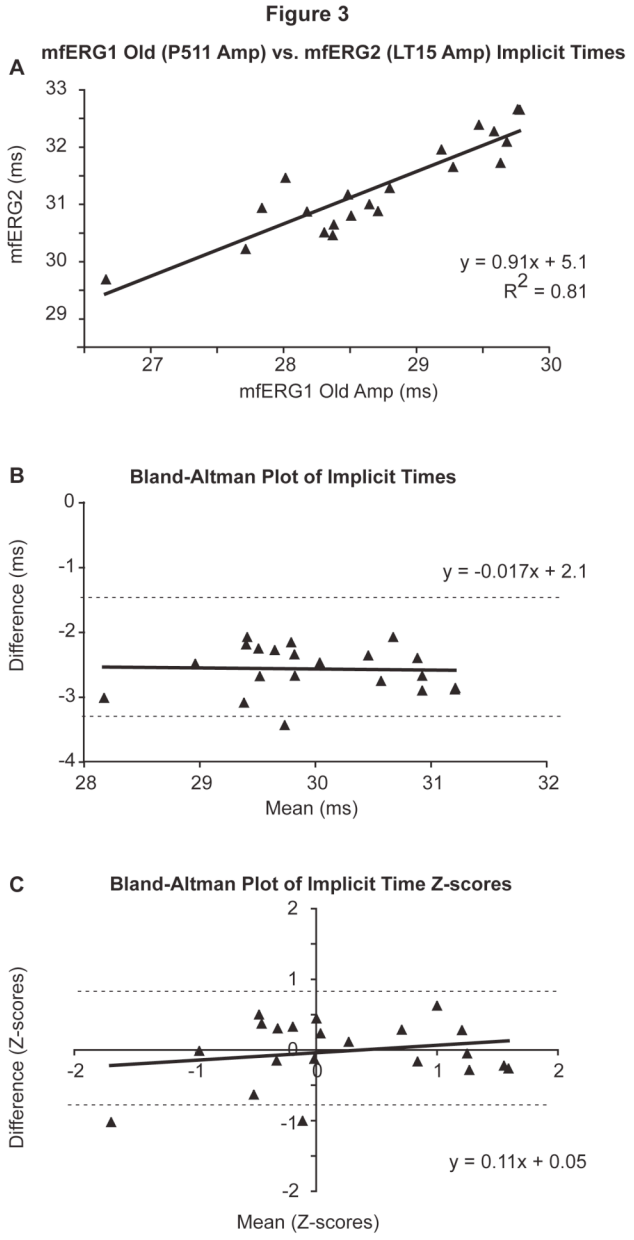

Figure 3-Implicit time comparison for the older amplifier of mfERG1 and mfERG2. A) Comparison of the implicit times (ms). Each point is a whole eye average of one subject from group 3 B) A Bland-Altman plot of that same data as A. The dashed lines on the plot indicate the 95% confidence interval and the solid line is the mean difference (2.5 ms for the range of the mean implicit time data observed) between the instruments for all 21 subjects. The slope of the line is not statistically different than zero ($p = 0.88$) C) The Bland-Altman plot of the Z-scores of the implicit time data with the dashed lines indicating the 95% confidence interval and the solid line indicating the mean difference (0.05 Z-score units) for the 21 subjects. The slope of this line is not different from zero ($p = 0.92$).

Figure 4A shows the whole eye average amplitude comparison for the 21 subjects in group 3 for the older amplifier of mfERG1 and for mfERG2. The mean amplitude of mfERG2 was 0.30 ± 0.07 μV compared to 0.20 ± 0.05 μV for mfERG1 Old Amp. Although the agreement of amplitudes between the instruments varies among the subjects ($R^2 = 0.43$), the two instruments are comparable as the 95% confidence interval of the slope of the regression line contains 1.0 (95% CI = 0.32-1.11). The Bland-Altman plot of the amplitude data (Figure 4B) shows an average difference of 0.07 μV across all values but with a large 95% confidence interval associated with this value (0.01 to -0.19 μV). The Z-score Bland-Altman plot (Figure 4C) also has a slope that is not significantly different from zero ($p = 0.29$) and the fact that the regression line passes through zero shows that the data is in better agreement with this conversion. The range of amplitude differences for these 21 subjects was large, ranging from 0.1 to 1.6 Z-scores. The 95% confidence interval of ±1.5 Z-scores indicates that amplitude is not as reproducible as implicit time.

Figure 4

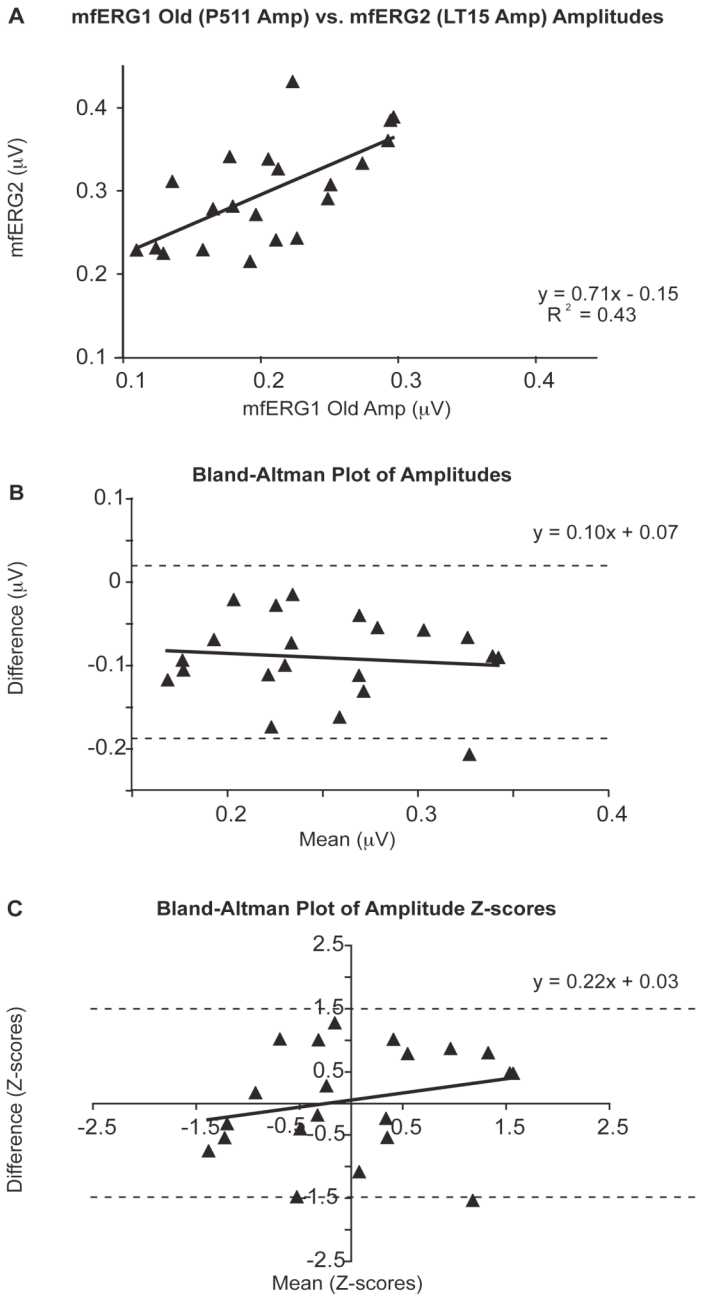

Figure 4-Amplitude comparison for the older amplifier of mfERG1 and mfERG2. A) Comparison of amplitudes (μV). Each point is a whole eye average of one subject from group 3. B) A Bland-Altman plot of that same data as A. The dashed lines on the plot indicate the 95% confidence interval and the solid line is the mean difference (0.07 μV) between the instruments for all 21 subjects. The slope of this line is not statistically different than zero ($p = 0.23$) C) The Bland-Altman plot of the Z-scores of the amplitude data with the dashed lines indicating the 95% confidence interval and the solid line indicating the mean difference (0.03 Z-score units) for the 21 subjects. The slope of this line is not different from zero ($p = 0.29$).

Data collected on the newer amplifier of mfERG1 (Grass model CP511) was also compared to data collected on mfERG2. As expected from their similar band-pass filtering characteristics, these two amplifiers exhibited raw implicit times that were similar (Figure 5A), with a mean difference of only 0.1 ± 0.34 ms between the two instruments (Figure 5B). The mean implicit time for mfERG1 New Amp was 31.40 ± 0.90 ms and the mean implicit time for mfERG2 was 31.30 ± 0.87 ms. Conversion of the data into implicit time Z-scores produced an even smaller mean difference between the instruments, making them more comparable (Figure 5C). The 95% confidence interval of the difference between the two similar amplifiers was ±0.74 Z-scores

Figure 5

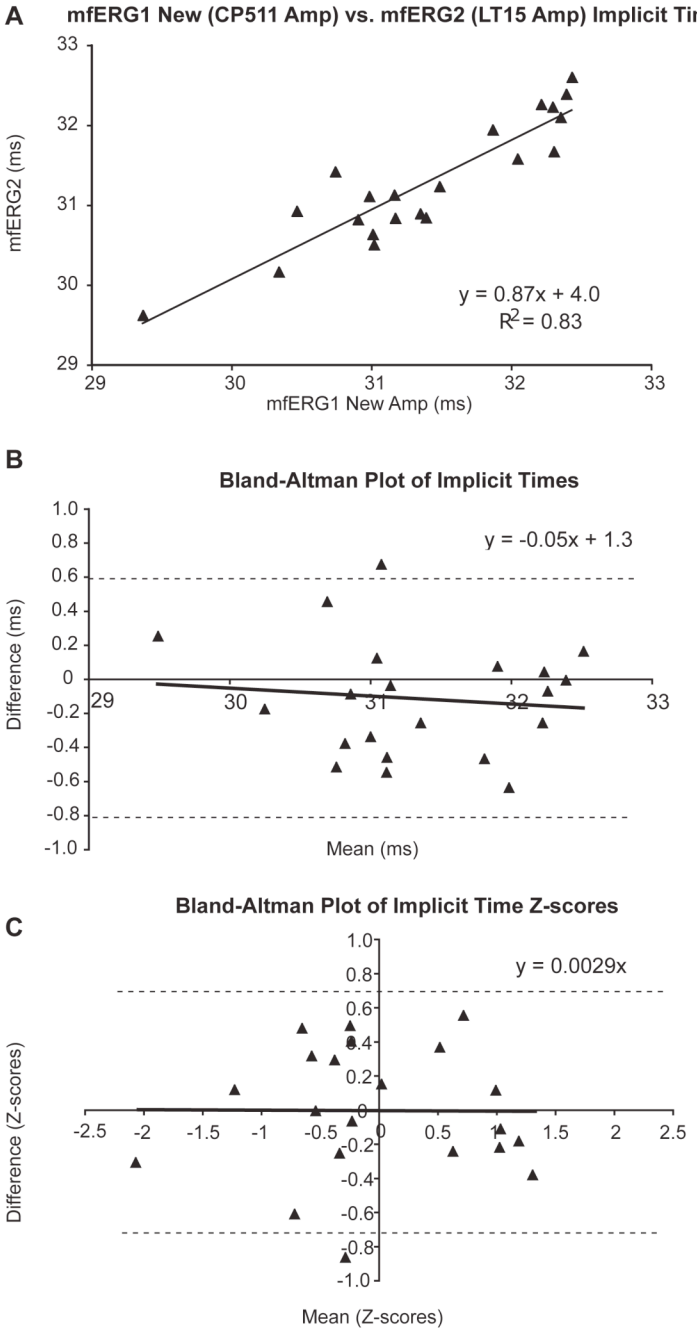

Figure 5-Implicit time comparison for the newer amplifier of mfERG1 and mfERG2. A) Comparison of the implicit times (ms). Each point is a whole eye average of one subject from group 3. B) A Bland-Altman plot of that same data as A. The dashed lines on the plot indicate the 95% confidence interval and the solid line is the mean difference (0.1 ms) between the instruments for all 21 subjects. The slope of this line is not statistically different than zero ($p = 0.65$). C) The Bland-Altman plot of the Z-scores of the implicit time data with the dashed lines indicating the 95% confidence interval and the solid line indicating the mean difference (0 Z-score units) for the 21 subjects. The slope of this line is not different from zero ($p = 0.98$).

The mfERG recordings performed to compare the instruments were not obtained in the same session, so the question arises as to how much of the observed difference is due to subject variation over time and how much is due to actual instrumentation and electrode differences. To address this, 9 subjects with normal vision were recorded on mfERG2 and retested 1 year later (±15 days). The results showed that the mean (of all 103 local response measurements) implicit time Z-scores differed from 0.04 to 0.76 Z-scores with a mean difference of 0.36 ± 0.28 Z-scores. The amplitude Z-score differences ranged from 0.02 to 2.60 Z-scores, with a mean difference of 0.85 ± 0.81 Z-scores. For these 9 subjects, coefficients of variation (CV) were also calculated for the raw data of each of the 103 hexagons for both implicit time and amplitude. The local implicit time CVs ranged from 2.2% to 4.3% with a whole eye average of 3.0 ± 0.5%. The local amplitude CVs ranged from 10.5% to 47.3% with a whole eye average of 23.7 ± 6.9 % (data not shown). This indicates that implicit time remains fairly stable over recording sessions but amplitudes are more variable.

The last analysis explored the similarity of implicit time and amplitude measures of the 103 *local* mfERGs obtained on the two instruments. The 95% confidence intervals of the difference between mfERG2 and the older amplifier of mfERG1 (the most different hardware configurations) were evaluated at all 103 retinal locations for the 21 subjects in group 3. The plots in Figure 6 show the individual Z-score confidence intervals, represented as vertical gray bars, and the mean confidence intervals, represented as dashed horizontal lines. For implicit time, the mean local difference between the two instruments was 0.01 Z-score. The dashed horizontal lines in Figure 6A indicate the mean 95% confidence interval (1.07 to -1.05). The locations near the blind spot (e.g., elements 48 and 59), are the most variable (up to 1.5 Z-scores in each direction). Based on these results, a local difference in implicit time must be greater than approximately 1 Z-score unit to differentiate it from inter-instrument variability and establish a significant functional change at a single retinal location. In this study, all of the subjects have normal vision (controls) so no actual retinal defects existed. Both instruments agreed that all of these subjects were normal, with no subject having more than 4 local implicit time Z-scores ≥ 2.0 ($p = 0.91$). Figure 6B shows the 95% confidence intervals for the difference in amplitude Z-scores for the same responses. While the mean difference for all of the hexagons is small (0.04 Z-scores), the local amplitudes had more variation than the implicit times. The average 95% confidence interval for the amplitude Z-scores was 1.63 to -1.54 with some hexagons having a 95% confidence interval > 2.0 Z-scores.

Figure 6

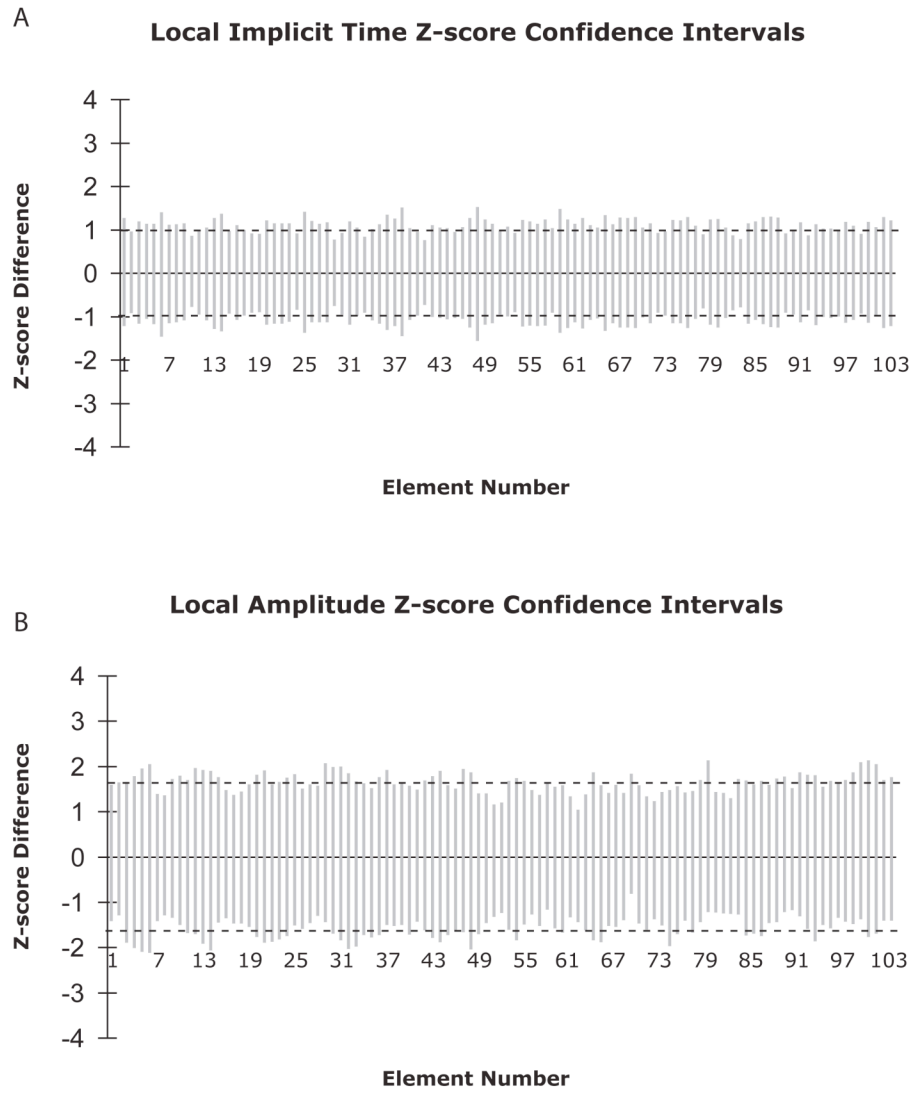

Figure 6- A) The 95% confidence intervals of the implicit time Z-score differences between the older amplifier of mfERG1 and mfERG2 for each of the 103 elements (gray vertical lines). The dashed lines indicate 1 Z-score in either direction which is the average 95% confidence interval for all 103 hexagons. B) The 95% confidence intervals of the amplitude Z-score difference between the older amplifier of mfERG1 and mfERG2 at of each of the 103 hexagons. The dashed lines indicate 1.6 Z-scores which is the average 95% confidence interval for this data.

3.6 Discussion

The purpose of this study was to evaluate the robustness and stability of the mfERG as it is recorded over both time and with different instrumentation from the same manufacturer. In this study, we used VERIS™ software and hardware. Although there have been several studies examining the repeatability and variability of the mfERG, the reproducibility of the mfERG across systems within a laboratory or across laboratories

had not been examined. Understanding this reproducibility is a key component in pooling and comparing data across laboratories and replacing all or parts of an mfERG instrument during a study. For multicenter mfERG studies the reproducibility, or agreement, of the response measures must be established first.

It is known from past work that there are many sources of possible variation in the mfERG. It has been shown that differences in luminance [20], contrast [21], pupil size [22], adaptation states [23, 24], and even less than full correction of refractive error [25, 26] can all cause alterations in the mfERG. Furthermore, the way the data are filtered and processed during the recording session is another potential source of variability from session to session and laboratory to laboratory [27, 28]. These past studies have shown that while there are many factors that can cause variability, if they are controlled within a laboratory, the repeatability of the mfERG responses can be good, particularly with implicit time measures. All of these factors were controlled in this study in both intra-session and inter-session recordings. Furthermore, the use of the Hood and Li template scaling method in this study may have helped to improve reproducibility. Compared to measurements of peaks and troughs made manually, the template scaling method is more objective and less affected by noise. The method's relative insensitivity to noise is due to the fact that the waveform template is fit to the response being measured, using a least-squares criterion, over an 80 ms epoch. Thus, random noise in the region of the P1 peak has relatively little effect on either its estimated amplitude or implicit time.

Overall, we found the mfERG Z-scores for amplitude were satisfactorily reproducible and Z-scores for implicit time were very reproducible across time and with different instrumentation. The ±0.86 Z-score confidence interval for mean implicit time corresponds to ±0.73ms, which is less than ±1 real-time signal sample in our recordings. However, differences in recording instrumentation can cause raw response measures to be very different between instruments. These raw response differences can exist even when systems are similarly calibrated and when band-pass filter settings are nominally the same. In our study the amplifiers on mfERG1 were set to the same band-pass settings and records were taken simultaneously; the raw amplitudes were similar but raw implicit times very different (2.5 ms mean difference) in the two channels. These implicit time differences are not surprising, given the different filter characteristics but it must be noted that 2.5 ms is a large difference, more than 2.0 Z-scores. This difference is large enough to cause concern in a longitudinal study or comparison of data across laboratories, if one was not aware of the filtering differences between amplifiers. This difference could also lead to a belief that an eye had improved or deteriorated even when there was no actual change.

By normalizing, using data from a normal population, mfERG measurements are more comparable and in reasonably close agreement across instruments, and the effects of differences in instrumentation are minimized. The normal subject samples should be similar and matched appropriately to the disease state and patient sample being studied, as was the case in this study. There are multiple normalization methods, including percentiles and Z-scores. We chose Z-scores for a number of reasons. They include the mean and variability of the normative data so they can be quickly used to identify abnormalities. But most importantly, they transform the measurements so that they are relative to the control data collected on specific instruments. Another possible approach to making data more comparable is to band-pass filter recordings over a larger frequency

range and then digitally filter the responses. This would likely remove some of the differences we observed in implicit time. Because digital filtering uses Fourier analysis there is no phase shift as there can be in analog filtering. However, digital filtering would likely not help in making amplitude data more reproducible.

For the first part of our study, we performed amplifier comparisons using parallel channels. We did this to avoid time-varying (test-retest) factors and to isolate differences in the instrumentation. When comparing both the same and different instruments across time in the second part of our study, we found that amplitudes were much less repeatable than implicit times. This is in agreement with earlier studies which have also found amplitudes to be more variable [29]. The CVs we found for both amplitudes and implicit times are in agreement with previous studies [12, 13, 15] when averaging over the whole eye. We also looked at CVs on a local level and found them to be fairly consistent across the retina when examining implicit time but highly variable for amplitudes. No CVs were calculated for Z-score data as CVs are poor estimates of variation when the mean of the data is near zero, which is the case for Z-scores of subjects with normal vision. However, the range of Z-score differences in amplitude measurements are also much more variable than it is for implicit times.

In general, comparison of different instruments involves true instrument differences (e.g., the hardware and software design) and test-retest variation. It appears that a large part of the variability between instruments that we observed, especially in amplitude, might come from inter-session rather then inter-instrument sources. Most of the response variation we observed between the instruments, after conversion to Z-scores, was of the same magnitude as test-retest on the same instrument with the same amplifier. Therefore, it appears that data collected on different set-ups can be compared more easily after conversion to Z-scores, at least when recording conditions are sufficiently equated.

Previous studies examining the repeatability of the mfERG have typically used ring averages to look at the differences between different sessions. This study uses comparisons among eye averages and also among local response measurements. In agreement with other studies [12, 13] we found, not surprisingly, that the local measures are less repeatable in comparison to whole eye averages. There are a number of reasons why local measurements can be less repeatable than eye averages, including a lower signal to noise ratio, small changes in stimulus placement on the retina, and changes in electrode placement in the case of amplitudes.

In conclusion, the mfERG is quite reproducible, even across different recording installations. This study suggests that it is possible to compare and/or combine data obtained from different instrumentation, provided that sufficiently large and similar normative data sets are collected on each instrument. Conversion of raw mfERG measurements to Z-scores based on normative data is an efficient and effective means to compare or combine measurements obtained with different instrumentation. Such comparisons and combinations are critical to multicenter studies, some longitudinal studies, and to following patients over years of care.

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Chapter 4: Multifocal Electroretinograms Predict Onset of Diabetic Retinopathy in Adult Patients with Diabetes

4.1 Prelude

This study builds on the previous research in our laboratory and creates a multivariate model for the prediction of the local onset of diabetic retinopathy in eyes which have had no previous retinopathy (i.e. first onset). Our lab has previously created models for the prediction of retinopathy in patients with diabetes with and without retinopathy. However, those models did not have enough patients with no retinopathy at baseline who developed retinopathy for the first time to create a successful model for the onset of retinopathy in eye that had never had any retinopathy. Most of the new retinopathy predicted in those models was for retinal patches in eyes that had some minimal retinopathy elsewhere in the retina. Thus gathering a larger sample size of patients with no retinopathy to follow was the logical next step in our work.

The onset of retinopathy is an important clinical transition in diabetes. Being able to predict who is going to make this transition and in which specific local retinal area would undoubtedly aid in clinical trials examining treatments of potential novel preventative treatments, and help clinicians working with "at risk" patients.

The predictive model we created revealed that the mfERG IT can predict the onset of retinopathy with good sensitivity (80%) and specificity (74%) as long as the type of diabetes is taken into account. This indicates that the mfERG IT is indeed a very sensitive early measure of the health of the retina in diabetes. The model also sets the stage for the use of the mfERG to predict diabetic edema (Chapter 7). The two models use the same approach in different patient groups.

This chapter has been published in the scientific literature but is presented here with an additional figure. Figure 2, the causal diagram, is not included in the original publication. It is included here as I feel the relationship between the factors in this study adds an important component. Creating a causal diagram is an aspect that I learned in preparing this model and I wanted to document it. Thus in the publication in *Investigative Ophthalmology and Vision Science*, there are only two figures and figure 3 here is the same as figure 2 in that publication. The manuscript and this chapter are otherwise identical.

 This study was also presented as a poster at the 2010 ARVO meeting.

Harrison WW, Bearse MA, Ng JS, Jewell NP, Barez S, Burger D, Schneck ME, Adams AJ. Multifocal Electroretinograms Predict Onset of Diabetic Retinopathy in Adult Patients with Diabetes. ***Invest Ophthalmol Vis Sci***.2011; 52(2) 772-777.

Harrison WW, Bearse MA, Ng J. Jewell NP, Barez S, Schneck ME, Adams AJ. Multifocal Electroretinograms are Predictive of the Onset of Diabetic Retinopathy in Adult Diabetics. ***Invest Ophthalmol Vis Sci***. 2010: 51 ARVO E-Abstract 4673

4.2 Abstract

4.2.1 Purpose

Our previous models predicted local formation of diabetic retinopathy (DR) in adults with diabetes (DM) and existing retinopathy. Here we derived a multivariate model for local prediction of DR onset in patients with no prior retinopathy.

4.2.2 Methods

Seventy-eight eyes from 41 DM patients were tested annually for several years. The presence or absence of DR at the last study visit was the outcome measure, and measurements of risk factors from the previous visit were used for prediction. Logistic regression was used to assess the relationship between DR development and 7 factors: multifocal ERG implicit time (mfERG IT) Z-score, gender, diabetes duration, blood glucose, HbA1c, age, and diabetes type. Thirty-five retinal zones, spanning 45 degrees, were constructed from the mfERG stimulus elements. The maximum IT Z-score for each zone was calculated based on data from 50 control subjects. ROC curve analysis, using 5-fold cross-validation, was used to determine the model's predictive properties.

4.2.3 Results

Mild DR developed in 80 of 2,730 retinal zones (3%), in 29 of 78 eyes (37%). Multivariate analysis showed mfERG IT to be predictive for DR development in a zone, after adjusting for diabetes type. The multivariate model has a sensitivity of 80% and specificity of 74%.

4.2.4 Conclusion

mfERG IT is a good predictor of DR onset, one year later, in patients with DM without DR. It can be utilized to assess the risk of DR development in these patients, and may be a valuable outcome measure in evaluation of novel prophylactic therapeutics directed at impeding DR.

4.3 Introduction

The number of patients with diabetes in the United States is expected to drastically increase, nearly doubling in the next 25 years.[1] As diabetes is the leading cause of new cases of preventable blindness in Americans of working age (20-74 years old),[2] the ongoing search for better and earlier treatments for diabetic eye disease has become even more important. The gold-standard treatment, laser photocoagulation,[3] is aimed only at the end stage of eye disease and has many side effects including decreases in peripheral and night vision.[4] Despite much research there is still nothing shown to be more effective for saving vision in the late stages of this disease.[5] Furthermore, even with several efforts, no successful ocular treatments for mild to moderate non-proliferative diabetic retinopathy (NPDR) have been found. However, patients at this stage of disease often report visual symptoms such as difficulty driving at night.[6]

Currently, patients with early NPDR are only counseled on blood sugar and blood pressure control, and monitored. While better blood sugar and blood pressure control has been shown to be effective at reducing retinopathy progression at early stages of disease,[5] it is often a difficult task for patients to accomplish, and is not successful in all patients. Some patients still progress to worsening retinopathy even with improved blood sugar control.[7-9] The vision of many patients could be preserved, at least for a longer period of time, if earlier treatments were available.

Previous studies that have examined candidate predictive factors for diabetic eye disease have mostly focused on those risk factors leading to the most severe non-proliferative diabetic retinopathy (NPDR) and treatable sight threatening proliferative diabetic retinopathy (PDR). They have found an association between severe NPDR and many factors including duration of diabetes, blood pressure, and smoking.[10] Furthermore, PDR has been linked to neuropathy, decreased visual acuity, elevated triglycerides, type 1 diabetes, and previous levels of retinopathy. [11, 12] PDR has also been linked with higher HbA1c % levels [13] and a reduction in HbA1c % reduces the need for and risks associated with laser photocoagulation.[14]

Changes in the retina are, however, detectable at a much earlier stage and studies have begun to focus on candidate predictive factors for earlier retinopathy development. The UKPDS study group found that even 1 or 2 microaneurysm are predictive of future worsening of retinopathy and should not be ignored.[15] Other factors such as microalbuminuria, hypertension, and neuropathy have also been found to increase the risk of earlier retinopathy.[16, 17] There are also many indications that neural changes take place in the retina during diabetes and that these changes take place before vascular changes are apparent.[18-21] These changes have been identified using several different electrophysiological tests and have been shown to worsen as diabetic retinal disease progresses. Several possible mechanisms for this progressive change have been suggested.[22] Neural changes are thus obvious candidates as risk factors for predicting retinopathy. Electrophysiological tests of neural function are fast, objective, and noninvasive.

We have previously developed multivariate models using the multifocal electroretinogram (mfERG) implicit time (IT), a local measure of retinal neural function, along with other diabetes health measures, to predict new local retinopathy development over 1-3 years in patients with DM and some retinopathy at baseline. [23-26] The present

study derives a new model to predict retinopathy development, within a one-year window, in a cohort of subjects who have no diabetic retinopathy at baseline. Prediction of the earliest clinically visible diabetic changes in the eye has implications for clinical care and tracking eye health. Perhaps more importantly the relatively short-term predictive measures allow rapid clinical trials of new drugs targeting the earlier stages of DR, while the alternative, visual acuity outcomes, demand more protracted studies.

4.4 Methods

4.4.1 Subjects

Forty-one adult subjects with diabetes completed the study. Both eyes were used in the analysis with the exception of 4 eyes that were excluded at the start of the study due to media opacities, intraocular lens implants, and myopic degeneration, leaving a total of 78 eyes. All subjects were between 25-65 years old with a mean age of 52.4 ± 10.8 years. There were 8 subjects with type 1 diabetes and 33 subjects with type 2 diabetes. Some of the subjects included in this study were part of our earlier predictive models but their data presented here represent more recent follow up visits that have not been previously reported. In addition, 50 healthy non-diabetic control subjects, who were between the ages of 21-67 with a mean age 43.7 ± 13.0 years participated, and their data were used for normalization, to create Z-scores and local templates for the mfERG analysis.

All subjects had 20/25 or better acuity, a refractive error between -6D and +4D and no retinopathy at the start of the study. Subject demographic data is shown in Table 1. All subjects provided informed written consent and the procedures were in compliance with the Declaration of Helsinki and the University of California Berkeley Committee for the protection of Human Subjects.

Table 1

Group	Patients	Gender Male: Female	Age (years)	Duration (years)	Blood Glucose (mg/dL)	Hb A1c (%)	Average mfERG Implicit Time (Z-scores)	Retino-pathy develop-ment
Total Diabetes Patients	N= 41	22:19	52.4 ± 10.4	9.1 ± 4.4	181.0 ± 86.0	8.44 ± 1.7	0.87 ± 1.66	20 Yes: 21 No
Type 1	N = 8	3:5	38.3 ± 10.6	13.0 ± 6.3	118.0 ± 46.9	7.8 ± 0.9	-0.30 ± 0.78	5 Yes: 3 No
Type 2	N= 33	19:14	55.8 ± 7.7	8 .0 ± 3.2	183.3 ± 91.8	8.6 ± 1.8	1.17 ± 1.65	15 Yes: 18 No
Control	N=50	21:29	43.7 ± 13.0	N/A	103.2 ± 20.8	N/A	0.00 ± 0.67	N/A

Table 1: Subject demographic data. Average mfERG implicit time includes all hexagons for that group at baseline when no retinopathy was present.

4.4.2 Study Timeline and Testing Procedures

All subjects with diabetes were followed over time and study visits occurred annually until they either developed retinopathy (n=20) or the study ended (n= 21). Recruitment was continuous with the average time in the study being 3 years, with a range of 1-6 years. Patients with and without retinopathy at the end of the study had the same range and mean for time followed. The mean for patients who developed retinopathy was 3.35 ± 1.2 years and those who did not had a mean of 3.23 ± 1.3 years.

Every study visit included a full medical history, non-fasting blood glucose reading (One Touch Ultra, Lifescan, Milpitas, CA), HbA1c % (At Home A1c, FlexSite Diagnostics Palm City, FL) measurement, dilated fundus examination with photos covering the central 50 degrees (Carl Zeiss Meditec, Dublin, CA), and mfERG (VERIS, EDI, Redwood City, CA).

The mfERGs were recorded as described before in Ng et al.[25], Han et al.[24] and Bearse et al.[26] Briefly, subjects were fully dilated with 1% tropicamide and 2.5% phenylephrine and a Burian-Allen contact lens electrode was used. A ground electrode was placed on to the right earlobe and the contralateral eye was occluded during the recording. A VERIS 4.3 system was used with a scaled 103 hexagon stimulus array displayed on a CRT at a frame rate of 75 Hz. The stimulus array subtended 45 degrees on the retina. An eye camera display refractor unit was used. This allowed the patient to self-adjust a cross in the center of the display to best focus. The monitor was calibrated every six months to insure quality measures over time. It remained very stable between calibrations Preamplifier filters were set to 10-100 Hz and retinal signals were amplified 100,000 times. The contrast of the stimulus display was set to 98% with the white elements at 200 cd/m^2 and the dark elements at < 2 cd/m^2. Seventeen percent spatial averaging was used with a single iteration of artifact removal.

First-order P1 kernel mfERG implicit times were measured with the template scaling method previously described.[27] For this method local templates were constructed from the mean local waveforms of the 50 control subjects. The template is scaled in both amplitude and time to fit a subject's corresponding local waveform by minimizing the least squares difference between the subject's local waveform and the local template. This information is then used to derive the P1 implicit time and N1-P1 amplitude. A statfit indicating the goodness of fit is generated for each response. Although no local response fits reached this criterion, a statfit of over 0.8 would have been rejected. Each local implicit time (IT) measure for the diabetic subjects was converted to a Z-score using the mean and standard deviation obtained from the controls. For our instrumentation and control data, one mfERG IT Z-score is equal to 0.9 ms when averaged over all measured retinal locations.[28]

To be spatially conservative, 35 retinal zones (each of which contained two or three neighboring hexagons) were constructed from the 103 stimulus elements. For each zone, a maximum IT Z-score was assigned from the 2 or 3 Z-scores from hexagons in that zone. All fundus photographs were graded in a detailed and masked fashion for the presence or absence of retinopathy without knowledge of other study results by a retinal specialist. The mfERG array was overlaid on to the digital photographs to match the location of retinopathy with any applicable mfERG zones (Figure 1).

Figure 1

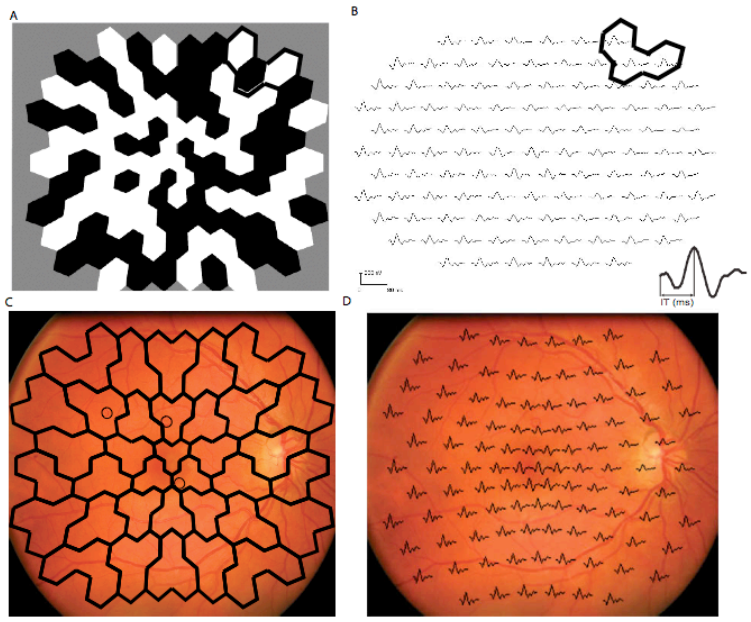

Figure 1: A: The mfERG array with a sample three hexagon zone highlighted. B: The array results in 103 mfERG traces, which were grouped by zone. A sample zone is again shown. The inset waveform shows the measurement of the P1 implicit time. C: The zones were also overlaid over fundus photos to determine which zones had retinopathy. The circles highlight the retinopathy that has developed D: The same photograph of one of our type 1 patients with their mfERG trace array overlaid.

4.4.3 Statistical analysis

Logistic regression[29] was performed to examine associations between new retinopathy development and seven baseline risk factors (measured 1 year prior to the retinopathy outcome for individuals who developed retinopathy, and one year prior to the last visit for those who remained retinopathy free): mfERG IT Z-score, diabetes duration, diabetes type, gender, blood glucose level, HbA1c, and age. The assumed relationship between the factors was determined before the analysis and is shown in Figure 2.

Figure 2

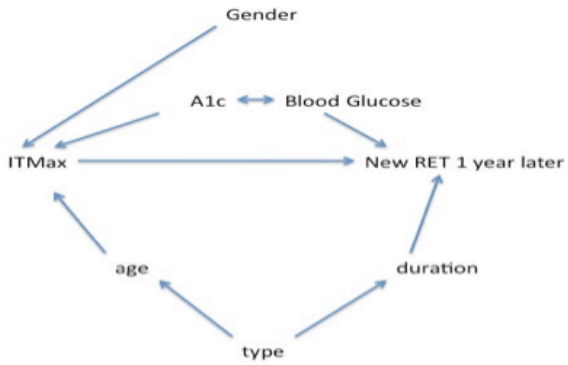

Figure 2: Causal Diagram showing the assumed relationship between the measured factors in the model.

Since correlations may exist between mfERG IT zones within the eye of any one subject and across eyes of the same subject, generalized estimating equations (GEEs) were used to estimate model coefficients. With the GEE approach, estimates allow for covariance between zones in the same subject, while assuming independence across subjects.[30] Observations on both eyes of a single subject were combined into a single

'cluster' to permit correlations across eyes. Robust variances were used for inference to accommodate any disparity between the assumed and true covariance structures.

For the logistic regression analysis, we first performed a univariate analysis of every risk factor, determining which factors were most likely to be predictive. Next, possible confounders of mfERG IT were identified and evaluated. Lastly, a final model was derived using a stepwise forward regression approach to determine if other factors strengthened the predictive power of the model.

Probabilities of new retinopathy development in a zone were calculated from the final model and used to construct receiver operating characteristic (ROC) curves.[31] A 5-fold cross-validation procedure was used, randomly dividing the data (grouped by eye) into 5 subsets. Each subset is used to validate a model created by the other 4 sets of data together. The five validations were averaged to determine the generalized predictive accuracy of the model.[32, 33]

4.5 Results

4.5.1 Retinopathy Development and Comparison of Type 1 and Type 2 Patients

Retinopathy developed in 20 of the 41 subjects. This occurred in 29 of the 78 eyes and 80 of the 2,730 retinal zones (3%). All of the retinopathy that developed was mild and was either a microaneurysm or a dot hemorrhage. No subject had retinopathy develop in more than five zones in the same eye. Seventeen of the 80 zones that developed retinopathy (21%) were in the 8 type 1 subjects in the study. When comparing type 1 and type 2 subjects, it was found that the type 1 subjects were younger, had longer diabetes durations, faster mfERG implicit times, and had better blood glucose control than the type 2 group (Table 1).

4.5.2 Model Creation

First we evaluated each potential risk factor in univariate models. The mfERG IT was found to be the most significant factor in the prediction of future retinopathy. The univariate analysis found that mfERG IT Z-score alone had an odds ratio of 1.16 (1.02-1.33). Diabetes duration was the only other factor that was significant in the univariate analyses. The duration of diabetes had an odds ratio of 1.07 (1.00-1.15) (Table 2). This means that mfERG IT Z-score alone and duration of diabetes alone are predictive of retinopathy. The odds ratios approximate relative risks, meaning that for every unit increase in mfERG IT Z-score, the risk of retinopathy onset increases by 16%, and for every year increase in diabetes duration the risk of the onset of retinopathy increases by 7%. All the other factors were not significant in univariate analysis.

Table 2

Variable	Coefficient	P-Value	Odds Ratio
mfERG Implicit Time	0.15	0.02	1.16 (1.02-1.33)
Duration	0.07	0.04	1.07 (1.00-1.15)

Table 2: Significant univariate models for the prediction of retinopathy. All other factors were not significant.

Next, the potential confounding of mfERG IT by other risk factors was examined. A confounder is a factor that correlates with mfERG IT as well as with retinopathy development. If a confounder is found, it must be included in the model so that it is properly accounted for. The type of diabetes was found to be a significant confounder of mfERG IT, changing the mfERG IT model coefficient by more then 10% and thus it must be included in the model. The mfERG IT coefficient is changed from 0.15 (P = 0.02; OR 1.16; 95% CI 1.02-1.33) when type is not accounted for in the model (see Table 2), to 0.18 (P = 0.012 OR 1.20 95% CI 1.04-1.53) when it is included. The more general model is:

$$log(p/1-p) = -3.8 + 0.18\,(IT\ Z\text{-}score) + 0.42\,(Diabetes\ Type)$$

where p denotes the probability of a given zone developing retinopathy one year following the measurements, and diabetes type is a binary factor with "0" for type 2 diabetes, and "1" for type 1 diabetes. Finally, other factors including diabetes duration and blood glucose level were evaluated to see if they improved the overall model fit but none reached significance at a 0.05 level. Thus the parsimonious model above was the ultimate choice.

The coefficient for mfERG IT in the multivariate model yields an odds ratio of 1.20 (95% CI: 1.04-1.53) which can again be interpreted as an approximate relative risk, meaning that for every unit increase in implicit time Z-score there is a 20% increase in the risk of developing retinopathy within one year.

4.5.3 Cross-Validation

A five-fold cross-validation was used to estimate the specificity and sensitivity of the selected model. This was done because the specificity and sensitivity estimates using the full data set are, by necessity, overly optimistic justifying the need for cross-validation. It yielded the 5 sets of coefficients (Table 3) whose average was 0.17 for implicit time and 0.41 for diabetes type, similar to that of the coefficients in the final model. Each of these five models yielded an ROC curve which had a range of sensitivities from 82-73% and a range of specificities from 80-69% (Figure 3). The average accuracy of these ROC curves indicates that the final model has validated sensitivity of 80 ± 4% and a specificity of 74 ± 4%.

Table 3

5-fold model number	IT Z-score coefficient	Type Coefficient	OR IT	OR Type	Sensitivity %	Specificity %
1	0.18	0.39	1.20	1.48	82	72
2	0.18	0.41	1.19	1.50	82	80
3	0.17	0.31	1.18	1.36	79	69
4	0.18	0.48	1.20	1.62	81	74
5	0.15	0.44	1.16	1.55	73	75
Average	**0.17**	**0.41**	**1.19**	**1.50**	**80**	**74**
All data	0.18	0.42	1.20	1.38	87	82

Table 3: Five-fold cross validation coefficients, odds ratios, and ROC parameters. Bolded line indicates averages of the five curves and gives overall parameters for the model.

Figure 3

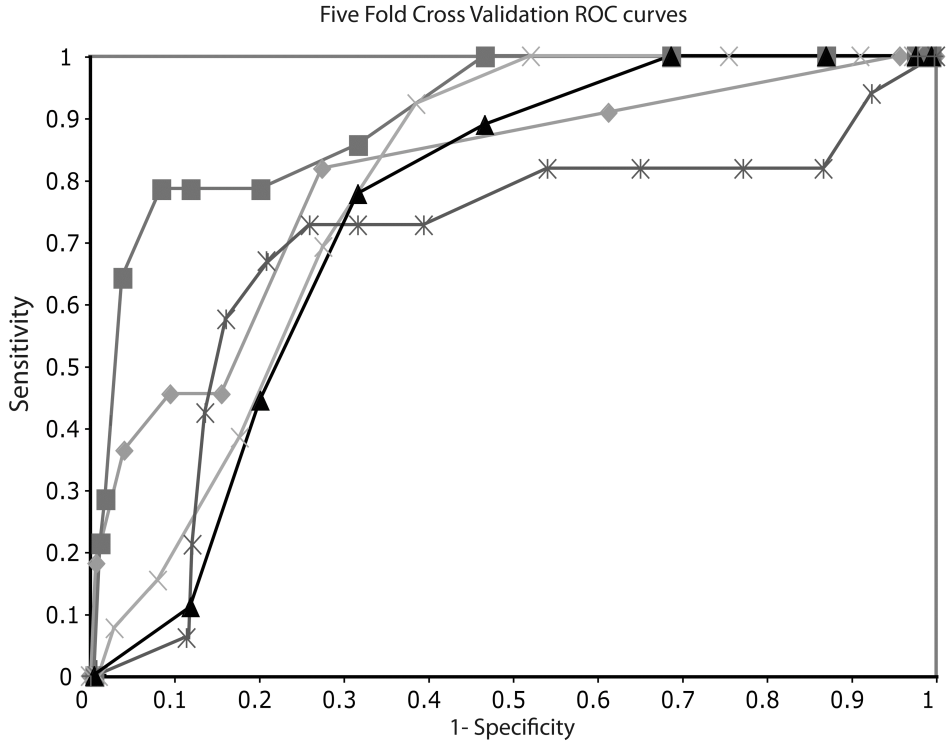

Figure 3: Receiver Operating Curves (ROC) based on the five fold cross validation subsets of the data. Each different line (symbol) is a curve constructed from 1/5 of the data using coefficients from the other 4/5. The average of the five curves yields a sensitivity of 80% and specificity of 74%.

4.6 Discussion

In this study, we created a multivariate model for the prediction of retinopathy onset in adult patients with diabetes. The main predictive risk factor in this model is a local retinal neural measure, implicit time of the mfERG. The mfERG IT has been shown in past studies to be delayed in patients with diabetes. While the exact mechanism causing the delays in diabetes remains unknown, hypoxia, local blood flow changes, or changes in local metabolism may be responsible for the effects observed.[22] In our model, a one unit Z-score increase in mfERG IT increases the risk of the onset of retinopathy in a retinal zone by 20% in these patients. Furthermore, we found that the power of the mfERG IT for predicting the development of retinopathy is different for adult patients with type 1 and type 2 diabetes; with patients having type 1 diabetes displaying a greater risk for the onset of retinopathy with smaller comparative delays in mfERG IT than the type 2 group. Consequently, type of diabetes must be adjusted for in these predictions and included in the predictive model.

Previously, our group developed models to locally predict new retinopathy development in patients with and without baseline retinopathy over a 1-3 year follow-up period. We have found several factors that are predictive of new retinopathy in these patients, including mfERG IT Z-score, diabetes duration, blood glucose, and the presence of DR at baseline. In our previous 1 year model,[24] the strongest factor predicting new retinopathy in a retinal zone was previous retinopathy in the eye. This is perhaps not surprising since, it is clinically accepted that an eye with some retinopathy has pathology, and is at high risk for more pathology. Our previous models did not have enough patients without baseline retinopathy who went on to develop retinopathy in the follow up period to make predictions about the onset of retinopathy in those eyes. Consequently, in this study, we investigated a group of patients with no retinopathy at baseline over a longer period of time. This model is a critical step for predicting the first fundoscopically obvious change in the retina at the typical interval between diabetic eye examinations. The clinical onset of DR (vasculopathy in the retina) signifies an important progression in the microvascular complications in diabetes, which may also be occurring in other organ systems. This progression can be an indicator for the physician and patient to consider more aggressive management, including shorter patient follow-up intervals.

Successful fitting of this model required a sufficient number of patients who developed retinopathy during the testing period and used a much larger sample size than previous predictive modeling studies. This is because the conversion rate to early retinopathy is low and retinopathy is scarce in the retinal tissue. Even with half of the patients developing retinopathy, the local retinopathy development in zones that was observed was relatively low overall (3%). Complicating the analysis and interpretation is the fact that even though it is known that the development of a microaneurysm is a process over time,[34] 50% of visible microaneurysms are transient.[15]

A one-year follow-up interval was utilized in this study in order to closely comply with standard clinical care follow-up guidelines of diabetic patients who have no DR or mild DR. The cross-validated sensitivity (80%) and specificity (74%) of this model is only slightly less than the 86% and 82% found in the earlier validated one year model using patients with baseline retinopathy and more severe retinopathy, even though the retinopathy observed in the current study was more scarce and more difficult to detect.[23]

Clearly the mfERG IT, when properly adjusted for diabetes type, is a very sensitive test for predicting even the earliest clinical retinal changes over a one-year window.

Associations between retinopathy development factors (such as duration of diabetes, blood glucose levels, type of diabetes, and blood glucose control) that were significant in previous retinopathy progression models from our group and other studies looking at retinopathy progression [10, 23, 25, 35], did not significantly contribute to the multivariate model for predicting retinopathy onset in conjunction with mfERG IT in patients who were retinopathy free. In our previous 3 year model,[25] diabetes type was found to be a possible predictive factor, however the small sample size of type 1 patients led to a large confidence interval for the odds ratio of that prediction. Because of the imprecision surrounding this factor, we did not include it in the previous 3-year model. In the present model diabetes type was not found to be directly predictive of retinopathy but instead confounded the mfERG IT's ability to predict the retinopathy. On average the type 1 patients developed more retinopathy but they also tended to have faster average implicit times than the type 2 patients. The differences between the two groups are accounted for by including diabetes type in this model, but additional studies on the early differences in neural function between the types of diabetes are needed. The overall differences between the present model and previous models likely stems not only from the difference in the retinopathy itself but also that this population of patients, who are developing the onset of retinopathy, is very different from the population of patients who already have retinopathy. Type 1 adult patients who have yet to develop retinopathy, for example, tended to have longer durations, younger ages, and better blood glucose control (several were using insulin pumps which have been shown to improve HbA1c% levels [36]) than type 2 patients in this same group. But these differences between type 1 and type 2 would not necessarily be observed in patients who already have retinopathy.

As the average age of type 1 patients at the time of diagnosis is younger and more definitive than type 2, the longer durations and younger ages we see in our study of adult patients with type 1 diabetes are to be expected. Most type 1 patients develop some retinopathy within 25 years of diagnosis[37, 38], and progression to proliferative diabetic retinopathy was also shown to be common in a study following type 1 patients over a 25 year period (42%).[39] On the other hand, perhaps because of a more ambiguous disease onset, type 2 patients appear to range more in their progression of disease. Many patients, (35%-45%), with type 2 diabetes have retinopathy at the time of their diabetes diagnosis[40] and so would not be eligible for this study. As many as 60% of patients with type 2 diabetes for over 20 years have retinopathy and 58% in the 11-20 year duration range also have retinopathy.[41] However, these figures include the patients with retinopathy at diagnosis, who were eliminated from our study. Our study represents a different, and likely healthier group of type 2 patients than are typically presented in the epidemiological data, which might partly account for why the type 2 group had a lower risk of first retinopathy development in the present study.

In summary, our new model for predicting of the onset of retinopathy, in eyes with no previous retinopathy, reveals that the mfERG IT is a useful tool for predicting retinopathy onset. It is objective, measures retinal function in about 8 minutes, and is reproducible.[28] The model could be used to identify higher risk patients to enroll for clinical trials or tests of newer medications aimed at delaying or preventing the earliest retinopathy well before visual acuity is affected. Our results also suggest that the mfERG

IT Z-score measurement when corrected for type of diabetes, could possibly act as a surrogate endpoint for studies where the preventative treatment of retinopathy is a primary goal. [42, 43]

4.7 References

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Chapter 5: Associations among Blood Pressure, Blood Glucose Control, Vessel Caliber, and Retinal Thickness in Patients with Type 2 Diabetes

5.1 Prelude

This study was originally designed to examine factors that alter optical coherence tomography (OCT) measurements in diabetic patients with and without retinopathy. The OCT measurements were taken as part of our longitudinal studies so that they could be used as one of our outcome measures, particularly for edema. In order to use the values in this capacity, it is important to understand which factors could confound the measurements. Once we properly identify these factors, we can control for the confounders in the model if necessary. Thus this experiment is a necessary prelude to chapters 6 and 7.

After examining many factors, we found that in patients with retinopathy blood pressure was positively and linearly associated with retinal thickness. The association was stronger with diastolic blood pressure than systolic blood pressure but both were associated. This indicates that blood pressure should be accounted for when examining retinal thickness measurements in patients with retinopathy and further implicates blood pressure as an important factor in these patients.

These results were presented in Abstract form at the American Academy of Optometry meeting (2008) and at ARVO (2009). Full publication of the main result is anticipated in the future.

Harrison WW, Ng J, Bearse MA, Neuville JM, Bronson-Castain K, Mesropian L, Barez S, Schneck ME, Adams AJ. Diastolic blood pressure and retinal thickness are related in diabetic eyes with retinopathy – a pilot study. *Optom Vis Sci.* 2008; 85: E-abstract 80045

Harrison WW, Bearse MA, Schneck ME, Ng JS, Bronson-Castain KW, Barez S, Adams AJ. Diastolic blood pressure and retinal thickness in patients with diabetes and hypertension. *Invest Ophthalmol Vis Sci.* 2009; 50: ARVO E-Abstract 1368.

5.2 Abstract

5.2.1 Purpose

Retinal thickness (RT) is an important diagnostic sign in diabetes, particularly related to the presence of retinal edema. We examine the role of blood pressure (BP) in RT in type 2 diabetes, with and without mild or moderate non-proliferative diabetic retinopathy (NPDR).

5.2.2 Methods

40 type 2 diabetes patients (22 without NPDR and 18 with NPDR) and 26 control subjects participated. Blood pressure, retinal thickness (Stratus OCT3), fundus photography, HbA1c, and blood glucose were measured. Correlations between BP, blood glucose measures, vessel caliber, and RT were evaluated.

5.2.3 Results

Blood pressure is positively and significantly associated with retinal thickness in patients with NPDR. This correlation is most significant in macular regions outside the fovea. Increased BP was not associated with decreased arteriole caliber in diabetes, but was in controls. Higher HbA1c was associated with higher BP but not with increased retinal thickness in patients with NPDR.

5.2.4 Conclusions

Even within the normal range of BP, increased BP is linearly associated with increased RT in patients with type 2 diabetes and NPDR. For diastolic pressures, every 10mmHg increase accounts for 5% RT increase; for systolic pressure a 10mmHg increase leads to a 2.5% increase in retinal thickness. Thus, blood pressure could be an important component to consider when evaluating the retinal thickness changes in diabetes.

5.3 Introduction

Diabetes is the leading cause of new blindness in people aged 21-74 in the United States, and is an increasing problem around the world today.[1] Macular edema, the leaking of fluid from vessels into the retinal tissue, is one of the leading causes of vision loss in these patients.[2-4] Preventing macular edema in at-risk individuals would help to save sight and represent a major advance in visual health.

Currently, most treatments for macular edema are aimed at slowing or reducing edema revealed through fundus examination, fluorescein angiography, or optical coherence tomography (OCT). Very recent reports suggest that combination pharmaceutical and laser treatments may be quite effective in treating macular edema in diabetes.[5] However, faster and earlier diagnosis of edema even at a sub-clinical level could be helpful in reducing the impact on vision, and lessen the need for these invasive treatments.[6, 7] Finding modifiable factors that influence retinal thickness and vessel permeability would be helpful in this process.

As macular edema is caused by a release of fluid and proteins from the retinal vessels, a co-existing hypertension is of particular importance. Hypertension puts additional strain on the small venules and arterioles of the body, which are already affected by hyperglycemia in diabetes. In subjects without diabetes, the retinal arterioles are affected by hypertension alone, tending to become smaller in caliber.[8] It has also been shown that the retina has a protective mechanism from hypertension in healthy subjects which consists of myogenic constrictions in arterioles, the Bayless effect, but this effect can be impaired by hyperglycemia in patients with diabetes.[9]

Other studies have found other links between diabetic retinal changes and increased blood pressure. In subjects with non-proliferative diabetic retinopathy (NPDR), an elevated systolic blood pressure has been shown to be predictive of an increase in retinopathy over a 4 year period.[10] Yet other studies found associations between higher systolic or diastolic blood pressure and retinopathy progression over time in type 1 subjects.[11, 12] Several studies have shown a relationship between blood pressure and macular edema, with high systolic blood pressure shown to be correlated with diffuse macular edema [13] and improved blood pressure control reducing the risk of macular edema.[14]

This study evaluates the effect of blood pressure on retinal structure in patients with type 2 diabetes with and without NPDR. The purpose is to determine whether higher blood pressure is associated with increased retinal thickness in patients with diabetes.

5.4 Methods

5.4.1 Subjects

Forty subjects with type 2 diabetes (22 with no retinopathy and 18 with moderate or mild non-proliferative diabetic retinopathy) and 26 healthy non-diabetic controls were included. All but 4 of the subjects with diabetes were taking hypertensive medications to control blood pressure, and 4 of the controls were also taking hypertensive medications prescribed. No interventions were performed to change blood pressure levels during the course of the study. Subject demographic data is shown in Table 1.

Table 1: Characteristics of Subject Groups

Group	Controls	No Retinopathy	Moderate or Mild Non-proliferative Retinopathy
Number of subjects	26	22	18
Age (Years)	54.6 ±10.4	52.7 ± 9.4	57.8 ± 7.2
Gender	12M : 14F	10M : 12F	12M: 6F
Blood Pressure (mm Hg)	117.6/73.3 ± (16.5/8.3)	120.2/75.3 ± (14.1/8.4)	126.2/76.0 ± (13.6/10.8)
Retinal thickness (microns)	245.5 ± 12.5	251.5 ± 13.9	249.4 ± 21.5
Number of subjects on blood pressure medications	4 (15% of subjects)	19 (86% of subjects)	17 (94% of subjects)
Blood Glucose (mg/dL)	**106.7 ± 25.2***	**151.4 ± 43.2***	**207.5 ± 83.9***
HbA1c (%)	N/A	**8.1 ± 1.4**	**9.9 ± 2.1** †

Table 1: Values are means ± SD. Bolded values are significantly different. *Blood Glucose was significantly different in all three groups ($p < 0.05$). † HbA1c different between patients with and without retinopathy ($p < 0.006$).

Blood pressure (LAS on Automatic cuff, Omron Model HEM-773, Bannockburn, IL), blood glucose (One Touch Ultra, Lifescan, Milpitas, CA), retinal thickness (Stratus OCT3, Carl Zeiss Meditec, Dublin, CA), and fundus photography covering the central 50 degrees (Carl Zeiss Meditec, Dublin, CA) were measured on all subjects. HbA1c (FlexSite Diagnostics, Palm City, FL) was also measured on all subjects with diabetes. All subjects provided written informed consent and procedures adhered to the tenets of the Declaration of Helsinki and the UC Committee for the Protection of Human Subjects.

All subjects had a best-corrected visual acuity of 20/25 or better and a spherical equivalent refractive error between -6D and +4D. All were dilated with 1% tropicamide and 2.5% phenylephrine to at least 7mm to insure high quality OCT and fundus photograph clarity. Fundus photographs were graded for level of retinopathy by a retinal specialist, and subjects with patches of edema in the central 50 degrees were not included in the study.

5.4.2 OCT

Two different OCT scanning procedures were used. First a standard fast macular scan was done which takes six 6mm scans of the central macula at one time. Second, a 12 radial scan protocol was employed to give better resolution. The 12 radial scans were taken sequentially with each scan comprised of 512 axial samples. This 12 scan technique has been fully described in Neuville et al.[15] and the same protocol and retinal groupings were used in this study. The retinal thickness was taken from the vitreo-retinal surface to the RPE/outer segment interface. The two scan modalities gave the same average retinal

thickness. The 12 scan protocol was used to provide greater resolution of the thickness of 37 retinal regions, as there are more data points captured with the 12 scan protocol. The 12 scans were interpolated to identify the thickness in 37 different macular locations. The macular region was averaged overall and also divided into 5 sections: central, nasal, temporal, superior, and inferior.

5.4.3 Blood Vessel Analysis

Fundus photographs of the optic discs of all subjects were used for the analysis of retinal blood vessels using the IVAN software (University of Wisconsin, Madison, WI). The software measures and summarizes the caliber of retinal venules and arterioles within 0.5 to 1 disk diameter around the optic nerve. We followed the standard protocol described previously in detail.[16-18]

5.4.4 Data Analysis

Linear regression was used to determine the significance of the correlations. The means of the subject groups were compared using Student t-tests. The significance level of linear regressions performed at multiple locations were corrected for multiple comparisons.

5.5 Results

5.5.1 Comparisons of Subject Groups

Perhaps surprisingly, the mean retinal thicknesses ($p = 0.71$) and the mean blood pressures ($p = 0.14$) of the three groups did not significantly differ (Table 1). However, blood pressures were well controlled in our diabetes population and fell within the normal range for most subjects. Only 4 subjects (2 with NPDR and 2 without) had a high diastolic blood pressure above 90mm Hg and 9 subjects (5 with NPDR and 4 without) had an elevated systolic blood pressure, above 130 mmHg. HbA1c measures ($p < 0.009$) and blood glucose ($p < 0.04$) at the time of OCT measurement were significantly higher in DM patients with NPDR compared to those without NPDR. (Table 1)

5.5.2 Blood Pressure and Retinal Thickness

There was no correlation between blood pressure and retinal thickness in the control group ($r^2 = 0.048$, $p=0.81$) or the patients without NPDR ($r^2 = 0.0016$, $p=0.92$). However, a strong positive correlation was found between mean retinal thickness and blood pressure in the NPDR group. This relationship was present for both diastolic ($r^2 = 0.45, p < 0.02$; Figure 1A) and systolic ($r^2=0.29, p < 0.05$) blood pressure (Figure 1B). Over a diastolic blood pressure range of 55 - 90 mm Hg the macula thickness increased on average by 18%. Over a systolic range of 110 - 145 mm Hg, the macula increased in thickness on average by 9%.

Figure 1

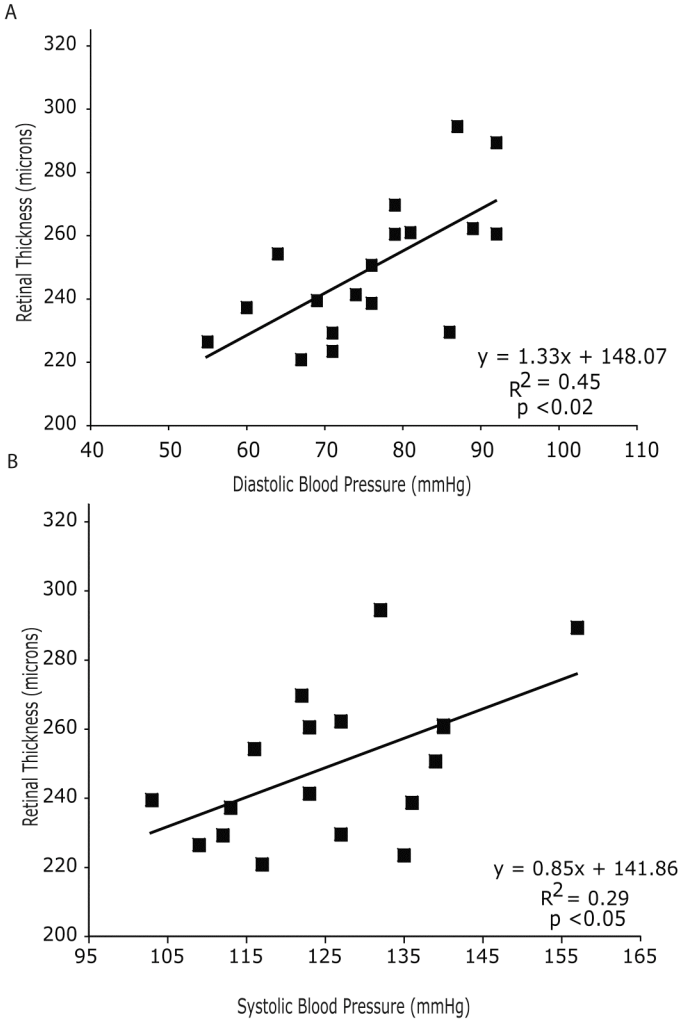

Figure 1: The correlation between retinal thickness (microns) and A) diastolic blood pressure (mm Hg) and B) systolic blood pressure (mm Hg) for the subjects with mild to moderate non-proliferative diabetic retinopathy.

Further examination of the association between diastolic blood pressure and retinal thickness in patients with retinopathy, in different macular areas, found that the correlation between diastolic blood pressure and retinal thickness was not significant in the central fovea. After correction for multiple comparisons, the significant p value was $p<0.013$, so the correlation was also not significant in the temporal and nasal macula. It was significant in the superior and inferior regions of the macula. Figure 2 highlights the significance of the correlations for each retinal area measured.

Figure 2

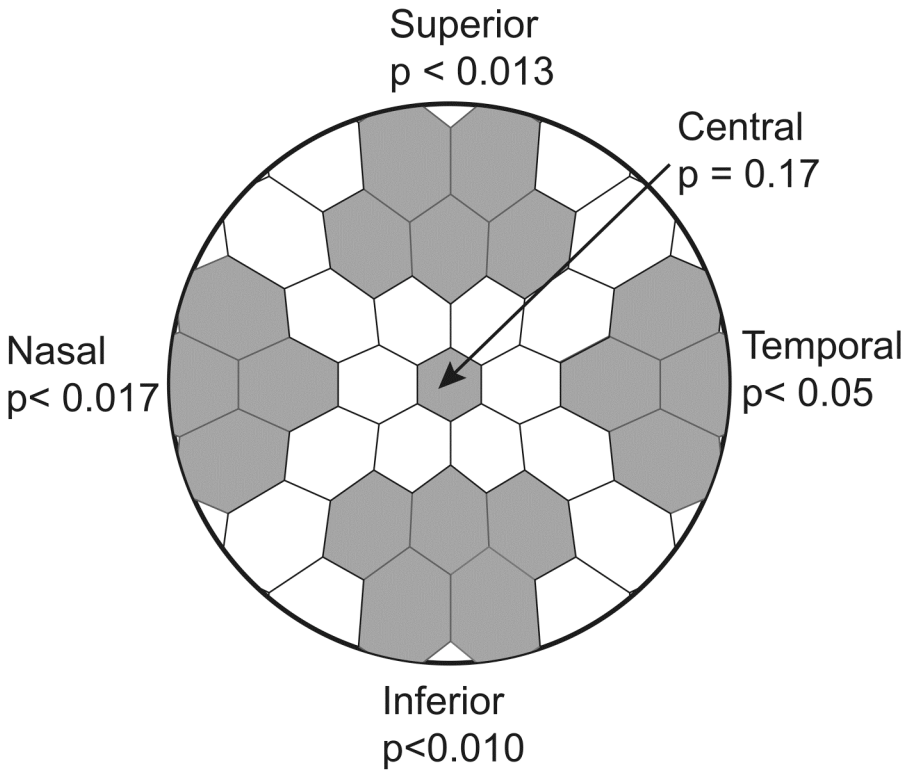

Figure 2: The circular area measured by the OCT divided into 37 hexagons. The shaded areas show the five sub-regions examined. The p-values for the significance of the correlation between that region's retinal thickness and the subject's blood pressure are shown.

5.5.3 Vessel Caliber Analysis

The previous analysis indicates that areas where blood vessels are located have more significant associations between blood pressure and retinal thickness, when compared to areas with less blood vessels. Thus, the caliber of the retinal blood vessels was examined for its contribution to this association. First, the vessel calibers were compared between the three groups. Second, associations between vessel calibers and blood pressure were assessed.

5.5.3.1 Comparison of Vessel Calibers Between Subject Groups

The venule sizes of the subject groups were different. There was a trend toward the venules becoming larger in diabetes. The subjects with retinopathy had significantly larger venules than controls ($p < 0.03$). There were no differences between the three groups with respect to arteriole size ($p=0.09$). Since the arteriole size was relatively

unchanged and the venules became larger in diabetes, as expected, the arteriole to venule ratio (AVR) was also smaller in the subjects with NPDR than the other groups ($p < 0.02$) (Table 2).

Table 2: Vessel Caliber size measurements.

Group	Controls	No Retinopathy	Moderate or Mild Non-proliferative Retinopathy
Arteriole Size (microns)	181.1 ± 15.6	190.0 ± 19.4	186.2 ± 19.4
Venule Size (microns)	**221.1 ± 25.9 ***	232.2 ± 25.9	**246.6 ± 31.7 ***
AVR	0.825 ± 0.083	0.828 ± 0.102	**.736 ± 0.092 †**

Table 2: Values are mean ± SD. Bolded values are significantly different. *Venules were significantly larger in patients with retinopathy compared to controls. ($p< 0.03$) † Patients with retinopathy have significantly smaller AVR than the other two groups. ($p<0.02$)

5.5.3.2 Associations between Blood Pressure and Vessel Caliber

As expected, a negative correlation was found between arteriole caliber and blood pressure only in the control group for both systolic and diastolic measures ($r^2 =0.31$, $p < 0.03$ systolic; $r^2= 0.27$, $p < 0.05$ diastolic) (Figure 3A and B). However, in contrast, when examining this correlation between blood pressure and vessel caliber, in the groups with diabetes, there were no associations between blood pressure and any vessel caliber measures for these subjects, with or without NPDR. Also, none of the subject groups demonstrated correlations between retinal thickness and any of the vessel measures.

Figure 3

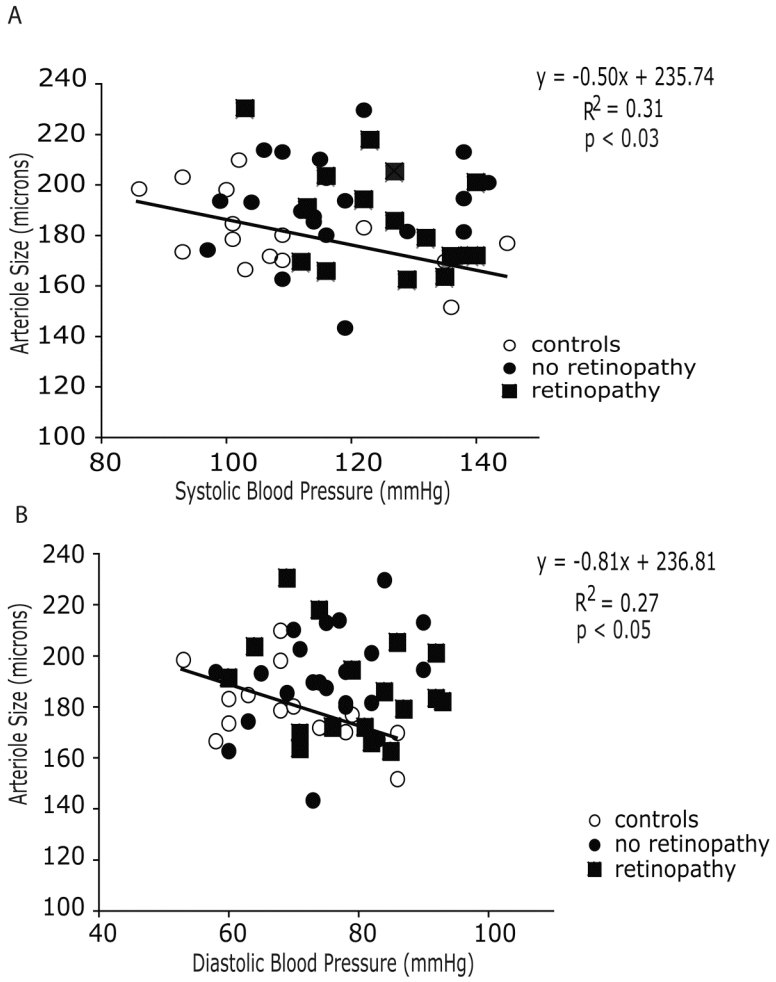

Figure 3: The correlation between arteriole size (microns) and A) systolic blood pressure (mm Hg) and B) diastolic blood pressure (mm Hg) for control subjects and subjects with diabetes, with and without non-proliferative diabetic retinopathy. The line highlights the significant relationship for control subjects. There was not a significant correlation for either subject group with diabetes.

5.5.4 Blood Glucose Measures

Neither blood glucose levels at the time of testing nor HbA1c were correlated with retinal thickness or blood pressure in any group. However, in the NPDR group, a positive correlation was found between systolic and diastolic blood pressure and HbA1c ($r^2 = 0.47$, $p < 0.001$ systolic and $r^2 = 0.33$ $p < 0.01$ diastolic).

5.6 Discussion

Macular edema is a leading cause of vision loss in subjects with diabetic retinopathy. Earlier diagnosis of this condition at a sub-clinical level could lead to better and more immediate treatment. It has been shown that while OCT is adequate for identifying edema,[19] OCT alone may not be able predict who will develop edema from a

sub-clinical state.[20, 21] Therefore, other modifiable factors that may be predictive should continue to be investigated.

In this study, the association between blood pressure and retinal thickness was examined. We found that blood pressure is positively and linearly associated with retinal thickness, in subjects with mild to moderate NPDR but no edema, even within the normal blood pressure range. This correlation is most significant for diastolic blood pressure in the non-central regions of the macula. For the highest blood pressures in these subjects, the level of retinal thickening is consistent with sub-clinical edema. This suggests that blood pressure could play an important role in the development of edema in these subjects.

To our knowledge, there is only one other study that looked at the relationship between blood pressure and retinal thickness in subjects with diabetes. Asefzadeh et al.[22] looked at patients with either mild NPDR or no retinopathy and found no correlation between the measures. This is consistent with our results, in as much as we found no correlation in subjects without retinopathy. A moderate NPDR group was not evaluated by Asefzadeh et al., and most of our retinopathy group had moderate NPDR, and only a few subjects had mild central NPDR.

There are a number of plausible explanations for the association between increased retinal thickness and higher blood pressure in subjects with NPDR. The first is that an increased blood pressure expands the retinal vessels causing them to take up more retinal space and consequently increase the retinal thickness. As with other studies which noted increased venular caliber in diabetes,[23-24] we noted that the venule size is greater in subjects with diabetic retinopathy. Some studies have also noted an increase in arteriole size in diabetes,[25 26] but we did not. This may be because, by dividing the subjects into groups by retinopathy status, we did not have a large enough sample in each group to have adequate statistical power for this measure.

In agreement with other studies, we found that higher blood pressure is correlated with decreased arteriole vessel caliber in control subjects (Bayless effect). Interestingly, this relationship was absent in subjects with diabetes, even in those subjects without retinopathy. This indicates that early changes to the retinal vessels are occurring, causing them to be larger than expected. Blum et al.[9] who also noted a lack of vessel constriction with increased blood pressure in subjects with type 1 diabetes. To our knowledge, this effect had not been previously documented in type 2 diabetes.

In the subjects with diabetes, we found no correlation between blood pressure and the venule size, arteriole size, and AVR, and no correlations between these measures and retinal thickness. Therefore, increased vessel caliber is an unlikely cause of the increased retinal thickness with increased blood pressure in patients with mild and moderate NPDR. While it is important to note that the blood vessel measures were obtained from the larger vessels around the optic disc, and the retinal thickness measures encompass areas of the macula with small capillaries, we have no reason to think that the changes to the vessels due to blood pressure and hyperglycemia would not be true in both retinal regions.

The severity of retinopathy cannot explain the relationship between increased retinal thickness and higher blood pressures, because the patients in this study were selected to be homogenous with respect to retinopathy status. Most patients had moderate non-proliferative diabetic retinopathy, and the patients who were included with mild

NPDR all had hemorrhages in the macular region. Furthermore, OCT has been shown to be unable to detect the difference between a minimal retinopathy group and a no retinopathy group, in type 1 patients. Therefore, it might be insensitive to minimal differences in retinopathy.[27]

We observed a correlation between increased blood pressure and higher HbA1c in the NPDR group. While HbA1c did not correlate directly with retinal thickness, this relationship between blood pressure and HbA1c indicates that patients with higher blood pressures may tend to be in poorer overall health. This poorer health could correlate with other factors that could increase the retinal thickness, such as inflammation in the retina, which we did not specifically measure in this study. These results agree with Klein et al.[28] who found that glycemic control and blood pressure were both associated with the risk of macular edema in type 1 diabetic patients. The relationship between these factors, even early in the disease, is complex and deserves further study.

A more likely explanation of the relationship between blood pressure and retinal thickness is that the breakdown of the blood-retinal barrier due to diabetes, and possibly earlier malignant hypertension, causes a leakage of fluid into the retinal layers. This leakage is exacerbated by increased blood pressure. While no fluid is clearly visible on the fundus photograph or in the OCT itself, a sub-clinical diffuse edematous event could be present. This is consistent with the correlation between blood pressure and retinal thickness being weakest in the central fovea and stronger in the areas with greater numbers of intraretinal vessels.

The hypertension and diabetes study (HDS) indicated that as many as 39 % of type 2 subjects have hypertension at the time of their diabetes diagnosis.[29] Several studies have looked at both the effects of hypertension as a co-morbidity factor in diabetes and the effects that the two diseases have together on the retina. The United Kingdom Prospectus Diabetes study (UKPDS) is the largest study to look at blood pressure intervention in type 2 diabetes subjects.[30, 31] It found that blood pressure (BP) intervention reduces the risk of microvascular complications, the most common of which was retinopathy. Improved blood pressure control also reduces the risk for photocoagulation at a 6 year follow up.[31] Previous studies have also looked at the importance of blood pressure control in the retinal health of subjects with diabetes. A recently published study by Beulens et al.[14] shows a two-fold decrease in the odds of macular edema with reduced blood pressure (OR = 0.50). The UKPDS found that a reduction of systolic BP by a median 10 mmHg and diastolic BP by 5 mmHg reduced microvascular disease by 37%.[31] If the results seen in our study are a manifestation of a sub-clinical edema caused by pushing of fluid out of the retinal vessels by a higher blood pressure, then proper control of blood pressure early in the diabetes process could be essential to reduce the risk of sight threatening clinically significant macular edema. Future studies examining blood pressure interventions and evaluating changes in retinal thickness with changes in blood pressure using a larger sample size of patients with moderate retinopathy are needed as a follow up to this work.

In conclusion, higher blood pressures are associated with greater retinal thickness in subjects with moderate or mild non-proliferative diabetic retinopathy. This occurs even when blood pressures are largely within the normal range. This indicates that blood pressure could play an important role in the mechanisms leading to increased retinal thickness and possibly retinal edema.

5.7 References

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Chapter 6: Local associations between Retinal Structure and Function in eyes with Diabetic Macular Edema

6.1 Prelude

This cross sectional study examined local associations between macular edema, retinal thickness (optical coherence tomography) measures, mfERG measures, and visual acuity. It was conceived to establish relationships between the mfERG measures and edema in our data. This is a necessary step before moving on to use the mfERG as a possible predictive measure for edema in the model in the next chapter (7). The literature indicates that there are relationships between mfERG implicit time, mfERG amplitude, and edema.[1-5]

We first examined the general associations between the mfERG and edema in our data to make sure they were in agreement with the literature. Second, we examined local associations, which has not been examined in previous studies. We also expanded our study beyond the literature, to look at local associations between the mfERG, edema, retinal thickness, type of diabetes, duration of diabetes, gender, blood pressure, HbA1c, visual acuity and contrast sensitivity. Our methods of data processing and analysis allows for examination of associations in 37 macular locations. All studies done examining these associations previously used the software provided by the OCT instrument in 9 larger sectors and averages of mfERG locations, usually in concentric retinal ring areas.

We found that the mfERG measures are locally associated with edema. Retinal thickness measures and visual acuity measures were also locally associated with mfERG implicit times. The mfERG amplitudes were associated with visual acuity but not with retinal thickness. Significant associations were found were between local edema and male gender, type 2 diabetes, visual acuity, contrast sensitivity and retinal thickness. Furthermore, retinal thickness and visual acuity and contrast sensitivity were associated, as well as visual acuity and female gender and contrast sensitivity.

This study confirms the relationships between mfERG measures and edema that have been shown in the literature. This sets up the mfERG as a candidate test for the prediction of edema, presented in Chapter 7. Furthermore, the studies in this chapter also establish local associations between all these measures. This is the first study to establish a local association between mfERG implicit time and visual acuity in edema.

This paper has not been published in any form but some cases, which are also included within the data in this chapter, were presented at the American Academy of Optometry meeting in Orlando in 2007.

Harrison, WW Bearse MA, Ng J. Davila O, Schneck ME, Adams AJ. Clinically Significant Macular Edema Cases Are Associated With Multifocal Electroretinogram Abnormalities *Optom Vis Sci.* 2007; 84: E-abstract 075228.

This project will be submitted as an abstract for the 2011 Academy meeting in Boston.

6.2 Abstract

6.2.1 Purpose

To evaluate, for patients with diabetic macular edema, associations between retinal function, measured with the multifocal electroretinogram (mfERG) and retinal structure, measured with fundus photography and optical coherence tomography (OCT). A secondary purpose was to measure associations between these factors, visual acuity, and other diabetes health indicators that may influence them.

6.2.2 Methods

Twenty-two eyes from 15 patients with diabetic macular edema were included. Thirteen of those patients had type 2 diabetes (19 eyes) and two had type 1 diabetes (3 eyes). Fifty-two controls were also included for comparison purposes and to normalize data. The multifocal electroretinogram (mfERG) (VERIS 5.2), Stratus OCT3 thickness measures, fundus photograph grading, HbA1c measures, blood pressure, visual acuity, contrast sensitivity, and an extensive history were performed for every patient. The results from the OCT, mfERG, and photographs for the macular region (central 6 mm) were overlaid and locally compared using logistic and linear regressions with general estimating equations to account for local correlations. Regression analysis was also used to evaluate associations between these local measures and other diabetes health indicators.

6.2.3 Results

Locally edema was associated with delayed mfERG implicit times (IT), decreased mfERG amplitudes (Amp), retinal thickening on an OCT, type 2 diabetes, male gender, decreased visual acuity and decreased contrast sensitivity. Similarly, increased local thickness on the OCT was associated with delayed mfERG IT, decreased visual acuity, decreased contrast sensitivity, and edema in a fundus photo at that location. Decreased visual acuity was associated with delayed IT, decreased Amp, decreased contrast sensitivity, increased OCT thickness, female gender, and edema on a fundus photograph.

6.2.4 Conclusion

Local associations exist between mfERG measures, OCT measures, visual acuity, and the presence of edema. These strong associations could be useful in studies aimed at the prevention and treatment of macular edema.

6.3 Introduction

Macular edema is a leading cause of vision loss in patients with diabetes.[6] Since edema can occur at any stage of diabetic retinopathy and the treatments for edema are currently invasive, preventing edema is an important clinical and research goal. Evaluating risk factors associated with macular edema could improve our understanding of this disease. One such risk factor that has been identified in previous studies is change to the multifocal electroretinogram (mfERG), a local measure of retinal neural function.

Studies evaluating the mfERG in diabetic macular edema have found that the mfERG is sensitive to the changes in the retinal tissue. In edema, the implicit time (IT) of the mfERG becomes delayed and the amplitude (Amp) becomes reduced.[1-5] The mfERG technique has also been widely used as a tool to evaluate retinal function in edema before and after diabetes surgeries. Previous work has evaluated photocoagulative and vitrectomy surgeries as well as injections for edema. They have found that the mfERG implicit time is a predictive factor for the outcome of vitrectomy, and that the mfERG results are different after surgery in eyes with and without visual improvement.[7-9] Furthermore, other studies found an improvement in mfERG amplitude after focal laser and bevacizumab injection for edema.[10, 11]

The focus of this study is to evaluate the local relationship between retinal structure, function, and visual acuity in diabetic macular edema. Retinal function was measured with the mfERG, and the retinal structure was evaluated with both optical coherence tomography (OCT) and fundus photography. We also evaluated other diabetes health factors that could influence these associations, as well as the relationship between retinal function and visual acuity and contrast sensitivity.

6.4 Methods

6.4.1 Patients

Twenty-two eyes from 15 patients (mean age 54.4 ± 9.5 years) with diabetes and macular edema (in the central 6 mm) were included in this study. In addition 52 healthy non-diabetic controls (mean age 43.1 ± 14.7 years) were evaluated. The control data was used for comparison purposes, to normalize data, and create Z-scores.

The study was cross sectional with all patients being evaluated once. The patients with edema had visual acuities ranging from 20/15 to 20/60, with an average of 20/25 (95 ETDRS letters). All controls had vision that was 20/20 or better. On average, the patients with diabetic edema had long durations of diabetes and poor blood sugar control (Table 1). All the patients with diabetes had moderate to severe nonproliferative diabetic retinopathy. Sixteen of the 22 eyes had macular edema that was classified as clinically significant (CSME) by the ETDRS standards.

Table 1 Patient Data

Patient	Age (years)	Sex (M:F)	Visual Acuity (OD/OS) Snellen Equivalent	HbA1c	Duration (years)
1	48.3	F	20/25 20/25	14.8	0.2 *
2	49.8	F	20/40	12.0	13
3	63.0	F	20/25	10.8	15.2
4	60.0	F	20/60	13.9	29.2
5	59.8	M	20/20 20/25	8.8	2.8
6	58.7	M	20/25 20/20	9.6	24.2
7	59.5	M	20/25	8.5	15.6
8	58.2	M	20/20 20/20	13.0	10.1
9	45.4	M	20/20	9.6	4.4
10	40.2	F	20/20	14.5	10.2
11	61.7	F	20/25 20/32	8.5	17.8
12	64.6	M	20/32 20/32	9.0	24.2
13	62.6	M	20/20	9.9	19
14	41.2	M	20/15 20/15	10.1	25.8
15	28.0	M	20/15	10.8	20.7
Average	**54.4 ± 9.5**	**9:6**	**20/25- ± 7 letters**	**10.7 ± 2.4**	**15.3 ± 8.8**
Controls	43.1 ± 14.7	23:29	20/20	N/A	N/A

* Patient 1 was diagnosed with Diabetes when her vision became blurry due to CSME.

6.4.2 Tests Performed

Retinal thickness (Stratus OCT3: Carl Zeiss Meditec Dublin CA), mfERG (VERIS 5.2: EDI Redwood City, CA), blood pressure (LAS on Automatic cuff :Omacron Model HEM-773 Bannockburn IL), HbA1c (At Home A1c: FlexSite Diagnostics; Palm City, FL), stereo fundus photographs covering the central 45 degrees (Carl Zeiss Meditec), visual acuity (ETDRS chart), contrast sensitivity (Pelli-Robson chart) and a detailed history were performed on every patient. All subjects provided written informed consent and procedures adhered to the tenets of the Declaration of Helsinki and the UC Committee for the Protection of Human Subjects.

6.4.3 Determining the location of edema

The location of the edema was determined by a retinal specialist who evaluated the fundus photographs and graded them in detailed fashion for the level of retinopathy and location of edema. The photographs were available as macular stereo pairs. The retinal specialist was blind to the results of the other tests. All subjects were also asked to undergo fluorescein angiography, which was also graded in the same detailed fashion. Eleven of the 15 subjects consented for the fluorescein angiography and that information was also used to verify the location of edema when available.

6.4.4 Optical Coherence Tomography

A customized 12 radial scan OCT protocol was employed when gathering OCT data.[12] This was done to increase the resolution of the data, compared to the 6 scan protocol available with the Stratus software. The 12 radial scans were taken sequentially with each scan comprised of 512 axial samples. They were then uploaded into a matlab (Mathworks; Natick, MA) program designed to calculate the average thickness for 37 hexagonal regions within the central 6 mm. These hexagons match up with the central 37 hexagons of the mfERG stimulus. An average of the 37 hexagons gives the average macular thickness, which was verified to be the same value as the average from the 6 scans from the OCT software. This 12 scan technique has been fully described in Neuville et al.[12] and the same protocol was used in this study. As is standard for the Stratus instrument, the retinal thickness was taken from the vitreo-retinal surface to the RPE/outer segment interface. The average thickness for each hexagon was calculated from the control patients. OCT values were examined as raw values and as differences from the control mean for each location. The difference calculation allows us to account for the topography differences across the macula.

6.4.5 mfERG recordings

Subjects were fully dilated with 1% tropicamide and 2.5% phenylephrine. A Burian-Allen contact lens electrode was used and a ground electrode was placed on to the left earlobe of each patient. The other eye, which was not currently being recorded, was occluded with an eye patch. A VERIS 5.2 system was used with a 103 hexagon CRT display. The display was 75 Hz and subtended 45 degrees on the retina. The unit had an eye-camera-refractor display which allowed the subject to self adjust the screen to best focus to correct for their refractive error while wearing the contact lens. Preamplifier filters were set to 10-100 Hz and retinal signals were amplified 100,000 times. The contrast of the stimulus display was set near to 100% with the light elements at 200 cd/m^2 and the dark elements at < 2 cd/m^2. Seventeen percent spatial averaging was used with a single iteration of artifact removal in waveform processing.

First order kernel mfERG P1 implicit times were measured with the template scaling method described by Hood and Li.[13] The local templates were constructed from the mean local waveforms of the 52 control subjects. The template was then scaled in amplitude and time to the subject's local waveform, minimizing the least square difference between the two. The program designates a statfit, which is a measure of the goodness of fit for each waveform. When this measure is over 0.8, it indicates a poor fit and was a criterion for rejection. No patients in this study were rejected for a poor statfit. Each local Amp measure and IT measure, for the patients with diabetes, was converted to

a Z-score using the mean and standard deviation of the 52 controls. For our instrumentation, an mfERG IT Z-score on average is equal to 0.9 ms and an mfERG Amp Z-score is equal to 0.19 μV.

6.4.6 Statistical Treatment of Data

The mfERG stimulus was overlaid onto the fundus photos and regions with edema were identified. (Figure 1) The Z-scores from the waveforms of all macular regions with edema were averaged together to find the average edema mfERG. The Z-scores from the remaining 37 macular hexagons were averaged to create the non-edema mfERG and T-tests were used to evaluate the differences between these groups. Hexagons outside the macula were not included in the analysis.

Linear and logistic regressions[14] were used to evaluate associations between variables. Univariate analyses determined the individual associations for each measure and are presented. To account for the fact that some patients had two eyes contributing to the data while others only had one eye, and all patients had 37 different retinal locations in each eye, general estimating equations with robust variances were used. These allow us to compensate for the correlations in the data that likely exist, and also to accommodate for differences between the true and assumed covariance structures. General estimating equations allow coefficients estimates to account for any covariance between hexagons in the same subject but assume independence across different subjects.[15]

Figure 1

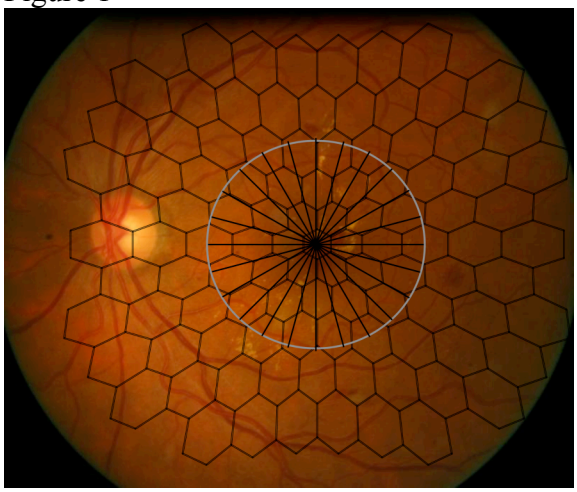

Figure 1: mfERG overlay and OCT overlay shown over the retina of one of the edema patients. The 37 hexagons inside the circle were evaluated for every patient.

6.5 Results

6.5.1 mfERG results in the areas with and without edema

Overall the eyes in this study displayed poor retinal neuronal health. The average macular mfERG IT was 2.32 ± 2.00 Z- scores and the average mfERG Amp was -0.60 ± 1.04 Z-scores. Thirty percent of the hexagons were identified as having edema. The

macular areas with edema showed longer delays, 3.08 ± 2.27 Z-scores on average, than the areas without edema, 2.00 ± 1.81 Z-scores ($p<0.001$). The amplitudes of the areas with and without edema ($p <0.001$) were also significantly different. The areas with edema had average decreased amplitudes of -0.94 ± 0.88 Z-scores, and the areas without edema were -0.46 ± 1.07 reduced on average.

6.5.2 Associations between edema and other factors

Logistic regression was used to determine associations between local edema on a fundus photograph and other factors. The factors which were evaluated were: mfERG IT, mfERG Amp, Retinal thickness (OCT) values, type of diabetes, gender, age, HbA1c, visual acuity, and contrast sensitivity. Edema was individually associated with delayed mfERG IT, decreased mfERG Amp, decreased VA, decreased contrast sensitivity, type 2 diabetes, and thickening on the OCT (Table 2).

Table 2

Factor	Coefficient (95% CI)	P value	Odds Ratio
mfERG IT (Z-scores)	0.236 (0.072, 0.400)	0.005	1.27
mfERG Amp (Z-scores)	-0.450 (-0.878, -0.021)	0.040	0.64
Visual Acuity (Letters)	-0.072 (-0.116, -0.028)	0.001	0.93
Contrast Sensitivity (Log CS)	-4.22 (-5.64, -2.80)	0.0001	0.015
Type of Diabetes	1.04 (0.44, 1.65)	0.001	2.82
Gender	-1.33 (-2.11, -0.56)	0.001	0.26
OCT (Microns from the mean)	0.012 (0.003, 0.022)	0.012	1.01

Table 2: Factors associated with macular edema

6.5.3 Associations between OCT and other factors

As OCT is used to identify regions of edema in clinical situations, we wanted to see if the same factors which were associated with the edema identified in a fundus photo, were associated with retinal thickening on an OCT. To account for the topography differences in the OCT over the macular area, the difference between the individual's local reading and mean of the control patients at that location were calculated. Linear regression was then used to examine the relationship between this calculated OCT measure and the other factors. Thickening on the OCT was associated with edema on the fundus photograph, as it was with mfERG IT delays, decreased visual acuity, and decreased contrast sensitivity. (Table 3)

Table 3

Factor	Coefficient (95% CI)	P value
mfERG IT (Z-scores)	7.56 (0.96, 14.1)	0.025
Edema	22.10 (2.48, 41.73)	0.027
Visual Acuity (Letters)	-1.62 (-2.81, -0.43)	0.007
Contrast Sensitivity (Log CS)	-86.50 (-142.68, -30.32)	0.003

Table 3: Factors associated with Retinal Thickening

6.5.4 Associations between visual acuity and other factors

Associations between visual acuity and other factors in these patients were also examined with linear regression. Although all of our patients had macular edema, most had good visual acuity on the ETDRS chart. Vision loss was associated with edema in the fundus photograph, delayed mfERG IT, decreased mfERG Amp, female gender, decreased contrast sensitivity, and increased thickness on an OCT. (Table 4)

Table 4

Factor	Coefficient (95% CI)	P value
Edema	-4.01 (-6.51, -1.64)	0.001
mfERG IT (Z-scores)	-1.89 (-3.5, -0.26)	0.023
mfERG Amp (Z-scores)	3.08 (0.14. 6.02)	0.040
Gender	-10.1 (-16.4, -3.78)	0.002
Contrast Sensitivity (Log CS)	35.6 (23.89, 47.47)	0.0001
Retinal Thickness (Microns from the mean)	-0.65 (-0.1148. -0.0159)	0.010

Table 4: Factors associated with visual acuity

6.6 Discussion

In this study we evaluated local associations between structure and function in patients with macular edema. In our study the local presence of edema was evaluated both with a fundus photograph and with the Stratus OCT, in 37 separate macular locations. The two measures were highly correlated and provided similar results. However there were some important differences. First, edema on a fundus photograph had stronger associations with the factors. And second, edema on the photo was also associated with more factors than retinal thickness (as shown in tables 2 and 3). mfERG Amplitude, diabetes type, and gender were associated with edema on a photo but not with the retinal thickness measures. Thus, this 'pilot' data indicates that in research situations where risk factors are being evaluated and possibly in trials where treatments for edema are being assessed, there should be caution in substituting values from an OCT in the place of evaluation of a fundus photograph for local edema. Important associations may be missed when using the OCT retinal thickness values. Previous studies have also offered this caution but because they have indicated that the agreement between fundus examination and OCT was the lowest in cases of mild edema.[16] Mild edema with good acuity is representative of our data set. Furthermore, other work found that overall agreement between OCT and photography for the presence or absence of edema was only moderate when the edema was mild.[17, 18] In our study the two measures were highly locally correlated to one another, but the associations with other diabetes health factors were not as strong on the OCT.

The difference in the associations seen for OCT and fundus grading deserves more thoughtful analysis. There are several reasons why the associations could differ with retinal thickness measures as opposed to the fundus photo evaluation. First, the OCT provides a finer quantitative scale of data compared to the photo. The photo either has edema in a local region or does not. The data from the OCT is sensitive not only to the areas of overt edema but also areas of subclinical edema and general retinal thickening that may not be visible on a fundus photo. While this is ideal for measuring changes in the retina over time, subclinical edema and general retinal thickening may, or may not, be associated with risk factors for macular edema which is vision threatening. Perhaps evaluating the OCT differently with set retinal thickness cut offs (in the manner of an ROC curve) would allow for a more equal comparison between the two measures and more insight into the actual associations. This may also be useful because in our analysis we used logistic regression to analyze the associations between edema on the photo and other factors and linear regression to evaluated the association between retinal thickness and other factors. If we used "cut offs" as described above, we could use logistic regression for both sets of associations which is advantageous due to the use of odds ratios, that all predicted values fall between 0 and 1, and the differences in the variance of the data is better accounted for.[14]

However such an analysis would also need to account for the fact that OCT measures are subject to confounding by a number of other variables that may, or may not, affect the interpretation of edema in a fundus photograph. For example, one of the factors that we noted as having a significant association with edema but not with retinal thickening was gender. It is accepted that the OCT readings of men and women are

different, with men having thicker retinas than woman.[19, 20 19, 20] Perhaps in our study the relatively small sample sizes of men and women limited revealing differences.

Another possibility to consider is that other studies have shown that in diabetes there can be local thinning and tissue loss. This has been shown in both type 1 and type 2 diabetics, and can occur very early in the disease process.[21-24] The thinning could occur in the same location as the subsequent edema, and if there is tissue loss in an area followed by edema, the values on the OCT might not be as compelling as viewing the fundus photographs.

We are aware of one other study that has looked at the association between structure and function in diabetic patients with diabetic retinopathy and with and without edema.[25] This study by Holm et al found that increased macular thickness is associated with increased IT in the central macula. Our results are in agreement with theirs in this regard.[25] Holm et al. also found a relationship between increased macular thickness and decreased mfERG amplitudes. We found a relationship between local edema and reduced local mfERG amplitudes but not with increased OCT thickness and reduced amplitudes. This may be because the measures in our study used 37 local retinal regions while the prior study averaged results in rings over many hexagons in comparison, and also because the patients in our study mostly had mild edema while the prior study had a wide variety of patients included.

In agreement with previous work,[1, 25] we did find that the patients in our study generally had reduced amplitudes, and that although the amplitude changes tended to be largest in the regions with edema, the reduced amplitudes were widespread over the entire retina. In general, the neuronal health of the patients in our study was very poor overall in all locations for both amplitude and implicit time. Another study by our group found that decreased local mfERG amplitudes are highly significant in the prediction of future local edema,[26] indicating that the relationship between mfERG amplitude decrease and edema is complex. It seems plausible given the available data on mfERG changes in diabetes and edema that changes to the mfERG amplitudes in diabetes happen at a more rapid rate and much later in the disease process. Implicit time changes on the other hand, begin very early in the disease process and change more gradually as the eye gets sicker.[3, 4, 27, 28] More studies on the timing and nature of amplitude changes in diabetic eye diseases need to be done.

Studies have noted that there is a correlation between increased foveal thickness and visual acuity loss in edema.[17, 29, 30] This correlation is one of the reasons that clinically the OCT is such a useful tool. We also confirmed a relationship between increased local macular thickness and visual acuity loss in our data. Since our patients generally had good acuity for a group of patients with edema, it is important to note that these correlations were present even in generally mild edema.

We also noted that although male gender was associated with edema, female gender was associated with vision loss in our study. However, this association between female gender and decrease visual acuity is likely idiosyncratic to our cross sectional study, this association is strongly biased the fact that the two patients with the most vision loss happened to be female. A larger sample is needed to evaluate if women really are more vulnerable to vision loss in edema compared to their male counterparts.

We found that decreased contrast sensitivity was more significantly associated with both edema and increased retinal thickness than visual acuity, indicating that

contrast sensitivity may be a better measure to assess visual changes in early edema. This is also in agreement with the literature, which has noted that contrast sensitivity is a very sensitive measure for retinal health in diabetes. Studies have noted that contrast sensitivity is decreased in edema and in diabetes earlier in the disease process.[31, 32] Sokol et al.[33] found that in diabetic patients with normal snellen acuity there can be contrast sensitivity loss.

We also found an association between changes to local retinal function, in the form of both IT delays and decreased Amp, and decreased acuity. In the literature we could not find any other studies that noted a correlation between delayed implicit time and decreased visual acuity. The study by Holm et al also looked at this relationship between foveal retinal thickness, implicit time, amplitude, and visual acuity. They found relationships exist between retinal thickness and visual acuity, and between mfERG amplitude and visual acuity, but not between implicit time and visual acuity. However, the association of visual acuity and implicit time in their study was close to significance, with a p value of $p = 0.054$.

When evaluating similarities and differences between our data and the previously published study on this topic, we found that our group largely verified the results of the previous study, adding to the literature which suggests that there are important associations between retinal function measured by the mfERG, visual acuity and edema. While the sample sizes of the two studies are similar (15 vs 18 patients), the differences that are present likely arise from the fact that all the eyes in our study had macular edema while the prior study evaluated eyes with and without edema. Also, the profile of our patients differed as the patients in our study generally have better visual acuities and a smaller range of retinal thickening compared to the patients in the prior study. In essence our patient population was more homogenous, representing those with early to moderate edema changes, while the previous study looked at a much wider range of diabetic patients. Our study also evaluated the associations on a local level taking into account correlations between the measures while the prior study largely used data averaged in rings.

The associations between visual acuity, mfERG IT and mfERG Amp in these patients with diabetic edema could allow the two measures to be used together more easily in studies and clinical trials. Visual acuity loss is an important measure in many clinical trials involving the eye. The mfERG has been shown to have changes in diabetes early in the disease process, even before clinical retinopathy is present, and now also correlations to vision loss late in the disease process. Thus, the mfERG could be very useful in trials for new treatments of macular edema in diabetic eyes.[4, 27]

6.7 References

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Chapter 7: Prediction, by retinal location, of the onset of diabetic edema in patients with nonproliferative diabetic retinopathy

7.1 Prelude

This longitudinal study follows up on all the previous chapters and describes the experiments that allow creation of a model to predict the specific local retina region of edema in patients with diabetic retinopathy. Since edema can be sight threatening, this model could potentially be used to predict changes that lead to vision loss, even specify where across the retina this edema will appear. The model was created in a similar style to the model created in chapter 4 and took advantage of the associations established in chapters 3, 5, and 6.

Chapter 6 established that the mfERG is locally associated with existing retinal edema. Given that the mfERG was also sensitive for the prediction of the onset of retinopathy in chapter 4, a model to evaluate if the mfERG is useful in the prediction of edema was a logical extension with considerable research and clinical management interest. Furthermore, chapter 5 established blood pressure as a measure that may be strongly associated with changes in the retina in patients with retinopathy. Thus, in evaluating the factors for prediction including blood pressure was important.

In this study, the patients were followed over time monitoring the health of their fundus as well as other diabetes health indicators. If edema developed we were able to evaluate data from the previous visit to see which local measures may be predictive of impending change. Eleven different risk factors were evaluated for their predictive properties. They included mfERG implicit time, mfERG amplitude, HbA1c, blood glucose, systolic and diastolic blood pressure, degree of retinopathy, age, duration of diabetes, type of diabetes and sex. Of these factors, only the mfERG measures have the potential to point to the specific location across the retina where edema is expected to arise.

We first created a model, which used only the mfERG measures to predict edema. We found that the mfERG alone could predict local edema with both 72% sensitivity and specificity. Next we created a multivariate model to predict local. That model found that mfERG implicit time, mfERG amplitude, sex, and systolic blood pressure, together, could predict local edema with 84% sensitivity and 76% specificity. Since edema can occur at any stage of diabetic retinopathy, a model to predict local edema could be an important first step in finding an earlier treatment for this disease, which remains a leading cause of vision loss in diabetic patients.

This study has been submitted for publication and is currently under revision. It has also been accepted for presentation at the 2011 ARVO meeting. Portions of this work were presented at the 2010 American Academy of Optometry meeting.

Harrison WW, Bearse MA, Schneck ME, Wolff BE, Jewell NP, Barez S, Mick AB, Dolan BE, Adams AJ Multifocal Electroretinograms, Systolic Blood Pressure, and Gender Together are Predictive of Local Onset of Diabetic Edema in Patients with Diabetic Retinopathy. *Invest Ophthalmol Vis Sc*i. 2011: 52 ARVO E-Abstract 558.

Harrison WW Bearse MA, Schneck ME Wolff BE, Jewell NP, Barez S, Adams AJ. Multifocal Electroretinograms and Blood Pressure Predict the Onset of Diabetic Retinal Edema within One Year. *Optom Vis Sci.* 2010: 87 E- abstract 100744

7.2 Abstract

7.2.1 Purpose

To formulate a model to predict the specific local retinal regions of the onset of diabetic retinal edema (DE) in adults with diabetic retinopathy (DR), at risk for DE.

7.2.2 Methods

46 eyes from 23 patients with DR were included. Subjects were followed semi-annually until DE developed or the study concluded. The presence or absence of DE within the central 45 degrees at the final visit was the outcome measure, and data from the prior visit was used as baseline. A logistic regression model was formulated to assess the relationship between DE development and: Multifocal electroretinogram (mfERG) implicit time (IT) Z-score, mfERG amplitude (Amp) Z-score, sex, diabetes duration, diabetes type, blood glucose, HbA1c, age, systolic (SBP) and diastolic blood pressure, and grade of retinopathy. 35 retinal zones were constructed from the mfERG elements and each was graded for DE. Data from 52 control subjects were used to calculate the maximum IT and minimum Amp Z-scores for each zone. ROC curves from a five-fold cross-validation were used to determine the model's predictive properties.

7.2.3 Results

Edema developed in 5.2% of all retinal zones, and in 35% of the eyes. The mfERG Amp, mfERG IT, SBP, and sex were together predictive of edema onset. Combined, these factors produce a model that has 84% sensitivity and 76% specificity.

7.2.4 Conclusions

Together mfERG, SBP, and sex are good predictors of local edema in patients with DR. The model is a useful tool for assessing risk for edema development, and a candidate measure to evaluate novel therapeutics directed at DE.

7.3 Introduction

Diabetes is the leading cause of preventable blindness in the US among adults 21-74 years of age.[1] Among these patients, a primary cause of vision loss is macular edema, caused from leaking of fluid out of the retinal vessels into the tissue.[2-4] Edema can occur at any stage of diabetic retinopathy, and can have devastating visual consequences. Thus, predicting and preventing macular edema in "at risk" individuals is an important clinical research and patient care goal. Currently the standard-of-care treatment for macular edema is focal laser. It involves using tiny laser burns in the macular area to inhibit the spread of fluid in the retina. This treatment does not restore lost vision but can reduce further vision loss.[5] Other treatments, such as injections of steroids and anti-VEGF agents have been successful in some patients.[6, 7] More recent studies suggest that a combination of these treatments may be even more effective in reducing vision loss,[8] but a preventative measure that is less invasive is still needed for these patients.

There have been a number of studies that have looked at factors associated with macular edema with a particular interest in modifiable risk factors. Edema has been associated with a longer duration of diabetes, higher systolic and diastolic blood pressure, Latino and African American ethnicity, prior amputation, and increasing retinopathy severity.[9, 10] Some studies have used the multifocal electroretinogram (mfERG) to evaluate diabetic macular edema and its treatments.[11, 12]

The mfERG has been shown to be sensitive to changes in diabetes even quite early in the disease process.[13, 14] Thus these neural function measures might identify and predict more severe changes, such as retinal edema. The mfERG measures are affected by long standing edema and increased foveal thickness. Studies have shown that the mfERG implicit time (IT) is prolonged and the mfERG amplitude (Amp) reduced with retinal edema.[15, 16] Furthermore, the mfERG has been shown to be a useful tool in evaluating the success of intravitreal injections for diabetes, and predictive of the functional prognosis for the results after surgeries for diabetic eye disease.[11, 12, 17]

We have previously developed multivariate models using the mfERG IT and other diabetes health measures to predict new local retinopathy development over 1-3 years in patients with DM both with, and without, nonproliferative diabetic retinopathy at baseline.[18-22] Here we create a model using the mfERG IT and Amp to specifically predict potentially sight-threatening edema in patients with existing retinopathy. The ability to identify those patients at highest risk for vision loss within the following year could have wide spread application in both clinical trials evaluating new treatments and in monitoring the care of patients with diabetic retinopathy.

7.4 Methods

7.4.1 Patients

Twenty-seven adult patients with diabetes completed the study. Four patients with type 2 diabetes were excluded from the analysis at the end of the study. Reasons for exclusion were outlined at the start of the study and were as follows: one patient was excluded due to poor mfERG fixation at baseline (resulting in a template-scaling measure, statfit, over 0.8), one patient developed a visually significant cataract requiring surgery, and two patients developed proliferative diabetic retinopathy with blood obscuring the retinal tissue in the final fundus photos and needing laser photocoagulation.

This left 23 patients that were included in the final analysis, and both eyes of each patient were used. All patients were between 25-65 years old with a mean age of 47.4 ± 12.1 years. There were 10 patients with type 1 diabetes and 13 with type 2 diabetes. In addition, 52 healthy non-diabetic controls (mean age 43.1 ± 14.7 years) participated, and their data was used for normalization, to create Z-scores and local waveform templates for the mfERG analysis. At baseline, all patients and controls had 20/25 or better acuity and all patients with diabetes had varying levels of nonproliferative diabetic retinopathy in at least one eye. All patients with media opacities, retinal edema in the central 45 degrees at baseline, or prior laser treatment anywhere in the retina were excluded from the study, and patient demographic data is shown in Table 1. All participants provided written informed consent and the procedures were in compliance with the Declaration of Helsinki and the University of California Berkeley Committee for Protection of Human Subjects.

Table 1

Group	Patient	Sex M:F	Type 1:2	Age years	Duration years	Blood Glucose mg/dL	Hb A1c %	Blood Pressure SBP/ DBP mmHg	Ret levels None: Mild: Mod-erate: Severe
Diabetes	N= 23	12: 11	10:1 3	47.4 ± 12.1	16.5 ± 8.5	172.5 ± 79.7	9.3 ± 1.9	128.9/78 .8 ± 25.8/11. 9	3: 18: 17: 6
Controls	N=52	23: 29	N/A	43.1 ± 14.7	N/A	105.6 ± 22.3	N/A	113.4/70 .3 ± 17.5/9.7	N/A

Table 1: Baseline patient demographic data

7.4.2 Study Timeline and Testing Procedures

All patients with diabetes were followed semi-annually over time until the study concluded or edema developed. Recruitment was continuous and the average time in the study was 2 years, with a range of 0.5-4 years. The last study visit was used as the outcome and the previous full study visit was used as the baseline for prediction.

Every year, each study subject would undergo a full study visit which included a full medical history, random blood glucose reading (One Touch Ultra, Lifescan, Milpitas, CA), and HbA1c (At Home A1c, FlexSite Diagnostics Palm City, FL) measurements, dilated fundus examination with photos covering the central 50 degrees (Carl Zeiss Meditec, Dublin, CA), a Stratus OCT3 (and Cirrus OCT (Carl Zeiss Meditec) for all patients visits after 11/2008), blood pressure reading (LAS on automatic cuff, Omron

Model HEM-773, Bannockburn, IL), and mfERG (VERIS, EDI, Redwood City, CA). In between full study visits, at a six-month follow up visit, all measures were repeated except the mfERG. There was no difference in the average time between the baseline and the outcome visit for patients who developed or did not develop edema. Patients who developed edema had an average study time between baseline and outcome of 9.0 ± 2.9 months. Patients who did not develop edema had a study time of 10.3 ± 2.9 months.

Patients who developed edema anywhere in the central 45 degrees at any visit were asked to return within 2 weeks for fluorescein angiogram (FA) to confirm the location and extent of the edema. All but two of the patients returned for the additional testing. The FA was graded in detailed fashion for the location of retinal edema and grade of overall retinopathy by a retinal specialist masked to the mfERG and all other results. The fundus photos, which were available to the retinal specialist as macular stereo photos, were graded in the same manner. A combination of the results of the FA, photos and OCT's were used to determine the exact location of edema. Patients with clinically significant macular edema (CSME) were referred to their ophthalmologist for evaluation and any necessary treatment.

7.4.3 mfERG recordings

Subjects were dilated to at least 7 mm with 1% tropicamide and 2.5% phenylephrine. A bipolar Burian-Allen contact lens electrode was used for recording. A ground electrode was placed on the left earlobe of each patient and the other eye, which was not currently being recorded, was occluded. A VERIS 5.2 system was used with a 103 hexagon display. A 75 Hz CRT display which subtended 45 degrees on the retina was used. The unit had an eye-camera-refractor display which allowed the subject to self-adjust the screen to best focus to correct for their refractive error. This display also allowed us to monitor the subject in real time for good fixation. Preamplifier filters were set to 10-100 Hz and retinal signals were amplified 100,000 times. The contrast of the stimulus display was set to near 100% with the light elements at 200 cd/m^2 and the dark elements at < 2 cd/m^2. When processing the waveforms, 17 % spatial averaging was used with a single iteration of artifact removal.

First order kernel mfERG P1 IT and Amp were measured with the template scaling method.[23] The local templates were constructed from the mean local waveforms of the 52 control subjects. The template was then scaled in time and amplitude to match the subject's local waveform, by minimizing the least square difference between the two. The program designates a measure of the goodness of fit for each waveform labeled a "statfit." A "statfit" over 0.8 indicates a poor fit and was a criterion for rejection, and one subject was rejected on this basis. Each local IT measure and Amp measure for the diabetic patients was converted to a Z-score using the local mean and standard deviation of the 52 controls. For our instrumentation, an mfERG IT Z-score on average is equal to 0.9 ms and an mfERG Amp Z-score is equal to 0.19 μV.

To be spatially conservative, 35 retinal zones, containing two or three neighboring hexagons, were constructed from the 103 mfERG stimulus elements. For each zone a maximum IT Z-score and a minimum Amp Z-score was assigned, selecting from the Z-scores of the mfERG for hexagons in that zone. All fundus photographs and FA's were graded in a detailed and masked fashion for the presence or absence of edema, and the degree of retinopathy on a clinical scale (none, mild, moderate, severe). The mfERG

array was overlaid on to the digital photographs to match the location of edema with the mfERG zones (Figure 1).

Figure 1

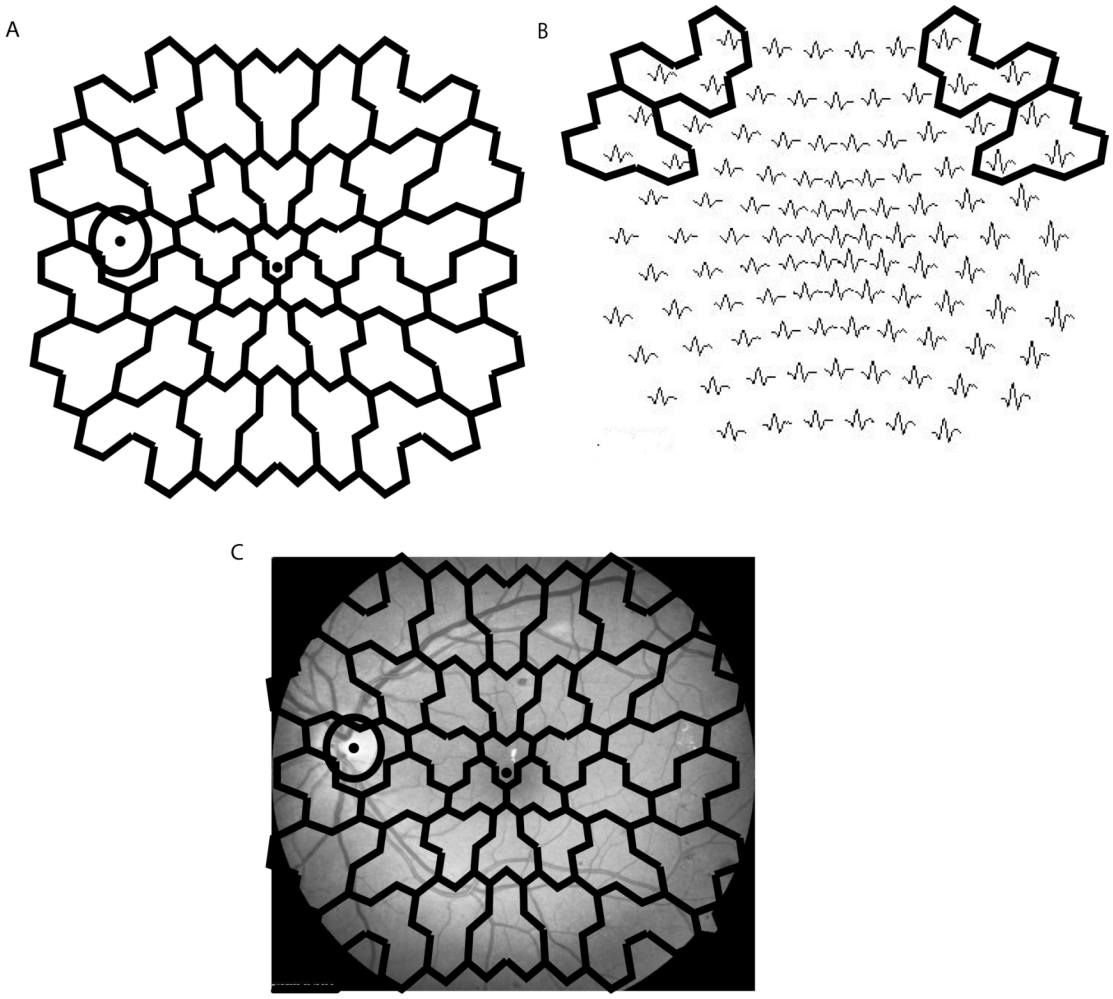

Figure 1: A: The 35 retinal zones that were constructed. B: The maximum mfERG IT Z-score and minimum mfERG Amp Z-score were assigned to the entire zone. C: The zones were overlaid on the fundus photographs to mark the location of the edema.

7.4.4 Statistical analysis

Logistic regression[24] was performed to examine associations between development of diabetic edema and eleven baseline risk factors. These factors were measured at the last full study visit (within 1 year prior to the outcome) for the individuals who developed edema, and also at the last full visit for patients who did not

develop edema. The baseline measures included in the modeling process are, mfERG IT Z-score, mfERG Amp Z-score, diabetes duration, diabetes type, sex, blood glucose level, HbA1c, systolic blood pressure, diastolic blood pressure, age, and degree of retinopathy.

The univariate relationship between edema and degree of retinopathy was also examined at the time of the outcome measurements (follow up), as well as the relationship between edema and change in retinopathy status. These were not included in the model but were evaluated separately as individual associations.

Since correlations likely exist in this data structure between both the mfERG measures in different zones within an eye of any one subject, and between eyes of the same subject, model coefficients were estimated with generalized estimating equations (GEEs). GEEs allow coefficient estimates to account for covariance between zones in the same subject, but assume independence across subjects.[25] As in previous models by our group,[20, 22] observations from a single subject were combined into a single cluster to permit correlations across eyes. Robust variances were used for inference to accommodate for any differences between the true and assumed covariance structures.

For the logistic regression analysis, we followed the steps of a standard stepwise forward regression. We first performed a univariate analysis of all eleven risk factors and determined which factors were most likely to be predictive. Second, possible confounders and interaction terms were evaluated. Lastly, two models were created. The first model used only mfERG measures to predict edema (labeled as mfERG only model-model 1), thereby ignoring all other factors. The second model evaluated the mfERG IT and mfERG Amp along with the additional 9 risk factors to create the best multivariate predictive model using all the data available (labeled as multivariate model-model 2). All logistic regressions used an independent correlation structure with robust estimates for inference as previously noted.

Receiver operating characteristic (ROC) curves were constructed from probabilities of new edema development calculated from the models.[26] The data were then randomly divided into 5 subsets and a five-fold cross-validation procedure was used to validate each model's results. Each of the five subsets was used to validate a model created by combining the other four subsets of data. The validations were averaged to determine the generalized predictive properties of each model.[27, 28]

7.5 Results

7.5.1 Edema Development and Location

Edema developed in 16 of the 46 eyes (35%), 10 of the 23 patients (43%), and 83 of the 1610 retinal zones (5.2%). Of the patients who developed edema, 7 had type 2 diabetes and 3 had type 1 diabetes.

The edema tended to form in the temporal or central macula, qualifying as CSME and potentially threatening sight. Overall, 11 of the 16 eyes (69%) that developed edema qualified as clinically significant. Edema was found in the two zones just temporal and infero-temporal to the central fovea (Figure 2), in 10 out of the 16 (63%) eyes that developed edema.

Figure 2

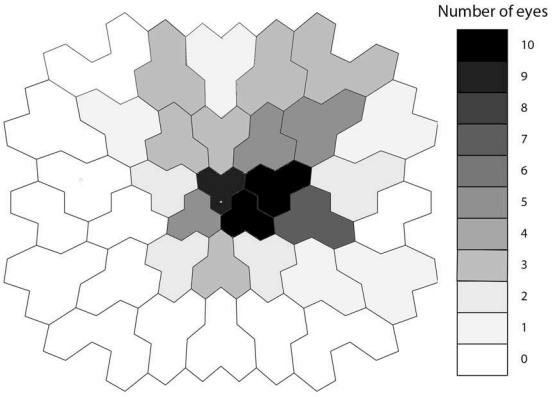

Figure 2: Retinal distribution of new edema development. Colors on the gray scale represent the number of eyes that developed edema in a particular zone when all eyes were displayed as left eyes. The darkest zones are the locations where the greatest number of patients developed new edema.

7.5.2 Relationship of Edema and Degree of Retinopathy

Edema development was found to be associated with the degree of retinopathy at the follow up visit, at the time the edema was clinically visible ($p < 0.0001$). However, degree of retinopathy at baseline was not predictive of future edema ($p = 0.19$). Given this result, we also examined change in retinopathy status between the two visits and its relationship to edema development. Although there was a trend toward worsening retinopathy being associated with edema development, in our sample it was not a significant association ($p = 0.06$). Eleven of the 46 eyes had retinopathy that worsened between the two study visits and about half (five) of these eyes developed edema. Two eyes had improvements in their retinopathy from baseline to the outcome visit. Neither of these eyes developed edema. The remaining 33 eyes had no changes in the overall amount of retinopathy as determined by clinical fundus grading. Of the unchanged eyes, 11 developed edema and 22 did not.

7.5.3 Univariate Analysis of Risk Factors

First we evaluated the individual predictive properties of each potential risk factor in univariate models. The mfERG Amp Z-score was found to be the most significant univariate factor for the prediction of edema. Other factors that were significant ($p<0.05$) were mfERG IT Z-score, and age (Table 2). This means that each of these factors could independently predict edema development. Thus, edema development was associated with delayed IT, decreased Amp, and older age. There were 5 baseline factors that were categorized as marginally predictive of edema with a p-value less than 0.2. These were degree of retinopathy, systolic blood pressure, type of diabetes, sex, and duration of diabetes. These factors were added first in the stepwise regression. The rest of the factors were not independently predictive, but were still evaluated for inclusion in the multivariate model discussed later.

Table 2: Significant univariate coefficients for the prediction of edema

Variable	Coefficient	p-Value	Odds Ratio
mfERG IT (Z-scores)	0.435	0.005	1.55 (1.14-2.09)
mfERG Amp (Z-scores)	-0.851	0.001	2.34 (1.41-3.90)
Age (years)	0.090	0.026	1.09 (1.01-1.18)

7.5.4 Location-Specific Prediction of Edema using only mfERG IT and mfERG Amp

Given that both mfERG measures were highly predictive in the univariate analysis, we were interested in how well the mfERG alone could predict future edema. First, the potential confounding of mfERG IT and Amp by the other risk factors was examined. No factors were found to confound in this model. Additionally, no interaction was found between mfERG IT and mfERG Amp. In the model (shown below), "p" is the probability of developing edema in a zone within one year. The model, which uses only mfERG IT Z-score and mfERG Amp Z-score, was highly significant for the prediction of edema.

log(p/1-p) =-3.79 + 0 .37 (IT Z-score) -0.88 (Amp Z-score).
(mfERG only model- model 1)

The coefficients here yield odds ratios that can be interpreted as approximate relative risks. For increasing mfERG IT, the odds ratio is 1.44 (95% CI: 1.05-2.11) and, for decreasing mfERG Amp, the odds ratio is 2.41 (95% CI: 1.30-3.86). This means, for example, that for every unit increase in mfERG IT Z-score the odds of developing edema increase by 44%, when the amplitude is held constant.

7.5.5 Cross-Validation

A five-fold cross-validation was used to estimate the validity and general accuracy of the mfERG only model. It yielded the 5 sets of coefficients (Table 3) whose average was 0.37 for mfERG IT Z-score, -0.88 for mfERG Amp Z-score, the same as the coefficients in the mfERG model before cross-validation. Each of these five validation models yielded an ROC curve, which had a range of sensitivities and specificities from 68-83% (Figure 3). The average accuracy of these ROC curves indicates that this mfERG only model has a cross-validated sensitivity and specificity of 72 %.

Table 3: 5-fold cross validation for mfERG only model.

5-fold model number	IT Z-score coefficient	Amplitude Z-score coefficient	Sensitivity %	Specificity %
1	0.406	-0.863	68	68
2	0.357	-0.873	74	74
3	0.371	-0.835	83	83
4	0.352	-0.883	68	68
5	0.376	-0.948	68	68
Average	**0.372 ± 0.02**	**-0.880 ± 0.04**	**72.2 ± 6.6**	**72.2 ± 6.6**

Figure 3

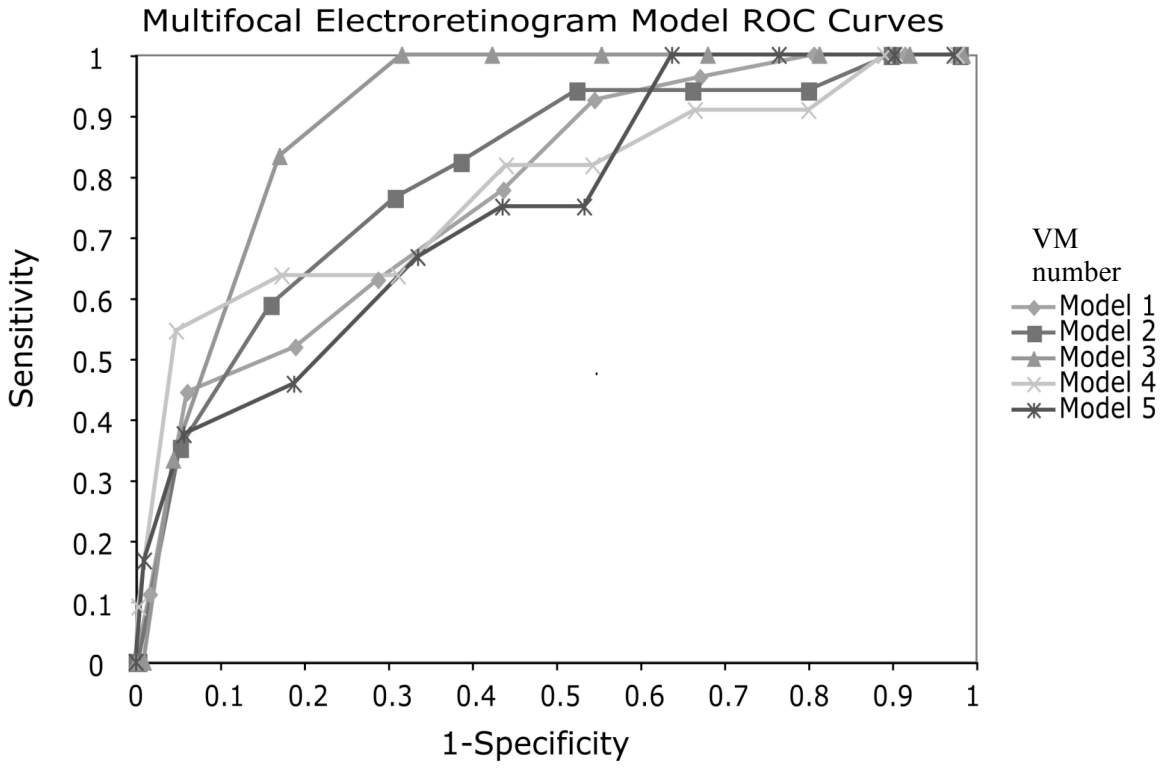

Figure 3: Five ROC curves from the five-fold cross-validation of mfERG only model (model 1). Each symbol represents a curve constructed from 1/5 of the data using coefficients modeled from the other 4/5 of the data. The validation model number (VM) matches with table 3.

7.5.6 Multivariate Model using mfERG and other Factors to Predict Local Edema

A stepwise forward regression was used to examine other measured factors to see whether they improved the model. Two additional factors, systolic blood pressure (SBP) and sex were found to be significant at a $p<0.05$ level and improved the model and its predictive abilities.
The selected multivariate model is:

log(p/1-p) =-5.39 + 0 .37 (IT Z-score) -0.87 (Amp Z-score) +0.017 (Systolic blood pressure) -1.93 (Sex)

(*multivariate model- model 2*)

In this model "p" is again the probability of developing edema in a given zone within 1 year. The coefficients give an odds ratio for mfERG IT Z-score of 1.44 (95% CI: 1.13-1.84), an odds ratio of 2.38 for decreasing mfERG Amp Z-score (95% CI: 1.55-4.48), an odds ratio for blood pressure of 1.02 (95% CI: 1.00-1.04) per mmHg change, and 6.89 for sex (95% CI: 2.32-20.09), all interpreted when holding all the other included factors constant. These can also be interpreted as approximate relative risks. In this model the coefficients for amplitude and sex are negative terms. This means that, as mfERG Amp decreases, the risk of edema increases. The negative sex term means that men were more than six times more likely to develop edema than woman in a given local retinal area.

7.5.7 Cross-Validation

A five-fold cross-validation was also used to estimate the validity and general accuracy of this model. There are 5 sets of coefficients (Table 4) whose average was 0.37 for mfERG IT, -0.87 for mfERG Amp, 0.017 for blood pressure, and -1.93 for sex. Each of these five models yielded an ROC curve, which had a range of sensitivities from 82-88% and a range of specificities from 65-84% (Figure 4). The average accuracy of these ROC curves indicates that the final model has a cross-validated sensitivity of 84.4% and a specificity of 75.8%.

Table 4: 5-fold cross validation for multivariate model

5-fold model number	IT Z-score coefficient	Amp Z-score coefficient	SBP coefficient	Sex coefficient	Sensitivity %	Specificity %
1	0.419	-0.837	0.017	-1.95	88	74
2	0.353	-0.873	0.018	-2.00	82	65
3	0.387	-0.837	0.017	-2.07	84	76
4	0.332	-0.896	0.018	-2.02	83	80
5	0.364	-0.914	0.013	-1.60	85	84
Average	**0.371 ± 0.03**	**-0.871 ± 0.03**	**0.017 ± 0.002**	**-1.93± 0.17**	**84.4 ± 2.1**	**75.8 ± 6.4**

Figure 4

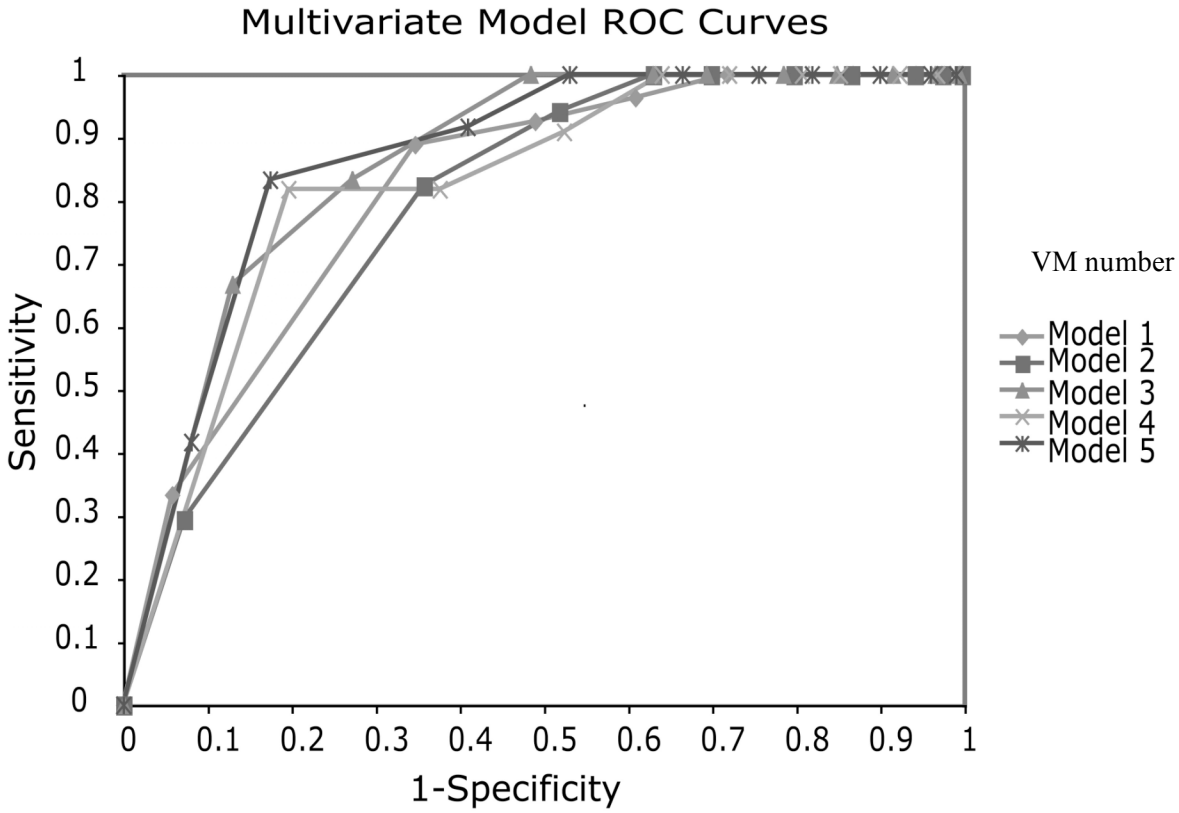

Figure 4: Five ROC curves from the 5-fold cross-validation of the selected multivariate predictive model (model 2). Each symbol represents a curve constructed from 1/5 of the data using coefficients modeled from the other 4/5 of the data. The validation model number (VM number) matches with the information in Table 4.

7.6 Discussion

We have created a multivariate model for the prediction of diabetic retinal edema onset in an at-risk patient group. This model shows that mfERG Amp, mfERG IT, systolic blood pressure, and sex are collectively predictive of edema onset at specific retinal locations within one year. The model has high sensitivity (84%) and specificity (76%).

Previously, we developed multivariate models to look at prediction of retinopathy in patients with diabetes, both with and without retinopathy. In those models we showed that the mfERG IT is highly predictive of new retinopathy in patients with early stage retinal complications.[18, 20-22] Most recently we reported that the mfERG IT has predictive capabilities for impending diabetic retinopathy, in eyes that have had no prior retinopathy.[22] In the present study we used the mfERG technique to successfully predict more serious vision-impacting edema onset in the retina. It is known from prior work that the mfERG implicit time is affected by previous retinopathy and presence of hard exudates in an eye,[13, 29] and that the mfERG is also able to differentiate between different kinds of retinopathy.[13, 30] In our study, most of the patients had abnormal mfERGs from their diabetes-induced retinal changes. More importantly our model was able to predict, with good sensitivity, the retinal areas about to undergo the more serious vision threatening retinal onset of edema.

With further analysis of the sensitivity and specificity of the multivariate model (model 2), we found that in the zones around the regions where the edema developed, the model produced a number of false positives (regions that were predicted to develop edema but did not). This indicates that the neural dysfunction seen in our study may extend beyond the region where the fundoscopic changes are seen. In fact, this difference between the area of neural dysfunction and the observed fundus changes decreases the specificity of the model (76%). This is in agreement with the work by Greenstein et al who also found that the mfERG changes extend beyond the areas where edema is present.[15] Consequently, it is plausible that the specificity could be considerably higher if we had averaged the mfERG responses of the eye and used that in this model rather than the 35 separate zones. However, such an approach sacrifices the ability to predict the specific retinal sites for the impending edema, a feature of our modeling results. Also, the resulting relatively small sample size would be less than ideal for such an analysis.

Our previous models have not included mfERG Amp Z-score as a predictive factor for diabetic change. We had not found it to be predictive of early retinal changes. This was probably because the measure is quite variable within the control population leading to insensitivity in prediction until more serious retinal changes are impending.[13, 19, 21, 31] However, given that previous work has shown that edema significantly changes mfERG amplitude,[15] we chose to evaluate it as part of this model. We found decreased amplitudes in zones that developed subsequent edema. Furthermore, decreased amplitudes had the most significant p-value ($p < 0.0001$) of the four predictive factors in the multivariate model and a very high odds ratio. As a point of caution regarding this statement, it is important to note that the odds ratios for measures in the model cannot be compared to each other since they are dependent on different scales. The odds ratio for blood pressure, for example, is per mmHg increase. More clinically meaningful differences in units of blood pressure (5-10 mmHg) would necessarily carry a much larger odds ratio but the significance of the prediction would remain the same.

Studies of the prediction and evaluation of sight-threatening retinopathy and macular edema note the importance of blood pressure control in reducing the risk of vision loss from diabetes. Improved blood pressure control reduces the risk of retinopathy and macular edema.[32, 33] Furthermore, elevated blood pressure has been shown to increase the risk for retinopathy progression.[34-36] The Wisconsin epidemiologic study of diabetic retinopathy (WESDR) study found that higher blood pressure at baseline, even in the absence of clinical hypertension, increased the risk of future edema.[37] Our study and model are in agreement with these studies and reveal higher blood pressure at baseline, regardless of the presence of hypertension, is an important risk factor for developing edema.

Our multivariate model also reveals that male sex is associated with an increased risk of local diabetic retinal edema. Ozawa et al,[38] in our lab, have found that there is a difference in the mfERG of diabetic males and females even before retinopathy develops, with females under age 50 having fewer neuroretinal defects than males of the same age. Several studies found that men have a higher risk for, or are more likely to have, diabetic retinopathy than women.[39-42] However, we could find no other studies in the literature showing a direct association between male sex and diabetic edema. It is worth noting though that studies evaluating edema and retinopathy frequently controlled for gender, raising the possibility that those authors considered sex to be an important confounder in their studies.[43, 44]

While retinal edema can occur at any stage of diabetic retinopathy, the severity of retinopathy increases the likelihood of edema and sight loss.[9, 10, 45] In our study, when looking at the levels of retinopathy in patients at the time the outcome measures were made, we also found that patients with more severe retinopathy were more likely to have edema. Based on the previous work, we targeted patients with moderate retinopathy to increase the likelihood that patients would develop edema in the follow up period. For the same reason, we selected patients with longer durations of diabetes (average duration of our patients was 16 ± 8.5 years). In effect, we truncated the range of durations of diabetes in our study population compared to our previous modeling studies. This selection of patients may be the reason that duration, as a potential risk factor, was not significant in our study. We also did not find retinopathy level at baseline to be statistically related to future edema despite other studies noting this trend.[46] Again, this may be due to our choice of patients with predominantly moderate retinopathy, and to our relatively small sample size.

Here we have predicted which local retinal regions were at the highest risk for new edema. Evaluating new edema development also gave us an opportunity to also examine which larger regions of the retina seemed to be most vulnerable to edema. We noted that most of the new edema occurred near the fovea and qualified as CSME. This is consistent with the ETDRS findings; patients with edema within 1 disc diameter of the macular center were more common there as well.[47] The WESDR study also looked at the incidence of macular edema in diabetic patients and found similar results.[46]

We noted a nasal-temporal asymmetry in the location of new edema, with most edema occurring in the temporal retina. However, with only 16 eyes developing new edema, this may be idiosyncratic to our study. Perhaps relevant is the finding of Hudson et al. who looked at blood flow in the macular region in patients with clinically significant edema and found that for patients with edema the blood flow temporally, but

not nasally, was slower than the blood flow in control patients.[48] So there may be a nasal-temporal asymmetry in edema development, but more studies with larger study groups are certainly needed to explore this.

In summary, mfERG Amp, mfERG IT, systolic blood pressure, and sex are, collectively, predictive of future sight-threatening edema in at-risk diabetic patients with retinopathy. Furthermore, with use of the mfERG, the predictions are specific to retinal locations. The usefulness of inclusion of blood pressure in the model is consistent with previous findings that that blood pressure is an important factor in the progression of diabetic eye disease.[33, 49] Our model also suggests that male sex is a risk factor for more severe changes in the eye beyond retinopathy. Our study establishes all these measures as candidates for selecting patients for targeted studies looking at prevention of edema.

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Chapter 8: Conclusions and Future Directions

8.1 Conclusions and Summary

Diabetes is the leading cause of blindness in working aged Americans.[1] When examining the reasons why this is the case, several become apparent. First, there is large population of diabetic patients in our country. There are 25.8 million people with diabetes in the United States according to the most recent figures. This is over 8% of the population, and the number is rapidly growing.[1] Second, diabetes is a difficult disease to manage. Patients with diabetes need constant vigilance with their diet and in monitoring their blood glucose control. There are many medications that must be balanced correctly in order to maintain good health and prevention of complications.[2-4] And third, the prevalence of blindness from diabetes is due, in part, to the fact that the treatments that are currently available for diabetic eye disease are aimed at the late stages of the disease when vision is already lost, or at risk of being lost, despite much research in this area.

There are currently no treatments for diabetic eye disease aimed at the earlier stages of retinopathy, even though clinically the damage can be detected early in the disease process. Furthermore, the treatments that are available, while effective, are invasive; laser surgeries and injections.[5, 6] Less invasive treatments, available earlier in the disease process are needed. This is a very important clinical and research goal.

There are many obstacles that stand in the way of developing this much needed preventative treatment. However, one thing is clear. When a preventative treatment is finally developed, there will be an ideal window for it to be administered. This window spans from the time that diabetes is diagnosed until before edema or proliferation develops, and there is a risk of vision loss. Thus, this window differs for every person but is typically years in length, especially in type 1 diabetes when the onset of the disease is clearly defined. However, the treatment window also contributes to the biggest obstacles. There are issues and concerns about treatments of diabetic eye disease at this point in the course of the disease, largely because most patients retain good vision during this early window. First, there is always concern about side effects from treatments in eyes with good vision, but as the treatment is intended to save future sight, this issue is comparably minimal. The bigger issue is that in diabetes the patient is often unaware of the changes going on in their eyes, making it difficult to detect, without repeated fundus examination, if a retina is changing from diabetes and how rapidly. Diabetes is a unique pathology in that a great deal of damage occurs to the retinal tissue before vision is affected. Also accompanying this problem is the issue that the onset of retinopathy and edema are rare events in the overall diabetic population. Clinical trials to test treatments aimed at these events therefore take very large numbers of patients, and lots of time and money.

The studies presented here, investigated the changes that are happening in the diabetic retina during this time between diagnosis and the development of macular edema. The overall goal was to use predictive models to gain more information about which local retinal regions are about to undergo important transformations to the next step of disease, either onset of retinopathy or macular edema. Prediction of diabetic eye disease will aid in overcoming some of the obstacles of clinical trials by identifying which regions are most at risk for progression. Knowing this information can aid in

patient care and also potentially in lowering the number of patients needed for a successful clinical trial aimed at these rare events.

The multifocal electroretinogram (mfERG) is a local test of neural retinal function. Changes to the mfERG have been shown to be associated with all stages of diabetes, and are predictive of impending eye disease in previous models created in our lab.[7-11] This makes the mfERG a candidate test for evaluating and predicting diabetic eye disease, both at its onset and in more serious vision impacting changes. The predictive models and studies in this thesis utilized the mfERG along with other diabetes health measures to evaluate the changes in the retina in diabetes at critical junctures.

This dissertation presented five related studies that evaluate diabetic retinopathy in adult patients with both type 1 and type 2 diabetes. Although predictive modeling of diabetic eye disease is certainly the main focus of this work, we have used the mfERG measures, retinal thickness measures, and other diabetes health indicators in both cross sectional and longitudinal studies to evaluate local changes in the diabetic retina.

Chapter 3 explored the reproducibility of the mfERG. This study was an important first step in this work because our group has two mfERG instruments, and using both instruments for data collection was essential to the success of the other projects. The literature did not provide a good solution for the best way to combine data from both of our VERIS instruments. We also wanted to examine the differences between the three different amplifier models that are used in our systems.

A group of 21 control patients were examined on the two mfERG instruments and a total of three amplifiers. We found that the raw data was not comparable between the two instruments, which gave similar amplitudes due to their calibrations, but very different implicit times. However, when the data was normalized with Z-scores, it could be easily compared between instruments and amplifiers. This work also confirmed that the mfERG implicit time (IT) is very stable with low variability over time in the control population but that amplitudes (Amp) are much more variable.[12]

Understanding the reproducibility of the instrumentation used in our studies is a very important component in gathering the very best data. It allowed us to be comfortable utilizing data collected on multiple instruments without adding variability to the measures, and in potentially comparing our data to the work of other laboratories. The information gained from this study aided the other studies presented in this thesis by allowing data to be complied over multiple mfERG instruments. It will also aid all future studies in our lab and others labs looking to do the same.

Chapter 4 created a multivariate model for the local prediction of the onset of diabetic retinopathy. This longitudinal study built on the past work of the lab, and was the logical next step to our previous studies. Ng et al. and Han et al. created multivariate models to predict new retinopathy in patients with and without prior retinopathy over a 1, 2 and 3 year period.[13, 14] However, in those past models there were not enough patients who had no retinopathy at baseline and then converted to retinopathy, to create a model for the onset of retinopathy. So, here we created a model to predict retinopathy in a group of patients who had no previous retinopathy, in a larger sample size of patients.

We followed a group of 41 diabetic patients with no retinopathy for several years, and every year measured their diabetes health indicators, and evaluated the health of their

retinas. We were then able to determine which patients and retinal locations developed retinopathy for the first time. We looked back to examine differences between those locations and the locations that did not develop retinopathy in the previous year, using a multivariate predictive model. This model found that the mfERG IT is predictive of the local onset of retinopathy with an 80% sensitivity and 74% specificity when taking the type of diabetes into account. Type of diabetes was an important confounder in this study. Patients with type 1 diabetes were younger with longer durations of retinopathy than the patients with type 2 diabetes. They developed more retinopathy but had mfERG results that were less delayed. Given that retinopathy was a very rare event in this study, with only 3% of retinal zones developing retinopathy, a model with such good sensitivity and specificity indicates that the mfERG is indeed very sensitive even to early retinal changes. [15]

Chapter 5 investigated the relationship between retinal thickness, blood pressure, and blood glucose in type 2 diabetic patients with and without retinopathy. This cross sectional study was performed in order to gain more information about factors that may alter retinal thickness in these diabetic patient groups. Since increased retinal thickness accompanies edema, these retinal thickness measures were candidate measures to be examined in our other studies evaluating and predicting edema, as a risk factor and an outcome measure. We wanted to understand how these measures could be altered or confounded by other diabetes health measures and co-morbidities outside of edema.

We found that in patients with retinopathy there is a significant positive linear relationship between blood pressure and retinal thickness. This relationship was most significant when examining the association with diastolic blood pressure. Systolic blood pressure also displayed this association but the significance was not as strong. Thus as diastolic blood pressure increased, so did retinal thickness, even to the point where some of the patients in that study even had retinal thicknesses that approached subclinical edema. This relationship does not exist in patients without retinopathy or without diabetes.

Furthermore, in this chapter we also investigated the possible cause behind this association. There were two candidate hypotheses for this association between blood pressure and retinal thickness. They were: 1) The retinal vessels are expanding due to higher blood pressure and taking up more space 2) The higher blood pressure is pushing more fluid into the retinal layers, due to alterations in the blood retinal barrier, causing the increased thickness. We examined the retinal blood vessels and found it is not changes to the caliber of the blood vessels that is responsible for the increased thickness. This decreases the likelihood that hypothesis one is the reason for the association. Only the arterioles of the control patients had changes in caliber that were associated with blood pressure, which was an expected and well-documented effect. The patients with diabetes did not have caliber changes to arteriole or venules that were associated with blood pressure. This indicates that hypothesis two is more likely, that the higher blood pressure is pushing more fluid into the retina causing the retinal thickening. There was no relationship between blood glucose and retinal thickness in any patient group in our study.

This study highlights blood pressure as an important confounder of retinal thickness measures in patients with retinopathy. It also indicates that there are not retinal

thickness alterations from blood pressure in patients without retinopathy or in control patients. We also confirmed that blood glucose levels are not associated with retinal thickness in the diabetic retina in any patient group.

Chapter 6 evaluated a small group of 22 patients with macular edema in a cross sectional study. While the literature shows relationships between edema and mfERG changes,[10, 16] we wanted to verify these associations in our own data set, as well as examine local correlations between retinal thickness, edema, and mfERG measures, which had not been reported in other studies.

We measured retinal structure and function in 37 separate local macular regions and found that retinal edema is associated with delayed mfERG IT, decreased mfERG Amp, type 2 diabetes, increased retinal thickness, and decreased visual acuity and contrast sensitivity. Many of these associations were also present when examining increased retinal thickness but only included mfERG IT, edema on a fundus photo, and decreased acuity and contrast sensitivity, and they were not as strongly significant as the associations with the photos. Lastly we evaluated which factors were associated with decreased visual acuity in these patients. The patients in this study represented a unique group of diabetics with macular edema whom, for the most part, retained good visual acuity on our ETDRS chart. On average their acuity was 95 letters. We found several factors were associated with vision loss even within this group with good acuity. These included the mfERG measures. This association between local mfERG changes particularly the mfERG IT, and vision loss in edema has not been reported previously, and this finding adds a new aspect to the use of the mfERG for the prediction and treatment of edema.

This study was able to confirm in our data set findings from the literature about the neuronal health in edema. It also adds to the literature by examining local associations between the mfERG, edema, and vision loss.

Chapter 7 is the second longitudinal study included in this thesis. This study created a model to predict changes in the retina that can cause vision loss, namely retinal edema. This study built on the previous success we have had in creating predictive models using the mfERG in diabetic eye disease. It followed a new cohort of patients who had nonproliferative diabetic retinopathy and were at risk for edema.

In this study a group of 23 patients with nonproliferative diabetic retinopathy were followed semiannually for up to 4 years. During their visits we measured diabetes health indicators and examined the health of the fundus. The patients in this study were targeted to have long durations of diabetes and poor control of their blood glucose. By following this at-risk group over time, we were able to observe which patients and local retinal regions developed diabetic edema and which did not. First we examined the edema that developed and found that most of the new edema was clinically significant edema by the ETDRS standards,[5] which could potentially threaten sight. This finding added importance to the predictive models, as they had the potential to have an impact on preventing sight loss.

We followed a similar strategy to the model in chapter 4. We used the health measures from the year before the edema developed to create predictive models for the local onset of retinal edema in these patients. First we created a model using only the

measures from the mfERG since they were highly predictive in a univariate analysis. We found that mfERG IT and mfERG Amp together could predict local diabetic edema with a sensitivity and specificity of 72%. We also created a multivariate model, which utilized the mfERG measures along with systolic blood pressure and sex to improve our prediction of local edema within one year. We found that our multivariate model had good sensitivity (84%) and specificity (76%) for the prediction of local diabetic edema.

In this study we used diabetes health measures and predicted edema that could cause sight loss with good sensitivity and specificity. Such a multivariate model could be an important first step in treatments aimed before edema develops.

8.2 Future Directions

There are several studies that represent the logical next steps to the work outlined in this dissertation. Here I will outline the follow up studies that I feel should be considered.

The first follow up study is a more in depth exploration into the neural function differences between type 1 and type 2 diabetes in patients with and without diabetic retinopathy. The model in chapter 4, predicting the onset of retinopathy in patients with no retinopathy, highlighted differences in the electrophysiological function between the two types of diabetes. Type 1 diabetics had mfERGs that appeared much healthier with less delays than their type 2 counterparts. However, this model only looked at patients without retinopathy, so differences in the electrophysiological function between the two types of diabetes in patients with various levels of retinopathy would also be useful to researchers in our area. Such a study would not be simple to conduct though, as it would be really important to control for the other confounders that affect these measures such as duration of diabetes and the age of the patients. These tend to be different between type 1 and type 2 patients with no retinopathy and could also differ in patients with retinopathy. A large sample of both types of diabetics would be also necessary for a successful study. As type 1 patients only compose 5-10% of all patients with diabetes, finding enough type 1 patients with varying levels of retinopathy could be challenging.

A second follow up study that should be considered is a more complete look at amplitude changes in patients with diabetic retinopathy, leading up to edema. Specifically a study to map out a time course of these amplitude changes would be interesting. It is clear from the work presented here that amplitude reduction is a risk factor for edema, and also that amplitudes are reduced in edema itself. We also know that amplitudes are highly variable and tend not be reduced in early diabetic changes. However there is a time between early diabetes and edema risk, where the amplitudes must be rapidly changing, that needs to be examined more carefully. The associations that amplitude has with nonproliferative diabetic retinopathy at various stages, and at what stage the amplitude becomes affected in diabetes, is still unclear after the work presented here.

Several of the studies in this thesis identify blood pressure as an important confounder and/or cofactor in the prediction and identification of diabetic edema. However there are still many unanswered questions about what roll blood pressure plays as a cofactor in the diabetic retina. It is not clear at what stage blood pressure becomes an important factor or to what extent blood pressure alone affects the mfERG measures. Follow up studies to evaluate subjects with diabetes with and without hypertension and

subjects with hypertension with and without diabetes, would be useful to evaluate the different effects of the two diseases. This could also prove challenging as the two diseases are co-morbid and finding enough subjects in these four groups many be difficult. The literature only has one study that has looked at changes to the mfERG in hypertension[17] and there has never been an attempt to evaluate the two separately and together. If blood pressure changes appear to play an additive role in changes in neural function, other co-morbities to diabetes such as high cholesterol and inflammation should also be examined for their effects.

Lastly, the most logical next step to the work presented here is to repeat the predictive model of local edema with more patients. While 23 patients provided significant results, it is a very small sample size for such an important clinical research problem. Furthermore, our study also had a relatively high rate of data that needed to be excluded compared to our sample size (3 of 26 patients). A larger sample size of patients would aid in reducing any bias in the data that is introduced from our exclusion criterion. More patients would also allow more factors to be measured and assessed for their predictive properties. Some other candidate factors which could be included in future models include other diabetes health measures such as inflammatory markers or cholesterol measures, and other measures of neural function such as electrooculograms or visual fields.

In conjunction, another group of patients at-risk for edema should be followed and used to validate this model. The 5-fold cross-validation gives good information about the accuracy of the model, but an independent validation would be the best way to assess how accurate the model is for clinical uses. As the original group of patients in this study was small, there were no additional patients available for an independent validation. In the future, when more patients have been assessed, this would be a good study to consider.

Because the group of recruited patients for this study is very specific, with long durations of diabetes, poor glucose control and nonproliferative diabetic retinopathy, a multi-center trial to increase the size of the model population and validate the model would be an ideal way to include a large number of patients. Based on the work in chapter 3, a multi-center trial should be feasible as long as all the sites have a large number of control patients to normalize the data and all the sites use the same inclusion and exclusion criterion and data processing.

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