The association between glycated haemoglobin levels and P100 visual evoked potentials in diabetes mellitus

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THE ASSOCIATION BETWEEN GLYCATED HAEMOGLOBIN LEVELS AND P100 VISUAL EVOKED POTENTIALS IN DIABETES MELLITUS

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Diabetes mellitus (DM) is a metabolic disorder which is characterised by hyperglycaemia resulting from defects in insulin secretion, insulin action or both. The long-term specific effects of DM include the development of retinopathy, nephropathy and neuropathy. Cardiac disease, peripheral arterial and cerebrovascular disease are also known to be linked with DM.

Type 1 diabetes mellitus (T1DM) accounts for approximately 10% of all individuals with DM, and insulin therapy is the only available treatment. Type 2 diabetes mellitus (T2DM) accounts for 90% of all individuals with DM. Diet, exercise, oral hypoglycaemic agents and occasionally exogenous insulin are used to manage T2DM. The diagnosis of DM is made where the glycated haemoglobin (HbA1c) percentage is greater than 6.5%.

Pattern-reversal visual evoked potential (PVEP) testing is an objective means of evaluating impulse conduction along the central nervous pathways. Increased peak time of the visual P100 waveform is an expression of structural damage at the level of myelinated optic nerve fibres.

This was an observational cross sectional study. The participants were grouped into two phases. Phase 1, the control group, consisted of 30 healthy non-diabetic participants. Phase 2 comprised of 104 diabetic participants of whom 52 had an HbA1c greater than 10% (poorly controlled DM) and 52 whose HbA1c was 10% and less (moderately controlled DM).

The aim of this study was to firstly observe the possible association between glycated haemoglobin levels and P100 peak time of pattern-reversal visual evoked potentials (PVEPs) in DM. Secondly, to assess whether the central nervous system (CNS) and in particular visual function is affected by type and/or duration of DM.

The cut-off values to define P100 peak time delay was calculated as the mean P100 peak time plus 2.5 X standard deviations as measured for the non-diabetic control group, and were 110.64 ms for the right eye.

The proportion of delayed P100 peak time amounted to 38.5% for both diabetic groups, thus the poorly controlled group (HbA1c > 10%) did not pose an increased risk for delayed P100 peak time, relative to the moderately controlled group (HbA1c ≤ 10%). The P100 PVEP results for this study, do however, reflect significant delay ($p < 0.001$) of the DM group as compared to the non-diabetic group; thus, subclincal neuropathy of the CNS occurs in 38.5% of cases. The duration of DM and type of DM had no influence on the P100 peak time measurements.

Keywords: Diabetes mellitus, glycated haemoglobin percentage, visual evoked potential.

Dedication

To Judy, Kira and Kelly.

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List of Abbreviations

ANS	-	Autonomic nervous system
Cd/m^2	-	Candela per square metre
CMV	-	Cytomegalovirus
CNS	-	Central nervous system
CVD	-	Cardiovascular disease
DAN	-	Diabetic autonomic neuropathy
DM	-	Diabetes mellitus
DR	-	Diabetic retinopathy
FPG	-	Fasting plasma glucose
FVEP	-	Flash visual evoked potential
HbA1c	-	Glycated haemoglobin
IDDM	-	Insulin-dependent diabetes mellitus
ICC	-	Intraclass correlation coefficient
IGT	-	Impaired glucose tolerance
ISCEV	-	International Society for Clinical Electrophysiology of Vision
NAION	-	Nonarteritic ischaemic optic neuropathy
NIDDM	-	Non-insulin dependent diabetes mellitus
NPDR	-	Non-proliferative diabetic retinopathy
OGTT	-	Oral glucose tolerance test

PNS	-	Peripheral nervous system
PPG	-	Post prandial plasma glucose
PVEP	-	Pattern-reversal visual evoked potential
T1DM	-	Type 1 diabetes mellitus
T2DM	-	Type 2 diabetes mellitus
VA	-	Visual acuity
VEP	-	Visual evoked potential
WHO	-	World Health Organization

Chapter 1

Introduction

1.1 Definition of diabetes mellitus (DM)

The World Health Organization (WHO, 1999) describes the term diabetes mellitus (DM) as referring to a 'metabolic disorder with heterogeneous aetiologies which is characterised by chronic hyperglycaemia and disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both'.

1.2 Effects of DM

The long-term pathological effects of DM include the development of retinopathy, nephropathy and neuropathy. Individuals with diabetes are also predisposed to other diseases including cardiac, peripheral arterial and cerebrovascular diseases (Alberti & Zimmet, 1998).

The International Diabetes Foundation (IDF) estimated that 387 million people worldwide were living with diabetes in 2014 with the majority (80%) of these residing in low- and middle-income countries (IDF, 2014). The IDF projects this number will increase to 592 million by the year 2035.

1.3 Classification of DM

The two most common forms of diabetes are Type 1 diabetes mellitus (T1DM) –previously described as insulin-dependent (IDDM) –and Type 2 diabetes mellitus (T2DM) –previously described as non-insulin dependent diabetes (NIDDM). Both Type 1 and Type 2 DM are caused by a combination of environmental and genetic factors that are briefly discussed next.

1.4 Epidemiology of Type 1 diabetes mellitus (T1DM)

Type 1 DM accounts for approximately 10% of all individuals with diabetes (Rewers, 1991). It is caused by the autoimmune destruction of the beta cells in the pancreas. Life-long exogenous insulin therapy administered in injection form is at present the only treatment available; without this treatment a person with T1DM will have a reduced healthy life expectancy (Imagawa et al., 2000). According to Holt (2004), an estimated 20 million people worldwide, mainly children and young adults, have T1DM. According to Holt (2004), asthma is the only chronic childhood disorder more prevalent than T1DM.

There is an increase of 2-5% per annum in the worldwide incidence rate of T1DM (Maahs et al., 2010). Epidemiologic studies have shown there is not a big difference in the incidence among individuals of different genders diagnosed before the age of 15 (Kyvik et al., 2004). The male-to-female incidence ratio is approximately 1.5 after the age of 25 years (Dorman & Bunker, 2000).

1.5 Environmental risk factors associated with Type 1 diabetes mellitus (T1DM)

Environmental risk factors are considered to be initiators or accelerators of beta cell autoimmunity. The most prevalent T1DM environmental risk factors are viruses and infant nutrition (Martin et al., 1991). Common viruses during childhood are the enteroviruses, including the Coxsackie viruses and polioviruses that have systemic effects on the pancreas (Dahlquist, 1998). Other viruses that have been linked with T1DM include the cytomegalovirus (CMV) (Pak et al., 1988), rubella (Menser & McIntosh, 1992), mumps (Hyoty et al., 1995) and the rotavirus (Honeyman et al., 2000).

Early exposure to the protein in cow's milk has been linked with T1DM as observed by Borch-Johnsen et al. (1984). These authors found that children with T1DM had been breastfed for a shorter time than their non-diabetic siblings or the non-diabetic children from the general population. It was therefore postulated by these authors that a shorter time of breastfeeding

may trigger incipient exposure to dietary proteins that induce an abnormal immune response in infants.

Delayed exposure to microorganisms due to higher standards of living may compromise the development of the immune system, and thus the role of hygiene has been hypothesised in the aetiology of T1DM (McKinney et al., 1997).

1.6 Epidemiology of Type 2 diabetes mellitus (T2DM)

Type 2 DM is the most prevalent form of the disease afflicting 90% of all individuals with diabetes. It is caused by altered insulin secretion and peripheral insulin resistance. Diet, exercise, oral hypoglycaemic agents and, occasionally, exogenous insulin is used to manage T2DM (Alberti & Zimmet, 1998).

According to the results of a study done by Wild et al. (2004) on the global prevalence of DM, the vast majority of T2DM are men and woman aged 45 to 64 years who live in developing countries. In 2000 it was approximated that 171 million people (2.8% of the world's populace) had DM and by 2030 this figure will more than double to 366 million (4.4% of the world's populace).

These figures were however drastically underestimated as reported by the IDF in their 2013 report, whereby 382 million people worldwide (8.3% of adult population) were reported to have DM, of which 90% being T2DM. The IDF projects that this figure could rise to 592 million, by 2035. The IDF (2013) report, rates China (109 million), India (69 million), USA (29 million), Brazil (14 million) and the Russian Federation (12 million) as the top 5 countries with the highest prevalence of DM. The UK DM prevalence as reported by the British Diabetic Association 2014/15 report, indicate that 4 million people have DM in the UK, this represents 6% of the UK population.

1.7 Environmental risk factors related to Type 2 diabetes mellitus (T2DM)

The main environmental risk factors for T2DM is obesity (body mass index $\geq$ 30kg/m^2) and leading an inactive lifestyle (Van Dam, 2003). In recent years the significant global rise in obesity has been associated with critical increases in the rates of T2DM (Zimmett et al., 2001). An estimated 80% of all new T2DM cases in adults and children are as a result of obesity (Lean, 2000).

A lack of regular physical exercise is a major risk factor for developing T2DM. Exercising not only helps to control a person's weight, but also helps to regulate the glucose and lipid metabolisms in the body thereby reducing the risk of T2DM. Making lifestyle changes that focus on a healthy diet and exercise reduce the risk of progression from impaired glucose tolerance (IGT) to T2DM by 60% whereas oral hypoglycaemic medication only reduces the risk of progression from IGT to T2DM by 30% (Pan et al., 1997).

1.8 Role of genetics in the development of DM

First-degree relatives have a greater chance (6%) of developing T1DM than unrelated members (1%) of the general population (Dorman & Bunker, 2000). First-degree relatives of T2DM are three times more likely to have the disease than those with no positive family history of T2DM (Flores et al., 2003; Hansen, 2003).

1.9 Glycated haemoglobin test (HbA1c) for the diagnosis for DM

The glycated haemoglobin test (HbA1c) that indicates the mean plasma glucose over the previous eight to 12 weeks (Nathan et al., 2007) has been in clinical use since the 1980s and has subsequently become a mainstay of clinical practice (Massi-Benedetti, 2006). A major advantage of the HbA1c test is that it can be taken at any time of the day and does not need to be taken while fasting.

The HbA1c test was recommended in 2009 by an International Expert Committee that included members of the European Association for the Study of Diabetes as the foremost diagnostic test to be used for the diagnosis diabetes (International Expert Committee, 2009). It was recommended by the Association that the HbA1c measurement should not only be used to diagnose DM, but also to identify those at risk of progression towards the disease. In 2011 it was recommended by the WHO that DM be diagnosed with HbA1c > 48 mmol/mol (> 6.5%) (WHO, 2011).

Glycation is the non-enzymic covalent chemical sourcing of glucose onto proteins through amino groups. The glycation of proteins occurs in tissues that are exposed to glucose. The magnitude of protein glycation is reliant on the level of exposure to glucose. The range of glycation is represented as a percentage of total haemoglobin (HbA) (Brownlee, 1994).

The HbA1c percentage depends on the average age of the erythrocytes in the blood specimen at the time of measurement; the percentage of HbA1c is higher in older cells (Bunn et al., 1978). The average red cell lifespan is 117 days in non-diabetics; females have a gender difference of around 11 days less (Diem & Lentner, 1970). The concentration of glucose in the blood can be changed by food intake, protracted fasting or exercise (Young et al., 2006). Most factors that influence fasting plasma glucose (FPG) do not alter HbA1c concentrations. Short-term lifestyle changes such as exercise and/or food intake has no influence on the HbA1c values (Bry et al., 2001).

Hyperglycaemia is the biochemical trademark of DM; however, the fasting and 2-hourly oral glucose tolerance test (OGTT) measures only a brief period in a single day. Cardiovascular disease (CVD) is the most common chronic complication of DM with a 5- to 10-times higher incidence rate compared to that of microvascular disease. Fasting plasma glucose is a poor indicator of future CVD events whereas HbA1c are good predictors; this further confirms the usefulness of HbA1c in diagnosing DM (Khaw et al., 2004).

HbA1c values of > 6.5% confirm the diagnosis of DM. If values are between 6.00 - 6.49% it indicates a high risk for DM and an effective prevention plan should be implemented. Individuals whose HbA1c values measure 5.50 - 5.99% and who also present with other diabetes risk factors such as central obesity, atherogenic dyslipidaemia, hypertension and metabolic syndrome should be made aware of their very high risk of developing DM (Bonora & Tuomilehto, 2011).

A diagnosis of diabetes is made when the HbA1c is greater than 6.5% (WHO, 2011). Higher amounts of glycated haemoglobin indicates poor control of blood glucose levels and this is associated with nephropathy, neuropathy and retinopathy (Hanssen et al., 1992). The reference range of HbA1c for healthy non-diabetics is 4 - 5.9%. A diabetic person with good glucose control has an HbA1c level that is close to the 4 - 5.9% reference range (Koenig et al., 1976).

1.10 Diabetic neuropathies

Diabetic neuropathy is a well-known complication of DM (Dyck et al., 1993). It may be diffuse (involving several body parts) or localised (involving a specific nerve and part of the body).

The nervous system consists of two major divisions: the central nervous system (CNS) that is made up of the brain, the cranial nerves, and the spinal cord. Secondly, the peripheral nervous system (PNS) comprises the nerves (that join the CNS with the sensory organs), the muscles, blood vessels, and glands of the body. The peripheral nerves can be motor, denoting that they are involved in motor activity such as walking, or they can be sensory which means they relay information back to the CNS. The CNS and the PNS combine to govern autonomic processes such as breathing, heartbeat, blood pressure, and visual function.

The neuropathies that ensue in DM are considered to be heterogeneous by their symptoms, the pattern of neurologic involvement, the course and pathologic alterations as well as the underlying mechanisms (Dyck et al., 1993; Llewelyn et al., 2005).

Peripheral diabetic neuropathy

Peripheral diabetic neuropathy, also known as 'distal symmetric neuropathy' or 'sensorimotor neuropathy', is a multifactorial condition involving metabolic changes (Greene et al., 1987), neurovascular dysfunction (Cameron & Cotter, 1994), and changes in trophic support (Brewster et al., 1994). Nerve damage occurs in the arms and legs; the feet and legs are generally affected before the hands and arms. Peripheral neuropathy can lead to muscle weakness and the loss of reflexes. A common complication is foot infections; if not treated quickly the foot infection may spread to the bone which often leads to the need for an amputation of the limb (Potter et al., 1998).

Diabetic autonomic neuropathy (DAN)

The condition known as 'diabetic autonomic neuropathy' (DAN) is not well understood in spite of the considerable negative influence it has on the quality of life and survival (Vinik & Erbas, 2001). Often classified as either clinically evident or subclinical, DAN can affect the entire autonomic nervous system (ANS). Diabetic autonomic neuropathy is evidenced by the dysfunction of one or more organ systems, for example, the cardiovascular, gastrointestinal, genitourinary, sudomotor or ocular systems (American Diabetes Association and American Academy of Neurology, 1988). However, of more significance is that DAN mostly occurs as a widespread disorder affecting all parts of the ANS because many organs are dually innervated by receiving fibres of the parasympathetic and the sympathetic divisions of the ANS (Ziegler, 1999).

Clinical symptoms of DAN do not usually occur for some time after the development of diabetes. Subclinical autonomic dysfunction in T2DM can occur within a year of diagnosis and within two years after diagnosis of T1DM (Pfeifer et al., 1984).

Focal neuropathy

Also described as 'cranial neuropathy', focal neuropathy is very rare – especially in individuals younger than 50 years. Its onset is thought to occur due to a microvascular 'infarct'. Such an episode can be painless or may be accompanied by a headache. The oculomotor nerve (CN III) is most frequently affected but pupil involvement is generally spared. The trochlear (CN IV) and the abducens nerves (CN VI) may also be affected (Asbury 1987; Thomas & Tomlinson, 1993).

Focal neuropathies are limited to the arrangement of single nerves and their inclusion is referred to as 'mononeuropathy' or 'mononeuritis multiplex'. Mononeuropathies are due to vasculitis and the ensuing ischaemia of the nerves (Vinik et al., 1992).

1.11 Central nervous system (CNS) involvement in DM

The central nervous system (CNS) is structurally and functionally linked to the peripheral nervous system (PNS). The CNS is afflicted by the metabolic and vascular ramifications of DM (Mijnhout et al., 2006). It has long been accepted that DM can lead to cognitive dysfunction (Miles & Root, 1922).

Cognitive dysfunction in DM has a prevalence of roughly 40% in long-standing or poorly controlled diabetes (Dejgaard et al., 2005). Hypoglycaemia and not hyperglycaemia has been associated with cognitive decline in insulin-treated diabetics (Deary & Frier, 1996). Cognitive dysfunction in DM has also been termed 'functional cerebral impairment', 'central neuropathy' and 'diabetic encephalopathy' (Mijnhout et al., 2006).

It has been shown that both cognitive dysfunction and peripheral neuropathy are present in individual persons with T1DM (Ryan et al., 1993). However, T2DM does not share the same mechanisms and risk factors. With regard to peripheral complications, hyperglycaemia is the

main protagonist while cerebral complications find their aetiologies more with a vascular component than with hyperglycaemia (Sheetz & King, 2002).

The pathological development in the CNS of individuals with diabetes is usually subtle whereas vascular trauma results in haemorrhage and infarction of the neuronal tissue. Compared to the blood-retinal barrier, the blood-brain barrier is less affected by uncontrolled hyperglycaemia. This may be due to the influence of the surrounding tissue and the richness of the cerebral microvessels in antioxidant enzymes (Tayaran et al., 1987) that help to protect the CNS from metabolic changes caused by hyperglycaemia (Mooradian & Morin, 1991). Compelling structural, haemodynamic, biochemical and physiological alterations occur in the cerebral microvessels of diabetics. It is these alternations that most likely lead to diabetes-related complications of the CNS (Mooradian, 1997).

1.12 Definition of visual evoked potential (VEP)

Visual evoked potentials (VEP) represents the response of the visual cortex to stimuli presented in the visual field. Visual evoked potential test the function of the visual pathway from the retina to the occipital cortex. The conduction of the visual pathways from the optic nerve, optic chiasm and optic radiations to the occipital cortex is measured. The information acquired from taking these measurements can thus give insight into the integrity of the CNS, and in particular into its relationship to visual function. The recording of visual evoked potentials to study the detail of the electrical activity of the brain has been possible for almost 50 years (Cobb & Dawson, 1960).

1.13 General description of VEP

Perfoming a VEP is a simple, sensitive and an objective technique for evaluating impulse conduction along the central nervous pathways. Increased peak time of the visual P100 waveform is an expression of structural damage at the level of myelinated optic nerve fibres. The visual cortex responds to various visual stimuli and this elicits different types of visual

evoked potential. The three main types of VEP are pattern-reversal (PVEP), pattern onset/offset and flash (FVEP). To record the VEP, the cortex needs to be stimulated as strongly as possible (Hubel & Wiesel, 1977).

Neurons in the cortex, unlike photoreceptors, are not strongly activated by stimuli that are homogenous over space and time. The stimulus has therefore to be structured in either space or time or both to produce a strong response. To meet these criteria the most common stimuli for VEP are short flashes or the appearance of contrast reversal of a visual pattern. Not all retinal positions are equally represented in the primary visual cortex. Visual resolution degrades from the fovea to the periphery, as do the density of the retinal ganglion cells. The magnification factor represents the size of the cortical representations as a function of position in the visual field (Drasdo, 1977). It therefore follows that the representation of the central 10^{0} radius represents more than half of the overall cortical representation of the visual world and this then dominates the VEP (Horton & Hoyt, 1991). A stimulus diameter of 15^{0} will elicit VEP of near maximal amplitude.

The standard clinical test entails the recordings of the pattern-reversal PVEP. The visual stimulus is a high contrast black and white checkerboard covering the central 20 – 30 ° of the visual field of which the black and white squares sequentially change places. The PVEP is then the averaged response to this reversal. The responses are recorded from three electrodes covering the occipital region with a mid-frontal electrode as the voltage reference. The signal at the midline occipital electrode usually contains a positive component which occurs roughly 100ms after the pattern-reversal; this is known as the P100. The P100 is preceded by a smaller negative component with a peak time of about 75 ms known as the N75 and, finally, another negative peak of N2 or N145 which occurs after the P100. The peak time of the P100 at the midline electrode is taken as the measure of the retinostriate conduction time (Walsh et al., 2005).

1.14 Factors influencing PVEP

The size of the checks displayed on the checkerboard screen affects the amplitude of the waveform and the peak time of the P100. Because the cells of the visual cortex are maximally sensitive to movement at the edges, a pattern-shift with a frequency of 2.0 ± 0.2 reversals per second is used (Odom et al., 2010). Additionally, pupillary size and gender as well as age all affect the PVEP. Visual acuity of up to 6/60 does not alter the response of the PVEP; instead, larger checks may be used. Certain drugs such as those used for epilepsy (carbamazepine and sodium valproate) prolong PVEP (Yuksel et al., 1995).

1.15 Optic nerve and central nervous dysfunction

Visual electrophysical studies of neurological disorders such as multiple sclerosis (MS) as well as Parkinson's disease (PD) have given new insights into the pathophysiology of such neurological diseases (Halliday et al., 1973). This has significantly improved knowledge of previously unknown aspects of the visual system and its organisation (Halliday et al., 1973).

Electrophysiological studies of the optic nerve using pattern PVEP tests have shown an increase in the peak time of the P100 wave. The P100 wave reflects the bioelectrical function of the optic nerve. Peak time increases in the P100 wave is a subclinical feature of optic nerve dysfunction and is usually noted without any pathological changes in the general ophthalmic examination. The dysfunction of the ganglion cells measured by electrophysiological tests precedes the structural changes observed in the ganglion cell layer. According to Pollock et al. (1989), this may explain the cause of visual disturbances reported by patients in the early stages of Alzheimer's disease who present with apparently normal eyes.

Visual evoked potential is a means of objectively evaluating the physiology and pathophysiology of the human visual system including the visual pathways and the visual cortex. It helps to detect abnormalities in patients with visual complaints who do not present with visible pathological ophthalmological changes. Such evoked potential abnormalities are called 'silent lesions'. Pattern-reversal stimuli are the most commonly used method to generate VEP in a checkerboard or grating pattern (Regan, 1989).

1.16 Overview and research rationale

The purpose of this study was to investigate whether there is a possible association between the P100 peak time of pattern-reversal visual evoked potential (PVEP) and the glycated haemoglobin percentage (HbA1c) in DM; to establish whether the central nervous system, in particular the visual function, is affected resulting in DM-related central neuropathy, and whether the type of diabetes and the duration that the participant has been diagnosed as a diabetic are relevant.

In DM the most common determinant of visual loss is retinopathy (Ewing et al., 1998). According to Karlica et al. (2010), this visual loss is a result of vascular and metabolic abnormalities of the retina. These researchers found VEP to be effective in identifying retinal dysfunction in that damage to retinal ganglion cells in DM can be detected where normal

visual acuity has been attained. Ganglion cell damage can be viewed as preclinical diabetic retinopathy where there is no ophthalmoscopic evidence of retinopathy (Karlica et al., 2010). Diabetic retinopathy (DR) is characterised as an advancing disease of the retinal vasculature (Antonetti et al., 2006). The clinical diagnosis of DR is made where lesions to the retinal vasculature are visible by observation means such as ophthalmoscopy or fundus photography (Early Treatment Diabetic Retinopathy Study [ETDRS], 1991). The clinical appearance of DR is due to an increased vascular permeability, vascular closure, and retinal ischaemia (Antonetti et al., 2006).

Visual evoked potential is a non-invasive procedure used to objectively examine the activity of the visual system as well as to investigate the integrity of the visual pathway in optic neuritis (Hood et al., 2000). Jones and Brusa (2003) suggest the resulting conduction change can be qualitatively evaluated by observing the peak time delay of the VEP. In the same study Jones and Brusa (2003) found that the original peak time delay of the VEP correlates to the size of the demyelinated area of the optic nerve.

The results of this study could impact on healthcare professionals involved in managing DM as well as all those individuals who have DM. The integrity of the participants' CNS will be established without the use of expensive and invasive medical procedures. Compared to previous studies that only referred briefly to the type of DM, and in which the control or duration of DM was not considered, this study will evaluate two distinct groups of participants, namely, those with poorly controlled DM (HbA1c > 10%) and those with moderately controlled DM (HbA1c ≤ 10%). Participants with other ophthalmic or neurological pathologies will be excluded so as to not influence the study results. It is thus by distinction of comparing two diabetic groups with no obvious ophthalmic pathology as well as by noting the duration the participants had their diabetes controlled that this study aimed to address the knowledge gap left open by previous studies.

1.17 Summary

This chapter covered brief descriptions of DM. The effects of DM and the involvement of the CNS were also mentioned. Visual evoked potential was also briefly described. In the next chapter a literature review will be presented.

Chapter Two

Literature Review: the role of visual evoked potential in diabetes mellitus

In the previous chapter the study was introduced. The introduction covered describing DM, the effects of DM and the involvement of the CNS. Visual evoked potential (VEP) was also described and explained. This chapter presents a more specific literature review on the role of the VEP in DM. The literature review was an important aspect of the research since it formed the basis of the methodology followed in this study.

2.1 Introduction

An in-depth literature review was conducted to determine the existing knowledge and current developments related to the association between glycated haemoglobin levels and P100 visual evoked potential (VEP) in diabetes mellitus (DM). The review also considers whether the central nervous system (CNS) and in particular visual function is affected by diabetes and, if so, the type of diabetes involved.

Relevant articles were identified covering DM and VEPs as well as central neuropathy that were published in peer-reviewed journals.

A search of the National Library of Medicine and the Web of Science database was done to identify all applicable articles published between 1970 and October 2014. Keywords and phrases used for the search to investigate the association between glycated haemoglobin levels and P100 visual evoked potential in DM included ‘diabetic neuropathy’; ‘diabetic encephalopathy’; ‘visual evoked potential and diabetes’; ‘diabetic central neuropathy and VEP’ and ‘glycated haemoglobin levels and vision’. Searches were restricted to ‘humans’.

In total 34 peer reviewed articles were identified. Of these 34 identified articles 28 were included in the literature review of this study. Six studies were excluded due to their unrelated testing techniques and protocols from the present study.

Table 2.1 lists the main studies used in this literature review and these were chosen to be included based on each individual study closely corresponding to the literature investigation carried out.

Studies denoted with an asterisk complied with the ISCEV standards for clinical visual evoked potentials.

Author and Year	Type of DM	Control of DM	Duration of DM	Diabetic Retinopathy	Number of Participants	P100 peak time
Kumar et al., 2014	T2DM	noted and compared	noted	no retinopathy NPDR	40 Control 40 T2DM	delayed in H1A1c > 7 %
Ismail, 2014	NIDD	not noted	noted	noted	30 Control 74 NIDD	no peak time delay
Shrivastava et al., 2014*	T1DM	noted	noted	nil	20 Control 20 T1DM	significant delay with positive correlation to duration of DM
Bhanu et al., 2012*	NIDD	noted	noted	nil	20 Control 20 NIDD	P100 peak time correlation with duration of DM
Gayathri et al., 2012*	T2DM	noted	noted	nil	20 Control 40 T2DM	delayed P100 significantly delayed HbA1c > 7%
Heravian et al., 2012*	T2DM	noted	noted	20 no retinopathy 20 NPDR	40 Control 40 T2DM	significant delay in both diabetic groups
Chopra et al., 2011*	T2DM	not noted	noted	nil	30 Control 90 T2DM	significant delay in both diabetic groups
Wolff et al., 2010	T2DM	noted	noted	2 no retinopathy 3 NPDR	13 Control 3 NPDR T2DM	both groups of participants P100 have peak time delay
Al-idani et al., 2009	T1DM and T2DM	noted	noted	nil	50 Control 20 T1DM 20 T2DM	Significantly delayed P100 of both diabetic groups

Author and Year	Type of DM	Control of DM	Duration of DM	Diabetic Retinopathy	Number of Participants	P100 peak time
Rajewski et al., 2007	T1DM and T2DM	noted	noted	noted	40 T1DM 40 T2DM	delayed P100
Dolu et al., 2003	T2DM	noted	noted	nil	30 Control 51 T2DM	increase of VEP peak time
Verrotti et al., 2000	T1DM	noted	noted	nil	30 Control 30 T1DM	significant P100 delay
Parisi et al., 1998	IDDM	noted	noted	nil	14 Control 14 IDDM	significantly delayed P100
Azal et al., 1998	IDDM, NIDDM	noted	noted	noted	20 Control 6 IDDM	P100 significantly delayed in diabetic group
Raman et al., 1997	T1DM and T2DM	noted	noted	nil	21 NIDDM 4IDDM 15 Control	Significantly delayed P100 of both diabetic groups
Seidl et al., 1996	T1DM	noted	noted	nil	53 IDDM 53 Control	increased P100 peak time
Parisi et al., 1995	IDDM	noted	noted	nil	10 Control 10 IDDM	increased P100 in diabetic participants
Moreo et al., 1995	T2DM	noted	noted	nil	35 Control 18 T2DM	increased peak time P100

Author and Year	Type of DM	Control of DM	Duration of DM	Diabetic Retinopathy	Number of Participants	P100 peak time
Uccioli et al., 1995	T1DM	not noted	noted	nil	10 Control 10 T1DM	significant delay in both diabetic groups
Ziegler et al., 1994	IDDM, NIDDM	noted	noted	noted	12 Control 7 IDDM	increased P100 in diabetics
Mariani et al., 1990	T1DM and T2DM	noted	noted	nil	35 Control 15 T1DM	increased peak time P100
Algan et al., 1989	T1DM and T2DM	noted	noted	noted	54 Control 31 T1DM	P100 significantly delayed in diabetics
Pozzessere et al., 1988	IDDM, NIDDM	noted	noted	nil	40 Control 11 IDDM	delayed VEP P100 in diabetic group
Anastasi et al., 1985	T1DM	noted	noted	nil	36 Control 50 T1DM	increased P100 peak time
Puvanendran et al., 1983	not noted	noted	noted	nil	35 Control 16 DM	increased peak time of P100 in diabetic group

2.2 Investigating the effects of DM and the CNS

The literature review revealed that although the peripheral nervous system (PNS) in DM has been extensively investigated, the pathophysiology of the CNS abnormalities associated with DM is still not well understood because few researchers have focused on this specific aspect in their studies as explained by Dolu et al. (2003). These authors support the statement of Moreo et al. (1995) who observed that in the nineties the neuroretinal function and the effects of DM on the CNS was an area that had received far less investigation than the pathophysiology of the PNS. In the same timeframe Dejgaard et al. (1991) found the use of neuroimaging techniques provided evidence that DM causes structural changes in the brain thereby confirming it is a long-term complication of DM and the CNS is indeed affected.

Imaging technology such as magnetic resonance imaging (MRI) principally remains an imaging, structural or anatomic evaluation revealing information about structural anomalies. Evoked potential (EP) testing determines functionality. Although it provides some insight into the physiology of a certain anatomic pathway, the understanding is less than the spatial or localising information provided by an MRI (Alessandrini et al., 1999).

It is therefore posited in this study that visual abnormalities in DM should be seen in the context of visual function: starting with the anterior chamber of the eye (including the retina, optic nerve, central pathways and occipital cortex) and ending up requiring higher cerebral function to perceive and respond to the stimulus.

2.3 Investigating the effect of DM on VEP

Electrophysiological investigation using visual evoked potential (VEP) is a very simple, sensitive and objective technique for evaluating impulse conduction along the CNS pathways (Azal et al., 1998). Visual loss resulting from DM occurs due to vascular disease as well as from metabolic anomalies affecting the retina and the visual pathways. VEP appraises the integrity of the neural pathways used for vision and is therefore an effective gauge of incipient visual changes in DM (Gayathri et al., 2012).

Increased peak time of the visual P100 waveform as observed in DM is an expression of structural damage at the level of myelinated optic nerve fibres (Bhanu et al., 2012; Azal et al., 1998). VEP is useful in diagnosing peripheral as well as central neuropathy in DM (Algan et al., 1989; Abraham et al., 1986). Many patients exhibit normal clinical examinations but show a decrease in nerve conduction velocity. The neural retina of diabetic eyes experience subtle functional changes before any microvascular lesions are detected by fundus photography (Lieth et al., 2000). Peak time delay of the VEP is a specific finding that evidences demyelinating diseases (Dolu et al., 2003; Cerizza et al., 1990; Carsten et al., 1989).

Two studies conducted in the mid to late 1990s (Azal et al., 1998; Moreo et al., 1995) reveal the P100 wave peak time were significantly larger in study patients with new onset non-insulin dependent diabetes mellitus (NIDDM) when compared with healthy control subjects. Moreover, the findings indicate optic nerve involvement may develop in patients with NIDDM even before the onset of any symptoms.

An interesting result occurred when the findings of various previous studies on the use of VEP and DM were investigated: the congruent finding was that, when compared to non-diabetic control participants, diabetic participants exhibited a definite increase in the P100 peak time (Kumar et al., 2014; Bhanu et al., 2012; Gayathri et al., 2012; Chopra et al., 2011; Algan et al., 1989). Of note though is that the percentages of participants who presented with abnormal

results differed quite significantly among the different study findings. It is proposed that these disparities were due to the differences in study populations and methods.

Ewing et al. (1998) state there is conclusive evidence showing abnormalities occur in the P100 waveform in DM before the development of retinopathy in newly diagnosed patients with IDDM when compared with those who have long-standing DM, as corrections of the blood-glucose levels improve P100 peak time delay measurements, however the P100 peak time values of long-standing DM, remained significantly delayed when compared to non-diabetic values. These researchers conducted a study on electrophysiological and psychophysical abnormalities and alterations in the vision of DM participants. They discovered a discrepancy exists as to whether there is a correlation as found by their study, with recent metabolic control and P100 wave peak time in poorly controlled DM.

Several studies have investigated the relationship between DM duration and P100 peak time. The Diabetes Control and Complications Trial (DCCT) Research Group (1993) reported conflicting information regarding this. Perros et al. (1996) further observe there are conflicting reports with regard to the abnormal VEP results in diabetic participants who did not have retinopathy. Despite some insightful findings, Perros et al. (1996) suggest the evidence found pertaining to the relationship between the duration of diabetes and VEP abnormalities has been limited. On the contrary, Ewing et al. (1998) state that a clear distinction has not been made between the diabetic types, duration of DM, presence of complication, visual acuity and quality of glycaemic control for the inclusion criteria of study subjects in many studies that compared the P100 peak time in DM.

In a more recent study Kumar et al. (2014) found VEP anomalies resulted from poor control and long duration of the disease. Both these factors correlated with significant VEP peak time delay as well as having a decreased amplitude of the waveform. Participants consisted of 40 T2DM without retinopathy and 40 T2DM with non-proliferative diabetic retinopathy (NPDR). The duration of diabetes varied in both groups from one to 10 years. A control group of 40 healthy non-diabetic patients was also investigated. Kumar et al. (2014) conclude their study

by stating as soon as tight metabolic control is achieved, the VEP abnormalities correct themselves. The authors suggest that this is because of these correlations taking place that VEP impairment is only functional and completely reversible. However Kumar et al. (2014) confirms that, VEP assessment is essential for the uncovering of pre-retinopathy changes that take place; it is plausible to reduce DM complications by using VEP as a testing procedure for diabetics.

The percentages of participants presenting with increased P100 peak time among the various studies examining VEP abnormalities as a consequence of DM range from 9 – 77% (Mariani et al., 1990; Algan et al., 1989; Anastasi et al., 1987; Ponte et al., 1986; Cirillo et al., 1984; Puvanendran et al., 1983). Collier et al. (1988) state they did find VEP abnormalities in diabetic patients with retinopathy but no abnormalities were found in any diabetic patients who did not have retinopathy. However, the findings of Mariani et al. (1990) indicate a prolongation of the P100 peak time in diabetic patients with no retinopathy present.

Uccioli et al. (1995) report impaired P100 peak time in VEP in newly diagnosed IDDM patients. Some research studies report a correlation between recent metabolic control and P100 wave peak time in diabetic patients, but not all the studies reviewed concur (Azal et al., 1998; Ziegler et al., 1994; Algan et al., 1989; Pozzessere et al., 1988).

Ponte et al. (1986) report there was an increase in the P100 peak time of 50 subjects with asymptomatic T1DM who had no retinopathy while Millinger et al. (1987) established that abnormal VEP can reflect maculopapular fibre or optic nerve involvement. More recently, in their study comprising 20 T1DM participants with no diabetic retinopathy (DR) Shrivastava et al. (2014) found results similar to those of Ponte et al. (1986). The diabetic participants in the study done by Shrivastava et al. (2014) exhibited a significant delay of the P100 as well as a correlation between the delay of the P100 and the duration of disease. However, they did not find a significant connection between the P100 peak time and the glucose level of the diabetic participants.

Central neuropathy in DM has not been appreciated as long as peripheral neuropathy has been. Describing clinical and pathological evidence for a diabetic myelopathy and encephalopathy, De Jong (1977) explains it was found that changes in the optic nerves occurred as frequently as changes in the peripheral nerve in DM; yet, there was no clinical evidence in any of the reviewed study cases of optic neuritis and the individual patients' visual acuity was normal.

Heravian et al. (2012) discovered abnormal peak time of the P100 wave in 60% of their DMT2 participants when compared to the control group. Forty diabetic patients (consisting of 20 diabetics having NPDR and 20 diabetic participants without NPDR) and a control group of 40 healthy, non-diabetic participants took part in this study. Interestingly, for both of the diabetic groups the peak time of the P100 wave was significantly delayed – even for those with no diabetic retinopathy. It was therefore concluded that an increased peak time of the P100 as observed in diabetic subjects can be interpreted as structural damage to the myelinated optic nerve fibres and, therefore, dysfunction of the CNS. Visual evoked potential can thus ascertain preclinical microvascular as well as neurodegenerative effects within the retina and along the visual tracts.

Li et al. (2001) report VEP irregularities in DM associate well with hyperglycaemia. Raman et al. (1997) found similar results to that of Al-idani et al. (2009) in that a positive correlation existed between glucose control and delayed P100 peak time, however no correlation was found in these two studies relating to duration of disease. However, Heravian et al. (2012) do not recognise the correlation between blood glucose levels and P100 wave peak time in diabetic participants as it did not reflect in their findings and neither did any association between the duration of diabetes and P100 wave peak time.

Bhanu et al. (2012) conducted a study with 20 participants with NIDDM and concluded the observed prolongation of P100 peak time could be the consequence of structural damage at the myelinated optic nerve fibres level or retinal ganglion cell damage in advance of possible

progression of diabetic retinopathy. Of note is that this particular study showed a compelling positive correlation with P100 peak time and the duration of DM, as did Seidl et al. (1996).

A significant correlation between the P100 peak time and the duration of DM was also observed by Chopra et al. (2011). These researchers concluded that VEP measurements in DM can be used to make an early diagnosis of central neuropathy thereby ensuring prompt medical care. Type 2 diabetes mellitus (T2DM) participants with different durations of the disease were used in the mentioned study by Chopra et al. (2011); however, there is no mention made in the method of glycaemic control of the participants. To determine if a correlation exists between the duration of DM and a prolonged P100 waveform, the blood glucose levels should be analysed as this will allow for a more decisive correlation to be adopted.

2.4 Rationale for study methodology

The majority of the studies reviewed with regard to examining the effects of DM and VEP report a significant increase in the P100 peak time of diabetic participants when compared to their non-diabetic controls. In fact, the different methodologies employed by the various research studies reviewed made it difficult to compare the study results and findings obtained because in many of them no distinction is made between the type and duration of DM, the presence of ophthalmic and neurological pathology, and the quality of glycaemic control.

There is a need to investigate the effect of the DM type, duration of diabetes, visual acuity, and level of glycaemic control on VEP whilst controlling for other ocular and neurological pathologies. Making use of two groups of participants and comparing poorly controlled HbA1c 10% and above measurements to the moderately controlled HbA1c 10% and less, will expand on the existing knowledge.

2.5 Summary

This chapter covered the literature review relevant to the association between DM and VEP. The rationale for the study design was discussed. In the next chapter the methodology of the study will be described in detail.

Chapter Three

Methods

In the previous chapter research relating to DM and VEP was presented. In this chapter the methods of the study will be covered. These will be based on the literature review studied from previous research to narrow the gaps in current knowledge.

3.1 Study design

An observational cross sectional study design was selected to observe the PVEP results of non-diabetic control and diabetic participants. The control participants were investigated in Phase 1 of the study and the diabetic participants in Phase 2. Observational cross sectional studies do not require follow-up consultation as data are obtained in a single, once-off consultation (Hennekens & Buring, 1987).

3.2 Aim of study

The objectives were to:

- determine whether there is a possible association between the P100 peak time of visual evoked potential (PVEP) and the glycated haemoglobin percentage
- to establish whether the central nervous system (CNS) in particular visual function is affected, resulting in DM-related central neuropathy as well as to the type of diabetes involved and the duration that the participant has been diagnosed as a diabetic.

3.3 Study plan

The study consisted of two phases.

In Phase 1 the aim was to determine the standards for the normal range of the PVEP instrumentation and thus determine the normative values for the P100 for this study. For the first phase healthy non-diabetic participants were recruited.

Phase 2 involved the recruitment of diabetic participants. These diabetic participants had been medically classified as either T1DM or T2DM. Particular attention was paid to the participants' glycated haemoglobin percentages. The diabetic participants were then formed into two groups: those having an HbA1c greater than 10% and those whose HbA1c was 10% and less (moderate control vs. poor control).

3.4 Setting

The study was conducted at the practice of Nagle and Louw Optometrists. The location is 12 Glen Gables, corner of Lynnwood Road and January Masilela Drive, Lynnwood Glen, Pretoria, Republic of South Africa (RSA). The above mentioned practice is an independent optometric practice owned and managed by the principal investigator, Mark Nagle.

3.5 Ethics

The Aston University Ethical Committee approved the conduction of this study on 10 April 2013. (See Appendix A1 - Ethics Application 421).

Permission to conduct the study was sought from the Pharma-Ethics Ethics Committee. Pharma-Ethics is an independent research ethics committee in the Republic of South Africa. Permission to conduct this study in the RSA was granted by the committee. (See Appendix A2).

3.6 Recruitment of participants

Recruitment was carried out in collaboration with Professor James Ker who is a specialist physician/cardiologist having his own private medical practice. This collaboration was necessary as it is beyond the scope of practice for an optometrist to order an HbA1c blood test.

The participants remained under the medical care of Professor Ker throughout the time of this study – from the time they were recruited until the study was completed.

The design of the study, data collection, data entry, data analysis and write-up were done by the optometrist, Mark Nagle.

3.7 Informed consent

Ethical considerations as well as the study procedures were explained to every potential participant individually before enrolment. It was explained to each individual that they had the right to choose to participate or decline participation without prejudice. If an individual chose to participate, he or she was advised that withdrawal from the study at any time was an option that would not be disputed or queried.

All questions from the participants were answered and queries clarified. They were made aware of their rights as participants, e.g. to willingly participate or decline participation without prejudice. They were assured their identities would remain anonymous and all data would remain confidential. Those who then voluntarily decided to participate in this study were requested to complete and sign a participation consent form. After signing the informed consent form, they were then included as participants in the study. Vulnerable groups, namely, children and mentally incapacitated participants, were not considered for participation or recruited as participants in this study.

Professor Ker kept the study data, relevant notes and original record file of each participant safely and securely locked away in his practice. Every individual participant was allocated a code (a computer generated file number) to guarantee her or his anonymity. Confidentiality of the participants' details as well as the data collected from them was upheld throughout the study process. These documents were transferred via e-mail and will be retained for a minimal period of 15 years. Calculation as to the time-period of 15 years was started after the last consultation .

3.7.1 Sample sizes.

The sample sizes for Phases 1 and 2 of the study were determined using nQueryAdvisor 7.0 Statistical Solutions Ltd. software – a one-sided testing using Fisher's exact test.

A sample of at least 30 participants is required to determine the normal range for P100 peak time from a control group. Accordingly, to determine the normal range for P100 peak time a sample of at least 30 participants is needed to be assessed. The determination of cut-off values to define the normal range assumes an underlying Gaussian distribution of the data. By convention this is a reasonable assumption when the sample size is at least 30. By making use of the Shapiro-Wilks test for normality (see Chapter 4), the P100 peak time values for both right and left eye did not deviate from a Gaussian distribution with p-values of 0.90 and 0.45 respectively.

For Phase 1 the control group comprised of 30 healthy non-diabetic participants. It was considered appropriate to compare two groups of participants, a moderate to good control group and a well-controlled group, to the mean control P100 peak time and also to the proportion of participants with delayed P100 peak time, i.e. the P100 peak time in excess of mean plus 2.5 standard deviation for the control group.

The sample size for Phase 2 was based on the comparison of proportions since a larger sample size than for a comparison of means is required. The proportions of participants with delayed P100 peak time in the moderate to well-controlled groups were expected to be about 30%. However, a sample size of 52 participants per group would have 90% power to detect a clinically relevant twofold increase in the poor control groups, i.e. 60%, using nQuery Advisor 7.0 Statistical Solutions Ltd. software.

3.7.2 Inclusion criteria for Phases 1 and 2

The inclusion criteria for both phases of the study were similar.

- Participants had to be older than 18 years.
- Both male and female participants were included as participants.
- All ethnic groups were included.
- A minimum visual acuity of 6/9 for distance and N5 for near for each eye (aided or unaided).

3.7.3 Exclusion criteria for Phases 1 and 2

The exclusion criteria for both phases of the study were similar.

- Participants presenting with ophthalmic pathology such as any media opacities in the anterior aspect of the eye (cornea, anterior chamber and crystalline-lens) as well as any posterior chamber media opacities (clear vitreous).
- All retinal pathologies (diabetic retinopathy, ARMD, retinal detachments to name a few) led to exclusion.
- Neuro/ophthalmic pathologies such as glaucoma led to exclusion.
- All neurological medical conditions such as multiple sclerosis (MS) led to exclusion from the study.

3.8 Study procedures

Phase 1 of the study was done with 30 healthy non-diabetic participants. In this phase the standards for the normal range of the PVEP instrumentation, therefore the normative values for the P100 waveform for this study, were determined. The results from the recent HbA1c blood tests of these 30 participants confirmed their HbA1c was between 4% and 5.9%. These results confirmed their non-diabetic status.

Phase 2 of the study was done with 104 diabetic participants. The diabetic participants were divided into two groups: group one comprised of 52 participants who had an HbA1c greater than 10% and group two consisted of 52 participants whose HbA1c measured 10% and less.

3.9 Data collection protocol Phases 1 and 2

- Confirmation of HbA1c measurement.
- Confirmation of signed informed consent forms.
- A comprehensive medical history taking that included making notes of all types of medications taken.
- Comprehensive ocular examination:
    - Including unaided visual acuities, ocular motilities, retinoscopy, pupil reactions, a subjective refraction, colour vision screening using an Ishihara pseudoisochromatic 24 plate, an Amsler Grid test, and a confrontational visual field screening.
    - A slit-lamp examination (Nikon NS IV) was performed starting with the lids/lashes, sclera, conjunctiva, and the cornea to examine the anterior structures of the eye. An anterior chamber depth grading was done using the Von Herrick technique. The crystalline lens examination from the anterior aspect to the posterior section with any media opacity was carefully documented.

- A posterior ocular examination was performed using both a direct and an indirect ophthalmoscope. An indirect ophthalmoscopy was performed using a Volk super field non-contact slit-lamp lens that enabled an undilated 95 degree field of view with a 0.76X magnification.
- An applanation tonometry (Nikon NS IV) was performed after instilling one drop (0.4%) Novesin Wander (Restan) in each eye. Three readings were taken for each eye. No fluorescein was instilled for this procedure.
- Fundus photography was done using a Topcon TRC NW200 (non-mydriatic retinal camera). All four retinal quadrants, i.e. superior nasal, inferior nasal, superior temporal and inferior temporal were photographed as well as the posterior pole for each eye.

3.10 Visual evoked potential (VEP) test methods Phases 1 and 2

3.10.1 Research VEP training

Basic PVEP usage was demonstrated by the Nihon Khoden agents before the study commenced. A qualified and registered neurophysiologist assisted in further PVEP usage training to ensure compliancy before the study began. The same neurophysiologist acted as the overseer of the control group during Phase 1 of the study.

3.10.2 VEP instrumentation room set-up

The main optometric consulting room was used to house the VEP unit. A Nihon Kohden (MEB 9400) neuropak (S1) 2-channel amplifier was used for storing and summating the waves. A 2-channel unit is used to measure the prechiasmal parts of the visual pathway, namely, the retina and optic nerve. Nihon Kohden neurophysiological technicians ensured that all the necessary installation and calibration procedures of the VEP unit were carried out in accordance to the (ISCEV Guideline 9B, 2008).

The temperature in the consulting room was kept to a constant 22°C. The air conditioner unit did not interfere with the noise level in the room. Additionally, all noise artifacts were removed from the room as measured with a Mastech (MS 6700) auto ranging digital sound level metre.

To ensure an equal darkened room for all participants, the room illumination was adjusted from one single light source to a pre-set rheostat level of 10 Lux. All other light sources from instrumentations were switched off. The room luminance was determined by measuring the luminance at four positions with a hand-held photometer (Goldilux) within 1 m from the stimulus-screen. The average of these measurements was used for the study. Each participant was given 5 minutes to adapt to the darkened light level in the room before the PVEP test began. No mobile phones or any other distracting devices were allowed in the main optometric consulting room.

Figure 3.1: PVEP participant set-up

3.10.3 PVEP screen

An Esquire 17-inch cathode ray tube (CRT)-60HZ screen (dimensions 330 mm x 275 mm) was used to display the stimulus. The screen was positioned 1 m from the seated participant resulting in a visual angle of 18.33 °and a check size of 1.164 °.The visual angle describes the

size of the image of one light or dark element at the retina and this commonly indicates the size of the squares on the checkerboard pattern.

The brightness as well as the contrast controls of the display screen were covered to prevent accidental adjustment thereby ensuring the same settings were used from the start of the study to its completion.

A screen stimulus luminance of 60 cd/$m^2$with a high contrast of 80% was maintained for all participants in this study.

In pattern-reversal PVEP the stimulus consists of a sudden change of all light pattern elements into dark ones and vice versa. This is used to avoid overall luminance changes. There was no overall change in the mean luminance of the screen during pattern-reversal. This is pivotal for PVEP as the peak time of the response increases when the mean luminance is decreased (Brigell et al, 2003).

The mean luminance of the stimulus screen was inspected after every 10 participants to ensure consistency in the study. A sheet of white paper was held in front of the stimulus-screen (distance 0.5 m from the screen) and with the lights set to testing conditions (10 Lux). The reflection of the black and white changing checkerboard did not indicate any transient luminance change with the pattern shifts.

3.10.4 PVEP instrument recording parameters

The electrode impedance did not exceed 5 kΩ.

Full field stimulus was employed; thus, the stimulus pattern was equal to both sides of the fixation point on the display screen. Full field testing is most sensitive in detecting changes in the visual system anterior to the optic chiasm. The checkerboard pattern-reversal consisted of black and white square checks numbering 16 x 12, measuring 19 mm x 19 mm, and having

a 4:3 width-to-height aspect ratio. Visual fixation was at the centre of the display screen. The reversal rate of 2 reversals per second was used to elicit the PVEP.

The bioelectrical signal was amplified and filtered by band-pass filters of 1-100HZ ($1H_Z$ = Lo-cut; $100H_Z$ = Hi-cut). With automatic rejection of artifacts and excluding artifacts given to each eye it averaged over 100 stimulus periods. This was repeated twice and the averages of the two waveforms were superimposed to demonstrate reproducibility. The signal-to-noise ratio was set at 5 microvolts.

The analysis time was 250 ms as this is the standard for transient PVEP for normal adults. For children and adult individuals with abnormally delayed PVEP, 500 ms is employed (Odom et al., 2009).

3.10.5 PVEP waveform and markings

The polarity refers to the positivity and negativity between the two electrodes connected to the inputs of the recording system. The relationship between the electric potential changes at the electrodes and the upward and downward deflections of the evoked potential tracing depends on where the electrode is connected (ISCEV, 2009).

The polarity convention used for this study was placing the positive (or active) electrode on the O_Z occipital region and the negative reference electrode on the Fp_z region 12 cm above the nasion. These electrode placements resulted in an upward deflection as a positive waveform (P100) and a downward deflection as a negative waveform (N75 and N145).

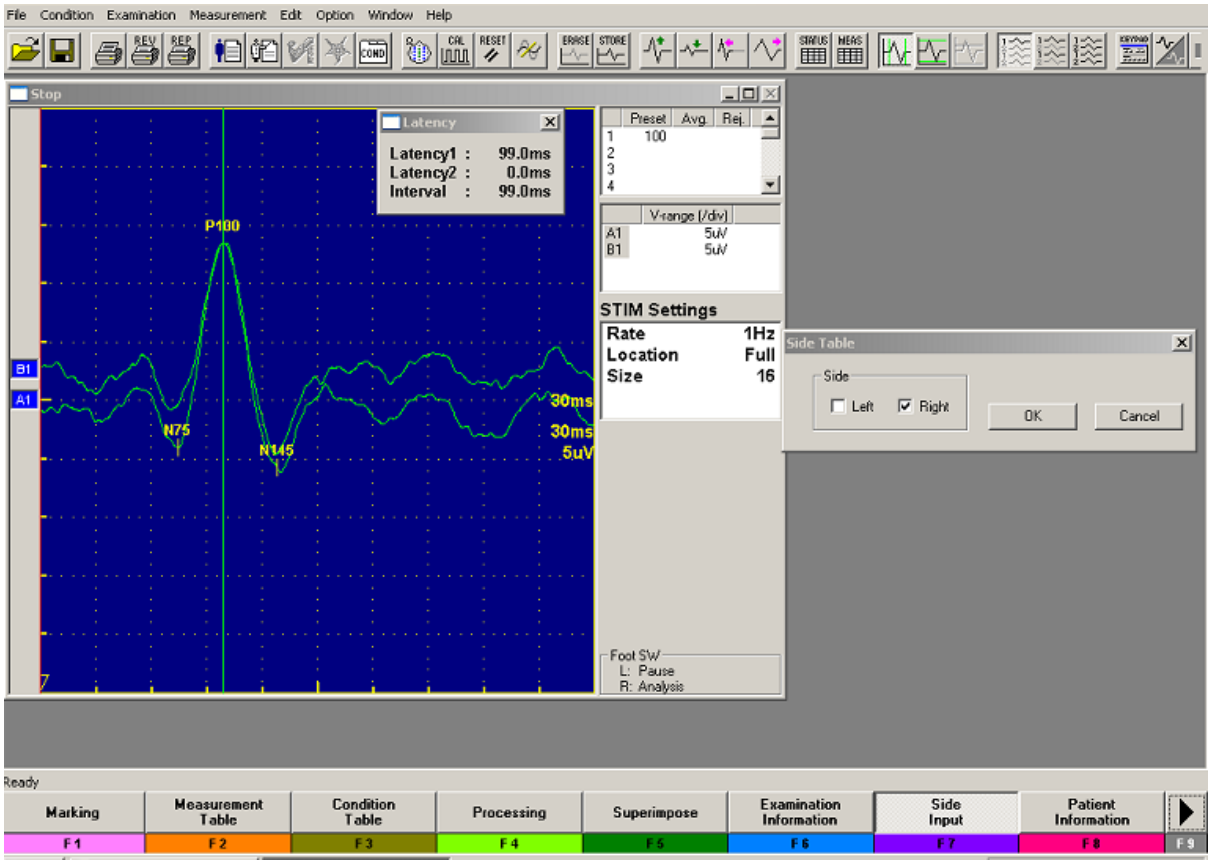

Figure 3.2: PVEP waveform demonstrating polarity

3.10.6 Participant preparation for PVEP test

The participant preparation was done in accordance with the ISCEV Standards (2009).
A 10-minute time interval was given to allow the participant time to recover from the eye test. None of the participants had their pupils dilated because an altered pupil size may change the stimulus luminance thereby affecting the PVEP amplitude and peak time. No participants presented with abnormal pupil sizes or with anisocoria.

The placing of the electrodes as well as the nature of PVEP testing was explained to each participant. A nylon 150 mm tape measure was used to take the participant's head measurements and markings on the scalp were made with a red greaseless marker pencil. These markings for placement of the electrodes were in accordance with the International 10/20 System (ISCEV Standards, 2009) as shown in Figure 1.2.

As shown in Figure 3.3 silver-silver chloride recording electrodes were placed on the scalp at the following reference points: 1) (O_z) occipital region for recording electrode; 2) the reference electrode (Fp_z) frontal or 12 cm above the nasion; 3) the ground electrode placed at the vertex (C_z).

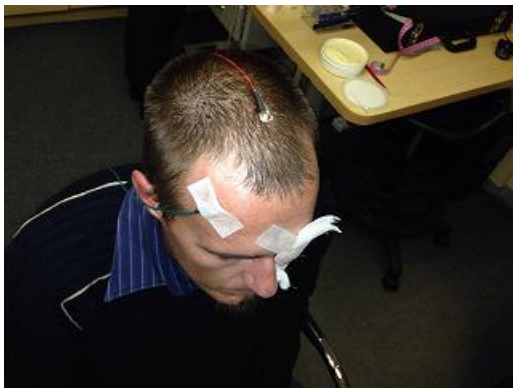

Figure 3.4:Electrode placing on participant

The International 10/20 System is established on head-size measurements (Jasper, 1958). The mid-occipital electrode location (O_z) is on the midline. The distance above the inion is determined by 10% of the distance between the inion and the nasion (for most adults this distance measures 3 – 4 cm).

The location of the ground electrode (C_Z) was marked as 40% of the inion-nasion distance above O_Z. On average this is 11 cm above the nasion for the 10/20 System (Chatrian et al., 1980). The reference electrode was placed on the right forehead area (Fp_z).

The polarity of O_z was positive and that of C_z negative resulting in the upward deflection of the P100 evoked potential and a downward deflection of the N75 and N145 evoked potentials.

Nihon Kohden Skinpure 135 g scalp preparation gel Y2-0019 was used to clean the scalp and remove dead skin cells and oils. To apply the electrodes, Nihon Kohden Elefix EEG contact paste Z-401CE was applied on the marked electrode locations to make sure a good, stable electrical connection between the scalp and the electrodes was made. Each electrode was pressed firmly into the Elefix contact paste onto the scalp. Micropore gauze was placed on top of the active electrodes to ensure their contact was maintained.

Figure 3.5: VEP participant preparation

The participant was comfortably seated at a distance of 1 m from the PVEP display screen. The distance was measured with a metre stick from the orbit to the centre of the display screen. The 1 m distance was maintained throughout the examination of each participant.

An eye patch with gauze (secured with a 24 mm x 10 m 3M Micropore 1530 dressing tape) was placed over the non-testing eye. It was explained to each participant that the occluded eye should not have to be forced closed by the eyelid and the natural blink reflex should continue under the eye patch. Therefore, each participant was asked if the occluded eye could see anything. For both Phases 1 and 2 the left eye of each participant was occluded first.

The electrode impedance was checked after the placements of the electrodes and again after the eye patch had been applied. If spectacles were worn it was placed over the eye patch. The electrode impedance did not differ by more than 20% between electrode sites (O_z and F_z) as seen in the image screen shot below.

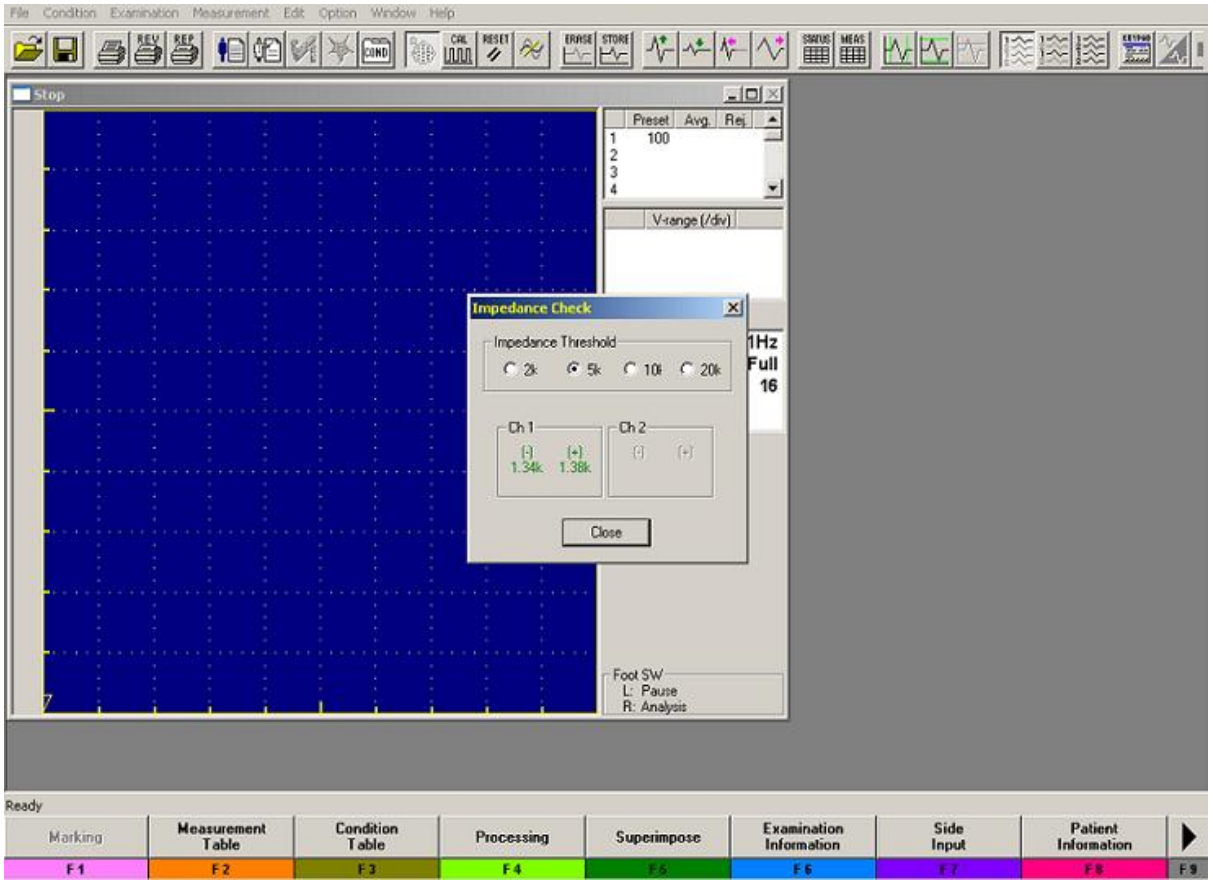

Figure 3.6: Electrode impedance screen shot

At the end of each procedure the participant's hair and scalp was cleaned of all the contact paste and the head markings made with the greaseless marker pencil. Each cup-shaped silver-silver electrode was scrubbed and washed with 0.5 g chlorhexidine and 70 ml propyl alcohol solution to ensure sterility before being reused.

3.10.7 PVEP instructions given to participants

The principal investigator, Mark Nagle, requested each participant to make sure she or he was feeling as comfortable and relaxed as possible when viewing the checkerboard screen. The participant was further requested to maintain a normal blink rate to ensure a clear optical image. Also, if the participant experienced any discomfort and/or defocus she or he was to mention it. The participant was assured that, if this should occur, the PVEP test would be paused so that she or he could regain her or his focus and concentration.
The participants were instructed to maintain their focus on the central white block in the middle of the display screen. They were encouraged to keep a silent count on the rate of the alternating black and white checks to help maintain their visual concentration.

No PVEP test was started before the participant had confirmed that he or she was ready and comfortable to begin.

3.11 Data analysis

For the analysis of the gathered data, the Stata Release 11 (Stata Corp LP, 2009) statistical software program was used.

The P100 peak time and HbA1c of the study groups were summarised using descriptive statistics thereby including the mean, the standard deviation, and the range. The cut-off value for delayed P100 peak time was determined as the mean +2.5 x standard deviation for the control group. The cut-point calculation assumes an underlying Gaussian distribution which was confirmed with the Shapiro-Wilks test for normality. Reliability was confirmed by doing

a second reading for 15 randomly selected participants from the control group. This second reading was done by repeating the whole PVEP procedure for the control group. The reliability was measured by the intraclass correlation coefficient (ICC) that was determined from a random effects maximum likelihood regression analysis.

With respect to the P100 peak time category (not delayed/delayed), the diabetic groups were compared using logistic regression and Fisher's exact test. From this logistic regression odds ratios along with 95% confidence intervals were reported.

For the comparison of the control and diabetic groups, a regression analysis, the analysis of covariance age was employed. Analysis of covariance (ANCOVA) with covariate age was used to determine the effects from this regression analysis. Testing was at the 0.05 level of significance.

3.12 Summary

This chapter described the design for this cross sectional study during which the possible association between the P100 peak time of pattern-reversal visual evoked potential (PVEP) and the glycated haemoglobin percentages were investigated. It included the study plan, a full description of the recruitment, the requirements for PVEP testing, and the preparation of the participants for this testing. The setting and instrumentation set-up for PVEP were described in detail. Ethical approval to conduct the study in the RSA was noted.

The next chapter will present the repeatability and reliability of Phase 1 – the non-diabetic control participants' PVEP findings as well as to determine the cut-points determining normal from delayed P100 peak time measurements for the DM groups.

Chapter Four

Reliability Analysis

The methods used to conduct this study were presented and discussed in the previous chapter. The participant selection, setting, PVEP testing were, inter alia, described in depth. It was further confirmed that ethical approval to conduct this study in the Republic of South Africa was obtained.

This chapter concerns the repeatability and reliability of the data analysis of Phase 1 that involved the non-diabetic participants.

4.1 Phase 1 – Reliability assessment of the non-diabetic group

Thirty non-diabetic participants were enrolled in Phase 1 of the study. The participants consisted of 10 males and 20 females with a mean age of 32.6 ± 8.52 years. The mean HbA1c values of 5.38% ± 0.17 confirmed all 30 participants' non-diabetic status (Koenig et al., 1976). The entire and exact PVEP testing procedure was repeated with each of 15 participants. Thirty observations is generally accepted to conform with the normal distribution theory (Pagano, 2004).

Repeatability of the P100 measurements was assessed in a subset of 15 control participants with the intraclass correlation (ICC) (on a scale of 0 to 1 where 1 represents perfect reliability and with no measurement error and 0 indicates no reliability) using random-effects maximum likelihood regression. The observations are displayed in Table 4.1.

The ICC equation used for this study was: ICC = (mean squares between patients – mean squares within patients)/(mean squares between patients + mean squares within patients). The ICC was the preferred reliability statistical analysis test for this study because intrarater agreement is best measured by ICC (Lachin, 2004).

Table 4.1: ICC as measure of repeatability of waveform markings P100, N75, N145 and amplitude – control (non-diabetic) group – Phase 1

Waveform	ICC	
	Right	Left
P100	0.928	0.831
N75	0.820	0.844
N145	0.931	0.931
Amplitude	0.933	0.933

Considering an ICC of 0.9 as excellent, the repeatability of the PVEP results for VEP unit (Nihon Kohden MEB 6400K) displayed in Table 4.1 was acceptable for this sample.

4.2 Normative data

The cut-points for PVEP peak time to determine normal from delayed peak time were determined as mean +2.5 x standard deviations, where exceeding the cut-point will imply delayed PVEP peak time. (See table 4.4; 4.5; 4.6; 4.7). The latter calculation assumed an underlying Gaussian distribution which was confirmed by the Shapiro-Wilks test for normality. The result confirms that the underlying distribution did not deviate from the Gaussian distribution for both the right and left eyes.

Table 4.2: Cut-point measurements for delayed peak time P100, N75, N145 – control (non-diabetic) group – Phase 1

	Right (ms)	Left (ms)
P100	110.64	111.86
N75	85.70	79.70
N145	157.02	158.97

Table 4.3: Shapiro-Wilks test for normal data p-values – control (non-diabetic) group – Phase 1

Variable	Observations	Right p-value	Observations	Left p-value
P100	30	0.90	30	0.45
N75	30	0.20	*28	0.14
N145	30	0.85	30	0.21

The left N75 produced an original p-value of 0.00071 using 30 observations; the data thus deviated from normality. Two outlier measurements of 81 ms and 89 ms respectively were identified and removed, resulting in a new but not significant p-value of 0.14 thereby showing that the new data did not deviate from normality.

4.3 Summary statistics P100 – control (non-diabetic) group – Phase 1

The right and left eyes did not differ significantly with respect to the P100 ($p = 0.63$; student's paired t-test).

Table 4.4: Descriptive statistics for P100 peak time – control (non-diabetic) group – Phase 1 (N = 30)

Side	Mean (ms)	Standard deviations	Min (ms)	Max (ms)
Right	101.22	3.77	92.7	109.5
Left	101.00	4.35	88.5	109.2

4.4 Summary statistics N75 – control (non-diabetic) group – Phase 1

The right and left eyes did not differ significantly with respect to the N75 (p = 0.39; student's paired t-test).

Table 4.5: Descriptive statistics for N75 peak time – control (non-diabetic) group – Phase 1 (N = 30)

Side	Mean (ms)	Standard deviations	Min (ms)	Max (ms)
Right	72.80	5.16	63.9	85.2
Left	71.44	3.30	67.2	78

4.5 Summary statistics N145 – control (non-diabetic) group – Phase 1

The right and left eyes did not differ significantly with respect to the N145 (p = 0.30; student's paired t-test).

Table 4.6: Descriptive statistics for N145 peak time – control (non-diabetic) group – Phase 1 (N = 30)

Side	Mean (ms)	Standard deviations	Min (ms)	Max (ms)
Right	133.50	9.41	114.3	155.1
Left	132.54	10.57	114	151.5

4.6 Summary statistics amplitude – control (non-diabetic) group – Phase 1

The right and left eyes did not differ significantly with respect to the amplitude (p = 0.35; student's paired t-test).

Table 4.7: Descriptive statistics for Amplitude – control (non-diabetic) group – Phase 1 (N = 30)

Side	Mean (μV)	Standard deviations	Min (μV)	Max (μV)
Right	12.67	5.52	4.7	25.5
Left	12.28	5.33	4.2	25.5

4.7 Summary

The reliability and repeatability of the data obtained from Phase 1, the non-diabetic control group, were assessed. The cut-points to determine normal from delayed P100 peak time for the DM group was established as right eye 110.64 ms and left eye 111.86 ms.

The next chapter will focus on Phase 2 of the study. It will include a data analysis of the diabetic group and the results of this study on DM groups.

Chapter Five

Results

The previous chapter covered the reliability and repeatability of the data obtained and analysed in Phase 1 – the non-diabetic control group of this study. The P100 peak time cut-points was established from the non-diabetic control group to distinguish the normal from the delayed P100 peak time. This chapter presents the results of the study of the DM groups.

5.1 Phase 2 – Diabetic group analysis

The results for this group are represented by data of the right eye only, as it has been demonstrated in chapter 4 that no statistical difference was found between the PVEP values of the right and left eyes.

The moderately controlled DM group (6.5% < HbA1c ≤ 10%) consisted of 52 participants; 33 males and 19 females. The mean age for this group was 56.1 ± 13.04 years and the mean duration in years for this group was 7.8 ± 5.9 years. The poorly controlled DM group (HbA1c > 10%) comprised of 52 participants: 29 males and 23 females. The mean age for this group was 45.2 ± 12.68 years and the mean duration in years for this group was 9.1 ± 6.3 years. DM group (moderate;poor) differed significantly with respect to age (Student's two-sample t-test: $p< 0.001$) but not with respect to disease duration (Student's two-sample t-test: $p = 0.294$). Furthermore the DM groups also did not differ with respect to gender distribution (Fisher's exact test: $p = 0.549$ Males: 63.5% ($^{33}/_{52}$) vs 55.8% ($^{29}/_{52}$)or Females: 36.5% ($^{19}/_{52}$) vs 44.2% ($^{23}/_{52}$)).

Table 5.1: Participant demographics – diabetic groups

HbA1c > 10%		6.5% < HbA1c ≤ 10%	
T1DM	T2DM	T1DM	T2DM
8 males	21 males	2 males	31 males
9 females	14 females	1 females	18 females

5.2 DM as a risk factor for P100 peak time delay

The P100 peak time values were binarized in the moderately controlled DM group (6.5% < HbA1c ≤ 10%) and poorly controlled DM (HbA1c > 10%) group using the cut-points for P100 based on the control group data and defines the threshold for peak time delay measured 110.64 ms for the right eye as summarised in Table 5.2.

Relative to the moderate control group (6.5% < HbA1c ≤ 10%) the poorly controlled (HbA1c > 10%) group were not at increased risk for delayed P100 peak time (Right: Odds Ratio = 1, ci: 0.454 – 2.204, p = 1.00 ; logistic regression).

Table 5.2: Categorised P100 values by DM group –Phase 2 – right eye*(N = 52 per group)

Group (DM)	**Eye**	**Proportion delayed**	**p-value***
Moderate control (6.5% < HbA1c ≤ 10%)	Right	38.5% (20/52)	1.000
Poor control (HbA1c > 10%)	Right	38.5% (20/52)	

* Fisher's exact test and p< 0.05.

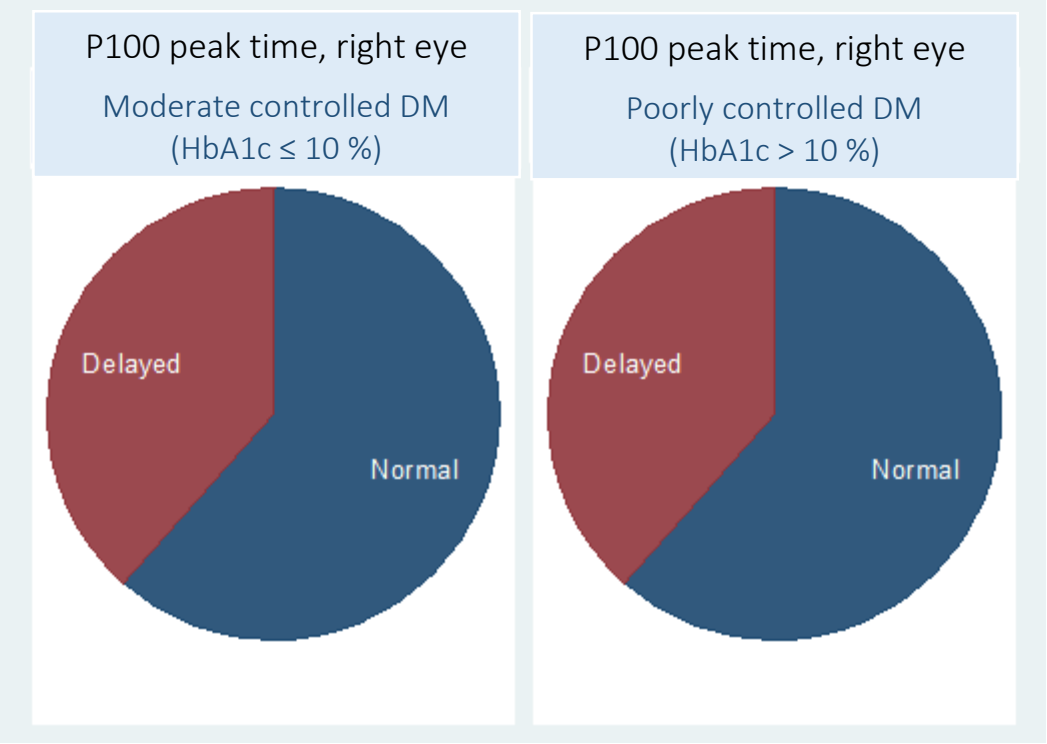

Figure 5.1: Pie graph – P100 peak time by DM group

5.3 DM as a risk factor for N75 peak time delay

Relative to the moderate control group (HbA1c ≤ 10%) the poorly controlled (HbA1c > 10%) group were not at increased risk for delayed N75 peak time (Right: Odds Ratio = 0.376, ci: 0.0696-2.033, p = 0.256; logistic regression).

Table 5.3: Categorised N75 peak time values by DM group –Phase 2 –right eye*(N = 52 per group)

Group (DM)	Eye	Proportion delayed	p-value*
Moderate control (6.5% < HbA1c ≤ 10%)	Right	9.62% (5/52)	0.437
Poor control (HbA1c > 10%)	Right	3.85% (2/52)	

* Fisher's exact test and p< 0.05

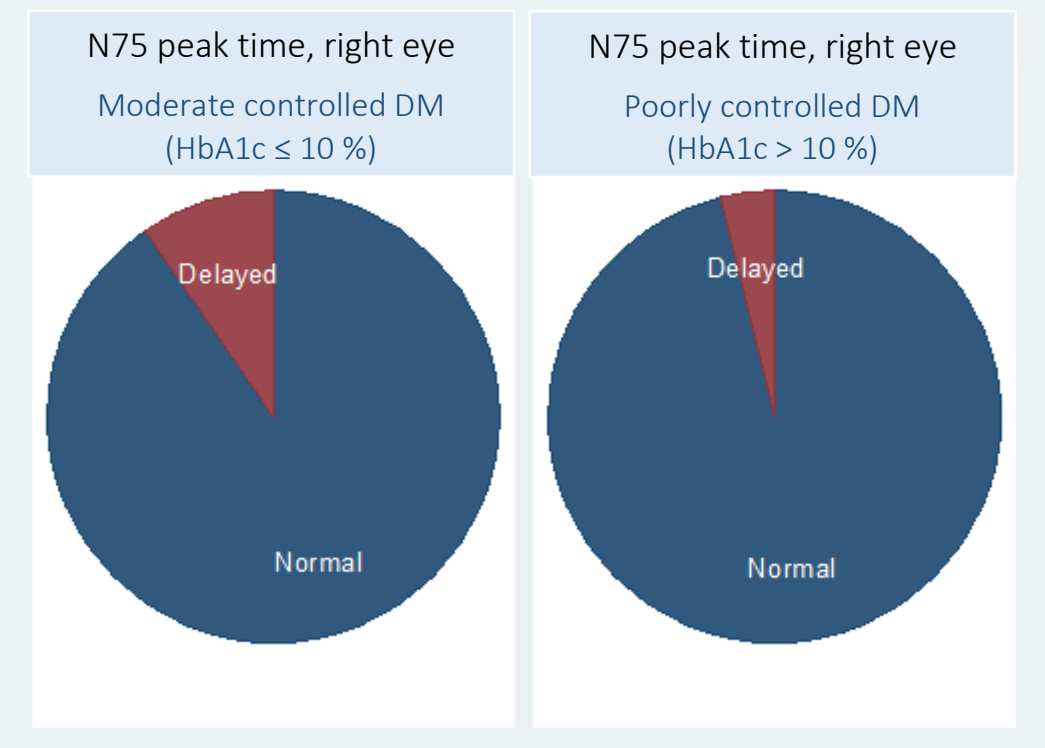

Figure 5.2: Pie graph – N75 peak time by DM group

5.4 DM as a risk factor for N145 peak time delay

Relative to the moderate control group (HbA1c ≤ 10%) the poorly controlled (HbA1c > 10%) group were not at increased risk for delayed N145 peak time (Right: Odds Ratio = 1.565, ci: 0.415-5.91, p = 0.509; logistic regression).

Table 5.4: Categorised N145 peak time values by DM group – Phase 2 – right eye*(N = 52 per group)

Group (DM)	Eye	Proportion delayed	p-value*
Moderate control (6.5% < HbA1c ≤ 10%)	Right	7.69% (4/52)	0.741
Poor control (HbA1c > 10%)	Right	11.54% (6/52)	

* Fisher's exact test and p< 0.05

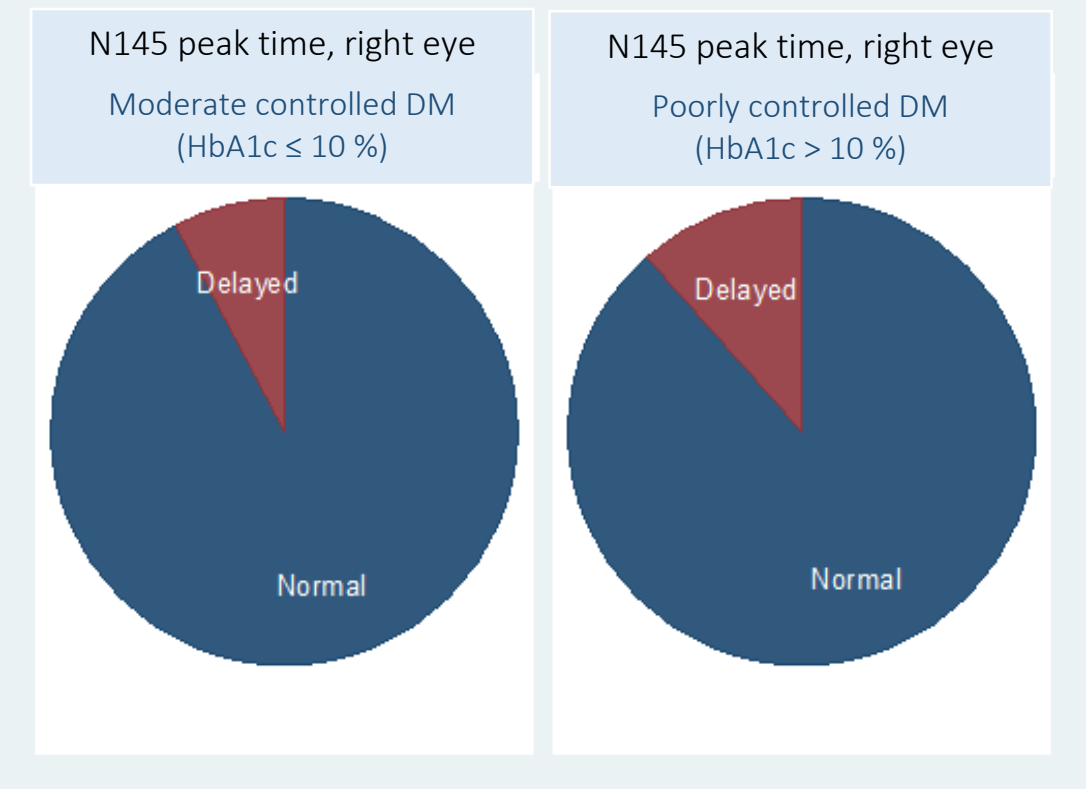

Figure 5.3: Pie graph – N145 peak time by DM group

5.5 DM as a risk factor for amplitude change

Relative to the moderate control group (HbA1c ≤ 10%) the poorly controlled (HbA1c > 10%) group were not at increased risk for a change in amplitude (Right: Odds Ratio = 1.151, ci: 0.406-3.260, p = 0.791; logistic regression).

Waveform abnormalities of the amplitude measurements are prone to alterations, due to participant co-operation, fixation and alertness (ISCEV Guideline 9B, 2008). The amplitude value is a reflection of concentration and is not used as an outcome measure due to greater inter-individual fluctuations (Regan, 1989).

Poor concentration was grouped as 0 – 5 μV.
Good concentration was grouped as 5 – 12 μV.

Table 5.5: Categorised amplitude values by DM group –Phase 2 –right eye*(N = 52 per group)

Group (DM)	Eye	Good concentration	Poor concentration	p-value*
Moderate control (6.5% < HbA1c ≤ 10%)	Right	84.62% (44/52)	15.38% (8/52)	1.000
Poor control (HbA1c > 10%)	Right	82.69% (43/52)	17.31% (9/52)	

* Fisher's exact test and p< 0.05

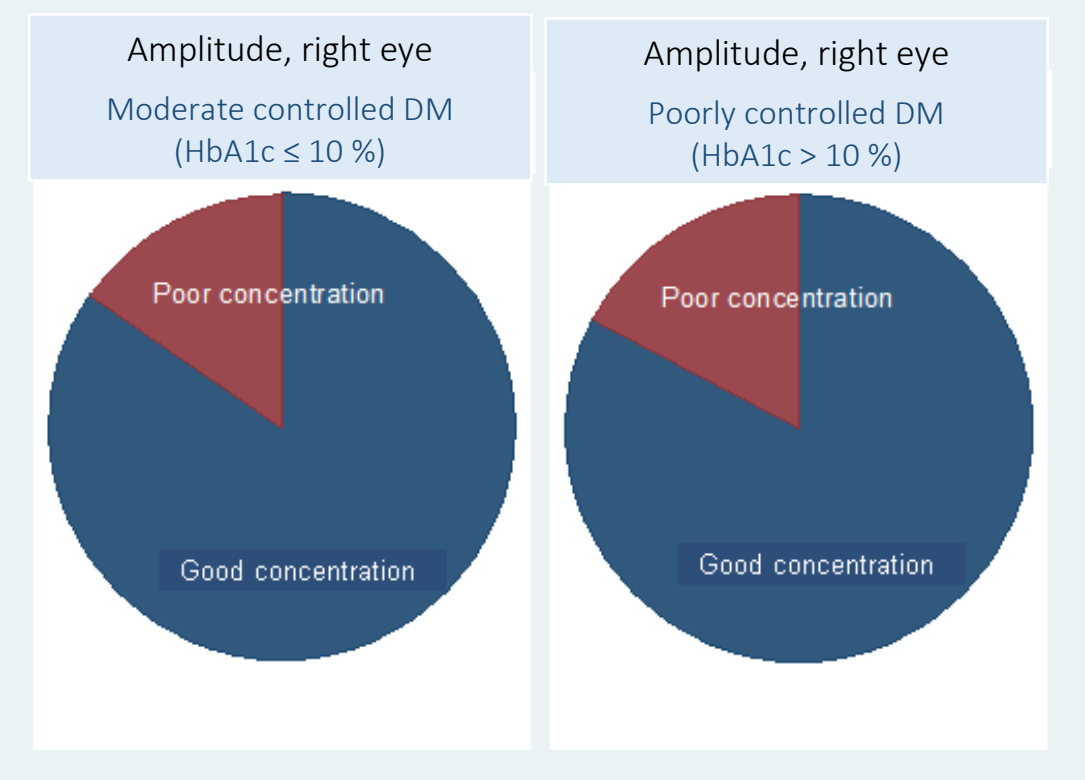

Figure 5.4: Pie graph – Amplitude by DM group

5.6 Study group comparisons

The P100 was assessed for this two-factored study design, with the main effects DM group (moderate control; poor control) and DM type (T1DM; T2DM) using analysis of covariance (ANCOVA) starting with the inclusion of an interaction term and possible covariates age and disease durations.

Graphically there was no relationship between P100 and for age of participant, neither for disease duration. These parameters as well as the interactions were also not significant in the ANCOVA, ie age ($p = 0.289$), duration of disease ($p = 0.836$) and interaction ($p = 0.758$).

Gender was not significantly associated with DM type ($p = 0.91$) and DM groups ($p = 0.76$).

The final ANOVA included the main effects only and neither was significant;
DM groups ($p = 0.822$; [HbA1c ≤ 10%] 109.59ms vs [HbA1c > 10%] 109.25ms).
DM type ($p = 0.630$; [T2DM] 109.59ms vs [T1DM] 108.65ms).

On the continuous scale (original) for P100 peak time, the three groups (control group, moderately controlled DM [HbA1c ≤ 10%], poorly controlled DM [HbA1c > 10%]) were compared when adjusting for age. The latter approach was adopted due to the generally younger age of the control participants (see Figure 5.5).

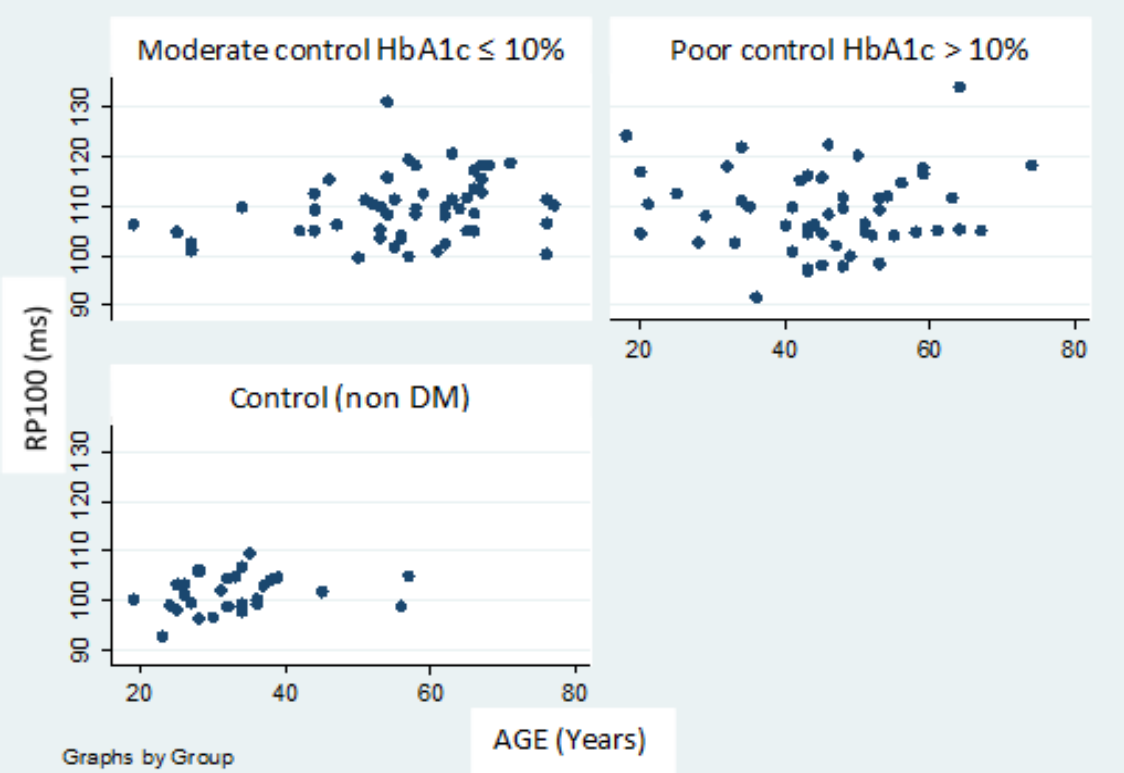

Figure 5.5: Scatter diagram by group for right eye P100 (ms) VS age (years)

5.7 Summary statistics and group comparisons for P100

The P100 measurements of the right eye are summarised by group in Table 5.6.

Table 5.6: Descriptive statistics for P100 peak time in the right eye by group

Group	N	Mean (ms)	Standard deviation (ms)	Adjusted mean P100 peak time (ms) when age is 46.64 years
Moderate control (6.5% < HbA1c ≤ 10%)	52	109.54	6.38	108.78
Poor control (HbA1c > 10%)	52	108.94	8.12	109.05
Control group	30	101.22	3.77	102.33

The adjusted means in the last column were derived when groups were compared with respect to right eye P100 peak time in a regression analysis which included groups as a main factor and age as covariate. The pairwise comparisons between groups, after adjusting for age, are given in Table 5.7.

Table 5.7: Group comparisons with respect to P100 peak time in the right eye and adjusted for age

Comparison	**Effect (ms)**	**p-value**
Moderate control (6.5% < HbA1c ≤ 10%) vs. control	6.451	0.001
Poor control (HbA1c > 10%) vs. control	6.719	< 0.001
Moderate control (6.5% < HbA1c ≤ 10%) vs. poor control (HbA1c > 10%)	-0.268	0.849

Note that, although the two DM groups did not differ (effect: -0.268 ms, p = 0.849), both the moderate as well as the poor control groups were significantly delayed compared to the control group (HbA1c ≤ 10% effect: 6.45 ms, p = 0.001 and HbA1c > 10% effect: 6.72ms, p < 0.001).

5.8 Summary statistics N75

The N75 measurements of the right eye are summarised by group and are given in the Table 5.8.

Table 5.8: Descriptive statistics for N75 in the right eye by group

Group	N	Mean (ms)	Standard deviation (ms)	Adjusted mean N75 peak time (ms) when age is 46.64 years
Moderate control (6.5% < HbA1c ≤ 10%)	52	74.70	8.14	74.57
Poor control (HbA1c > 10%)	52	72.50	8.10	72.47
Control group	30	72.80	5.20	72.96

The groups were compared with respect to right eye N75 peak time in a regression analysis which included groups as a main factor and age as covariate. The results are given in Table 5.9.

5.9 N75 – age adjusted peak time results

The two DM groups did not differ from each other (effect: 2.10 ms, p = 0.194) but they also were not significantly delayed when compared to the control group (HbA1c ≤ 10%: 1.61 ms, p = 0.460 and HbA1c > 10%: 0.48 ms, p = 0.800).

Table 5.9: Group comparisons with respect to N75 in the right eye and adjusted for age

Comparison	Effect (ms)	p-value
Moderate control (6.5% < HbA1c ≤ 10%) vs. control	1.611	0.460
Poor control (HbA1c > 10%) vs. control	0.484	0.800
Moderate control (6.5% < HbA1c ≤ 10%) vs. poor control (HbA1c > 10%)	2.095	0.194

5.10 Summary statistics N145

The N145 measurements of the right eye are summarised by group and are given in the Table 5.10.

Table 5.10: Descriptive statistics for N145 in the right eye by group

Group	N	Mean (ms)	Standard deviation (ms)	Adjusted mean N145 peak time (ms) when age is 46.64 years
Moderate control (6.5% < HbA1c ≤ 10%)	52	142.14	11.36	142.41
Poor control (HbA1c > 10%)	52	142.70	13.01	142.61
Control group	30	133.50	9.41	133.10

The groups were compared with respect to right eye N145 peak time in a regression analysis which included groups as a main factor and age as covariate. The results are given in Table 5.11.

5.11 N145 – age adjusted peak time results

Therefore, although the two DM groups did not differ (effect: -0.200 ms, p = 0.936), both the moderate as well as the poorly controlled DM groups were significantly delayed when compared to the control group (HbA1c ≤ 10%: 9.31 ms, p = 0.006 and HbA1c > 10%: 9.51ms, p = 0.001).

Table 5.11: Group comparisons with respect to N145 in the right eye and adjusted for age

Comparison	Effect (ms)	p-value
Moderate control (6.5% < HbA1c ≤ 10%) vs. control	9.311	0.006
Poor control (HbA1c > 10%) vs. control	9.512	0.001
Moderate control (6.5% < HbA1c ≤ 10%) vs. poor control (HbA1c > 10%)	-0.200	0.936

5.12 Summary statistics amplitude

The amplitude measurements of the right eye are summarised by group and are given in the Table 5.12.

Table 5.12: Descriptive statistics for amplitude in the right eye by group

Group	N	Mean (μV)	Standard deviation (μV)	Adjusted mean amplitude (μV) when age is 46.64 years
Moderate control (6.5% < HbA1c ≤ 10%)	52	10.35	5.01	10.68
Poor control (HbA1c > 10%)	52	10.01	5.08	9.96
Control group	30	12.67	5.52	12.19

The groups were compared with respect to right eye amplitude in a regression analysis which included groups as a main factor and age as covariate. The results are given in Table 5.13.

5.13 Amplitude- measurements as adjusted by age

The two DM groups did not differ significantly (effect: 0.72 μV, p = 0.513) nor did the DM group differ significantly from the control group (HbA1c ≤ 10%: -1.51 μV, p = 0.306 and HbA1c > 10%: 2.23 μV, p = 0.082).

Table 5.13: Group comparisons with respect to amplitude in the right eye and adjusted for age

Comparison	**Effect (μV)**	**p-value***
Moderate control (6.5% < HbA1c ≤ 10%) vs. control	-1.51	0.306
Poor control (HbA1c > 10%) vs. control	2.23	0.082
Moderate control (6.5% < HbA1c ≤ 10%) vs. poor control (HbA1c > 10%)	0.72	0.513

5.14 Summary

P100 peak time measurements for both DM groups, moderate control (6.5% < HbA1c ≤ 10%) and poorly controlled (HbA1c > 10%), were significantly delayed for the right eye when compared to an age adjusted control group (Phase 1).

The next chapter will be the discussion chapter in which the study results are assessed.

Chapter Six

Discussion

The previous chapter dealt with the results of the DM groups. This chapter presents the discussion and conclusion of the study.

6.1 Re-visiting purpose and objectives

The purpose of this study was to determine the association between glycated haemoglobin levels and P100 pattern-reversal visual evoked potential (PVEP) in diabetes mellitus (DM). To achieve the purpose, the following objectives were set:

- to determine whether there is a possible association between the P100 peak time of pattern-reversal visual evoked potential (PVEP) and the glycated haemoglobin percentage, and
- to establish whether the central nervous system (CNS), and in particular visual function, is affected resulting in DM-related central neuropathy, and whether the type of diabetes involved and the duration that the participant has been diagnosed as a diabetic.

6.2 Main outcomes

This was an observational cross sectional study conducted in two phases. The participants were grouped into Phase 1, the control group, that consisted of 30 non-diabetic participants. Phase 2 comprised of 104 diabetic participants of whom 52 had an HbA1c greater than 10% and 52 whose HbA1c was 10% and less (moderate diabetic control vs. poor diabetic control).

Phase 1 – Control (non-diabetic) group – P100 PVEP results

Determining the P100 peak time measurements for the 30 healthy non-diabetic participants was necessary to establish the normative values for this study. The cut-points for the P100 peak time measurements to determine normal from delayed were determined as the mean +2.5 x standard deviations.

The recommended standards for PVEP consider abnormal P100 peak time as 2.5 x standard deviations above the mean for a comparable age control sample selected from the normal population (ISCEV Guideline 9B, 2008).

The cut-points determining normal from delayed for the P100 peak time in the right eye was 110.64 ms and 111.86 ms for the left eye. Peak time abnormalities are an indication of visual pathway dysfunction once ocular and retinal pathologies have been eliminated as a cause upon examination (ISCEV Guideline 9B, 2008).

Phase 2 – Diabetic group – P100 PVEP results

Phase 2 of this study consisted of two diabetic groups. One was a moderately controlled group (HbA1c ≤ 10%) and the other a poorly controlled group (HbA1c > 10%).

The concordance between poorly controlled DM and the increased odds of developing polyneuropathy has been established using the HbA1c cut-point of 7% (El-Salem et al., 2009). The American Diabetes Association (2010) also advocates for HbA1c levels less than 7%. El-Salem et al. (2009) further state nerve conduction anomalies in diabetic patients are highly correlated to HbA1c levels in the subclinical stages of polyneuropathy. It is for this reason that the HbA1c cut-point of 10% was chosen for the poorly controlled diabetic group in this study. Moreover, choosing HbA1c < 7% as the cut-point for the current study might have suggested that only having early or subtle nerve disease could lead to developing polyneuropathy; thus, potentially missing the diagnosis. However, choosing HbA1c levels of 10% and over as the cut-

point ensured that participants with an advanced disease participated which made the comparison between the two DM groups more compelling.

The cut-points for P100 delayed peak time were established in Phase 1 of this study with the right eye measuring 110.64 ms; therefore, any measurements above this defined the abnormal P100 peak time.

The proportion of delayed P100 peak time for the moderately controlled diabetic group (HbA1c ≤ 10%) totalled 38.5% of participants for the right eye. Incidentally, the poorly controlled group (HbA1c > 10%) measured their P100 peak time for the right eye at 38.5%. The data reflect the poorly controlled (HbA1c > 10%) group did not pose an increased risk for delayed P100 peak time relative to the moderately controlled group (HbA1c ≤ 10%).

The visual system encounters functional declines as a result of non-pathological aging. Alterations in the retina and its neuro-connections lead to visual decline; VEP helps to evaluate these changes to the visual system (Phurailatpam et al., 2014)

Visual evoked potential helps to assess the functional changes of the sensory systems during various stages of life (Onofrj et al., 2001). Age must be considered an important factor when conducting VEP studies as it has been shown in previous studies that the P100 peak time increase with age (Phurailatpam et al., 2014; Onofrj et al., 2001; Emmerson-Hanover et al., 1994; Celesia et al., 1987; Allison et al., 1984).

The mean age of Phase 1 non-diabetic participants (control group) in this study was 32.6 ± 8.52 years. In Phase 2 the two diabetic age groups were 56.1 ± 13.04 years for the moderately control group (HbA1c ≤ 10%) and 45.2 ± 12.68 years for the poorly controlled group (HbA1c > 10%). Because age is an important variable in the measurement of P100 peak time, and in this study the average age for the control group was younger than that of the two diabetic groups, regression analysis was employed among the three groups as a main factor and age was a

covariate. The adjusted mean age for the comparison of P100 peak time measurements among the three groups was 46.64 years.

The results of the P100 measurements in this study indicated the measurements of the two DM groups did not differ significantly: the moderately controlled group (HbA1c ≤ 10%) measured 108.78 ms and the poorly controlled group (HbA1c > 10%) measured 109.05 ms; thus, the effect was (-0.268 ms, $p = 0.849$) between the two DM groups. These two DM groups were, however, significantly delayed compared to that of the control group (HbA1c ≤ 10%: 6.4 ms, $p = 0.001$ and HbA1c > 10%: 6.72 ms, $p < 0.001$).

The primary objective of this study was to establish whether an association existed between the P100 peak time measurements and the glycated haemoglobin percentage when comparing two diabetic groups (a moderately controlled group [HbA1c ≤ 10%] with a poorly controlled group [HbA1c > 10%]) by using cut-points obtained from a non-diabetic control group.

The results reflect that diabetic control has no connection in increasing the P100 peak time measurements because the moderately control group (HbA1c ≤ 10%) presented results nearly identical to that of the poorly controlled group (HbA1c > 10%) for P100 measurements in the right eye. The proportion of delayed P100 measurements of the participants' right eyes was 38.5% for both DM groups.

Conversely, the P100 results of Phase 2, the DM group, reflect a significant delay ($p<0.001$) when compared to the non-diabetic control group in Phase 1 of the study. This result correlates with the finding of Heravian et al. (2012) in that 40% of their diabetic participants without retinopathy presented with P100 peak time above the normal range cut-points. Heravian et al. (2012) did not appreciate any correlation with P100 abnormalities and glycemic control. Algan et al. (1989) found 28% of their DM participants showed a significantly delayed P100 in comparison with their control group with no correlation to metabolic control.

The secondary objective of this study was to establish whether the type of DM involved and the duration since the participants' diagnoses as diabetics had an effect on the P100 measurements, and if this was the case, whether it affected the central nervous system (CNS), particularly the visual function. The Phase 1 control group was not included in this section. A two-way ANOVA was carried out on the DM group and no effect was found between DM types ($p = 0.630$) (T1DM and T2DM) or between DM groups ($p = 0.822$) (HbA1c > 10% and HbA1c ≤ 10%); hence, no interaction was observed between DM groups and DM types.

It was also observed in the results of this study that the duration of DM had no influence on the P100 peak time measurements($p = 0.836$). Ismail (2014), Hervian et al. (2012), Ziegler et al. (1994) and Algan et al. (1989) indicated results similar to these of the present study in that the participants in their studies showed no correlation between P100 peak time and the type or duration of DM. On the other hand, in various other studies conducted by authors such as Kumar et al. (2014), Shrivastava et al. (2014), Bhanu et al. (2012), Chopra et al. (2011), Dolu et al. (2003) and Azal et al. (1998) it was found a significant positive correlation existed between the duration of DM and the P100 peak time.

There was no sign of decreased visual function in the participants in this study. Specifically, the DM group did not reflect any sign of decreased visual function or, more particularly, an exaggerated P100 peak time such as evidenced in an optic neuropathy pathology.

6.3 Limitations

The study did not present with too many unforeseen difficulties. Some minor problems were encountered, such as the time factor. The time factor for recruiting the poorly controlled diabetic group participants (HbA1c > 10%) took longer than expected. Another challenge was that some of the diabetic participants then did not qualify for enrolment in the study due to having ocular pathology such as cataracts or diabetic retinopathy (all ocular pathology was excluded by the study protocol as mentioned in Chapter 3).

These study limitations were however unrelated to any equipment or system failure.

Seven participants from the moderately controlled group (HbA1c ≤ 10%) were found to be unsuitable participants due to ocular pathology in the form of cataracts. No other diabetic related ocular pathology was detected in this group of participants and all 52 participants met the study inclusion requirements.

Eight participants from the poorly controlled group (HbA1c > 10%) were found to be unsuitable participants due to ocular pathology in the form of cataracts and diabetic retinopathy. Two participants presented with cataracts and six participants presented with diabetic retinopathy. Four participants presented with moderate non-proliferative diabetic retinopathy and two with mild non-proliferative retinopathy. The necessary ophthalmological referrals were all made for those participants who presented with ocular pathology. All 52 participants met the study inclusion requirements.

The average age of the moderately controlled group (HbA1c ≤ 10%) was 56.1 ± 13.04 years. This group consisted of 33 male and 19 female participants. The average age for the poor control group (HbA1c > 10%) was 45.2 ± 12.68 years with 29 male and 23 female participants. It was to be expected that cataract formation would be present in this age group (Jeganathan et al., 2008). The duration of each participant being diagnosed as a diabetic in years was similar in both groups, namely, HbA1c ≤ 10%: 7.8 ± 5.9 and HbA1c > 10%: 9.1 ± 6.3. This factor would also have contributed to cataract development.

The mean age of the control group (non-diabetics) was 32.6 ± 8.52 years whereas the mean age for the poorly controlled group (HbA1c > 10%) was 45.2 ± 12.68 years. For the moderately controlled group (HbA1c ≤ 10%) the mean age was 56.1 ± 13.04 years. The differences in the mean age resulted in a non-aged matched comparison which necessitated using regression analysis among the three groups as a main factor and age a covariate.

The study design of having one referral source for all the participants of this study was a private medical specialist physician practice, and therefore the amount of diabetic participants may have been limited. Having a larger diabetic participant referral source , such as a dedicated diabetic clinic may have made the recruitment process of all the participants easier as well as potentially having a better age match for the control group (non-diabetic group) and the two diabetic groups used in this study.

6.4 Confounding variables

The study results of Phase 1 did not produce any confounding results, but there was a slight difference between the right eye and left eye of the P100 ICC repeatability measurements (0.928 and 0.831 respectively). A possible explanation for this could be participant fatigue as the study protocol was to always start with the right eye first. However, these ICC results are still repeatable and therefore reliable.

The Phase 2 study results produced identical results for the right eye P100, whereby 38.5% of the participants produced a delayed P100 peak time of $p = 1.00$ for both the HbA1c $\leq$ 10% and the HbA1c > 10% groups.

6.5 Improvements and future work

The results of this study clearly demonstrate a proof of concept that DM has an influence on the optic nerve conduction and on all levels of control; thus, an effect on the CNS. A large randomised trial is needed to explore this concept further whereby other components of the optic nerve need to be measured and the physiology of the optic nerve be assessed to determine whether the P100 peak time delays are episodes of exacerbations or whether it is a stable peak time delay.

Randomised trials have long been considered as the gold standard when it comes to clinical research (Akobeng, 2005), and this trial could take place in a diabetic department of a medical training hospital, having an ophthalmic as well as a neurophysiological departments.

6.6 Discussion

Peripheral neuropathy is one of the first complications to arise in DM. This is possibly due to damaging of the small diameter nerve fibres which consist of 70 – 90% of all the peripheral nerve fibres (Smith & Singleton, 2008). Neuropathic pain results from injury to the small-fibre nerves and this is one of the most disabling symptoms of diabetic neuropathy (Gandhi et al., 2010). The Oslo study was a long-term follow-up study examining T1DM of long duration. Large- and small-nerve fibre function and their association with HbA1c were investigated. The Oslo study investigators emphasise the importance of having good glycaemic control, especially for long-term T1DM, to preserve large- and small-fibre function because it plays a vital role in neuropathy development. In the Oslo study it was concluded that HbA1c is a significant risk factor associated with the progression of large- and small-fibre damage in long-term T1DM (Sveen et al., 2013).

The Diabetes Control and Complications Trial (DCCT) research group demonstrated a clear link between glycaemia and the advancement of microvascular complications in T1DM where the HbA1c measures 6 – 11% after a period of six years (DCCT, 1996).

In their study on T2DM, Stratton et al. (2000) observed a highly significant link between the advancement of diabetes-related complications with hyperglycaemia as measured with the mean HbA1c in the reference range of < 6 HbA1c > 10%. These researchers furthermore found a 1% reduction in HbA1c reduced the risk for microvascular complications by 37% as well as having a 21% lower risk of death related to diabetes.

A secondary aim of this study was to investigate the possible effect of DM and its effects on the central nervous system (CNS), in particular the effects on visual function.

Nonarteritic anterior ischaemic optic neuropathy (NAION) is the most prevalent acute optic neuropathy in individuals aged 50 years and older (Hattenhauer et al., 1997). The aetiology of NAION is multifactorial; however, vascularpathic disorders such as DM are frequently cited as a risk factor (Jacobson et al., 1997).

A systematic review and meta-analysis was undertaken by Chen et al. (2013) to investigate the associated increased risk of NAION and DM. The conclusion of Chen et al. (2013) was that, according to their meta-analysis, DM increases the risk of NAION (OR = 1.64; 95% CI = 1.17 – 2.30; p = 0.004). These authors state the proper control of blood glucose levels could reduce the risk of developing NAION; they stress the importance of neuro-ophthalmic investigations that focus on diabetics who have visual symptoms indicative of NAION.

The findings of Chen et al. (2013) concur with those of Lee et al. (2011) who used a large study sample of 25 515 diabetics and an equal number of age- and gender-matched non-diabetics to determine the development of the incidence of NAION among DM patients and among the elderly. The study results of Lee et al. (2011) indicate a significantly increased risk of NAION in DM.

Visual evoked potential (VEP) is a non-invasive instrument used to examine the activity of the visual system. The effect of nerve conduction change can be assessed by noting the peak time delay of the VEPs (Jones & Brusa, 2003). The P100 peak time prolongation represents the size of the demyelinated optic nerve fibres; therefore, VEP provides a highly sensitive instrument to measure demyelination of the optic nerve (You et al., 2011).

Chapter Seven

Conclusions

The results of the present study are significant in terms of the clinical relevance of measuring the PVEP P100 peak time in people having DM of all classifications and control. The proportion of P100 delayed peak time of 38.5% for both DM groups may lead to further questions and studies researching whether the optic nerve as a component of the CNS has a greater form of protection against damage resulting from hyperglycaemia. Ewing et al. (1985) found that in DM the CNS cells were less afflicted than the PNS cells. They ascribe this finding to the possibility that, due to the blood-brain barrier, transport for glucose decreases in the CNS and the levels of glycation products are lower in the CNS compared to its level in the peripheral nerves.

This study revealed that the P100 PVEP peak time is indeed associated with the HbA1c measurements of diabetics. Whether poorly controlled or moderately controlled DM, demyelination of the CNS occurs in 38.5% of cases. In adition, the use of PVEP in DM is a useful non-invasive procedure to detect retinal dysfunction at the ganglion cell level, and can thus be considered as preclinical diabetic retinopathy screening, therefore PVEP in DM has the potential to reduce DM complications.

The important question to ask is in how many diabetic clinics is the physiology of the optic nerve examined. DM is a chronic lifestyle disease with far-reaching implications for millions of people who suffer from it as well as being a condition which can lead to both acute and chronic complications and even death. With the looming possibility that the already excessively high global number of people living with DM may increase to 592 million by the year 2035, diabetic clinics need to include an examination of the physiology of the optic nerve in DM patients; DM patients need to be examined from foot to eye to prevent complications and improve their condition.

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Appendix A - Research Ethics Board Approval

The study was approved by the Aston University Ethical Committee on 10th April 2013. (Ethics application number 421).

The primary ethical issues raised by the design of this protocol are the recordings of the visual evoked potentials of two groups of diabetic participants.

It is necessary to put the following additional precautions for the research participants in place.

Anonymity

This will be preserved by the removal of particiants' identities and not using their personal identification (ID) numbers. A coding system will be introduced and each participant will be allocated a specific code(a number 1-134) which can be linked back to Prof. Ker'soriginal record cardof the participant, should cross-referencing be needed when writing up of the study.

Risks and Benefits

There is no apparent foreseen risks to the study participants. Apart from applanation tonometry and the placing of the electrodes to the subject's head which some may or may not exprience as a little uncomfortable, all other tests are non-invasive. Should a mydriatic agent be needed 0.5% tropicamide will be instilled only after the necessary precautions have been carried out to ensure the safe installation of the dilating drops. Apart from minor stinging and the cycloplegia for six hours there will be no other posssible risk to the participants.

On the other hand, the participants may benefit from this study in that they will be made aware of whether there is any damage to their optic nerves as measured by their P100 wavelengths.

Informed Consent

After the purpose and study method have been explained to every individual participant they will be given the opportunity to decide whether they want to participate or not. From those who voluntarily decide to participate verbal and signed written consent will be obtained. Additional protection of the research participant is the informed consent giving Mr MD Nagle access to Prof. Ker's clinical record card of every participant.

Voluntary Participation

Participation will be voluntary. No partipants will be pressurised or coerced to participate in this study. No remuneration for participation will be offered or accepted. The participants will be assured that they can decide to withdraw from the study at any time without stating a reason and without prejudice.

Privacy and Confidentiality

Prof. Ker will be recruiting the participants from clients in his private practice. A file number will be allocated to every participant and will be used as his or her code number throughout the study process. Also, the presentation of the results of the study will omit names or any other information that can link the participant to the study. The possibility that a participant can be identified or that she or he can be linked to the data is not negotiable and therefore the participants' confidentiality and anonymity will be maintained and guaranteed at all times during and after the study has been completed.

The research data will be analysed by both Prof. Ker and Mr Nagle. Prof. Ker willhave the responsibility to keep all the data and images captured confidential.

By instituting these additional protections, the risks have been appropriately minimised and a reasonable and ethically acceptable balance between risks and benefits has been established.

Appendix A1 – Aston University

Aston University Ethics Committee
Aston University
Aston Triangle
Birmingham
B4 7ET
Telephone +44 (0)121 204 3000
Fax +44 (0)121 204 3696

Chairperson: Ms Nichola Seare

Secretary: Mr John Walter

10th April 2013

Dr Hannah Bartlett

School of Life and Health Sciences

Dear Hannah

Study Title: 'The association between glycated haemoglobin levels and P100 visual evoked potentials in diabetes mellitus'

REC Reference: Ethics Application 421

Protocol Number:

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

The project is approved until the completion date specified on the form (October 2 2014) provided it is commenced within two years of the date of this letter and you are required to notify the Committee when the project is completed.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	EC Review Date
University Ethics Application Form	One	23/11/2012
Consent Form Phase 1 of the study	One	23/11/2012
Consent Form Phase 2 of the study	One	23/11/2012
University Ethics Application Form	Two	12/02/2013
Consent form phase 1 post review	Two	12/02/2013
Consent form phase 2 post review	Two	12/02/2013
Consent form phase 1 post review 09_04_13	Three	09/04/2013

Consent form phase 2 post review 09_04_13	Three	09/04/2013

Statement of compliance

The Committee operates in accordance with the Aston University Ethics policy and procedures:

http://www1.aston.ac.uk/registry/for-staff/reqsandpolicies/ethics-policy-and-procedures/

Reporting Requirements

The details of the investigation will be placed on file. You should notify the Secretary of the University Ethics Committee of any adverse events which occur in connection with this study and/or which may alter its ethical consideration, and/or any difficulties experienced by the volunteer subjects.

If you intend to make any future protocol amendments these must be approved by the Ethics Committee prior to implementation. You should also seek approval for any extension of the approved completion date.

Membership

The members of the University Ethics Committee present at the meeting are listed below:

- Professor Pawan Budhwar, Associate Dean Research
- Professor Richard Booth, Professor of Occupational Health & Safety, Aston University
- Dr Robert Morse, Lecturer on the B.Sc. Audiology programme
- Ms Nichola Seare, AHRIC Director, Aston University
- Mr John Walter, Director of Governance, Aston University

REC reference: **Ethics Application 421**
Please quote this number on all correspondence

With the Committee's best wishes for the success of the project

Yours sincerely

J. G. Walter

Secretary of the Ethics Committee

Email: j.g.walter@aston.ac.uk

Appendix A2 – Pharma-Ethics

ETHICS COMMITTEE APPROVAL FORM

Ethics Reference No.	**14045595**	**Date of Meeting**	Wednesday, April 16, 2014	
Principal Investigators:	Prof J Ker	**Investigators:**	Mr D Nagle	Investi
Protocol Title:	THE ASSOCIATION BETWEEN GLYCATED HAEMOGLOBIN LEVELS AND THE EFFECTS ON THE CENTRAL NERVOUS SYSTEM ASSESSED BY THE P100 VISUAL EVOKED POTENTIALS IN DIABETES MELLITUS			

DOCUMENTS REVIEWED			Tick As Appropriate	Yes	No
Protocol Name	1				
Protocol / Amendment No. (and/or)	N/D	Date:		✔	
Investigator's Brochure					
Subject Information/Consent Form	Consent Form	N/D		✔	
Advertisements					✔
Questionaires					
Relevant Trial Hospital/(s)					✔
Insurance	SAOA Proof of Insurance, Policy No: SPL/SLFG/000002402	Valid From: 01 Jan 2014 To: 31 Dec 2014		✔	
Research Unit					✔
Synopsis of Study/Trial Summary					✔
Other / Document Submitted	Pharma-Ethics Application Form and Covering Letter dated 4 April 2014			✔	
	Aston University Ethics Committee Confirmation of Ethical Opinion, dated 10 April 2013			✔	
	Reference Letter dated 1 July 2010			✔	

DETAILS OF COMMITTEE	
Name	Pharma-Ethics Independant Research Ethics Committee
Address	123 Amcor Road, LYTTELTON MANOR, 0157

DETAILS OF MEETING	Yes	No
Is the Investigator a member of the committee ?		✔
If "Yes" did he/she vote ?		✔
Is the Committee organised and operated according to applicable laws and regulations together with ?	✔	
Local GCP requirements ?	✔	
ICH GCP requirements ?	✔	
FDA GCP requirements ?	✔	
Progress reports required on a Yearly basis ?	✔	

DECISION ON APPROVAL : is approval given to conduct the trial ?	Tick As Appropriate
Yes - with no conditions	✔
Yes - with conditions	
Specify conditions :	
No	
Specify reasons	

SIGNATURES		
I confirm that the details on this form are correct:		Date
Name: **Dr C.S.J. Duvenage** **Chairperson of Committee**	**Signature:**	**Wednesday, July 09, 2014**

PROTOCOL NUMBER **1** ETHICS REF.: **14045595**

Appendix B – Sample Consent Form

Mark Nagle-Student number: 109089665

Consent form:

Research workers, school and subject area responsible:

- Professor James Ker- Specialist Physician/ Cardiologist, Department of Internal Medicine, University of Pretoria, South Africa.
- Mr. Mark Nagle- Optometrist, Life and Health Sciences, Vision Sciences, Aston University, United Kingdom.

Project Title:

The association between glycated haemoglobin levels and P100 visual evoked potentials in diabetes mellitus.

Invitation: Phase 1 of the study

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully.

What is the purpose of the study?

The purpose of this study is to monitor the changes in diabetes mellitus that take place within the central nervous system, particularly its relationship to vision. Visual evoked potentials are used as a simple, sensitive test that allows one to investigate the central nervous system without needing any input from the participant. The test is able to detect early changes to the nerves before any symptoms arise. There are no direct benefits by taking part in this study, apart from gaining the knowledge of how diabetes affects the visual system.

Why have I been chosen?

You have been chosen to participate in our study, as part of our control group. The results from the control group will be compared with the results from two groups of diabetic participants. One group will have glycated haemoglobin percentage values that are equal to or greater than 10 %. The other group will have glycated haemoglobin percentage values that are less than 10 %. Certain people will not be able to take part in the study, and these include those with existing ocular pathology, namely cataracts or any other media opacity or optic nerve disease or any retinal pathology.

What will happen to me if I take part?

By volunteering to be a participant in our study you will have a comprehensive eye examination,
including photography of the back of your eye. This is in order to exclude any ocular pathology which could influence the results of our study.

The study itself will be completed in one visit, except if pupil dilation is required, then a second visit will be required. You will be asked to observe a black and white alternating checker board pattern in a semi-dark room. Three electrodes will be carefully placed on your head; these electrodes are commonly used in medical environments and will not cause any pain or discomfort. They are used to measure signals from your central nervous system.

All you will be asked to do is to pay close attention to the video monitor at all times. This test will take no longer than 15 minutes, and together with the comprehensive eye examination the total visit time should be no more than one hour.

Are there any potential risks in taking part in the study?

The risks in participating in the study are minimal as all tests are used in the course of clinical practice.
The use of eye drops may result in minor stinging that could last for up to 15 seconds. The effects of the dilating eye drops could result in your eyes being extra light sensitive and not able to focus comfortably for six hours after installation, and this may affect your ability to drive or to operate heavy machinery. Therefore, it is recommended that you do not do either of these activities for at least six hours from the installation of the dilating eye drops.

Very rarely, patients experience more severe side effects from the eye drops. However the investigator will monitor you closely following instillation of the drops to ensure that any treatment that might be required is administered appropriately.

It would be helpful if you did not apply any hair spray or other hair products on the day of the study, as this could make placement of the electrodes more difficult.

Do I have to take part?

No, you do not have to participate if you do not wish to do so. You are free to withdraw from the study at any time. The researchers may suggest that you withdraw from the study if they consider it appropriate.

Expenses and payments:

There are no expenses or payments for participation in this study.

Will my taking part in this study be kept confidential?

Privacy and confidentiality will be protected vigorously to the extent permissible by law. All data in written form as well as electronic form will be kept by Prof J Ker, along with your original clinical records held by this practice. All the necessary information obtained by your participation in this study will be in anonymous form. Prof Ker will allocate a code to your research file, and this code can be used by him to link back to your clinical records held by him. All your records pertaining to this study will be kept for a minimum of 15 years, thereafter all data would be disposed of in accordance to the data protection act of the GOC. We cannot, however, guarantee privacy or confidentiality.

What will happen to the results of the research study?

The results of the study would be used in order to complete the doctorate degree of Mr M.D Nagle. We also aim to publish the results of this study. However, there will be no reference to any individual's performance in any publication. The results of the study can be obtained directly from Prof J Ker, however each participant will be given a full explanation of their results.

Who is organizing and funding the research?

Mr M.D Nagle is organizing the study, however there is no external funding for this research project.

Who has reviewed the study?

The study has been reviewed by the Aston University Research Ethics Committee.

Who do I contact if something goes wrong or I need further information?

Please feel free to contact Prof James Ker (jker@wol.co.za), +2712 341 0078

Who do I contact if I wish to make a complaint about the way in which the

research is conducted?

If you have any concerns about the way in which the study has been conducted, then you should contact the Secretary of the University Research Ethics Committee on j.gwalter@aston.ac.uk or telephone 0121 204 4665.

Volunteer consent form

Title of project:The association between glycated haemoglobin levels and P100 visual evoked potentials in diabetes mellitus.

Name of Chief Researcher: Prof James Ker

		Initial
1	I confirm that I have read and understand the information sheet for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
2	I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.	
3	Should it be necessary, I give consent for Mr MD Nagle to view my clinical records held by Prof J ker.	
4	I agree to take part in the above study.	

Name of participant		Name of researcher	
Date		Date	
Signature		Signature	

Mark Nagle-Student number: 109089665

Consent form:

Research workers, school and subject area responsible:

- Professor James Ker- Specialist Physician/ Cardiologist, Department of Internal Medicine, University of Pretoria, South Africa.
- Mr. Mark Nagle- Optometrist, Life and Health Sciences, Vision Sciences, Aston University, United Kingdom.

Project Title:

The association between glycated haemoglobin levels and P100 visual evoked potentials in diabetes mellitus.

Invitation: Phase 2

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully.

What is the purpose of the study? The purpose of this study is to monitor the changes in diabetes mellitus that take place within the central nervous system, particularly its relationship to vision. Visual evoked potentials are used as a simple, sensitive test that allows one to investigate the central nervous system without needing any input from the participant. The test is able to detect early changes to the nerves, before any symptoms arise. There are no direct benefits by taking part in this study, apart from gaining the knowledge of how diabetes affects the visual system.

Why have I been chosen? You have been chosen to participate in our study, as your glycatedhemoglobin percentage meets the requirement criteria of our study. One hundred and four participants are needed for the successful completion of the study. The participants will be grouped into two groups of fifty two each, those who's HbA1c is greater or equal to 10%, and those that are below 10%. Certain people will not be able to take part in the study, and these include those with diabetic retinopathy, or existing ocular pathology, including cataracts, optic nerve disease or any retinal pathology.

What will happen to me if I take part?By volunteering to be a participant in our study you will have a comprehensive eye examination, including photography of the back of your eye. This is in order to exclude any ocular pathology which could influence the results of our study.

The study itself will be completed in one visit, except if pupil dilation is required, then a second visit will be required. You will be asked to observe a black and white alternating checker board pattern in a semi-dark room. Three electrodes will be carefully placed on your head; these

electrodes are commonly used in medical environments and will not cause any pain or discomfort. They are used to measure signals from your central nervous system.

All you will be asked to do is to pay close attention to the video monitor at all times. This test will take no longer than 15 minutes, and together with the comprehensive eye examination the total visit time should be no more than one hour.

Are there any potential risks in taking part in the study? The risks in participating in the study are minimal as all tests are used in the course of clinical practice. The use of eye drops may result in minor stinging that could last for up to 15 seconds. The effects of the dilating eye drops could result in your eyes being extra light sensitive and not able to focus comfortably for six hours after installation, and this may affect your ability to drive or to operate heavy machinery. Therefore, it is recommended that you do not do either of these activities for at least six hours from the installation of the dilating eye drops.

Very rarely, patients experience more severe side effects from the eye drops. However the investigator will monitor you closely following instillation of the drops to ensure that any treatment that might be required is administered appropriately.

It would be helpful if you did not apply any hair spray or other hair products on the day of the study, as this could make placement of the electrodes more difficult.

Do I have to take part? No, you do not have to participate if you do not wish to do so. You are free to withdraw from the study at any time. The researchers may suggest that you withdraw from the study if they consider it appropriate.

Expenses and payments: There are no expenses or payments for participation in this study.

Will my taking part in this study be kept confidential? Privacy and confidentiality will be protected vigorously to the extent permissible by law. All data in written form as well as electronic form will be kept by Prof J Ker, along with your original clinical records held by this practice. All the necessary information obtained by your participation in this study will be in anonymous form. Prof Ker will allocate a code to your research file, and this code can be used by him to link back to your clinical records held by him. All your records pertaining to this study will be kept for a minimum of 15 years, thereafter all data would be disposed of in accordance to the data protection act of the GOC. We cannot, however, guarantee privacy or confidentiality.

What will happen to the results of the research study? The results of the study would be used in order to complete the doctorate degree of Mr M.D Nagle. We also aim to publish the results of this study. However, there will be no reference to any individual's performance in any publication. The results of the study can be obtained directly from Prof J Ker, however each participant will be given a full explanation of their results.

Who is organizing and funding the research? Mr M.D Nagle is organizing the study, however there is no external funding for this research project.

Who has reviewed the study? The study has been reviewed by the Aston University Research Ethics Committee

Who do I contact if something goes wrong or I need further information? Please feel free to contact Prof James Ker (jker@wol.co.za), +2712 341 0078

Who do I contact if I wish to make a complaint about the way in which the research is conducted? If you have any concerns about the way in which the study has been conducted, then you should contact the Secretary of the University Research Ethics Committee on j.gwalter@aston.ac.uk or telephone 0121 204 4665.

Volunteer consent form

Title of project:The association between glycated haemoglobin levels and P100 visual evoked potentials in diabetes mellitus.

Name of Chief Researcher: Prof James Ker

		Initial
1	I confirm that I have read and understand the information sheet for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
2	I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.	
3	Should it be necessary, I give consent for Mr MD Nagle to view my clinical records held by Prof J Ker.	
4	I agree to take part in the above study.	

Name of participant		Name of researcher	
Date		Date	
Signature		Signature	

Appendix C

Data Analysis for Left Eye

Phase 2 DM Group

1 Phase 2 – Diabetic group analysis

The results for this group are represented by data of the left eye only.

The moderately controlled DM group (6.5% < HbA1c ≤ 10%) consisted of 52 participants; 33 males and 19 females. The mean age for this group was 56.1 ± 13.04 years and the mean duration in years for this group was 7.8 ± 5.9 years. The poorly controlled DM group (HbA1c > 10%) comprised of 52 participants: 29 males and 23 females. The mean age for this group was 45.2 ± 12.68 years and the mean duration in years for this group was 9.1 ± 6.3 years. DM group (moderate;poor) differed significantly with respect to age (Student's two-sample t-test: p< 0.001) but not with respect to disease duration (Student's two-sample t-test: p = 0.294). Furthermore the DM groups also did not differ with respect to gender distribution (Fisher's exact test: p = 0.549 Males: 63.5% ($^{33}/_{52}$) vs 55.8% ($^{29}/_{52}$)or Females: 36.5% ($^{19}/_{52}$) vs 44.2% ($^{23}/_{52}$)).

The P100 peak time values were categorised in the moderately controlled DM group (6.5% < HbA1c ≤ 10%) and poorly controlled DM (HbA1c > 10%) group using the cut-points for P100 based on the control group and defines the threshold for peak time delay measured 111.86 ms for the left eye as summarised in the Table 1.

2. DM as a risk factor for P100 peak time delay

Relative to the moderate control group (6.5% < HbA1c ≤ 10%) the poorly controlled (HbA1c > 10%) group were not at increased risk for delayed P100 peak time (Left: Odds Ratio = 0.722, ci 0.327 – 1.596, p = 0.421).

Table 1: Categorized P100 values by DM group - Phase 2 –left eye*(N = 52 per group)

Group (DM)	Eye	Proportion delayed	p-value*
Moderate control (6.5% < HbA1c ≤ 10%)	Left	42.3% (22/52)	0.546
Poor control (HbA1c > 10%)	Left	34.6% (18/52)	

* Fisher's exact test and $p < 0.05$.

3. DM as a risk factor for N75 peak time delay

Relative to the moderate control group (6.5% < HbA1c ≤ 10%) the poorly controlled (HbA1c > 10%) group were not at increased risk for delayed N75 peak time (Left: Odds Ratio= 0.698, ci: 0.266-1.832, p = 0.465; logistic regression).

Table 2: Categorized N75 peak time values by DM group - Phase 2 –left eye*(N = 52 per group)

Group (DM)	Eye	Proportion delayed	p-value*
Moderate control (6.5% < HbA1c ≤ 10%)	Left	23.08% (12/52)	0.626
Poor control (HbA1c > 10%)	Left	17.31% (9/52)	

4. DM as a risk factor for N145 peak time delay

Relative to the moderate control group (6.5% < HbA1c ≤ 10%)the poorly controlled (HbA1c > 10%) group were not at increased risk for delayed N145 peak time (Left: Odds Ratio= 2.130, ci: 0.503-9.02, p =0.304; logistic regression).

Table 3: Categorized N145 peak time values by DM group - Phase 2 –left eye*(N = 52 per group)

Group (DM)	Eye	Proportion delayed	p-value*
Moderate control (6.5% < HbA1c ≤ 10%)	Left	5.77% (3/52)	0.488
Poor control (HbA1c > 10%)	Left	11.54% (6/52)	

* Fisher's exact test and $p < 0.05$.

5. DM as a risk factor for amplitude change

Relative to the moderate control group (6.5% < HbA1c ≤ 10%)the poorly controlled (HbA1c > 10%) group were not at increased risk for a change in amplitude (Left: Odds Ratio= 1.725, ci: 0.611-4.870, p =0.303; logistic regression).

Waveform abnormalities of the amplitude measurements are prone to alterations, due to participant co-operation, fixation and alertness (ISCEV Guideline 9B, 2008). The amplitude value is a reflection of concentration and is not used as an outcome measure due to greater inter-individual fluctuations (Regan, 1989).

Poor concentration was grouped as 0-5 μV
Good concentration was grouped as 5-12 μV

6. Study group comparisions

The P100 was assessed for this two-factored study design, with the main effects DM group (moderate;poor) and DM type (T1DM; T2DM) using analysis of variance (ANCOVA) starting with the inclusion of an interaction term and possible covariates age and disease durations.

Graphically there was no relationship between P100 and either of age and disease durations and these parameters as well as the interactions were also not significant in the ANCOVA, ie age ($p = 0.212$), duration of disease ($p = 0.961$) and interaction ($p = 0.763$).

The final ANOVA included the main effects only and neither was significant;
DM groups ($p = 0.402$; [HbA1c ≤ 10%] 110.31ms vs [HbA1c > 10%] 109.03ms).
DM type ($p = 0.904$; [T2DM] 110.31ms vs [T1DM] 110.54ms).

Table 4: Categorized Amplitude values by DM group - Phase 2 –left eye*(N = 52 per group)

Group (DM)	**Eye**	**Good concentration**	**Poor concentration**	**p-value***
Moderate control (6.5% < HbA1c ≤ 10%)	Left	86.54% (45/52)	13.46% (7/52)	0.438
Poor control (HbA1c > 10%)	Left	78.85 (41/52)	21.15% (11/52)	

* Fisher's exact test and $p < 0.05$.

7. Summary statistics P100

The P100 measurements of the left eye are summarised by group and are given in the Table 5.

Table 5: Descriptive statistics for P100 peak time in the left eye by group

Group	N	Mean (ms)	Standard Deviation (ms)	Adjusted mean P100 peak time (ms) when age is 46.64 years
Moderate control (6.5% < HbA1c ≤ 10%)	52	110.32	6.10	109.68
Poor control (HbA1c > 10%)	52	109.11	8.30	109.20
Control group	30	101.00	4.35	102.00

The groups were compared with respect to left eye P100 peak time in a regression analysis which included groups as a main factor and age as covariate. The results are given in the Table 6.

8. P100 – age adjusted peak time results

The two DM groups did not differ significantly (effect: 0.47ms, p = 0.738), however both moderate and poorly controlled were significantly delayed compared to the control group (HbA1c ≤ 10%: 7.73ms, p < 0.001 and HbA1c > 10%: 7.25ms, p < 0.001).

Table 6 Group comparisons with respect to P100 peak time in the left eye and adjusted for age

Comparison	Effect (ms)	p-value*
Moderate control (6.5% < HbA1c ≤ 10%) vs. control	7.730	<0.001
Poor control (HbA1c > 10%) vs. control	7.254	<0.001
Moderate control (6.5% < HbA1c ≤ 10%) vs. Poor control (HbA1c > 10%)	0.473	0.738

9. Summary statistics N75

The N75 measurements of the left eye are summarised by group and are given in the Table 7.

Table 7 Descriptive statistics for N75 in the left eye by group

Group	N	Mean (ms)	Standard Deviation (ms)	Adjusted mean N75 peak time (ms) when age is 46.64 years
Moderate control (6.5% < HbA1c ≤ 10%)	52	76.32	8.10	75.90
Poor control (HbA1c > 10%)	52	73.11	9.45	73.18
Control group	28	71.44	3.30	71.95

The groups were compared with respect to left eye N75 peak time in a regression analysis which included groups as a main factor and age as covariate. The results are given in the Table 8.

10. N75 – age adjusted peak time results

The two DM groups differed from each other by (effect: 2.84ms, p = 0.96), they were not significantly delayed when compared to the control group (HbA1c ≤ 10%: 2.84ms, p = 0.089 and HbA1c > 10%: -1.21ms, p = 0.552).

Table 8: Group comparisons with respect to N75 in the left eye and adjusted for age

Comparison	Effect (ms)	p-value*
Moderate control (6.5% < HbA1c ≤ 10%) vs. control	2.84	0.089
Poor control (HbA1c > 10%) vs. control	-1.21	0.552
Moderate control (6.5% < HbA1c ≤ 10%) vs. Poor control (HbA1c > 10%)	2.84	0.096

11. Summary statistics N145

The N145 measurements of the left eye are summarised by group and are given in the Table 9.

Table 9: Descriptive statistics for N145 in the left eye by group

Group	N	Mean (ms)	Standard Deviation (ms)	Adjusted mean N145 peak time (ms) when age is 46.64 years
Moderate control (6.5% < HbA1c ≤ 10%)	52	143.57	11.33	144.03
Poor control (HbA1c > 10%)	52	143.45	13.61	143.38
Control group	30	132.54	10.57	131.87

The groups were compared with respect to left eye N145 peak time in a regression analysis which included groups as a main factor and age as covariate. The results are given in Table 10.

12. N145 – age adjusted peak time results

Thus although the two DM groups did not differ (effect: 0.650ms, p = 0.802), both moderate as well as the poorly controlled DM groups were significantly delayed when compared to the control group (HbA1c ≤ 10%: 12.16ms, p = 0.001 and HbA1c > 10%: 11.51ms, p < 0.001).

Table 10: Group comparisons with respect to N145 in the left eye and adjusted for age

Comparison	Effect (ms)	p-value*
Moderate control (6.5% < HbA1c ≤ 10%) vs. control	12.160	0.001
Poor control (HbA1c > 10%) vs. control	11.513	< 0.001
Moderate control (6.5% < HbA1c ≤ 10%) vs. Poor control (HbA1c > 10%)	0.650	0.802

13. Summary statistics amplitude

The amplitude measurements of the left eye are summarised by group and are given in the Table 11.

Table 11: Descriptive statistics for amplitude in the left eye by group

Group	N	Mean (µV)	Standard Deviation (µV)	Adjusted mean amplitude (µV) when age is 46.64 years
Moderate control (6.5% < HbA1c ≤ 10%)	52	9.87	4.43	9.88
Poor control (HbA1c > 10%)	52	9.57	4.80	9.57
Control group	30	12.28	5.33	12.27

The groups were compared with respect to left eye amplitude in a regression analysis which included groups as a main factor and age as covariate. The results are given in Table 12.

13. Amplitude – measurements as adjusted by age

The two DM groups did not differ significantly (effect: 0.307 μV, p = 0.803) compared to each other, however the poorly controlled DM group (HbA1c > 10%) was significantly different from the control group, but this was not so for the moderate DM group (HbA1c ≤ 10%: -2.40 μV, p = 0.103 and HbA1c > 10%: 2.70 μV, p = 0.024).

Table 12: Group comparisons with respect to amplitude in the left eye and adjusted for age

Comparison	**Effect (μV)**	**p-value***
Moderate control (6.5% < HbA1c ≤ 10%) vs. control	-2.402	0.103
Poor control (HbA1c > 10%) vs. control	2.700	0.024
Moderate control (6.5% < HbA1c ≤ 10%) vs. Poor control (HbA1c > 10%)	0.307	0.803

14. Summary

P100 peak time measurements for both DM groups, moderate control (6.5% < HbA1c ≤ 10%) and poorly controlled (HbA1c > 10%), were significantly delayed for the left eye when compared to an age adjusted control group (Phase 1).

Appendix D

Raw Data

Data table for Control Participants-Phase 1

NO	GENDER	AGE	HbA1c	R EYE	N75 (ms)	P100 (ms)	N145 (ms)	AMP (uV)	L EYE	N75 (ms)	P100 (ms)	N145 (ms)	AMP (uV)
1	MALE	39	5.30%	R EYE	85.2	104.7	119.4	9.3	L EYE	75.6	104.7	122.1	8.1
2	MALE	34	5.40%	R EYE	75.9	98.7	146.4	4.7	L EYE	75.9	98.7	146.7	4.2
3	FEMALE	36	5.40%	R EYE	72.3	99.3	127.2	14.4	L EYE	70.8	98.7	121.2	11.8
4	FEMALE	26	5.10%	R EYE	67.5	101.1	155.1	24.3	L EYE	67.2	101.1	151.5	21.8
5	FEMALE	45	5.00%	R EYE	66.3	101.7	130.8	14.6	L EYE	67.2	100.8	130.2	14.8
7	FEMALE	28	5.60%	R EYE	76.8	105.6	138.6	7.7	L EYE	72.6	101.4	141.3	6.6
8	FEMALE	28	5.20%	R EYE	67.8	96.3	147.6	25.5	L EYE	69.0	95.7	148.5	25.5
11A	FEMALE	30	5.20%	R EYE	69.6	96.6	127.8	14.7	L EYE	69.0	96.3	128.1	14.0
11B	FEMALE	30	5.20%	R EYE	71.4	95.7	124.2	14.7	L EYE	70.2	98.7	128.4	14.0
12A	MALE	31	5.30%	R EYE	72.0	102.0	133.5	17.1	L EYE	78.0	99.0	136.8	14.6
12B	MALE	31	5.30%	R EYE	76.8	100.8	135.6	17.1	L EYE	76.8	102.9	135.9	14.6
13	FEMALE	23	5.50%	R EYE	67.5	92.7	114.3	10.1	L EYE	67.2	88.5	114.0	12.2
16A	FEMALE	27	5.40%	R EYE	67.2	99.6	128.7	6.7	L EYE	69.9	103.5	129.9	9.3

16B	FEMALE	27	5.40%	R EYE	67.5	102.3	129.0	6.7	L EYE	68.7	101.4	137.1	9.3
20A	MALE	32	5.40%	R EYE	69.0	104.4	134.7	16.6	L EYE	78.0	108.3	141.0	18.2
20B	MALE	32	5.40%	R EYE	74.1	104.7	140.4	16.6	L EYE	74.7	105.0	144.0	18.2
22	MALE	35	5.80%	R EYE	84.3	109.5	150.6	9.0	L EYE	89.1	109.2	149.7	8.0
23A	FEMALE	33	5.60%	R EYE	72.3	104.7	138.9	11.6	L EYE	72.3	106.8	147.0	13.2
23B	FEMALE	33	5.60%	R EYE	72.3	105.9	135.9	11.6	L EYE	71.7	106.5	144.3	13.2
24	FEMALE	56	5.20%	R EYE	73.5	98.7	124.5	5.9	L EYE	72.6	100.5	126.6	8.5
25A	MALE	34	5.30%	R EYE	80.4	106.8	134.1	7.0	L EYE	77.7	105.6	131.4	5.9
25B	MALE	34	5.30%	R EYE	80.4	104.4	133.5	7.0	L EYE	86.4	106.2	129.3	5.9
26	FEMALE	37	5.40%	R EYE	70.8	102.9	143.7	14.0	L EYE	72.3	100.2	136.2	17.3
27A	MALE	36	5.40%	R EYE	72.6	100.2	129.9	18.7	L EYE	73.8	102.0	127.8	12.8
27B	MALE	36	5.40%	R EYE	71.7	98.7	129.0	18.7	L EYE	73.2	102.6	126.6	12.8
28A	FEMALE	34	5.20%	R EYE	69.6	99.3	129.9	13.8	L EYE	67.8	98.4	121.5	14.7
28B	FEMALE	34	5.20%	R EYE	69.3	99.0	129.3	13.8	L EYE	67.2	97.8	123.6	14.7
29A	FEMALE	25	5.30%	R EYE	73.5	103.2	131.4	16.2	L EYE	71.1	104.1	126.0	9.9
29B	FEMALE	25	5.30%	R EYE	69.6	103.5	128.7	16.2	L EYE	70.8	104.4	124.2	9.9
31A	FEMALE	25	5.50%	R EYE	73.5	98.1	125.1	9.0	L EYE	67.2	99.3	117.3	9.0

31B	FEMALE	25	5.50%	R EYE	75.6	98.1	125.4	9.0	L EYE	67.5	100.2	123.3	9.0
32	MALE	38	5.40%	R EYE	76.2	104.1	134.1	20.1	L EYE	71.1	105.0	139.8	20.7
35	FEMALE	32	5.50%	R EYE	68.1	98.7	128.7	12.0	L EYE	69.9	95.7	121.2	10.2
36A	MALE	57	5.50%	R EYE	81.0	105.0	139.2	6.7	L EYE	81.0	104.1	129.6	6.1
36B	MALE	57	5.50%	R EYE	81.9	106.2	142.2	6.7	L EYE	82.5	101.1	134.1	6.1
48A	MALE	19	5.40%	R EYE	71.1	100.2	138.0	16.8	L EYE	70.8	103.2	140.4	17.8
48B	MALE	19	5.40%	R EYE	70.5	101.1	138.6	16.8	L EYE	71.7	103.2	140.4	17.8
50	FEMALE	28	5.20%	R EYE	76.2	106.2	137.7	8.9	L EYE	70.5	99.3	136.5	7.6
53A	FEMALE	28	5.30%	R EYE	63.9	96.3	117.6	12.2	L EYE	68.4	96.3	121.8	10.4
53B	FEMALE	28	5.30%	R EYE	67.8	94.8	115.5	12.2	L EYE	65.4	97.5	120.0	10.4
54A	FEMALE	26	5.40%	R EYE	75.0	103.2	139.8	6.9	L EYE	69.3	105.3	144.3	7.3
54B	FEMALE	26	5.40%	R EYE	72.6	103.8	137.4	6.9	L EYE	67.5	103.5	143.7	7.3
55A	FEMALE	34	5.30%	R EYE	70.5	97.8	129.3	6.4	L EYE	73.5	99.9	123.6	8.4
55B	FEMALE	34	5.30%	R EYE	68.4	97.2	128.1	6.4	L EYE	70.8	98.4	127.2	8.4
117	FEMALE	24	5.30%	R EYE	74.4	99.0	128.1	19.2	L EYE	71.7	98.4	124.2	19.5
AVERAGE		**32**	**5.36%**	**R EYE**	**72.8**	**101.2**	**132.8**	**12.5**	**L EYE**	**72.3**	**101.3**	**132.4**	**12.0**

10 Males

20 Females

Data table for diabetic participants HbA1c ≤ 10%

NO	GENDER	AGE	TYPE	YRS	HbA1c %	R EYE	N75 (ms)	P100 (ms)	N145 (ms)	AMP (uV)	L EYE	N75 (ms)	P100 (ms)	N145 (ms)	AMP (uV)
6	FEMALE	27	DMT_2	1	6.50%	R EYE	77.4	102.6	151.6	10.8	L EYE	73.5	102.9	152.1	10.6
10	FEMALE	47	DMT_2	1	6.50%	R EYE	63.9	106.2	142.2	18.8	L EYE	69.9	105.3	144.0	15.6
14	FEMALE	62	DMT_2	12	7.00%	R EYE	75.3	108.0	151.8	11.7	L EYE	70.8	115.8	145.5	7.2
15	FEMALE	44	DMT_2	10	7.00%	R EYE	90.0	112.5	147.3	8.7	L EYE	94.8	110.4	142.5	7.8
33	MALE	56	DMT_2	5	6.20%	R EYE	82.5	103.5	126.9	6.2	L EYE	88.8	108.6	133.8	6.5
34	MALE	52	DMT_2	5	7.20%	R EYE	83.1	110.4	159.9	16.1	L EYE	85.2	113.1	166.2	17.4
37	FEMALE	53	DMT_2	2	5.80%	R EYE	87.0	105.3	135.9	7.8	L EYE	75.6	103.2	132.3	10.5
38	MALE	57	DMT_2	5	7.20%	R EYE	98.1	119.4	142.8	10.6	L EYE	97.8	120.3	149.7	10.9
39	FEMALE	57	DMT_2	5	8.30%	R EYE	63.6	99.9	132.9	22.4	L EYE	66.3	100.8	130.5	20.6
41	FEMALE	44	DMT_1	20	7.00%	R EYE	80.1	109.2	154.2	2.6	L EYE	79.8	119.7	163.8	3.6
44	MALE	55	DMT_2	2	8.90%	R EYE	75.9	101.7	124.8	11.8	L EYE	72.9	106.5	126.9	11.6
47	MALE	53	DMT_2	6	8.00%	R EYE	72.0	109.8	140.7	7.6	L EYE	74.4	114.6	155.4	8.2
49	FEMALE	53	DMT_2	24	8.20%	R EYE	72.0	103.5	123.9	10.2	L EYE	78.9	99.9	122.1	7.7
52	MALE	42	DMT_2	1	7.10%	R EYE	74.7	105.0	123.6	5.9	L EYE	75.0	108.0	156.3	7.9
59	MALE	54	DMT_2	6	8.60%	R EYE	78.0	131.1	166.8	5.9	L EYE	72.3	124.8	166.2	6.8
60	MALE	62	DMT_2	10	6.80%	R EYE	64.5	109.8	156.6	13.1	L EYE	60.6	109.2	156.3	11.7
61	MALE	58	DMT_2	7	7.20%	R EYE	84.9	118.2	149.1	4.8	L EYE	89.7	119.7	152.1	6.5
62	MALE	68	DMT_2	12	7.30%	R EYE	81.6	118.2	147.0	11.1	L EYE	75.6	115.6	155.4	12.5
63	FEMALE	58	DMT_2	18	6.80%	R EYE	75.6	109.5	152.1	14.0	L EYE	77.1	109.5	151.5	12.4
65	FEMALE	62	DMT_2	10	6.20%	R EYE	65.7	102.3	121.2	19.4	L EYE	69.9	102.0	124.5	17.9
66	MALE	50	DMT_2	1	5.50%	R EYE	72.3	99.6	124.2	12.2	L EYE	71.7	103.8	126.9	9.9
68	MALE	63	DMT_2	2	6.80%	R EYE	67.8	111.3	149.1	4.7	L EYE	69.0	112.2	150.3	3.2
69	MALE	65	DMT_2	1	7.40%	R EYE	72.9	111.6	151.8	7.8	L EYE	79.5	119.7	152.7	7.7

73	FEMALE	77	DMT_2	16	7.00%	R EYE	86.1	110.1	164.1	8.2	L EYE	89.1	111.3	153.9	7.0
74	MALE	46	DMT_2	4	6.70%	R EYE	87.6	115.3	144.0	6.5	L EYE	91.5	114.9	150.0	4.2
76	MALE	19	DMT_1	12	6.80%	R EYE	71.4	106.2	140.7	14.0	L EYE	75.9	107.4	132.9	10.4
77	FEMALE	76	DMT_2	4	7.00%	R EYE	72.9	111.3	145.8	13.2	L EYE	78.0	113.1	148.2	18.2
78	MALE	76	DMT_2	16	6.40%	R EYE	77.1	100.2	123.9	4.4	L EYE	78.3	102.0	130.2	3.4
79	MALE	76	DMT_2	4	6.90%	R EYE	46.8	106.5	149.4	26.7	L EYE	63.9	107.7	145.2	22.0
80	MALE	25	DMT_1	23	8.00%	R EYE	77.1	104.7	124.5	2.9	L EYE	75.9	105.0	120.6	4.1
81	FEMALE	66	DMT_2	20	7.00%	R EYE	74.1	105.0	137.4	13.1	L EYE	69.0	105.0	146.7	12.6
82	MALE	56	DMT_2	5	7.00%	R EYE	69.9	104.1	126.9	9.7	L EYE	71.4	104.4	127.5	9.9
84	MALE	55	DMT_2	2	9.00%	R EYE	74.1	111.3	135.3	6.7	L EYE	75.0	108.9	135.0	5.6
85	MALE	66	DMT_2	10	8.50%	R EYE	78.9	108.6	139.8	15.6	L EYE	86.1	110.7	135.9	9.3
87	FEMALE	27	DMT_2	6	6.50%	R EYE	70.2	101.1	128.7	13.6	L EYE	71.4	101.4	128.7	10.1
89	MALE	66	DMT_2	7	7.50%	R EYE	80.7	117.3	150.0	6.1	L EYE	88.8	117.3	150.0	8.6
92	FEMALE	67	DMT_2	8	7.00%	R EYE	67.5	112.8	145.5	16.2	L EYE	69.9	112.2	145.5	12.6
93	MALE	54	DMT_2	7	7.00%	R EYE	69.3	108.3	144.0	5.3	L EYE	66.9	108.3	145.2	6.4
94	MALE	64	DMT_2	12	6.00%	R EYE	72.6	109.5	135.6	8.9	L EYE	68.4	109.8	147.3	9.8
95	FEMALE	61	DMT_2	7	6.70%	R EYE	68.4	100.8	129.9	16.6	L EYE	73.2	100.5	131.1	17.3
96	FEMALE	58	DMT_2	15	8.00%	R EYE	73.8	108.3	142.5	8.6	L EYE	76.8	108.6	142.2	7.8
97	MALE	71	DMT_2	7	7.30%	R EYE	69.9	118.8	154.8	10.3	L EYE	70.2	116.1	151.8	10.2
98	MALE	34	DMT_2	2	8.10%	R EYE	75.3	109.8	136.2	3.5	L EYE	75.6	109.2	134.7	3.1
100	MALE	59	DMT_2	8	7.00%	R EYE	71.7	112.5	143.1	4.7	L EYE	73.2	113.1	139.5	5.0
104	MALE	51	DMT_2	6	7.90%	R EYE	83.7	111.3	145.2	4.3	L EYE	87.0	111.9	145.2	4.1
107	MALE	67	DMT_2	14	6.90%	R EYE	67.5	115.5	143.4	9.8	L EYE	76.5	115.2	143.1	10.7
110	FEMALE	65	DMT_2	2	6.00%	R EYE	69.3	105.0	137.4	13.9	L EYE	72.0	105.6	138.6	13.3

123	MALE	67	DMT_2	6	7.40%	R EYE	72.3	118.2	150.0	8.8	L EYE	74.7	116.1	151.8	9.3
128	MALE	54	DMT_2	7	7.20%	R EYE	69.9	115.8	158.1	9.5	L EYE	74.7	116.4	156.6	9.6
133	FEMALE	66	DMT_2	5	6.70%	R EYE	71.1	113.4	149.7	12.4	L EYE	70.5	113.4	148.8	12.9
140	MALE	63	DMT_2	5	8.20%	R EYE	82.2	120.6	152.1	9.4	L EYE	87.0	120.3	146.4	10.4
141	MALE	44	DMT_2	1	8.20%	R EYE	72.6	105.0	137.1	11.1	L EYE	68.4	105.3	136.2	10.8
AVERAGE ≤10%		**56.1**		**7.8**	**7.18%**	**R EYE**	**74.7**	**109.5**	**142.1**	**10.3**	**L EYE**	**76.3**	**110.3**	**143.6**	**9.9**

HbA1c ≤10%

DMT2 - 49 Participants

DMT1 - 3 Participants

Males - 33 Participants

Females - 19 Participants

DMT1 - 2 Males

- 1 Female

DMT2 - 31 Males

- 18 Females

Data table for diabetic participants HbA1c > 10%

NO	GENDER	AGE	TYPE	YRS	HbA1c %	R EYE	N75 (ms)	P100 (ms)	N145 (ms)	AMP (uV)	L EYE	N75 (ms)	P100 (ms)	N145 (ms)	AMP (uV)
17	FEMALE	53	DMT_2	6	10.60%	R EYE	66.0	98.4	127.5	8.4	L EYE	66.0	99.9	125.7	8.1
35	MALE	55	DMT_2	2	10.60%	R EYE	75.3	104.1	134.7	3.0	L EYE	67.2	98.7	120.9	3.8
40	MALE	63	DMT_2	6	10.10%	R EYE	85.2	111.6	135.6	5.4	L EYE	90.0	114.3	147.3	9.1
42	MALE	46	DMT_2	3	10.50%	R EYE	95.4	122.4	155.7	3.1	L EYE	99.3	122.7	149.1	3.6
45	FEMALE	53	DMT_2	7	10.10%	R EYE	81.0	109.2	124.2	7.2	L EYE	70.8	105.3	129.9	3.8
51	MALE	34	DMT_1	15	11.00%	R EYE	79.5	111.0	154.2	18.3	L EYE	88.2	116.7	153.9	11.5
70	FEMALE	43	DMT_1	25	10.10%	R EYE	66.3	97.2	147.9	10.9	L EYE	61.5	98.4	148.2	13.0
71	MALE	47	DMT_2	3	11.00%	R EYE	76.2	102.0	125.7	7.8	L EYE	73.8	101.0	123.0	5.6
75	MALE	21	DMT_1	10	13.30%	R EYE	85.2	110.4	141.5	8.7	L EYE	85.8	112.5	133.2	4.5
86	MALE	43	DMT_2	1	11.00%	R EYE	69.9	104.4	141.0	10.5	L EYE	73.8	105.0	153.6	10.4
88	MALE	46	DMT_2	14	10.10%	R EYE	81.3	108.3	138.6	14.4	L EYE	86.4	111.0	146.7	11.3
91	FEMALE	43	DMT_2	7	13.0%	R EYE	63.3	96.9	144.6	10.5	L EYE	49.5	98.7	144.9	13.6
99	FEMALE	74	DMT_2	15	11.50%	R EYE	79.8	118.2	151.8	12.2	L EYE	80.7	117.9	158.7	12.4
101	FEMALE	20	DMT_1	7	15.70%	R EYE	74.4	104.4	150.0	24.4	L EYE	74.4	103.8	150.0	20.9
102	MALE	20	DMT_2	1	16.90%	R EYE	75.3	117.0	142.2	4.6	L EYE	71.1	117.9	152.7	5.4
103	FEMALE	34	DMT_1	13	13.60%	R EYE	67.8	121.8	170.1	6.4	L EYE	66.3	121.2	172.2	5.0
105	FEMALE	42	DMT_2	2	11.00%	R EYE	70.5	115.2	162.6	12.0	L EYE	78.3	115.8	164.1	12.3
106	MALE	56	DMT_2	3	11.10%	R EYE	70.2	114.6	145.5	7.6	L EYE	75.0	115.2	148.8	6.9
108	FEMALE	25	DMT_2	12	10.50%	R EYE	73.8	112.5	154.5	12.4	L EYE	73.5	112.5	162.3	10.4
109	FEMALE	29	DMT_1	12	10.10%	R EYE	62.1	108.0	149.1	19.8	L EYE	59.1	108.0	151.5	18.5
111	MALE	59	DMT_2	15	11.60%	R EYE	73.2	117.6	158.4	2.0	L EYE	73.5	118.2	153.3	4.0
112	FEMALE	45	DMT_2	1	10.10%	R EYE	65.4	98.1	129.9	20.0	L EYE	66.3	97.8	132.0	22.8
113	MALE	58	DMT_2	10	10.10%	R EYE	74.4	104.7	132.3	7.2	L EYE	77.7	105.3	136.8	9.0

114	FEMALE	50	DMT_2	10	12.30%	R EYE	65.7	120.2	152.8	4.6	L EYE	66.5	119.5	148.5	5.1
115	MALE	48	DMT_1	8	14.10%	R EYE	72.3	109.5	146.4	15.3	L EYE	72.6	109.2	135.6	12.3
116	FEMALE	32	DMT_1	22	11.40%	R EYE	70.2	117.9	165.6	3.4	L EYE	68.7	118.2	167.7	4.3
118	FEMALE	48	DMT_1	15	11.40%	R EYE	63.6	97.8	120.0	10.9	L EYE	67.8	97.8	126.9	10.0
119	MALE	61	DMT_2	6	12.00%	R EYE	74.4	105.0	134.4	12.0	L EYE	75.6	105.6	133.8	9.1
120	FEMALE	33	DMT_1	12	10.20%	R EYE	74.1	102.6	139.5	15.2	L EYE	71.1	102.3	140.7	12.7
121	MALE	59	DMT_2	15	11.00%	R EYE	73.8	116.4	147.0	3.9	L EYE	75.3	115.8	142.2	3.2
122	MALE	40	DMT_2	6	11.10%	R EYE	74.4	105.9	136.8	12.4	L EYE	74.1	106.2	132.9	10.9
124	FEMALE	51	DMT_2	14	10.20%	R EYE	76.5	104.7	134.4	9.2	L EYE	76.2	104.4	131.7	11.1
125	MALE	45	DMT_2	8	12.30%	R EYE	79.5	115.8	145.5	1.9	L EYE	79.8	115.2	143.7	4.3
126	MALE	43	DMT_2	9	13.60%	R EYE	72.0	105.6	134.1	8.7	L EYE	74.1	106.2	143.4	7.2
127	MALE	41	DMT_2	7	12.00%	R EYE	75.9	109.8	149.7	9.1	L EYE	73.8	109.2	156.3	7.6
129	MALE	54	DMT_1	1.5	10.20%	R EYE	75.0	111.9	138.3	5.2	L EYE	78.9	111.3	138.0	3.0
130	MALE	53	DMT_2	20	10.10%	R EYE	67.8	111.6	141.3	14.4	L EYE	77.4	111.0	142.8	10.3
131	MALE	48	DMT_2	20	10.50%	R EYE	56.7	111.6	140.7	6.1	L EYE	78.0	111.0	134.7	4.8
132	MALE	49	DMT_2	1	11.70%	R EYE	71.1	99.9	132.0	13.2	L EYE	70.8	99.0	129.9	13.7
134	MALE	64	DMT_1	7	10.70%	R EYE	63.0	105.3	136.2	13.2	L EYE	67.5	105.6	141.0	11.2
135	MALE	51	DMT_1	12	10.60%	R EYE	75.0	106.5	134.4	5.6	L EYE	80.1	108.0	145.2	6.0
136	FEMALE	67	DMT_2	20	10.30%	R EYE	62.7	105.0	134.1	11.7	L EYE	48.0	104.7	135.0	16.7
137	FEMALE	18	DMT_1	6	13.30%	R EYE	65.7	124.2	179.4	9.0	L EYE	68.1	124.8	180.9	6.7
138	MALE	44	DMT_1	1	10.10%	R EYE	61.5	106.2	141.0	17.4	L EYE	60.0	105.9	139.5	16.6
139	FEMALE	45	DMT_2	20	10.10%	R EYE	65.4	104.4	136.5	8.0	L EYE	68.7	105.3	136.2	9.8
142	MALE	64	DMT_2	8	12.70%	R EYE	101.4	134.1	165.9	3.9	L EYE	97.2	133.8	163.5	2.6
143	FEMALE	36	DMT_2	1	10.30%	R EYE	69.9	91.5	111.9	15.7	L EYE	67.8	91.2	113.4	18.1
144	FEMALE	28	DMT_1	1	14.00%	R EYE	66.0	102.6	142.5	8.1	L EYE	75.0	103.2	139.5	7.4
145	MALE	35	DMT_1	16	10.50%	R EYE	75.6	109.8	153.9	13.6	L EYE	69.9	110.1	152.1	12.6
146	FEMALE	41	DMT_2	10	13.00%	R EYE	70.2	100.8	125.1	17.0	L EYE	71.4	101.1	122.7	14.4

147	MALE	43	DMT_2	2	10.50%	R EYE	67.2	116.1	149.7	7.8	L EYE	68.1	116.4	150.0	10.8
148	FEMALE	52	DMT_2	5	11.00%	R EYE	69.6	104.1	131.1	7.2	L EYE	71.1	103.8	132.8	9.4
AVERAGE >10%		**45.2**		**9.1**	**11.44%**	**R EYE**	**72.5**	**108.9**	**142.7**	**10.0**	**L EYE**	**73.1**	**109.1**	**143.5**	**9.6**

HbA1c >10%

DMT2 - 35 Participants

DMT1 - 17 Participants

Males - 29 Participants

Females - 23 Participants

DMT1 - 8 Males

- 9 Females

DMT2 - 21 Males

- 14 Females