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Novel Measures Of Retinal Health And Their Inter- relationships In Early Diabetes

Author
Dhamdhere, Kavita P.

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Novel Measures Of Retinal Health
And Their Inter- relationships In Early Diabetes

By

Kavita Prashant Dhamdhere

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Committee in Charge:

Professor Anthony J. Adams, Chair
Professor Gunilla Haegerstrom-Portnoy
Professor Nicholas P. Jewell

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Abstract

Novel Measures Of Retinal Health In Early Diabetes

And Their Inter- relationships

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Over last three decades, diabetes emerged as a leading cause of vision loss. Diabetic retinopathy and diabetic macular edema (DME) are common retinal complications in patients with diabetes. These end-organ complications have a sudden, and debilitating impact on vision eventually leading to blindness. The present standard of care for management and treatments of these complications are inherently destructive, associated with unavoidable side effects, and not universally effective in reversal of visual loss. They are expensive and invasive late stage treatments that are administered once structural retinal damage is clinically obvious. Early detection and better understanding of early stages of diabetic retinopathy is crucial to delay the onset and to slow progression of diabetic retinopathy.

Predictors of incipient retinopathy are needed for early diagnosis and for development of effective preventatives and curatives to save sight and to replace invasive treatments. This depends on the discovery and use of sensitive testing measures. In this thesis we use one such technique mfERG; it can be used to evaluate the health of retinal neurons at 103 retinal locations across the central 45 degrees of retina and is a very sensitive tool for detecting neural changes that are predictive of onset and progression diabetic retinopathy and edema.

Abnormalities revealed by the mfERG correlate well with the degree of clinical presentation of vascular retinopathy. Understanding the relationships between retinal neural changes and other retinal health indicator changes in diabetes may prove helpful to broaden knowledge about structural and functional changes in the retina that are exceptionally important for any new advances in curative as well preventative medicines and to define novel sensitive endpoints for clinical trials. In this dissertation I present four studies focused respectively on, revealing relationships between the mfERG measures of neural function and retinal thickness (Chapter 1), revealing relationships between mfERG measures of neural function and letter contrast sensitivity (Chapter 2), revealing early microvascular and photoreceptor changes in diabetes (Chapter 3) and finally, understanding how retinal health indicators (structural and functional) change in the patients with DME- a potentially sight threatening retinal vascular event.

The first study presented as the third chapter in this dissertation, tests if changes in retinal thickness and if mfERGs have any spatial correspondence in patients that are at

an early stage of diabetes and do not have any retinopathy. Furthermore we are interested to see if there is any spatial agreement in the occurrence of abnormalities in both measures. It would be a very important finding if the study could shed some light on whether these two techniques could be used as surrogates of each other.

After testing the relationships of retinal neural function with retinal structure in the first study, I studied the possible relationships of retinal neural function with vision function assessed using letter contrast sensitivity in diabetes. The relationship between structure and function, a theme of the thesis, is also of interest in sensory function like letter contrast sensitivity. I examine whether retinopathy status influences the relationship between neural function and vision function. This study, as a first step, examines whether letter contrast sensitivity is different between participants with diabetes and the controls and whether the type, duration and control of diabetes and the presence of background retinopathy impacts the vision function measured here.

In the third study I examine, using adaptive optics scanning laser ophthalmoscopy (AOSLO), parafoveal cone photoreceptors and the retinal parafoveal capillary network in adult patients with type 2 diabetes and no retinopathy. Here again the rationale is to look at detailed and novel; structural aspects of the diabetic retina as an initial step in relating to function. Specifically, the focus of the study is to examine whether the cone photoreceptor spacing and retinal parafoveal capillary network are altered prior to the onset of vascular diabetic retinopathy. The study aimed to test whether AOSLO is a viable method for detection and characterization of microscopic signs of diabetic retinal damage in cone photoreceptors and capillaries in the parafovea before it is reflected in clinical examination.

Finally the fourth study is a longitudinal study to examine the vision, neuro-retinal function, retinal thickness, diabetes control and blood pressure of the subjects who are at risk for developing DME. Then the study proceeds to examine the natural history of the neural and vision function by tracking a group of participants beyond the onset of their DME; this is the main purpose in this study.

Collectively, across the 4 studies, I found the mfERG measures to be sensitive to diabetic changes in the function of neural retina at all stages of diabetic eye disease. However, there was no clear relationship between mfERG and other retinal health measures in the absence of clinical signs of retinopathy. AOSLO measures revealed clinically non-evident alterations in cone photoreceptor spacing and capillaries. The sensitive nature of the mfERG neural function measure and AOSLO structural assessment could eventually be used to help clinicians and researchers to develop the tools and means to preserve vision, in response to the burgeoning epidemic of blindness due to diabetes.

Table of Contents

List of Tables

List of Figures

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The material in the dissertation is based on the following a series of publications and conference presentations. The dissertation author is the primary investigator or co-investigator and thanks co-authors for their contributions, described in more detail at the end of each chapter. The publications and presentations are listed below, listed alongside the most relevant chapter of the dissertation.

Refereed Journal Publications

Chapter 3	**Kavita P. Dhamdhere**, Marcus A. Bearse, Jr., Wendy W. Harrison, Shirin Barez, Marilyn E. Schneck, Anthony J. Adams. Associations Between Local Retinal Thickness and Function in Early Diabetes. Invest Opthalmol Vis Sci. 2012
Chapter 5	Johnny Tam, **Kavita P. Dhamdhere**, Pavan Tiruveedhula, Silvestre Manzanera, Shirin Barez, Marcus A. Bearse, Jr., Anthony J. Adams, and Austin Roorda, "Disruption of the Retinal Parafoveal Capillary Network in Type 2 Diabetes Prior to the Onset of Diabetic Retinopathy," Investigative Ophthalmology and Visual Science 52(12): 9257-9266, 2011.
Chapter 5	Johnny Tam, **Kavita P. Dhamdhere**, Pavan Tiruveedhula, Brandon J. Lujan, Robert N. Johnson, Marcus A. Bearse, Jr., Anthony J. Adams, and Austin Roorda. Subclinical Capillary Changes in Non Proliferative Diabetic Retinopathy. OVS 2011

Conference Abstracts and Presentations

Chapter 6	**Kavita Dhamdhere**, Wendy Harrison, Marcus Bearse, Marilyn Schneck, Shirin Barez, Anthony Adams Changes in neuro-retinal and vision function after occurrence of diabetic macular edema: A pilot study AAO 2012 E-abstract SA-30369
Chapter 4	**Kavita P. Dhamdhere**, Marcus A. Bearse, Jr., Brian E. Wolff, Wendy W. Harrison, Maria Cardenas, Shirin Barez, Marilyn E. Schneck, Anthony J. Adams. Associations Between Contrast Sensitivity And Multifocal Electroretinograms In Type2 Diabetes. Invest Opthalmol Vis Sci. 2011; 52: ARVO E-abstract 1271/A21
Chapter 4	**Kavita P. Dhamdhere**, Marcus A. Bearse, Jr., Wendy W. Harrison, Shirin Barez, Marilyn E. Schneck, Anthony J. Adams. Contrast Sensitivity And Multifocal Electroretinograms Associations In Adult Patients With Diabetes. AAO 2011 E-abstract 115573
Chapter 3	**Kavita P. Dhamdhere**, Marcus A Bearse Jr, Wendy W Harrison, Kevin Bronson-Castain, Shirin Barez, Marilyn E Schneck, Anthony J Adams Local Associations Between Retinal Function And Thickness Changes In Diabetes Without Retinopathy. AAO 2010 E-abstract 105891

Chapter 1: Introduction and Literature Review

1.1 Diabetes

1.1.1 Overview and problem statement

The purpose of this chapter is to emphasize the magnitude and impact of diabetes, its implications on the global health, its financial burden and to review the work done in this field as it relates to the chapters covered in this thesis. Over the past three decades, the number of people with diabetes mellitus has more than doubled globally, making it one of the most important public health challenges to all nations. From 1995 to 2010, the prevalence of diabetes in the US increased from 4.5 to 26.4 per 1000 people, a six-fold increase and it still continues to increase (Fig. 1.1).

Figure 1.1

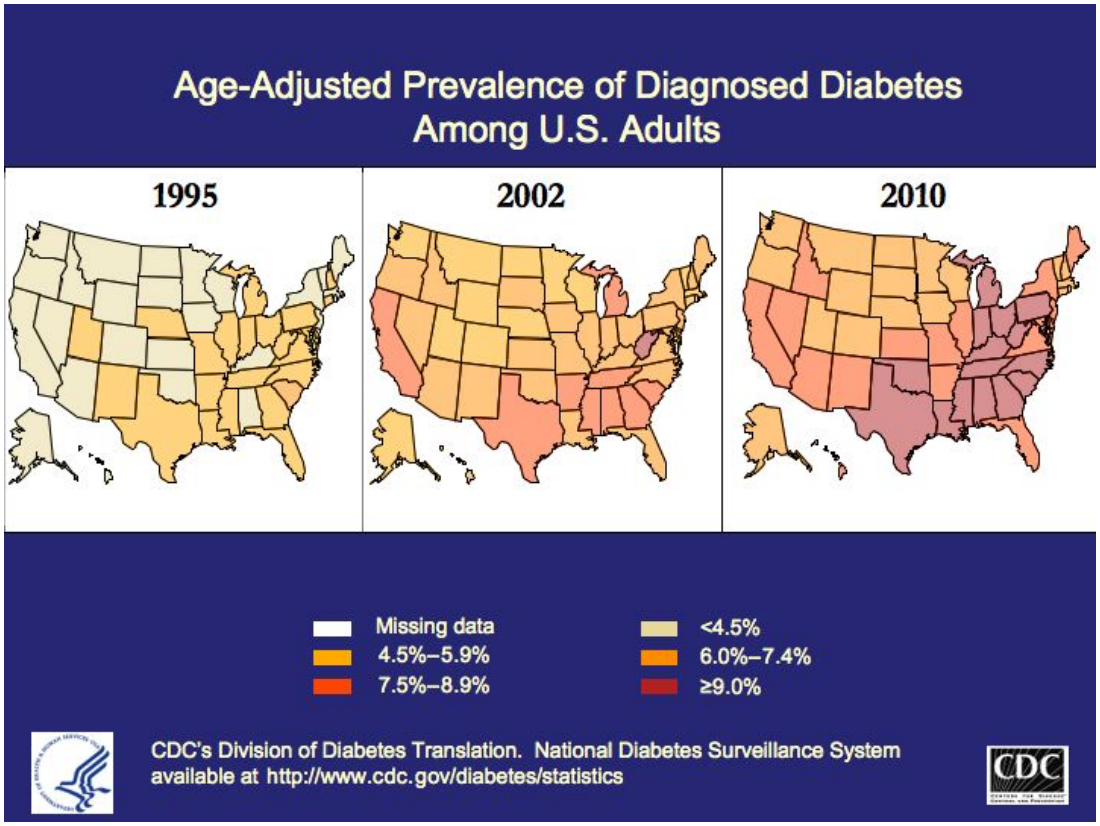

Figure 1.1: Diagrammatic representation of dramatic rise in the Diabetes prevalence in United States since 1995: CDC's Division of Diabetes Translation: http://www.cdc.gov/diabetes/statistics.

The incidence of diabetes mellitus has risen dramatically in the United States since 1940.[1] According to American Diabetes association in 2011, 25.8 million children and adults in the United States, i.e 8.3% of the population, have diabetes. An additional 57 million people are pre-diabetic and are high risk to become diabetic if they continue

their life style.[2] In 2007; the U.S. wide economic cost of diabetes was estimated to be $174 billion.[2] Health care and medical expenditures related to diabetes totaled $116 billion. People with diabetes had medical expenses that were 2.3 times higher than those without diabetes. Diabetes is the fifth leading cause of death in United States.[3] In 2007, diabetes accounted for 15 million work days of absence, 120 million work days with reduced performance, 6 million reduced productivity days for those not in the workforce, and an additional 107 million work days lost due to unemployment disability attributed to diabetes. In summary diabetes not only affects the people who have it but it as well has huge impact on overall health, economy and the productivity.[4]

Diabetes-related morbidity and mortality are rooted in its multi systemic nature. Diabetes of long duration affects almost every tissue in the body. Chronic complications of diabetes include accelerated atherosclerosis and its associated vascular disease processes of chronic heart disease, stroke, peripheral vasculopathies and neuropathies, renal impairment and failure and ocular diseases leading to vision loss. The studies in this thesis are centered on the retinal complications of diabetes and it is important to understand diabetes as a multisystemic disease to get a better understanding of its end organ damage.

1.1.2 Pathophysiology of Diabetes

Diabetes mellitus (DM) is a group of clinically heterogeneous disorders with glucose intolerance in common. It is a syndrome characterized by chronic hyperglycemia and other disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Diabetes has probably been known to medical science longer than any other ailment, yet, in many respects it is poorly understood. The pathophysiology of diabetes is related to the hormone insulin, which is secreted by the beta cells of the pancreas. Understanding the physiology of insulin is extremely important for understanding the pathophysiology of diabetes. The etiology behind insulin failure plays an important role in determining the type of diabetes, especially the major types, Type 1 and Type 2 diabetes. It is important to get an overview about the pathophysiology of diabetes to understand the basic differences in the nature of Type1 and Type2 diabetes that affect the ocular health differently.

In a healthy person, insulin is produced in response to increases in the level of glucose in the bloodstream, and its major role is to control glucose concentration in the blood. Insulin allows the body's cells and tissues to use glucose as a main energy source. Also, this hormone is responsible for conversion of glucose to glycogen for storage in the muscles and liver cells. This way, sugar level is maintained at a stable amount.

In a diabetic person, there is abnormal metabolism of the insulin hormone. The cause for this malfunction differs according to the type of diabetes. Whatever the cause, the body cells and tissues do not make use of glucose from the blood, resulting in elevated blood glucose; a typical symptom of diabetes called hyperglycemia. This condition is also exacerbated by the conversion of stored glycogen to glucose, i.e., increased hepatic glucose production. Over a period of time, this hyperglycemia leads to severe complications affecting almost all the systems in the body, including the eye.

Most of the pathological conditions of diabetes mellitus can be attributed to one of the following three major effects of lack of insulin 's action

(1) Decreased utilization of glucose by the body's cells, with a resultant increase in blood glucose concentration
(2) Markedly increased mobilization of fats from fat storage areas causing deposition of lipids in the vascular walls due to abnormal fat metabolism and resulting atherosclerosis and abnormal breakdown of fats leading to the formation of ketone bodies
(3) Depletion of protein in the tissues of the body.

1.1.3 Classification of Diabetes Based on Etiology

In 1997, the American Diabetes Association revised the nomenclature for the major types of diabetes. It is important to understand types of diabetes as the previous studies have shown that different types of diabetes have a very different disease course, pathogenesis and they affect the retina differently. Some of the studies included in this thesis will shed some light on these differences.

1.1.3.1 Type 1 diabetes

Type 1 diabetes was previously known as IDDM (insulin dependent diabetes mellitus) or juvenile-onset diabetes. About 5-10% of patients with diabetes have type 1 diabetes.[4] It affects 3 in 1000 children and its incidence is increasing worldwide both in low and high prevalence populations.[5] It is primarily a disease of the young given its peak incidence at the age of 10 to 12 years for girls and 12 to 14 years for boys; however, the disease can occur at any age, but most patients are diagnosed before age 20. In Type 1 diabetes, the pancreas cannot synthesize the amount of insulin required by the body. The pathophysiology of Type 1 diabetes mellitus suggests that it is an autoimmune disease, wherein the body's own immune system generates secretion of substances that attack the beta cells of the pancreas. Consequently, the pancreas secretes little or no insulin. This lack of insulin (natural or artificial) leads to fat burning and eventually to life threatening ketoacidosis. The onset of type 1 diabetes is typically sudden with symptoms such as increased urination, thirst, and fatigue.[2]

1.1.3.2 Type 2 diabetes

Type 2 diabetes mellitus is a heterogeneous disorder characterized by peripheral insulin resistance, impaired regulation of hepatic glucose production, and declining pancreatic β-cell function, eventually leading to β -cell failure.[6,7] β-cell dysfunction is initially characterized by impairment in insulin secretion and may come before the onset of glucose intolerance.[8]

Initiation of the insulin response depends upon the trans-membranous transport of glucose and coupling of glucose to the glucose sensor. The glucose/glucose sensor complex then induces an increase in glucokinase by stabilizing the protein and impairing its degradation. The induction of glucokinase serves as the first step in linking intermediary metabolism with the insulin secretory apparatus. Glucose transport in β -cells of type 2 diabetes patients appears to be greatly reduced.[9,10] There could be defective glucose potentiation and a decreased conversion of proinsulin to insulin.[11,12] Autoimmune destruction of pancreatic β-cells may be a factor in type 2 diabetic patients and has been termed the syndrome of latent autoimmune diabetes in adults.[13-15]

Chronic hyperinsulinemia inhibits both insulin secretion[16] and action,[17] and hyperglycemia can impair both the insulin secretory response to glucose[18] as well as cellular insulin sensitivity.[19] Prospective studies have demonstrated the presence of either insulin deficiency or insulin resistance before the onset of type 2 diabetes.[20] Two studies have reported the presence of insulin resistance in nondiabetic relatives of diabetic patients at a time when their glucose tolerance was still normal.[21]

The ability of insulin to suppress hepatic glucose production both in the fasting state and postprandially is impaired, and glucose production increases as type 2 diabetes progresses. Hepatic insulin resistance is characterized by a marked decrease in glucokinase activity and a catalytic increased conversion of substrates to glucose despite the presence of insulin.[22] Thus, the liver in type 2 diabetes is programmed to both overproduce and underuse glucose. The elevated free fatty acid levels found in type 2 diabetes may also play a role in increased hepatic glucose production.[23] In addition, recent evidence suggests an important role for the kidney in glucose production via gluconeogenesis, which is unrestrained in the presence of type 2 diabetes.[24]

1.1.3.3 Gestational diabetes

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy.[25] GDM is the most common medical complication and metabolic disorder of pregnancy, occurring in 1–14% of patients depending on the population described and the criteria used for diagnosis.[26] Early in pregnancy, maternal estrogen and progesterone increase and promote pancreatic ß-cell hyperplasia and increased insulin release.[27] Increases in peripheral glucose utilization and glycogen storage with a concomitant reduction in hepatic glucose production result in lower maternal fasting glucose levels.[28] As pregnancy progresses, increased levels of human chorionic sommatomammotropin (hCS), cortisol, prolactin, progesterone, and estrogen lead to insulin resistance in peripheral tissues. The mechanism of insulin resistance is likely a postreceptoral defect, since normal insulin binding by insulin-sensitive cells has been demonstrated.[29] The pancreas releases 1.5–2.5 times more insulin in order to respond to the resultant increase in insulin resistance.[30]

1.1.3.4 Other specific types of diabetes

Maturity-onset diabetes of the young (MODY) is a clinically heterogeneous group of disorders characterized by nonketotic diabetes mellitus, an autosomal dominant mode of inheritance, an onset usually before the age of 25 years and frequently in childhood or adolescence, and a primary defect in the function of the beta cells of the pancreas. MODY can result from certain gene mutations. All these genes are expressed in beta cells, and mutation of any of them leads to beta-cell dysfunction and diabetes mellitus. These genes are also expressed in other tissues, and abnormalities in liver and kidney function may also be evident in some forms of MODY. Factors that affect insulin sensitivity, such as infection, puberty, pregnancy, and (in rare cases) obesity, may trigger the onset of diabetes and increase the severity of hyperglycemia in patients with MODY, but otherwise, nongenetic factors have no important role in the development of this disorder.

Diabetes can occur due to genetic defects in insulin action. Insulin exhibits impaired receptor binding because of mutation in the insulin gene. Mutation of the insulin receptor is often associated with acanthosis nigricans (thickening and discoloration of skin) and some forms of polycystic ovarian syndrome.

Damage of the exocrine pancreas can lead to diabetes. It takes extensive damage to pancreas for diabetes to occur. This damage includes trauma, infection, chronic necrotizing pancreatitis and pancreatic carcinoma, cystic fibrosis and hemochromatosis

Endocrinopathies like acromegaly, Cushing's syndrome, glucagonoma and pheochromocytoma caused by excess secretion of hormones which antagonize insulin including growth hormone, cortisol, glucagon and epinephrine.

Many drugs may impair insulin resistance or insulin secretion leading to diabetes in predisposed individuals. Major drugs include synthetic glucocorticoids, cyclosporin A, nicotinic acid, interferon, pentamidine, occasionally thiazide diuretics

Infections like congenital rubella is the most common virus implicated in the development of diabetes Coxsackievirus B, adenovirus, mumps and cytomegalovirus have all been implicated in inducing certain cases of the disease**.**

1.1.4 Diagnosis

The diagnosis of diabetes is based on one of four abnormalities: hemoglobin A1C, fasting plasma glucose, random elevated glucose with symptoms, or abnormal oral glucose tolerance test. Patients with impaired fasting glucose and/or impaired glucose tolerance are referred to as having increased risk for diabetes. People over age 45 should be tested for prediabetes or diabetes. If the first blood glucose test is normal, they should be re-tested every three years. People under age 45 should consider getting tested for prediabetes or diabetes if they have a body mass index of greater than or equal to 25 and have one or more of the risk factors.

A diagnosis of diabetes is made when fasting plasma glucose of greater than or equal to 126 mg/dL or random plasma glucose of greater than or equal to 200 mg/dL or oral glucose tolerance test (OGTT) value of greater than or equal to 200 mg/dL. The OGTT is obtained 2 hours after a drink containing glucose has been consumed, which occurs after fasting for at least 8 hours with the symptoms of diabetes or A1C greater than or equal to 6.5 percent. For HbA1c measures, pre-diabetes is the range from 5.7%-6.4%.

The date of diagnosis is usually very precise for type 1 diabetes, but type 2 diabetes is often diagnosed serendipitously, and 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.

1.1.5 Treatment of Diabetes

Treatment of diabetes comprises diet therapy, exercise, insulin, and noninsulin agents, including oral medications and the non-insulin injectable drug. Every patient with diabetes needs dietary recommendations, explained by an expert. Diet control and weight loss may be sufficient to control type 2 diabetes in many patients if used early in the disease process.[31]

Insulin therapy is required for all patients with type 1 diabetes and for those patients whose type 2 diabetes is not adequately controlled or is unresponsive to diet and oral medications. The goal of therapy is to maintain normal or near-normal blood glucose levels throughout the day.[32] There are several different types of insulin. Sulfonylurea insulin stimulates the pancreatic islets' secretion of insulin, reduces hepatic glucose production and increase in the number of insulin receptors. The nonsulfonylurea insulin secretagogues repaglinide and nateglinide bind to a specific site on the sulfonylurea receptor and increase insulin secretion, although they are short-acting agents. Biguanides, such as metformin, block hepatic glucose production. Glucosidase inhibitors, such as acarbose and miglitol, block starch, sucrose, and maltose absorption. Thiazolidinediones, such as pioglitazone and rosiglitazone, decrease insulin resistance by enhancing insulin-mediated glucose disposal by muscle. Because of idiosyncratic liver damage and liver failure, the Food and Drug Administration (FDA) removed one of the thiazolidinediones, troglitazone, from clinical use in the United States in 2000.[33]

Among the incretinmimetic agents, sitagliptin is a dipeptidy peptidase-IV inhibitor that blocks the inactivation of native glucagon-like peptide. Another incretinmimetic, exenatide, administered by subcutaneous injection twice daily, stimulates insulin secretion, suppresses hyperglucagonemia, delays gastric emptying, and depresses appetite.

The use of combination oral therapies and oral therapies combined with insulin is common and increasing. A combination approach offers the patient the potential benefit from the synergistic actions of different medications while reducing adverse effects. Fixed-dose combinations are now emerging for treatment of type 2 diabetes. Also available are insulin preparations such as the basal insulin glargine and a rapid-acting insulin, insulin aspart. These advances enable the initiation of more effective basal bolus insulin therapy, which can result in better glycemic control.

1.1.6 Complications of diabetes

Diabetes can cause a wide range of health complications, affecting almost every part of the body. Most of these diabetic complications are brought on by the long-term effects of high blood sugar, and can be prevented, minimized or at least delayed by proper control of the patient’s blood glucose levels. However, many of these complications can develop and become quite severe before the patient even realizes them. The complications could be acute or chronic. This study is focused on the retinal complications of diabetes. However it is important to get a complete picture of diabetes damage as it is hard to overlook and not take into account the damage to other systems.

1.1.6.1 Risk Factors

All the clinical forms of diabetes are characterized by hyperglycemia and the risk of specific complications. There is considerable evidence from animal models that demonstrate a causal relation between hyperglycemia and complications. In humans, a durable relation between the level of glucose control and the prevalence and incidence of retinopathy supports the glucose hypothesis.[34] However, some research challenges the theory of hyperglycemia as the cause of diabetic complications. The fact that 40% of diabetics who carefully control their blood sugar still develop neuropathy,[35] and that some

of those with good blood sugar control still develop nephropathy indicate that hyperglycemia is not the sole cause of these complications.[36] In our later chapters we will focus on the risk factors for the retinal damage in diabetes. Since currently available treatments cannot reliably control glycemia at a stipulated level, the only testable glucose hypothesis in human diabetes compares the ability of different treatments rather than that of different glucose levels to prevent or ameliorate long-term complications. Controlled clinical trials like Diabetes Control and Complications Trial study have shown a decrease in the progression from incipient nephropathy to overt proteinuria with intensive diabetes therapy.[37-39] The another risk factor is the duration of diabetes.[40] The duration after puberty is of particular importance.[41] Hypertension is associated with the development and progression of nephropathy. The principal risk factor for coronary artery disease in diabetes is nephropathy.

1.1.6.2 Acute complications of diabetes

Low insulin levels cause the liver to turn to ketone for fuel; ketone bodies are intermediate substrates in that metabolic sequence. Elevated levels of ketone bodies in the blood decrease the blood's pH, leading to diabetic ketoacidosis (DKA). This can lead to dehydration, and rapid breathing. Abdominal pain is common and may be severe. Ketoacidosis can easily become severe enough to cause hypotension, shock, and death. DKA is always a medical emergency and requires medical attention. Ketoacidosis is much more common in type 1 diabetes than type 2.

Hyperosmolar nonketotic state is another acute complication. During very high blood glucose levels, water is osmotically drawn out of cells into the blood and the kidneys eventually begin to dump glucose into the urine. This results in loss of water and an increase in blood osmolarity. This will eventually lead to dehydration. The body's cells become progressively dehydrated as water is taken from them and excreted. Electrolyte imbalances are also common and are always dangerous.

Hypoglycemia is an acute complication of several diabetes treatments. The patient may become agitated, sweaty, weak, and have many symptoms of sympathetic activation of the autonomic nervous system resulting in feelings akin to dread and immobilized panic. Consciousness can be altered or even lost in extreme cases, leading to coma, seizures, or even brain damage and death. In patients with diabetes, this may be caused by several factors, such as too much or incorrectly timed insulin, too much or incorrectly timed exercise or not enough food (specifically glucose-containing carbohydrates).

It is more accurate to note that iatrogenic hypoglycemia is typically the result of the interplay of absolute (or relative) insulin excess and compromised glucose counterregulation in type 1 and advanced type 2 diabetes. Decrements in insulin, increments in glucagon, and increments in epinephrine are the primary glucose counterregulatory factors that normally prevent or (more or less rapidly) correct hypoglycemia. In insulin-deficient diabetes (exogenous) insulin levels do not decrease as glucose levels fall, and the combination of deficient glucagon and epinephrine responses causes defective glucose counterregulation.

Diabetic coma can occur because of severe diabetic hypoglycemia, diabetic ketoacidosis advanced enough to result in unconsciousness from a combination of severe hyperglycemia, dehydration and shock, and exhaustion or hyperosmolar nonketotic coma.

An estimated 2 to 15 percent of diabetics will suffer from at least one episode of diabetic coma in their lifetimes as a result of severe hypoglycemia.

The immune response is impaired in individuals with diabetes mellitus. Hyperglycemia both reduces the function of immune cells and increases inflammation. The vascular effects of diabetes also tend to alter lung function, all of which leads to an increase in susceptibility to respiratory infections. Several studies also show diabetes associated with a worse disease course and slower recovery from respiratory infections. [42]

Diabetes is associated with periodontal disease (gum disease) [43] and may make diabetes more difficult to treat.[44] Gum disease is frequently related to bacterial infection by organisms such as Porphyromonas gingivalis and Actinobacillus actinomycetemcomitans.[45] A number of trials have found improved blood sugar levels in type 2 diabetics who have undergone peridontal treatment.

1.1.6.3 Chronic complications of diabetes

Chronic elevation of blood glucose level leads to angiopathy. The endothelial cells lining the blood vessels take in more glucose than normal, since they do not depend on insulin. They then form more surface glycoproteins than normal, and cause the basement membrane to grow thicker and weaker. This results in microvascular disease and macrovascular disease. Neuropathy is another chronic complication. It has been discovered that the serum of diabetics with neuropathy is toxic to nerves even if its blood sugar content is normal.[46] It is still not clear if neuropathy comes prior to the angiopathy/ vasculopathy or vice a versa. Chronic complications of diabetes can occur throughout the body and being a major focus of this thesis I will deal with the ocular complications in detail in separate subsections.

1.1.6.3.1 Cardiovascular Disease

The prevalence of the cardiovascular disease is markedly increased with an increased incidence at a younger age in patients with diabetes.[47] In coronary artery disease the usual protective effect of female sex is eliminated by diabetes. Atypical anginal symptoms and congestive heart failure are common in those with diabetes. Mortality from first or subsequent myocardial infarctions is higher in patients with diabetes than patients without diabetes.[48] Patients with type 2 diabetes are commonly obese and have hypertension and dyslipidemia. However, independently of these variables, diabetes remains a major risk factor for coronary artery disease.[49]

1.1.6.3.2 Nephropathy

Nephropathy is the diabetes-specific complication associated with the greatest mortality. Nephropathy develops in only 35 to 45 percent of patients with type 1 diabetes and less than 20 percent of those with type 2 diabetes.[50,51] The first sign is the development of microalbuminuria (30 to 300 mg of albumin per 24 hours), which may occur as early as five years after the onset of diabetes followed by overt proteinuria (>500 mg of protein per liter) in another 5 to 10 years. Hypertension invariably develops during this period. In the next 5 to 10 years, the nephrotic syndrome develops and the glomerular

filtration rate falls, resulting in end-stage renal disease. The mean durations of type 1 and type 2 diabetes before the development of end-stage renal disease are 17 years and 23 years. Nephropathy in patients with type 2 diabetes is complicated by the uncertain duration and the high prevalence of coexisting hypertension, which may cause nephrosclerosis. Microalbuminuria appears to precede nephropathy in patients with type 2 diabetes.

1.1.6.3.3 Neuropathy

The clinical manifestations of neuropathy in patients with diabetes fluctuate. A peripheral, symmetric sensorimotor neuropathy is the most common form of diabetic neuropathy, whose other forms include cranial and peripheral motor neuropathies and autonomic neuropathy types. Although neuropathy is also more common with longer duration of diabetes, a relatively severe, early-onset polyneuropathy has been described.[52] Electrophysiologic studies demonstrate subclinical abnormalities, including slowed motor- and sensory-nerve conduction in most patients, after 5 to 10 years of diabetes.[53] Symptoms, including paresthesia, a risk factor of foot trauma and diabetic ulcers, are characteristically worse at night. Radiculopathies may also occur, mimicking disk disease. Autonomic neuropathy can affect gastric or intestinal motility, erectile function, bladder function, cardiac function, and vascular tone. Subclinical changes can often be detected within 5 to 10 years after the onset of type 2 diabetes. Diabetic diarrhea and incontinence are rare but can be disabling. Impotence is the most common clinical manifestation of autonomic neuropathy, affecting more than 50 percent of men with diabetes. Cardiac autonomic neuropathy may result in resting tachycardia and postural hypotension.

1.1.6.3.4 Other Systemic Complications

Other long-term complications are a tendency for certain infections (e.g., pseudomonas “malignant” external otitis, monilial skin infections, and rhinocerebral mucormycosis) and cognitive impairment. Dupuytren's contractures and periarticular thickening of the skin leading to decreased mobility of the fingers are also more common in patients with diabetes.[54] Evidence shows that gas exchange is adversely impacted and forced expiratory volume worsens with diabetes duration and poorer blood glucose control.[55]

1.1.7 Ocular Complications

Diabetes can affect the eyes in more than one way. The ocular complications arise from the alteration in vascular and neural structure and function. Diabetes mellitus is one of the leading causes of irreversible blindness worldwide, and, in the United States, it is the most common cause of blindness in people younger than 65 years of age. At least 50,000 Americans are legally blind from this condition. Diabetes is also responsible for 5,800 of the new cases of blindness reported annually.[56] The incidence of all ocular manifestations of diabetes increases with age and duration of the disease. Approximately 5 percent of the population with diabetes may develop glaucoma, compared with about 2

percent of the general population.[57] Cataracts are 2 to 4 times more prevalent, occur at younger ages, and progress more rapidly in patients with DM than in the general population.[58] With increasing prevalence of DM, diabetic retinopathy is the leading cause of preventable vision loss.[59]

1.1.7.1 Risk factors for ocular complications in diabetes

It is important to understand the risk factors for ocular complications in diabetes. Apart from all the risk factors for systemic complications following are the specific factors that have established causal relationship with the occurrence of ocular complications.

- Obesity: >120% desirable body weight or body mass index >25 kg/m2.
- Genetic predisposition i.e. having a first-degree relative with diabetes eye disease.
- Ethnicity: African American, Hispanic or Native American.
- Gestational diabetes with a baby weighing more than 9 pounds at birth.
- Hypertension (blood pressure >140/90).
- Hyperlipidemia: HDL cholesterol level <35 mg/dl and/or a triglyceride level >250 mg/dl.

1.1.7.2 Glaucoma

People with diabetes are twice as likely to develop glaucoma as are non-diabetics. Both glaucoma and diabetes are diseases with vascular components, which may be the common denominator to understanding the ocular effects in patients with both conditions. Diabetes may influence risk of primary open angle glaucoma via hyperglycemia-related vascular constriction leading to increased intraocular pressure[60] and increased susceptibility to glaucomatous optic nerve damage. According to Sato and Roy[61], high glucose levels in the aqueous humor of patients with diabetes may increase fibronectin synthesis and accumulation in the trabecular meshwork. High blood sugar can cause the lens of the eye to swell leading to obstacle in aqueous drainage. There are contradictory findings such as those of the Ocular Hypertension Treatment Study (OHTS) found that diabetes is protective against glaucoma.[62]

1.1.7.3 Lenticular complications

Individuals with diabetes suffer from episodes of transient refractive changes. The mechanism behind these changes is obscure. There is a shift towards myopia or hyperopia in association with hyperglycemia or hypoglycemia, respectively.[63,64] In diabetic patients the change is commonly towards myopia, especially after starting of treatment. In addition to refractive changes, recent onset diabetic patients also exhibit changes in accommodation. Waite and Beetham reported transient paralysis of accommodation in 21% of diabetic patients, most commonly in those between 20 and 50 years of age.[63] In diabetes excess glucose in the crystalline lens is converted in sorbitol through the action of aldose reductase. Sorbitol, being poorly permeable across cellular membranes, accumulates in the lens longer. This creates difference in the osmotic pressure leading to influx of water from the aqueous humor into the lens, causing

swelling with myopic refractive changes. Abrupt plasma glucose reduction leads to transient difference in osmotic pressure inside and outside the eye and that may alter the composition of lens, aqueous humor and vitreous. These changes may cause reduction in the refractive index causing hyperopia.

Cataracts are a well-known cause of visual impairment in diabetic patients. The Framingham Eye Study reported increase in the prevalence of cataracts in diabetic patients.[65] Many studies, including the large population-based Blue Mountains Eye Study[66] reported an increased prevalence and incidence of posterior subcapsular cataracts in diabetic patients. There have also been reports of an increased incidence of cortical cataracts in diabetic patients. The Beaver Dam Eye Study found that a 1% increase in HbA1c increased the risk of nuclear cataracts by 15% in diabetic patients.[67] Snowflake cataracts, which are white subcapsular opacifications, have been described in young type 1 diabetic patients. The enzyme aldose reductase catalyzes the reduction of glucose to sorbitol through the polyol pathway. Intracellular accumulation of sorbitol leads to osmotic changes resulting in hydropic lens fibers that degenerate and form sugar cataracts.[68] This creates a hyperosmotic effect that results in an infusion of fluid to countervail the osmotic gradient and a collapse and liquefaction of lens fibers, which ultimately results in the formation of lens opacities. The osmotic stress in the lens induces apoptosis in lens epithelial cells to the development of cataract. The role of osmotic stress is particularly important for the rapid cataract formation in young patients with type 1 diabetes mellitus due to the extensive swelling of cortical lens fibers. Osmotic stress induces stress in the endoplasmic reticulum leading to the generation of free radicals. That generates reactive oxygen species and causes oxidative stress damage to lens fibers.

1.1.7.4 Optic nerve and extra-ocular nerve damage

Diabetes-related damage to the nerves is reflected in the eyes at three levels, retinal neural cell damage, the vascular related optic nerve damage, and damage to the extra-ocular muscles. This thesis mainly concerns the retinal neural damage but it is worthwhile to review the optic nerve damage. Anterior ischemic optic neuropathy is an acute vascular condition of the optic nerve. Studies suggest that up to 25% of patients with this condition have a history of diabetes.[69] In patients with diabetes, diabetic microvascular disease affecting the anterior part of the optic nerve causes ischemia that could lead to secondary neuronal degeneration.

Diabetic papillopathy is an uncommon optic nerve condition characterized by acute disc edema and mild vision loss. Diabetic papillopathy is a risk factor for the progression of diabetic retinopathy.[70] There could be a toxic effect of abnormal glucose metabolism on the optic nerve in individuals with diabetes. The significance of this condition is that misdiagnosis of telangiectasia at the optic disc in diabetic papillopathy can be mistaken as neovascularization in the optic disc as part of proliferative diabetic retinopathy, leading to uncalled-for laser photocoagulation.

Extraocular motility disorders may occur in patients with diabetes, secondary to diabetic neuropathy, involving the third, fourth, or sixth cranial nerve.[71] Diabetes is the underlying cause in 25–30% of patients aged 45 years and older who develop acute extraocular muscle palsy. Patients with extraocular palsies present with binocular

diplopia. Patients with diabetes also commonly have reduced sensitivity in their corneal neurons.[72]

1.1.7.5 Retinal neuropathy

Retinal neural damage as a result of diabetes is the main focus of this dissertation. The changes in the retinal neurons tested using psychophysics and electrophysiology will be discussed in detail. However other changes to neurons in the eye are also related and merit brief discussion.

There is evidence of early neuronal degeneration in diabetes in which the patients with diabetes were seen to have thinner inner retinal layers when compared to controls. In the retinal neurons in animal studies there was 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.[73] There is evidence of an early and persistent neural apoptosis in diabetes.[72] In STZ rats the thickness of the nerve fiber layer in the superior polar hemisphere of the retina was found to be significantly reduced compared with the control group, indicating a loss of axons in this region and implying an accompanying loss of retinal ganglion cells.[74] Studies suggest glutamate excitotoxicity or glycosylation end products (AGE) are possible causes for neuronal changes in retina.[75]

1.2 Diabetic Retinopathy

1.2.1 Overview

Diabetic retinopathy remains a leading cause of visual impairment in working age and can reach its more advanced stages in the almost total absence of symptoms. The prevalence of diabetic retinopathy is about 70% in patients with type 1 diabetes and 40% among those with type 2, with no differences by gender.[55] The prevalence increases with disease duration and practically all patients with type 1 diabetes develop retinopathy, proliferative retinopathy half the time, within 20 years of the diagnosis. The most serious forms of retinopathy, proliferative retinopathy and macular edema, occur in 23% and 14% of patients with type 1 and type 2 diabetes respectively.

Alterations of retinal capillaries are the basis of diabetic retinopathy and include multiple occlusions, increased permeability of the vessel wall and, in the proliferative type, growth of newly formed vessels. Occlusions cause areas of ischemia and focal (microaneurysms) or generalized dilatation of the capillaries. Dilated, fragile and hyperpermeable vessels result in microhaemorrhages and leakage of serum and lipoproteins in the neuroretina, with the formation of edema and hard exudates. Occlusion of vessels may result in focal retinal ischemia, which may manifest as white-grayish areas with blurred margins, or cotton wool spots. The presence of these lesions defines non-proliferative retinopathy, which can be mild, moderate or severe, and can develop into two forms at high risk of visual loss: diabetic macular edema and proliferative diabetic retinopathy (figures 1.2, 1.3).[76]

When the lesions of diabetic retinopathy involve the macula severe functional vision impairment may result. Diabetic macular edema affects primarily patients with

type 2 diabetes who represent 90% of the diabetic population. It is now the main cause of visual impairment in diabetes. Progressive ischemia of the peripheral retina can cause proliferative diabetic retinopathy, with growth of new vessels that may invade the vitreous and give rise to vitreous haemorrhages and development of fibroglial tissue. The latter, by contracting, may cause retinal detachment.

Although diabetic retinopathy is considered predominantly a pathology of microvessels, increasing evidence points at degeneration of the neuroretina, mainly apoptosis of ganglion cells and glial activation, as an early event which may predate and perhaps contribute to microcirculatory abnormalities.[77-79] Damage of the neuroretina may result in loss of color discrimination and contrast sensitivity, as detectable by electrophysiological studies in patients with short diabetes duration[80,81,74],and delayed multifocal electroretinographic implicit time may predict the development of early microangiopathy.[82] Metabolic and signalling pathways involved in retinal neurodegeneration may be shared with, and/or activate mechanisms involved in, the pathogenesis of microangiopathy.[83]

The patients studied for this dissertation are at the various stages of retinopathy from no retinopathy to the severe forms and complications like diabetic macular edema.

1.2.2 Diagnosis and grades

The main symptom of diabetic retinopathy is reduced vision, but this occurs only when the condition is advanced and may be irreversible. Early changes in diabetic retinopathy are generally asymptomatic, and treatment may be needed long before patients are aware of any loss of vision. All patients over the age of 11 years with type 1 diabetes need a retinal examination annually or more frequently if clinically indicated. Type 2 diabetes may have been present for years before diagnosis; so all patients with type 2 diabetes should have a retinal examination at least every 12 months, starting as soon as the diagnosis is made.[84]

Early non-proliferative retinopathy (Fig. 1.2) is comprised of the microaneurysm as the first ophthalmoscopically detectable signs of the retinopathy seen as small red dots in the middle layers of the retina. When the walls of the capillaries are weakened enough, it may rupture, giving rise to an intraretinal hemorrhage. If the hemorrhage is deep i.e. in the inner nuclear layer or outer plexiform layer, it usually is round or oval and called a dot or blot hemorrhage. It is very difficult to distinguish between the dot hemorrhage and a microaneurysm using ophthalmoscopy. Fluorescein angiography helps to distinguish a small dot hemorrhage from a microaneurysm as dot hemorrhages leak dye. However angiography cannot distinguish clotted blood from aneurysms. If the hemorrhage is superficial in the nerve fiber layers it appears to be a flame shaped hemorrhage. Macular edema or retinal thickening is an important manifestation of the non-proliferative retinopathy and is the important cause of the legal blindness in Type 2 diabetes.

Advanced non-proliferative retinopathy (Fig. 1.2) shows signs of increasing inner retinal hypoxia including multiple retinal hemorrhages, cotton wool spots, venous beading and loops, intra-retinal microvascular abnormalities and large areas of capillary nonperfusion depicted on fluorescein angiography.

Figure 1.2

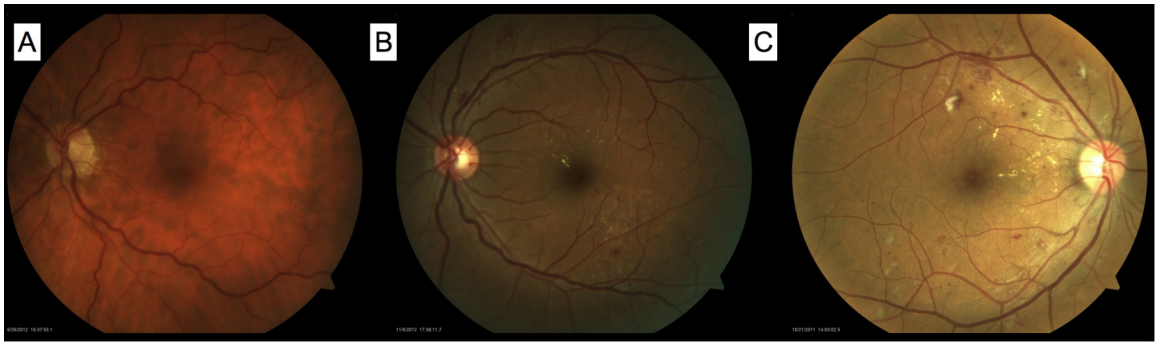

Figure 1.2: Non-proliferative diabetic retinopathy. A. Mild non-proliferative diabetic retinopathy showing early retinal changes with occasional hemorrhages. B. Moderate non-proliferative diabetic retinopathy showing more advanced retinal changes C. Severe non-proliferative diabetic retinopathy showing advanced retinal changes like cotton wool spots and extensive hemorrhages.

Cotton wool spots, also called soft exudates or nerve fiber infarcts, result from ischemia, non exudation. Local ischemia causes effective obstruction of axoplasmic flow in the normally transparent nerve fiber layer; the subsequent swelling of the nerve fibers gives cotton-wool spots their characteristic white puffy appearance. Fluorescein angiography shows no capillary perfusion in the area corresponding to a cotton wool spot. Microaneurysms frequently surround the hypoxic areas.

Venous beading is an important sign of sluggish retinal circulation. Venous loops are almost always near the areas of capillary nonperfusion. Intra-retinal microvascular abnormalities are dilated capillaries, which seem to function as collateral channels. They frequently are difficult to differentiate from surface retinal neovascularization. Capillary hypo-perfusion always surrounds intra-retinal microvascular abnormalities.

The ETDRS found that intra-retinal microvascular abnormalities, multiple retinal hemorrhages, venous beading and loops, widespread capillary nonperfusion, and widespread leakage on fluorescein angiography were all significant risk factors for the development of proliferative retinopathy. Interestingly, cotton wool spots are not.[85]

Proliferative diabetic retinopathy (Fig. 1.3) is the more severe form of progressed or advanced retinopathy that can lead to severe vitreous hemorrhage or retinal detachment. Approximately 50% of patients with very severe non-proliferative diabetic retinopathy progress to proliferative diabetic retinopathy within one year.[86] Proliferative vessels usually arise from veins and often begin as a collection of multiple fine vessels. When they arise from within the one disc diameter of the optic nerve head they are referred to as neovascularization of the disc and if they arise from further away from the optic nerve head they are referred to as the neovascularization elsewhere. Unlike the normal blood vessels these new vessels leak fluorescein into the vitreous.

Once the stimulus for the growth of new vessels is present, the path of subsequent growth taken by neovascularization is along the route of least resistance. For example the absence of the true limiting membrane on the disc could explain the prevalence of the new vessels there. Neovascularization seems to grow more easily on preformed connective tissue and so the shallowly detached posterior vitreous face is a frequent site for growth of new vessels. These changes could lead to more severe complications like

retinal detachment and gross vitreous hemorrhages.

Figure 1.3

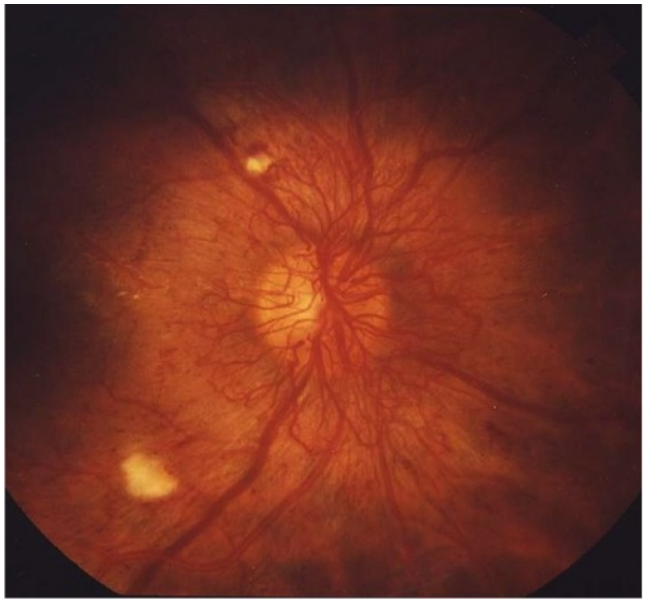

Figure 1.3: Picture taken from (http://www.nei.nih.gov/photo/eyedis/index.asp) Proliferative diabetic retinopathy showing massive neovascularization.

Macular edema (Fig. 1.4) or retinal thickening is an important manifestation of non-proliferative retinopathy and proliferative diabetic retinopathy. It is the main cause of the legal blindness in diabetes. The intercellular fluid comes from leaking microaneurysms or from diffuse capillary incompetence. Clinically macular edema is best detected by biomicroscopy. The edema causes scattering of light by multiple interfaces it creates in the retina by separated retinal cells. This decreases the retina's translucency such that normal retinal pigment epithelial and choroidal background pattern is blurred. Finally the pockets of the fluid in the outer plexiform layer, if large enough, can be seen as cystoid macular edema. Usually cystoid macular edema is seen in eyes that have other signs of severe non-proliferative retinopathy such as numerous hemorrhages or exudates.

If the leakage of the fluid is severe enough, lipid may accumulate in the retina; again, the outer plexiform layer is first to be affected. In some cases, lipid is scattered through the macula. In others, it accumulates in a ring around a group of leaking microaneurysms, or around the areas of capillary non-perfusion. This pattern is called circinate retinopathy.

1.2.3 Treatment

Evidence from large randomized trials and long-term follow-up studies show that primary prevention, early detection, and effective treatment reduce the risk of visual loss. Educating patients as to why they should attend regular screenings and accept treatment is important because the disease is usually asymptomatic until the late stages. Patients should understand that good diabetic control improves their chances of retaining good

vision. However, even patients with “good” control develop complications 20 years after the onset of diabetes.

Figure 1.4

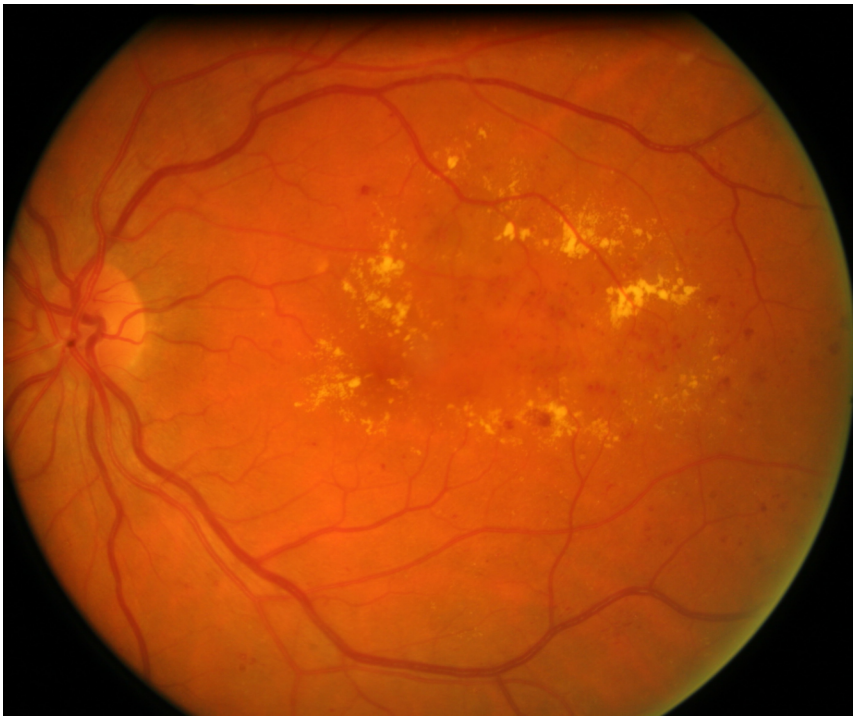

Figure 1.4: Clinically significant diabetic macular edema showing hard exudates, hemorrhages and other vascular changes in retina.

Primary prevention can be achieved by optimal glycemic control. The target is a glycated hemoglobin (HbA1c) of 6.5% as intensively lowering blood glucose to a target 6.0% has been associated with increased mortality.[87,88] Improved glycemic control can reduce but not abolish the risk of retinopathy. The Diabetes Control and Complications trial found that intensive treatment reduced the risk of development and progression of diabetic macular edema by 26% compared with usual management over nine years of follow-up.[88] The UK Prospective Diabetes Study published in 1997 examined the role of tight control in patients with type 2 diabetes. It found a 17% reduction in the risk of progression of retinopathy, a 29% reduction in the need for laser treatment, and a 16% reduction in the risk of legal blindness in the intensively treated group compared with those randomized to usual management at 10 years.[89] In practice, however, perfect glycaemic control is unattainable in type 1 diabetes because of unpredictable hypoglycaemia during ultra-tight control. Perfect control is also unattainable in most people with type 2 diabetes, and control tends to deteriorate with time.[89]

Another important aspect in early prevention is to control the blood pressure and lower the lipid levels. Target blood pressure is 140/80 mm Hg or lower.[89] The UK Prospective Diabetes Study randomized patients with hypertension either to tight control of blood pressure (<150/85 mm Hg) with a β blocker or an angiotensin converting

enzyme inhibitor (and other agents if needed) or to less tight control (<180/105 mm Hg) with- out the use of these agents. After seven years' follow-up, the progression of diabetic retinopathy was reduced by 35% in the tight control group compared with the less tight group. At nine years, the risk of moderate visual loss and the need for laser treatment were reduced by 47% and 35%, respectively, in the tight control group compared with the less tight group. There is no clear evidence that one method of lowering blood pressure is superior to another.[90,91] The Diabetic Retinopathy Candesartan Trial was a large randomized trial designed to assess whether blockade of the renin-angiotensin system reduced the incidence or progression of diabetic retinopathy in normo-albuminuric normotensive patients. Candesartan had no effect on the incidence of new diabetic retinopathy in patients with type 1 diabetes and no effect on the progression of established retinopathy in patients with type 1 and type 2 diabetes.

Observational data from the Early Treatment of Diabetic Retinopathy Study suggest that lipid lowering agents may decrease the risk of vision loss in patients with diabetic retinopathy.[92] The target for total cholesterol is less than 4 mmol/l or low density lipoprotein cholesterol less than 2.0 mmol/l.[90] The mechanisms by which such agents improve exudative diabetic retinopathy are unclear, although laboratory evidence suggests that oxidized low density lipoprotein cholesterol is toxic to retinal endothelial cells.[93]

Protein kinase C inhibitors are commonly used to prevent the further retinal damage in diabetes especially in diabetic macular edema.[94] Ruboxistaurin was designed as an orally active protein kinase C-β inhibitor, but in a large randomized controlled trial it failed to reduce the progression of maculopathy in patients with early diabetic retinopathy.[95] A post hoc analysis showed that it did reduce the incidence of moderate visual loss. More effective agents to prevent retinopathy may become available in the future.

Regular screening and early detection are very important steps involved in diabetic retinopathy management. Diabetic retinopathy is an appropriate target for screening: it is an important health problem with a recognizable pre-symptomatic state, appropriate treatment is available once retinopathy is present, and screening is cost effective.[96] Current screening techniques now rely on departments set up to perform digital retinal photographs, which are then visually inspected, a labor intensive process that produces different levels of detection depending on the screener's expertise. This calls for reliable objective techniques for detection, monitoring and screening of diabetic retinopathy. In this study I will discuss in detail such novel techniques that are proven reliable and sensitive to pick early subclinical functional and structural retinal changes in diabetes. One such technique is optical coherence tomography that uses near infrared light to image cross sections of the retina. Optical coherence tomography is more sensitive than clinical examination or stereoscopic fundus photography for the detection of retinal thickening and clinically relevant macular edema.[97] However, the role of optical coherence tomography in screening is uncertain because it is not clear that treatment of subclinical diabetic macular edema would improve the prognosis of patients with diabetic retinopathy.[97]

It is timely to get an overview of the current treatments for established retinopathy. Most patients with retinopathy do not need treatment and can be monitored safely by annual retinal examinations.

Laser photocoagulation is a well-established treatment for diabetic retinopathy in the presence of macular edema or new vessel growth and has undergone only small modifications in the past three decades. The main aim of this technique is to induce regression of new blood vessels and reduce central macular thickening and thus prevent visual loss from proliferative diabetic retinopathy and diabetic macular edema, respectively.

Photocoagulation has been shown to be effective in many studies. The Diabetic Retinopathy Study (DRS), a trial of laser treatment for proliferative diabetic retinopathy, found a 50% reduction in severe visual loss after scatter laser photocoagulation for new vessels at the optic disc.[98] The Early Treatment of Diabetic Retinopathy Study (ETDRS) compared the effect of laser photocoagulation with observation for diabetic macular edema. At three years the risk of moderate visual loss was reduced by 50% (from 24% to 12%) in the laser group.[99] Visual acuity improved in only 3% of patients. However, diabetic macular edema still causes visual loss, and in some patients even the most aggressive treatment cannot prevent loss of vision.[100] More treatment options are needed.

Intravitreal injection of corticosteroids has been used to treat inflammatory eye disease for many years, although the mechanism of action is not fully understood. A large randomized trial comparing intravitreal injection of 4 mg triamcinolone with standard laser photocoagulation found that steroids were initially more effective than laser but by two years eyes treated with laser had better visual acuity and less macular edema. In addition, intravitreal steroid increased the risk of cataract formation and raised intraocular pressure.[101]

Anti-vascular endothelial growth factor (ant-BVEGF) treatments are heavily used as newer treatment options. Concentrations of vascular endothelial growth factor are raised in the vitreous of eyes with diabetic macular edema.[96] Anti-vascular endothelial growth factor drugs including pegaptanib (Macugen), bevacizumab (Avastin), and ranibizumab (Lucentis) may therefore be useful in the management of diabetic macular edema.

Vitrectomy i.e. surgical removal of the vitreous is important in the treatment of proliferative diabetic retinopathy. It aims to improve vision by removing any blood in or behind the vitreous, reattaching detached areas of retina, and reducing the stimulus for neovascularization by complete pan-retinal laser photocoagulation. Recurrent vitreous cavity hemorrhage is one of the most common complications. Theoretically, vitrectomy should be beneficial in diabetic macular edema that does not respond to laser treatment, but this has shown little effect.[102] In situations like vitreous traction, vitrectomy may be beneficial.

1.3 Diabetic macular edema (DME)

1.3.1 Overview

With an increasing prevalence of diabetes mellitus, diabetic retinopathy remains the leading cause of preventable vision loss.[59] In 2011, of an estimated 380 million people worldwide with diabetes[103,104], over one-third had signs of diabetic retinopathy, and a third of these are afflicted with vision-threatening retinopathy, like severe non-proliferative diabetic retinopathy or DME.[105] In the USA, an estimated 29% of adults

with diabetes have diabetic retinopathy and 3 % have DME.[106] In the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) in the U.S.A., about 14-25% of patients with type 2 diabetes developed DME over a 10-year follow-up period.[107] Data from the 25-year follow-up of the WESDR diabetes cohort show that virtually all patients (97 %) developed retinopathy over time, with a third to a half going on to develop vision-threatening disease (29 % developed DME, 17 % developed clinically significant DME and 42 % developed PDR).[108,109] DME is more prevalent in type 2 diabetes and is the primary cause of moderate to severe visual loss for diabetic patients given the high prevalence of type 2 diabetes.[110,111] Apart from the effects on vision, the presence of DME is a marker of concomitant diabetes complications in other organs and is associated with increased rate of mortality.[112-114] Its role in the process of vision loss in diabetic patients and its occurrence are being increasingly studied. Even in the absence of severe retinopathy DME can cause distortion of vision and may cause a significant decrease in visual acuity. Although it is a common complication of diabetic retinopathy it does not necessarily follow the regular course of diabetic retinopathy progression. It may occur at any stage of diabetic retinopathy, whether mild or more advanced.[115]

1.3.2 Pathophysiology of DME

DME occurs following breakdown of the blood–retinal barrier (BRB) due to leakage of dilated, hyperpermeable capillaries and microaneurysms. It comes with an increase of fluid in the retinal tissue, resulting in an increase in its thickness. This increase in fluid may be intracellular i.e. cytotoxic or extracellular i.e. vasogenic [116] In DME, extracellular edema resulting from breakdown of the BRB is generally present. The BRB regulates fluid movements into and out of the retinal tissue. If the BRB breaks down, it enables increased movements of fluids and molecules into the retina, with extracellular accumulation of fluid and deposition of macromolecules. The vascular disruptions of DME are characterized by abnormal vascular flow, disruptions in permeability, and/or nonperfusion of capillaries. Endothelial cells are responsible for maintaining the BRB, and damage to them results in increased vascular permeability. In early stages of DME, breakdown of the inner BRB occurs, resulting in accumulation of extracellular fluid in the macula.[117,118] Abnormal vessel permeability results in leakage of water, blood cells, proteins, and lipoproteins into the surrounding retinal tissue, and subsequent dysfunction of the macula resulting in decreased vision.

Pericytes of the capillaries are essential cellular components in the regulation of retinal capillary perfusion, and damage to these cells in diabetes mellitus leads to altered retinal hemodynamics, including abnormal autoregulation of retinal blood flow.[119] In diabetes, there is increased retinal leukostasis that affects retinal endothelial function, retinal perfusion, angiogenesis, and vascular permeability. In particular, leukocytes in diabetes are less deformable and they may be involved in capillary non-perfusion, endothelial cell damage, and vascular leakage in the retinal microcirculation.[120] As a result of occluded capillaries, retinal ischemia stimulates a pathologic neovascularization mediated by angiogenic factors, such as vascular endothelial growth factor (VEGF), which plays an important role in the development of DME.[121] One common feature of diabetic retinopathy is the thickening of the capillary basement membrane and increased deposition of extracellular matrix components. This may contribute to the development of

abnormal retinal hemodynamics[121], including abnormal autoregulation of retinal blood flow.

1.3.3 Location of Macular Edema

In the healthy physiologic state, water is produced as a metabolic byproduct within the neuronal retinal tissue.[122] The normal intraocular pressure of the eye continuously forces fluid into the retina and keeps the retina attached to the RPE.[123] When healthy the inner retina is dehydrated by glial cells such as Muller cells, whereas the subretinal space and outer retina are kept dry by the pumping mechanism of the RPE. Intraretinal fluid collection may develop as a result of enhanced fluid leakage due to breakdown of the BRB or by the impaired removal of fluid resulting in fluid accumulation within the retina and in the subretinal space. Generally, the edema may develop due to fluid collection in interstitial spaces and then cause cellular compression or it may collect within cells and result in cellular swelling. Once excess fluid has entered the retinal interstitium, its further spread is limited by 2 high-resistance barriers, the inner and outer plexiform layers.[124] These layers may act as barriers as the extracellular space within them is highly convoluted and very narrow, thereby preventing free passage of fluid and solutes.

A prevailing view is that cyst formation secondary to macular edema is formed by swollen and dying Muller cells. In the case of intracellular edema, leakage will not be seen on angiography, as the fluid is intracellular. An explanation for intracellular edema in diabetes is one provided by hypoxia-induced ionic channel disturbances leading to an osmotic gradient. This gradient drives water from the blood and vitreous into the glial cells and causes glial swelling, edema, and cyst formation.[125,126] When present as cysts, the fluid-filled cysts are predominantly located in the inner nuclear layer and the Henle fiber layer. This fluid accumulation results in cell displacement and the splitting of the perifoveal neuroretina within these layers. On optical coherence tomography imaging and on histology, Muller cell fibers are seen spanning these fluid filled compartments.[127]

1.3.4 Incidence and Prevalence of DME

The incidence and prevalence of DME appears to vary in different epidemiologic studies depending on the type, treatment and the mean duration of diabetes. Although DME can develop at any stage of retinopathy, it is frequently related with increase in duration and severity of retinopathy.

DME prevalence, indicated in the WESDR, is only about 3% in mild NPDR but increases to 38% in moderate to severe NPDR and is 71% in eyes with PDR. According to WESDR reporting, the incidence of clinically significant DME was 4.3% in type 1 diabetes and 5.1% in type 2 diabetes with insulin and 1.3% in those without insulin. At 10 years, the rate of developing DME was 20.1% in patients with diabetes type 1 and 25.4% in patients with type 2 diabetes needing insulin and 13.9% in those without insulin.[128]

1.3.5 Clinical Evaluation of Macular Edema

Clinical evaluation of macular edema has been characterized by its difficulty and subjectivity. Direct and indirect ophthalmoscopy may only show an alteration of the foveal reflexes. Stereoscopic fundus photographs (SFP) and slit-lamp fundus stereo biomicroscopy have been the standard clinical methods to evaluate changes in retinal volume in the macular area, but they are dependent on the observer experience, and the results do not offer a reproducible measurement of the volume change.[129] Nevertheless, together they are useful to visualize signs correlated with retinal thickening, such as hard and soft exudates, hemorrhages, and microaneurysms. The introduction of imaging methods, such as optical coherence tomography (OCT), made macular edema evaluation more precise and reliable.

1.3.6 Diagnosis and Classification

The ETDRS defined clinically significant DME based on clinical grounds by SFP as edema satisfying any one of the following three criteria: 1) any retinal thickening within 500 μm of the center of the macula, 2) hard exudates within 500 μm of the center of the macula with adjacent retinal thickening, or 3) retinal thickening at least 1 disc area in size, any part of which is within 1 disc diameter of the center of the macula. The Global Diabetic Retinopathy Project Group based its definition of DME on clinical examination results alone, without reference to the terms focal or diffuse. This group defined DME as present or absent based on thickening or lipid exudates in the macula. When present, DME was subclassified into mild, moderate, or severe, depending on the distance of the thickening and exudates from the fovea.[130]

1.3.6.1 Color Fundus Photographs

Color fundus photography has been a state of the art technique to define DME. It refers to the area involved to define the severity and stage of the DME. Laursen et al state that "Diffuse macular edema was defined as having two or more disc areas of retinal thickening and involving the center of the macula" and that "Focal edema was defined as an area of retinal thickening less than 2 disc areas in diameter not affecting the center of the macula."[131] Kang et al chose diffuse DME to mean an area of retinal thickening greater than 1 disc area rather than 2 disc areas and did not require center involvement.[132] Some authors imply that increased lipid exudates correlate with a more focal type of DME. Others have defined focal edema in terms of having circinate rings of exudation. In general, the fundus photographic criteria mirror the characteristics of published ophthalmoscopic definitions of focal and diffuse DME.

1.3.6.2 Fluorescein angiography

Fluorescein angiography (FA) has been an important method to evaluate DME, and although not considered a screening exam, it provides important information about retinal perfusion, BRB integrity, and new vessel growth. The source of fluorescein leakage is graded categorically by proportion of leakage originating from

microaneurysms for classification of edema as focal or diffuse. Eyes with 67% or more of leakage associated with microaneurysms are classified as focal, those with 33% to 66% of leakage associated with microaneurysms are classified as intermediate, and those with less than 33% of leakage associated with microaneurysms are classified as diffuse.[132]

Angiographic classifications of DME have included noncystoid and cystoid macular edema (CME) [133] and focal or diffuse DME[134]. Focal macular edema is characterized by the presence of localized areas of retinal thickening associated with focal leakage of individual microaneurysms or clusters of microaneurysms or dilated capillaries. Diffuse macular edema is a more generalized and chronic form of edema, visualized as widespread macular leakage and pooling of dye in cystic spaces.

1.3.6.3 Optical Coherence Tomography (OCT)

OCT is a noninvasive method that is analogous to B-scan ultrasound imaging, except it uses infrared light reflections instead of ultrasound. It produces reliable, reproducible, and objective cross-sectional images of the retinal layers and the vitreoretinal interface. It allows quantitative measurements of retinal thickness (RT) and helps to diagnose and guide treatment strategies in DME. OCT scans may demonstrate diffuse thickening of the neuro-sensory retina and loss of the foveal depression; cystic retinal changes, which manifest as areas of low intraretinal reflectivity; and serous retinal detachment, in DME. The possibility to quantify retinal thickness by OCT is based in the distance between the anterior and posterior highly reflective boundaries of the retina, using appropriate algorithms.[135]

To evaluate and quantify macular thickening, a patient's data are compared with a normative database. Normal retinal thickness in the macular area, measured with time-domain devices, has been calculated to be 200–250 μm, and physiological foveal depression has a mean thickness of 170 μm[136]. Based on these reference values, retinal thickness is considered normal, borderline, or thickened, in the setting of edema. [136]

OCT images of DME depict the presence of low intraretinal reflectivity, due to fluid accumulation in the extracellular space of the retina. The process begins as a diffuse retinal thickening with sponge-like appearance of the retinal layers, showing increase in the extracellular spaces advancing to the typical image of cystoid spaces.[137] Another finding in DME is an outer retinal thickening, characterized by an ill-defined, widespread hyporeflective area, which can be distinguished from serous retinal detachment, by the absence of a highly reflective anterior boundary. Hard exudates are visualized as spots of high reflectivity with low reflective areas behind them (shadow effect), located in the outer retinal layers.[138] They are due to protein or lipid deposition, secondary to the breakdown of the BRB. Hemorrhages also block the reflections from deeper retinal layers.

DME can assume different morphologic patterns on OCT. Kim et al. proposed five morphologic patterns.[139]

Patterns of macular edema:

1. Edema of the inner retinal layers: Breakdown of inner/outer BRB
2. Cystoid spaces in the retina: Overall involvement: Breakdown of inner/outer BRB
3. Sub-retinal fluid accumulation: Breakdown of outer BRB
4. Tractional retina edema: Breakdown of inner BRB

5. Combination of all patterns mentioned above

In summary, OCT is today the only method that allows an objective follow-up of the major characteristics of DME. It allows a clear identification of the intraretinal fluid distribution and the presence or absence of vitreous traction. It is an excellent method to document these findings. Furthermore, OCT allows a quantitative diagnosis of ME, as it is used to obtain numerical representation of the retinal thickness.

CSME may be diagnosed using only biomicroscopy, but CSME with minimal increase in retinal thickness is difficult to recognize without OCT. Different studies demonstrated that OCT may identify DME in patients with normal biomicroscopy. In diabetic patients with increased retinal thickness between 200 and 300μm, considering abnormal values if they are above 200 μm, only 14% are detected by ophthalmoscopy. It corresponds to a subclinical form of macular edema.[140]

1.3.7 Treatment of DME

Contemporary treatment strategies for DME are hugely based on the microvascular complications of diabetes. Systemic metabolic abnormalities are the major cause behind the microvasculopathies leading to severe complications in retina due to diabetes.

1.3.7.1 Control of systemic metabolic abnormalities

The DCCT and the UKPDS studies proved that optimal metabolic control could reduce the incidence and progression of DR and thus of DME. [141,13] Optimal metabolic control is an important treatment goal and should be implemented early and maintained for as long as it is safely possible. [142] Tight control of hypertension is also effective in reducing disease progression.[141] Hyperlipidemia has been linked to the presence of retinal hard exudates in patients with DR. ETDRS had indirectly found that elevated cholesterol resulted in doubling the risk of retinal hard exudates at baseline, increasing the risks of developing hard exudates during follow-up and moderate vision loss at 5 years by 50%, each.[92] Also, some evidence suggests that lipid-lowering therapy may reduce hard exudates and microaneurysms. [90] The Action to Control Cardiovascular Risk in Diabetes (ACCORD) study recommends values for hemoglobin A1c (HbA1c < 6.5%–7%) and blood pressure (< 130/< 85 mm Hg). [143,144]

1.3.7.2 Non-pharmacologic therapies for DME

Laser photocoagulation therapy has proven effective in treating DME. The goal of macular laser photocoagulation for DME is to limit vascular leakage through a series of focal laser burns at leaking microaneurysms or grid laser burns in regions of diffuse breakdown of the blood-retinal barrier and macular areas with capillary non-perfusion. The rationale of focal/grid laser treatment is to reduce the leakage from the microaneurysms and hence reduce the macular edema, reduce the macular area in the inner retina which is ischemic and hypoxic, and perhaps to allow oxygen from the choriocapillaris to diffuse to the hypoxic inner retina near it. ETDRS results showed that macular laser photocoagulation reduced the risk of vision loss by 50% at 5 years for patients with CSME.[145]

Vitrectomy may be of benefit in the management of DME. An ongoing study by the Diabetic Retinopathy Clinical Research Network, is currently evaluating the role of focal/grid laser compared with vitrectomy for DME.[146] Given the risk of blindness without treatment, laser photocoagulation and/or vitrectomy will continue to have a major role in the management of DR/DME. Both laser photocoagulation and vitrectomy improve quality of life for patients with DR and are cost-effective.[147-149] However, these interventions are indicated only when DR has progressed to a measurably advanced stage in which some visual acuity may already be lost. Side effects, such as loss of peripheral, night, or color vision are noted in some photocoagulation treated patients. Vitrectomy can accelerate cataract formation and includes risks of retinal detachment and endophthalmitis, which are fortunately rare.[150] In some patients treated with photocoagulation, DME can reoccur.

1.3.7.3 Pharmacologic therapies in DME

Due to the limitations of non-pharmacologic treatments, the use of current available medicinal therapies is increasing, while other agents targeting the underlying biochemical mechanisms that cause DME are being researched. Corticosteroids are known to reduce vascular permeability, reduce BRB breakdown, downregulate vascular endothelial growth factors (VEGF) production, and inhibit certain matrix metalloproteinases.[151] There are several steroids like triamcinolone acetonide[152], that are being investigated for the treatment of DME and its complications.

Use of anti VEGFs in DME is becoming very popular. There are at present three major anti VEGFs which are being used for DME; Pegaptanib sodium, ranibizumab, and bevacizumab. Pegaptanib is an aptamer that binds to the VEGF. The study found Pegaptanib to cause either a temporary regression of neovascularization on fundus photographs or regression or absence of fluorescein leakage from neovascularization.[153] Ranibizumab and bevacizumab are affinity matured antibody fragments that bind to all of the isoforms of VEGF. These drugs currently are under clinical trials.[154-156]

Experimental studies have shown that protein kinase C (PKC) activity and levels of diacylglycerol (DAG), an activator of PKC, are increased following exposure of vascular tissues to elevated glucose.[157,158] Ruboxistaurin, a specific inhibitor of PKC[159], has been shown to prevent and reverse microvascular complications in animal models of diabetes[160], to block neovascularization associated with retinal ischemia[161], and to inhibit the effect of VEGF on retinal permeability and endothelial cell growth[162].

1.4 Visual function changes in diabetes

Several studies have shown that visual function changes occur in diabetes. They are present even before any structural changes are seen in the retina. Here I mention the findings from many past studies that formed the foundation of my current work.

1.4.1 Visual acuity and contrast sensitivity

Routinely Snellen visual acuity tests or Bailey-Lovie visual acuity tests are used to examine vision function. These tests fail to detect any functional changes early in

diabetes retinal complications and seemingly are affected only if macular edema has occurred.[163] Contrast sensitivity tests, on the other hand, indicate visual functional abnormalities at an early stage of retinopathy. For type 2 diabetes, in the absence of retinopathy, contrast sensitivity has not been observed to be different than the controls. However, in type 2 diabetes with mild to moderate retinopathy, contrast sensitivity is significantly lower at 6 and 12 c/deg spatial frequencies.[164,165] For type 1 diabetes, the contrast sensitivity function is decreased from spatial frequencies of 1.5 to 18 c/deg, especially in the middle spatial frequencies of 6 and 12 c/deg before visual acuity is abnormal and there are any clinical signs of retinopathy. The drop in contrast sensitivity showed a correlation with the duration of diabetes.[166,167]

Contrast sensitivity and its relationships with structural and neuroretinal function changes are an important part of my thesis. I will discuss in detail how contrast sensitivity tested with Pelli-Robson charts differs between patients with diabetes and non-diabetic controls and will shed light on its relationships with the other structural and functional changes in diabetic retina.

1.4.2 Color vision

Color vision has long been tested in patients with diabetes and many researchers found it to be altered in diabetes. On the 100-hue test, high error scores were observed by Lakowski et al. in early diabetes in1970.[168] Further Adams' group studied this phenomenon using incremental threshold methods. They found abnormal chromatic spectral sensitivity functions with decreased sensitivity of the short-wavelength pathway but preserved middle and long wavelength functions in the patients with diabetes but no retinopathy.[169] Many further researchers observed similar changes in color sensitivity in the patients with diabetes at the various stages of retinopathy. Later studies used tests like heterochromatic matching, wavelength discrimination, psychophysical measurements and a blue flash-on-flash method against a yellow background.[170,171]

Besides the subjective measures reviewed above, objective examinations of color vision mainly in fovea was performed by measuring color visual evoked potentials. Diabetes control seems to affect S-axis VEP latencies.[172] In the 1980s, using a subjective measure, short wavelength automated perimetry, local S-cone function across the retina was tested and it was reported to be abnormal in patients with diabetes and retinopathy, especially when clinically significant macular edema was present.[173,174]

In this study we used the Adams desaturated D-15 color test[175] and detected worsening of color discrimination in adults with diabetic retinopathy. Furthermore this worsening is correlated with reduced contrast sensitivity and mfERG amplitude and implicit time abnormalities. The details will be discussed in chapter 4 of this thesis.

1.4.3 Electrophysiology in diabetes

Electrophysiology has long been used to study neuro-retinal function changes in diabetes. The photopic a-wave and b-wave amplitudes of conventional electroretinograms (ERG) were found to be not sensitive for the prediction and detection of early retinal changes in diabetes.[176,177] In patients with mild non proliferative diabetic retinopathy there is evidence of scotopic b-wave implicit time delays and 30-Hz flicker amplitude

reduction.[176,177] It has been reported that abnormalities in photopic b-wave amplitude and implicit time occur only in severe forms of non proliferative diabetic retinopathy and proliferative diabetic retinopathy.[178-180] In the studies with full field flash ERG in diabetes a reduction in oscillatory potential (OP) amplitudes and increase in OP implicit time (particularly the first OP) was revealed. In more severe diabetes there is a loss of amplitude and increase in implicit time of the full field flash b-wave under both photopic and scotopic conditions.[181,182] Furthermore, changes in OP's have been reported to predict the progression of diabetic retinopathy in one study.[183] The focal ERG is recorded with a specialized technique that allowed recording of local electrical retinal responses to focal light stimuli. The focal ERG implicit time and amplitude were significantly delayed and reduced, respectively, in patients with type 2 diabetes and no retinopathy.[184] In recent studies a more advanced and location-specific technique is in use for testing electrical neuroretinal function. The technique is called multifocal electroretinogram and was developed by Sutter et al. in 1992.[185]

1.4.4 Multifocal Electroretinogram (mfERG)

The mfERG is a newer technology that allows ERG responses to be studied objectively from multiple local areas in central retina. The technique has made it possible to obtain mapping of photopic activities from hundreds of retinal locations in response to the flicker pattern formed using a pseudo-random m-sequence. Cross-correlation methods are used to extract the local ERG responses from a continuous output signal. The stimulus pattern and its projection on retina are shown in Figure 1.5. 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 mostly from ON bipolar cells. The rods, ganglion cells, and inner retinal cells contribute very little to the mfERG. [186] 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 simultaneously and rapidly, and is reproducible. 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.[123]

The mfERG is ideal to assess neuroretinal function in diabetes for several reasons. There have been many studies assessing the mfERG in diabetes by our group and others. It is very sensitive to functional changes even before retinopathy is apparent. Changes to the mfERG in diabetes and its relationships with various other structural and vision function changes is the focus of this thesis.

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.[187,188] Implicit time delays have been shown to be correlated with ischemic areas in the retina.[189] The oscillatory potentials and amplitude have also shown change and decrease in diabetes. 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 measurements in this disease process and should be considered. [190]

Our group first used the mfERG for evaluation of retinal changes. We demonstrated that the mfERG worsens locally in areas of the retina with changes from diabetes. More recently we have focused on the use of the mfERG for the prediction of retinopathy and macular edema. Predictive models for patients with and without retinopathy have been created for one, two and three years into the future.[191-193] Recently our group has established models for retinopathy prediction in patients without diabetic retinopathy[194] and established a macular edema prediction model in patients with diabetic retinopathy.[195]

Figure 1.5

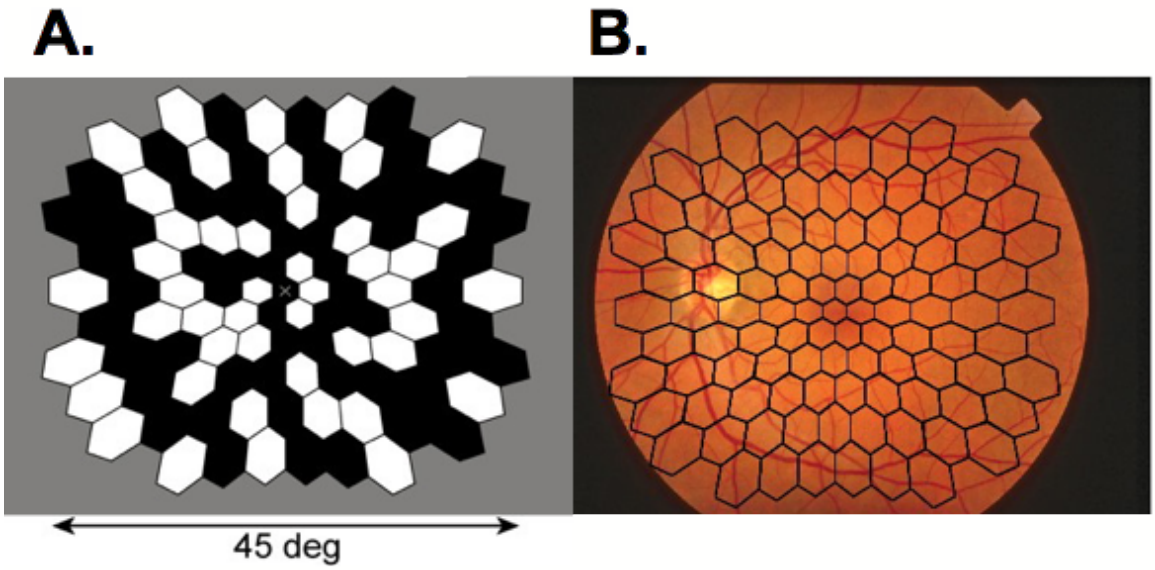

Figure 1.5: mfERG stimulus and its projection on retina. A. mfERG stimulus made up of 103 flickering hexagons. B. Projection of hexagonal pattern on central 45 deg of retina.

1.5 Prior studies of prevention and prediction of diabetic retinopathy and diabetic macular edema

Since the treatments for diabetic retinopathy are very invasive and do not result in full recovery from the vision loss, there is a pressing need for research on prevention and prediction of diabetic retinal damage. The contemporary diagnostic techniques for established diabetic retinopathy are insensitive to early subclinical changes in diabetic retinopathy. They are useful to monitor the prognosis of the retinal damage in advanced stages of diabetes i.e. when retinal vascular changes are evident clinically.

It is important to understand the retinal function and structure changes and their interrelationships in early diabetes. This understanding will help to further research on prevention and prediction of retinal damage in diabetes, to create an early detection protocol, to invent newer sensitive and reliable endpoints for clinical trials and to establish better screening tests that are easy, inexpensive and sensitive to identify patients at risk. The focus of this thesis is to examine these relationships in diabetic eye disease. Very little work has been done for the earlier stages of retinopathy.

Previous studies on the prediction of both which patients will develop diabetic

macular edema, and its prevention, 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 advanced retinopathy and diabetic macular edema and progression of existing retinopathy.[141] The DCCT found that intensive insulin therapy in patients with type 1 diabetes might lower the risk of diabetic retinopathy by 76% and macular edema by 50%.[196] High systolic blood pressure has been shown to be correlated with diffuse macular edema and improved blood pressure control reduces the risk of macular edema.[197,198]

1.5.1 Important cohort studies in diabetic retinopathy

In the last three decades many important studies focused on the prediction and prevention of retinopathy. These studies provided milestones to define the current standard of care for the treatment of diabetic eye disease. The main studies are the ETDRS, UKPDS, DCCT, and WESDR studies. They are extensive efforts that represent group effort of statisticians, epidemiologists, clinicians, and researchers. It is appropriate to go over these important studies as they continue to be a guideline for many upcoming studies in the field of early diabetes like studies included in this thesis. Apart from the important contribution of guiding future research, these studies provide a resource of statistical and epidemiological information about diabetes and its retinal complications. The endpoints used in these studies (presence or progression of retinopathy and a decrease in visual acuity) serve as a guideline for diabetes and vision related studies.

1.5.2 The Early Treatment Diabetic Retinopathy Study (ETDRS)

ETDRS was a multicenter, randomized clinical trial designed to evaluate argon laser photocoagulation and aspirin treatment in delaying or preventing progression of early diabetic retinopathy to more severe stages of visual loss and blindness. It was focused on determining the best time to initiate photocoagulation treatment in diabetic retinopathy, to monitor closely the effects of diabetes mellitus and of photocoagulation on visual function, and to produce natural history data that can be used to identify risk factors and test etiologic hypotheses in diabetic retinopathy.[145] A total of 3,711 patients were recruited to be followed for a minimum of 4 years to provide long-term information on the risks and benefits of the treatments under study. This study was conducted beginning in 1980.

The study produced many interesting results. Aspirin did not affect the progression of retinopathy or vision. However it did not increase the risk of vitreous hemorrhage and was associated with a decreased risk of cardiovascular disease. The study therefore concluded that for patients with mild to severe non-proliferative or early proliferative diabetic retinopathy, aspirin has no effect on the progression of retinopathy. However, the aspirin does not seem to have any harmful effects for diabetic patients with retinopathy and therefore it may be used when required for cardiovascular disease or other medical indications without any ocular contraindications.[199] With respect to photocoagulation for the focal treatment for macular edema, the ETDRS demonstrated that photocoagulation definitely reduced the risk of moderate vision loss, especially for those eyes with macular edema that involved or are at risk of impairing the center of the macula.[87] There was an

increase of moderate visual gain in those eyes that received focal treatment as well as a decrease in the amount of retinal thickening. The study also demonstrated a statistically significant reduction in severe visual loss for those eyes with early treatment, especially in type 2 diabetes. The study concluded that eyes with clinically significant macular edema should be considered for focal photocoagulation. Lastly the study concluded that early vitrectomy should be considered for advanced active proliferative diabetic retinopathy.

Before current treatments, the prognosis for patients with proliferative diabetic retinopathy was blindness within 5 years for more than 50 percent of patients. Rates of blindness in ETDRS patients following the development of proliferative retinopathy are remarkably lower. Legal blindness is reduced to less than 5 percent in 5 years for patients with proliferative retinopathy. Severe vision loss is reduced to 1 percent.

The visual acuity was used as an endpoint in this study but it is now well established that retinopathy does not alter visual acuity until the very late stages of the disease and when the central macula is involved. Thus, there is a need to establish sensitive, reliable surrogate endpoints. One of the long-term interests of my research is to define novel measures of retinal health that may help to well identify the plausible surrogate endpoints for clinical studies.

1.5.3 United Kingdom Prospective Diabetes Study (UKPDS)

The United Kingdom Prospective Diabetes Study (UKPDS) was the largest and longest study of patients with type 2 diabetes done in the United Kingdom. It started in 1977 and continued until 1997. The study led to more than 85 publications. The focus was to determine whether treatments that reduced blood sugar levels to near-normal levels would decrease the risk of the development or progression of long-term end organ diabetic complications, such as retinopathy. The treatments studied were insulin, first or second generation sulfonylurea oral diabetes medicines, and another oral diabetes medicine called metformin. The study found that all treatments were better than diet alone at reducing blood sugar levels, and were equally effective at reducing blood sugar levels. In order to keep blood sugar levels near normal, additional medicines or insulin needed to be added about every 4 years.[200]

Furthermore the study also examined the advantages and disadvantages of sulfonylurea medicines or insulin and their effectiveness for reducing the risk of diabetic retinopathy. As a result the study showed, mono-therapy with sulphonylureas, insulin or metformin all proved ineffective over time. Also to study the role of blood pressure in diabetes end organ damage, people in the study who had type 2 diabetes and high blood pressure were divided into two groups. For one group, the goal was to keep blood pressure levels below 180/105 millimeters of mercury (mm Hg). For the other group, the goal was levels below 150/85 mm Hg with medicine (tightly controlled). Results showed that tightly controlled blood pressure reduced the risk of mortality, diabetes complications and progression of diabetic retinopathy and declining eyesight.[201] The blood pressure medications are now routinely included in the management of diabetic retinopathy.

The study also revealed 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 12 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.

1.5.4 The Diabetes Control and Complications Trial (DCCT)

The Diabetes Control and Complications Trial (DCCT) was a carefully conducted study designed as a prospective, randomized controlled clinical trial to determine whether intensive treatment, with the goal of maintaining blood glucose concentrations close to the normal range, could decrease the frequency and severity of diabetic microvascular and neurologic complications. A total of 1,441 subjects with type 1 diabetes were enrolled between 1983 to 1993.[106] Of these, 726 subjects were recruited as the primary prevention cohort within the first five years after developing diabetes and had no evidence of diabetic retinopathy nor of microalbuminuria at baseline. Another 715 subjects were recruited as the secondary intervention cohort within the first fifteen years after developing diabetes and had mild-to-moderate background diabetic retinopathy with either normoalbuminuria or microalbuminuria. Glycemia was assessed by quarterly measurements of HbA_{1C}. Retinopathy was assessed by seven field fundus photography every six months. Renal function was assessed by annual measurement of creatinine clearance and albumin excretion rate on timed 4-hour urine samples. Neuropathy was evaluated by clinical examination (neurologic history and physical examination), electrophysiology (peripheral nerve conduction velocities), and autonomic nerve testing, at baseline, five years, and study end. Subjects were followed for a minimum of four years and up to nine years. Retinopathy progression is used as an endpoint in this study that comes with the challenge of coordinating fundus grading.

The results show unambiguously that intensive therapy effectively delays the onset and slows the progression of diabetic retinopathy, diabetic nephropathy, and diabetic neuropathy in patients with type 1 diabetes. [202,203] 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%[106].The conclusion is that patients should attempt the best possible glucose control as early as possible in the course of the disease.

1.5.5 The Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR)

The Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) began in 1979 with the purpose to describe the frequency and incidence of complications associated with diabetes, and to identify risk factors (such as poor glycemic control, smoking, and high blood pressure) which may contribute to the development of these complications. Another purpose of the WESDR is to examine health care delivery for people with diabetes.

WESDR involved patients with younger-onset type 1 diabetes (996 people) and older-onset persons mostly with type 2 diabetes (1370 people) who were first examined from 1980 to 1982. The examination involved refraction and measurement of best

corrected visual acuity, examination of the front and back of the eye, measurement of the pressure in the eye, fundus photography, blood tests for HbA1c and random blood sugar, and urine testing for protein in the urine. There were 5 follow-up examinations of the cohort in 1984-86, 1990-92,1995-96, 2000-01and 2006-07. More than 200 publications resulted from the study that demonstrated the associations of good control of blood sugar with less risk of incidence and progression of diabetic retinopathy and diabetic kidney disease. In the 2006-2007 follow-up of the WESDR group examined the relationship of novel risk factors such as inflammation and endothelial dysfunction to the incidence and progression of retinopathy and other diabetic complications and the relationship of diabetic complications to depression and changes in quality of life in persons with type 1 diabetes[204-207].

1.6 Summary and Rationale behind current work

Diabetes mellitus affects more than 8% of the total population in United States. It is not only contributing to morbidity and mortality but is directly affecting the country financially. Diabetic retinopathy is the leading cause of blindness in the working age population. Visual loss occurring due to retinal damage in diabetes is generally irreversible but is largely preventable. The contemporary treatments like photocoagulation and intravitreal steroidal injections are aimed primarily at limiting further visual loss and, due to their invasive nature, are usually are associated with side effects. Thus early detection and prediction of retinal damage in diabetes is essential to avoid further retinal complications leading to vision loss.

Ideally early detection measures should be minimally invasive, sensitive, reproducible, inexpensive, objective and very easy to perform. Such measures are more likely to be used if they do not come with any specialized requirements. Unfortunately, the current trials of new medications use visual acuity as the end point of clinical trials. Visual acuity is a coarse and subjective measurement of retinal function. There is a need for establish newer objective endpoints and surrogates for visual acuity. In the past our lab, as well many other groups, have found the mfERG to be a sensitive and reliable measure to detect and to predict early retinal changes due to diabetes. The goal of my study is to understand the relationship of the mfERG with other sensitive and reliable structural and functional measures of retinal health. I believe understanding this relationship will help to advance the broader aim of diabetes research.

1.6.1 Current thesis studies: overview

This dissertation includes five studies revealing retinal function changes and retinal anatomy changes in early type 1 and type 2 diabetes at various stages of retinopathy. Furthermore it aims to understand relationships between retinal structure, retinal function and diabetes control measurements. Finally this thesis discusses the established macular edema and changes in vision function, retinal structure and mfERG with progression or regression of clinically evident macular edema. The major testing module used is mfERG. The studies are interdependent and utilize from research in our lab, including data collected by me.

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 studies type 1 and type 2 diabetes patients with no retinopathy, with retinopathy and with macular edema cross-sectionally as well as longitudinally. In Chapter 2, general methodology of mfERG recording and other testing and subject recruiting information are discussed in detail. Chapter 3 focuses on a cross-sectional study done in controls, patients with type 1 and patients with type 2 diabetes to study the relationships of mfERG with retinal thickness in absence of retinopathy. In Chapter 4 the letter contrast sensitivity and its relationships with mfERG will be discussed. Chapter 5 focuses on the health of the parafoveal photoreceptors and capillaries in patients with type 2 diabetes and no retinopathy. The focus of Chapter 6 is on patients with established macular edema and the changes in retinal structure and function with the regression and progression of edema.

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Chapter 2: General Methods and techniques

2.1 Overview of the study design

In this chapter I provide the general outline of the techniques and methodologies that are applicable to all the studies included in this thesis as individual chapters. Repetitions are unavoidable, as each chapter will have its own preface, introduction, methods, analysis, results, conclusion and discussion prepared in a journal publication format. This chapter discusses the general protocol run on the subjects included in all of the studies. The number of tests involved in each subsequent chapter depends on the question of interest.

As briefly mentioned during the introduction this dissertation is composed of 4 studies each as individual chapters. Three of these studies, chapters 3, 4, and 5 are cross sectional studies, looking at data at one point in time and comparing different groups of subjects. The study discussed in chapter 6 is a longitudinal study for which a group of patients was followed over time. All studies were observational with no medicinal or surgical interventions.

2.2 Subjects

2.2.1 Groups

Subjects involved in this dissertation were studied as two basic groups, controls and patients with diabetes. The diabetes group is further subdivided into two groups: type 1 and type 2. In turn each of these groups retinopathy is further divided based on the retinopathy grade as:

1. Patients with diabetes and no retinopathy,
2. Patients with diabetes and retinopathy, and
3. Patients with diabetic macular edema.

The number of subjects involved in the individual studies varies because cross sectional studies were done on different timelines for an ongoing longitudinal study.
Patients were recruited from Kaiser Hospital Oakland, San Francisco Veterans Administration Hospital, The Eastmont Wellness Center, and the UC Berkeley Optometry Clinic.

Study participants were paid $25 per visit for participation in each visit. 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.

2.2.2 Inclusion criteria

General inclusion criteria are the same for all the study participants. All the subjects were between the ages of 21 and 68. They had clear crystalline lenses and were free from any media opacities; there was no history of previous surgeries or injuries to their eyes.

The acceptable refractive error ranged between +6.00 D and -6.00 D. All the subjects were free from any other retinal disease. All the subjects except patients with diabetic edema had to have a best-corrected visual acuity of 20/25 or better at the beginning of the study. Group specific details are as follows.

There were over 100 healthy control study subjects combined across all studies. The number of controls involved in individual studies varied depending on study design. All control patients were confirmed to have normal blood glucose levels at the time of testing. Controls recruited after 2008 were tested for HbA1c as a confirmatory test to rule out diabetes/ hyperglycemia at the time of testing. Any potential control participant with elevated random blood glucose at the time of testing (over 200 mg/dl) or HbA1c higher than 7.0% (for the participants recruited after 2008) was not included as a control participant. An mfERG was obtained from the better seeing eye of each control participant. If both eyes had equal acuity the eye with less refractive error and clear lens was chosen; in the case of equal refractive error in each eye, the left eye was chosen.

Approximately 50 patients with diabetes and no retinopathy were included in the studies in chapter 3, 4 and 5. The exact number of patients involved in individual studies varies. They had to have at least 7 years of diabetes duration. These patients were studied in cross section to study the relationships between various retinal health measures. Several of these patient and control participants were recruited for prior studies and were being seen longitudinally for 1-10 years. Part of their earlier data was reported earlier (Han et al.,[1] Ng et al.[2], Harrison et al.[3,4]).

Patients with diabetes and retinopathy were included in studies in Chapters 5 and 6. There were approximately 40 such patients. These patients had mild to moderate non-proliferative diabetic retinopathy and were also targeted to have duration of diabetes over 8 years or an HbA1c over 8%; many of the patients met both criteria. Some of these patients were previously recruited as patients with no retinopathy and were observed for the longitudinal study looking at retinopathy development. Once they developed the retinopathy they were moved to this group.

Chapter 6 studied patients with macular edema. 12 patients who had edema at the entry point were examined. Retina specialist on our team, Shirin Barez MD, evaluated fundus photos of all the patients and established them as patients with clinically significant macular edema (CSME). There were 7 patients with type 2 diabetes and edema, and 5 patients with type 1 diabetes. These 12 patients were studied longitudinally and the data in the year before the time of edema development has already been published.[4] All patients had edema in the central 6 mm (macula) of the retina. Patients with retinal edema were included regardless of visual acuity or duration of diabetes but the other inclusion/exclusion criteria were applied. For chapter 6 these patients were followed for three visits 6 to 12 months apart from each other to study retinal health measures with progression or regression of macular edema.

2.3 Testing

All the study participants were invited to the UC Berkeley School of optometry to test as per the study protocol. The testing protocol was comprehensive and involved vision function tests, dilation of eyes, blood work for diabetes, general health measures like height, weight and blood pressure, electrophysiological tests, non-invasive imaging of retina (optical coherence tomography and fundus photography), slit lamp bio-microscopy and autorefraction. Upon arrival to our lab the patients were asked to sign the consent forms, HIPPA statement and were asked to fill out their medical history. Copies of the signed forms were provided to them.

2.3.1 Vision function tests

Best-corrected visual acuities were obtained from each study participant after signing the consent form and compensating them for study participation. High contrast and low contrast visual acuities were tested for each eye from 10 feet using ETDRS chart. Pinhole testing was used whenever needed. Contrast sensitivity was measured using the Pelli-Robson chart at 10 feet for each eye. Patients with high contrast visual acuities worse than 20/25 were excluded from the study except for patients with edema.

Near spatial vision under conditions of reduced contrast and reduced luminance was performed on approximately 50 participants (controls and patients with diabetes) recruited after October 2011, using a simple new clinical test, the Smith-Kettlewell Institute Low Luminance (SKILL) Card. The study is still in progress and so the results are not included in this thesis. The SKILL Card consists of two near acuity charts mounted back to back. One side has a chart with black letters on a dark gray background designed to simulate reduced contrast and luminance conditions. The other side has a high-contrast, black-on-white letter chart. The SKILL score is the acuity loss (number of letters) between the light and dark sides. A higher SKILL score is indicative of greater vision function loss.

Monocular color vision test was performed for each subject using the Adams desaturated D-15 color vision (DSAT) color caps.[5] The cap order was noted and analyzed to get a color confusion score. Larger scores resulted from a greater number of errors in color cap arrangement. It has been shown in many past studies that color vision is abnormal in patients with diabetes.[6-8] Our lab has already studied the relationships of color vision with mfERG in type 2 diabetes and the results were presented at the AAO meeting in 2010.

All the participants' pupils were dilated fully with 1.0% tropicamide and 2.5% phenylephrine. The cornea was anesthetized with 0.5% proparacaine. For the participants that never had any past dilation of pupils, preliminary assessment of anterior chamber angle was done with a penlight. Corneas were tested for integrity of epithelial membrane with fluorescein dye and the blue filter light.

2.3.2. Blood tests

The fingertip prick method was used to test the blood for three different kinds of blood glucose level documentation. Random blood glucose levels were tested using the One Touch Ultra glucometer in control participants to rule out diabetes and in diabetics to determine the blood glucose level at the time of testing. (It has been shown that blood glucose levels at the time of recording could affect mfERG results[9]). Thirdly, HbA1c a measure of three-month blood glucose average, was tested on diabetic patients. For the patients recruited before 2008 the Immuno Assay Method was used to document HbA1c percentage (Flexsite Diagnostics Palm City FL). Patients recruited after October 2010 were tested by the monoclonal antibody method for HbA1c (DCA-2000 analyzer, Bayer Diabetes Care, Terrytown NY with testing reagents by Siemens Inc, Washington DC). Blood pressure (Left Arm Sitting) was also tested on every patient. An automatic cuff (Omron HEM- 773) was used to measure blood pressure for consistency across examiners.

2.3.3 Retinal imaging

2.3.3.1 Optical Coherence Tomography

Optical Coherence Tomography (OCT) has improved our ability to detect and monitor retinal thickness changes and is used to study retinal structure in diabetes. It was first developed by Huang et al (1991)[10] and was developed for commercial/clinical use with Stratus OCT 3 (Time domain OCT, Carl Zeiss Meditec Dublin CA) and Cirrus HD spectral domain OCT (Carl Zeiss Meditec); both were used in the current thesis. OCT is able to produce cross-sectional imaging of retinal layers at the micrometer scale. The instrument applies an interferometer that measures and compares the light reflected from the retinal tissue to a reference set of known reflectance measures. The inferometric measurement of amplitude and depth can be extracted from the coherence between the sample and reference waveforms. Light reflected from more superficial layers of the retina takes less time to return than light reflected from the deeper retinal structures. Along with the time delay, different layers of the retina have different levels of reflectance and thus will produce different levels of amplitude. OCT allows for sampling of the retina every 10μm over 2mm in depth, as the wavelength of the light used for imaging is 9-10μm. Axial scans are combined to form a cross-sectional image of retinal layers. The amplitude of the response at each axial sample can be illustrated as an image (Figure 2.1).

Figure 2.1

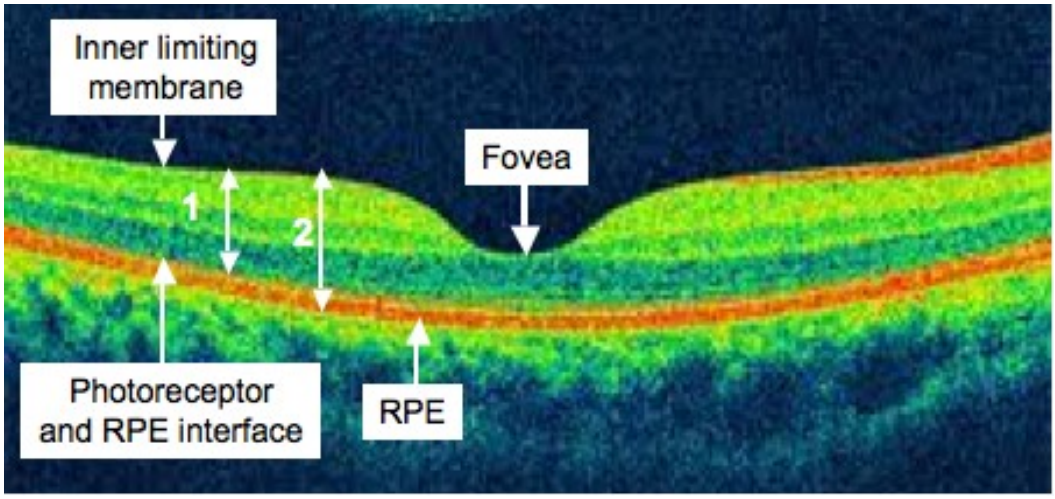

Figure 2.1: High definition retinal scan. 1) Retinal thickness measured on Stratus OCT 3 machine delineating internal limiting membrane to Photoreceptor and RPE inteface. 2) Retinal Thickness measured on Cirrus OCT machine delineating internal limiting membrane to RPE.

Participants included in the study before November 2008 were imaged on Stratus OCT 3. Each 6 mm scan is composed of 512 (11μm transverse resolution) scans centered at fovea. The Stratus software delineates the vitreoretinal interface and pigment epithelial/photoreceptor outer-segment interface and measures the difference between the two at each axial sample. This measurement results in a retinal thickness at each of the 512 axial samples for each scan. Up to 12 radial scans can be acquired sequentially through manual acquisition in less than 2 seconds per scan.[11] Analysis of the retinal thickness will be discussed in more detail in chapter 3.

Participants tested after November 2008 were imaged also with the Cirrus OCT instrumentation. The Cirrus OCT performs scans at a velocity of 27,000 axial scans per second with an axial resolution of 5μm. It is less depended on patient fixation and is faster than the Stratus OCT. It measures whole retinal thickness from the retinal pigmented epithelium to the inner limiting membrane registering higher values of retinal thickness as compared to Stratus OCT. [12,13] The Cirrus OCT measures whole retinal thickness using a 6 mm square, 200-by-200 sample grid centered on the foveola. Average retinal thickness values were calculated within 9 zones following the ETDRS pattern: a 1-mm diameter central zone, four 3-mm diameter perifoveal zones, and four 6-mm diameter peripheral zones. A circular scan 12 degree in diameter centered on the optic nerve head measured the retinal nerve fiber layer in 12 sectors around the optic nerve. Analysis of the retinal thickness will be discussed in more detail in chapter 6.

2.3.3.2 Fundus Photography and vessel analysis

Digital fundus photos (50° field) were taken through a dilated pupil from all participants at the time of testing. A Zeiss camera (Model- Visucam pro NM) was used

to capture three 45° photos within the central 50°. These pictures were uploaded on to the Eyepacs web server developed by Jorge Cuadros et al[14] and were graded by a retina specialist who was masked to the individuals' identity, to exclude the presence of diabetic retinopathy in controls and to grade the retinopathy in patients with diabetes. Subjects were subscribed to subsequent groups based on these grading. These pictures were used to overlay mfERGs and to assign to the damaged locations. (Figure 2.2)

Fundus photographs of the optic discs of all participants were used for the analysis of retinal blood vessels using the Vasculomatic a la Nicola (IVAN) software (University of Wisconsin, Madison, WI). The software measures retinal vessel diameters of the six largest arterioles and six largest venules located 0.5–1.0 disc diameters from the disc margin (Figure 2.2). Each image takes about 20 min of trained user input for analysis. We followed the standard protocol previously described in detail.[15-17]

Figure 2.2

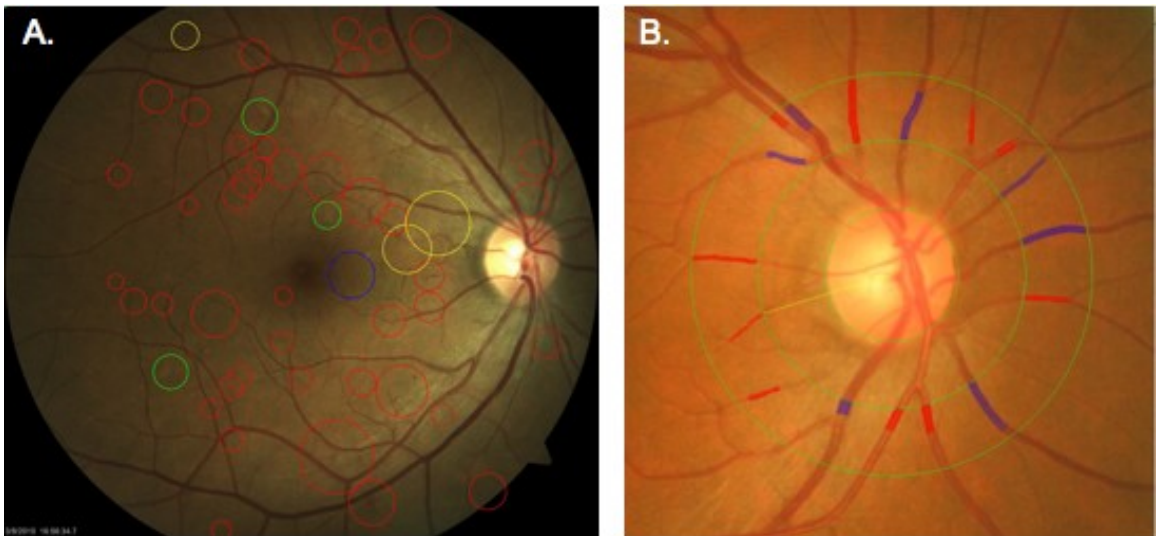

Figure 2.2: A. Grading of a fundus photo of a patient with retinopathy and edema. The colors indicate various retinal lesions. Red: hemorrhages, Blue: edema, Yellow: exudates, Green: cotton wool spots, Purple: IRMA. B: Vessel analysis using IVAN software. Blue indicate venules and red indicates arterioles.

2.3.3.3 Adaptive optics laser ophthalmoscope (AOSLO)

Data presented in chapter 5 was acquired using the AOSLO[18,19] technique to primarily study the health of the photoreceptors and foveal avascular zone in type 2 diabetes. In collaboration with UCBSO Roorda lab, using a noninvasive method to visualize and assess the human parafoveal capillary network[20,21],we were able to assess hemodynamics of specific capillaries in relation to the surrounding capillary network in patients with type 2 diabetes and no retinopathy.[22-24]The detailed images generated using the AOSLO enabled us to detect changes in individual capillaries that were previously undetectable. The same detailed images were used to study photoreceptors that were not studied in diabetes previously.

30 participants were recruited: 15 adult patients diagnosed with type 2 diabetes for at least seven years, with no diabetic retinopathy in at least one eye, and 15 adult age-matched control participants. All the participants were tested on mfERG and AOSLO imaging scheduled, as two different visits spaced no longer than a month apart. For the AOSLO visits, a series of overlapping videos were acquired near the foveal region of the retina. Individual photoreceptors could be resolved in the videos; the flow of individual leukocytes through retinal capillaries could also be seen. Videos were processed to generate high contrast images of photoreceptors. We analyzed photoreceptors using the AOSLO videos. To analyze the photoreceptors, locations of individual cones were labeled, and cone spacing was quantified as described before (Figure 2.3).[25,26]

2.3.4 mfERG recordings

2.3.4.1 Data acquisition

mfERGs were recorded using a visual Evoked Recording Imaging System from two different machines using 4.3 or 5.2 software (VERIS, EDI, San Mateo, CA), depending on the study design. Both the systems are similar and use 9-inch CRT display, which is a 640 x 480 pixel display. Pupils were fully dilated with 1.0% tropicamide and 2.5% phenylephrine. After the cornea was anesthetized with 0.5% proparacaine, a Burian Allen bipolar contact lens electrode (Hansen Ophthalmic, Solon City, IO) was placed on the eye and a ground electrode was clipped to the earlobe. The fellow eye was occluded.

An array of 103 hexagonal elements (Figure 2.4) was displayed on retina by an eye camera unit (EDI, San Mateo, CA) at a 75 Hz frame rate. The hexagons were modulated between bright (200 cd/m^2) and dark (<2 cd/m^2) according to an m-sequence with a base interval of approximately 13.3 msec during the 7.5 min recordings. Subjects adjusted the focus such that central fixation target became clear. The stimulus pattern was projected into the participant's eye on central 45° of retina. 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.

Recordings were made as sixteen 30-second long segments. Real time display with the eye camera monitored recording quality and eye movements, respectively. Signals were amplified (gain, 10^6), band-pass filtered (10-100 Hz and 10-300 Hz) and recorded with a sampling interval of 0.83 msec (16 X per video frame). Contaminated segments containing artifacts were discarded and re-recorded. mfERGs were processed in the usual way with one iteration of artifact removal and spatial averaging with 1/6 of the surrounding responses.

After the recording, the contact lens electrode was washed by hard contact lens cleaner and disinfected by using 10% bleach solution for 5 minutes. Then it was rinsed in regular water first and soaked in a cup of distilled water for another hour to remove any residual bleach.

Figure 2.3

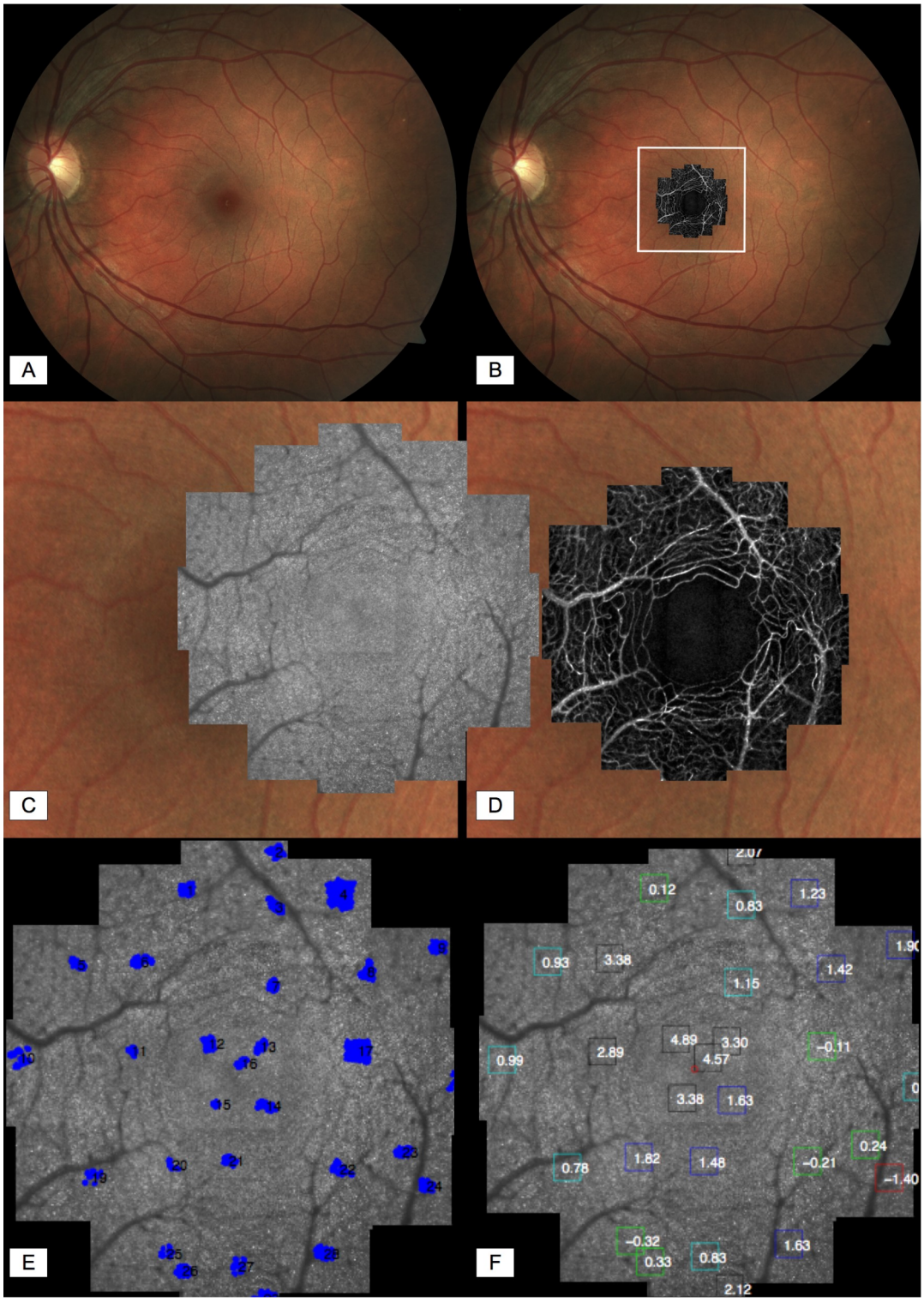

Figure 2.3: Example of AOSLO imaging for one diabetic subject. In this example, overlapping videos were taken in 21 different locations on the retina, processed to generate capillary images, and then compiled to generate a montage of the parafoveal photoreceptors and capillary network. (**A**, **B**) A 45° fundus

photograph, with and without AOSLO images. (**C**, **D**) Higher magnification of in (**B**), showing a portion of the fundus photograph, with and without AOSLO images. (**E**, **F**) Parafoveal locations where cones were assigned using cone marker software (**E**), The boxes indicate the mean location of the selected cones and the number indicates the Z-score, which is the number of standard deviations the current cone spacing differs from a normal eye at that location. Z-scores higher than 2 indicate significant increases in cone spacing. In this patient, cone spacing is normal, except at the fovea center, where the spacing is higher than expected (i.e. cone density is lower than expected).

Figure 2.4:

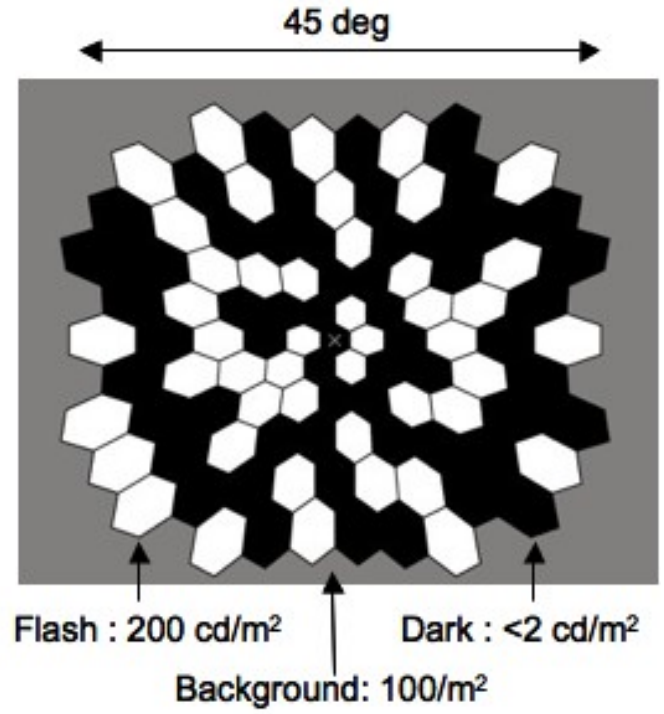

Figure 2.4: The VERIS stimulus from both mfERG machines as it was displayed on the retinas to the patients in the study.

The mfERG system was calibrated every month. Following the instructions from EDI, the calibrator was connected to DB25 connector in the back of the computer in place where usually amplifier is connected. The sensor of the calibrator was put onto the VERIS eye camera. Then VERIS software was started and auto calibration was selected from the menu. The white hexagon was calibrated to have a luminance of 200 cd/m^2, the black hexagon to have a luminance of less than 2 cd/m^2 and the surrounding area was set to 100 cd/m^2.

2.3.4.2 Extracting mfERG response

The implicit time and the amplitude of the local first-order mfERG responses were analyzed by a stretching method described in detail by Hood and Li (Figure 2.5). [27]

Figure 2.5

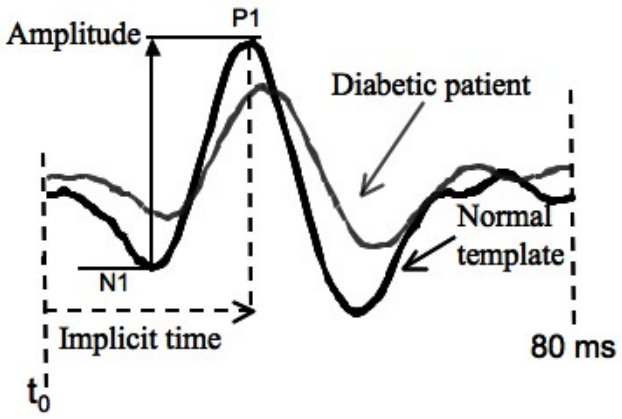

Figure 2.5: mfERG Response Measurement

A normal waveform template was obtained by averaging the mfERGs recorded from the control subjects at each stimulus location. For the normal template, the amplitude was calculated as the voltage difference between the first trough and the first peak and implicit time was measured to the first prominent response peak (P1). Individual responses from the eye of all subjects were compared with the normal template for the first 80ms. The templates were independently scaled in amplitude (A-scale) and time dimensions by multiplying the amplitude and the time vector by a scale factor for each parameter (T-scale) until the best least-square fit to the local patient response was obtained. Local response amplitude was then calculated by multiplying the A-scale and the corresponding un-scaled template amplitude. The local response implicit time was derived by multiplying the T-scale and the corresponding un-scaled template implicit time. (Figure 2.6)

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Figure 2.6

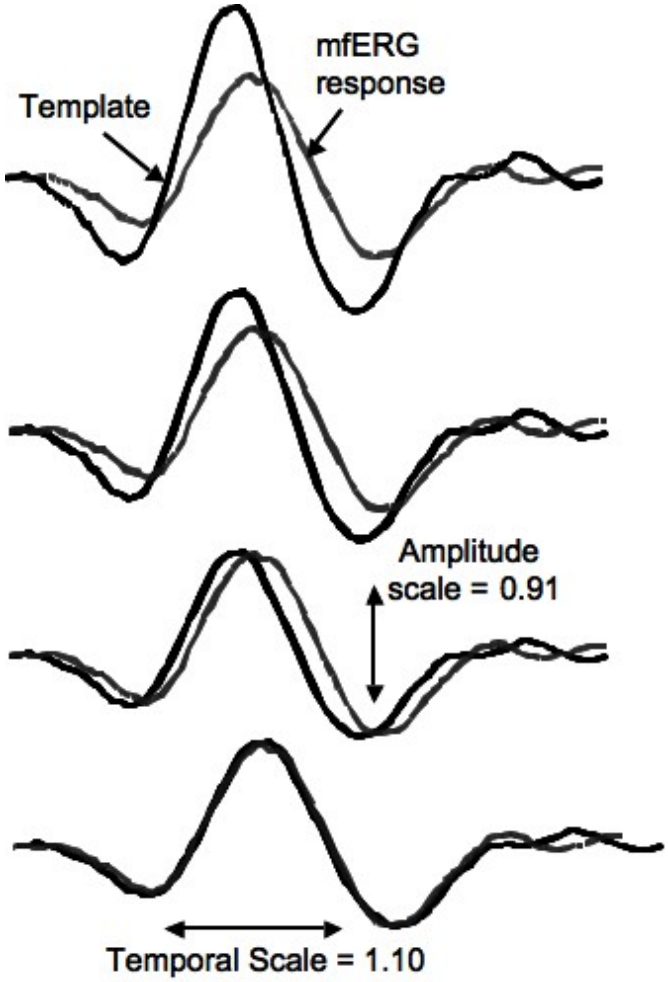

Figure 2.6: Template scaling method: Hood and Li 1997

Multiplicative scaling over time i.e. stretching have been proved a superior fit than simple shifting of template in diabetes.[28] The quality of the fits was controlled by goodness of fit measures, a parameter called statfit and is generated by the stretching program. 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.[26]

2.3.4.3 Z-score calculation

103 mfERG responses were calculated for controls. 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.

2.3.4.4 mfERG responses at the locations with macular edema and retinopathy

For the study presented in Chapter 6, the spatial correspondence between mfERG stimulus array and fundus photographs was defined based on the location of the optic disc and fovea (Figure 2.7). The data taken from 103 hexagons was grouped into 35 zones. The rationale behind comparing diseased retinal locations with 35 zones instead of individual hexagons is to lower the probability of erroneous calculations. Because the size visible in the fundus photo could be smaller than the actual anatomical lesion on the retina creating zones helps account for this possibility. The location of retinopathy might not lie directly over the site of the actual anatomic lesion. The grouping of the individual locations helps to get rid of the possible mismatch between the retinal locations and the mfERG recordings. This allowed us to be more spatially conservative especially in the central retina where, because of the cone density scaling, the hexagons are smaller. A zone's composition will be discussed more in detail in Chapter 6.

Figure 2.7

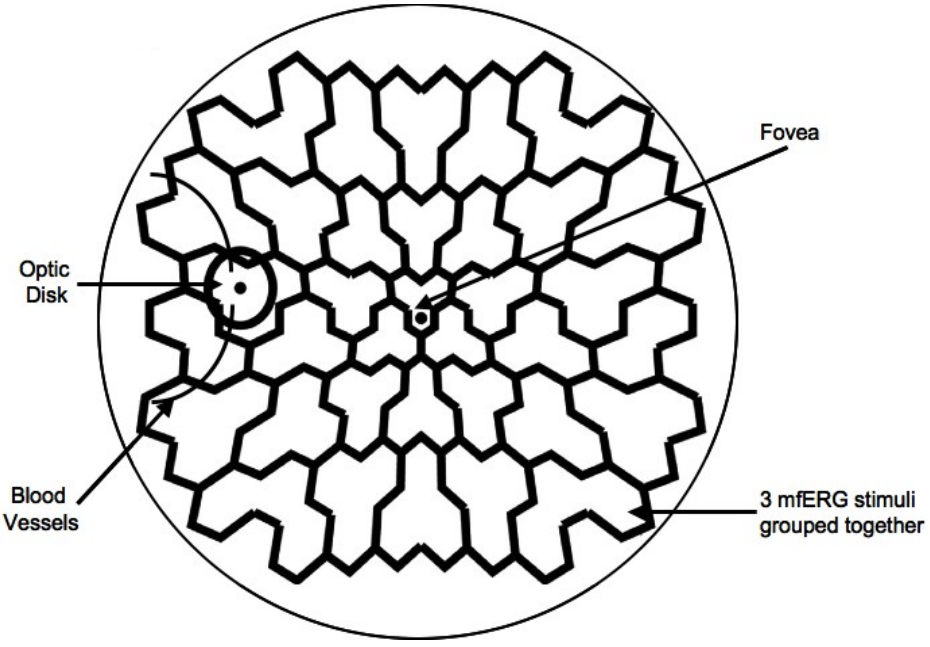

Figure 2.7: Spatial correspondence of mfERG stimulus array divided in 35 zones and the fundus photos

The mfERG implicit time for a zone is defined by the maximal Z-score i.e. most delayed within the zone, and the mfERG amplitude for a zone is defined by the minimal Z-score, i.e. most reduced, within the zone.

2.3.4.5 Statistical analysis

The statistical treatment of the data was very dependent on the experimental issues addressed in each study and is therefore discussed in each of individual chapters and is discussed in detail where appropriate. In most of the studies logistic regression methods were used to examine the relationships between various measures of retinal health. Logistic regression[29] allowed 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: Log (p/1-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), retinal thickness (categorical), gender (binary), age (continuous), and mfERG IT and mfERG Amp (both continuous).

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Chapter 3: Relationships of mfERG with retinal thickness in absence of retinopathy

3.1 Preface

This study is done in cross section to evaluate the relationship between retinal thickness and neuroretinal function. Retinal thickness is measured using oculo coherence tomography (Stratus OCT) and neuroretinal function is assessed using multifocal electroretinograms (mfERG). In previous studies done in our lab we have shown mfERGs to be sensitive to the retinal changes in diabetes. OCT is a state of art technique to evaluate changes in retinal thickness. Our interest is to study if changes in retinal thickness and in mfERGs have any spatial correspondence or coincidence in patients that are at an early stage of diabetes and do not have any retinopathy. Furthermore we are interested to see if there is any spatial agreement in the occurrence of abnormalities in both measures. It would be a very important finding if the study could shed some light on whether these two techniques can be used as surrogates of each other or not.

In conclusion, we see no difference in retinal thickness among the study groups. Also there is a lack of correlation or association between retinal thickness and neuroretinal function. In the future, we will analyze individual layers of retina to reveal changes due to diabetes. We plan to test the relationship between individual retinal layer changes and neuroretinal function.

This study was presented as a poster at the 2010 ARVO meeting and also presented with more subjects in the 2010 AAO meeting. Abstracts included in Appendix of this thesis. This chapter has also been published in the scientific literature in *Investigative Ophthalmology and Vision Science*. The manuscript and this chapter are very much identical.

a) Kavita P. Dhamdhere, Marcus A Bearse Jr, Wendy W Harrison, Kevin Bronson-Castain, Shirin Barez, Marilyn E Schneck, Anthony J Adams Local Associations Between Retinal Function And Thickness Changes In Diabetes Without Retinopathy. AAO 2010 E-abstract 105891

b) Bearse MA, Dhamdhere K, Harrison WW, Bronson-Castain K, Barez S, Schneck ME, Adams AJ. Local Relationships Between Retinal Thickness And Functional Changes In Diabetes. Invest Opthalmol Vis Sci. 2010; 51: ARVO E-abstract 5070.

c) Dhamdhere KP, Bearse MA Jr, Harrison W, Barez S, Schneck ME, Adams AJ. Associations Between Local Retinal Thickness and Function in Early Diabetes. Invest Ophthalmol Vis Sci. 2012 Aug 7.

3.2 Introduction

Diabetes affects the eye in many ways, and diabetic retinopathy is the most common and serious ocular complication.[1] As the worldwide prevalence of diabetes continues to increase, diabetic retinopathy remains a leading cause of vision loss and blindness in many countries.[2] Of the 285 million people worldwide with diabetes, about a

third have signs of diabetic retinopathy, and a third of these might develop vision-threatening retinopathies. In the United States alone, 24,000 new cases of preventable blindness due to diabetes occur every year.[3]

Contemporary treatment modalities for diabetic retinopathy are aimed at preserving vision once diabetic retinopathy is clinically evident, as opposed to preventing diabetic retinopathy. Laser photocoagulation and intraocular injections of steroids and anti-VEGF agents remain the mainstay of ophthalmic therapy for vision-threatening retinopathies. Despite the often remarkable efficacy of these therapies in slowing further vision loss, they are either associated with significant ocular side effects or they are invasive and destructive. Additionally, even with adequate therapy, reversal of vision loss and prevention of further retinal damage is uncommon. Therefore, researchers continue to search for new and increasingly effective therapeutic strategies, aiming to improve and save vision at an earlier or even subclinical stage of the disease.

The pathophysiology of diabetic retinopathy has two important components, functional and structural. However, the current definitions of retinopathy, its treatment protocol and the endpoints for clinical trials focus on either the structural aspects of the disease or visual acuity. There is a growing body of clinical and experimental evidence pointing to the presence of neuronal and glial abnormalities in early stages of diabetes, before vascular lesions are clinically apparent, resulting in dysfunction and even degeneration of these retinal cells.[4-8] By the time neuronal injury is reflected in routine clinical visual acuity testing, retinopathy has usually progressed to advanced stages. Unfortunately, it is unclear at this time, how changes in neuronal function or vision function are related to structural changes in retinopathy like retinal thickness changes.

Our previous work along with other studies have shown that the mfERG is sensitive and specific to the neurodegenerative or functional changes that precede clinical signs of diabetic retinopathy. [9-13] Significant mfERG IT delays are locally predictive of future non-proliferative retinopathy.[13-16]

OCT is a sensitive and reliable technique that enables us to acquire high-resolution, in vivo images of the central retina. It has the potential to enhance early diagnosis and objective evaluation of structural damage in retinopathy. OCT non-invasively generates tomographic images that are similar to tissue sections and permits visualization of morphological changes in the retina due to diabetes that had previously only been possible with histopathology.[17,18] It has recently become the state of art technique to evaluate retinal thickness and changes in diabetes and other diseases.

Despite the fact that both mfERG and OCT techniques are effective and sensitive in detecting "subclinical" functional and structural changes in the retina due to diabetes, their relationships, especially at pre-retinopathy stages of the disease, have not been explored. A better understanding of their possible relationships is important for advancing the development of better diagnostics, preventatives and therapeutic interventions at early stages of diabetic retinopathy.

In this study we compare these techniques, mfERG to assess the functional neural health of the retina, and OCT to assess retinal thickness. One of our motivations is to determine whether mfERG and OCT can serve as surrogate measures for each other of early retinal effects of diabetes. We investigate potential local relationships between these two measures and examine whether the type of diabetes influences any relationship. Specifically, we test whether mfERG and retinal thickness abnormalities show spatial

agreement with each other. We analyze local retinal thickness with novel methodology developed in one of our previous studies that allows us to compare retinal structure and function at 37 locations in the central 20°.[19]

3.3 Methods

3.3.1 Subjects

This cross-sectional observational study involved 76 participants made up of 29 non-diabetic healthy controls (Control group; 13 males and 16 females), and 47 patients (20 males and 27 females) with clinical diagnosis of diabetes and no diabetic or other form of retinal or ocular pathology (Table 3.1). 10 patients with Type 1 diabetes (Type 1 group) and 37 patients with Type 2 diabetes (Type 2 group) were examined. All subjects were between 25 to 65 yrs of age with a mean age of 47.0±12.8 yrs, 46.6 ± 13.2 and 53.2 ± 8.7 yrs for controls, Type 1 group and Type 2 group, respectively. Subjects had best-corrected visual acuity of 20/20 or better, clear ocular media, and refractive errors within the range of ±6D. The purpose of the study and potential risks were explained to the subjects before obtaining their informed written consent to participate in the study. The protocol is based on the tenets of the Declaration of Helsinki and was approved by the University of California Committee for Protection of Human Subjects.

Table 3.1: Characteristics of study participants

Subject Group	Gender		Mean Age (yrs)	Mean DM Duration (yrs)	Mean HbA1c %
	Males	Females			
Control	13	16	47.0 ± 12.8	NA	NA
Type 1	3	7	46.6 ± 13.2	17.2 ± 13.5	8.2 ± 1.4
Type 2	17	18	53.2 ± 8.7	7.81 ± 4.8	8.6 ± 1.6

3.3.2 mfERG Recordings

mfERGs were recorded using a visual evoked response imaging system (VERIS Science 4.3; EDI, San Mateo, CA) and dilated pupils (6 – 8 mm diameter). Dilation was achieved using 1.0% tropicamide and 2.5% phenylephrine hydrochloride. The stimulus array consisted of 103 hexagonal elements that were displayed at a 75 Hz frame rate on a monochrome CRT. The sizes of the hexagons were scaled with eccentricity to account for cone density (Fig.3.1). The luminance of each hexagon was independently alternated between black (<2cd/m^2) and white (200cd/m^2) according to a pseudorandom binary m-sequence. The stimulus array subtended approximately 45° on the retina, centered on the fovea. Each recording was made up of 16 segments of approximately 25 seconds each. Retinal activity was recorded with a Burian-Allen bipolar contact lens electrode (Hansen Ophthalmic, Solon City, IA, USA) filled with 1% carboxymethylcellulose sodium (Refresh Celluvisc, Allergan Inc., Irvine, CA, USA), which was placed on the anesthetized (0.5% proparacaine) cornea. A ground electrode was clipped to the right

earlobe and electrode impedance was kept bellow 5 kΩ. Fixation was controlled by a fixation target 'X' in the center of the stimulus and by monitoring displacements of the lens and eye movements using an in-line infrared camera. Contaminated recording segments were discarded and rerecorded. The signals were amplified 100,000 times, filtered 10 - 100 Hz and sampled at 1200 Hz. The fellow eye was covered with an eye patch.

3.3.3 mfERG Analysis

Each of the 103 first-order local mfERG responses was analyzed with one iteration of artifact removal and spatial averaging with 1/6th of the surrounding responses. The responses were measured using the template scaling method described in detail by Hood and Li.[20] Each of the local responses was compared to a waveform template constructed by averaging the corresponding local waveforms of the control subjects (right eye response arrays were converted to left eye orientation). Each template was independently scaled in amplitude and time dimensions such that subject's local waveform and local template had minimal least-squares difference. Statfit, a measure of goodness of fit, was generated and responses with statfits greater than or equal to 0.8 were not included in analysis. The first negative peak (N1) and the first positive peak (P1) of the local mfERG response waveforms were identified and the N1- P1 amplitudes (AMP) and P1 implicit times (IT) were measured. The P1 implicit times were measured from the onset of the local stimulus flash to the P1 peak. The Control group's mean and standard deviation for each local mfERG measure were used to compute Z-scores for the patients' corresponding mfERG responses.

3.3.4 Retinal Thickness Measurements

The Stratus OCT3 (Zeiss Meditec, Dublin, CA) was used to measure retinal thickness. Twelve radial B-scans (6 mm long- central 20°, 512 A-scans, 10 μm axial and 11 μm longitudinal resolution) centered at the foveola were acquired from the eyes fixating a green target (Fig. 3.1). Scans contaminated by fixation loss, blink or eye movement were discarded and recaptured. Only scans with a signal strength of ≥ 6 out of a possible 10 were included for analysis (mean signal strength was 7.1± 1.3). The scan acquisition time ranged from 1 to 2 seconds. Retinal thickness was calculated as the distance between the first signal from the vitreoretinal interface and the signal from the outer border of the retinal pigment epithelium.

3.3.5 Retinal Thickness Analysis

Retinal thickness was analyzed as previously described (Neuville, et al. 2009). Thickness measurements were exported in left eye orientation from the OCT instrument and processed in Matlab (MathWorks, Natick, MA). A continuous 360° pseudocolor retinal thickness map was generated by interpolating the points between each scanned point using triangle-based linear interpolation and fitting this to a 1024×1024 grid. The thickness map was then divided into 37 hexagons such that they correspond to the central 37 hexagons in the mfERG stimulus (Figure 3.1). Thickness values falling on the

boundary of hexagons were excluded in a six-sample-wide uniform exclusion area. The number of A-scans within each hexagon ranged from 63 at a peripheral location to 707 at the center hexagon. The total number of data points within a hexagon, including interpolated points, ranged from 5,020 to 17,637. An average thickness of each hexagon was computed. Retinal thickness values were converted to percentile ranks based on the retinal thickness values from controls (which were not normally distributed).

Figure 3.1

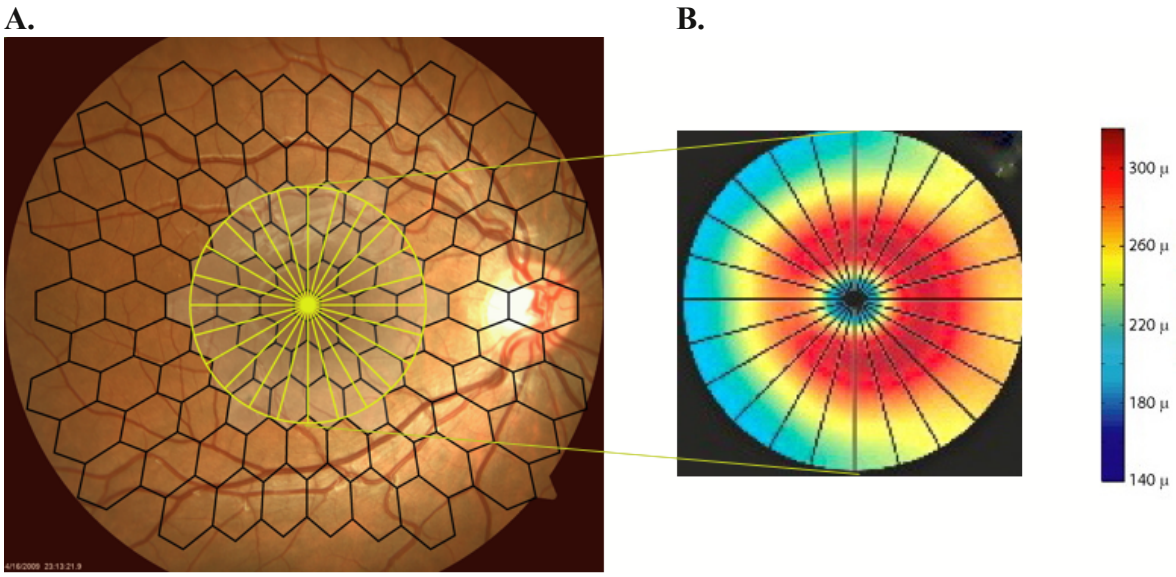

Figure 3.1: The central 37 mfERG stimulus hexagons corresponding to the central 20° of OCT radial scans (A). Pseudo-color map of retinal thickness generated by interpolating the points between each scan using triangle-based linear interpolation (B).

3.3.6 Statistical Analysis

T-tests were used to examine whether the subject groups differed significantly from each other in any of the measurements (IT, AMP, and retinal thickness). An abnormal mfERG response for each hexagon was defined as a Z-score value ≥ 2 ($P < 0.023$) for IT, and a Z-score ≤ -2 ($P < 0.023$) for AMP. Retinal locations with thickness values beyond or equal to the 97.7th percentile of the Control group were defined as abnormally thick and values below or equal to the 2.3rd percentile of the Control group were defined as abnormally thin. Eyes having 4 or more abnormal locations ($P < 0.023$) were defined as abnormal eyes for each particular measure (e.g., 4 abnormally thin retinal locations made the eye abnormally thin). Proportions tests were performed to examine whether the subject groups differed in their frequencies of abnormalities.

One eye of each subject was included in the analysis, the eye with both a better statfit value (defined in mfERG methods section above) and a better OCT scan quality (minimum signal strength of 6). If both eyes of a subject had equal statfit and scan strength values, the left eye was included. Spatial associations between the mfERG and retinal thickness were examined locally within the subject groups and also within

individual subjects. A 2 × 2 cell Fisher exact test for significance was performed to identify any association between local retinal thickness and either IT or AMP. Linear regression analysis was not used because a linear relationship was not assumed.

In a second analysis, abnormal retinal thinnesses or thicknesses were examined to investigate their spatial agreement with either abnormally delayed IT or abnormally diminished AMP. A 2 × 2 cell Fisher exact test for significance was performed to identify any spatial agreement between the abnormalities.

3.4 Results

To preview the main findings of this study, retinal function in the Type 2 group was worse than the Type 1 group, and the two groups differed in retinal thickness abnormalities. There were no local associations between retinal thickness measured with OCT and retinal neural function measured with mfERG in adult patients with diabetes who had no diabetic retinopathy. There was also no spatial correspondence of functional and anatomical abnormalities. These results were obtained in subject group comparisons and also within individual eyes.

3.4.1 Subject group differences

Potential differences between the three subject groups were examined. The Type 1 group was significantly younger ($P< 0.001$) with almost double the duration of diabetes than the Type 2 group. The Type 1 and Type 2 subject groups were significantly different ($P< 0.001$) from each other in both IT and AMP measures (Fig. 3.2). The Type 2 group had significantly delayed IT and diminished AMP ($P< 0.001$) compared to the controls, but subjects with Type 1 diabetes and controls had similar IT and AMP. Mean retinal thickness was similar in all the three subject groups.

Figure 3.3 shows the percentages of abnormalities for the different functional and anatomical measures. Abnormalities are defined as explained in the method section. We found only one eye with mixed thickness abnormalities (2 abnormally thin and 2 abnormally thick hexagons in same eye). The frequency (percentage) of mfERG IT and AMP abnormalities in the Type 2 group was greater than that in the Control and Type 1 groups (Fig. 3.3; $P < 0.001$ for all). The Type 1 group had a larger percentage of AMP abnormalities ($P < 0.001$), but not IT abnormalities, than the Control group. Type 1 and Type 2 diabetes were observed to affect retinal thickness differently. The Type 2 group had a larger percentage of retinal thinning abnormalities than the Control group ($P < 0.001$). In contrast, the Type 1 group had a larger proportion of retinal thickening "abnormalities" compared to the Control and Type 2 groups ($P < 0.001$ for both).

Figure 3.2

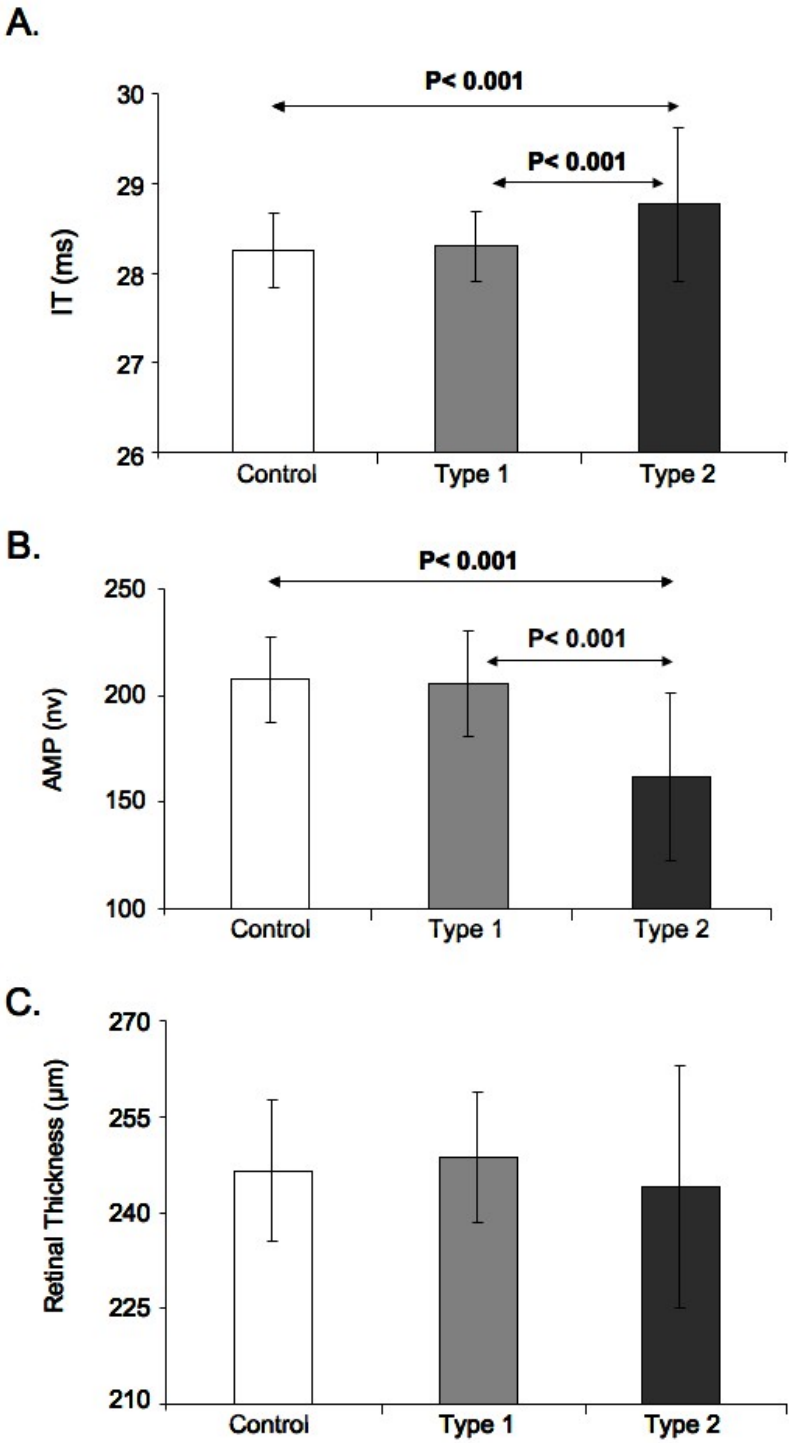

Figure 3.2: Group differences for IT, AMP and retinal thickness. The Type 2 group had longer IT and lower AMP than the Type 1 and Control groups. Retinal thicknesses of the three groups were similar.

Figure 3.3

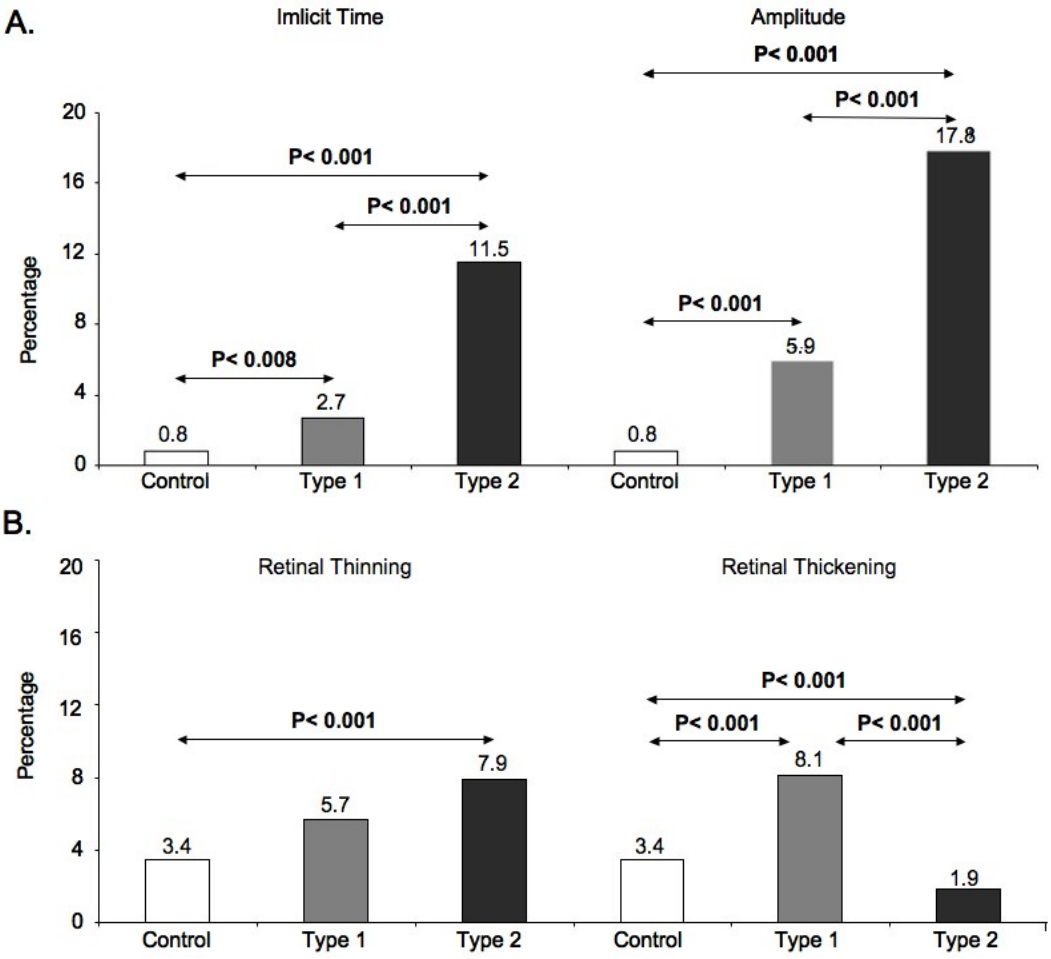

Figure 3.3: mfERG and retinal thickness abnormality percentages

3.4.2 Associations between local retinal thickness and local mfERG IT

The potential local retinal associations between retinal function and retinal thickness were examined separately for each subject group. No spatial associations between retinal thickness and IT were found in any subject group. Figure 3.4 shows the results from the Type 2 subject group as an example. In this figure, IT Z-scores from 37 retinal locations are plotted against retinal thickness percentiles in the 37 corresponding locations for all patients with Type 2 diabetes. This distribution does not reveal an association. This finding represents what we also observed in the Control and Type 1 subject groups.

Figure 3.4

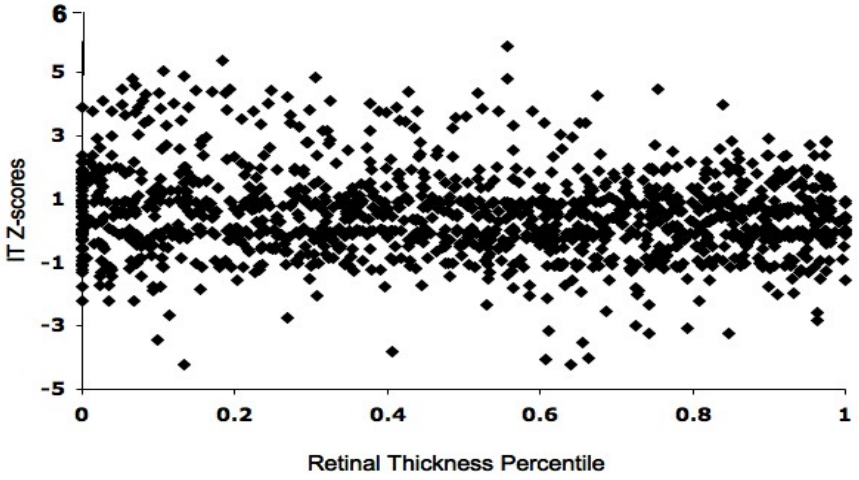

Figure 3.4: Association between retinal thickness and mfERG IT in the Type 2 group

When individual retinas were examined, no consistent associations were found between local retinal thickness and local mfERG measurements within any subject group. None of the subjects in the Type 1 group had a significant association between local retinal thickness and IT. Some of the subjects in the Type 2 and Control groups showed a significant association but the association was positive for some subjects (retinal thickening is associated with longer IT or larger AMP and retinal thinning is associated with fast IT or reduced AMP) and negative (retinal thinning is associated with longer IT or larger AMP and retinal thickening is associated with fast IT or reduced AMP) for others. In fact, the number of subjects with a positive association was equal to the number of subjects with a negative association in the Control group. Table 3.2 summarizes the percentage of subjects in each group who had significant associations (p-value< 0.001) between retinal thickness and IT.

Table 3.2: Percentages of eyes with significant associations between retinal thickness and mfERG IT

Subject Group	Positive association (%)	Negative association (%)
Control	10.3	10.3
Type 1	0.0	0.0
Type 2	5.7	11.4

3.4.3 Associations between local retinal thickness and local mfERG AMP

Spatial associations within the subject groups were examined first. There were no significant spatial associations between retinal thickness and AMP in any of the three subject groups. Figure 3.5 shows the results obtained from the Type 2 group as an example.

AMP Z-scores from 37 retinal locations are plotted against the retinal thickness percentiles of the 37 locations for all subjects with Type 2 diabetes. The distribution does not reveal an associative trend. This was also observed in the Type 1 and Control subject groups.

Figure 3.5

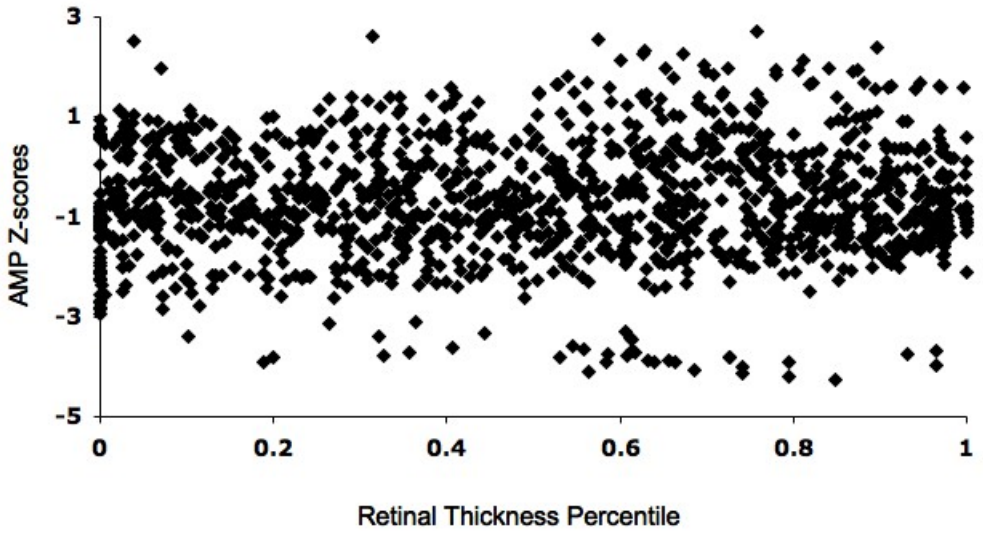

Figure 3.5: Association between retinal thickness and mfERG AMP in the Type 2 group

Next, potential associations between local retinal thickness and AMP were tested within each individual within each group. Some of the subjects in each group showed a significant association but this finding was not consistent. For example, the percentages of subjects with positive and negative associations were equal (10.0%) in the Type 1 group. The percentages of subjects with a positive association were also low and approximately equal to the number of subjects with a negative association in the Control and Type 2 subject groups, though in these groups positive associations were slightly more likely than negative ones. Table 3.3 summarizes the percentages of subjects in each group who had significant associations (p-value< 0.001) between retinal thickness and AMP.

Table 3.3: Percentages of subjects showing significant associations between local retinal thickness and mfERG AMP

Subject Group	Positive association (%)	Negative association (%)
Control	10.3	6.9
Type 1	10.0	10.0
Type 2	17.1	11.4

3.4.4 Spatial associations between retinal thickness abnormalities and mfERG abnormalities

Within all subject groups, retinal thickness abnormalities (both thinning and thickening) did not show evidence of significant spatial agreement with either IT or AMP. The p-values for the Fisher exact analysis test results are shown in Table 3.4. The occurrence of abnormalities was low, as shown earlier in Fig. 3.3, and not consistent across the individual subjects. Therefore, the total number of abnormalities in the different measures was too small to allow for statistically valid examination of potential associations within individual subjects.

Table 3.4: P-values for the Fisher exact analyses are shown for the abnormality associations

Subject Group	IT vs Thinness	IT vs Thickness	AMP vs Thinness	AMP vs Thickness
Control	0.99	0.99	0.99	0.99
Type 1	0.99	0.53	0.62	0.66
Type 2	0.09	0.16	0.11	0.10

3.5 Discussion

Our results indicate that there is no significant spatial correlation or association between full retinal thickness and either mfERG AMP or mfERG IT. In addition, the absence of agreement of spatial location of abnormalities suggests that significant mfERG response changes in diabetic patients without retinopathy are not related to significant retinal thickness changes. The subjects included in this study have fairly well preserved retinal function and structure, with no clinical signs of retinopathy. Therefore, the lack of spatial agreement between retinal thickness and mfERG abnormalities could be related to the fact that these patients are at early stages of diabetic eye disease.

In our study, Type 2 patients have worse mfERG retinal function as compared to Type 1 patients despite the fact that their duration of diabetes is about half the duration of diabetes of Type 1 patients and their glucose control assessed by HbA1c is similar. The finding is in agreement with other studies done in our lab. It is known that Type 1 and Type 2 diabetes have very different presentations and that the two diseases affect vascular and neural health with different time courses and natural histories. The reasons for the retinal function differences we observed between the Type 1 and Type 2 groups, therefore, could be related to differences in the pathophysiologies and co-morbidities of the two diseases.

It appears that, in both Type 1 and Type 2 diabetes without retinopathy, retinal thickness measurements and mfERG findings are not identifying similar aspects of retinal changes. The two measures also differ in their capabilities to identify significant early retinal changes as diabetes affects structure and function, with more abnormalities observed for the mfERG than for retinal thickness. Perhaps the most important conclusion from these results is that the mfERG and OCT measurements of full retinal

thickness cannot be treated as surrogates of each other in the evaluation of retinal changes produced by diabetes, at least before the onset of diabetic retinopathy.

To our knowledge, our study is the first to quantitatively analyze the potential associations between local retinal thickness changes assessed by OCT and local functional changes assessed by mfERG in patients with diabetes and no retinopathy. Previous studies performed to correlate retinal function and structure have provided mixed results. Some have found significant correlation[21], others found weak association.[22] Most of the previous studies have examined eyes with advanced stages of retinopathy, and insensitive psychophysical vision measures, such as visual acuity, have been used as a measure of retinal function. To date, no other study has examined local retinal structure-function relationships in early diabetes before signs of diabetic retinopathy can be seen clinically. Apart from the selection criteria for patients, the main difference between this study and the other studies is the method used to analyze retinal thickness. In our study, we used a co-registration technique to overlay topographic structural and functional maps of the retina that facilitate the local evaluation of 37 different locations in central 20° of the retina. This allowed us to verify our results in multiple small sectors of retina.

The primary limitation of our study was the relatively small number of patients in the Type 1 subject group. In addition, since this was a cross-sectional study, the results of a similar study focused on patients with more advanced, clinical stages of diabetic retinopathy might yield different results. Despite these potential limitations, we have observed that local functional changes are not associated with local retinal thickness changes in patients with diabetes without retinopathy.

A recent study found a strong correlation between specific retinal layer thickness (not full thickness) and visual acuity in late stages of diabetic retinopathy.[23] New knowledge and understanding of local structure-function relationships will help create effective therapies that could save vision at an earlier or even subclinical stage of diabetic eye disease and revolutionize care of diabetic retinopathy. We are planning in the future to use segmentation techniques to examine potential relationships between the mfERG and the thickness of different retinal layers in early diabetes.

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Chapter 4: Associations between letter contrast sensitivity and multifocal electroretinograms in diabetes

4.1 Preface

In this chapter I discuss a cross-sectional study to explore the relationships between letter contrast sensitivity vision function and multifocal electroretinograms (mfERG) neural function of the retina in patients with diabetes. Letter contrast sensitivity was tested in all study participants using Pelli-Robson charts, and mfERGs were recorded from the central 45 degrees of the retina at the same visit. Log contrast sensitivity was compared with mfERG implicit time (IT) and mfERG amplitude (AMP) using linear regression. 46 non-diabetic healthy controls, 40 participants with type 2 diabetes and no retinopathy, 28 participants with type 2 diabetes and non-proliferative retinopathy and 12 participants with type 1 diabetes and no retinopathy participated in the study.

It is well established that diabetes has an effect on electrophysiological neural retina and psychophysical measures of vision. During the course of the disease diabetes affects the vascular and neural health globally leading to end organ damage all over body. Retinopathy is one of major end organ complications. Diabetes and diabetes-induced retinal changes underlie the abnormalities in vision function and the neural electrical activity in the retina long before any clinically detectable morphological changes occur.[1-3] Past studies from our lab and others have shown that mfERG IT is delayed by changes to the retina due to diabetes. Diabetes has been shown to affect contrast sensitivity.[4,5]

The primary purpose of this study is to examine the possible relationships between letter contrast sensitivity with mfERG IT and the mfERG AMP measures of neural function of the retina in diabetes. We examine whether retinopathy status influences the relationships. This study first examines whether letter contrast sensitivity is different between participants with diabetes and the controls and whether the type of diabetes and the presence of background retinopathy influence this relationship. We also examine whether letter contrast sensitivity is related to the duration of diabetes and blood glucose control.

To briefly summarize the results, we found that mfERG IT delays in the central retina (Central 45 degrees) were associated with reduced letter contrast sensitivity in adults with type 2 diabetes and retinopathy. This was observed even though most of the retinopathy was mild non-proliferative retinopathy and most of the central retina was retinopathy-free in the majority of the eyes. In type 1 diabetes, we saw no significant relationship between letter contrast sensitivity and mfERG. The full study is not yet published but was presented at Association for Research in Vision and Ophthalmology 2011 and at the American Academy of Optometry meeting in 2011. Abstracts are included in the appendix of this thesis.

a) Kavita P. Dhamdhere, Marcus A. Bearse, Jr., Brian E. Wolff, Wendy W. Harrison, Maria Cardenas, Shirin Barez, Marilyn E. Schneck, Anthony J. Adams. Associations Between Contrast Sensitivity And Multifocal Electroretinograms In Type 2 Diabetes. Invest Opthalmol Vis Sci. 2011; 52: ARVO E-abstract 1271/A21

b) Kavita P. Dhamdhere, Marcus A. Bearse, Jr., Wendy W. Harrison, Shirin Barez, Marilyn E. Schneck, Anthony J. Adams. Contrast Sensitivity And Multifocal Electroretinograms Associations In Adult Patients With Diabetes. AAO 2011 E-abstract 115573

4.2 Introduction

Early detection of diabetic damage to the retina has become a focus of research, as the worldwide prevalence of diabetes is increasing at an alarming rate.[6] Diabetic retinopathy remains the number one cause for preventable blindness.[7] Researchers have proven that early interventions can prevent vision loss and retinal structural damage in later stages of diabetes that can lead to blindness.[8] With the advent of pharmacological therapies aimed at slowing down or halting the progression of diabetic retinopathy in its early stages, the demand for early detection and better understanding of early effects of diabetes on retinal structure and function becomes increasingly important.[9,10]

Currently the assessment of retinal function loss in patients with diabetes, especially in early stages, relies heavily on visual acuity as a gold standard for vision function screening. Unfortunately, by the time the patients with diabetes start experiencing the visual loss, massive subclinical damage to the retina has already occurred. In our previous studies we have shown that the multifocal electroretinogram (mfERG) is sensitive and specific to detect such subclinical damage to the neural retina that precede clinical signs of diabetic retinopathy.[11-15]

Contrast sensitivity is a sensitive measure of subtle retinal function changes in diabetic retinopathy and diabetic macular edema.[16-18] It is quick to perform and cost effective.[4,5,19] Contrast sensitivity seems to be affected in asymptomatic stages of retinopathy as a result of changes in the retinal neurons and is associated with the presence of retinopathy. [4,5]

A better understanding of the relationship between mfERG and letter contrast sensitivity may prove important for advancing the development of better diagnostics, screening, preventatives, management and therapeutic interventions especially at early stages of diabetic retinopathy.

The purpose of this study is to determine, in patients with diabetes with normal visual acuity, the nature of the association, if any, between retinal electrophysiological function assessed using mfERG and retinal psychophysical function assessed using a letter contrast sensitivity test. We want to test whether letter contrast sensitivity can serve as a surrogate measure for mfERG in the detection of early effects of diabetes on the neural retina. We investigate potential local relationships between these two measures and examine whether the type, duration and control of diabetes influences any of these measures or their relationships.

4.3 Methods

4.3.1 Study participants

This cross-sectional, observational study included single eyes of 126 participants. Forty participants with type 2 diabetes and no retinopathy (T2NoRet group; 20 males and 20 females) and 12 retinopathy free participants with type 1 diabetes (T1Noret group; 5 males and 7 females), 28 participants with type 2 diabetes and mild to moderate non-

proliferative diabetic retinopathy (NPDR group; 13 males and 15 females), and 46 age matched controls (Control group; 19 males and 27 females) were studied. Their mean ages were 53.6 ± 8, 37 ± 9.6, 53.3 ± 7 and 49.7 ± 11.4 yrs for the T2NoRet, T1Noret, NPDR and control groups, respectively. Diabetes duration was 7.7 ± 4, 14 ± 6.2 and 8 ± 3.8 yrs for the T2NoRet, T1Noret and T2NPDR group, respectively. (Table 4.1) All participants had visual acuity of at least 20/20, refractive error ranging between ± 6 D and no lenticular opacities. All the participants were free of any ocular pathology except for the participants in NPDR group who had mild to moderate retinopathy at the time of study. The retinopathy in most eyes was in the form of a couple dot or blot retinal hemorrhages and or a couple of microaneurisms. The purpose of the study, the entire protocol, participants' rights as a research subject and potential risks were explained to every participant before obtaining their informed written consent to participate in the study. The protocol adheres to the tenets of the Declaration of Helsinki and was approved by the internal review board of University of California Committee for Protection of Human Subjects.

Table 4.1: Participant characteristics

Group	Number of Subjects (Females/Males)	Mean Age (yrs ± SD)	Mean Diabetes Duration (yrs)	Mean HbA1c %
Control	46 (27/19)	49.7 ± 11.4	NA	5.8 ± 0.9
T1NoRet	12 (7/5)	37.0 ± 9.6	14.0 ± 6.2	7.8 ± 1.8
T2NoRet	40 (20/20)	53.6 ± 8.0	7.7 ± 4.0	8.2 ± 1.5
T2 NPDR	28 (15/13)	53.3 ± 7.0	8.0 ± 3.8	8.9 ± 1.3

4.3.2 Letter contrast sensitivity testing and analysis

Visual acuity was measured in each eye using a high-contrast Bailey-Lovie chart[20] and participants with visual acuity 20/20 or better were included in the study. Letter contrast sensitivity was tested in each eye using a Pelli-Robson letter chart (Figure 4.1).[21] Participants wore the appropriate habitual prescription for testing. The Pelli-Robson chart includes letters of the same size grouped into triplets, where two groups appear on each line of the chart. It tests contrast sensitivity for the 3 to 5 cycles/deg spatial frequency. The participants were 10 feet away from the chart. They were required to start at the top left-hand corner of the chart and to read aloud each letter. The test was continued until participants got 2 letters wrong in a triplet. Guessing was encouraged and each letter read correctly was scored as 0.05 log units.

Slitlamp biomicroscopy was conducted to confirm transparency of the crystalline lens. 45° fundus photos were graded to identify the presence or absence of retinopathy and in case of its presence to identify the locations of non-proliferative retinopathy.

Figure 4.1

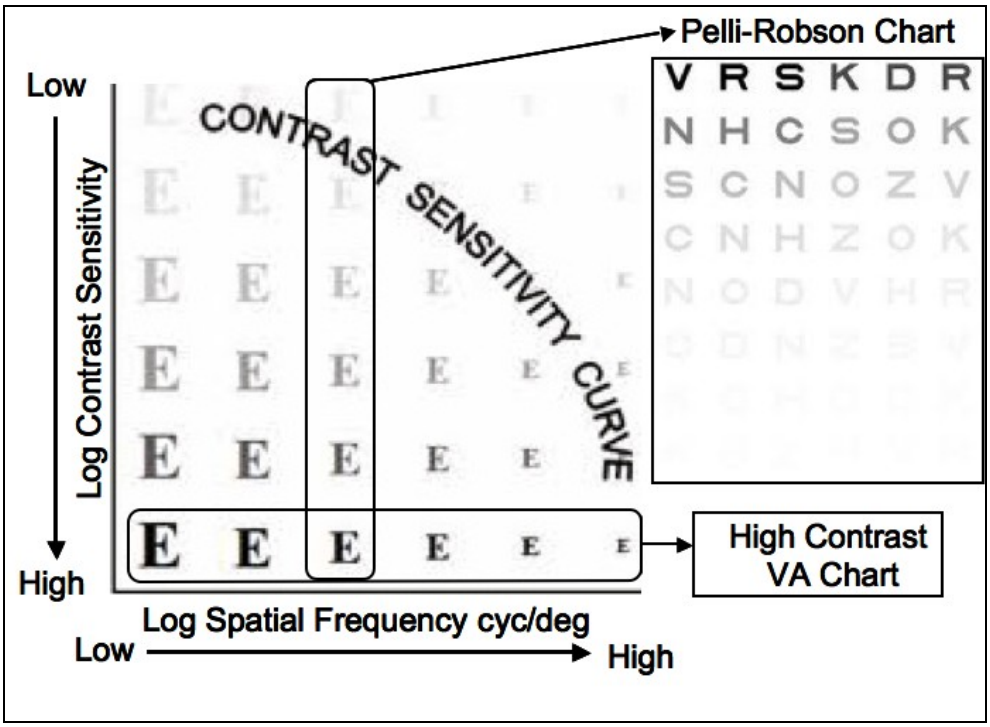

Figure 4.1: Pelli-Robson Contrast sensitivity chart. Letter size used to test the contrast sensitivity is testing sensitivity for contrast at peak sensitive spatial frequency

4.3.3 mfERG Recordings

Participants' pupils were dilated using 1.0% tropicamide and 2.5% phenylephrine hydrochloride for mfERG recording. Dilated pupil size ranged from 6 to 8 mm diameter) using a visual evoked response imaging system (VERIS Science 4.3; EDI, San Mateo, CA). Approximately the central 45° of retina was tested using a stimulus array consisting of 103 hexagonal elements displayed at a 75 Hz frame rate on a monochrome CRT. The size of the hexagons was scaled with eccentricity according to cone density (Figure 4.2). The luminance of each hexagon was independently alternated between black (<2cd/m^2) and white (200cd/m^2) according to a pseudorandom binary m-sequence. A Burian-Allen bipolar contact lens electrode (Hansen Ophthalmic, Solon City, IA, USA) was filled with 1% carboxymethylcellulose sodium (Refresh Celluvisc, Allergan Inc., Irvine, CA, USA) and was placed on the anesthetized (0.5% proparacaine) cornea and retinal activity was recorded for approximately 8 minutes using 16 recording segments. A ground electrode was clipped to the right earlobe and electrode impedance was kept bellow 5 kΩ. Fixation was controlled by a fixation target 'X' in the center of the stimulus and by monitoring displacements of the lens and eye movements using an in-line infrared camera. Contaminated recording segments were discarded and rerecorded. The signals were amplified 100,000 times, filtered 10 - 100 Hz and sampled at 1200 Hz. The fellow eye was covered with an eye patch.

4.3.3.1 mfERG Analysis

Each of the 103 first-order local mfERG responses was analyzed with one iteration of artifact removal and spatial averaging with 1/6th of the surrounding responses. The amplitude and implicit time of responses were measured using the template scaling method described in detail by Hood and Li.[22] Each of the local responses was compared to a waveform template constructed by averaging the corresponding local waveforms of the control participants (right eye response arrays were converted to left eye orientation). Each template was independently scaled in the amplitude and time dimensions such that the participant's local waveform and local template had minimal least-squares difference. Statfit, a measure of goodness of fit, was generated and responses with statfits greater than or equal to 0.8 were not included in analysis. The first negative peak (N1) and the first positive peak (P1) of the local mfERG response waveforms were identified and the N1- P1 amplitudes (AMP) and P1 implicit times (IT) were measured. The P1 implicit times were measured from the onset of the local stimulus flash to the P1 peak.

Figure 4.2

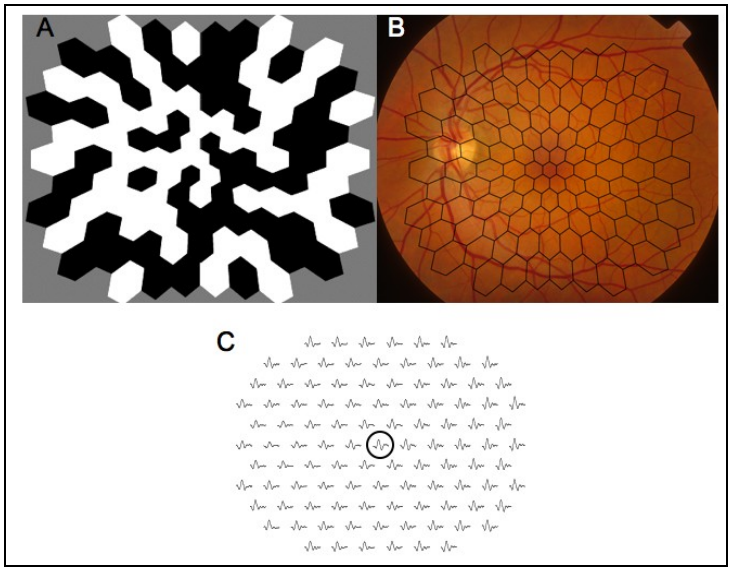

Figure 4.2: A. mfERG stimulus array consisted of 103 hexagonal elements scaled to photoreceptor density across eccentricity. B. Test area on the retina: Approximately the central 45° centered on foveola. C. 103 first-order local mfERG responses with the central response highlighted with a circle corresponding to central 2.4°

Only responses corresponding to the letter contrast sensitivity test area on retina i.e. mfERG IT and AMP coming from the very central 2.4° hexagon (Figure 4.2) were used for all statistical comparisons to test the group differences and to test the correlation with letter contrast sensitivity.

4.3.4 Statistical treatment

T-tests were used to examine whether the subject groups differed significantly from each other on any of the measurements (IT, AMP, and letter contrast sensitivity). The letter size of Pelli-Robson chart is much smaller (approximately 0.8° on retina) than the central testing zone of the mfERG stimulus array. Contrast sensitivity score in log units was compared to mfERGs from the central 2.4° using linear regression. Effects of diabetes duration and HbA1c on letter contrast sensitivity and mfERG were tested using linear regression.

4.4 Results

In summary, participants with type 2 diabetes and retinopathy (NPDR group) had the worst letter contrast sensitivity and most delayed mfERG IT. In the central 2.4°, mfERG IT in participants free of retinopathy (T2NoRet and T1NoRet groups) was similar to the controls and did not significantly differ from the controls. The patient participant groups had mfERG AMP similar to controls except being significantly reduced in the NPDR group compared to controls. Reduced letter contrast sensitivity was correlated with delayed IT only in the NPDR group. Letter contrast sensitivity was not correlated with AMP in any participant group

4.4.1 Subject group differences

Potential differences between the three subject groups were examined using t-tests. The Type 1 group was significantly younger ($P< 0.001$) with almost double the duration of diabetes than the Type 2 group. Letter contrast sensitivity was significantly different between all groups ($P<0.001$). Letter contrast sensitivity in the NPDR group was lowest (Figure 4.3), despite the fact that only 2 of these 28 participants had retinopathy within the central 2.4°. The no retinopathy groups (T2NoRet and T1NoRet) had similar IT in the central 2.4° of retina and were very similar to controls (Figure 4.3). However, the NPDR group had significantly longer IT (response delays) than the other groups ($P<0.001$). AMP in the central 2.4° of retina was significantly lower ($P<0.001$) in NPDR group than in controls.

The no retinopathy groups did not differ from one another or from the control group (Figure 4.3). Duration of diabetes and HbA1c did not have any statistically significant effect on letter contrast sensitivity (Table 4.2).

Table 4.2: Effects of diabetes duration and HbA1c on letter contrast sensitivity

Group	Duration of diabetes vs Contrast Sensitivity	HbA1c vs Contrast Sensitivity
Control	NA	$P > 0.99$
T1NoRet	$P = 0.46$	$P = 0.59$
T2NoRet	$P = 0.91$	$P = 0.81$
NPDR	$P = 0.26$	$P = 0.95$

Figure 4.3

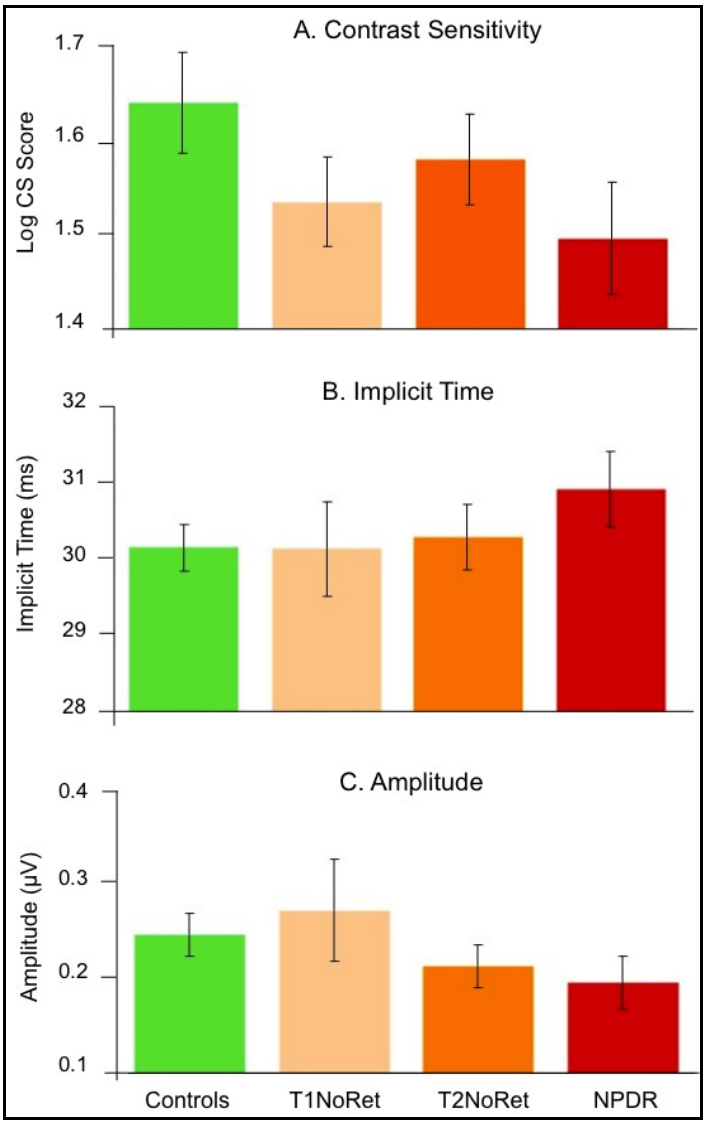

Figure 4.3: Group differences for Letter contrast sensitivity, IT and AMP. **A.** Letter contrast sensitivity is significantly different between all groups (P<0.001) and is lowest in the NPDR group **B**. IT is similar in controls and other no retinopathy groups but is significantly longer in NPDR group than the other groups (P<0.001) **C.** Controls and other no retinopathy groups have similar AMP, but in the NPDR group AMP is significantly lower than controls (P<0.001). The error bars are the standard errors.

4.4.2 Correlation of letter contrast sensitivity with mfERG IT and AMP

mfERG IT from the central 2.4° of retina and letter contrast sensitivity did not show any correlation in controls or in patients with either type 1 diabetes or type 2 diabetes in the absence of retinopathy. Table 4.3 summarizes P-values of all the comparisons. Patients in the NPDR group show a negative correlation between letter contrast sensitivity and mfERG IT. In other words, reduced letter contrast sensitivity was correlated (P<0.0001) with longer (more abnormal) IT from the central 2.4° of retina (Figure 4.4).

mfERG AMP from central 2.4° of retina and letter contrast sensitivity did not show any correlation in controls or in patient groups. Presence of retinopathy did not modify this non-correlation. Table 4.3 summarizes P-values of all the comparisons.

Figure 4.4

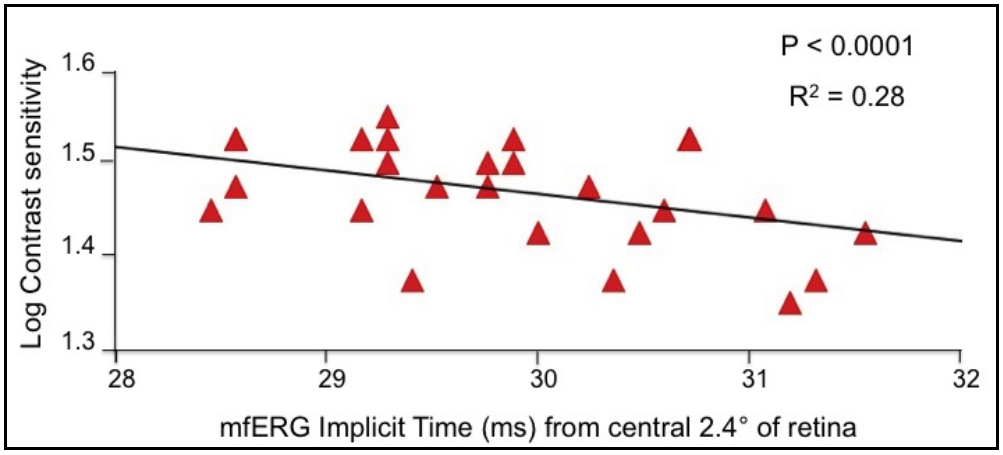

Figure 4.4: Correlation between Implicit time from central 2.4° of retina and letter contrast sensitivity. Reduced letter contrast sensitivity was correlated negatively with longer IT from the central 2.4° of retina in the NPDR group. Each data point represents an individual participant with type 2 diabetes and NPDR.

Table 4.3: T-test P-values: Statistically significant P-values are highlighted in bold

Measure	Letter Contrast Sensitivity	IT	AMP
Control vs T1NoRet	**P < 0.001**	P = 0.97	P = 0.40
Control vs T2NoRet	**P < 0.001**	P = 0.59	P = 0.04
Control vs NPDR	**P < 0.0001**	**P < 0.01**	**P = 0.005**
T1NoRet vs T2NoRet	P = 0.19	P = 0.69	P = 0.06
T1NoRet vs NPDR	P = 0.32	P = 0.06	**P = 0.02**
T2NoRet vs NPDR	P = 0.03	P = 0.06	P = 0.32

4.5 Conclusion and discussion

The results of this study indicate no significant correlation or association between letter contrast sensitivity and either mfERG AMP or mfERG IT from central 2.4° of retina in the absence of retinopathy. This is true for both types (Type 1 and 2) of diabetes.

The participants included in this study have fairly well preserved retinal function. The lack of correlation between letter contrast sensitivity and mfERG function could be related to the fact that these patients are at the early stages of diabetic eye disease. Once diabetic damage to the retina becomes clinically evident in type 2 diabetes, letter contrast sensitivity shows statistically significant negative correlation with mfERG IT but not with mfERG AMP. This was observed even though most of the retinopathy was mild and the central retina was retinopathy free in most eyes (93%).

In our study, mfERG retinal function is worse in patients with NPDR. All the participants with diabetes, irrespective of type of diabetes or status of retinopathy have reduced amplitude, but participants in NPDR group have longer IT and the lowest AMP. Letter contrast sensitivity is reduced in all participants with diabetes and the finding remains the same in both type of diabetes. Letter contrast sensitivity appears to further decline once retinopathy becomes clinically evident in type 2 diabetes. Duration of diabetes and diabetes control do not alter this finding. The reduction in letter contrast sensitivity is interesting, as all these participants have normal visual acuities and otherwise well preserved retinal function like low contrast visual acuity, color vision and mfERG.

It appears that, in both type 1 and type 2 diabetes without retinopathy letter contrast sensitivity, measured using Pelli-Robson charts, and mfERG findings are not identifying similar aspects of retinal neural changes. Though the letter contrast sensitivity varies over a range of 8 letters in these participants, the mfERG function does not have much variability. This restriction of range may be influencing the lack of correlation. The two measures also differ in their sensitivity to significant early retinal changes, with abnormalities observed for letter contrast sensitivity while the mfERG function in central 2.4° of retina is not significantly reduced compared to normal. Perhaps the most important conclusion from these results is that the mfERG and letter contrast sensitivity function cannot be treated as surrogates for each other in the evaluation of changes in neural retinal function produced by diabetes, at least before the onset of diabetic retinopathy.

Presence of retinopathy in type 2 diabetes affects retinal neural function both electrophysiologically and psychophysically as reflected in delayed mfERG IT, reduced mfERG AMP and reduced letter contrast sensitivity in central 2.4° of retina. Once the neural function, as measured by letter contrast sensitivity and mfERG is abnormal, the two measures establish a relationship. Unfortunately this study did not include any participants with type 1 diabetes and retinopathy. We do not know how the patients with type1 diabetes would behave in terms of letter contrast sensitivity and mfERG function once retinopathy is established. The study of Type 1 diabetes without retinopathy is also limited, in this study, by the small number of participants without diabetes.

To our knowledge, our study is the first to quantitatively analyze the potential associations between letter contrast sensitivity assessed by Pelli-Robson charts and local neural functional changes assessed by mfERG in patients with diabetes. Most of the previous studies have examined eyes with advanced stages of retinopathy, and relatively insensitive psychophysical vision measures, such as visual acuity, have been tested to determine relationships with either mfERG or letter contrast sensitivity. New knowledge and understanding of relationships between two extremely sensitive macular function indicators (mfERG and letter contrast sensitivity) in diabetes will help create effective

retinal screening strategies in diabetes. It may prove important in the development of therapies that could preserve vision at an earlier or even subclinical stage of diabetic eye disease and significantly affect care of diabetic retinopathy. In the future we will use other macular function indicators like D-15 color vision tests and examine those foveal vision functions relationship to letter contrast sensitivity.

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Chapter 5: Health of parafoveal capillaries and cone photoreceptors in type 2 diabetes and no retinopathy

5.1 Preface

In this chapter we study, using adaptive optics scanning laser ophthalmoscopy (AOSLO), parafoveal cone photoreceptors and the retinal parafoveal capillary network in adult patients with type 2 diabetes and no retinopathy. The focus of the study is to examine whether the cone photoreceptor spacing and retinal parafoveal capillary network are altered prior to the onset of diabetic retinopathy. The study aimed to test whether adaptive optics scanning ophthalmoscopy (AOSLO) is a viable method for detection and characterization of microscopic signs of diabetic retinal damage in cone photoreceptors and capillaries in the parafovea before it is reflected in clinical examination.

11 control participants and 12 adult participants with type 2 diabetes and no retinopathy were included in this study. AOSLO videos were acquired in the parafoveal region of one eye from every participant. Detailed images of the parafoveal capillary network were generated using custom motion contrast enhancement algorithms. The combination of AOSLO images and videos enabled the simultaneous assessment of structural integrity of cone density and several features of the parafoveal capillary network.

The goal of the cross-sectional study was to establish whether the cone photoreceptor spacing is increased and the retinal parafoveal capillary network is altered to examine whether prior to the onset of diabetic retinopathy in adult patients with type 2 diabetes.

To summarize, we found that using a novel application of AOSLO imaging, it is possible to noninvasively visualize and quantitatively assess diabetes-induced changes in cone photoreceptor spacing and parafoveal capillaries. The photoreceptor spacing in participants with diabetes is significantly increased as compared to the controls included in the study. The parafoveal capillaries appear to be altered in type 2 diabetes even in the absence of clinical signs of retinopathy.

This study was presented as a paper at the 2011 ARVO meeting in Florida (Tam et al, 2011a). Abstract is included in the appendix of this thesis. Some of the findings in this chapter have also been published in the scientific literature in Investigative Ophthalmology and Vision Science (Tam et al, 2011b). Some of the description of the methods, and some figures are republished here with the permission of ARVO, the copyright holder of the IOVS manuscript, and under the permission of the University of California Berkeley Graduate Division.

a. Johnny Tam, Kavita P. Dhamdhere, Pavan Tiruveedhula, Silvestre Manzanera, Shirin Barez, Marcus A. Bearse, Jr., Anthony J. Adams, Austin Roorda Noninvasive Assessment Of Parafoveal Capillaries In Type 2 Diabetes Prior To Onset Of Diabetic Retinopathy 11-A-4790-ARVO

b. Tam J, Dhamdhere KP, Tiruveedhula P, Manzanera S, Barez S, Bearse MA Jr, Adams AJ, Roorda A. Disruption of the retinal parafoveal capillary network in type 2 diabetes before the onset of diabetic retinopathy. Invest Ophthalmol Vis Sci. 2011 Nov 29; 52(12): 9257-66.

The study presented in this chapter is based on two PhD theses. Much of the technique described in the methods for generating the custom motion contrast

enhancement algorithms, creating the AOSLO images, highlighting the capillaries and analyzing the capillary health was developed as part of a PhD Thesis (Johnny Tam) in the research laboratory at UC Berkeley where most of this collaboration took place. The results from the capillary health analysis are already published in our publication (b) mentioned above. However this thesis author was involved in acquiring AOSLO videos, working on some of the early stages of capillary image forming like trimming the video frames and applying the techniques and all methods described here, and all the data analysis on receptor spacing studies.

5.2 Introduction

Vascular remodeling and capillary alteration have long been identified in diabetes. As diabetes affects capillaries globally throughout the body, it affects one of the most vulnerable capillary beds in the body, i.e. retina. Diabetic damage to the retina clinically manifests as retinopathy and is present in nearly 80% of patients who have had type 2 diabetes for 15 or more years.[1,2] Although the natural progression of diabetic retinopathy from early changes into the late stages has been well characterized[3], the early microscopic changes that precede retinopathy are not as well understood. Small size and low optical contrast of retinal capillaries make it very difficult to assess the capillary network in living human retina. Fluorescein angiography (FA) remains the gold standard for visualizing human retinal capillaries. It is invasive, not completely free of adverse effects and so is not performed in patients with diabetes and retinopathy unless clinically justified in advanced stages of disease.[4]

Though diabetic retinopathy is characterized based on the vascular lesions, it is a dual disorder that includes microvascular complications and neurodegeneration of the retina.[5,6] Vascular and neural changes lead to compromised retinal function. The neural function loss of retina in diabetes is very well recognized.[7,8] But the specific cell types undergoing retinal neuropathology in diabetes remain unidentified in living human retina. Previous studies examining neural function and its deficits in diabetes provide evidence for rod and cone receptoral and postreceptoral deficits in patients with retinopathy.[9] Electrophysiological studies in eyes with diabetes and no retinopathy reveal significant reductions in the b-wave amplitude of cones. [7,8,10,11] Some animal studies suggest that, in diabetes, attenuated ERG responses result from perturbed cone photoreceptor morphology followed by cone degeneration.[12] It is reported in human retina that hyperglycemia significantly enhances cone metabolism leading to degenerative changes.[13] Though the function of photoreceptors is well studied in diabetes, diabetes' effects on photoreceptor morphology remain unknown, especially in early stages when no clinical signs of retinal damage are evident.

In previous studies assessment of the human parafoveal capillary network has been commonly quantified using a macroscopic metric such as the foveal avascular zone (FAZ) size and shape[14-18], or perifoveal intercapillary area[17]. The large intersubject variability noticed in these studies[15,16,17] makes it difficult to detect alterations, especially in the early stages of the disease. Moreover, macroscopic metrics used in these studies mask several unique microscopic topological features of the parafoveal capillary network like interdigitating arrangement of arterioles and venules[19,20] , circumferential variation in capillary density[19-22] and variation in capillary density in the radial direction[20]. In the

parafoveal capillary network, capillary density is more dependent on proximity from the edge of the FAZ than on eccentricity. Any of these topological features could become affected in diabetic retinopathy with little consequence on a macroscopic metric like FAZ. Thus, there is a need for more sensitive imaging biomarkers to characterize diabetic retinopathy.

We hypothesized that there exist specific capillary channels within the parafoveal capillary network that are affected in diabetic retinopathy. Disruption of such channels would lead to a change in the distribution of blood flow through the network, which could lead to the development of clinical signs of retinopathy and morphological changes in photoreceptors. Recently, Johnny Tam developed noninvasive methods to visualize and assess the human parafoveal capillary network[23,24], with the ability to assess hemodynamics of specific capillaries in relation to the surrounding capillary network[25], using an adaptive optics scanning laser ophthalmoscope (AOSLO)[26,27].

In this chapter, we use a novel application of the AOSLO to determine the relationship between capillary channels, capillary network topology, capillary hemodynamics and farafoveal photoreceptor spacing in patients with type 2 diabetes and no retinopathy. The detailed images generated using the AOSLO enabled us to detect changes in individual capillaries and parafoveal photoreceptors that were previously undetectable. This chapter highlights issues of receptor spacing; our publication noted above was primarily directed at the capillary hemodynamics and its relationship to the development of vascular anomalies.

5.3 Methods and imaging

The research protocol adhered to the tenets of the Declaration of Helsinki. After a detailed explanation of procedures, written informed consent was obtained from all participants. The research protocols were approved by the University of California, Berkeley Committee for Protection of Human Subjects.

5.3.1 Study participants

The study involved 23 participants: 12 adult patients with type 2 diabetes for at least 7 years (Table 5.1), with no diabetic retinopathy (T2DM group) in at least one eye, and 11 adult age-matched control subjects with no history of diabetes (control group). All participants were free of any ocular pathology with a best-corrected visual acuity of at least 20/20. Any participant with history of prior ocular surgery, lenticular or media opacities, or were pregnant or lactating, were excluded.

Table 5.1: Participant characteristics

Group	Gender (N) Males/Females	Mean Age (yrs)	Mean DM Duration (yrs)	Mean HbA1c (%)
Controls	11 (7/4)	52.1 ± 10.2	NA	NA
T2DM	12 (5/7)	55.5 ± 7.8	9.7 ± 2.8	7.6 ± 1.8

5.3.2 AOSLO Imaging

One eye from each subject was selected for imaging. In a few participants, only one eye satisfied all inclusion criteria. If both eyes satisfied all inclusion criteria, then the eye with the better refraction was selected for imaging. The selected eye was dilated (2.5% phenylephrine hydrochloride, 1% tropicamide). A dental impression mount was used to stabilize head position. The AOSLO system uses a low-coherence, 840-nm light source, a Shack-Hartman wavefront sensor, and a 140-actuator microelectro-machined (MEMS) deformable mirror (Boston Micromachines Corporation, Watertown, MA).[25] Overlapping AOSLO videos were acquired in the parafoveal region (1.8 deg field size, 40 seconds, 60 Hz). The subject's pulse was measured using a photoplethysmograph (MED Associates Inc., St. Albans, VT, USA), and simultaneously recorded in a data file during acquisition of all videos.

5.3.3 AOSLO image analysis

Custom motion-contrast enhancement algorithms, developed by Johnny Tam, were applied offline to generate capillary perfusion images from each of the acquired AOSLO videos, and the resulting capillary perfusion images were assembled to generate a montage of the parafoveal capillary network, showing the foveal avascular zone (FAZ), surrounding parafoveal capillaries and parafoveal photoreceptors (Figure 5.1). Distortions in images caused by eye movements were eliminated with the use of customized software.[28,29] After correction; static frames were averaged to enhance the signal-to-noise ratio.

Figure 5.1

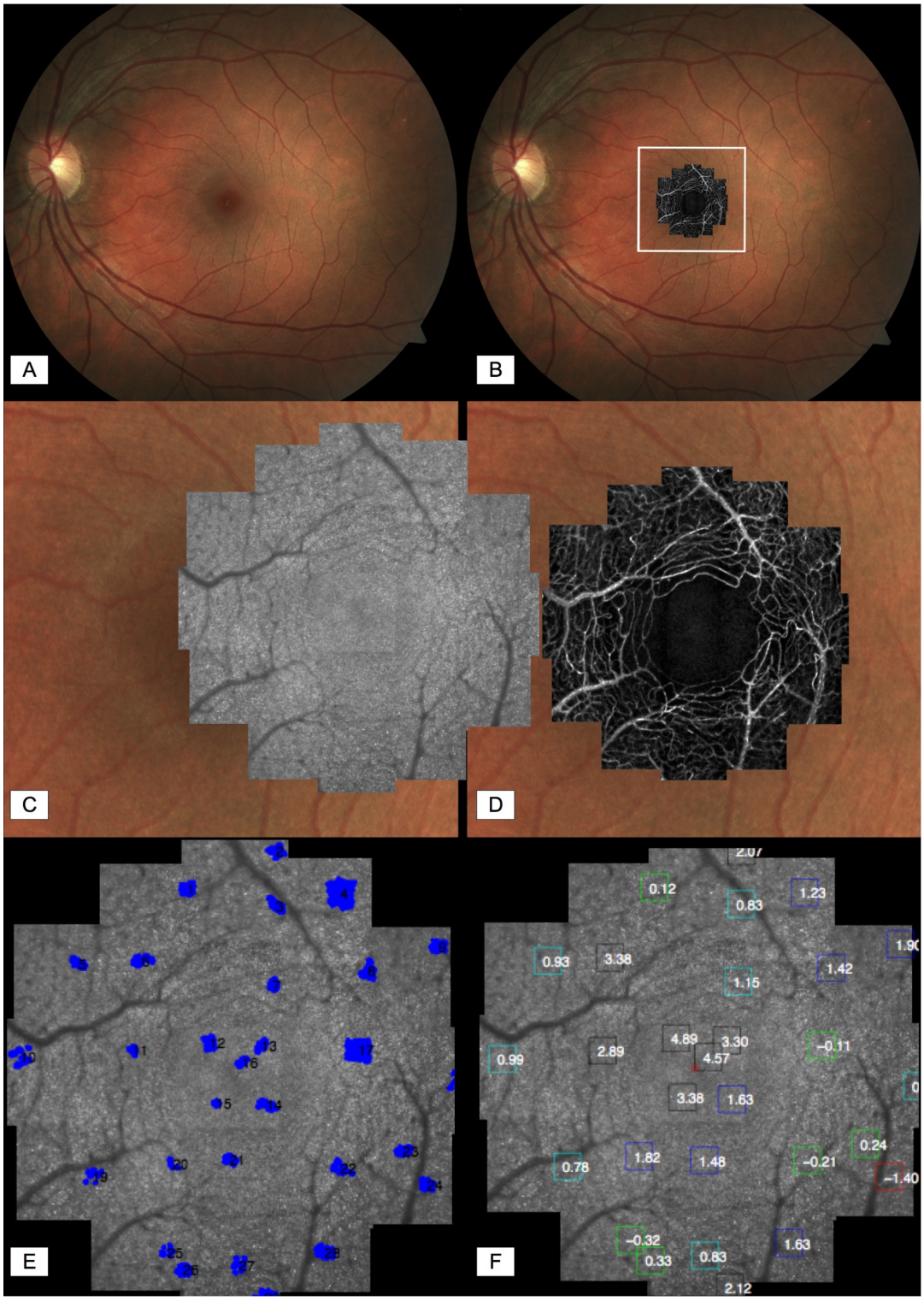

Figure 5.1: Example of AOSLO imaging for one subject with type 2 diabetes and no retinopathy. In this example, overlapping videos were taken in 29 different locations on the retina, processed to generate capillary images, and then compiled to generate a montage of the parafoveal photoreceptors and capillary

network. (**A**, **B**) A 45° fundus photograph, with and without AOSLO images. (**C**, **D**) Higher magnification of image from (**B**), showing a portion of the fundus photograph, with and without AOSLO images. (**E**, **F**) Parafoveal locations where cones were assigned using cone marker software (blue) (**E**), The boxes indicate the mean location of the selected cones and the number indicates the Z-score, which is the number of standard deviations the current cone spacing differs from a normal eye at that location. Z-scores higher than 2 indicate significant increases in cone spacing.

5.3.4 Cone Spacing Analysis

Image scales were computed from calibration images of a model eye, recorded before each imaging session. Regions of the image in which a contiguous cone mosaic was clearly visible were selected, and the power spectrum was computed using Image J software (version 1.3 6b with Java 1.5.0_06; http://rsb.info.nih.gov/ij; National Institutes of Health, Bethesda, MD). To analyze the photoreceptors, locations of individual cones were labeled, and cone spacing was quantified.[30,31]

Spacing was estimated by manually selecting cone centers in contiguous unambiguous patches of cones and inferring the spacing from a histogram of all inter-cone distances within that set. Average nearest-neighbor spacing was determined from the first peak in the density recovery profile described by Rodieck to quantify the spatial arrangement of cells.[30,31] Briefly, density recovery profile plots the average histogram of density of all cells surrounding each cell in the mosaic as a function of distance from the central cone. Cone spacing was chosen as a parameter because it provided the most robust and conservative measurement for cone density comparison among eyes.

Cone spacing for each respective location was compared with expected spacing from a database of 27 normal eyes. Deviations from normal were quantified as z-scores, or the number of standard deviations from the normal mean at that location (Figure 5.1). A z-scores value ≥ 2 ($P < 0.023$) is considered to be abnormal.

5.3.5 Parafoveal Capillary analysis

Images of the parafoveal capillary network in 12 T2DM participants and 11 controls were generated. Images of perfused capillaries were used to compare the two groups for qualitative differences. Specifically, we examined AOSLO images for subclinical capillary peculiarities, such as capillary bends and possible precursors to microaneurisms, as well as possible breakdown of the topological organization of the capillary network. We identified arterio-venous channels and calculated tortuosity, recognized and assessed capillary dropouts, and assessed capillary hemodynamics using AOSLO capillary images.

5.3.5.1 Identification of Arterio-Venous (AV) Channels

AV channels (defined here as the simplest, most direct capillary paths connecting arteries to veins) were identified, and tortuosity was calculated (Figure 5.2).

The locations of arterioles and venules were identified by overlaying AOSLO images with fundus photographs. Paths representing candidate AV channels, starting at an arteriole and ending at a venule, were drawn. A set of candidate AV channels was generated and three least tortuous AV channels were identified and tortuosity was

measured. The tortuosity was calculated by dividing total squared curvature of a line with length of the line.[32] We calculated the average tortuosity of three least tortuous AV channels we identified.

Figure 5.2

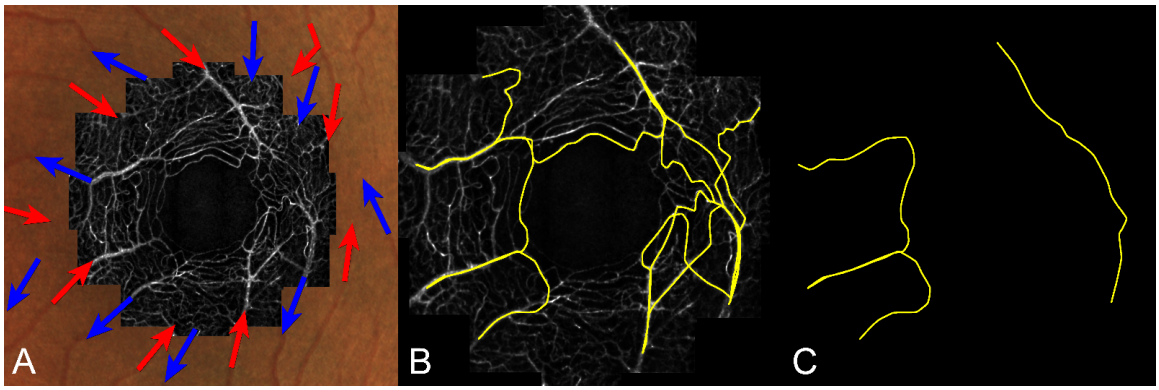

Figure 5.2: Taken from Tam et al. 2011.[33] Identification of AV channels on AOSLO images. The steps are: (A) identify locations of arterioles (red) and venules (blue), (B) identify candidate AV channels satisfying the branch selection rule, and (C) select the three least tortuous AV channels.

5.3.5.2 Measures of Capillary Dropout

Three measures of capillary dropout were used: FAZ size, FAZ shape, and capillary density.

FAZ Size and Shape

The borders of the FAZs were extracted using a semi-automated algorithm[22]. We identified the FAZ as the largest avascular zone near the fovea. The area of the extracted region was quantified in pixels^2 and then converted to mm^2 using a model eye parameterized by the biometry measurements from each subject. The effective diameter of the FAZ was calculated as the diameter of the circle with equal area.

We measured the shape of the FAZ as acircularity calculated by dividing perimeter of FAZ with perimeter of a circle with same area. A perfectly circular FAZ has an acircularity equal to 1. Deviations from a circular shape increase the value of this acircularity metric. The quality of the combined AOSLO images was sufficient to enable FAZ shape measurements in 11 out of 11 control subjects and 9 out of 12 T2DM participants.

Capillary density

The centerlines of all vessels in a region of interest (ROI) within 0.15 degrees of the edge of the FAZ were extracted using a semi-automated extraction process, as described previously[22]. The inner border of the ROI was defined as the edge of the FAZ, and the outer border was defined as the contour spaced 0.15 degrees from the edge of the FAZ. Capillary density was measured by dividing total length of all extracted capillaries by the area of the ROI. The quality of the AOSLO images was sufficient to enable capillary density measurements in 8 out of 11 control subjects and 9 out of 12 T2DM participants (Figure 5.3)

Figure 5.3

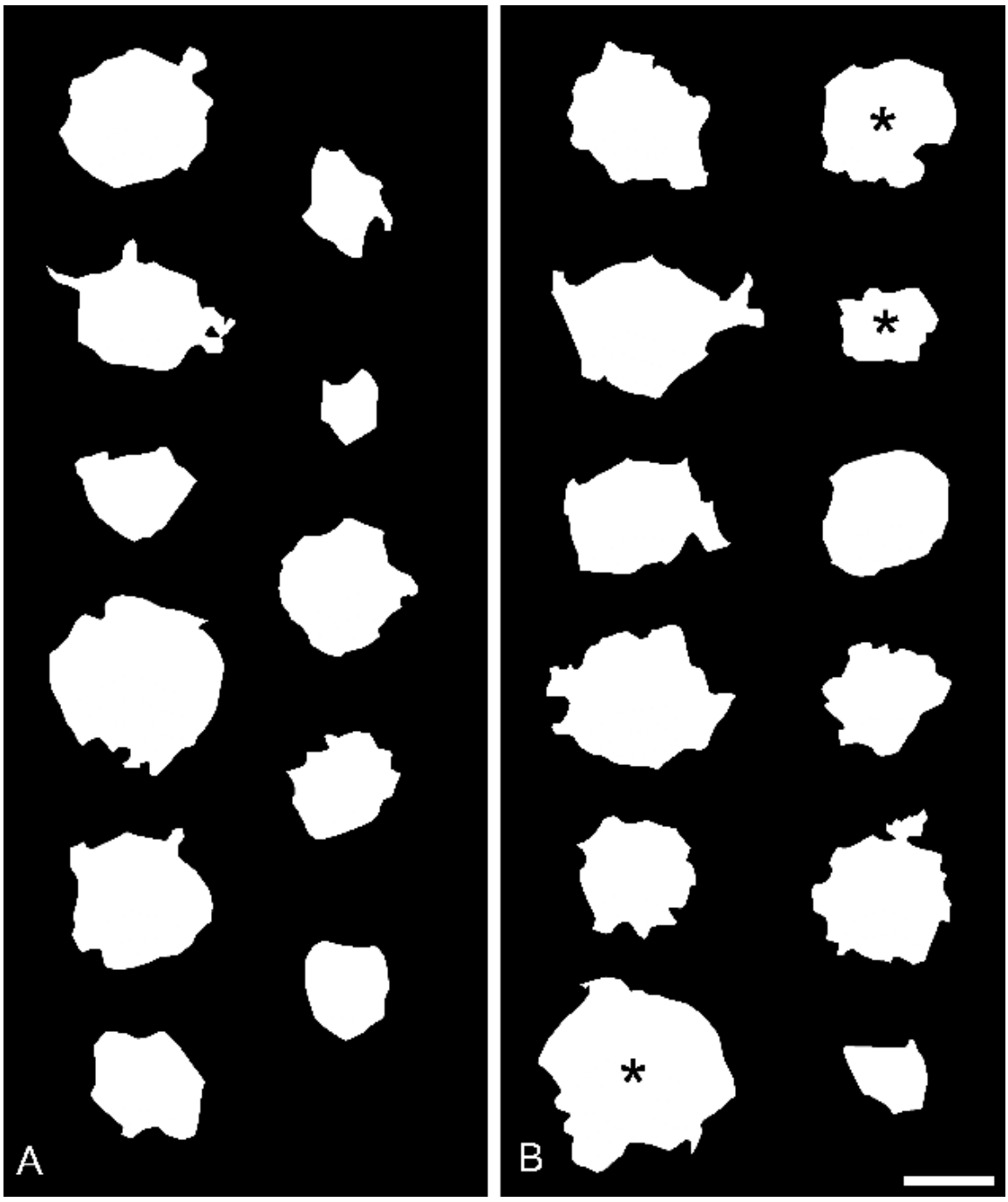

Figure 5.3: Taken from Tam et al. 2011[33]. Extracted FAZs for (A) control and (B) T2DM group. Three FAZs could not be extracted due to poor data quality, as described in the text. Of the extracted FAZs, three FAZs were not used for quantification of FAZ shape, due to poor quality data in one or more videos showing the edge of the FAZ (asterisk). For these FAZs, the extracted FAZ was estimated from the AOSLO image and quantified for size but not shape. Scale bar, 500 µm

5.3.5.3 Capillary Hemodynamics

We calculated two measures of capillary hemodynamics, leukocyte speed and pulsatility index.

Leukocyte speed

We quantified the speed of leukocytes through selected AV channels. We identified the least tortuous AV channel that also contained many leukocytes, and measured the speed of all leukocytes that could be clearly identified in the corresponding 40 second AOSLO video. The speed of each leukocyte was quantified directly, incorporating corrections for raster scanning and eye motion, as described previously[24]. We then calculated the average leukocyte speed by plotting leukocyte speed vs. relative cardiac cycle determined from the subject's pulse, dividing the cardiac cycle into 5 bins, calculating the average speed of each bin, and then taking the average speed of the 5 bins. The quality of the AOSLO videos was sufficient to enable leukocyte speed measurements in 8 out of 11 control subjects and 7 out of 12 T2DM participants.

Pulsatility index

We calculated the pulsatility index (PI) for leukocytes using a method described previously[25]. Briefly, leukocyte speeds were plotted vs. relative cardiac cycle, and divided into 5 bins. We defined V_{max} as the bin with highest average speed, V_{min} as the bin with the lowest speed, and V_{mean} as the average speed of all 5 bins. PI was calculated as, $PI = (V_{max} - V_{min}) / V_{mean}$.

We calculated PI only when there were at least two leukocytes identified in each bin. Applying this criterion, we calculated PI in 7 out of 11 control subjects and 5 out of 12 T2DM participants.

5.3.5.4 Statistical analysis

We compared the two groups using two-tailed un-pooled t-tests with a significance level of 0.05. For cone analysis, proportions test was performed to examine whether the subject groups differed in their frequencies of abnormalities.

5.4 Results

5.4.1 Cone Spacing

The average cone spacing in T2DM group was significantly increased as compared to the controls ($p<0.05$). In other words cone density in participants from T2DM group was reduced as compared to the controls. We measured cone spacing in about 20 ± 3 contiguous unambiguous cone mosaic locations in each retina. The percentage of abnormal locations in eyes from the T2DM group is significantly higher than the percentage of abnormal locations (reduced cone density) in eyes in the control group ($p <0.0002$) (Table 5.2). Most of the abnormal locations were in the central 1° of macula.

Table 5.2: % of locations with abnormally reduced cone density

Group	Total number of locations assessed	Locations with z-scores value ≥ 2	% of abnormal locations
Controls	250	19	7.6
T2DM	270	84	31.11

5.4.2 Parafoveal Capillaries

In general, there were no obvious homogeneous differences that could be observed between the T2DM and control groups. The interdigitating arteriole and venule organization was maintained in both groups. It appeared that there were areas of focal capillary disruption, notably around areas of capillary bend formation. Interestingly, capillary bends were present in both groups, suggesting that some aspects of capillary disruption may be present even in healthy subjects (Figure 5.4).

Figure 5.4

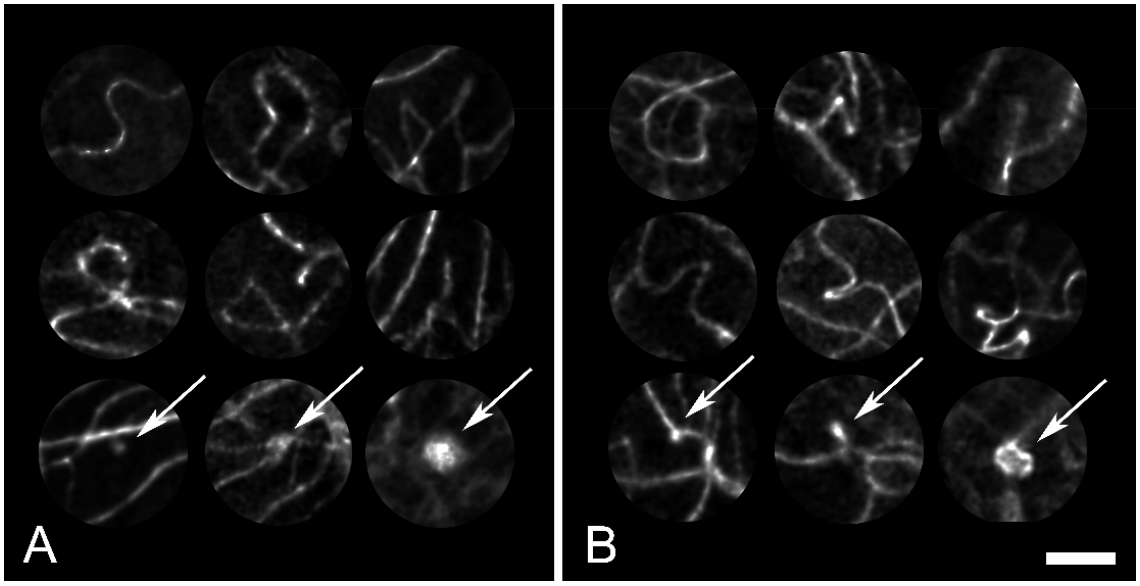

Figure 5.4: Taken from Tam et al. 2011[33]. Examples of capillary abnormalities, in (A) control and (B) T2DM_NoDR subjects. There were capillary bends and dead-end capillaries present in both groups (top two rows), as well as objects of various sizes that were similar in appearance to microaneurisms (arrows in bottom row), despite the absence of microaneurisms on color fundus photographs. Scale bar, 100 μm.

There were also objects that might be precursors to microaneurisms present in both groups; such objects were not clinically identified as microaneurisms based on fundus photography. The average AV channel tortuosity was increased by 26% in T2DM group compared to controls ($p<0.05$). There were no statistically significant differences in capillary dropout or capillary hemodynamics (Figure 5.5). Comparing T2DM to

controls, the average FAZ size was 7.4% higher, FAZ shape 3.4% higher, capillary density 3.7% lower, leukocyte speed 14.4% lower, and pulsatility index 25% higher.

Figure 5.5

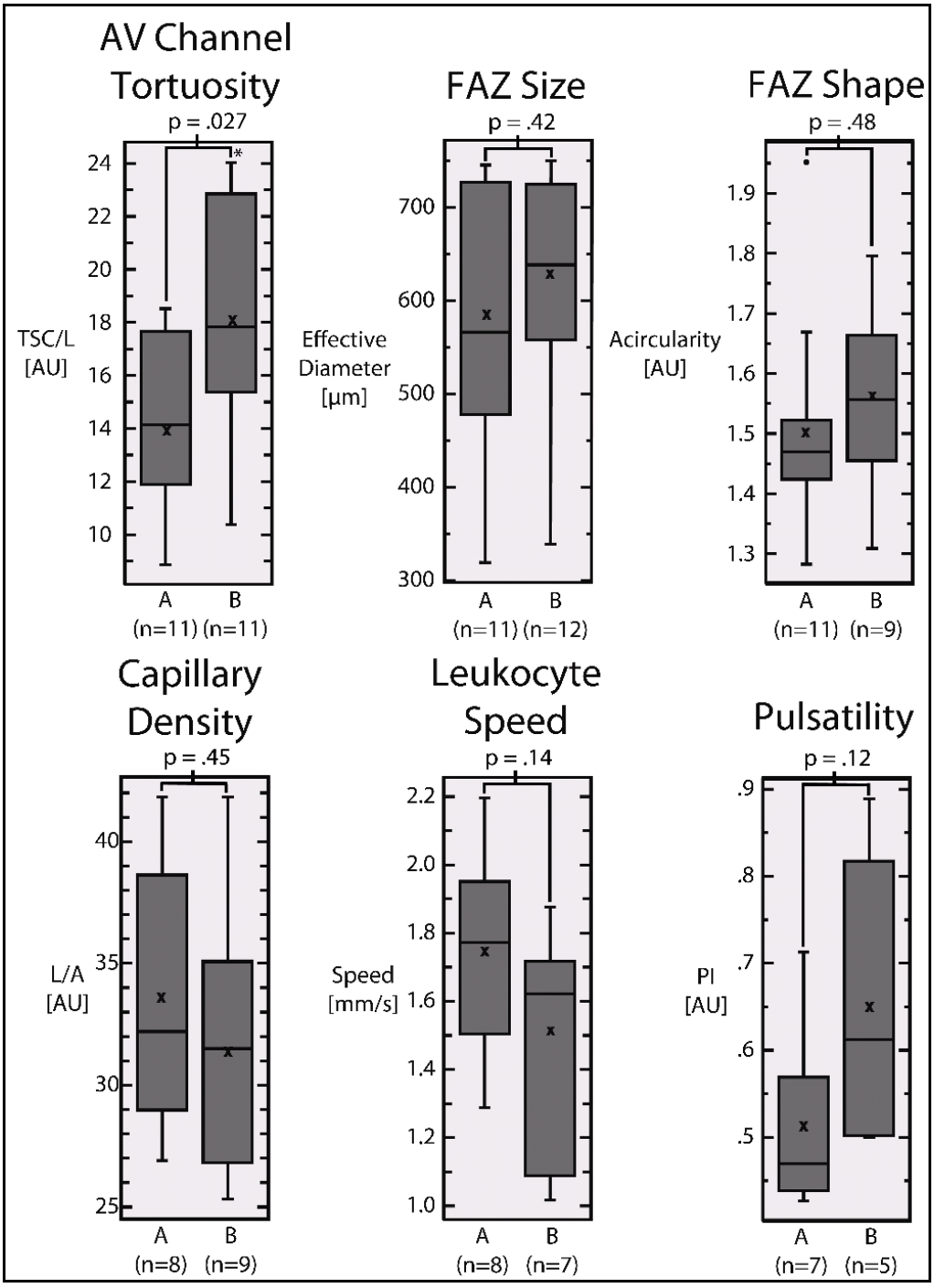

Figure 5.5: Taken from Tam et al. 2011[33]. Results from statistical analyses, for control (A) and T2DM_NoDR (B) groups. AV channel Tortuosity was significantly increased in the T2DM_NoDR group compared to the control group ($p<0.05$). Error bars are Standard errors of mean

5.4.3 Conclusion and discussion

The dual nature of diabetic retinopathy with a vascular and neural component has been studied extensively[6,34] with results suggesting affected neural retinal function in various stages of diabetes[10,11,12]. The evidence of neural damage in diabetes suggests that the photoreceptors may also be affected. The cone photoreceptor mosaics were found to be altered in the presence of retinopathy and edema[35] The state of cone photoreceptor spacing in the absence of retinopathy remains unknown. Unfortunately, we were not able to reach meaningful quantitative conclusions about photoreceptor spacing with this small sample, though the results show significant differences in cone spacing of controls and the T2DM group, with increased spacing in the T2DM group.

The ability to image photoreceptors in living human subjects has only been available with fairly recent advances in imaging technology. However, in participants of this study, there was no evidence that the photoreceptor layers were disrupted in any of the optical coherence tomography (OCT) images. The increase in cone spacing observed by the AOSLO may be a microscopic change that is too small to be detected by other methods. Further studies are needed to address the issue of quantifying cone spacing.

As we discuss in detail in our publication[33] the human retinal capillary network appears to be altered in type 2 diabetes prior to development of clinically identified retinopathy. We think it is important to consider microvascular changes and hemodynamics as a causal relationship with clinical signs of retinopathy. We found that AV channels in the retina are disrupted in diabetes, even before any signs of retinopathy. We propose that the ongoing cycle of AV channel disruption leads to the redistribution of leukocytes out of AV channels into exchange capillaries[36] that may not be suitable for increased leukocyte traffic. Specifically, the passage time for leukocytes through exchange capillaries is likely to be much higher than for leukocytes through AV channels. This would lead to an overall accumulation of leukocytes in the network[37] leading to focal capillary dropout, for which many leukocyte-based mechanisms have been proposed[38]. This may be triggered by a decrease in the deformability and increase in activation of diabetic polymorphonuclear leukocytes[39,40]. As the cycle of AV channel disruption continues, these microvascular changes could lead to more evident clinical signs of retinopathy and photoreceptor disruption.

Our results regarding no significant change in FAZ size and shape are consistent with previous studies, which have investigated capillary dropout by quantifying the size of the FAZ (larger FAZ corresponding to capillary dropout in the parafovea).[15,16,17,18] Some found more acircular FAZ only after the presence of diabetic retinopathy. [14,16] Our findings of no change in capillary density are similar to the findings in previous studies that found increase in perifoveal intercapillary area i.e. reduced parafoveal capillary density only in presence of diabetic retinopathy.[17,41] This suggests that macroscopic capillary dropout is likely to occur only after the manifestation of clinical signs of diabetic retinopathy.

Parafoveal capillary velocity has been found to be decreased in patients with diabetes[39], consistent with the data in this study. Finally, studies investigating the pulsatility of blood in choroidal vessels in the earlier stages of diabetes, have found inconsistent results, with decreases[42], no change[43], and increases[44] shown. In our study, leukocyte speed was 14% lower, and the pulsatility index 25% higher. Decreased

leukocyte speed could be a result of increased AV channel tortuosity, since leukocytes must deform to travel through small capillaries in single-file[45], and any increase in tortuosity is likely to require additional deformations for leukocyte passage. Although the results of statistical testing for these hemodynamic measures were not significant (i.e. inconclusive), such metrics may still be of clinical importance.

The small sample size did not allow us to examine correlations of capillary changes and photoreceptor spacing alterations with variables such as HbA1c, disease duration, or age and it is certainly one of the limitations of this study. Despite the small sample size, AV channel tortuosity and cone spacing was significantly different when comparing the two groups, suggesting that these parameters are highly sensitive.

Small sample size is a limitation of this study. The ethnicities of the two groups differ. While there are no studies to suggest that the parafoveal capillaries and cone distribution are different with respect to ethnicity, it is possible that differences attributable to ethnicity exist. Finally, the process of AOSLO imaging combined with video and image analysis is time consuming, and requires specialized equipment that is not yet commercially available. In this study, AOSLO imaging for one eye from each subject required about 2.5 hours, followed by about 24 hours of offline processing per eye to generate images of parafoveal capillaries, photoreceptor montages and to quantify the parameters described in this study.

In conclusion, we demonstrate a method to noninvasively assess retinal capillaries, leukocytes and photoreceptors in patients with type 2 diabetes and no retinopathy. Although there are now several methods to noninvasively visualize capillaries in humans[46-48], the system of imaging and analysis described in this paper is unique in that capillaries leukocytes and photoreceptors can be analyzed from the same dataset. These methods may be useful for assessing the microcirculation in other diseases, particularly in cases when FA is not performed, or for establishing a normal database of parafoveal capillaries. In the future, AV channel tortuosity may be important as an imaging biomarker for evaluating the efficacy of a therapeutic agent, or as a tool to assess the onset and progression of DR.

The ability to assess change in photoreceptor density might help in better designing the use of vascular therapeutic agents especially on a background of results suggesting mixed effects of common pharmacologic treatments controlling neural function like VEGF on photoreceptor health (In one report, decrease in VEGF expression in the retina resulted in photoreceptor degeneration[49]. In another report, overexpression of VEGF in the retina resulted in photoreceptor degeneration[50]).

Diabetic retinopathy is a complex disorder and revealing novel aspects of its pathophysiology may facilitate discovery of improved therapeutic intervention that prevent vascular pathology or that protect from neuropathology and more effectively block disease progression.

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Chapter 6: Changes in neuroretinal and vision function associated with diabetic macular edema

6.1 Preface

This longitudinal study examined the vision, retinal health, diabetes control and blood pressure of the subjects who were at risk for developing diabetic macular edema (DME). The subjects were followed over time until and after DME developed. The subjects who developed DME were studied at three visits over an average period of 22 months. The visit prior to DME development was considered as visit 1, the visit when DME was diagnosed was considered as visit 2 and the subjects with DME were tested again at a follow up visit 3 within a time window of 6 to 9 months after the diagnosis of DME. The main interest in this study is to understand how DME influences function and structure of retina over the course of the disease.

Previous studies using similar techniques to examine DME either were cross sectional, designed to study the effect of DME on retinal health[1,2] or were longitudinal, to predict DME in patients at risk of developing edema[3]. Studies done to examine mfERGs in diabetes have shown mfERG implicit time delays and mfERG amplitude reduction as an effect of changes in the neural retina resulting from diabetes and from DME.[1,2,4-6] Some of these studies reported reduction in vision function (high contrast visual acuity[2]) as an effect of location (proximity to the fovea) and extent of DME. A recent study by Harrison et al. in our lab has shown mfERG amplitude (AMP), mfERG implicit time (IT), systolic blood pressure, and gender to be collectively predictive of edema onset at specific retinal locations within 1 year.[3] To our knowledge no study has investigated how retinal health changes with time after DME is diagnosed and established, prior to the treatment. In this study we examined the retinal thickness, vision function and mfERGs prior to DME development, close to the time of DME development and some months after the DME was established, to see how these structural and functional measures in retina change as the DME progresses, regresses or remains steady.

At all three visits mfERGs were recorded. High contrast visual acuity, low contrast visual acuity, contrast sensitivity and color vision were tested. Blood pressure, random blood glucose at the time of exam and HbA1c were also measured. Finally, the retina was imaged using optical coherence tomography (OCT) and fundus photography. These retinal and general health measures were compared between the visits to examine any changes associated with the status of DME. In this study DME was diagnosed using color fundus photos graded by a retina specialist and DME was confirmed by fluorescein angiography in most patients- as called for by the participant's physician. This study is not yet published; part of the study was presented at the American Academy of Optometry meeting in Phoenix in October 2012.

> Kavita Dhamdhere, Wendy Harrison, Marcus Bearse, Marilyn Schneck, Shirin Barez, Anthony Adams. "Changes in neuro-retinal and vision function after occurrence of diabetic macular edema: A pilot study" AAO 2011 E-abstract 120369

The abstract for this presentation are in the Appendix of this thesis.

6.2 Introduction

With increasing prevalence of diabetes mellitus, diabetic retinopathy remains the leading cause of preventable vision loss.[7] In 2011, of an estimated 380 million people worldwide with diabetes[8,9], over one-third had signs of diabetic retinopathy, and a third of these were afflicted with vision-threatening retinopathy, such as severe non-proliferative diabetic retinopathy, PDR or DME.[10] In the USA, an estimated 29% of adults with diabetes have diabetic retinopathy and 3 % have DME.[11] In the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) in the U.S.A., 14-25% of patients with Type 2 diabetes developed DME over a 10-year follow-up period.[12] Data from the 25-year follow-up of the WESDR diabetes cohort show that virtually all patients (97 %) developed retinopathy over time, with a third to a half going on to develop vision-threatening disease (29 % developed DME, 17 % developed clinically significant DME (CSME) and 42 % developed PDR).[13,14] DME is more prevalent in Type 2 diabetes and is the primary cause of moderate to severe visual loss for diabetic patients given the high prevalence of Type 2 diabetes. [15] Apart from the effects on vision, the presence DME is a marker of concomitant diabetes complications in other organs and is associated with increased rate of mortality.[16-18]

DME can occur at any stage of diabetic retinopathy and currently is not treated until it impairs vision or reaches a stage of CSME. (In the ETDRS, DME was characterized as "clinically significant" if any of the following were noted: retinal thickening within 500 microns of the fovea, hard exudates within 500 microns of the fovea if associated with adjacent retinal thickening, or an area of retinal thickening 1 disc diameter or larger if any part of it is located within 1 disc diameter of the fovea[19]). Even though early diagnosis and treatment produce better results, there is a high prevalence of patients who become legally blind. (A person is considered to be legally blind if he or she has a best corrected vision of 20/200 or worse in their better seeing eye[20,21]) despite receiving timely treatment.

Therefore, it is clinically and scientifically important to establish a better treatment strategy for DME. Unfortunately, although there are significant advancements being made in the early diagnosis and treatment of patients, the number of patients at risk for the development of vision loss or blindness due to diabetic retinopathy is still thought to be increasing since the worldwide incidence of diabetes is increasing. Some estimates have projected that by the year 2050, there will be 50 million or more diagnosed and undiagnosed diabetic patients in the United States, of whom as many as half or 25 million may have diabetic retinopathy.[22,23]

The contemporary treatments for edema are invasive and are aimed at preventing further structural and functional retinal damage once significant damage is already present. If applied in a timely manner, these treatments often help to partially restore central visual loss but not non-foveal vision. Rather these treatments may impact peripheral vision negatively.[24] Owing to the negative consequences of the existing treatments, clinicians remain conservative in treating the patients with DME and prefer to wait until CSME; a more vision-threatening stage is present. The patients with macular edema insufficient to get a recommendation of medical intervention deserve appropriate consideration. DME often is present for many years without vision loss and it becomes challenging for the physicians to instruct the patient for the need for treatment or

examination when progression to foveal involvement occurs. Better structural and functional characterization of DME during the course of the disease are crucial to address questions like how DME develops into CSME, how frequently the patient should be examined after DME is diagnosed, and how patients should be assessed using sensitive, reliable and objective novel measures that are more specific than traditional assessment based on visual acuity.

Currently in patients with DME OCT, stereoscopic color fundus photos and FA are the main techniques to monitor structural prognosis. The functional impact of DME and its prognosis are quantified and monitored by visual acuity.[25-27] These tests remain the mainstay for the endpoints of most of the clinical trials of treatments for DME. These tests are reliable, non-invasive, easy to conduct, reasonably affordable, fulfilling these requirements to be standard end points.[28,29] However they are insensitive and do not test all aspects of retinal health. Visual acuity represents only one aspect of macular function. For the evolution of medicine in the field of DME and to better plan the strategies to monitor its course, it is required that these standard assessments be continually reviewed and potentially updated.

This longitudinal study examined the retinal health and function, diabetes control and blood pressure of the subjects who were at risk for developing DME. The subjects were followed over time until DME developed and then followed for months after development of DME. The participants who developed DME were studied at three visits over an average period of 22 months. The visit prior to DME development was considered visit 1, the visit when DME was diagnosed was considered visit 2 and the participants with DME were tested again at visit 3 within a time window of 6 to 9 months after diagnosis of DME. The main interest in this study is to understand how DME influences function and structure of the diabetic retina with the evolution of DME. At all three visits mfERGs were recorded; high contrast visual acuity, low contrast visual acuity, contrast sensitivity and color vision were tested; blood pressure, random blood glucose at the time of exam and HbA1c were measured; and the retina was imaged using optical coherence tomography (Cirrus OCT, Zeiss) and fundus photography. These retinal and general health measures were compared between the visits to examine any changes in these measures associated with the status of DME.

In the preliminary brief presentation in Phoenix October 2012, the focus was on the neuro-retinal function changes in Type 2 diabetes with edema. It was summarized as follows:

7 patients with Type 2 diabetes were recruited at the time of edema development (3 participants with CSME and 4 with retinal edema elsewhere) and were examined again after 6-9 months of edema development. The changes in neuro-retinal function were examined at retinal locations with edema and at retinal locations that were edema free. At the follow-up visit we found that 4 of 7 eyes were resolved. The 3 eyes that had CSME at visit 1 did not resolve at follow-up visit. Overall neuro-retinal retinal function was reduced at the time of edema development and at follow-up visit. There was no improvement in the neuro-retinal function despite resolution of edema in 4 of 7 eyes. The finding was true for both, edematous locations and edema free locations. Following are the findings:

1. Implicit time (mfERG IT)

mfERG IT was significantly delayed at both edematous and edema free locations at

both visits as compared to controls. Though edema regressed in 4 of 7 eyes from visit 1 to visit 2, implicit time neither seem to neither improved nor got worse, in other words, remained unchanged over two visits irrespective of edema status.

2. Amplitude (mfERG AMP)

mfERG AMP behaved similarly. AMP was significantly reduced at both edematous and edema free locations at both visits as compared to controls. Though edema regressed in 4 of 7 eyes from visit 1 to visit 2, AMP neither improved nor it got worse; in short it remained unchanged over two visits irrespective of edema status.

Based on these findings we concluded that neuro-retinal function did not improve in eyes with resolved edema at visit 2, nor got worse in eyes that did not resolve at visit 2. A longer follow up and larger sample size may well temper these conclusions.

6.3 Materials and Methods

6.3.1 Subjects

One eye of each of 12 patients with DME was included and was studied at three visits approximately spanning over 1½ years. Visit 1 was studied retrospectively in patients who developed DME. The patients were tested at the time of DME development (visit 2) and were followed 6 to 9 months later (visit 3). There were 5 patients with Type 1 diabetes (Type 1 group) and 7 with Type 2 diabetes (Type 2 group). The mean ages were 34.1 ± 8.9 and 58.6 ± 7.6 yrs and diabetes durations of 25.6 ± 7.4 and 13.8 ± 6.2 yrs, respectively (Table 6.1). All patients had poor diabetes control (HbA1c≥8.2), moderate to severe non-proliferative retinopathy at visit 1, non-proliferative retinopathy and DME at visit 2, and non-proliferative retinopathy and/or DME at visit 3.

In addition, 52 healthy non-diabetic controls with an age range of 21 to 65 years (mean age, 43.1 ± 14.7 years) were tested. All subjects (controls and patients) had clear ocular media, no other retinal pathology and refractive errors within the range of ±6D. Fundus photos, high contrast visual acuity, low contrast acuity contrast sensitivity, color vision, mfERG (VERIS 5.2), OCT retinal thickness (Cirrus), random blood glucose (BGL (One Touch Ultra glucometer)) and blood pressure LAS (BP (Omron HEM- 773)) were obtained at all visits in all patients. HbA1c (DCA-2000 analyzer, Bayer Diabetes Care, Terrytown NY with testing reagents by Siemens Inc, Washington DC) was measured in patients with diabetes at all visits. mfERG implicit time (IT) and amplitude (AMP) were converted to Z-scores using mean and standard deviation of control subjects, and grouped into 35 zones (as will be discussed later). Differences between visits were evaluated.

All procedures performed in this study adhered to the tenets of the Declaration of Helsinki. After a detailed explanation of the procedures, purpose of the study and potential risks, written informed consent was obtained from all participants. The research protocol was approved by the University of California, Berkeley Committee for Protection of Human Subjects.

Table 6.1 Patient Demographic Data at Visit 1

Characteristic	Type 1	Type 2	Controls
Number of subjects (M/F)	(5) 1/4	(7) 5/2	(52) 23/29
Age (yrs)	34.1±8.9	58.6 ± 7.6	43.1 ± 14.7
DM Duration (yrs)	25.6 ± 7.4	13.8 ± 6.2	N/A
HbA1c %	10.41 ± 1.67	8.36 ± 1.02	N/A
Blood Glucose (mg/dL)	150.2 ± 21.3	215.0 ± 85.6	105.6 ± 22.3
Blood Pressure SBP/ DBP (mm Hg)	111.6/72.6 ± 6.6/4.6	140.0/75.0 ± 25.5/7.0	113.4/70.3 ± 17.5/9.7

6.3.2 mfERG

6.3.2.1 Recording parameters

All subjects were pharmacologically dilated (tropicamide 1 % and phenylephrine hydrochloride 2.5 % eye drops) at all visits. mfERGs were recorded from both eyes (right eye first) with dilated pupils (6 – 8 mm diameter) using a visual evoked response imaging system (VERIS Science 5.2; EDI, San Mateo, CA). The non-tested eye was covered with an eye patch. A stimulus consisting of 103 hexagons scaled according to eccentricity to account for cone density and to produce responses with similar signal to noise ratio (SNR) was presented on a 9-inch CRT display, which is a 640 x 480 pixel display.[30] Frames were presented at 75 Hz (13.33 milliseconds/frame) under photopic conditions. In every frame each hexagon had an approximately 50 % chance of being illuminated according to a pseudo-random M sequence resulting in recording sessions approximately 8 min long. The luminance of bright frames was 200cd/$m^{2;}$ the luminance of the dark frames was <2 cd/m2. The stimulus was centered on the fovea and stimulated approximately 45°of the retina. Each session was split into 16 segments of equal length. Fixation was controlled by a fixation target 'X' in the center of the stimulus and was monitored for displacements of the lens and eye movements using an in-line infrared camera. Segments with fixation loss were repeated.

Retinal potentials were recorded using a bipolar Burian-Allen electrode (Hansen Ophthalmic, Solon City, IA, USA), amplified by a factor of 100,000 and analogue band-pass filtered 10 - 100 Hz and were sampled at 1200 Hz (0.83 ms/sample). The electrode was filled with 1% carboxymethylcellulose sodium (Refresh Celluvisc, Allergan Inc., Irvine, CA, USA), and was placed on the anesthetized (0.5% proparacaine) cornea. 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) and electrode impedance was kept bellow 10 kΩ.

6.3.2.2 mfERG 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. The 103 waveforms were then exported from VERIS. The first negative peak (N1) and the first positive peak (P1) of the local mfERG response waveforms were identified and the N1- P1 amplitudes (AMP) and P1 implicit times (IT) were measured. The P1 implicit times were measured from the onset of the local stimulus flash to the P1 peak. The template stretching method of Hood and Li[31] was used to quantify the P1 implicit time and N1-P1 amplitude. A template constructed from the mean local waveforms of the control subjects was fitted to the first 80 milliseconds of each subject's corresponding local response using a least squares fit (right eye response arrays were converted to left eye orientation). Each template was independently scaled in amplitude and time dimensions such that subject's local waveform and the local template had minimal least-squares difference. The goodness of fit, statfit, was generated and responses with statfits greater than or equal to 0.8 were not included in analysis. The Control subjects' mean and standard deviation for each local mfERG measure were used to compute Z-scores for the patients' corresponding mfERG responses. 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.

The spatial correspondence between mfERG stimulus array and fundus photographs was defined based on the location of optic disc and fovea. To reduce correspondence errors between the response array and the fundus photographs and to be spatially conservative, data taken from 103 hexagons were grouped into 35 retinal zones combining two or three neighboring elements (Fig. 6.1). The mfERG implicit time for a zone is defined by the maximal Z-score i.e. the most delayed within the Z-scores of the mfERG for hexagons in that zone, and the mfERG amplitude for a zone is defined by the minimal Z-score i.e. most reduced within the Z-scores of the mfERG for hexagons in that zone.

A retina specialist masked to the individuals' identity and status (diabetic or not), graded all fundus photographs and FAs for the presence or absence of edema, degree of edema (DME or CSME) and the degree of retinopathy on a clinical scale (none, mild, moderate, severe). The graded fundus pictures at the time of edema development (visit 2) were overlaid on the mfERG array to map the locations of DME onto the mfERG responses (Fig. 6.1). The retinal zones were assigned for having edema and for being edema free. Based on this assignment 35 zones were divided into two categories:

1. Retinal locations with edema at visit 2: Edema locations
2. Retinal locations free of edema at visit 2: Edema free locations

Figure 6.1

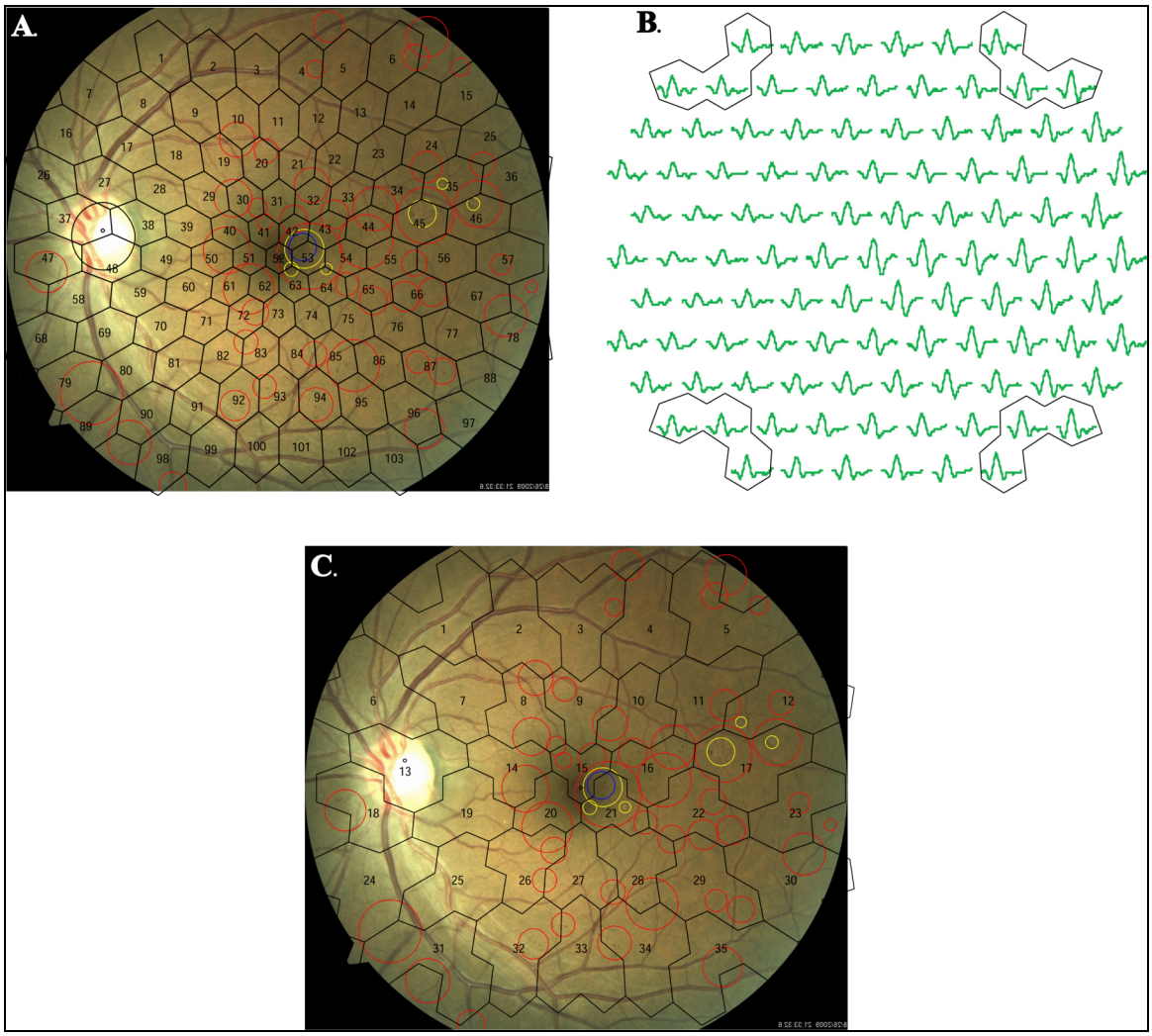

Figure 6.1: Fundus pictures graded by the retina specialist overlaid with 103 hexagonal pattern and making of 35 zones from the data collected from 103 elements. A) mfERG pattern with 103 elements overlaid on a graded fundus photo B) Sample waveforms with example of four highlighted zones C) Formation of 35 zones across the retina.

6.3.3 Retinal thickness measurements and analysis

Retinal thickness was measured using the scans captured by Cirrus HD spectral domain OCT (Carl Zeiss Meditec, Dublin CA). Signal strengths of OCT scans were not lower than 7. This instrument measures whole retinal thickness from the retinal pigment epithelium to the inner limiting membrane (Fig 6.2).[32]

The Cirrus OCT performs laser scans over a 6mm X 6mm area centered on the foveola (Fig 6.3A), capturing a cube of data consisting of 200 A-scans from 200 linear B-scans (40 000 points) at a rate of 27,000 axial scans per 1.5 seconds with an axial resolution of 5um and a transverse resolution of 15 microns.[33]

Figure 6.2

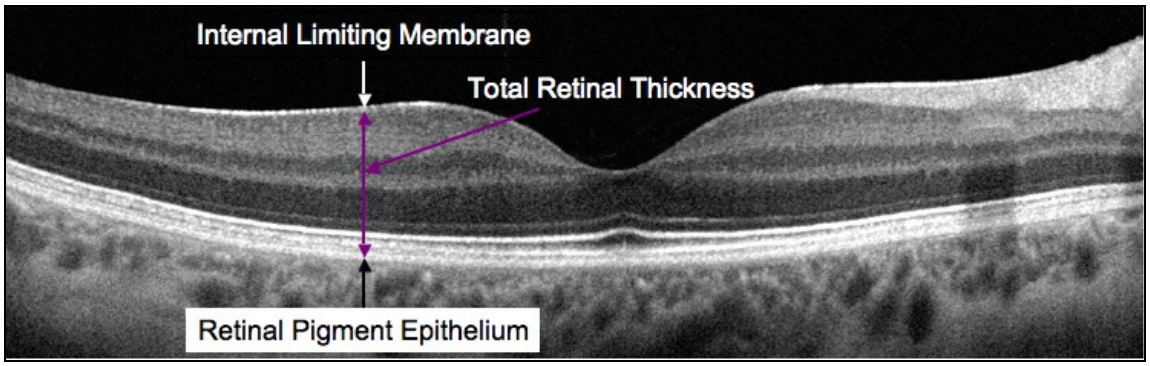

Figure 6.2: High-resolution retinal scan captured using Cirrus HD OCT. Total retinal thickness is measured from retinal pigment epithelium to inner limiting membrane.

Average total retinal thickness values were calculated within 9 zones following the ETDRS pattern: a 1-mm diameter central zone, four 3-mm diameter perifoveal zones, and four 6-mm diameter peripheral zones (Fig 6.3B). Average total retinal thickness values from each zone were compared using T-test to examine difference between the three visits in the Type 1 and Type 2 diabetes groups.

Figure 6.3

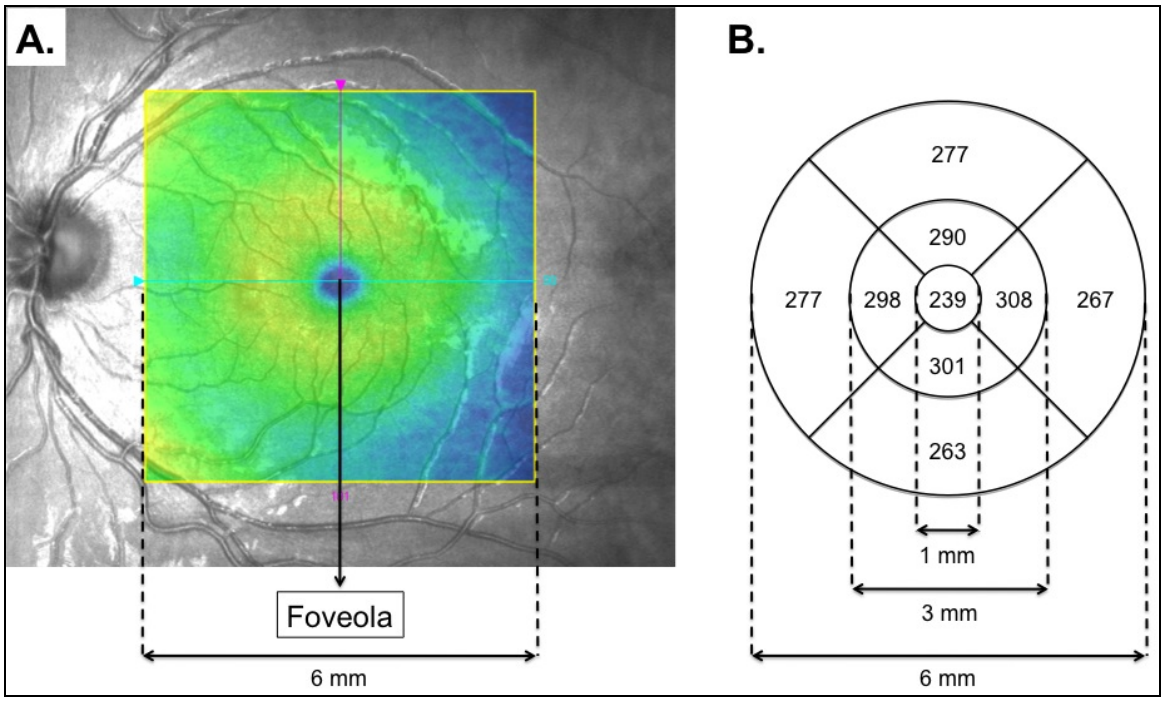

Figure 6.3: A) Cirrus OCT laser scan over a 6mm X 6mm area centered on the foveola. B) Example of average total retinal thickness values from 9 zones following the ETDRS pattern

A circular scan 12 degree in diameter centered on the optic nerve head (Fig 6.4) measures the retinal nerve fiber layer in 12 sectors around the optic nerve (Fig 6.4). The optic nerve head center is defined by mapping the central dark spot in the break in the retinal pigment epithelium. A circle of data with a radius of 1.73mm (12 degree) from the central dark spot is processed via bilinear interpolation and smoothing. Averaging of these A-scan measurements is automatically performed by a Carl Zeiss Meditec analysis algorithm developed for Cirrus HD-OCT, which does not involve user interaction. Average RNFL thickness of each sector was compared using T-test to examine difference between three visits in Type 1 and Type 2 groups.

Figure 6.4

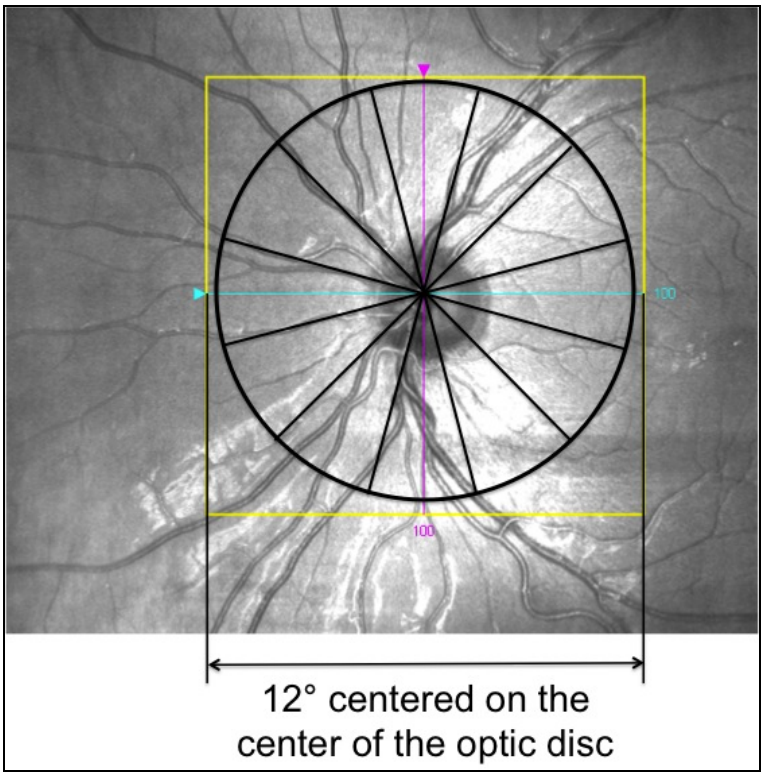

Figure 6.4: A circular scan 12 degree in diameter (1.73mm) centered on the optic nerve head measuring the retinal nerve fiber layer in 12 sectors around the optic nerve.

6.3.4 Vision function measures and analysis

Habitually corrected monocular visual acuities were obtained from each subject at each visit. High contrast and low contrast visual acuities were tested for each eye from 10 feet using Bailey-Lovie chart. Habitually corrected monocular contrast sensitivity was measured using the Pelli-Robson chart (Clement Clarke Inc., Harlow, UK) at 10 feet for each eye. Each letter correctly read was scored as 0.05 log units and the test was terminated when two letters of the triplet with same contrast were not identified correctly. Habitual correction was worn.

Monocular color vision testing was performed for each subject using the Adams desaturated D-15 color vision (DSAT) color caps under a Macbeth lamp (Illuminant C, 100 lux).[34] The cap order was noted and analyzed to compute a color confusion score using an automated program that analyzes the cap orders. The color confusion score represents the distance traveled in the color space in excess of a perfect arrangement expressed as a percentage.[3]

6.3.5 Statistical Analysis

In both patient groups (Type 1 and Type 2), t-tests were used to examine whether the three visits differed significantly from each other in any of the retinal structural health measurements (whole retinal thickness and retinal nerve fiber layer (RNFL) thickness), retinal neuro-functional health measurements (mfERG IT and mfERG AMP), and vision function measurements (contrast sensitivity, color vision, high contrast visual acuity and low contrast visual acuity). The change in neuro-retinal function over three visits was examined in both retinal locations, Edema locations and Edema free locations. In short we examined the retinal locations with edema and without edema at visit 2, to see how their neuro-retinal function was prior to development of edema and how their neuro-retinal function evolved after edema remained or resolved after some time (6-9 months).

The patient groups were compared to the controls using the T-test to see if they were different in any of the above measures at the baseline. Linear regression was performed at visit 1 to examine the effect of diabetes control (HbA1c and BGL), diabetes duration and blood pressure on retinal health measures.

6.4 Results

6.4.1 Summary

To summarize the main findings of this study, all 12 subjects had DME at visit 2; in 8 eyes (4 of the Type 2 group and 4 of the Type 1 group) the DME was resolved at visit 3. Three eyes of the Type 2 group and 1 eye of the Type 1 group, that had CSME at visit 2, did not resolve at visit 3 and continued to have CSME. Diabetes control (HbA1c and BGL at the time of exam), diabetes duration, and blood pressure did not show statistically significant association with retinal health measures (Linear regression analysis p-value > 0.5).

Central (foveal) vision function measures (VA, contrast sensitivity, color vision) though reduced appeared to be unaffected in the Type 1 group over the course of the DME. In other words vision function measures taken at visit 1 remain unaltered at the time of edema onset (visit 2) and did not seem to be influenced by the status of edema at visit 3. Vision function neither improved in eyes that were resolved (no edema) at visit 3, nor it got worse in eyes that remained unresolved (have edema) at visit 3. The same findings were seen in the Type 2 group except for color vision. Four patients in Type 2 group that had color vision defects (high color confusion scores) at visit 1 continued to worsen over the period of 3 visits irrespective of edema status.

No changes were observed in the retinal structure assessed by OCT in either group over the period of three visits. In both Type 1 and Type 2 participants, neuro-retinal

function was abnormal at the time of recruitment and it got even worse with the edema development. Despite edema resolution in 7 of 12 eyes, the neuro-retinal function was not restored. The locations having edema at visit 2 and locations free of edema at visit 2 were studied separately in both groups. Below are the findings:

6.4.2 Neuro-retinal function (mfERG)

6.4.2.1 Type 1 group

mfERG IT and AMP data extracted by stretching were used to examine possible differences in three visits in the Type 1 group. mfERG IT and AMP were examined to see change over three visits in Edema locations and in Edema free locations. Figure 6.5 summarizes results of mfERG responses in Type 1 group for all three visits. Following are the findings:

1. All the participants in Type 1 group had abnormal mfERGs (delayed IT (Z-score ≥ 2) and reduced AMP (Z-score ≤ 2)) in most retinal locations at visit 1 (prior to edema development).
2. mfERG IT:
   The retinal locations with edema at visit 2 had similar IT as prior to edema development (visit 1) and after the edema was established (visit 3). The retinal locations free of edema at visit 2 gave similar results. IT remained unchanged ($p > 0.5$) over three visits in both Edema locations and in Edema free locations. Despite 4 of the 5 eyes resolved at visit 3, there was no improvement in IT.
3. mfERG AMP:
   The retinal locations with edema at visit 2 had similar AMP prior to edema development (visit 1) and after the edema was established (visit 3). The retinal locations free of edema at visit 2 had significantly reduced AMP ($p < 0.005$) as compared to visit 1 (prior to edema development) but it stopped worsening and remained unchanged in visit 3 (after edema was established). Despite 4 of the 5 eyes resolved at visit 3, there was no improvement in AMP.

6.4.2.2 Type 2 group

mfERG IT and AMP data extracted by stretching were used to examine possible differences in three visits in the Type 2 group. mfERG IT and AMP were examined to see change over three visits in Edema locations and in Edema free locations. Figure 6.6 summarizes the results for mfERG responses in the Type 2 group for all three visits. Following are the findings:

1. All the participants in Type 2 group had abnormal mfERGs (delayed IT (Z-score ≥ 2) and reduced AMP (Z-score ≤ 2)) in most retinal locations at visit 1 (prior to edema development).
2. mfERG IT:
   The retinal locations with edema at visit 2 had significantly delayed IT as compared to visit 1 (prior to edema development). The retinal locations free of edema at visit 2 gave similar results. IT was significantly delayed in Edema locations as well in Edema-free locations as compared to visit 1 (both $p <$

0.00001). After the edema developed and remained for 6-9 months, IT appeared to remain unchanged in both Edema locations and in Edema-free locations. IT was significantly delayed from no edema stage to edema development in retinal locations with edema and in retinal locations free of edema. But once the edema developed, it stopped worsening further. There was no statistically significant difference in IT between visit 2 and visit 3 at both Edema locations and Edema-free locations. Despite the fact that 4 of the 7 eyes resolved at visit 3, there was no improvement in IT.

3. mfERG AMP:

   All retinal locations (Edema locations and Edema-free locations) had significantly reduced ($p < 0.00001$) AMP at visit 2 (edema development visit) than prior to edema development visit (visit 1). After the edema was established (visit 3) AMP continued to reduce ($p < 0.00001$). The reduction was greater in the retinal locations that were edema free at visit 2. Despite that 4 of the 7 eyes resolved at visit 3, there was no improvement in AMP.

Figure 6.5

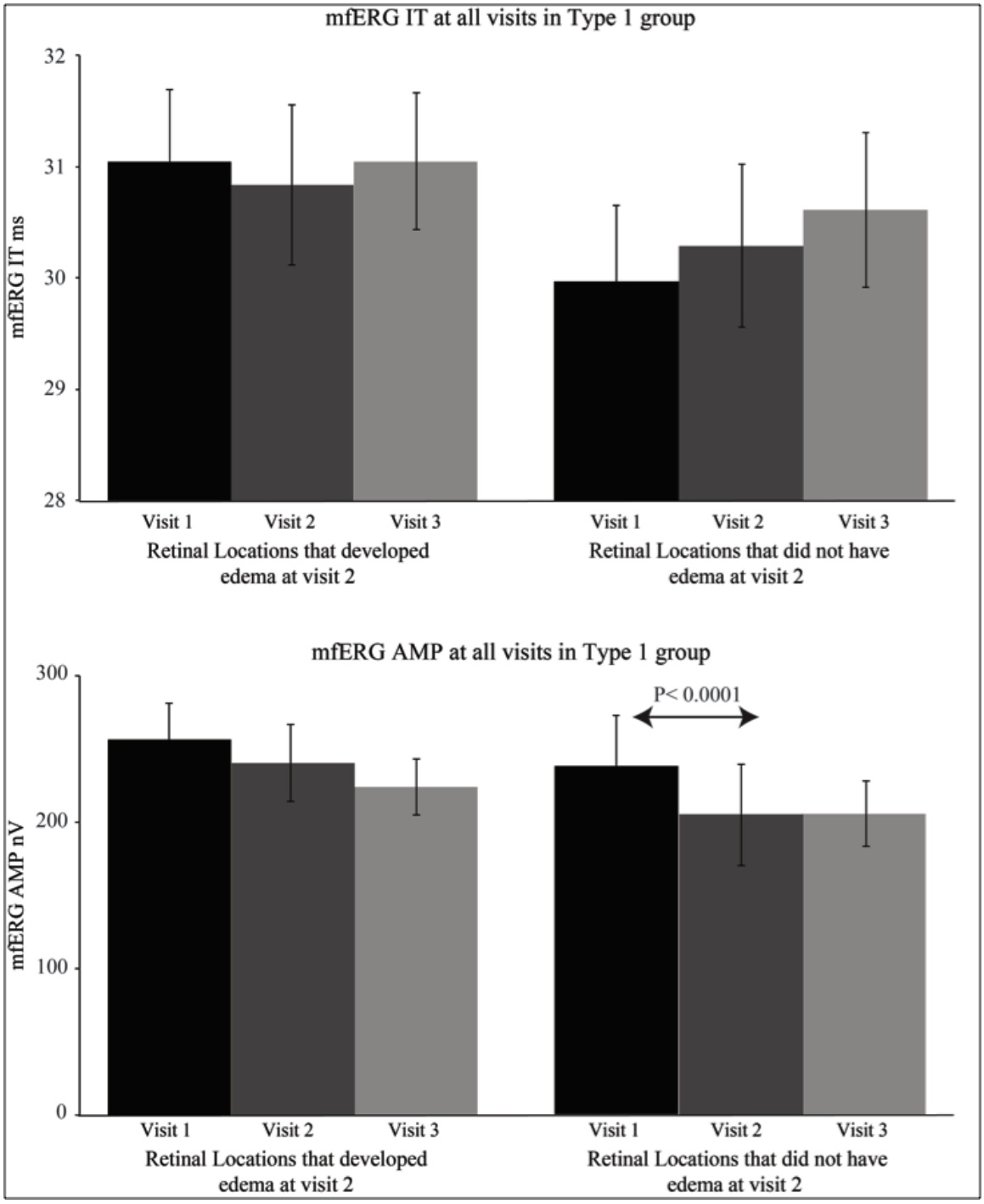

Figure 6.5: mfERG responses in Type 1 group for all three visits. mfERG IT did not changed significantly from visit 1 to visit 2 or from visit 2 to visit 3 in the locations with and without DME. mfERG AMP significantly reduced from visit 1 to visit 2 only in the locations that remained DME free at visit 2. AMP did not change further further from visit 2 to visit 3. Error bars represent 2*standard error of mean.

6.4.3 OCT Scans: Retinal thickness measures

Total retinal thickness computed from the central 6 mm area centered on the foveola was compared between the three visits in Type 1 and Type 2 groups. T-tests were used to examine change in the total retinal thickness values during the three visits. There was no significant difference seen in the total retinal thickness across the central 20 degrees of retina between visit 1, visit 2 and visit 3 in either group (Fig 6.7).

RNFL thickness was calculated in 12 sectors from an approximately 3.5 mm (12 degree diameter) area centered on the optic disc in every patient. T-tests were used to examine change in the RNFL thickness between the three visits. There was no significant difference seen in the RNFL thickness between visit 1, visit 2 and visit 3 in either group (Fig 6.8).

Figure 6.6

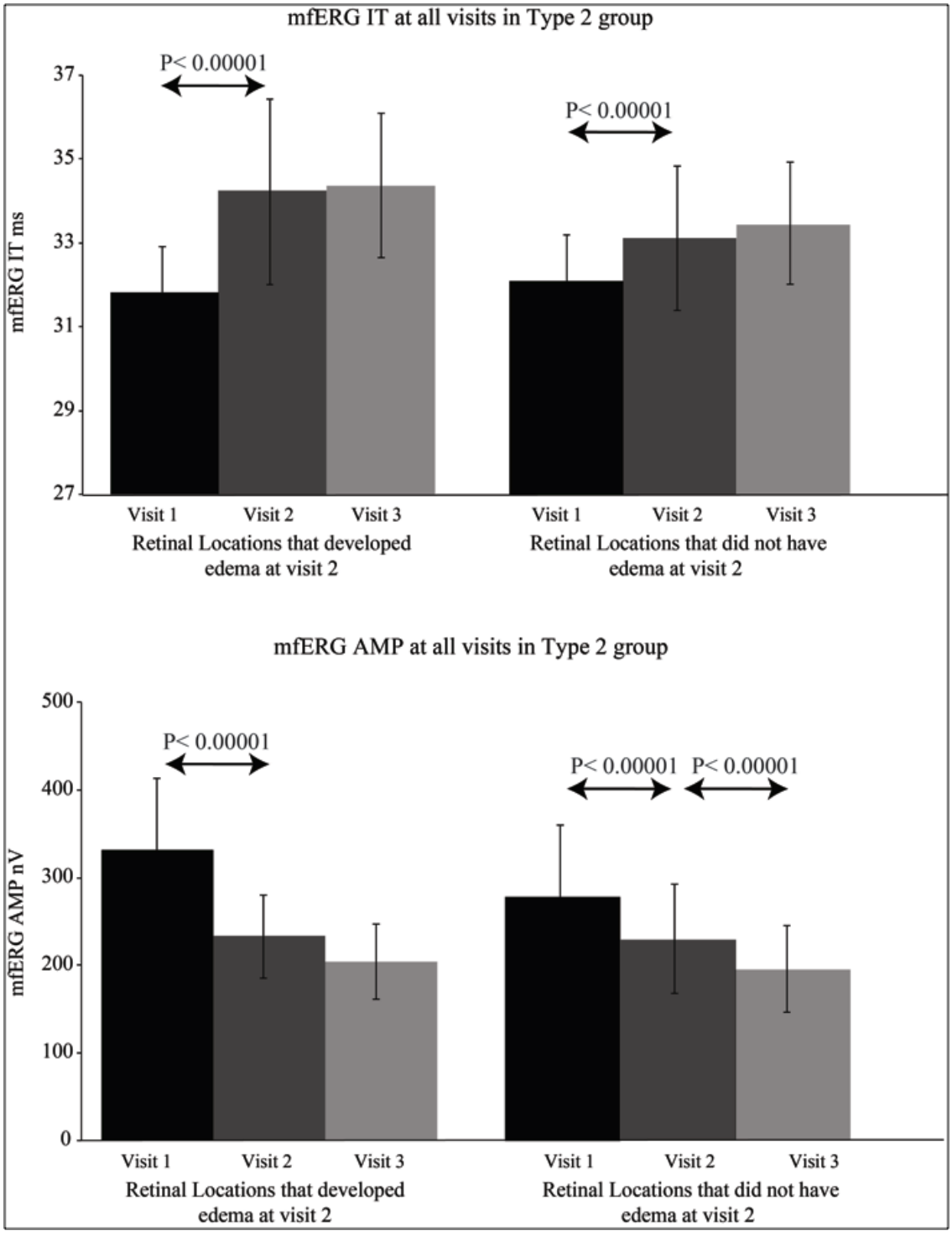

Figure 6.6: mfERG responses in Type 2 group for all three visits. mfERG IT was significantly delayed from visit 1 to visit 2 in the locations with and without DME at visit 2. mfERG AMP was significantly reduced from visit 1 to visit 2 in the locations with and without DME and decreased further in visit 3 only in the DME free locations. Error bars represent 2*standard error of mean.

Figure 6.7

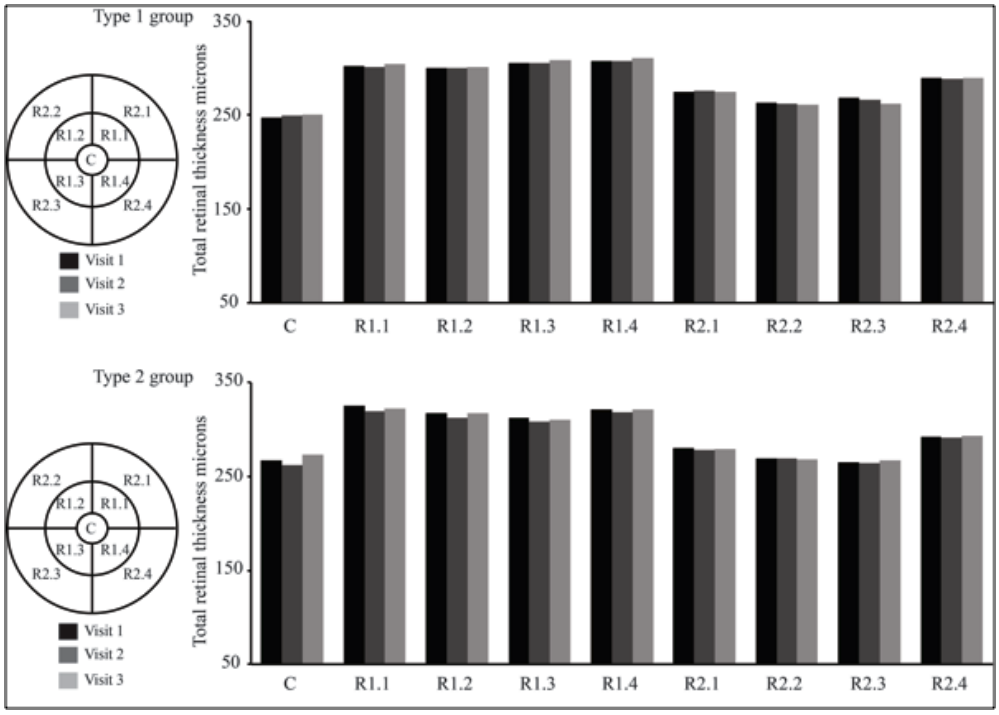

Figure 6.7: Total retinal thickness across the central 20 degrees of retina. No significant differences were observed between visit 1, visit 2 and visit 3 in either group

Figure 6.8

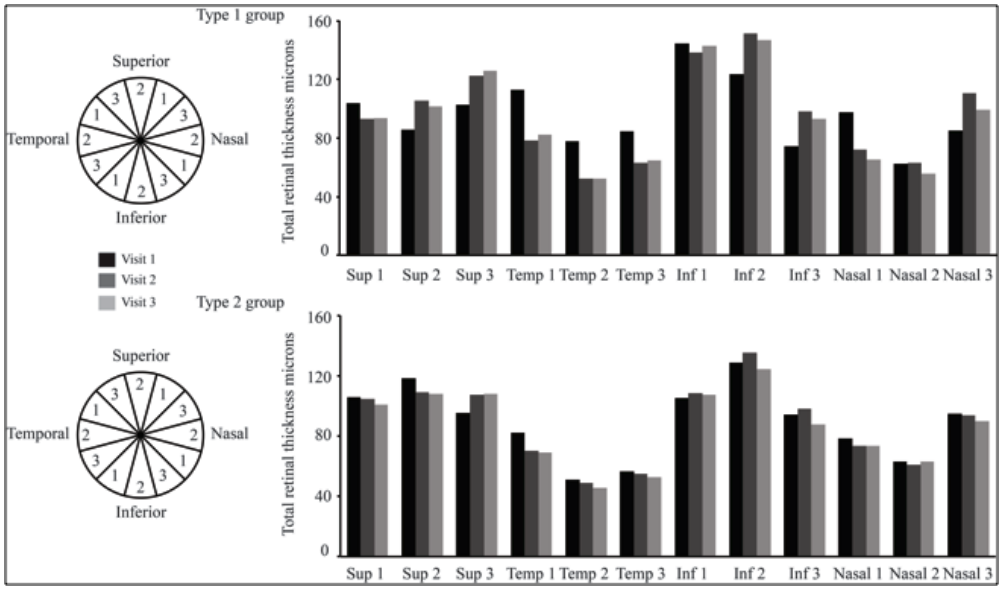

Figure 6.8: RNFL thickness from 12 sectors from an approximately 3.5 mm (12 degree diameter) retina centered on the optic disc. No significant differences were observed between visit 1, visit 2 and visit 3 in either group

6.4.4 Vision Function measures

Potential differences in vision function measures between consecutive visits in both diabetic patient groups were examined using paired t-tests. High contrast visual acuity, low contrast visual acuity and contrast sensitivity remain unchanged between the three visits in both diabetic groups. High contrast visual acuity and low contrast visual acuity in both patient groups were not significantly reduced at visit 1 from the controls, nor did it change significantly during the course of DME in either patient group (Fig 6.9). Contrast sensitivity score at baseline (visit 1) was significantly reduced in both patient groups as compared to the controls (Type 2: $p < 0.0001$; Type 1: $p < 0.05$). But as DME developed and advanced in time, contrast sensitivity score did not decrease further in either patient group (Fig 6.9). Four of the Type 2 patients had abnormally high color confusion score at visit 1 that worsened over visit 2 and visit 3 (Fig 6.10). In the Type 1 group color vision looked normal at visit 1 and did not change over the three visits.

6.5 Conclusions and discussion:

This study was motivated by a desire to examine how patients with DME, that do not qualify yet to receive medical intervention, behave in terms of their retinal health during the course of DME before treatment. Such non-clinically significant DME does not inevitably progress. When it does progress, it is uncertain how long it will remain unchanged in magnitude and how fast it will progress to become more vision threatening. A significant number of patients show spontaneous improvement in DME over time.

Our results indicate that progression or regression of DME not located near the fovea does not appear to have major effects on high contrast visual acuity, low contrast visual acuity and letter contrast sensitivity. The fact that most of the patients in the study have DME in the peripheral retina might be a reason to not find any effect on these measures where the maximum area tested on the retina is not larger than 1° at the fovea.[35-37] The only vision function found to deteriorate over the course of study was color discrimination. 50% patients in the Type 2 group who had abnormal color confusion scores at the baseline visit 1 continued to worsen over the three visits. All theses patients have their central retinas free of DME.

Total retinal thickness from an area 20° in diameter centered on the foveola and RNFL thickness of any area 18° in diameter centered on the optic disc, did not change with time. The fact that in 8 of 12 patients the central 20° of retina remained edema free might have influenced this finding. As the time course of DME progression is not known we believe that following these patients further in time might reveal some changes in the thickness. Also measuring the total retinal thickness could be a potential limitation; that possibility is raised in one of our recent studies.[38] It may be that examining the morphology and thickness of individual retinal layers will reveal more interesting information about the disease prognosis.

Figure 6.9

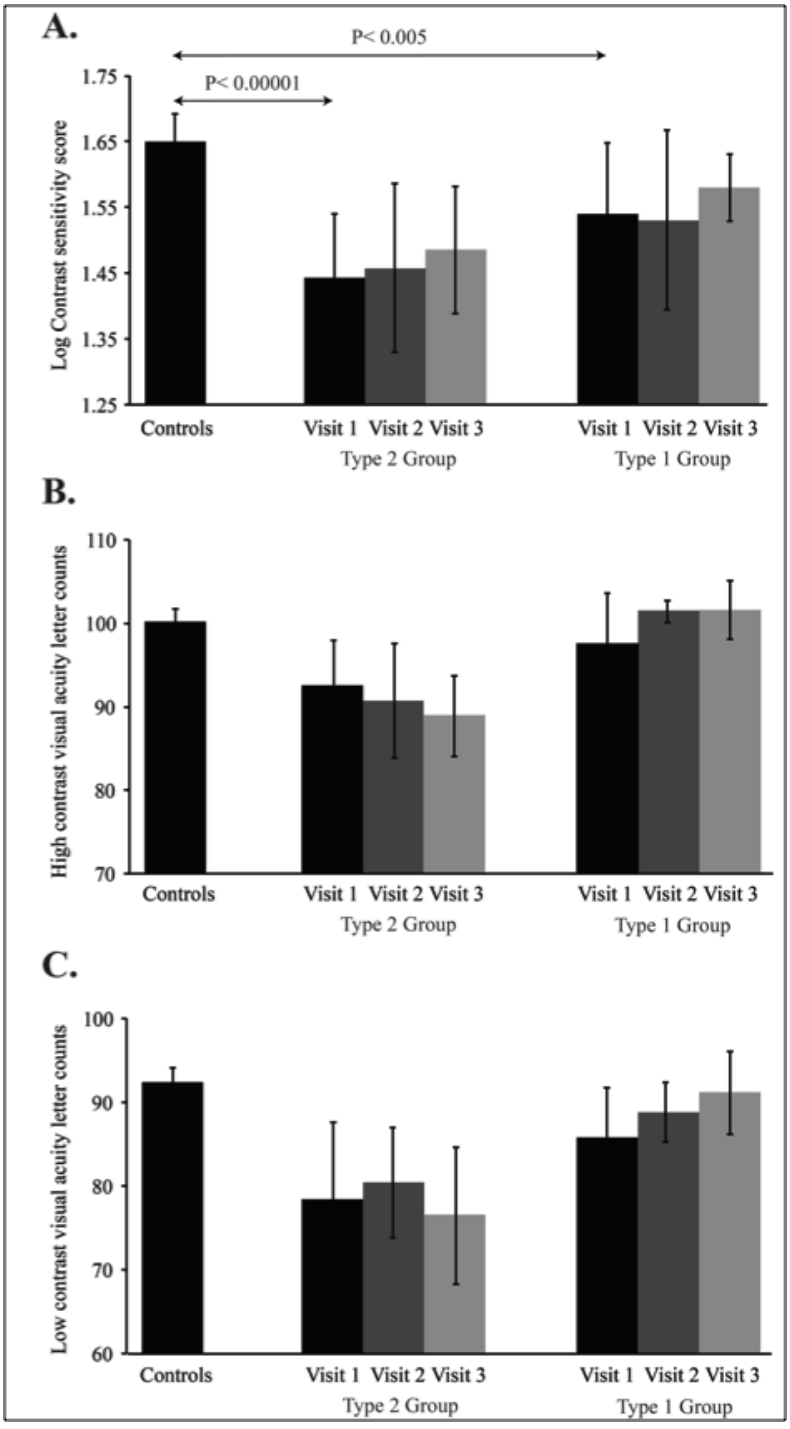

Figure 6.9: Changes in vision function measures. A) In both diabetes groups contrast sensitivity is significantly reduced (P-values are shown in the chart) at baseline visit 1 from controls. It does not change further in visit 2 and visit 3. B) High contrast visual acuity is reduced in both diabetes groups compared to controls but does not change during the next visits. C) Low contrast visual acuity is reduced in both diabetes groups compared to controls but does not change during the next visits.

Figure 6.10

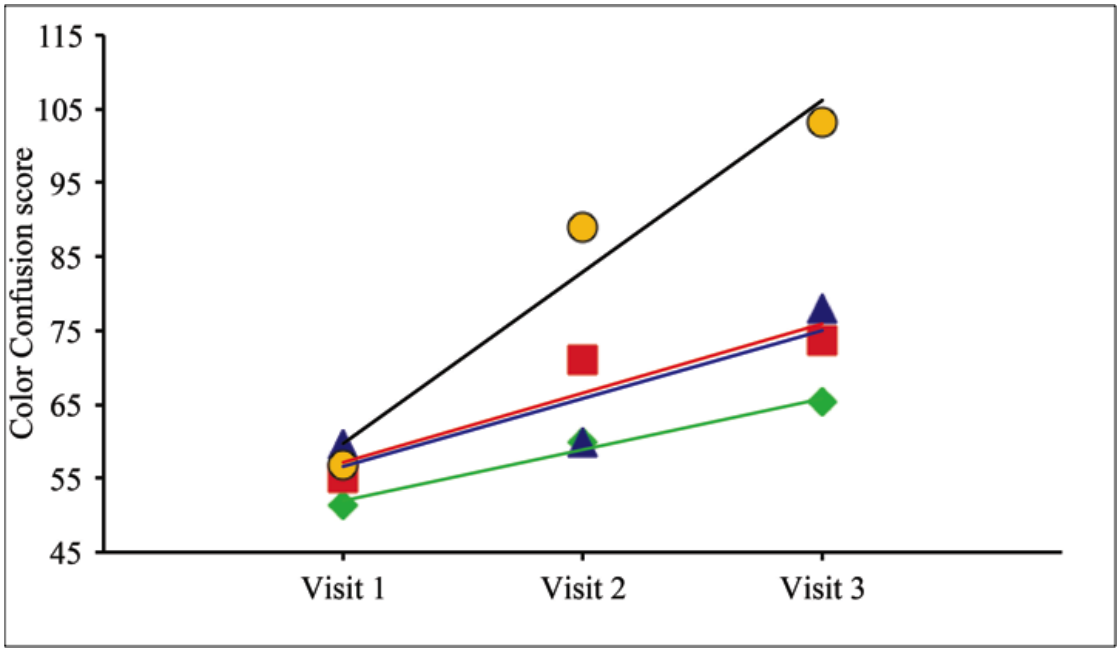

Figure 6.10: Changes in color discrimination score in the four patients who had abnormally high color confusion scores at baseline visit 1. Each colored symbol represents an individual patient who had abnormal color confusion score at visit 1 from the Type 2 group.

Neuroretinal function was altered in all patients at the time of recruitment. Type 1 and type 2 diabetes affects neuroretinal function differently and this is consistent with our findings in past studies.[4,38,39] mfERG function was not restored in the eyes that resolved the edema over time. The absence of any evidence of regained neural function of the retina could be due to the relatively short follow-up time; perhaps it was not long enough to allow any repair in neural function.

Our results for the Type 2 diabetes group clearly indicated that the mfERG IT is significantly delayed with the development of edema and it remains unchanged after edema is established (at least over the following 6-9 months). In fact there is also no change in the mfERG, over time, in non-edematous retina.

It has been demonstrated in prior studies that retinopathy and other diabetes-induced retinal changes affect IT.[4,6] Also, IT was shown to be predictive of new retinopathy in patients with or without early-stage retinal complications with high specificity and sensitivity.[40-42] The fact that the general retinopathy status for the whole eye, in most of the patients in this group, remained unchanged clinically after edema developed is consistent with the lack of IT change. More likely it could be because of a "floor effect" whereby the mfERG transmission time (IT) is so severely altered by the edema that there really is no further delay that can be reflected in the mfERG neural delay measure.

However, in contrast, the mfERG AMP continually worsened (reduced) with development of edema and after edema remained reduced for 6 to 9 months. These Type 2 participants had abnormally reduced AMP in most retinal locations before edema

developed and it continued to decrease when DME developed and after it was established. It will be interesting to follow these patients even further in time to see if the DME worsens, whether the diabetic subjects develop newer DME in the retinal locations that are currently disease free and if DME redevelops at the same or other retinal locations in patients where the DME has resolved.

For Type 1 diabetes, admittedly with a smaller number of participants, the results are a little different. Again the mfERG IT was unaffected over the course of the study, for both the retinal locations with or without edema. Part of the explanation for this may be that most patients had only moderate retinopathy in the periphery that did not change clinically over the study period. More likely perhaps, is that only 20% of patients in the Type 1 group developed CSME and it remained clinically constant over the 6 to 9 months. IT though abnormal to begin with did not get more delayed with development of edema.

Interestingly, for the Type 1 participants, the mfERG AMP was significantly reduced only in retinal locations that remained edema-free. This could be because the regions with DME might be subjected to a "floor effect", i.e. the amplitudes were so reduced initially that they could not deteriorate further. The reduction in the AMP in the DME free locations may be because these locations are still in a process of developing edema. This finding agrees with the recent publication from our lab in which we showed in the retinal zones that were retinopathy free and developed subsequent edema, mfERG amplitudes were reduced.[3]

A small sample size, variations in the follow-up time owing to the observational nature of the study and full retinal thickness evaluation as a measure of structural health of the retina, are the main limitations of the study. However, the results of this study suggest that the morphological presentation (retinal thickness) of DME may not correlate well with neuroretinal function. In other words, lack of change in the clinical presentation of DME or of full retinal thickness does not reliably give us detailed information about neuroretinal function or some vision functions such as color vision. Studying the changes in retina during a longer course of DME natural history may provide insight into mechanisms of DME and a better understanding. The various retinal health measures we have studied could also suggest the presence of different structural and functional DME phenotypes that may prove useful in tailoring DME treatment and forming a structured strategy of follow-up, which may lead to better visual outcomes for patients.

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Chapter 7: Conclusions and Future Directions

7.1 Summary and conclusions

Over the past three decades, the number of people with diabetes mellitus has more than doubled globally, making it one of the most important public health challenges to all nations. From 1995 to 2010, the prevalence of diabetes in the US increased from 4.5 to 26.4 per 1000 people, a six-fold increase and it still continues to increase.[1] The incidence of diabetes mellitus has risen dramatically in the United States since 1940.[2] According to the American Diabetes association, in 2011, 25.8 million children and adults in the United States, i.e. 8.3% of the population, have diabetes. Diabetes is the fifth leading cause of death in United States.[3]

While diabetes continues to be a huge health concern, diabetes-related morbidity and mortality are raising at an alarming rate. Diabetes is the leading cause of blindness in working aged Americans.[4] Diabetes results in chronic neuro-vascular damage leading to devastating vision loss and blindness. Current treatments are aimed at slowing the progression of sight loss once structural and functional retinal damage is clinically obvious. The treatments are expensive and invasive late stage treatments, with many complications and side effects.[5,6]

Research presented in this dissertation involves identifying predictors of incipient retinopathy that are needed for early diagnosis and for development of effective preventatives and curatives to save sight and to replace invasive treatments. This will promote the discovery of sensitive testing and eye health monitoring measures. The main technique I used, the mfERG, evaluates the health of retinal neurons at 103 retinal locations and is a very sensitive tool for detecting neural changes that are predictive of onset and progression diabetic retinopathy and edema.[7-13] It is crucial to recognize relationships between neural changes and other vision and structure changes in diabetes to create an early detection protocol. This understanding will help to broaden knowledge about structural and functional changes in retina that are exceptionally important for any new advances in curative as well as preventative medicines and to define novel sensitive endpoints for clinical trials of candidate therapies.

This dissertation presented four closely related studies that evaluated retinal health in adult patients with both type 1 and type 2 diabetes, especially at an incipient stage of diabetic retinal damage. We have used the mfERG measures, retinal thickness measures, vision function measures, AOSLO images of parafoveal capillaries and photoreceptors and other diabetes health indicators in both cross sectional and longitudinal studies to evaluate local changes in the retina of participants with diabetes.

Chapter 3 evaluated, in cross section, the relationship between retinal thickness and neuroretinal function. Retinal thickness was measured using oculo coherence tomography (OCT) and neuroretinal function was assessed using multifocal electroretinograms (mfERG). In previous studies done in our lab we have shown mfERGs to be sensitive to the retinal changes in diabetes.[9-13] OCT is a state of art technique to evaluate changes in retinal thickness.[14,15] We studied plausible relationships between changes in retinal thickness and changes in mfERGs to explore any spatial correspondence or coincidence in patients that are at an early stage of diabetes and do not

have any retinopathy. We also determined if there were any spatial agreement in the occurrence of abnormalities in two measures. The study was aimed at finding out whether these two techniques can be used as surrogates of each other or not.

We studied 76 participants (29 non-diabetic healthy controls, 10 patients with Type 1 diabetes and 37 patients with Type 2 diabetes). In conclusion, we found no difference in retinal thickness among the study groups. Also there was a lack of correlation or association between full retinal thickness and neuroretinal function at 37 locations measured. Furthermore, the absence of a relationship between specific spatial location abnormalities, for mfERG and retinal thickness, reinforces that there really is no significant association of mfERG with full retinal thickness, at least in patients without retinopathy. The two measures also differed in their capability to identify significant early retinal changes; more abnormalities were observed for the mfERG than for retinal thickness. Perhaps the most important clinical conclusion from these results is that the mfERG and OCT measurements of full retinal thickness cannot be treated as surrogates of each other in the evaluation of retinal changes produced by diabetes, at least before the onset of diabetic retinopathy.[16] This does not preclude the possibility that changes in thickness in individual layers may, in larger populations of participants, be related to mfERG function. That question could not be addressed in this research study.

We believe that new knowledge and a better understanding of local structure-function relationships may help development of effective therapies that could save vision at an earlier or even subclinical stage of diabetic eye disease and revolutionize care of diabetic retinopathy. In fact a better understanding of the structure-function relationships is important for advancing diagnostics, preventatives and therapeutic interventions at early stages of diabetic retinopathy.

Currently the assessment of retinal function loss in patients with diabetes, especially in early stages, relies heavily on visual acuity as a gold standard for vision function screening. Unfortunately, by the time the patients with diabetes start experiencing the visual loss, massive subclinical damage to the retina has already occurred. Researchers have proven that early interventions can prevent vision loss and retinal structural damage in later stages of diabetes that can lead to blindness.[17] This calls for the early detection and better understanding of early effects of diabetes on retinal structure and function.[18,19] For early detection we need more sensitive retinal health markers.

Chapter 4 presented findings from a study examining the relationship of contrast sensitivity with mfERG. This study first examined whether contrast sensitivity is different between participants with diabetes and the controls and whether the type of diabetes and the presence of background retinopathy influence this relationship. It was a cross-sectional study to explore the relationships of contrast sensitivity vision function and multifocal electroretinograms (mfERG) neural function of the retina in patients with diabetes. 46 non-diabetic healthy controls, 40 participants with type 2 diabetes and no retinopathy, 28 participants with type 2 diabetes and non-proliferative retinopathy and 12 participants with type 1 diabetes and no retinopathy; i.e. a total of 126 participants participated in the study.

mfERG retinal function in the central 2.4° of retina, was reduced (though within normal limits) in patients with retinopathy compared to other study participants. Contrast sensitivity was reduced in all participants with diabetes (Type 1 and Type 2) even in the

absence of clinically evident retinopathy. Contrast sensitivity appeared to further decline once retinopathy became clinically evident in type 2 diabetes. We think the reduction in contrast sensitivity is interesting, as all these participants have normal visual acuities and otherwise well preserved retinal function like low contrast visual acuity, color vision and, in some, even mfERG. Letter contrast sensitivity (which is a central foveal function) is affected in patients with retinopathy even though most of the retinopathy was mild to moderate and in the periphery of retina.

mfERG IT delays in the central retina (central 2.4 degrees) were associated with reduced letter contrast sensitivity in adults with type 2 diabetes and retinopathy. This was observed even though most of the retinopathy was mild non-proliferative retinopathy and most of the central retina was retinopathy-free in the majority of the eyes. There was a lack of correlation between contrast sensitivity and mfERG function in patients that were free of retinopathy. Once diabetic damage to the retina becomes clinically evident in type 2 diabetes, contrast sensitivity showed a negative correlation with mfERG IT. In type 1 diabetes we saw no significant relationship between contrast sensitivity and mfERG.

It appears that, in both type 1 and type 2 diabetes without retinopathy, letter contrast sensitivity, measured using Pelli-Robson charts, and mfERG findings are not identifying similar aspects of retinal neural changes. The two measures also differ in their sensitivity to detect significant early retinal changes with abnormalities observed for contrast sensitivity while the mfERG function in the central 2.4 degrees of retina is not significantly reduced compared to normal. This study indicated that letter contrast sensitivity discriminated between our study groups, especially the group of participants with type 2 diabetes and retinopathy were significantly worse than other groups. This result confirms that letter contrast sensitivity could be used as a powerful and sensitive screening measure of early retinopathy in diabetes.

Chapter 5 involved a study of 11 control participants and 12 adult patients with type 2 diabetes and no retinopathy. The goal of the cross-sectional study was to establish whether the retinal parafoveal capillary network is altered and to examine whether the cone photoreceptor spacing is increased prior to the onset of diabetic retinopathy in adult patients with type 2 diabetes. We wanted to test if AOSLO could be a reliable technique to image parafoveal capillary network and cone photoreceptors in patients with diabetes. The work was part of a larger collaborative study that has now been published.

> Tam J, Dhamdhere KP, Tiruveedhula P, Manzanera S, Barez S, Bearse MA Jr, Adams AJ, Roorda A. Disruption of the retinal parafoveal capillary network in type 2 diabetes before the onset of diabetic retinopathy. Invest Ophthalmol Vis Sci. 2011 Nov 29; 52(12): 9257-66.

To summarize, we found that using a novel application of AOSLO imaging, it is possible to noninvasively visualize and quantitatively assess diabetes-induced changes in parafoveal capillaries and cone photoreceptors. The parafoveal capillaries appear to be altered in type 2 diabetes, even in the absence of clinical signs of retinopathy. The photoreceptor spacing in participants with diabetes is significantly increased as compared to the controls included in the study. Though the findings were dramatically impressive, in few participants we had poor quality images that made parafoveal capillary and cone assessment impossible. This technique is really at the very early stage for clinical application and needs further development.

Chapter 6 involved a longitudinal study of the vision, retinal health, diabetes control and blood pressure of the 12 subjects (7 with Type 2 diabetes and 5 with Type 1 diabetes) who were at risk to develop diabetic macular edema (DME). The subjects were followed over time until and after DME developed. The subjects who developed DME were studied at three visits over an average period of 22 months. The main interest in this study was to understand how DME influences function and structure of retina over the course of the disease. Previous studies using similar techniques to examine DME either were cross sectional, designed to study the effect of DME on retinal health[20,21] or were longitudinal, to predict DME in patients at risk of developing edema[22]. This study has investigated how retinal health changes with time after DME is diagnosed and established, prior to treatment. We also examined what happens to the retinal health if DME resolves in the natural course of the disease.

In 4 of 7 patients in the Type 2 group and 4 of 5 patients in the Type 1 group, the DME resolved during the study period. Central (foveal) vision function measures (visual acuity and contrast sensitivity), though reduced from normal, appeared to be unaffected over the course of the DME. Vision function neither improved in eyes that were resolved, nor got worse in eyes that remained unresolved. Four of the 7 patients in the Type 2 group that had color vision defects (high color confusion scores) at the time of recruitment continued to worsen over the study period, irrespective of edema status. No changes were observed in the retinal structure assessed by OCT over the period of three visits. In both Type 1 and Type 2 participants, neuro-retinal function was abnormal at the time of recruitment and it got even worse with the edema development. Neuro-retinal function did not improve in eyes that resolved with time after the edema onset nor get worse in eyes that did not resolve the DME.

In the 3 studies presented in chapters 3, 4 and 6 we observed that Type 1 and Type 2 diabetes affects neuroretinal function differently and this observation is consistent with findings in our past studies.[8,16,23] In these current studies, Type 2 patients have worse mfERG retinal function compared to Type 1 patients despite the fact that their duration of diabetes is about half the duration of diabetes of Type 1 patients and their glucose control assessed by HbA1c was similar.

7.2 Future Directions

There are many areas for future work based on the work presented in this dissertation. We describe five potential directions:

(i) further investigation of neural function differences between Type 1 and Type 2 diabetes in patients with and without diabetic retinopathy and with DME,

(ii) examination of the relationship between retinal layer thickness and mfERGs

(iii) longitudinal studies with longer follow-ups to explore functional and structural behavior of retina in patients with DME,

(iv) studies of the relationship between parafoveal cone photoreceptor alteration and neuroretinal function

(v) exploration of the relationship between various central foveal functions like contrast sensitivity and color vision, which may be helpful in understanding the natural history of the retinal complications of diabetes.

The first follow up study (i) would involve a more comprehensive exploration of the retinal function and structure differences between type 1 and type 2 diabetes. All studies included in this dissertation that included both Type 1 and Type 2 patients, noted differences in the electrophysiological function between the two types of diabetes. Differences in neural function have been noted in our prior publications of our research group.[24-26] Patients with Type 1 diabetics had mfERGs that appeared much healthier with less delayed responses than patients with type 2 diabetes. The sample size in studies comparing Type 1 and Type 2 in this thesis is perhaps too small to allow meaningful distinctions between the two Types. The retinal structure-function study looked only at patients without retinopathy (12 patients). Though the contrast sensitivity study included patients with retinopathy, there were no patients with Type 1 diabetes and retinopathy in the study.

While the DME longitudinal study had only 5 patients with Type 1 diabetes and DME, a larger sample of both types of diabetics would be necessary for a definitive statements and conclusion about the differences between the two diabetes Types.

Differences in the electrophysiological function, retinal thickness changes (ii) and other vision function between the two types of diabetes in patients with various levels of retinopathy and macular edema would also be interesting. It will be important to control for the confounding factors like age, diabetes duration, age of onset of diabetes and diabetes control.

In the study presented in chapter 3 where we studied the relationships of full retinal thickness and mfERG, we did not find any special associations between these two measures. It remained unanswered if there exists any relationship between any particular retinal layer thickness and the neuro-retinal function assessed with mfERG. It will be interesting to know if diabetes has any affinity for certain retinal layers and if change in a particular retinal layer's thickness is associated with change in mfERG function.

The third potential follow-up study (iii) could be focused on patients with diabetes and DME. We found no change in retinal health indicators over a period of 22 months in patients with DME who had yet to receive any treatments. We also found that neural (mfERG) function was not restored after DME was resolved in those cases. The lack of any change in function could be because of the small sample size and relatively short follow-up time. It would be interesting to see in a larger sample how various type of diabetic retinal edema (diffuse, local, clinically significant) affect the retinal health in Type 1 compared to Type 2 diabetes after a year, and beyond, following edema development.

Understanding the differences between Type 1 and Type 2 diabetes presentations in terms of retinal health might prove crucial in planning pharmacological interventions and treatments strategies focused at halting the vision loss in diabetes.

We found that even in absence of clinically evident retinopathy there are obvious alterations in cone spacing and parafoveal capillaries in Type 2 diabetes. It will be interesting to examine if these findings also occur in Type 1 diabetes as well. We did not study the relationship of cone spacing alteration with neuro-retinal function. It could be a potential future study (iv) to explore the relationship of increased cone spacing and the mfERG. Revealing these relationships might suggest newer sensitive and objective retinal

health markers. Such novel retinal health indicators might be worthy endpoints for clinical trials.

We examined the relationships of contrast sensitivity and mfERGs. The other foveal vision functions like color vision are well studied in diabetes and have been shown to be altered. But the inter-relationships of various foveal psychophysical functions have not been well studied. Do all vision function change equally, simultaneously or even sequentially and are there any relationships among them? Such individual or combined data may provide more effective screening.

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Appendix

1. Abstract:

Kavita P. Dhamdhere, Marcus A Bearse Jr, Wendy W Harrison, Kevin Bronson-Castain, Shirin Barez, Marilyn E Schneck, Anthony J Adams Local Associations Between Retinal Function And Thickness Changes In Diabetes Without Retinopathy. AAO 2010 E-abstract 105891

Purpose: To examine, in adults with diabetes (DM) and no retinopathy, local relationships between retinal thickness measured with optical coherence tomography (OCT) and retinal function assessed with the multifocal electroretinogram (mfERG).

Methods: We studied 36 patients without retinopathy (9 type 1 DM; 27 type 2 DM; 50.0 +/- 11.8 y.o.) and 29 age-matched controls. P1 implicit time (IT) and N1-P1 amplitude (AMP) of each of the central 37 first-order mfERGs were obtained using a template scaling method. These were compared to spatially corresponding measurements of total retinal thickness (Stratus OCT 3). To account for variations with eccentricity, IT and AMP were converted to Z-scores, and retinal thickness was converted to percentile rank based on control data. Local abnormalities were defined as P-values <= 0.023. IT and AMP Z-scores were compared to retinal thickness percentiles using Chi-square and Fisher's exact analyses to assess their spatial associations.

Results: No significant spatial associations between either IT or AMP and retinal thickness were observed after accounting for inter-subject differences. This was true for both retinal thinning and thickening. The DM patients had a greater number of abnormal retinal locations than the control subjects in all measures. However, IT and AMP abnormalities were not spatially associated with retinal thickness abnormalities except within the type 2 DM group, where abnormally low AMP was associated with abnormally thin retinal tissue (P=0.01).

Conclusion: In adults with diabetes and no retinopathy, total retinal thickness changes are not spatially associated with retinal function changes as reflected by mfERG implicit time and amplitude. This suggests that change in retinal function precedes measurable changes in retinal thickness, and that mfERG and OCT do not provide comparable information in early diabetic retinal dysfunction.

2.Abstract:

Bearse MA, Dhamdhere K, Harrison WW, Bronson-Castain K, Barez S, Schneck ME, Adams AJ. Local Relationships Between Retinal Thickness And Functional Changes In Diabetes. Invest Opthalmol Vis Sci. 2010; 51: ARVO E-abstract 5070.

Purpose: To study the local relationships between retinal thickness assessed by optical

coherence tomography (OCT) and retinal function measured with multifocal electroretinography (mfERG) in adult patients with diabetes (DM) and no retinopathy.

Methods: We studied 36 patients with no retinopathy (9 with type 1 DM; 27 with type 2 DM; mean age = 50.0 +/- 11.8 yrs) and 29 healthy control subjects (47.0 +/- 12.8 yrs). The central 20 deg of one eye of each subject was examined. The amplitude (AMP) and P1 implicit time (IT) of each of the central 37 first order mfERGs were derived using a template scaling method (Hood & Li, 1997). These were compared to spatially corresponding retinal thickness measurements (Stratus OCT 3). To account for variation with eccentricity, retinal thickness was converted to percentile rank, and AMP and IT were converted to Z-scores based on control data. Local abnormalities were defined as P-values <= 0.023. Retinal thickness was compared to AMP and IT using Chi-square analyses to determine whether structure and function were locally associated.

Results: IT was positively associated with retinal thickness in the type 1 group ($P<0.001$) and marginally in the type 2 group ($P=0.025$), but not in the control group ($P>0.05$). AMP was positively associated with retinal thickness in the type 1 group ($P<0.001$) and the control group ($P<0.005$) but not in the type 2 group ($P>0.05$). As expected, the type 1 and type 2 groups had more abnormal locations than the control group. IT abnormalities were not spatially associated with retinal thickness abnormalities in any subject group ($P>0.05$). Abnormally thin retinal locations were associated with abnormally small AMP ($P=0.012$) in the type 2 group but not in the other subject groups.

Conclusions: Relationships between local retinal thickness measured with OCT and function assessed with mfERG are complex. For example, although longer mfERG implicit times are associated with retinal thickening in diabetes, there is not a significant spatial agreement between the two measurements in their classification of abnormalities. In addition, the associations are not necessarily monotonic, especially in type 2 diabetes.

3.Abstract:

Kavita P. Dhamdhere, Marcus A. Bearse, Jr., Brian E. Wolff, Wendy W. Harrison, Maria Cardenas, Shirin Barez, Marilyn E. Schneck, Anthony J. Adams. Associations Between Contrast Sensitivity And Multifocal Electroretinograms In Type 2 Diabetes. Invest Opthalmol Vis Sci. 2011; 52: ARVO E-abstract 1271/A21

Purpose: To examine the relationship between contrast sensitivity (CS) and the multifocal electroretinogram (mfERG) in adult patients with type 2 diabetes mellitus (T2DM).

Methods: Single eyes of 40 adult T2DM patients without retinopathy (NoRet group), 28 with mild to moderate non- proliferative diabetic retinopathy (NPDR group) and 46 controls were studied. Their mean ages were 53.6± 8.5, 53.3± 7.7 and 49.7± 11.4 yrs for the NoRet, NPDR and control groups, respectively. T2DM duration was 7.7± 4.0 for the

NoRet group and 8.0± 3.8 yrs for the NPDR group. All the subjects had 20/20 or better visual acuity. CS was tested using Pelli Robson charts. mfERGs were recorded and local N1-P1 amplitude (AMP) and P1 implicit time (IT) were derived using a template scaling technique (Hood & Li 1997). 45 deg fundus photos were graded for all subjects to identify the presence and location of NPDR. T-tests were performed to examine whether the subject groups differed. CS was compared to IT and AMP within two zones: C1 (the center mfERG from 0-1.2 deg eccentricity) and C2 (the average of 7 mfERGs from 0- 4.5 deg eccentricity) using linear regression.

Results: CS was significantly different between all subject groups ($P<0.001$). CS in the NPDR group was lowest, despite the fact that only 2 of these 28 patients had a lesion within the central 2.4 deg. IT in the NoRet and control groups did not differ in both zones. However, the NPDR group had significantly longer IT than the other groups in both zones ($P<0.001$). In the two zones, the NoRet and NPDR groups had similar AMP but were significantly lower than the controls ($P<0.001$). CS was negatively correlated with IT in both retinal zones in the NPDR group ($P<0.001$) but not in the other groups. CS was not correlated with AMP in any groups.

Conclusions: Longer mfERG IT in the central retina is significantly correlated with reduced contrast sensitivity in adults with T2DM and NPDR. This was observed even though most of the retinopathy was mild NPDR and the central retina was retinopathy free in majority of the cases. It will be interesting to monitor CS in NoRet patients who will develop NPDR in the future and to examine possible associations with mfERG.

4.Abstarct:

Kavita P. Dhamdhere, Marcus A. Bearse, Jr., Wendy W. Harrison, Shirin Barez, Marilyn E. Schneck, Anthony J. Adams. Contrast Sensitivity And Multifocal Electroretinograms Associations In Adult Patients With Diabetes. AAO 2011 E-abstract 115573

Purpose: To establish the relationship between contrast sensitivity function (CS) and retinal neural function assessed by multifocal electroretinogram (mfERG) in adult patients with diabetes (DM)

Methods: Single eyes of 40 type 2 DM (T2NoRet group) and 12 type 1 DM (T1Noret group) patients without retinopathy, 28 type 2 DM patients with mild to moderate non-proliferative diabetic retinopathy (NPDR group) and 46 controls were studied. Their mean ages were 53.6±8, 37±9.6, 53.3±7 and 49.7±9.4 yrs for the T2NoRet, T1Noret, NPDR and control groups, respectively. DM duration was 7.7±4,14±6.2 and 8±3.8 yrs for the T2NoRet, T1Noret and NPDR group, respectively. All subjects had 20/15 or better visual acuity. CS was tested with Pelli-Robson charts. mfERGs were recorded and local N1-P1 amplitude (AMP) and P1 implicit time (IT) were derived using a template scaling method. 45° fundus photos were graded to identify the presence and location of NPDR.

T-tests were performed to examine whether the subject groups differed. CS was compared to mfERGs from the central 2.4° using linear regression.

Results: CS was significantly different between all groups (P<0.001) and was lowest in the NPDR group, despite the fact that only 2 of these 28 patients had retinopathy within the central 2.4°. IT did not differ between NoRet groups and was very similar to controls. However, the NPDR group had significantly longer IT than the other groups (P<0.001). The diabetic groups had similar AMP but were significantly lower than the controls (P<0.001). CS was negatively correlated with IT only in the NPDR group (P<0.001). CS was not correlated with AMP in any subject group.

Conclusions: Longer mfERG IT in the central retina is correlated with reduced contrast sensitivity in adults with T2DM and NPDR. This was observed even though most of the retinopathy was mild NPDR and the central retina was retinopathy free in most of the eyes. There were no significant relationships between CS and mfERG in T1DM but this may be due to the smaller number of subjects in the group or the fact that none had NPDR.

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5. Abstract:

Johnny Tam, Kavita P. Dhamdhere, Pavan Tiruveedhula, Silvestre Manzanera, Shirin Barez, Marcus A. Bearse, Jr., Anthony J. Adams, Austin Roorda Noninvasive Assessment Of Parafoveal Capillaries In Type 2 Diabetes Prior To Onset Of Diabetic Retinopathy 11-A-4790-ARVO

Purpose: To investigate the appearance of the parafoveal capillary network in adult subjects with Type 2 diabetes prior to the onset of diabetic retinopathy, using adaptive optics scanning laser ophthalmoscopy (AOSLO).

Methods: 12 adult subjects with Type 2 diabetes and no diabetic retinopathy (age 55.5 +/- 7.8) were compared to 11 control subjects with no diabetes (age 52.2 +/- 10.6). AOSLO videos were acquired in the parafoveal region of one eye from each subject; for the subjects with diabetes, the absence of retinopathy was confirmed by grading of color fundus photographs by a retina specialist. Images of the parafoveal capillary network were generated using offline motion contrast analysis. These images were used to compare the two groups. The comparison included qualitative assessment of vascular features (loops and microaneurisms) as well as quantitative assessment of foveal avascular zone (FAZ) size and shape, capillary density (CD), and tortuosity of arterio-venous (AV) channels. AV channels were identified as the least tortuous capillary paths connecting arteries to veins.

Results: Loops and microaneurysm-like features were observed in both groups. In general, diabetic retinas (D) had more total features than control retinas (C). Comparing D to C, FAZ size was increased, but not significantly (p=0.42; n=12D, 11C); FAZ shape

was altered, but not significantly (p=0.48; n=9D, 11C); and capillary density within 0.15 degrees of the edge of the FAZ was decreased, but not significantly (p=0.48; n=9D, 8C). The tortuosity of AV channels was significantly increased ($p<0.05$; n=11D, 8C).

Conclusions: It is often difficult to find consistent changes in the microvasculature due to large intersubject variability. However, AOSLO imaging can be used to noninvasively visualize parafoveal capillaries and identify AV channels, which appear to be altered in Type 2 diabetes even before onset of diabetic retinopathy.

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6. Abstract:

Kavita Dhamdhere, Wendy Harrison, Marcus Bearse, Marilyn Schneck, Shirin Barez, Anthony Adams. "Changes in neuro-retinal and vision function after occurrence of diabetic macular edema: A pilot study" AAO 2011 E-abstract 120369

Purpose: The multifocal electroretinogram (mfERG) is a sensitive predictor of diabetic macular edema (DME) in at-risk patients. However, it is unclear how vision function continues to change after DME develops. Here we study changes in neuro-retinal and vision function after DME is established.

Methods: One eye of 12 patients with DME was included and was studied when DME developed (V1) and 6 to 12 months later (V2). There were 7 patients with type 2 diabetes (T2DM) and 5 with type 1 diabetes (T1DM). The mean ages were 58.6±7.6 and 34.1±8.9 yrs and DM durations of 13.8±6.2 and 25.6±7.4 yrs, respectively. All subjects had poor DM control (HbA1c≥8.2) and moderate to severe retinopathy (DR) and DME at V1, and DR and/or DME at V2. Fundus photos, visual acuity (VA), low contrast VA (LCVA), mfERG (VERIS 5.2), contrast sensitivity (CS), color vision (CV), OCT retinal thickness (Cirrus) and HbA1c were obtained at both visits. mfERG implicit time (IT) and amplitude (AMP) were converted to Z-scores, and grouped into 35 zones. Differences between V1 and V2 were evaluated.

Results: All subjects had DME at V1, resolving in 8 eyes at V2. CV appeared unaffected in the T1DM group but 4 of the T2DM subjects had high color confusion scores (CCS) at V1 that worsened at V2. mfERG IT did not worsen from V1 to V2 in either group. mfERG AMP was reduced from V1 to V2 only in the T2DM group ($p<0.0001$) in both retinal locations with or without DME. VA, LCVA and CS remained unchanged from V1 to V2 ($p>0.43$; $p>0.36$ and $p>0.16$). No changes were observed in total retinal thickness and retinal nerve fiber layer thickness ($p>0.58$ and $p>0.82$).

Conclusions: Progression or regression of DME does not appear to have major effects on vision function (VA, LCVA and CS). In T2DM patients, mfERG AMP worsens over time in established edema regardless of status or location of edema. In patients with T2DM who have abnormal CCS at the time of DME development, color vision continues to worsen over time.