

**EXERCISE AND TYPE 2 DIABETES MELLITUS: PERIPHERAL SENSORY
NEUROPATHY IN HUMANS AND REGULATION OF BLOOD GLUCOSE CONTROL
IN RODENTS**

Louise Stirk, B. Tech Pod (SA)

SUPERVISOR:

Professor E. Peters-Futre

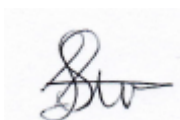
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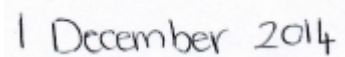
DECLARATION

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CONGRESS PRESENTATIONS

42nd Annual Conference of the Physiological Society of South Africa
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Oral Presentation by L Stirk:

Stirk L, Peters EM: EFFECTS OF AN 8-WEEK ENDURANCE EXERCISE PROGRAM ON GLYCAEMIC CONTROL AND PERIPHERAL SENSORY NEUROPATHY IN TYPE 2 DIABETIC PARTICIPANTS

Co-author of Oral Presentation by Peters, EM:

Peters EM, Naidoo P, Stirk L, Islam MS: EFFECTS OF A 5-WK ENDURANCE EXERCISE PROGRAMME ON GLYCAEMIC CONTROL IN DIABETIC AND PRE-DIABETIC RATS

ABSTRACT

PART ONE

A prospective cohort study was conducted on eight previously inactive diabetic volunteers (34 – 47 yr) with a mean pre-trial HbA1c of 7.45 (± 1.34) %, who completed an 8-week endurance exercise intervention of moderate intensity (Active Group, AG) as well as two passive controls (age 45-46 yr) with a sedentary lifestyle and one active control (AC; age 40yr) who continued with a prior exercise programme. All participants were requested to maintain their usual diet during the exercise intervention. The intervention in the AG included a 30-minute cycling program, during which the heart rate fluctuated between 60-80 % of age-predicted maximum, repeated three times per week. The effects of this endurance exercise program on signs and symptoms of Diabetic Peripheral Neuropathy (DPN) and possible associations between exercise-induced changes in mean resting heart rate (RHR), blood pressure (BP), body mass index (BMI), waist circumference, percentage body fat, eight subjective ratings of symptoms of pedal discomfort (SRPD), six objective neurological measures of DPN and changes in HbA1c, plasma brain-derived neurotrophic factor (BDNF) and adiponectin concentrations were determined in all participants ($n=11$). The exercise programme resulted in significant improvements ($p<0.05$) in the mean RHR, systolic BP and patellar and ankle reflexes in AG ($n=8$). It is concluded that the 8-week endurance exercise programme which was not combined with dietary intervention, was not successful in improving BMI and abdominal fat indexes, glucose homeostasis and neurological measures of DPN, but improved reflex action in the T2DM patients with DPN.

PART TWO

Six-week old male Sprague-Dawley rats (BW: 276.3 \pm 14.5g) were divided into three groups as Normal Control (NC, $n=7$), Diabetic Control (DBC, $n=6$) and Exercising Diabetic (DBE; $n=7$) groups. Diabetes was induced in the animals in DBC and DBE groups by feeding a 10% fructose solution *ad libitum* for first two weeks followed by an intraperitoneal injection of streptozotocin (STZ) (40 mg/kg BW), while the animals in NC group were given normal drinking water and injected with vehicle buffer. One week after the STZ injection, animals with 3 h fasting blood glucose (3hFBG) of more than 16 and 12 mmol.L⁻¹ were considered as diabetic and pre-diabetic, respectively. After the confirmation of diabetes, a progressive 5-week endurance exercise programme (20-22 m/min, 0-4° incline for 60 minutes.day⁻¹) was implemented for five days per week in the DBE group and a prediabetic (PDBE) rat, only. Daily food and water intake, weekly 3hFBG and body mass, and post-trial plasma insulin, fructosamine, adiponectin and BDNF concentrations were measured in all rats. Following the exercise programme, elevated 3hFBG, plasma fructosamine and BDNF concentrations ($p>0.05$), but hypoinsulinaemia ($p<0.05$) with unchanged plasma adiponectin concentrations ($p>0.05$) were recorded in the DBE group ($n=6$), while elevated plasma insulin concentrations was recorded in the PDBE rat with reduced 3hFBG and plasma fructosamine and elevated plasma adiponectin and BDNF levels. Data of this study suggest that although endurance exercise when combined with fructose withdrawal, is not effective in reversing STZ-induced diabetes, it may be effective in improving glycaemic status in the pre-diabetic stage.

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LIST OF ABBREVIATIONS

3hFBG	3-hour Fasting Blood Glucose
A	Alpha
B	Beta
AC	Active Control
AG	Active Group
ATP	Adenosine Tri-phosphate
AGEs	Advanced Glycation End products
ANOVA	Analyses of Variance
ApoB	Apolipoprotein B
BP	Blood Pressure
BMI	Body Mass Index
BDNF	Brain Derived Neurotrophic Factor
CVD	Cardiovascular Disease
CG	Control Group
CHD	Coronary Heart Disease
CIR	Corrected Insulin Response
CRP	C-reactive protein
DBC	Diabetic Control
DBE	Diabetic Exercise
DM	Diabetes Mellitus
DPN	Diabetic Peripheral Neuropathy
ER	Endoplasmic Reticulum
ELISA	Enzyme Linked Immunosorbent Assay
EDTA	Ethylenediaminetetraacetic Acid
FBG	Fasting Blood Glucose
FFAs	Free Fatty Acids

G6Pase	Glucose-6-phosphatase
GLUT4	Glucose transporter isoform 4
GLUT2	Glucose transporters- type 2
HbA1c	Glycosylated haemoglobin
HR	Heart rate
HDL	High Density Lipoprotein cholesterol
HOMA	Homeostasis Model Assessment
HOMA-IR	Homeostasis Model Assessment – Insulin Resistance
IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
IL-6	Interleukin-6
LIF	Leukemia Inhibitory Factor
LDL	Low Density Lipoprotein
MHR	Maximum heart rate
VO ₂ max	Maximum oxygen consumption
mRNA	Messenger Ribonucleic Acid
MetS	Metabolic Syndrome
ml	Milliliter
Ng	Nanogram
NADH	Nicotinamide Adenine Dinucleotide
NO	Nitric Oxide
NC	Normal Control
NF-β	Nuclear Factor β
NF-κβ	Nuclear Factor Kappa β
OR	Odds Ratio
OGTT	Oral Glucose Tolerance Test
PEPCK	Phosphoenolpyruvate Carboxykinase
PAI-1	Plasminogen Activator Inhibitor-1

K ⁺	Potassium
PDBE	Pre Diabetic Exercise
PKC	Protein Kinase C
RBG	Random Blood Glucose
RCT	Randomized Controlled Trials
RPE	Rate of perceived exertion
ROS	Reactive Oxygen Species
RAGE	Receptor of Advanced Glycation End products put into alphabetical order
RHR	Resting Heart Rate
SD	Standard Deviation
STZ	Streptozotocin
SRPD	Subjective ratings of Symptoms of Pedal Discomfort
TCA	Tricarboxylic Acid Cycle
TNF	Tumour Necrosis Factor
T1DM	Type 1 Diabetes
T2DM	Type 2 Diabetes

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Most common of the neuropathies experienced by diabetic individuals is chronic sensorimotor distal symmetric polyneuropathy, more simply known as diabetic peripheral neuropathy (DPN). DPN is defined as “the presence of symptoms and/or signs of peripheral nerve dysfunction in individuals with diabetes after the exclusion of other causes.” It is a great cause for concern as it can lead to other serious complications such as pedal ulcers and possible gangrene and amputation (American Diabetes Association (ADA), 2012). Although there is much evidence confirming the beneficial effects of different exercise modalities on glucose control in the Type 2 diabetic patient (Boulton *et al.*, 2005), only one research report focusing on the benefits of exercise on DPN has been completed to date (Gurney, 2011). Gurney (2011) was the first to demonstrate quantifiable changes in sensory function as a result of a low-impact balance and strength training intervention in diabetic participants with DPN.

Exercise has been considered a cornerstone of diabetes management, along with diet and pharmacological intervention (Sigal *et al.*, 2006). It is recommended that an accumulation of >30 minutes of moderate intensity activity on most days of the week or at least 150 minutes per week of moderate-intensity aerobic exercise, or resistance exercise 3 times a week has the most beneficial in managing diabetes (Sigal *et al.*, 2006; ADA, 2012). However accumulating evidence suggests that interval training has a greater effect on many of the cellular mechanisms (Haram *et al.*, 2009). It has been successfully used in younger and older patients who are in a higher risk category and has been shown to offer greater benefits compared to the traditional aerobic exercise interventions (Earnest, 2008).

The beneficial effects of exercise are attributed to an exercise associated decrease in the regulation of blood glucose concentration as reflected by the glycosylated haemoglobin concentration (HbA1c) resulting from improved glucose uptake (Earnest *et al.*, 2008). Glucose transporter isoform 4 (GLUT4) is a key signaling protein which is involved in glucose uptake and removal from the circulation and is a key regulator of whole-body glucose homeostasis (Huang and Czech, 2007). It is important in insulin-regulated glucose transport and is found in adipose tissues, skeletal and cardiac muscle. Earnest *et al.* (2008) proposed that GLUT4 protein expression increases with regular endurance exercise and that impairment in GLUT4 transport/ translocation may be the cause of insulin resistance and impaired regulation of blood glucose concentration.

The resultant increase in HbA1c has been associated with a higher risk of developing retinopathy, nephropathy, cardiovascular disease and/or damage to nervous tissue or neuropathy (Topiwala, 2012). Endurance, resistance and a combination of both exercise modalities have been associated with a similar reduction in HbA1c (Umpierre *et al.*, 2011), but little evidence exists of an association between physical exercise and sensory neuropathy (Gurney, 2011).

In 2007 Cuppinin published one of the first papers reporting an exercise-induced elevation blood BDNF concentrations attributed to the additional release of BDNF by contracting skeletal muscle. The beneficial effects of elevations in brain derived neurotrophic factor (BDNF) are well described (Nakagawa *et al.*, 2000; Cuppinin *et al.*, 2007; Krabbe *et al.*, 2007). These include i) effective lowering of blood glucose concentration by enhancing the glucose uptake by skeletal muscle, ii) enhanced sensitivity of insulin and iii) increased thermogenic activity and resting metabolic rate (Nakawaga *et al.*, 2000).

As Type 2 Diabetes Mellitus (T2DM) is characterized by both diminished insulin sensitivity and deficient insulin secretion, measuring insulin concentration alone in fasting plasma samples is also very informative. While it alerts the physician to possible reasons for the hypoglycaemia common in Type 1 diabetic patients, it provides feedback regarding the body's sensitivity to insulin in T2DM patients. Even with normal blood glucose concentrations, hyperinsulinaemia may indicate that the pancreas is working harder than normal to reduce the blood glucose concentrations and the presence of greater resistance to insulin's effect, a condition known as "insulin resistance syndrome".

Adiponectin has been reported to play an important role in the modulation of carbohydrate and lipid metabolism in insulin-sensitive tissues. Decreased circulating adiponectin levels have been reported by Yamauchi *et al.* (2001) in genetic and diet-induced murine models of obesity and by Arita *et al.* (1999) in diet-induced obesity in humans. Low levels of adiponectin have also been reported in the development of insulin-resistance in both obese and lipoatrophy mouse models (Yamauchi *et al.*, 2001). In human studies it has been reportedly shown that plasma levels of adiponectin are significantly lower in insulin resistant state of T2DM and that adiponectin is a major modulator of insulin action (Weyer *et al.*, 2001).

There is currently only one publication that explores the effects of exercise on DPN (Gurney, 2011). The possible associations between effects of exercise on DPN and between exercise-induced changes in DPN and exercise-induced changes in both HbA1c, adiponectin and BDNF levels are thus an open area for future investigation.

Due to the difficulties experienced in obtaining compliance from a large number of human participants and controlling the large number of variables that could affect the results, a laboratory study on rodents, in which (i) diabetes was induced using a combination of fructose supplementation and a streptozotocin (STZ) injection, (ii) exercise routines could be more strictly controlled and (iii) fasting blood

samples could more easily be obtained, was used to supplement the pilot work study on humans.

1.2 AIMS

Part One

To determine the effects, of an 8-week endurance exercise programme on peripheral sensory neuropathy in T2DM patients and the possible association of these signs and symptoms with quantitative measures of glucose control and change in the concentration of BDNF and adiponectin in blood.

Part Two

To determine the effects of a 5-week exercise programme, in combination with withdrawal of fructose supplementation, on blood insulin, fructosamine, BDNF and adiponectin concentrations and possible mechanisms of systemic regulation of glycaemic status in diabetic and prediabetic rats.

1.3 OBJECTIVES

Part One

- To determine the effects of an 8-week endurance exercise program on signs and symptoms of peripheral sensory neuropathy in T2DM patients
- To establish whether an 8-week program of regular endurance exercise which is not accompanied by a change in dietary intake, results in a significant effect on HbA1c and blood BDNF and adiponectin in T2DM patients with DPN

Part Two

- To determine the effects of fructose withdrawal, together with a 5-week exercise program in pre-diabetic and diabetic rats on glucose control as reflected by systemic concentrations of fructosamine, insulin, BDNF and adiponectin
- To establish the possible relationships between the various systemic measures of glucose control in pre-diabetic and diabetic rats

1.4 HYPOTHESES

Part One

As only one scientific experimental work has been published on the effects of exercise on DPN and the prior published evidence of an increase in BDNF concentrations and better controlled HbA1c following endurance exercise training, is not conclusive, the following null hypotheses were set:

- Regular endurance exercise does not improve DPN
- Regular endurance exercise alone does not reduce HbA1c in T2DM participants with DPN
- The attenuation of HbA1c is not related to exercise-induced changes in blood BDNF concentrations
- Resting blood BDNF and adiponectin concentrations do not change following an 8-week program of regular endurance

Part Two

As no study has yet been performed on the effect of exercise training on rats in which diabetes has been induced using the Fructose+ 40 mg STZ /kg model (Islam and Du Toit, 2009), the following null hypotheses were set:

- Regular endurance exercise in combination with fructose withdrawal, will not improve glucose control as reflected by circulating fructosamine, insulin, BDNF and adiponectin concentrations in fructose and STZ-induced diabetic rats

CHAPTER TWO

REVIEW OF THE RELATED LITERATURE

2.1 An Introduction to Diabetes Mellitus

Diabetes Mellitus (DM) is a chronic multifaceted metabolic condition characterised by consistent hyperglycaemia which manifests in humans with a fasting blood glucose $>7 \text{ mmol.l}^{-1}$ or 126 mg.dL^{-1} and an HbA1c $> 6.5\%$ (ADA, 2005). As insulin is the hormone that is primarily responsible for glucose uptake into myocytes under normal conditions, the hyperglycaemia is caused by insulin insensitivity and/or the decreased production of insulin by the pancreas (ADA, 2005; Desphande *et al.*, 2008; Mayo Clinic, 2014).

Depending on the aetiology, DM is categorised as either Type 1 (T1DM) or Type 2 (T2DM). T1DM is typically occurs at a young age and is a condition that involves pancreatic β -cell destruction leading to absolute insulin deficiency (ADA, 2005; Desphande *et al.*, 2008). T2DM, which, according to the most recent report of the WHO (WHO, 2014), currently accounts for 90–95% of those with DM, ranges from predominant insulin resistance with relative insulin deficiency in early stages, to a predominant insulin secretory defect with insulin resistance in more prolonged or severe cases (ADA, 2005, WHO, 2014).

2.2 Insulin Secretion and Glucose Uptake

Glucose metabolism is the process in which the body converts carbohydrate in food that is consumed, into glucose and used as an energy source for the body (Ogunjimi, 2013). The uptake of glucose into cells is aided by insulin that is exclusively produced by β -cells which are located within the Islets of Langerhans of the pancreas. In the protein assembly of insulin, the messenger RNA transcript is translated into an inactive protein called preproinsulin. Preproinsulin contains an amino-terminal signal sequence that is needed in order for the precursor hormone to pass through the membrane of the endoplasmic reticulum (ER). Upon entering the ER, the preproinsulin signal sequence is proteolytically removed to form proinsulin. The end product of the biosynthesis is a mature and active insulin. Finally the insulin is packaged and stored in secretory granules, which accumulate in the cytoplasm, until its release is triggered (Cartailler, 2014).

As is shown in Figure 2.1, the process by which insulin is released from the β -cells in response to changes in blood glucose concentration, is a mechanism involving Type 2 glucose transporters (GLUT2) that mediate the entry of glucose into the β -cells. This glucose is phosphorylated by the enzyme glucokinase. The modified glucose becomes effectively trapped within the β -cells and is further metabolized to create

adenosine tri-phosphate (ATP), the energy molecule. The increased ATP:ADP ratio causes the ATP-gated potassium (K^+) channels in the cellular membrane to close, preventing K^+ from being shunted across the cell membrane. The subsequent rise in positive charge inside the cell due to the increased concentration of K^+ , leads to the depolarization of the cell. The net effect is the activation of the voltage-gated calcium channels which results in the abrupt increase in intracellular calcium concentrations and triggers activation of the insulin-storing granules and the release of insulin from the β -cells and its diffusion into nearby blood vessels (Cartailler, 2014).

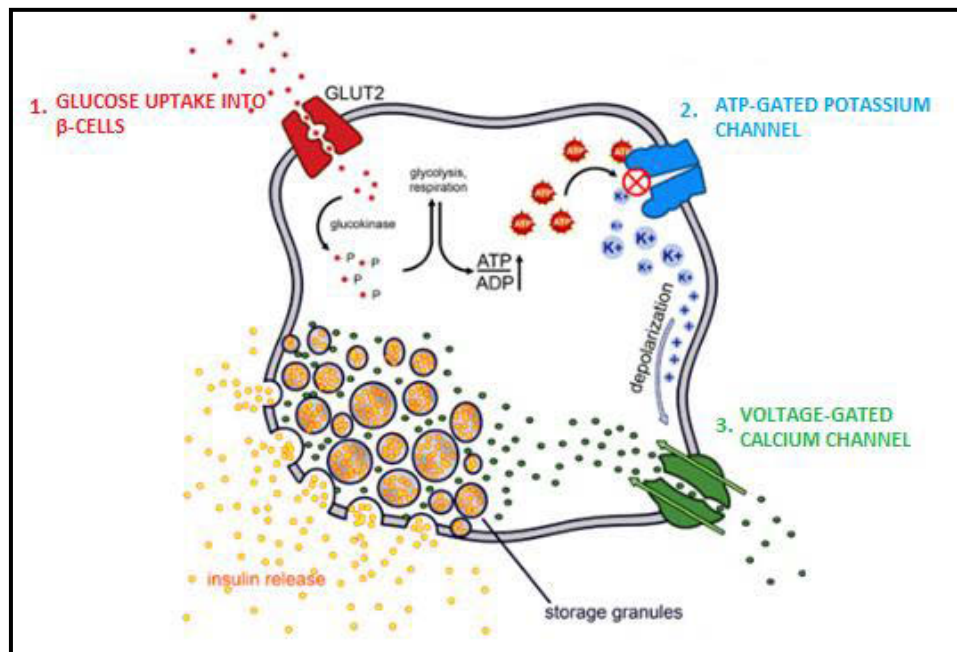


Figure 2.1: The mechanism of insulin secretion from the β pancreatic cells (Adapted from Cartailler, 2014)

Insulin molecules circulate throughout the blood stream until they bind to their associated insulin receptors. The insulin receptors promote the uptake of glucose into tissues that contain type 4 glucose transporters (GLUT4), such as the skeletal muscles and fat. The initial binding of insulin to its receptor initiates a signal transduction cascade that results in the removal of glucose from blood plasma (Figure 2.2). These changes can last from minutes to hours (Cartailler, 2014).

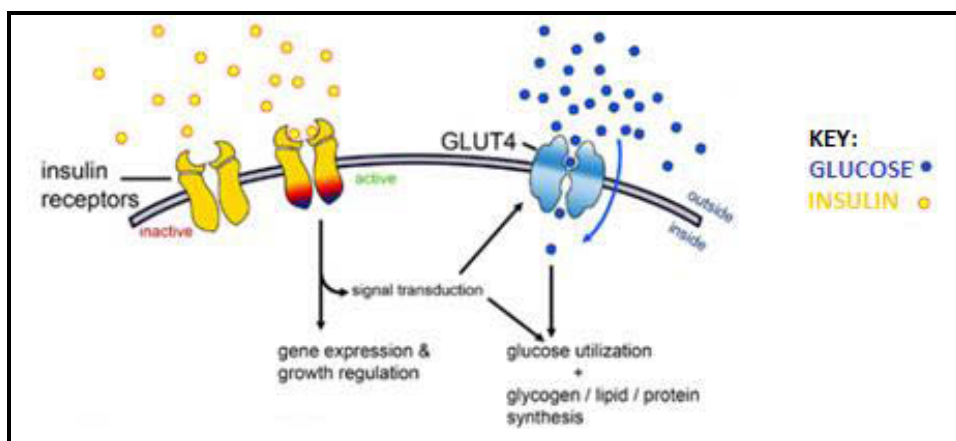


Figure 2.2: Insulin mediated glucose uptake (Adapted from Cartailler, 2014)

The body's response to blood glucose therefore requires the coordination of an array of mechanisms. Failure of any one component involved in insulin regulation, secretion, uptake or breakdown can lead to abnormally high levels of blood glucose resulting in DM (Cartailler, 2014).

2.3 Lipid Metabolism

The inability of the body to produce and/or utilize insulin can also affect the metabolism of lipids (Ogunjimi, 2013). From the findings of Boden *et al.* (2002) it is now clear that elevation of plasma free fatty acids (FFAs) plays an important role in the development of T2DM by causing insulin resistance. Adipocytes take up and store FFAs and during periods of low energy intake the body uses its fat reserves i.e. FFAs being released for other tissues to be used as fuel. However, if plasma FFA levels are elevated for more than a few hours, they will cause insulin resistance (Boden *et al.*, 2002).

The mechanisms by which elevated FFA levels result in insulin resistance have been determined in human skeletal muscle, where most insulin-stimulated glucose uptake, glucose transport or phosphorylation occurs. It has been suggested that it is not the accumulation of fat in muscle cells that causes insulin resistance, but rather the accumulation of other metabolites, including diacylglycerol, that occur (Boden *et al.*, 2002). It has also been proposed that FFAs can cause insulin resistance by increasing oxidative stress (Ceriello, 2000). Reactive oxygen species have been shown to activate protein kinase C (PKC) and the nuclear factor β (NF- β) pathway, thereby contributing to insulin resistance (Ceriello, 2000). Thirdly, FFAs also affect the functioning of insulin in the liver and thus contribute to hepatic overproduction of glucose and to elevated circulating blood glucose levels (Ceriello, 2000).

An increase in visceral fat is however believed to cause insulin resistance by mechanisms that do not directly involve FFAs. Adipose tissue is a source of inflammatory adipokines, which also act as mediators, such as tumor necrosis factor (TNF), interleukin (IL)-6 and peptides that include resistin, leptin and adiponectin (Boden and Laakso, 2004).

Of the above-mentioned adipokines, adiponectin is most likely to affect insulin sensitivity. Produced exclusively in adipocytes, it stimulates FFA oxidation, decreases plasma triglycerides, and improves glucose metabolism by increasing insulin sensitivity (Weyer *et al.*, 2001). The plasma levels of adiponectin in Caucasians and Pima Indians are negatively correlated with % body fat and plasma insulin levels and are positively correlated with insulin-mediated glucose uptake (Weyer *et al.*, 2001). In addition, plasma levels of adiponectin are lower in individuals with T2DM than in age and body mass matched individuals without DM. Thus the impaired release of adiponectin that occurs in obesity is thought to also contribute to insulin resistance and development of T2DM although the mechanism leading to decreased adiponectin levels in obesity is not clear (Weyer *et al.*, 2001).

2.4 Protein Metabolism

Insulin is also responsible for the development of new proteins, transporting amino acids into cells and guarding against the breakdown of proteins (Ogunjimi, 2013). Pereira *et al.*, (2008) demonstrated that T2DM men have insulin resistance of protein metabolism. The study further showed that insulin resistance may result in inhibition of modest stimulation of protein synthesis that occurs following insulin and amino acid infusion. Studies by Stump *et al.* (2003) and Asmann *et al.* (2006) showed impaired insulin-stimulated glucose disposal, muscle mitochondrial ATP production, and a reduction in mitochondrial protein synthesis in T2DM patients.

These findings are important as they indicate that T2DM involves defective protein metabolism and that the finding of impaired insulin plus amino acid stimulated protein synthesis in T2DM men may be of clinical importance (Pereira *et al.*, 2008).

2.5 Signs and Symptoms of DM

The increased blood glucose levels affecting fat, carbohydrate and protein metabolism lead to symptoms that are associated with DM. These typically include polyuria, polydipsia, weight loss, polyphagia, blurred vision and impairment of growth and susceptibility to certain infections (ADA, 2005; Guyton and Hall, 2011).

When blood glucose concentrations rise and pass the 'renal threshold' (between 180 and 220 mg.dL⁻¹), the kidneys excrete the glucose with the urine. This causes an osmotic shift of extra water into the urine from the blood, leading to polyuria. The resulting dehydration causes excessive thirst or polydipsia (ADA, 2005; Guyton and Hall, 2011).

Increased appetite or polyphagia and a loss of body mass has been reported to occur when glucose from the blood cannot enter the tissue cells resulting in starvation of the tissues and increased hunger. Insufficient insulin reduces the diffusion of glucose into tissue cells and its' use as glycolytic substrate. When this occurs, the body starts burning fat and muscle for energy, causing a reduction in overall body weight (ADA, 2005; Guyton and Hall, 2011).

Blurred vision can also be attributed to the high blood glucose concentrations. During the initial stages of retinopathy, the damage is limited to micro aneurysms in the blood vessel walls. This occurs as a result of the conversion of glucose to sorbitol during the intracellular polyol pathway, which increases the osmotic stress. Although these can leak blood and fluid, they do not usually affect vision unless there is oedema in the macula. At more advanced stages, there is blockage of the blood vessels that supply the retina (Guyton and Hall, 2011). In an attempt to restore blood supply, new blood vessels start to form.

However, these are weak and result in hemorrhages, which can cause blurred and patchy vision. Over time, this bleeding leads to scar tissue formation, which can result in retinal detachment, and if untreated, blindness (NHS, 2014).

DM also leads to poor blood circulation due to increased predisposition to atherosclerosis and arteriosclerosis. Creager *et al.* (2003) explains that in normal endothelial cells, biologically active vasodilatory substances are synthesized and released. This facilitates adequate blood flow and nutrient delivery while preventing thrombosis and leukocyte diapedesis. Among the important molecules synthesized by the endothelial cell is nitric oxide (NO). The bioavailability of NO represents a key marker in vascular health as it causes vasodilation of vascular smooth muscle cells and protects the blood vessel from endogenous injury (atherosclerosis) by preventing platelet and leukocyte interaction with the vascular wall and inhibit vascular smooth muscle cell proliferation and migration. Therefore the loss of endothelium-derived NO allows increased activity of proinflammatory transcription factor nuclear factor kappa β (NF- $\kappa\beta$), resulting in expression of leukocyte adhesion molecules and production of chemokines and cytokines. This promotes monocyte and vascular smooth muscle cell migration into the intima and formation of macrophage foam cells, characterizing the initial stages of atherosclerosis. Endothelium-dependent vasodilation is therefore abnormal in patients with T1DM and T2DM and the body's ability to repair tissue and heal wounds is affected. This can result in wounds remaining open and unhealed for months, increasing the risk of fungal and bacterial infections and even gangrene in patients with DM (Guyton and Hall, 2011).

Although T2DM remains a leading cause of cardiovascular disorders, blindness, end stage renal failure (ADA, 2005), a further complaint amongst diabetic patients is the development of chronic sensorimotor distal symmetric polyneuropathy. It is the most common of the neuropathies, more simply known as diabetic peripheral neuropathy (DPN) and has been defined as "the presence of symptoms and/or signs of peripheral nerve dysfunction in people with DM after the exclusion of other causes" (Boulton *et al.*, 2005).

2.6 Pathogenesis of DM and DPN

T2DM

In early disease stages of the T2DM patient, insulin production is normal or increased in absolute terms, but the insulin sensitivity is usually reduced. In most patients with T2DM, especially the obese, insulin resistance in target tissues (liver, muscle, adipose tissue and the myocardium) is a prominent feature. This results in both glucose overproduction and underutilization (ADA, 2012). Abnormal Islet cell function is a key and essential factor of T2DM. In addition, pancreatic α -cells of the patient with chronic T2DM also hypersecrete glucagon, resulting in further hepatic glucose production (ADA, 2012).

DPN

The factors that lead to the development of diabetic neuropathy are not completely understood and a few biochemical mechanisms will be discussed.

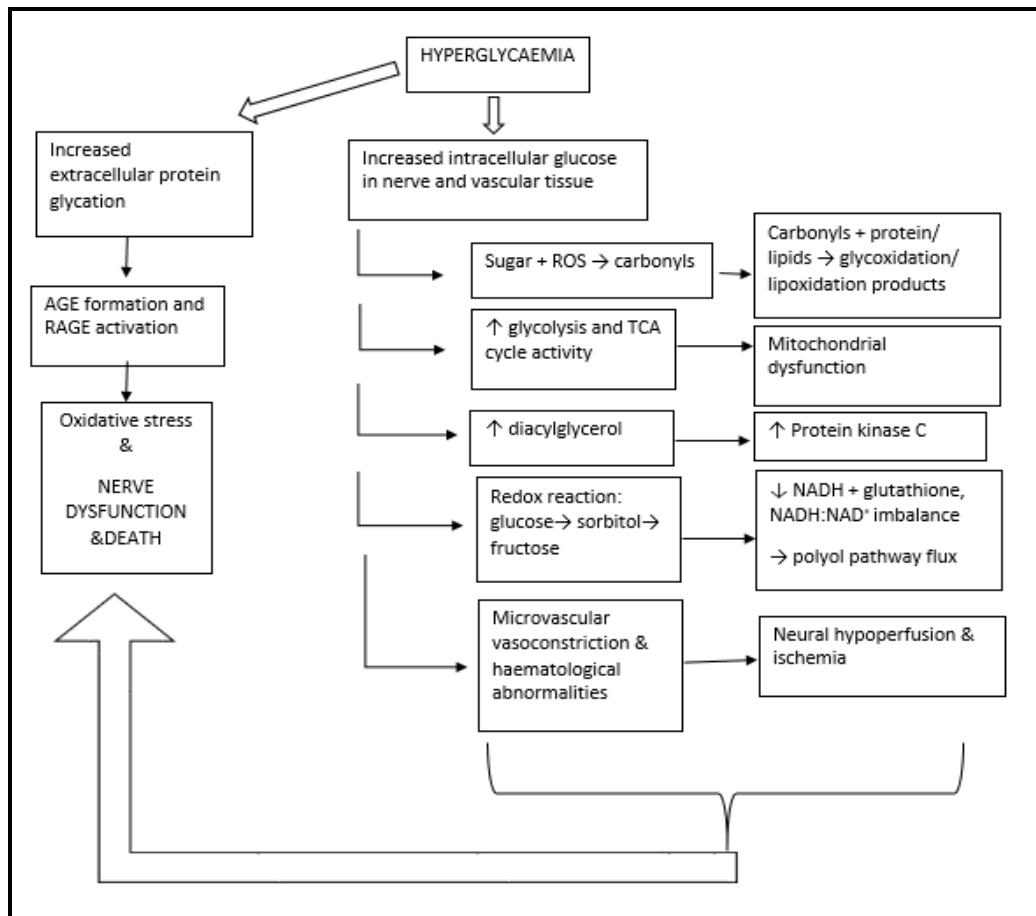


Figure 2.3: General Overview of Diabetic Neuropathy (Adapted from DUBY *et al.*, 2004; AGEs Advanced Glycation End products, RAGE Receptor of Advanced Glycation End products, ROS Reactive Oxygen Species, TCA Tricarboxylic Acid Cycle, NADH Nicotinamide Adenine Dinucleotide)

As is shown in Figure 2.3, DM promotes elevated intracellular concentrations of glucose that can take part in a number of different pathological processes. Glucose can react with ROS to form carbonyls that can react with proteins or lipids to produce glycooxidation or lipoxidation compounds, respectively. Glucose and its metabolites can also create carbonyl complexes with proteins, producing AGEs that contribute to oxidative stress. Alternatively, glucose metabolism itself creates free-radicals during normal production of ATP. The presence of excessive glucose may lead to increased production of reducing agents (i.e. NADH and FADH_2) through glycolysis and the Tricarboxylic acid cycle. The excess of electron donors may result in a dangerous imbalance in the mitochondrial electron transport chain that could accelerate the production of superoxide, a highly reactive free radical (Lin, 2012). Superoxide anions are capable of intense tissue damage and may contribute to the activation of PKC (a superfamily of 12 isoenzymes that act in the transduction of intracellular signalling and are activated by phosphorylation and subsequent binding to the second messenger diacylglycerol). Glucose and other sugars can also nonenzymatically form covalent bonds with proteins to produce Schiff bases and Amadori products,

which can further degrade or react to produce AGEs. This process occurs in euglycaemic individuals and normally affects only longer-lived proteins, but hyperglycaemia provides an excess of substrate i.e. glucose that may accelerate the reaction. The glycation of essential proteins could alter their structure and impair their function (Lin, 2012).

Receptors for AGEs have also been identified as contributors to oxidative stress and activate signal-transduction pathways, such as PKC and mitogen-activated protein. The polyol pathway can induce and drive secondary metabolic processes that promote intracellular instability and decay. It is an alternative catabolic pathway that is activated by elevated intracellular glucose levels. The first redox reaction of the polyol pathway couples the reduction of glucose by the enzyme al-dose reductase with the oxidation of NADPH to NADP⁺, producing sorbitol. Sorbitol is further oxidized to fructose by sorbitol dehydrogenase, which is coupled with the reduction of NAD⁺ to NADH. A high rate of "flux" of glucose through the polyol pathway has been hypothesized to be pathogenic, primarily by increasing the turnover of cofactors NADPH and NAD⁺ (Duby *et al.*, 2004; Lin, 2012). The reduction and regeneration of glutathione require NADPH, and depletion of glutathione could contribute to oxidative stress and the accumulation of toxic species. An imbalance in the NADH: NAD⁺ ratio could also result in increased production of AGEs and the activation of diacylglycerol and PKC. There is mounting evidence that PKC may therefore be a critical conductor in the metabolic pathologies associated with diabetic neuropathy (Duby *et al.*, 2004; Lin, 2012).

Other causes may be altered gene expression with altered cellular phenotypes, changes in cell physiology relating to endoskeletal structure or cellular transport, or reduction in neurotrophins e.g. Brain Derived Neurotropic factor (BDNF) (Lin, 2012).

2.7 Risk factors for DM

Fixed risk factors

Certain racial/ethnic groups have increased risk of developing DM. Greater insulin resistance has been shown in the Latino population and more β -cell dysfunction has been seen in East Asians (Inzucchi *et al.*, 2012). If both parents have T2DM, the child's risk is about one in two to develop diabetes (Diabetes.co.uk, 2014).

Physical inactivity

Pederson (2009) explains that physical inactivity leads to the accumulation of visceral fat. This visceral fat acts as an endocrine gland subsequently activating a network of inflammatory pathways resulting in chronic systemic inflammation which in turn is associated conditions including T2DM, cardiovascular diseases, colon cancer, postmenopausal breast cancer, dementia and depression.

Physical inactivity may induce negative effects on relatively fast-acting cellular processes in skeletal muscles or other tissues, elevating harmful plasma concentrations of triglycerides and lowering serum high density lipoprotein cholesterol (HDL) concentrations. Even prolonged sitting time is a high-risk factor for diseases like coronary artery disease or glucose metabolism in those with T2DM. As sitting for prolonged periods results in reduced cumulative energy expenditure it may have chronic effects on the predisposition to become overweight (Owen *et al.*, 2009).

Increased BMI/ Central Obesity

BMI is calculated by dividing mass (kg) by height (m) square. In Table 2.1 values for each class of obesity and the relative risk of comorbidities and Figure 2.4 shows relative risk of mortality, cardiac heart disease and T2DM to BMI.

Table 2.1: Body Mass Index and risk of co-morbidities (Adapted from WHO, 2000)

	BMI (kg.m ⁻²)	Risk of Comorbidities
Healthy weight	18.5 – 24.9	Normal
Overweight	25.0 – 29.9	Increased
Obese Class I	30.0 – 34.9	High
Obese Class II	35.0 – 39.9	Very High
Obese Class III	≥ 40.0	Extremely High

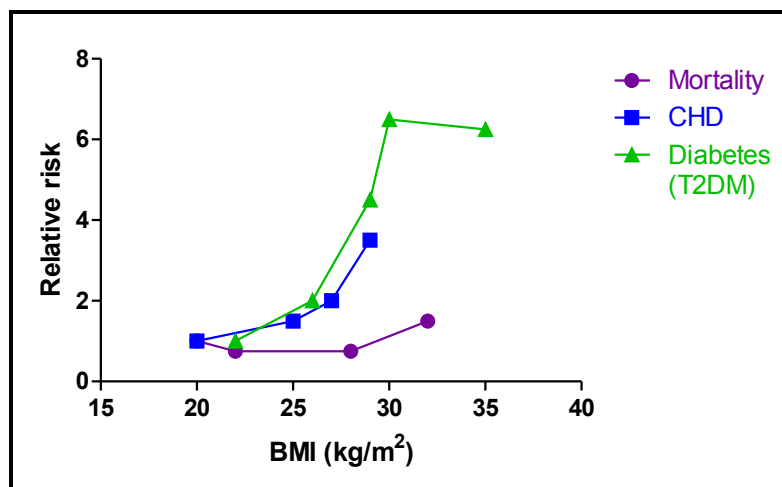


Figure 2.4: Relative risk of mortality, coronary heart disease (CHD) and T2DM according to body mass index (BMI) (Adapted from Manson *et al.*, 1995).

There is however evidence that intra-abdominal (visceral) obesity is the most dangerous obesity phenotype and that waist circumference is better than BMI to predict cardiometabolic risk (International Chair on Cardiometabolic Risk, 2013). Central obesity is defined as waist circumference ≥ 94 cm for Caucasian men and ≥ 80 cm for Caucasian women and for Asian men ≥ 90 cm and for Asian women ≥ 80 cm. It is of such concern and predisposes to so many other co-morbidities that was introduced by the

International DM Federation (IDF) in 2005 as a prerequisite for the diagnosis of metabolic syndrome (MetS) in addition to any two of the following risk factors listed below (Alberti *et al.*, 2005).

MetS also known as insulin resistance syndrome, is a cluster of three or more of the following common co-morbidities;

- Fasting plasma glucose concentration $\geq 6.1\text{mmol.l}^{-1}$
- Serum triglyceride concentration $\geq 1.69\text{mmol.l}^{-1}$
- HDL concentration $< 1.04\text{mmol.l}^{-1}$
- Blood pressure $\geq 130/85$ mmHg or controlled with medication
- Waist circumference of $> 88\text{cm}$ in women and 102cm in men

Metabolic studies have shown that among equally obese patients, subjects with an excess of intra-abdominal adipose tissue have the worst metabolic risk profile. These participants show insulin resistance and compensatory hyperinsulinaemia as an indication of insulin resistance. The presence of visceral obesity and insulin resistance have been associated with dyslipidaemic profile that includes hypertriglyceridaemia, low HDL cholesterol concentration, elevated apolipoprotein B as a marker of an increased concentration of atherogenic lipoproteins, and an increased concentration of small, dense LDL particles. In addition to the dyslipidaemic insulin resistant profile, these individuals present with impaired fibrinolysis, an increased susceptibility to thrombosis, endothelial dysfunction and an inflammatory profile (Lemieux *et al.*, 2000; Després and Lemieux, 2000).

A close relationship between visceral obesity and elevated C-reactive protein (CRP) levels has also been reported (International Chair on Cardiometabolic Risk, 2013). This could be due to macrophage infiltration of the adipose tissue of abdominally obese patients. These macrophages can produce inflammatory cytokines such as TNF- α and IL-6, which can have a local impact on adipose tissue metabolism as well as systemic effects. This can exacerbate the dysmetabolic profile seen among patients with an excess of intra-abdominal adipose tissue. For instance, the TNF- α could make adipose tissue insulin resistant, and it also has an inhibitory effect on the production of adiponectin (an important adipose tissue-derived cytokine that has been suggested to have anti-atherogenic and anti-diabetic properties). In addition, the release of IL-6 by fat cells is known to stimulate the production of CRP through the liver (International Chair on Cardiometabolic Risk, 2013).

The hyperlipolytic state and pro-inflammatory profile of intra-abdominal obesity could therefore explain the collection of metabolic abnormalities found in intra-abdominally obese patients (International Chair on Cardiometabolic Risk, 2013).

Stress

When the human body is under threat it elicits a number of neuroendocrine responses. These include an increased secretion of catecholamines from the adrenal gland and the activation of the sympathetic nervous system. Tsatsoulis and Fountoulakis (2006) describe how stress allows for a “fight or flight” response to occur by mobilizing endogenous substrate and inducing a state of insulin resistance in the liver and skeletal muscles. The release of the stress hormone, cortisol, also increases glucose in the bloodstream, in an enhancement of gluconeogenic processors (Tsatsoulis and Fountoulakis, 2006).

2.8 Diagnostic Laboratory Tests

2.8.1 Fasting Blood Glucose (FBG)

FBG measures blood glucose after an 8 hour fast (preferably done 1st thing in the morning after an overnight fast). This is often the first test done to diagnose DM (WebMD, 2012).

Interpretation of the results of a fasting test with respect to glucose levels in the human body are as follows (Diabetes.co.uk, 2014):

- Normal: 3.9 to 5.5 mmol.l⁻¹ (70 to 100 mg.dl⁻¹)
- Pre-diabetes or Impaired Glucose Tolerance: 5.6 to 7.0 mmol.l⁻¹ (101 to 126 mg.dl⁻¹)
- Diagnosis of DM: more than 7.0 mmol.l⁻¹ (126 mg.dl⁻¹)

2.8.2 2 hour Postprandial Blood Glucose

This measures the blood glucose exactly 2 hours after the start of eating a meal. This test is not used in the diagnosis of DM, but rather in understanding the effects of food choices of blood glucose. Ideally it is recommended that values remain less than 7.8mmol.l⁻¹ for people 50 years or younger, less than 8.3mmol.l⁻¹ for persons aged 50-60 years and less than 8.9 mmol.l⁻¹ in persons older than 60 (WebMD, 2012).

2.8.3 Random Blood Glucose (RBG)

This is the measurement of blood glucose at any time of the day regardless of what was ingested. This is not used to diagnose DM (WebMD, 2012). A level of 11.1 mmol.l⁻¹ or more in the blood sample indicates DM (Diabetes.co.uk, 2014).

2.8.4 Oral Glucose Tolerance Test (OGTT)

This is considered the gold standard test to be carried out for the diagnosis of DM. It involves drinking a sugar solution (i.e. water with 75g of sugar added) after an 8 hour fast. The blood glucose concentrations are measured prior to ingesting the sugar solution and then again 2 hours post (MedicineNet.com, 2014).

Impaired glucose tolerance (IGT) is diagnosed when the fasting plasma glucose is less than 7 mmol.l⁻¹ and the two hour glucose level is between 7.8 and 11 mmol.l⁻¹. This is also commonly referred to as "pre-diabetes" as IGT is associated with a higher risk of developing DM (MedicineNet.com, 2014). DM is diagnosed when two diagnostic tests done on different days show that the blood glucose concentrations are either the two hour level is greater than 11.1 mmol.l⁻¹ or the fasting glucose is noted as greater than 7 mmol.l⁻¹. A glycosylated haemoglobin (HbA1c) level of 6.5% or more also supports a diagnosis of DM (MedicineNet.com, 2014).

2.8.5 HbA1c

HbA1c is a laboratory blood test that represents the average level of blood glucose over the previous 3 months (Topiwala, 2012). It is linearly related to the average blood glucose over the past 1-3 months (but it is somewhat weighted to the past 2-4 weeks) (MDRTC, 2013). It ranges from less than 5.7% in non-diabetic individuals, to 5.7% -6.4% in the pre-diabetic population and > 6.5% in patients with DM (Topiwala, 2012). An increase in HbA1c above 6.5% has been associated with a higher risk of developing retinopathy, nephropathy, cardiovascular disease (CVD) and/or damage to nervous tissue or neuropathy (Topiwala, 2012).

2.9 Other relevant systemic biochemical measures

2.9.1 Plasma Fructosamine

Fructosamine is a glycosylated protein formed when carbohydrate and protein, primarily albumin, bond non-enzymatically. It can be used as an assessment for glycaemic control and has been shown to be proportional to blood glucose over a longer period (half-life about 17 days in the circulation). Fructosamine therefore represents a shorter term glycaemic control than HbA1c (Rigg, 2012), but may be preferred in rodents or humans with hemoglobin variants or altered red blood cell lifespans that can skew HbA1c readings (College of American Pathologists, 2010).

2.9.2 Insulin Concentration/ HOMA IR/ Insulogenic index

T2DM is characterized by both diminished insulin sensitivity and deficient insulin secretion. Insulin sensitivity can be measured by the hyperinsulinaemic-euglycaemic 'clamp' or the insulin response to an intravenous glucose infusion and, although labour intensive, these are often considered the "gold standards" for assessing insulin sensitivity and secretion (Bonora *et al.*, 2002).

The Homeostasis Model Assessment (HOMA) is commonly used to estimate the steady state of β cell function and insulin sensitivity as percentages in humans. The degree of insulin resistance is estimated at the baseline by computing an insulin resistance score (HOMA IR) using the following formula:

$$\text{HOMAIR} = \frac{\text{fasting plasma glucose (mmol.l-1)} \times \text{fasting serum insulin (mU/l)}}{22.5}$$

Low HOMA IR values indicate high insulin sensitivity, whereas high HOMA IR values indicate low insulin sensitivity (insulin resistance) (Bonora *et al.*, 2002)

The corrected insulin response (CIR) however appears to be the most useful index in humans, particularly when measured in the 30-minute post load sample (Hanson *et al.*, 2000). CIR is a measure of postprandial insulin secretion, but can be only calculated using the following formula for time points after the oral glucose load has been administered.

$$\text{CIR} = \frac{\text{Insulin} * 100}{\text{Glucose (Glucose - 70)}}$$

Measuring insulin concentration alone in fasting plasma samples is however according to Hanson *et al.* (2000) also very informative. While it alerts the physician to possible reasons for the hypoglycaemia common in T1DM patients, it provides feedback regarding the body's sensitivity to insulin in T2DM patients. Even with normal blood glucose concentrations, hyperinsulinaemia may indicate that the pancreas is working harder than normal to reduce the blood glucose concentrations and the presence of greater resistance to insulin's effect, a condition known as "insulin resistance syndrome."

Normal fasting insulin concentrations in human plasma lie within the range of 5- 20 mcU.mL⁻¹ (micro unit per milliliter), while normal concentrations in rodents are 0.5-10ng.ml^l (Hanson *et al.*, 2000). In addition to the aforementioned increased insulin resistance syndrome which is characteristic of T2DM and associated central obesity, an abnormally high concentration may indicate ingestion of an excessive amount of insulin or sulfonylurea-induced hypoglycaemia.

An ultra-sensitive insulin Enzyme Linked Immunosorbent Assay (ELISA) is a common method for the evaluation for the quantitative determination of insulin concentrations in plasma. The solid phase two site enzyme immunoassay is based on direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. During incubation insulin in the sample reacts with peroxidase-conjugated anti-insulin antibodies and anti-insulin antibodies bound to the microtitration well. A simple washing step removes unbound enzyme labelled antibody. The bound conjugate is detected by reaction with 3,3', 5,5'- tetramethylbenzidine. The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically (DRG International, 2012).

2.9.3 Plasma Adiponectin

Researchers have shown that adipose tissue is not merely an inert storage depot for lipids, but is an important endocrine gland that plays a role in endocrine, metabolic and inflammatory signals for the control of energy homeostasis. The proteins released are known as adipocytokines which include leptin, tumor necrosis factor (TNF)- α , plasminogen activator inhibitor type 1 (PAI-1), adiponectin, resistin and adiponectin (Maju, 2003).

Adiponectin is an important collagen-like protein that is synthesized in and secreted from differentiating white adipose tissue and circulates in high concentrations in the serum (Maju, 2003). Although it has anti-inflammatory properties that include suppression of macrophage phagocytosis, TNF- α secretion and blockage of monocyte adhesion to endothelial cells (Maju, 2003), adiponectin also plays an important role in the modulation of carbohydrate and lipid metabolism in insulin-sensitive tissues and may be critically important in high-risk cohorts, in whom augmentation of the anti-inflammatory and antidiabetic properties is required (Simpson and Sing, 2008).

In addition to significant inhibitory effects on TNF- α -induced monocyte adhesion and adhesion molecule expression, suggesting an increased risk of adverse health effects at serum concentrations below plasma levels of 5–25 $\mu\text{g/ml}$ (Ouchi *et al.*, 1999), it has paradoxically been reported to be of lower concentration in obese individuals (Arita *et al.*, 1999). Kappes and Loffler (2000) attribute this to the development of a negative feedback mechanism which regulates the expression of adiponectin in obese persons. While low levels of adiponectin in obese individuals have also been related to the development of insulin-resistance in obese mouse models (Yamauchi *et al.*, 2001), it has been shown that plasma levels of adiponectin are significantly lower in insulin resistant states of T2DM and a major modulator of insulin action in humans (Weyer *et al.*, 2001). Infusing recombinant full-length adiponectin has been shown to acutely improve insulin-induced suppression of hepatic glucose production in mice via an associated reduction in expression of gluconeogenic enzymes phosphoenolpyruvate carboxykinase (PEPCK) and Glucose-6-phosphatase (G6Pase; Combs *et al.*, 2001). It is however important to note that although development of hyperinsulinaemia is a possible mechanism for the suppression of adiponectin levels, it seems an unlikely mediator of low concentrations as it remains low in later stages of T2DM when insulin levels decrease (Maju, 2003).

A systematic review of the relationship between exercise and adiponectin levels was completed by Simpson and Sing in 2008. In five reported cross-sectional studies which supported a significant direct relationship between physical activity and adiponectin levels in serum, exercise training twice or more often per week was associated with adiponectin concentrations of $> 4 \mu\text{g.ml}^{-1}$. Adiponectin concentrations in subjects with impaired glucose tolerance ($4.34 \pm 0.28 \mu\text{g.ml}^{-1}$) and T2DM ($3.42 \pm 0.38 \mu\text{g.ml}^{-1}$) were significantly lower than in those with normal glucose tolerance ($8.88 \pm 0.39 \mu\text{g.ml}^{-1}$; $p < 0.001$) and significantly higher levels of physical activity (Blucher *et al.*, 2006).

Eleven uncontrolled studies investigating the effects of exercise training with average trial durations of 19.6 ± 13.4 weeks and consisting of 3.1 ± 1.2 h/week of aerobic exercise (Ryan *et al.*, 2003) or power/resistance training (Oberbach *et al.*, 2006) of moderate-to-high-intensity and low-to-moderate intensities reported significant increases in only 3 (27.3%) of the trials. These positive trials revealed an average increase in adiponectin of 1.33 ± 2.83 $\mu\text{g}\cdot\text{ml}^{-1}$ in post-training adiponectin levels (Hsieh and Wang, 2005; Bluher *et al.*, 2006; Oberbach *et al.*, 2006). Two non-randomized 3-week controlled trials of supervised low-to moderately intense cycle ergometer exercise (40 min/day, 5 days/week) in addition to walking 10,000 steps per day, however showed negligible changes in adiponectin concentrations (Yokoyama *et al.*, 2004).

Eight RCTs which averaged 15.3 ± 5 weeks with a mean exercise session time of 2.2 ± 0.5 h/week and used moderate–high intensity aerobic exercise (Boudou *et al.*, 2003; Marcell *et al.*, 2005; Balagopal *et al.*, 2005; Brekke *et al.*, 2005; Giannopoulou *et al.*, 2005), resistance training (Fatouros *et al.*, 2005) or combinations of aerobic and resistance exercise (Troseid *et al.*, 2005; Hara *et al.*, 2005), only reported an intensity-based dose–response effect in two studies (Fatouros *et al.*, 2005; Marcell *et al.*, 2005). Adiponectin levels rose significantly by 21.7 and 61.4% using moderate and high intensity resistance training, respectively, while the low-intensity resistance training did not significantly augment adiponectin levels (Fatouros *et al.*, 2005). Marcell *et al.* (2005) found that high-intensity aerobic exercise (80–90% maximum heart rate) was associated with an increase in adiponectin of 0.9 $\mu\text{g}\cdot\text{ml}^{-1}$, whereas moderate intensity aerobic exercise elicited an increase of only 0.7 $\mu\text{g}\cdot\text{ml}^{-1}$.

2.9.4 Plasma Brain Derived Neurotrophic Factor

BDNF is a neurotrophin which enhances the survival, growth, maintenance and differentiation of central and peripheral neurons (Mattson *et al.*, 2004). Although it has been shown to be produced primarily by the brain, it is also produced by peripheral non-neuronal tissues which include vascular endothelial cells, T cells, B cells, monocytes and smooth muscle cells (Rasmussen, 2009). It has been detected in both human plasma and human serum with the brain being the main source of circulating BDNF concentrations, contributing as much as 70-80% which is known to be able to cross the blood-brain barrier (Rosenfeld *et al.*, 1995; Rasmussen, 2009; Zoldaz and Pilca, 2010).

Functions

The favorable effects of elevations in brain derived neurotrophic factor (BDNF) are well described (Nakagawa *et al.*, 2000; Cuppinin *et al.*, 2007; Krabbe *et al.*, 2007). These include i) effective lowering of blood glucose concentration by enhancing the glucose uptake by skeletal muscle, ii) enhanced sensitivity of insulin and iii) increased thermogenic activity and resting metabolic rate (Nakawaga *et al.*, 2000).

In one of the first animal studies, Nakagawa *et al.* (2000) showed that administration of a small dose of exogenous BDNF was found to be effective in lowering blood glucose concentration by enhancing the hypoglycaemic action of insulin as well as enhancing energy expenditure. BDNF was also shown to enhance insulin sensitivity and improve insulin resistance. This is accomplished by BDNF acting on the brain and central nervous system, especially by acting on its receptor (TrkB) in the hypothalamus.

Krabbe *et al.* (2007) reported that low levels of BDNF in humans, compared to Nakawaga *et al.* (2000) who used rodents, were accompanied by impaired glucose metabolism and severity of insulin resistance concluding that decreased BDNF may be a pathogenetic factor involved in T2DM. It has also been shown that serum BDNF concentrations in T2DM patients are significantly lower than in non-diabetic controls. This possibly provides another explanation as to why diabetic patients have associated complications such as nerve damage as compared to non-diabetics. Interestingly, it has also been reported that the concentration of BDNF may be an early marker of pathological metabolic changes in the body (Krabbe *et al.*, 2007).

Exercise

In 2007 Cuppinin published one of the first papers reporting an exercise-induced elevation blood BDNF concentrations due to the additional release of BDNF by human contracting skeletal muscle. BDNF mRNA was found to increase 24-48 hours post exercise and return to normal values by 72 hours (Cuppinin *et al.*, 2007). These researchers concluded that BDNF up regulation in exercised muscle may be involved in muscle vascularization remodeling and maintenance of neuromuscular junctions.

Although it has been repeatedly shown that single bouts of exercise increase circulating concentrations of BDNF (Gold *et al.*, 2003; Rojas-Vega *et al.*, 2006; Tang *et al.*, 2008) and that the magnitude of the increase is dependent on the intensity of the exercise (Ferris *et al.*, 2007) with regular training, increments are less consistent. Schulz *et al.* (2004.a) demonstrated no effect after 8 weeks of aerobic bicycle training in individuals with multiple sclerosis whereas Castellano and White (2008) only found a temporary increase in BDNF after 4 weeks which returned to pre-training levels after 8 weeks of training. However the results reported by Zoldaz *et al.* (2008) demonstrated a significant increase in BDNF concentrations after a 5 week moderate intensity training in young healthy men. Before training the BDNF concentrations at rest were $10.3 \pm 1.4 \text{ pg. ml}^{-1}$ and post the 5-week training period resulted in a significant ($p = 0.01$) increase in resting BDNF concentrations to $16.8 \pm 2.1 \text{ pg.ml}^{-1}$ and BDNF concentration exercise induced from $10.9 \pm 2.3 \text{ pg.ml}^{-1}$ to $68.4 \pm 16.0 \text{ pg.ml}^{-1}$ after training.

2.9.5 Blood Glucose in Rats

The most common method of determining blood glucose concentration in rats is using a portable glucometer on blood collected from a tail vein. Jiu (2010) however showed that the blood glucose tested using a glucometer was not comparable with the glucose oxidase method with a glucose reading

difference of 7-11 mmol.l⁻¹. The difference of blood glucose between fasting for 5h and 11 h was 0.8 mmol.l⁻¹. Blood glucose from abdominal aorta was 40% higher than that from tail. The reduction of blood glucose was 8% in 60 min and over 50% in 120 min after blood being sampled. Therefore Jiu (2010) suggests that the upper limit for FBG and PBG be expanded to 7.5 mmol.l⁻¹ and 10.4 mmol.l⁻¹, respectively.

2.10 Diagnosis of DPN

Up to 50% of DM patients will experience symptoms of peripheral neuropathy such as burning pain, shooting or stabbing sensations, paraesthesia, hyperparathesiae and deep aching pain (Gordois *et al.*, 2003, Boulton *et al.*, 2005). DPN can present before the diagnosis of DM and it has been estimated that patients can have DM for several years prior to their diagnosis (ACFAS, 2013). Neuropathic pain is typically worse at night and symptoms are most commonly felt in the feet and lower limbs, although in some cases the hands may be affected (Boulton *et al.*, 2005). Examination of the lower limb usually exposes sensory loss of vibration, pressure, pain, temperature perceptions and absent reflexes. Patients with DM should be screened annually for DPN by examining pin prick, temperature, vibration perception, pressure perception and reflexes (Lawrence *et al.*, 2004; Boulton *et al.*, 2005; ADA, 2012). Loss of pressure perception and reduced vibration perception predict foot ulceration (Boulton *et al.*, 2005), while loss of sensation leads to infection, ulceration and amputation. It has been reported that 80-85% of amputations result from non-healing ulcers in diabetic patients with sensory peripheral neuropathy (Aszmann *et al.*, 2004).

To determine the degree of peripheral sensory neuropathy the following objective tests can be carried out:

2.10.1 Pressure Perception

The 10g monofilament is considered one of the most useful tools a clinician can use to test for DPN and is recommended by the International DM Federation and the International Working Group on the Diabetic Foot. It remains a useful clinical tool for detecting severe neuropathy and hence identifying patients at increased risk of ulceration and amputation (Merriman and Turner, 2002; Tan, 2010). The nerve fibers tested are the A-β group. The monofilament is applied at right angles to the skin surface with just enough pressure to deform the filament into a 'C' shape and held for 2 seconds (Figure 2.5, Diagram A & B). The patient is asked to close his/her eyes and to state which foot was touched while the podiatrist touches the specified areas as seen in Figure 2.5. Inability of the patient to detect the monofilament indicates neuropathy of the large fibers (Merriman and Turner, 2002).

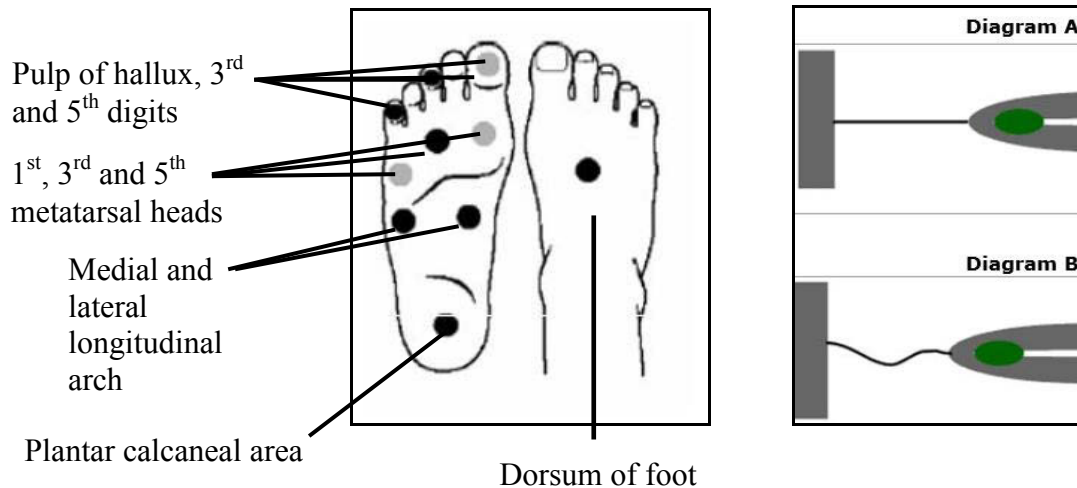


Figure 2.5: Using a 10g Monofilament to detect light pressure (Adapted from Diabetes India, 2014)

2.10.2 Vibration Perception

The nerves fibers tested with a 128Hz tuning fork are the large-diameter A β -fibers that terminate in Pacinian corpuscles and are sensitive to rapidly changing pressures or vibrations. They are coated with layers of connective tissue, which has the ability to absorb low-frequency pressure changes, so that only high-frequency vibrations such as those produced by the tuning fork would reach the receptors. As is shown in Figure 2.6, the vibrating tuning fork is placed on the skin above a bony prominence such as the apex of the hallux, malleolus and the first metatarsal phalangeal joint. The patient is to describe what they feel, without any prompts, as most of the patients are able to feel pressure, but not necessarily vibratory sensation (Singh *et al.*, 2005; Merriman and Turner, 2002).

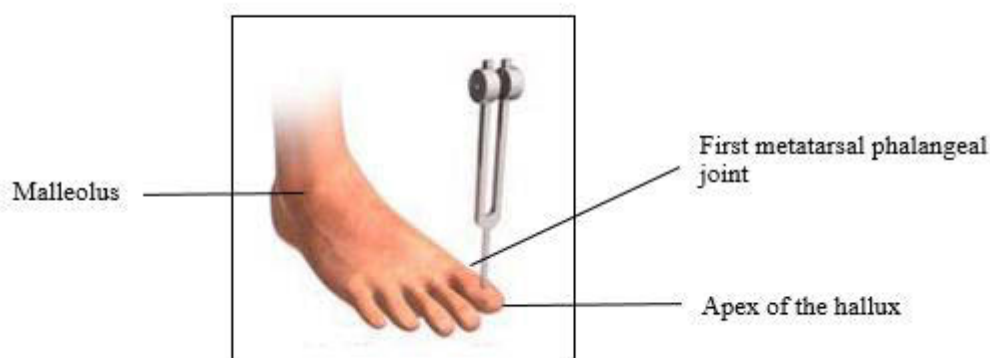


Figure 2.6: The three bony prominences on which sensitivity to the vibration of a Tuning fork is tested (Adapted from Caring for Diabetes, 2014).

2.10.3 Sharp/blunt pain Discrimination

A Neuropen, which is a spring-loaded device delivering a stimulus at a force of 40g and is the safest and most reliable method used to test pinprick sensitivity. As the sharp end cannot pierce the skin (Figure 2.7) this device assesses the sharp pain pathway, which begins with free nerve endings in the dermis, the A-delta fibres, then travels into the spinal cord and synapses in the dorsal horn. The postsynaptic fibres cross to the contralateral anterolateral columns which travel to the brain. The patients are asked to keep

their eyes closed and to state which is sharper, the first or the second sensation. The two ends are presented in random sequence to avoid correct guessing by the anxious-to-please participant (Merriman and Turner, 2002).

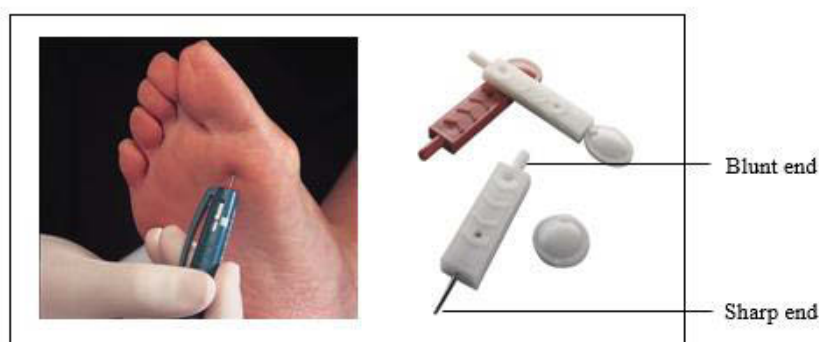


Figure 2.7: A neuropen which is used to assess the integrity of the sharp pain pathway. (Adapted from Owen Mumford, 2014)

2.10.4 Temperature Discrimination

A Tip Therm (a rod-shaped instrument with two ends of different temperatures; Figure 2.8) tests the integrity of the temperature receptors that feed the anterior part of the anterolateral columns. The nerve fibers involved are the narrow diameter A-delta and C fibers. The patients are asked to keep their eyes closed and are asked to state which side of the Tip Therm feels cooler (Merriman and Turner, 2002).

2.10.5 Light Touch Perception

The nerves tested are the large-diameter A- β fibres which ascend the dorsal columns that transmit light touch perception. The receptors (Meissner's corpuscles) lie in the superficial dermis. The patient is asked to keep their eyes close and to state when their foot is lightly stroked with piece of cotton wool and to indicate which foot they feel it on (Figure 2.8) (Merriman and Turner, 2002).

2.10.6 Reflexes

A patella hammer (Figure 2.8), is used to test for ankle and patella reflexes. The patellar reflex tests the integrity of the spinal reflex pathway (L3, L4) and exhibits descending influences on the ventral horn cell. The patient is to relax as much as tension can affect the test and is to sit sideways on the examination couch with his/her feet clearing the ground. A gentle tap on the patellar tendon with the hammer should elicit a knee jerk. The Achilles reflex tests the spinal reflex pathway of the sacral spinal nerves S1 and S2. The response is best elicited when the patients' foot is in slight dorsiflexion and the Achilles tendon is tapped with the hammer (Merriman and Turner, 2002).



Figure 2.8: A Tip Therm placed on the dorsum of the foot. (Adapted from Baehr, 2014) [LEFT]; Cotton wool stroked over the dorsum of the foot (Adapted from Soresen *et al.*, 2014) [MIDDLE]; Patella Hammer (Adapted from Medipost, 2014) [RIGHT].

2.11 Physical activity/ exercise and T2DM

As mentioned in the introductory chapter, Sigal *et al.* (2006) recommend that an accumulation of moderate intensity activity >30 minutes on most days of the week, or at least 150 minutes per week, or resistance exercise 3 times a week (ADA, 2012), is the most beneficial in managing DM.

In one of the first reported studies, Yeater *et al.* (1990) examined the effect of a low-to-moderate-intensity (69 per cent of maximal heart rate) walking program on lipids, glucose, insulin, HbA1c and cardiovascular fitness in randomly assigned control and exercise groups which walked for 40-45 minutes three times per week for two months while continuing their usual diets. A significant improvement was recorded in VO_{2max} (from 1.65 to 1.95 $l \cdot min^{-1}$) with a decrease in resting systolic blood pressure (141 to 130 mmHg), resting heart rate (88 to 81bpm) and HbA1c in 86% of the exercised group ($n=8$) after training.

2.11.1 Intensity, frequency and duration of exercise programs

There is a clear causal relationship between the amount of physical activity and coronary artery disease, T2DM and MetS (WHO, 2010). The STRIDE study (Slentz *et al.*, 2005; 2007a) demonstrated that body weight and visceral fat responded in a dose-response manner to total energy expenditure and that changes in exercise intensity alone did also not appear to have any effect on body weight or abdominal fat response. As is summarized in Table 2.2, exercise interventions that have repeatedly shown a decrease in HbA1c vary in terms of the duration required to bring about optimal change in HbA1c levels which appears to exceed 8 weeks. The ten studies described in Table 2.2 confirmed significantly improved glycaemic control following endurance and or resistance training interventions ranging in length from 8 weeks to 4 months. This range in duration is also confirmed in the meta-analysis of Thomas *et al.* (2009) on 14 randomized controlled trials in T2DM participants in which the duration of trials ranged from 8 weeks to 12 months and the exercise interventions significantly improved glycaemic control which are attributed to exercise associated decrease in HbA1c as a result of improved glucose utilization. A meta-

analysis of controlled clinical trials conducted by Boule *et al.* (2001) also showed that post-intervention HbA1c values were significantly reduced in moderate aerobic exercise trials of 8 weeks or less. The post-intervention values were 0.66% lower in the exercise group compared to the non-exercise group. These authors stated that a reduction of this magnitude is clinically significant and close to the difference of 0.9% between conventional therapy and intensive glucose-lowering therapy in the United Kingdom Prospective DM Study (UKPDS).

2.11.2 Interval training

In terms of the recent trend of incorporating high intensity interval training, WHO (2010) initially warned that T2DM patients are at a significantly increased risk for coronary artery disease and that a high-intensity exercise program is an unrealistic choice of management due to the risks associated with obesity, older age and sedentary lifestyle (WHO, 2010). Accumulating more recent evidence (Earnest, 2008; Little *et al.*, 2011; Diabetes.co.uk, 2014) however suggests that interval training has a greater effect on many of the cellular mechanisms and offers greater benefits than the traditional aerobic exercise intervention involving longer periods of conventional cardiovascular exercise such as continuous jogging. It has also been successfully used in younger and older patients who are in a higher risk category. These include patients with T2DM (Karstoft *et al.*, 2012; 2014).

Table 2.2: Exercise Interventions that have confirmed a statistically significant reduction in HbA1c and other markers of glycaemic regulation following an aerobic exercise interventions [Kelley and Kelley, 2007; Raz *et al.*, 1994; Maoirana *et al.*, 2002; Cauza *et al.*, 2005; Sigal *et al.*, 2007; Ng *et al.*, 2010; Pan *et al.*, 1997; Laaksonen *et al.*, 2005; Mc Auley *et al.*, 2002). T2DM: Type 2 Diabetes; DM: Diabetes Mellitus; IGT: Impaired glucose tolerance; OGTT: Oral glucose tolerance test]

	<i>Intervention</i>	<i>Participants</i>	<i>Variables examined</i>	<i>Results</i>
Raz <i>et al.</i> (1994)	12 wk moderate exercise training: 45 min, 3 x wk	40 participants with T2DM, randomly assigned to active/control group	Triglycerides, fructosamine, HbA1c	Reduction in all plasma levels in exercise group (p<0.05)
Maoirana <i>et al.</i> (2002)	8 wk, circuit training program: aerobic and resistance exercise	16 participants with T2DM	HbA1c, FBG	Decreases in HbA1c, FBG (p<0.05)
Cauza <i>et al.</i> (2005)	Strength vs. endurance training group for 4 months, three times a week	Randomized controlled trial of 22 patients with T2DM doing resistance training or aerobic training	HbA1c	Average decrease of 0.3% in HbA1c of aerobic training group
Sigal <i>et al.</i> (2007)	4 exercise groups: <ul style="list-style-type: none"> • resistance • aerobic • resistance + aerobic • sedentary control 	Randomized trial of previously inactive patients with T2DM	HbA1c	0.43% reduction in HbA1c of in aerobic training group. No change in HbA1c levels with a further increase in duration (p>0.05)
Knowler <i>et al.</i> (2002)	3 groups: <ul style="list-style-type: none"> • Placebo • Metformin • lifestyle-modification program 	3234 participants at high risk of developing DM; randomly assigned to groups	Incidence of onset of DM by OGTT	Modest increases in physical activity reduces the incidence of DM by 58%. Lifestyle change is twice as effective as MF therapy in preventing the progression to DM
Ng <i>et al.</i> (2010)	Resistance exercise vs. aerobic exercise (control) groups	Randomized trial of 60 T2DM participants	HbA1c	Decrease of 0.3% over the 8 week period in aerobic exercise group
Pan <i>et al.</i> (1997)	3 groups: <ul style="list-style-type: none"> • control • exercise only (EG) • diet only (DO) • diet and exercise group (DE) 	577 randomized subjects with IGT	Incidence of DM	Incidence of DM decreased by 33%, 47% and 38% in the DO, EG and DE groups respectively. Efficacy of diet similar to that of exercise; no additional benefit gained from combining diet and exercise
Laaksonen <i>et al.</i> (2005)	Leisure time-physical activity (LTPA) >4 hours/week of moderate exercise and diet modification	Randomized control study of 487 participants with impaired glucose tolerance	Incidence of T2DM	Reduced risk of developing DM by 58%
Mc Auley <i>et al.</i> (2002)	4 intervention groups: <ul style="list-style-type: none"> • control • modest diet & exercise • intensive diet & exercise (ID&E) 	Randomized control study of 79 overweight insulin-resistant normoglycaemic participants	Insulin sensitivity (euglycaemic insulin clamp)	Greater improvement in insulin sensitivity in ID&E group than in modest lifestyle change group

2.11.3 The effect of exercise alone versus combined dietary and exercise intervention?

Although a number of studies detailed in Table 2.2 have supported the effectiveness of exercise alone in improving glycaemic control in T2DM participants, the majority have combined the exercise intervention with a dietary intervention which consisted of a low fat, low refined carbohydrate or prudent diet (Pan *et al.*, 1997; Mc Auley *et al.*, 2002; Knowler *et al.*, 2002; Laaksonen *et al.*, 2005). Umpierre *et al.* (2011) confirmed that physical activity is only beneficial if combined with dietary recommendations, and structured exercise of greater than 150 minutes per week was associated with the greatest decrease in HbA1c. A Cochrane review (Oronzo *et al.*, 2009) on preventing T2DM concluded that physical activity interventions (on average, at least 150 minutes each week of brisk walking, cycling or jogging) with diet modification reduced incidence of DM by 37% in high-risk groups such as people with MetS and reported improvements in body mass, waist circumference and blood pressure. Carroll and Dudfield (2004) in a meta-analysis review of randomized control trials of metabolic abnormalities and exercise concluded that there is evidence that long-term exercise, without weight reduction, only modestly decreases abdominal adipose tissue and improves insulin action among overweight obese adults. In addition, these authors provided evidence that when exercise alone has shown to be effective in improving impaired glucose control and reducing the incidence of T2DM, it was associated with modest, but clinically relevant lipoprotein profile improvements in raising HDL-C and lowering triglycerides and modest reductions in blood pressure with greatest changes evident in overweight individuals with hypertension. However, exercise did not alter total cholesterol and LDL-C concentrations. It was concluded that more evidence is required on the effects of exercise alone (Carroll and Dudfield, 2004).

2.12 HbA1c and Exercise

The exercise-training associated decrease in HbA1c reported in the studies summarized in Table 2.2, has been associated with improved glucose utilization via previously described GLUT4-mediated glucose uptake and insulin-regulated glucose transport in adipose tissues, skeletal and cardiac muscle (Earnest *et al.*, 2008). While an acute bout of endurance exercise has been shown to provide a strong stimulus for mitochondrial biogenesis that increases GLUT4 concentration as contraction of the skeletal muscle stimulates the cell to translocate GLUT4 receptors to the surface, thus facilitating muscle glucose transport (Huang and Czech, 2007; Earnest *et al.*, 2008), it is also proposed that GLUT4 protein expression increases with regular endurance exercise and that impairment in GLUT4 transport/translocation may be the cause of insulin resistance (Earnest, 2008). Apparent cross talk between inflammatory and metabolic signaling by means of downstream mediators and signaling pathways, has also been reported (Earnest, 2008). These signaling effects are associated with impaired phosphorylation of Akt substrate 160 which is the first step identified in the insulin signaling cascade regulating GLUT4 translocation and glucose uptake which attributes to DM (Earnest, 2008).

The possible mechanisms responsible for the reduction in HbA1c (improved glucose control) due to exercise are further explained by Jensen and Richter (2012). Plasma glucose and muscle glycogen are important fuels for exercise and the increase in muscle glucose uptake during exercise is dependent on the delivery of glucose as a result of increased capillary perfusion and plasma glucose concentration as well as increased permeability of the muscle membrane. Muscle glycogen utilization is dependent on exercise intensity and duration and is controlled by the activity of glycogen phosphorylase and the concentration of glycogen and inorganic phosphate ions. In post-exercise recovery, muscle glucose uptake has increased sensitivity to insulin therefore increasing post prandial glucose uptake by previously active muscles. The molecular mechanism during exercise involves GLUT4 transporter which enables the movement of glucose into cells without the necessity of insulin being present. Furthermore, insulin sensitization may be attributed to an exercise-induced phosphorylation of proteins (TBC1D4 and p38 MAPK), which remain phosphorylated for hours post exercise (Jensen and Richter, 2012).

Although, as detailed in Table 2.2, ten studies confirmed a decrease in HbA1c and the incidence of the onset of DM after ≥ 8 weeks of exercise training alone, numerous studies (Oronzo *et al.*, 2009; Umpierre *et al.*, 2011) have failed to confirm an improvement in glycaemic control in T2DM participants following an intervention consisting solely of exercise. Burns *et al.* (2007) have explained that certain DM susceptibility genotypes might predispose individuals to respond or not to respond positively to exercise. It is possible that participants with early-onset T2DM (and severe obesity) have either genetic or acquired factors that result in resistance at the level of the skeletal muscle and are related to mitochondrial dysfunction. Burns *et al.* (2007) also reported that the failure to increase VO_2 max in diabetic participants is consistent with the presence of mitochondrial dysfunction.

Further studies are needed to be conducted to address these conflicting results, which are so important for the development of effective treatments for these high-risk patients.

2.13 Physical activity/ exercise for the treatment of DPN

It is important to decrease HbA1c levels as it is associated with a higher risk of developing retinopathy, nephropathy, cardiovascular disease and/or damage to nervous tissue or neuropathy (Topiwala, 2012; Boulton *et al.*, 2005). The first step in managing DPN is therefore to achieve and maintain optimal glycaemic homeostasis (Boulton *et al.*, 2005).

Although there is much evidence confirming the beneficial effects of different exercise modalities on glucose control in T2DM patient, little evidence exists of an association between physical exercise and sensory neuropathy and, to the authors knowledge, only one research report focusing on the benefits of exercise on DPN has been completed to date. A study by Gurney (2011) demonstrated quantifiable changes in sensory function as a result of a low-impact balance and strength training intervention in

diabetic participants with DPN. A significant improvement in sensory threshold was noticed at three of the four tested foot sites as well as for the average foot sensory threshold. The finding of improved sensory threshold in this study is unique, as it is the first study in which a group of neuropathic individuals have shown quantifiable improvements in sensory function following a low-impact exercise intervention.

2.14 Conclusion

The possible associations between the effects of exercise on DPN and between exercise-induced changes in DPN and exercise-induced changes in circulating HbA1c, BDNF and adiponectin concentrations levels are thus an open area for future investigation. As preliminary work has not yet been completed, the need for rodent study was required.

CHAPTER THREE

METHODOLOGY

This work consisted of two components. Firstly a clinical trial was conducted on human participants and thereafter elucidation of possible mechanisms was investigated following an exercise intervention on rodents.

PART ONE: CLINICAL TRIAL ON HUMAN PARTICIPANTS

3.1 Research Design of Human Study

The study design was a prospective cohort design with diabetic participants and a CG. Pre and post-intervention measurements were taken and every attempt was made to regulate possible extraneous variables which could invalidate the results of study or result in a type 11 error.

Table 3.1: Outline of longitudinal period study design

Baseline Assessment TEST 1	8 week exercise intervention	Post-trial Assessment TEST 2
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3.2 Ethical Approval and Inclusion Criteria for Human Study

Ethical approval to undertake this study (Appendix A) was obtained from the Biomedical Research Ethics Committee of the University of KwaZulu –Natal (Clearance No: BFC 055/13). The following inclusion criteria were set for participants in the study:

- Type 2 diabetic participants diagnosed with DPN
- Age Range: 34 and 48 years
- Using medication for the treatment of existing co-morbidities and oral anti-diabetic agents for the management of T2DM
- Less than an hour a week of exercise in the 3 months prior to the study
- Willingness to participate in the entirety of the trial, which includes the regular exercise intervention and laboratory testing
- Residing in the Ethekewini municipality and surrounding areas

Participants were randomly chosen from the available database of the private podiatry company for which the PI worked (Anette Thompson and Associates).

The selected 79 participants were phoned and asked if they would like to be a part of the study. Once verbal consent was obtained from the participants they were invited to attend the baseline assessment in the Exercise Physiology Laboratory of the Division of Human Physiology of the School of Laboratory Medicine and Medical Sciences of the University of KwaZulu-Natal.

3.3 Pre-Trial Assessment

3.3.1 Initial recruitment

Each participant was firstly given an information sheet (Appendix B) which fully outlined the purpose of the study, detailed the commitments expected, assured the participants that they may leave the trial at any time and required any illness or adverse effects of the trial to be reported urgently. An informed consent form (Appendix C) was signed by each participant and thereafter, further more careful screening was conducted on those who met the inclusion criteria and had enrolled for the study.

3.3.2 Further Screening

This firstly included completion of a general baseline questionnaire to assess their i) general health status, ii) lifestyle and iii) physical activity and whether they were able to exercise without causing exercise-induced complications (Appendix D). The information provided was verified by a medical practitioner who interviewed each participant and conducted a brief physical examination and assessment of vital signs, before the questionnaire was submitted.

3.3.3 Laboratory Testing

An exercise physiologist recorded the anthropometric measures which included body mass (kg) and height (cm) in light clothing without shoes and stature (cm) using an AdamLab electronic scale (JPS-2030, (Adam equipment, Connecticut, USA)) and custom-built stadiometer, respectively. Waist circumference was measured as the lowest reading of the girth, midway between the lateral lower ribs and iliac crest using a tape measurer. Percentage body fat was calculated from the sum of four skinfold thicknesses (biceps, triceps, subscapular and suprailiac) using the technique described by Durnin and Womersley (1974).

The physician recorded the participants' vital signs i.e. blood pressure, resting heart rate, temperature and respiratory rate. Other examinations included assessing the cardiovascular system for abnormal heart rhythm and murmurs and the respiratory system for abnormal breathing sounds. Possible infections and any dermatological conditions or lesions were also noted. The physician then examined the participants'

for any musculoskeletal problems that may have affected the compliance in the 8-week exercise intervention.

A 5ml venous blood was drawn by the physician into two Ethylenediaminetetraacetic Acid (EDTA) vacutainer tubes. One sample of whole blood was sent in to the local pathological laboratory (Ampath Laboratories, Westridge, Durban) and analysed for HbA1c. The remaining blood sample was immediately spun at 3000rpm for 5 min at 4°C and the plasma was stored at -80°C for later analysis of BDNF and Adiponectin concentrations using ELIZA kits (R&D Systems, Minneapolis, MN, USA and ADIPOQ, Abnova Corporation, Taiwan).

3.3.4 Nerve Function Testing

Thereafter peripheral sensory neuropathy status was assessed by a qualified podiatrist (B. Tech Pod). This included a questionnaire to assess their subjective perception of the participants in terms of the degree of sensory peripheral neuropathy symptoms they were experiencing at the onset of the trial (i.e. burning sensation, pins and needles, numbness and/or aching pain). The signs of sensory peripheral neuropathy were subsequently quantitatively assessed using the tests described below.

The Monofilament test

A 10g monofilament was applied at right angles to the skin surface with just enough pressure to deform the filament into a 'C' shape. The monofilament was held for 2 seconds at each of the ten locations specified in Figure 3.1. Participants were asked to keep their eyes closed and to state which foot was touched while each of the areas specified in Figure 3.1 was tested for integrity of the A β group of nerves and its sensitivity to the application of light pressure. The number of positive findings for each of the 10 sites tested on both feet ($n=20$), was used to allocate a score for this test (Table 3.2).

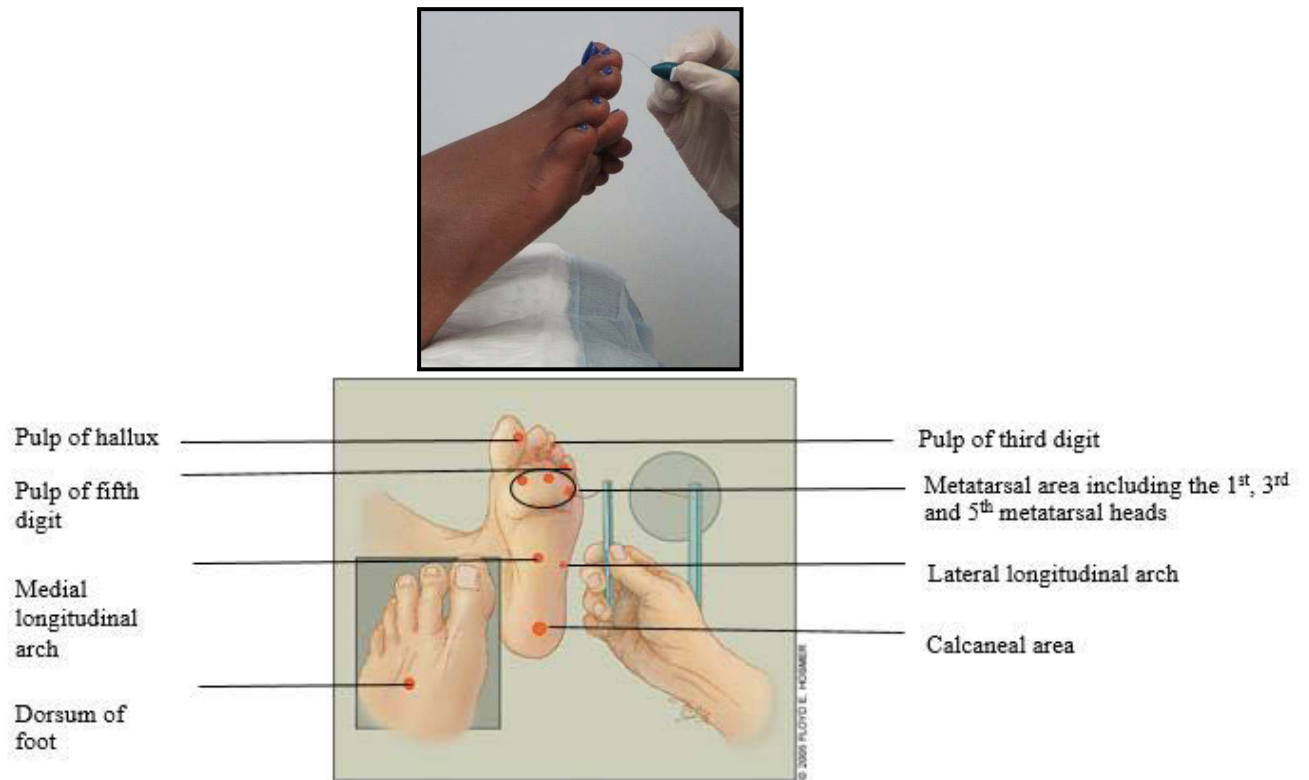


Figure 3.1: Using a 10g Monofilament (ABOVE) at appropriate sites (BELOW)

The Vibration test

A 128Hz tuning fork was used to test the ability to identify vibratory sensation. The vibrating tuning fork was placed on the skin above the bony prominences of the apex of the hallux, the medial malleolus and the first metatarsal phalangeal joint (Figure 3.2). The participant described what they felt, without any prompts, in order permit the tester to differentiate between the ability of participants to feel pressure, versus feeling the actual vibratory sensation. The number of positive findings for a total of 6 sites on the two feet was used to allocate a score for this test (Table 3.2). These sites include the 1st metatarsophalangeal joint, medial malleoli and apex of halluces.

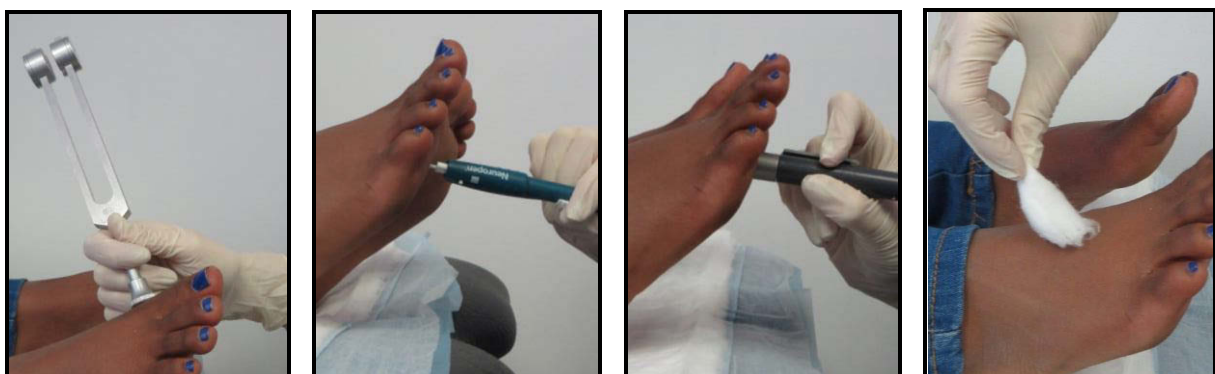


Figure 3.2: The use of a Tuning fork on a bony prominence [LEFT]; Neuropen using the sharp/ blunt application [MIDDLE-LEFT]; Tip Therm placed on the dorsum of the foot [MIDDLE-RIGHT]; testing dull sensation by stroking cotton wool on the foot [RIGHT]

Sharp Pain Pathway Test

A Neuropen which has a sharp and a blunt end (Figure 3.2) was used to assess the integrity of the sharp pain pathway. The participants were asked to keep their eyes closed and to state which of the two ends presented, felt sharper. The two ends were presented in random sequence to avoid correct guessing by the anxious-to-please participant. The number of positive findings for a total of 4 sites (dorsum and plantar of each foot) on the dorsal and plantar surfaces of the two feet was used to allocate a score for this test (Table 3.2)

Temperature Test

A Tip Therm with warm and cool ends (Figure 3.2) was placed on the dorsum and plantar surface of the foot in order to test the integrity of the temperature receptors. The participants were asked to keep their eyes closed and state whether the first or the second end placed on the dorsal and plantar surfaces of the foot, felt cooler. The number of positive findings for a total of 4 sites on any part of the dorsal and plantar surfaces of the two feet was used to allocate a score for this test (Table 3.2)

Light Touch Test

The participants were asked to keep their eyes closed. They were then asked to indicate whether they felt the light stroking of a piece of cotton wool on the left or the right foot (Figure 3.2). The number of positive findings for a total of 4 sites on any part the dorsal and plantar surfaces of the two feet was used to allocate a score for this test (Table 3.2)

Ankle and Patella Reflex Test

A patella hammer (Figure 3.3), was used to test for ankle and patella reflexes in the lower leg and foot. The participants were asked to sit sideways on the examination couch with their feet clearing the ground and to be relaxed as possible. A gentle tap on the patellar tendon with the hammer was used to determine whether it elicited a knee jerk. The intactness of the ankle reflex was tested by holding the foot in slight dorsiflexion and tapping the Achilles tendon with the hammer.



Figure 3.3: The patella hammer used to test for the presence of patella and ankle reflexes

The number of positive findings for a total score of 8 of both feet for this test. If the reflex was absent a score of 0 was given, if diminished a score of 1 was given and if the reflex was present a score of 2 was given. (Table 3.2)

Quantification of nerve function

As is shown in Table 3.2 , the values obtained for the above-mentioned six tests were added and presented as a percentage of the sum ($n=46$) in order to reach an overall value which reflected overall nerve function in the feet of the participants.

Table 3.2: Quantification of nerve function

Instrument used	Findings marked out of (per foot/ leg)	Total possible points allocated for both feet
10g Monofilament	10 sites	20
Vibration	3 sites	6
Neuroopen	2 sites	4
Tip Therm	2 sites	4
Cotton wool	2 sites	4
Patella Reflex	Present – 2	Present – 4
	Diminished – 1	Diminished – 2
	Absent – 0	Absent – 0
Ankle Reflex	Present – 2	Present – 4
	Diminished – 1	Diminished – 2
	Absent – 0	Absent – 0

3.4 The Exercise Intervention

Those participants who qualified for participation the study, were then invited to begin with the exercise-intervention. Their age-related maximum heart rate (MHR) was calculated according to the formula of Tanaka *et al.* (2001) i.e. $MHR = 208 - 0.7 \times \text{age}$, in order to facilitate the monitoring of the exercise intensity of the participants. The exercise programme consisted of low impact interval type exercise on spinning bikes which had cleated pedals that ensure that the pressure on the toes was minimized while cycling (Figure 3.4).

A 30-minute spinning class was completed three times a week for 8 weeks under the supervision of a qualified spinning instructor. This was completed by the group on “spinning bikes” in an environmental controlled exercise laboratory in the Prime Human Performance Institute at the Moses Mabida Stadium. Participants wore a Polar heart rate monitor and the PI rotated through the class to observe and randomly

record the heart rates and RPE during the class. Monitoring of the participants heart rate ensured that the heart rate was kept within a range of 60-80% of their previously calculated age-related maximum heart rate. The basic structure of a typical class session is provided in Table 3.3.

Table 3.3: Training protocol used by the active participants

PHASE	DURATION	DESCRIPTION
Warm-up	± 5 min	Relaxed cycle with low resistance against flywheel and comfortable cadence(55-65 rpm) maintaining 60% MHR and RPE of 4-6
Training phase A	± 2-3 min	Seated climb: every 20 seconds the participant increased the resistance by 1 level until reaching a level which elicited a heart rate of 80% MHR and RPE of 8-10
Training phase B	5 - 8 min	Active recovery interval: Resistance reduced until workload was equal to that of warm-up. Duration was dependant on time taken for heart rate to drop to 60% MHR and RPE to reach 4-6
Training phase C	4-10 min	Short fast spinning repetitions: 3-6 x 30sec repetitions of rapidly increasing cadence until close to maximal RPE (>8) and 80% MHR was reached with 20-60 sec recovery intervals permitting the heart rate to drop to 60% MHR and RPE to reach 4-6
Warm-Down	5-8 min	Relaxed cycle with low resistance against flywheel and comfortable cadence, maintaining 60% MHR and RPE of 4-6

rpm: revolutions per minute; MHR: maximum heart rate (Tanaka *et al.*, 2001) ; RPE: Rate of Perceived Exertion (Borg, 1982)

As the participants became fitter the level of difficulty of the spinning class was increased, by increasing the workload intensity, reducing the duration of recovery intervals and increasing the number of training repetitions. At all times, a medical doctor, specialized in Sports Medicine, and was on standby to manage any adverse event or possible complications that may have arisen.



Figure 3.4: The positioning of the foot within the pedal that has straps to ensure that pressure on the toes of the diabetic foot is avoided.

3.5 Post-Trial Assessment

The post-assessment was concluded 4 days after the exercise intervention had been completed. A post-trial questionnaire (Appendix E) was completed, the anthropometric measures taken at baseline were repeated and each participant was re-assessed by a physician. The level of peripheral sensory neuropathy was determined by the same qualified podiatrist who did the pre-training assessments (page 31). A 5ml venous blood sample was drawn into the EDTA tubes by the physician for the later assessment of blood HbA1c, plasma BDNF and adiponectin concentrations. The samples were processed as previously described on page 31 for the pre-assessment.

3.5.1 Biochemical Analyses

3.5.1.1 Plasma BDNF

Plasma BDNF was determined using a sandwich enzyme immunoassay kit (BioAssay™ ELISA Kit) (United States Biological, Swampscott, Massachusetts).

The procedure was as follows:

1. The stock Standard solution was made by adding 1.5ml of Standard/Sample diluent to the BDNF Standard. 950µl of standard/sample diluent was placed into an eppendorf tube #1 and 50 µl of the stock Standard solution added. Six further even serial dilutions were then prepared by adding assay diluent to the stock standard as shown in Figure 3.5.



Figure 3.5: Serial Dilution of Standard

2. 100uL of each standard (500, 250, 125, 62.5, 31.25, 15.63, 7.82 pg/mL) and sample diluted with sample diluent (1:2 dilution factor) were then placed into the wells of the plate that had been pre-coated with rabbit anti-human BDNF polyclonal antibody in duplicate.
3. The plate was sealed with a plate sealer and incubated at 4°C on a shaker overnight.

4. The plate washed 4 times by placing 250ul of diluted wash buffer/ diluted water using a multi-channel pipette and then blotted on clean paper towel.
5. Immediately before use the B2700-08E: Mab (Biotin) was be prepared. The biotinylated was diluted 1:1000 with B2700-08C. 100ul of the diluted Biotin was added to each well. The plate was then covered and incubated at room temperature for 2-3hours on a shaker. Step 4 was repeated.
6. Immediately before use the B2700-08F: Streptavidin was diluted 1:1000 with B2700-08C. 100ul of the diluted streptavidin (HRP) solution was added to each well. The plate was covered and incubated at room temperature on a shaker for 1 hour. Step 4 was repeated.
7. The B2700-08G: TMB/E Solution was warmed to room temperature and 100ul of TMB/E Substrate was added to each well. The plate was incubated at room temperature for 15 minutes. Then 500pg/ml standard was added creating a deep blue colour. The reaction was then stopped by adding 100ul B2700-08H. Stop Solution to each well. The blue colour changed to yellow. The plate was immediately read at 450nm on a plate reader (Spectrostar nano BMG Labtech, Germany).
8. The results were calculated with using a plate reader/ plate interface. The data was entered into the computer program curve fitting software. A fit was obtained for the standard samples with a linear regression analysis (Figure 3.7).

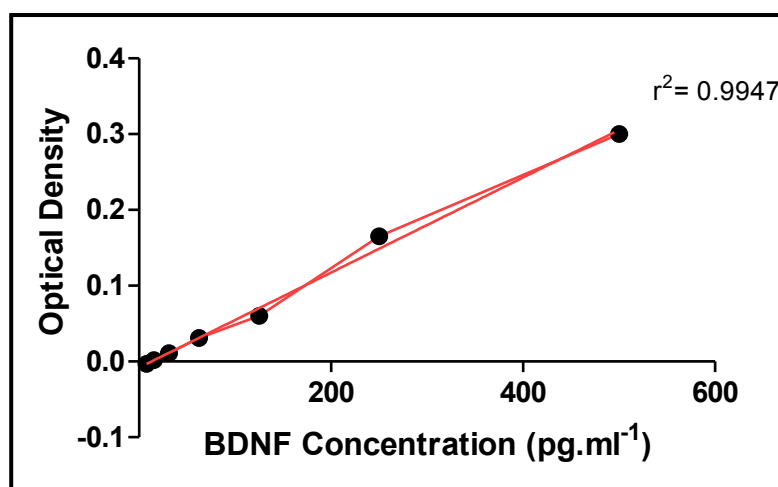


Figure 3.6: Standard Curve for BDNF assay

3.5.1.2 Plasma Adiponectin

In the ADIPOQ (Human) ELISA Kit, adiponectin concentration was determined using a sandwich enzyme immunoassay kit (Abnova Corporation, Taiwan).

The procedure for each plate was as follows:

1. 100 μ l of Standards (5-100 ng.ml⁻¹ for plasma samples), Dilution Buffer (Blank) and human plasma samples (diluted 300x with the Dilution Buffer prior to the assay) was pipetted into the appropriate wells in duplicate.

2. The plate was incubated at room temperature (25°C) for 1 hour, and placed on an orbital microplate shaker at 300 rpm.
3. The wells were washed 3 times with Wash Solution (0.35 ml per well).
4. 100 μ l of Conjugate Solution was added into each well. The plate was incubated at room temperature for 1 hour and placed on an orbital microplate shaker at 300 rpm.
5. The wells were washed again 3 times with Wash Solution (0.35 ml per well).
6. 100 μ l of Substrate Solution was added into each well and the plate was incubated for 10 minutes room temperature. The colour development was stopped by adding 100 μ l of Stop Solution.
7. The absorbance of each well was determined using a microplate reader set to 450 nm, with the reference wavelength set to 630 nm. The readings at 630nm were subtracted from the readings at 450 nm and the regression line shown in Figure 3.8 was used to calculate the concentrations.

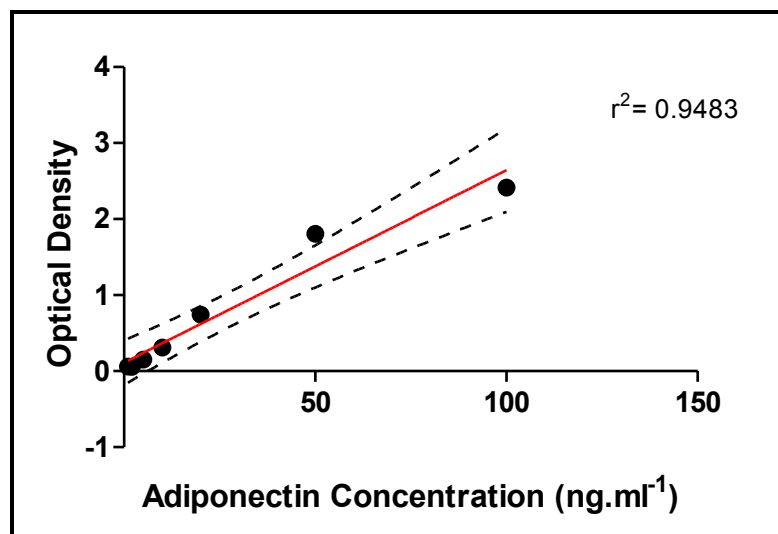


Figure 3.7: Standard Curve obtained for Adiponectin assay

3.5.2 Statistical Analyses

In the AG, data obtained were expressed as means \pm SD as well as range and analysed using pre-post paired *t* tests and an SPSS version 15.0 (SPSS Inc., Chicago, Illinois, USA) program. Alpha was set at 0.05 and the level of confidence at 95%. As directionality could not be assumed beforehand, two-tailed tests were used.

No statistical analyses were conducted on the control subjects ($n=2$). Data were presented as mean (\pm SD) only and the actual values were given for the AC.

PART TWO: ANIMAL WORK FOCUSING ON POSSIBLE MECHANISMS

3.6 Ethical Clearance for Rodent Work

This protocol was approved by the Animal Ethics Sub-committee, University of KwaZulu-Natal Ethics Committee, Pietermaritzburg Campus and ethics number 102/13/Animal was allocated to this project.

3.7 Housing, Grouping and Induction of Diabetes in Rats

3.7.1 Housing and Grouping

Six-week-old Sprague-Dawley rats with a mean body mass of 219.3 ± 7.0 g were obtained from the Biomedical Research Unit at the University of KwaZulu-Natal. They were randomly divided into 3 groups: (1) Normal control (NC), (2) Diabetic control (DBC), and (3) Diabetic exercise (DBE) (Table 3.4). The animals were housed in transparent polycarbonate cages in a temperature and humidity-controlled room with a 12 h dark-light cycle in accordance to the rules and regulations of the University of KwaZulu-Natal Experimental Animal Ethics Committee. The animals were fed rodent ripe meadow feed *ad libitum* during the 10 week experimental procedure.

3.7.2 Induction of Diabetes

NC were supplied with normal drinking water *ad libitum* whilst DBC and DBE were supplied with 10% Fructose (FR10) solution *ad libitum* for the initial 2 weeks only, and thereafter were given normal drinking for the rest of the experimental procedure.

Table 3.4: Summary of animal models

GROUP 1 <i>n</i> =7	GROUP 2 <i>n</i> =6	GROUP 3 <i>n</i> =7
Non-Diabetic Control (NC)	Diabetic Control (DBC)	Diabetic Exercise (DBE)
No Diabetes	Diabetes	Diabetes
No exercise	No exercise	Exercise

At the start of week 3, NC group was injected with a vehicle buffer only (*i.p*) whilst DBC and DBE were injected with 40mg/kg body mass (BM) of Streptozotocin (STZ) after an overnight fast.

3.8 Confirmation of Diabetes

A week following STZ injections, blood glucose levels were measured after a 3 h fast using a portable glucometer (ONETOUCH Select™) via blood collected from a tail vein. All animals with a blood glucose level >16.7 mmol.l⁻¹ were considered to be diabetic (Wilson and Islam, 2012), whereas the cut off

for pre-diabetes was $>12 \text{ mmol.l}^{-1}$. Weekly 3 h fasting blood glucose (3HFG) concentrations were obtained throughout the remainder of the experimental procedure.

3.9 Fluid and food intake and body mass

Daily food and water intake were obtained from each rat throughout the experiment, but averaged per week to note any changes observed. Mass was measured every week.

3.10 Treadmill training of rats

A motorized custom-made treadmill obtained from the Department of Physiology at the University of KwaZulu-Natal Westville campus, was used for the exercise protocol. DBE and PDBE rats were given a 2 week acclimation period which began with a speed of 16 m.min^{-1} maintained for 15min. Thereafter the speed and duration were increased reaching 20 m.min^{-1} for 55min by the end of the 2nd week after which the 5-week exercise program commenced.

3.11 Collection of blood

After a 6/7 hour fast all animals were euthanized using Halothane to the extent of the 4th stage of anaesthesia. The blood collected via cardiac puncture was equally dispensed into pre-cooled Vacutest tubes (EDTA tubes). Plasma blood samples were immediately centrifuged at 3000rpm at 3-4°C for 4min. Plasma was then pipetted off and dispensed into eppendorf tubes and dropped into liquid nitrogen. Thereafter plasma samples were placed in an ultrafreezer from the Department of Physiology until analysis was done.

3.12 Biochemical Analyses

3.12.1 Plasma BDNF

Plasma BDNF was determined using a sandwich enzyme immunoassay kit (BioAssayTM ELISA Kit) (United States Biological, Swampscott, Massachusetts) as described in 3.5.1.1. The procedure for each plate was identical to that used for human plasma samples excepting for a change in the dilution factors. Rodent plasma samples were diluted using a 1: 3 dilution factor and added to the wells in triplicate.

3.12.2 Plasma Fructosamine

Plasma fructosamine was analyzed using LabMax Plenno Automated Chemistry Analyzer. Plasma samples were transferred to eppendorf tubes and placed into the refrigerated compartment of the analyzer which presented results in mmol.l^{-1}

3.12.3 Plasma Insulin

A solid phase two site ELISA based on direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule, was used (DRG Ultrasensitive Rat Insulin ELISA kit-(EIA-2943; DRG International Incorporated).

1. After 25uL of prepared standards and undiluted plasma samples had been added to the 96 microtitration wells precoated with anti-insulin antibodies in triplicate, 100uL of peroxidase-conjugated mouse monoclonal anti-insulin (enzyme conjugate 11X; brought to RT) was added to each well.
2. The plate was incubated on an automatic shaker (150rpm) for 2 hours at room temperature to allow for the insulin in the standards and samples to react with the anti-insulin antibodies bound to the well.
3. After the reaction volume was discarded, 350uL of wash buffer solution was added to each well using a multipipette. The plates were tapped firmly several times against absorbant paper to remove excess liquid. This washing step which removed remaining unbound enzyme labelled antibody, was repeated 5 times.
4. 200 uL of TMB/E solution substrate (brought to RT) was added and the plate was incubated for 15 minutes at room temperature in order to detect the bound conjugate remaining on the walls of the wells.
5. 5 uL of stop solution (0.5M H₂SO₄) was added to each well and after the plate was placed on the shaker for 5 seconds to ensure mixing, the optical density was measured at 450 nm on a microplate spectrophotometer (Spectrostar nano BMG Labtech, Germany).
6. Using the plate reader/ plate interface, the data was entered into a computer program curve fitting software (Graphpad Prism 5.01). A good fit was obtained with a linear regression analysis (Figure 3.9).

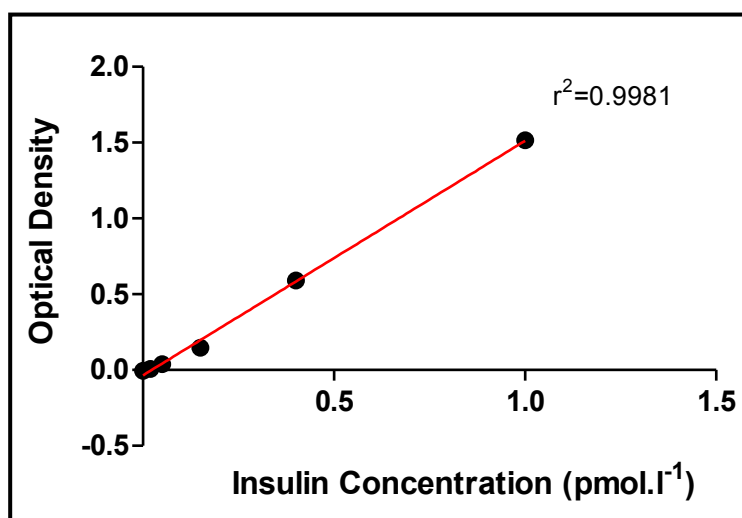


Figure 3.8: Standard curve for insulin assay

3.12.4 Plasma Adiponectin

As previously described for humans, an ADIPOQ ELISA Kit (Abnova Corporation, Taiwan) was used. The procedure for each plate was identical to that used for human plasma samples excepting for a change in the dilution factors. Rodent samples were diluted using a 1:100 dilution factor and the samples were added to the wells in triplicate.

3.13 Statistical analyses of data in rodent work

All data was presented as means (\pm SD) and analysed using Graph Pad Prism 5 software (version 5.01, 2007). For comparison of the means of weekly measurements in the three groups, a repeated measure analyses of variance (ANOVA) was used, whereas a one way ANOVA was used for comparison of the post-trial means of the NC, DBC and DBE groups. In the case of significance being detected, ANOVAs were followed by a Bonferroni *post-hoc* correction in order to establish which of the means differed.

CHAPTER FOUR

RESULTS

PART ONE: CLINICAL TRIAL ON HUMAN PARTICIPANTS

4.1 Participants

Of the 79 diabetic patients from the private podiatry practice (Anette Thompson and Associates) who met the inclusion criteria and received a phone call inviting them to participate in the study, only thirteen (ten active and 3 controls) agreed to enroll for the programme. Of these thirteen diabetic participants who were originally recruited as participants in the study, one control participant was unable to continue with the program due to health reasons, one active participant acknowledged having been active prior to the study and was permitted to continue with the study as an active control (AC) and by the end of the 7th week an active participant was unable to complete the exercise program due to work commitments. The final numbers of compliant participants therefore constituted an active group (AG) of eight, a control group (CG) of two and one active control (AC). Participants in the AG ranged from 34-47 years with a mean (\pm SD) age of 40.1 (\pm 4.6) years, the CG ranged from 45-46 years with a mean (\pm SD) of 45.5 (\pm 0.71) years and AC was 40 years of age.

4.2 Medical History and Lifestyle

From the baseline questionnaire, it was ascertained that all participants were not experiencing symptoms indicating the presence of viral or bacterial infections at the start of the exercise program. Only two of the active participants and one control participant reported experiencing symptoms of a general cold, three months prior to the study.

None of the participants had been diagnosed with conditions which have been shown to result in sensory peripheral neuropathy, but not be related to diabetes, such as heavy metal poisoning, vitamin B₁₂ deficiency, renal disease, chronic inflammatory demyelinating neuropathy, inherited neuropathies or vasculitis.

All participants in the study ($n=11$) made use of prescribed oral anti-diabetic medication which included a hypoglycemic agent which suppresses hepatic glucose production (Metformin HCL; $n=7$) and/or Glipizide ($n=4$) which acts to increase insulin sensitivity by binding to sulphonylurea receptors on the surface of the pancreatic β -cells. They undertook not to change the brand of medication, dosage and timing of ingestion as prescribed by their physicians prior to the start of the study. Of the AG ($n=8$), one

reported being previously diagnosed with hypertension and using anti-hypertensive medication. Another two participants were previously diagnosed with hypothyroidism and treated with Eltroxin. None were smokers. Three (37.5%) reported to drink an average of six tots of spirits and/or six beers a week. The caffeine intake included six participants (75%) drinking between 1-3 cups of coffee per day and four (50%) participants consuming 1-3 cups of tea a day. All, excepting AC, confirmed not to have previously taken part in scheduled exercise for more than one hour per week. Three participants consumed a high carbohydrate and low fat diet, four a prudent diet (low refined carbohydrates, transfat and saturated fat content) and one was a lacto-vegetarian.

The two control participants were both diagnosed and managed for hypertension prior to the study. One was a smoker, smoking up to five cigarettes a day and one control participant only drank during social occasions. One participant reported drinking 1-3 cups of coffee per day and two participants drank 1-3 cups of tea a day. One consumed a prudent diet and the other consumed a high carbohydrate- low fat diet, but both were cautious regarding their intake of refined carbohydrates/ high sugar containing foods.

In terms of their post-trial questionnaire, participants confirmed that they had maintained a similar diet throughout the intervention. The AG confirmed they had complied with exercise requirements of the program, whereas the control participants had refrained from participating in any regular structured exercise before or during the program and AC had maintained his usual thrice weekly endurance exercise programme.

Of the eight neurological symptoms experienced in the feet, the AG reported 50% improvement, however the CG reported worsening in 7 of the 8 neurological symptoms. The AC reported no change (Table 4.1).

Table 4.1: Reported number of participants expressed as percentage incidence of pre and post study neurological symptoms experienced in the feet in the active ($n=8$), control participants ($n=2$) and active control ($n=1$)

SYMPTOMS	AG		CG		AC	
	PRE - POST		PRE - POST		PRE - POST	
	% (N)		% (N)		% (N)	
BURNING PAIN	25 ($n=2$)	37.5 ($n=3$)	100 ($n=2$)	50 ($n=1$)	0 ($n=1$)	0 ($n=1$)
SENSITIVE TO TOUCH	25 ($n=2$)	12.5 ($n=1$)	50 ($n=1$)	0 ($n=0$)	0 ($n=1$)	0 ($n=1$)
PINS AND NEEDLES	25 ($n=2$)	12.5 ($n=1$)	50 ($n=1$)	0 ($n=0$)	0 ($n=1$)	0 ($n=1$)
ABLE TO DISTINGUISH BETWEEN HOT AND COLD WATER ON FEET	25 ($n=2$)	37.5 ($n=3$)	50 ($n=1$)	0 ($n=0$)	0 ($n=1$)	0 ($n=1$)
NUMBNESS	12.5 ($n=1$)	12.5 ($n=1$)	50 ($n=1$)	0 ($n=0$)	0 ($n=1$)	0 ($n=1$)
SYMPTOMS WORSE AT NIGHT	12.5 ($n=1$)	25 ($n=2$)	50 ($n=1$)	0 ($n=0$)	0 ($n=1$)	0 ($n=1$)
ABLE TO SENSE FEET WHEN WALKING	12.5 ($n=1$)	25 ($n=2$)	50 ($n=1$)	50 ($n=1$)	0 ($n=1$)	0 ($n=1$)
LEGS HURT WHEN WALKING	37.5 ($n=3$)	37.5 ($n=3$)	50 ($n=1$)	0 ($n=0$)	0 ($n=1$)	0 ($n=1$)

4.2.1 General Medical Assessment

Results of the medical examination and assessment of their vital signs, conducted by a medical physician at the start of the baseline assessment, indicated that participants did not present with symptoms which excluded them from the program.

One of the active participants, presented with angular stomatitis around the mouth, one with anhidrotic feet and slight L4/L5 facet joint pain. A control participant had mentioned the possible diagnosis of Morton's neuroma and another control participant reported to have paraesthesia in both feet and the doctor identified fine crepitation during breathing. All participants ($n=10$) were permitted to continue with the study.

4.2.2 Physical Characteristics

The physical characteristics of the active and control participants at baseline (pre-trial) and post-trial are presented in Table 4.2 and 4.3, respectively. Although pre-exercise intervention mean (\pm SD) body fat percentage and BMI dropped marginally for the active participants following the intervention, these reductions in the active participants were not statistically significant ($p>0.05$). The mean (\pm SD) resting heart rate measured in the active participants prior to the exercise intervention was 79.0 bpm \pm 7.9 and dropped significantly to 73.8 bpm \pm 8.0 post exercise ($p=0.012$). The mean (\pm SD) resting blood pressure

measured in the active participants prior to the exercise intervention was 136/94mmHg ($\pm 10.2/13.4$) which reduced to post exercise intervention to 126/91mmHg ($\pm 7.4/13.4$) and showed a significant change in the systolic resting blood pressure (SBP; $p=0.043$).

Table 4.2: Physical Characteristics of the Active Group (AG) ($n=8$)

Characteristics	Mean (\pm SD)	Range	Mean (\pm SD)	Range
	PRE	PRE	POST	POST
Mass (kg)	91.5 \pm 22.5	61.4-130.4	91.4 \pm 22.6	62.5-131.7
Stature (cm)	172 \pm 0.1	155-190	172 \pm 0.1	155-188
Percentage Body Fat*	34.2 \pm 5.7	27.5-43.0	33.4 \pm 4.6	26.0-38.6
Body Mass Index (kg.m ⁻²)	30.9 \pm 6.4	24.1-44.2	30.0 \pm 4.8	23.9-38.6
Resting Heart Rate (b.min ⁻¹)	79.0 \pm 7.9	68-90	73.8 \pm 8.0**	62-84
Resting SBP (mm Hg)	136.3 \pm 10.2	120-150	126.3 \pm 7.4**	115-140
Resting DBP (mm Hg)	94.4 \pm 13.4	82-125	91.1 \pm 13.4	80-122
Waist Circumference (cm)	101.5 \pm 17.2	78.0-136.7	100.4 \pm 15.2	77.8-130.5
Hip Circumference (cm)	107.0 \pm 10.5	93.0-126.8	105.8 \pm 13.7	89.0-136.0

SBP: Systolic Blood Pressure DBP Diastolic Blood Pressure * Derived from the sum of triceps, biceps, suprailiac and subscapular skinfold thickness ** $p<0.05$, versus pre-trial, students t test

Table 4.3: Physical Characteristics of Control Group (CG) ($n=2$) and Active Control (AC) ($n=1$)

Characteristics	CG				AC	
	Mean (\pm SD)	Range	Mean (\pm SD)	Range	PRE	POST
	PRE	PRE	POST	POST		
Mass (kg)	86.3 \pm 28.8	65.9-106.6	85.6 \pm 30.0	64.4-106.8	94.5	91.3
Stature (cm)	165 \pm 0.01	164-165	165 \pm 0.01	164-165	1.75	1.75
% Body Fat	35.2 \pm 12.5	26.4-44.0	35.0 \pm 11.4	26.9-43.0	27.5	26
BMI (kg.m ⁻²)	31.9 \pm 10.9	24.2-39.7	31.7 \pm 11.4	23.7-39.7	30.78	29.83
Heart Rate(b/min ⁻¹)	82 \pm 2.8	80-84	80.5 \pm 5.0	77-84	68	62
SBP (mm Hg)	136 \pm 5.7	132-140	140 \pm 0.0	140-140	132	130
DBP (mm Hg)	94 \pm 19.8	80-108	95 \pm 14.1	85-105	92	90
Waist Circumference (cm)	92 \pm 9.90	85-99	92.75 \pm 12.4	84.0-101.5	96	93
Hip Circumference (cm)	111.6 \pm 21.9	96.1-127	111.5 \pm 24.8	94-129	106	104

BMI: Body Mass Index SBP: Systolic Blood Pressure DBP Diastolic Blood Pressure
* Derived from the sum of triceps, biceps, suprailiac and subscapular skinfold thickness

As is evident in Table 4.3, there were no clinically significant changes in the physical characteristics of the AC or the CG over this time period.

4.3 Hematological Characteristics

The results of the hematological analysis performed on the venous blood samples taken at the baseline and post-trial assessments are presented in Table 4.4 below and a graphical representation of BDNF concentration changes is given in Figure 4.1.

Table 4.4: Hematological Analyses of Blood of the Active Group (AG) ($n=8$), Control Group (CG) ($n=2$) and Active Control (AC) ($n=1$).

Characteristics	Mean (\pm SD) PRE	Mean (\pm SD) POST	<i>p</i> Value
<u>Active Group ($n=8$)</u>			
HbA1C (%)	7.45 (\pm 1.34)	7.66 (\pm 1.58)	0.32
BDNF (pg.ml ⁻¹)	13.65 (\pm 3.34)	16.37 (\pm 4.46)	0.12
Adiponectin (μ g.ml ⁻¹)	1.37 (\pm 1.32)	1.24 (\pm 1.26)	0.74
<u>Sedentary Control Group ($n=2$)</u>			
HbA1C (%)	6.75 (\pm 1.20)	6.65 (\pm 1.20)	-
BDNF (pg.ml ⁻¹)	20.38 (\pm 3.38)	18.93 (\pm 1.92)	-
Adiponectin (μ g.ml ⁻¹)	1.73 (\pm 2.30)	1.61 (\pm 1.57)	-
<u>Active Control Group ($n=1$)</u>			
HbA1C (%)	7.00	7.70	-
BDNF (pg.ml ⁻¹)	10.40	6.73	-
Adiponectin (μ g.ml ⁻¹)	2.15	2.38	-

$p>0.05$, versus pre-trial, Students *t* test; - no comparative statistical analysis due to small sample size

Of the 8 participants in the AG, 7 (87.5%) had a pre-trial HbA1c reading of higher than 6.5% with the values ranging from 5.3 to 9.3%. As shown in Table 4.4 and Figure 4.1, the mean (\pm SD) HbA1c measured in the active participants prior to exercise intervention was 7.45% (\pm 1.34) and increased to 7.66% (\pm 1.58) post exercise intervention, which was not statistically significant ($p> 0.05$).

In terms of the change in mean (\pm SD) of plasma BDNF concentrations (Table 4.4 and Figure 4.1) the individual plasma BDNF concentrations increased in 4 of the 8 active subjects (50%). However the change in mean (\pm SD) as a result of the 8-week interval exercise intervention, did not reach statistical significance ($p=0.116$) and ranged from -6.06 to 3.92 pg.ml⁻¹.

There was no statistical change in the AG for plasma adiponectin concentrations ($p=0.74$) which ranged from 0.10-3.20 μ g.ml⁻¹. As there were only two participants in the CG, statistical significance of pre-post changes was not determined (Table 4.4 and Figure 4.1). The same applied to AC in whom HbA1c change and small reduction in plasma BDNF and adiponectin were detected.

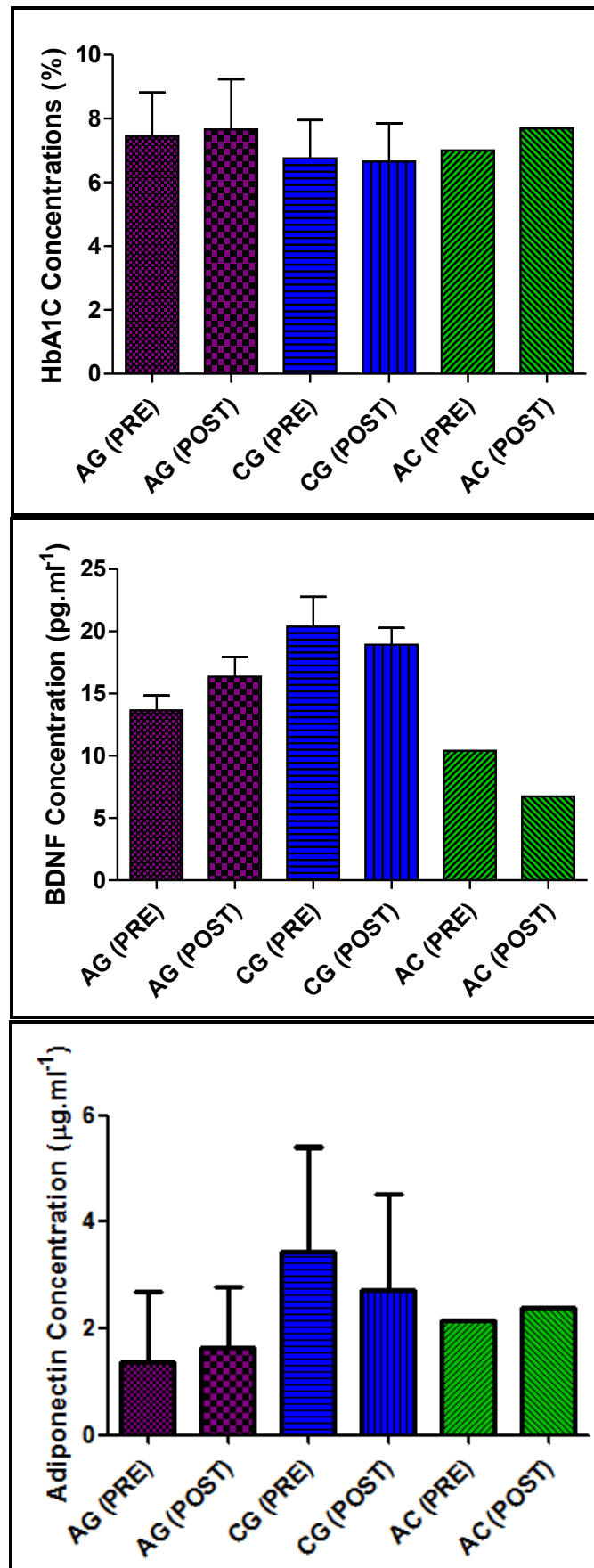


Figure 4.1: Mean \pm SD HbA1c (%) [TOP], plasma BDNF (pg.ml⁻¹) [MIDDLE] and adiponectin (μ g.ml⁻¹) [BOTTOM] concentrations in the active group (AG), control group (CG) and active control (AC) after the 8-week exercise program.

4.4 Diabetic Foot Assessment

Pedal pulses (i.e. Dorsalis Pedis Pulse, Posterior Tibial Pulse) were present in all participants ($n=11$) and described as strong before and after the intervention in 91% ($n=10$). However one participant in the AG had a weaker Posterior Tibial Pulse that had been identified at the onset of the study and showed no change following the exercise intervention.

The results of the quantitative objective assessment of sensory perception in the feet of the active participants are described in Table 4.5. Although there was not a significant increase in the total value representing nervous function which increased from 64.05% (± 33.56) to 82.61 (± 12.17), statistically significant increases were only observed in patellar ($p=0.0166$) and ankle reflexes ($p=0.0016$) of the AG. As is shown in Table 4.6, improvement was also noted in the AC.

Table 4.5: Diabetic Foot Assessment of Active group (AG) ($n=8$). Results expressed as means (\pm SD)

Characteristics	Mean (\pm SD)	Range	Mean (\pm SD)	Range
	PRE	PRE	POST	POST
Pressure Perception (%)	99.4 \pm 1.8	95-100	95.0 \pm 14.1	60-100
Vibration Perception (%)	89.6 \pm 19.7	50-100	89.5 \pm 23.6	33-100
Sharp/ Blunt Discrimination (%)	65.6 \pm 39.9	0-100	68.8 \pm 22.2	50-100
Temperature Perception (%)	59.4 \pm 32.6	0-100	78.1 \pm 20.9	50-100
Dull/ Light Touch (%)	93.8 \pm 11.6	75-100	100.0 \pm 0.0	100-100
Patella Reflex (%)	28.1 \pm 36.4	0-100	71.9 \pm 36.4*	0-100
Ankle Reflex (%)	12.5 \pm 23.1	0-50	75.0 \pm 26.7*	50-100
Total % nerve function	64.1\pm33.6	12.5-99.4	82.6\pm12.2	0-100

*Students' Paired t test ($p<0.05$)

Table 4.6: Diabetic Foot Assessment of Control Group (CG) ($n=2$) and Active Control (AC) ($n=1$)

Characteristics	CG		CG		AC	
	Mean (\pm SD)	Range	Mean (\pm SD)	Range	PRE	POST
	PRE	PRE	POST	POST		
Pressure Perception (%)	100 \pm 0.0	20-20	100 \pm 0.0	100-100	100	100
Vibration Perception (%)	100 \pm 0.0	100-100	100 \pm 0.0	100-100	100	100
Sharp/ Blunt Perception (%)	62.5 \pm 17.7	50-75	62.5 \pm 17.7	50-75	50	100
Temperature Perception (%)	75 \pm 35.4	50-100	87.5 \pm 17.7	75-100	75	75
Dull/ Light Touch (%)	100 \pm 0.0	100-100	100 \pm 0.0	100-100	100	100
Patella Reflex (%)	50 \pm 70.7	0-100	50 \pm 70.7	0-100	25	75
Ankle Reflex (%)	25 \pm 35.4	0-50	75 \pm 35.4	50-100	25	50
Total % nerve function	73.2\pm29.3	50-100	82.1\pm20.2	50-100	80.4	91.3

PART TWO: ANIMAL WORK FOCUSING ON POSSIBLE MECHANISMS

4.5 Compliance with Study Protocol

Using the previously–reported cut-off of a glucose concentration $>16.7\text{mmol.l}^{-1}$ ($>300\text{mg.dl}^{-1}$; Islam and Du Toit, 2009) as criterion for the existence of diabetes, 6 of the 7 rats were classified as diabetic. In the 7th rat 3h FBG concentration only reached 12.3mmol.l^{-1} . As this animal did therefore not meet the requirements of a diabetic model, it was decided that it will continue with the exercise program, but will be regarded as a Pre-Diabetic Exercise (PDBE) rat on which a case study would be done.

During the 8th week of the investigation, one rat in the DBC group sustained an injury during handling and had to be put down. All remaining animals (as shown in Table 4.7), completed the full 5-week exercise program and the full duration each exercise session.

Table 4.7: Summary of animal models after inducing diabetes using FR-10 STZ induction

GROUP 1		GROUP 2	GROUP 3	CASE STUDY
$n=7$		$n=6$	$n=6$	$n=1$
Non-Diabetic Control (NC)	Control	Diabetic Control (DBC)	Diabetic Exercise (DBE)	Pre-Diabetic Exercise (PDBE)
No Diabetes		Diabetes	Diabetes	Pre-Diabetes
No exercise		No exercise	Exercise	Exercise

As shown in Figure 4.2, the weekly water intake and blood glucose was significantly higher in DBE and DBC groups than in the NC group ($p > 0.05$) with that of PDBE rat lying well above the mean of NC group too. While the increase in body mass was significantly less in DBE group than both DBC and NC groups, that of the PDBE rat was less affected by the STZ injection than those in DBE and DBC groups. The 3hFBG was considerably lower in PDBE rat than in DBC and DBE rats with values dropping close to those in the NC group during the exercise program.

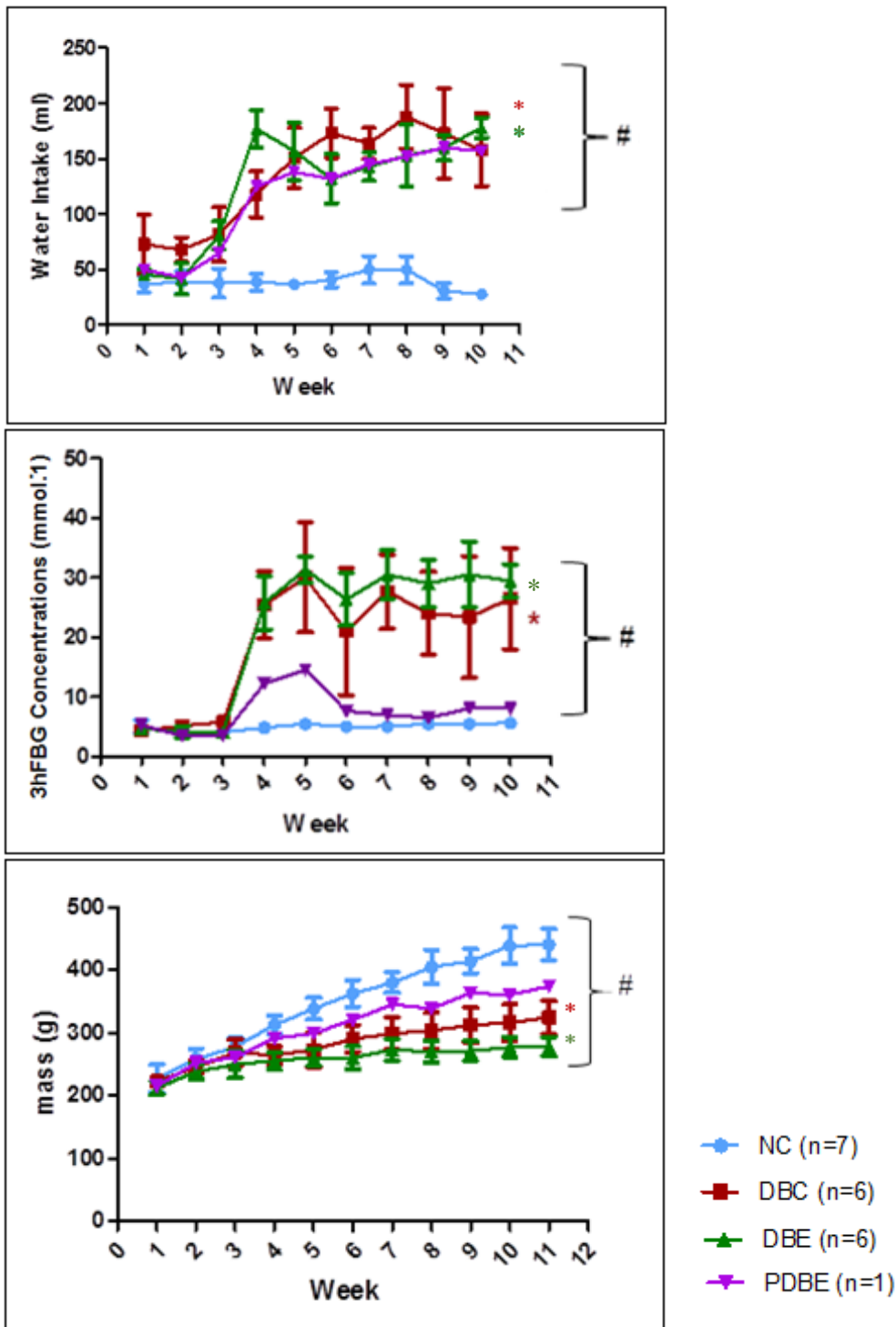


Figure 4.2: Mean weekly water intake (ml) [TOP] and 3 hour fasting blood glucose (3hFBG) concentrations (mmol.l⁻¹) [MIDDLE] and body mass (g) [BOTTOM] for Normal Control (NC), Diabetic Control (DBC) and Diabetic Exercise (DBE) groups and Pre-diabetic Exercise (PDBE) rat calculated weekly over the 10 week investigation. Data is represented with mean \pm SD per group # $p < 0.0001$, Repeated measures ANOVA, * $p < 0.05$ vs NC, post-hoc Bonferroni's Multiple Comparison Test.

The plasma fructosamine, BDNF and adiponectin concentration in the NC, DBC and DBE groups and as well as PDBE rat, are shown in Figure 4.3. The fructosamine was significantly higher in DBC and DBE groups than in NC group and lowest in PDBE rat, while adiponectin was highest in the PDBE rat.

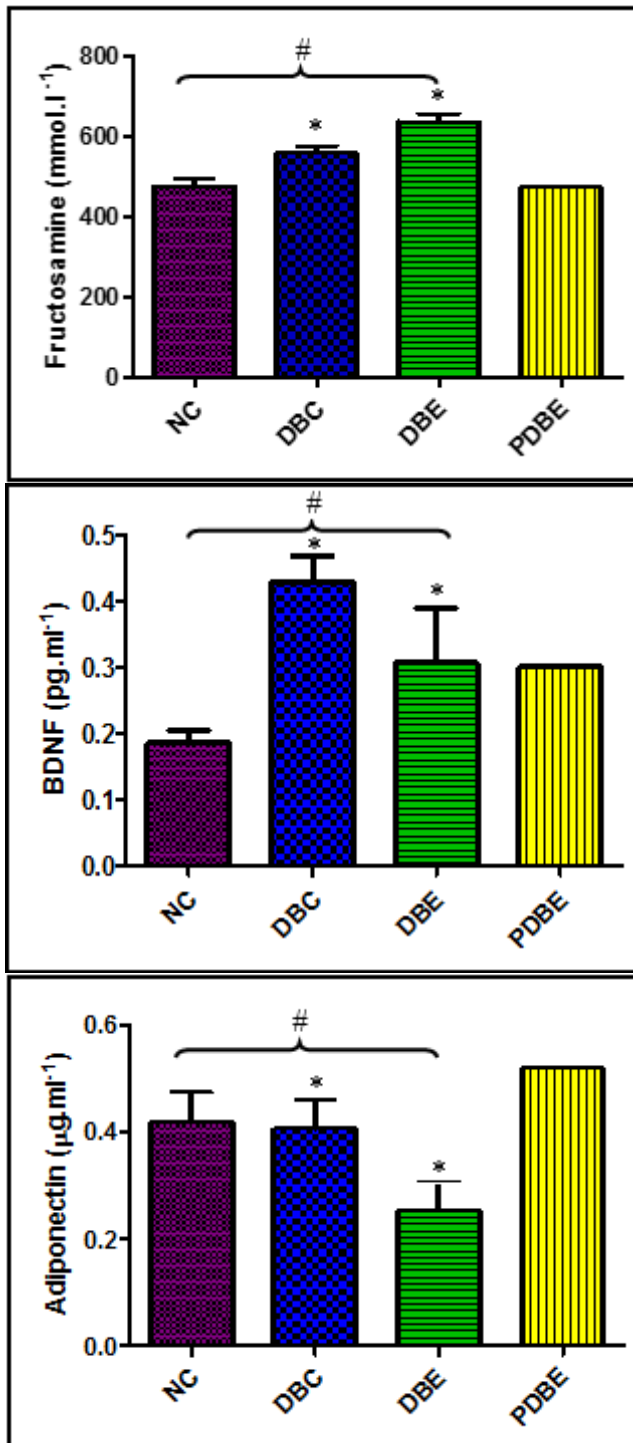


Figure 4.3: Mean \pm SD plasma fructosamine (mmol.l⁻¹) [TOP], BDNF (pg.ml⁻¹) [MIDDLE] and adiponectin concentrations (μ g.ml⁻¹) [BOTTOM] in the Normal Control (NC), Diabetic Control (DBC), Diabetic Exercise (DBE) groups and Pre-Diabetic Exercise (PDBE) rat after the 5-week exercise program. Data is represented with mean \pm SD per group # $p < 0.0001$, Repeated measures ANOVA, * $p < 0.05$ vs NC, *post-hoc* Bonferroni's Multiple Comparison Test.

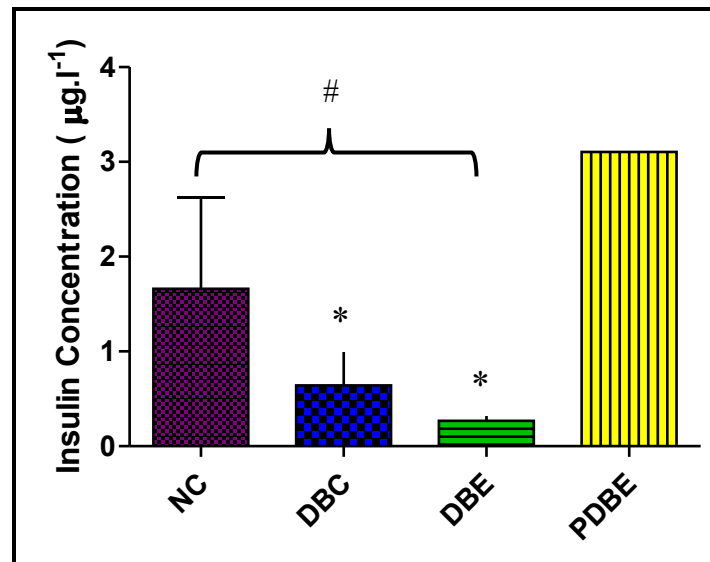


Figure 4.4: Mean \pm SD plasma insulin concentrations ($\mu\text{g.l}^{-1}$) in the Normal Control (NC), Diabetic Control (DBC) and Diabetic Exercise (DBE) groups and Pre-diabetic Exercise (PDBE) rat after the 5-week exercise program. Data is represented with mean \pm SD per group # $p < 0.0001$, Repeated measures ANOVA, * $p < 0.05$ vs NC, *post-hoc* Bonferroni's Multiple Comparison Test.

There was a significant difference in plasma insulin concentrations between the NC, DBC and DBE groups ($p = 0.0347$) (Figure 4.4). The DBE and DBC groups had the lowest insulin concentrations, the pre-diabetic rat presented with an insulin concentration higher than in NC group. The mean concentration of both DBC and DBE were significantly lower ($p = 0.0276$) than that of NC group.

Mean \pm SD plasma adiponectin concentrations ($\mu\text{g.l}^{-1}$) revealed a significant difference between the groups ($p = 0.0111$) and a positive correlation with insulin concentrations ($r = 0.7473$; $p < 0.05$).

4.6 Conclusion

While the exercise program in the human clinical study showed a significant decrease in mean heart rate and blood pressure ($p > 0.05$), the only parameters that were significantly affected by the exercise training were the ankle and knee reflexes ($p > 0.05$).

In the rodent work, the PDBE rat presented with a considerably higher post-exercise-training blood insulin and lower blood glucose concentrations than in the DBC and DBE groups. Circulating adiponectin concentrations were also considerably higher in the PDBE rat than in DBC and DBE groups following the exercise intervention.

CHAPTER FIVE

DISCUSSION

PART ONE: CLINICAL TRIAL ON HUMAN PARTICIPANTS

5.1 General Observations and Challenges

Although T2DM is prevalent in today's western society and a total of 79 potential participants, who possessed DPN and met all other inclusion criteria, were approached and invited to participate in this study, the small eventual sample size must be acknowledged as a major short coming of this pilot work. Several factors may have accounted for the difficulty in obtaining a large enrollment for the study and compliance from the participants.

Firstly, as detailed on page 20 and by the ADA (2012), the presence of symptoms of DPN implies that the diabetic condition has persisted for some time and become a chronic condition. Although participants were required to continue with their routinely prescribed medication for the control of the blood glucose concentration, the HbA1c levels which exceeded 6.5% in 86% of AG ($n=7$), do indicate that either the dosage of the medication was not sufficient to maintain normal concentrations in the AG, or a lack of adherence to the prescription. Furthermore, the physiological response to chronically elevated blood glucose concentrations resulting in DPN (Figure 2.3) would suggest that pharmacological glucose regulation has been inadequate over a long period leading to probable pancreatic cell dysfunction (ADA, 2012) which may have involved both failure of the β cells to produce sufficient insulin to regulate glucose metabolism as well as possible further glucose production due to α cell dysfunction and excess production of glucagon (ADA, 2012). Although manageable, this condition is widely accepted as being irreversible and often results in a negative state of mind and a set of behavioral habits becoming ingrained in patients possessing symptoms of DPN (ADA, 2005; Desphande *et al.*, 2008). This may therefore have contributed to lack of interest in participating in the study in so large a percentage of the patients originally approached ($n=79$).

The study was also restricted to participants who had been referred to a podiatry practice due to complaints of symptoms of DPN and were in the age range of 34-47 years. A commitment to a regular exercise program did not appear to be a priority to the patients at this stage of their lives as this age group are generally at the peak of their careers. Financial demands and family commitments are also commonly a first priority in this age group and despite the availability of exercise facilities, flexible training times and the prospect of an improvement of their general health status, considerable difficulty was experienced

by the researcher when attempting to persuade the diabetic patients at a podiatry practice to take part in this clinical trial.

T2DM is also widely accepted to develop in response to a sedentary lifestyle combined with a high carbohydrate and fat diet, high levels of stress and pressurized time-lines (Diabetes.co.uk, 2014). The dietary intake was not specifically controlled, but in order to ensure that diet did not have a confounding effect, each trialist was expected to undertake not to change his/her diet and to keep this consistent throughout the eight weeks. Hence this study investigated whether exercise alone would have a beneficial effect on glucose regulation and symptoms of DPN. As the dietary intakes were not carefully monitored by means of regular dietary record keeping, this aspect must however be acknowledged as a further potential limitation of the study.

A variance in stress levels could also not be controlled in the human participants other than via a questionnaire in which participants described that in terms of lifestyle, sleep pattern and stress levels, in which no significant change occurred in both groups. Although caffeine intake did not appear to be excessive in any of these participants, the quantity of beer some consumed was a concern due to its high carbohydrate and alcohol content (Diabetes.co.uk, 2014). From the information obtained from pre- and post-questionnaires and interaction with the participants during the course of the training programme, it can however be assumed that this potentially negative variable did not change over the 8-week period of the study.

A strength of the program was that the eventual AG of 8 compliant patients were given individual attention, their training routines were carefully monitored, and they received continual feedback and encouragement to stay in the program and not change their lifestyle and/or dietary intake while on the programme. Although the exercise programme may have been more effective in eliciting greater physiological changes had the exercise intervention lasted longer than 8 weeks and permitted greater increases in training intensity as the cardiovascular endurance of the participants in AG improved, this was unfortunately not possible within the scope of this pilot work for a Masters by course work.

Another limitation of this study was the failure to obtain a matched CG of similar size to the AG. Due to lifestyle and stress related factors specified above, which were also applicable to the CG, it was very difficult to obtain compliance from non-active participants without additional financial support for the study which would have enabled the remuneration of participants for their time and effort. Hence, a statistical comparison between pre-post data within the CG and between the control and AG was not possible. Data are reported as mean \pm SD, but no comparative inter-group statistical analyses were undertaken. This study will therefore be classified as an uncontrolled study which only examined the pre-post trial differences in the AG.

5.2 The participants

The active and control participants were selected strictly according to the inclusion criteria in order to minimise confounding factors and to maintain internal validity. These included the age-range, pharmacological control of blood glucose concentration, absence of infection or the diagnosis with conditions which have been shown to result in sensory peripheral neuropathy, but are not related to diabetes (e. g. heavy metal poisoning, vitamin B12 deficiency, renal disease, chronic inflammatory demyelinating neuropathy, inherited neuropathies or vasculitis). All participants possessing chronic lifestyle associated disorders or co-morbidities of diabetes, provided evidence of receiving appropriate medication and did not change the dosage shortly before and during the duration of the study.

5.3 The results of the exercise intervention

Although the exercise intervention included relatively high intensity interval type training and does appear to have been of optimal duration or intensity, there was a statistically significant improvement in pre-trial resting HR and BP ($p < 0.05$). Mass, waist and hip circumference did, however, not change significantly within the AG ($p > 0.05$), but only showed improvement in the AC. In addition, mean body fat percentage (33.4 ± 4.6) and BMI of the active participants (30.0 ± 4.8) which was well above the healthy range (WHO, 2000) in both women and men failed to drop significantly ($p > 0.05$) in both active and control participants. The likely explanation for this is that it has been shown that weight loss is the result of combined dietary and exercise interventions. As Macfarlan and Thomas (2009) conclude, “although it is clear that an appropriate exercise regimen produces a clinically significant benefits in weight loss when used in conjunction with caloric restriction, the dominant contributor is typically likely to be dietary intake (80% of the weight loss), with enhanced physical activity contributing to the remainder (20%).”

Most importantly, the exercise intervention which consisted primarily of interval training, resulted in a significant drop in mean resting heart rate ($p = 0.012$) from 79.6 to 73.8 bpm in the AG, although still elevated above the accepted normal readings of 60-70bpm. As reading above 80bpm may indicate a weak myocardium and therefore exercise would be indicated, none of the participants presented with tachycardia (a resting heart rate above 100bpm) which should be investigated for underlying conditions (Thomas and Draper, 2011).

The mean resting systolic blood pressure measured in the active participants prior to the exercise intervention also showed a significant mean reduction post exercise intervention ($p = 0.043$). No change was seen in the resting blood pressure of the CG who remained in a hypertensive state ($> 140/90$ mm Hg).

All participants were therefore referred back to their medical practitioners with detailed feedback after the trial to facilitate the making of adjustments to their prescribed medication, if needed, after the trial.

5.4 Hematological Characteristics

The mean HbA1c measured in the active participants (AG) prior to exercise intervention ($7.25 \pm 1.34\%$) did not change ($p > 0.05$) remaining above the normal HbA1c range of non-diabetic individuals (3.9 - 5.5%) and above the threshold used to be diagnosed with diabetes ($>6.5\%$). Only 3 of the 8 participants (37.5%) and one of the participants in the CG adhered to the recommendation of ADA (2005) that once diagnosed and treated for diabetes, the HbA1c reading must remain close 6.5%. Interestingly, in 62.5% ($n=5$) of the participants, an HbA1c reading close to 7% was recorded. This coincides with a blood glucose value greater than 8.6 mmol.l^{-1} and has been associated with damage to organs due to hyperglycaemia (Diabetes Outreach Country Health, 2011),

Although, as explained by Huang and Czech (2007) and Earnest *et al.*, (2008), an acute bout of endurance exercise training has been shown to provide a strong stimulus for mitochondrial biogenesis that increases GLUT4 concentration, thus facilitating muscle glucose transport (Huang and Czech, 2007; Earnest *et al.*, 2008) and it is proposed that GLUT4 protein expression increases with regular endurance exercise and that impairment in GLUT4 transport/ translocation may be the cause of insulin resistance (Earnest, 2008), the current 8-week exercise intervention alone appeared not to be intense and long enough to support a beneficial effect on glucose regulation.

Furthermore, the current study also failed to support the contention that a graded endurance exercise programme and improved endurance training status attenuates the glucagon and epinephrine release at rest and a given exercise load (Galbo *et al.*, 1975), hence reducing HbA1c in the long term.

The possible explanation for the failure of glucose regulation, as represented by HbA1c which represents the mean blood glucose concentration over a 3month period, to change following the 8-week exercise intervention, is two-fold:

Firstly it is most probably related to the chronic nature of the diabetes and the permanent pancreatic dysfunction that is most likely to have occurred in these participants who had already developed DPN. Unfortunately a limitation of this work is that it was not possible to obtain fasting blood samples from the participants which would have been required to measure plasma insulin concentration and hence permitted calculation of HOMAIR (Bonora *et al.*, 2002). The glucose-insulin ratio was however addressed in the follow-up rodent work completed in part two of this thesis.

Although Raz *et al.* (1994) emphasize that exercise should be an integral part of management in T2DM patients, the exercise intensity of the interval training should range between 60-80% of age-related HR

max (Kelley and Kelley, 2007). As the members of the AG had a mean (\pm SD) BMI of 30.9 ± 6.45 with 50% falling into the obese category and entered the programme with a prior history of sedentary lifestyle, the intensity of the exercise program was relatively low. Hence it can be concluded that in the convenience sample of T2DM patients who presented with DPN, the exercise training intervention of 8-weeks in duration was not sufficiently intense to result in an improvement in HbA1c and hence glucose control. Although it does therefore not confirm the findings of Cauza *et al.* (2005), Sigal *et al.* (2007), Ng *et al.* (2010) and Laaksonen *et al.* (2005), it does support the findings of Knowler *et al.* (2002), Pan *et al.* (1997), Mc Auley *et al.* (2002) and Umpierre *et al.* (2011) that exercise is only beneficial if combined with dietary recommendations.

A second possible reason for no change in HbA1c values in the current study is aligned with the findings of Burns *et al.*, (2007). He explains that there are certain DM susceptibility genotypes that might predispose individuals to respond or not to respond to exercise. Unfortunately it was beyond the scope of this study to include an investigation of the genetic profile of the AG.

Although the failure of the plasma BDNF concentrations which rose in only 50% ($n=4$) of the AG, to increase significantly ($p= 0.12$; Table 4.4) following the 8-week intervention, may in part be attributed to the low statistical power of the study and high standard deviations within the group, it does also appear to support previous findings of Schulz *et al.* (2004a), Levinger *et al.* (2008) and Goekint *et al.* (2010). The data therefore fails to support the findings of Cuppinin (2007) who reported an exercise-induced elevation blood BDNF concentrations attributed to the additional release of BDNF by contracting skeletal muscle. As this result was possibly also due to the inability to implement a sufficiently intense exercise programme, further research is needed to investigate the possible effects of chronic exercise on BDNF concentrations when the exercise training intervention is longer than 8-weeks in duration and a higher training intensity can be achieved as the participants improve their level of fitness.

HbA1c as mentioned, did not decrease in the AG. As HbA1c has been shown to be inversely proportional to plasma BDNF (Nakawaga *et al.*, 2000), this supports the observation that plasma BDNF did not rise significantly ($p>0.05$). As previously mentioned, greater intensity, frequency and duration of the exercise program may have however produced a greater enhancement of these BDNF concentrations which may, in turn, have positively affected HbA1c in the AC.

Plasma adiponectin concentrations also demonstrated no significant change in AG. These findings correlate with no change in the HbA1c concentrations as these parameters have also been shown to be inversely proportional to one another (Schulz *et al.*, 2004b). The findings of this study thus confirm the findings of the two non-randomized controlled trials which showed no change in circulating adiponectin (Yokoyama *et al.*, 2005; Kondo *et al.*, 2009) following an exercise intervention alone.

5.5 Diabetic Foot Assessment

The most important finding focuses on one of the primary objectives of the study, which was to determine the effects of an interval exercise program on DPN in T2DM patients, was that sensory peripheral neuropathy measured in the active participants showed significant improvement in knee and ankle reflexes as compared to that of prior to the 8-week exercise intervention. Interestingly, the two members of the CG demonstrated further loss of mean nerve function over the 8-week period.

This improvement in knee and ankle reflexes however may not be attributed to glucose control as these parameters showed no change and may lend support to the hypothesis of Paulsen *et al.*(2012) for improvement in reflex strength following exercise training. These researchers propose that exercise results in the increase in epinephrine concentrations which interacts with the β 2-adrenergic receptors, resulting in a sympathetic response. This response causes an increase in contractile activity and metabolic enzyme activity with resultant increased reflex strength (Paulsen *et al.*, 2012). Further work is, however, required to confirm this theory and to confirm no link between glucose control and these reflexes and the findings of this study which was only based on the data obtained from a small sample ($n=8$).

The findings of this pilot work did furthermore not confirm those of Gurney (2011) who demonstrated quantifiable changes in sensory function as a result of a low-impact balance and strength training intervention in diabetic participants with DPN and a significant improvement in sensory threshold at three of the four tested foot sites as well as for the average foot sensory threshold.

PART TWO: ANIMAL WORK FOCUSING ON POSSIBLE MECHANISMS

Due to the limitations of the pilot work conducted on a small sample of human subjects and the practical restrictions of working with humans including their unavailability to provide fasting blood samples and the difficulties associated with controlling life-style variables such as food, alcohol and caffeine intake, stress levels, environmental factors and general activity when not participating in the exercise programme, it was decided to look more deeply into the responses of diabetic rodents to a 5-week endurance exercise programme in combination with withdrawal of fructose supplementation.

5.6 Confirmation of Diabetes

Using the cut-off of a 3hFBG concentration $> 16.7 \text{ mmol. l}^{-1}$ ($>300 \text{ mg. dl}^{-1}$) as criterion for the existence of diabetes (Islam and Du Toit, 2009), 6 of the 7 rats were classified as diabetic. In the 7th rat 3h FBG concentration only reached a 3hFBG concentration of 12.3 mmol.l^{-1} . As this animal did therefore not meet the requirements of a diabetic model, it was decided to continue with the exercise program with this rat, but to classify it as a Pre-Diabetic Exercised (PDBE) rat on which a case study would be conducted comparing its responses to the exercise plus fructose withdrawal regime, to those of the rats in the DBC and DBE groups in which pancreatic damage had been induced.

5.7 Effects of the Intervention

In both DBC and DBE groups as well as the PDBE rat, polydipsia, polyphagia and mass loss was confirmed, supporting previous findings (ADA, 2005; diabetes.co.uk, 2014). The slower rate of increase in the mean body mass (g) as the rats matured in DBC and DBE groups ($p < 0.001$), is a well-documented consequence of the untreated diabetic state, which was, in this case, induced by a combination of dietary fructose intake and the STZ injection. It is well known that insulin is an anabolic hormone which stimulates glycogen and triglyceride synthesis (Guyton and Hall, 2011). If insufficient, the diffusion of glucose into the muscle cells and its metabolism to create ATP is slowed down. When this occurs, the body starts burning fat and muscle for energy, causing a reduction in overall body mass (NINDS, 2013).

Of great importance in terms of the findings of this study, are the blood fructosamine concentrations. As described in the literature review, it is a glycosylated protein formed when carbohydrate and protein, primarily albumin, bond non-enzymatically and can be used as an assessment for glycaemic control and blood glucose concentrations over a longer period (half-life about 17 days in the circulation). Fructosamine therefore represents a shorter term glycaemic control than HbA1c (Rigg, 2012), hence it is more appropriate for use in studies on rodents. Confirming the trend seen in the 3hFBG concentrations during the exercise intervention, the mean \pm SD plasma fructosamine levels (mmol. l^{-1}) were significantly higher ($p=0.0015$) in DBC and DBE groups than in the NC group and well correlated with final 3hFBG concentrations ($r=0.7070$), confirming the findings of Ladan *et al.* (2011).

In terms of metabolic regulation of glucose homeostasis, the mean \pm SD plasma BDNF concentrations (pg. ml^{-1}) in the DBE group which were significantly higher ($p=0.0201$) than in the DBC and NC groups and support the findings of a study by Cuppinin (2007) who published one of the first papers reporting an exercise-induced elevation in blood BDNF concentrations attributed to the additional release of BDNF by contracting skeletal muscle. Although these findings supported a milieu which promotes blood glucose

regulation (Nakawaga *et al.*, 2000), this was only apparent in the PDBE rat and did not occur in the DBE group in which pancreatic dysfunction had been induced by injection of STZ.

Most interesting was the significant difference between the mean \pm SD plasma insulin concentrations post-trial (pmol. l^{-1}) between the three groups ($p=0.0347$) and the fact that the highest plasma insulin concentration was recorded in the PDBE rat, with DBC and DBE groups presenting with the lower plasma insulin concentrations than in the NC group. Pinhas-Hamiel and Zeitler (2013) explain that the development of diabetes from normal glucose tolerance is a continuous process. The first stage is characterized by insulin resistance accompanied by a compensatory increase in insulin secretion, as seen in the PDBE group. Those with both IFG and IGT have insulin resistance, but the site of their predominant insulin resistance differs. Those with IFG have predominantly hepatic insulin resistance, whereas those with IGT have predominantly muscle insulin resistance. In the second stage, β cells fail to compensate for increased insulin resistance and hyperglycaemia develops, progressing from prediabetes to diabetes as β cell failure increases, resulting in progressive loss of β cell secretion as was seen in the DBC group who presented with lower plasma insulin concentrations. It must be acknowledged that the latter however probably results from a combination of genetic, environmental and biochemical determinants (Pinhas-Hamiel and Zeitler, 2013).

Furthermore the difference noted between the mean \pm SD plasma insulin concentrations ($\mu\text{g. l}^{-1}$) in DBC and DBE groups can be explained as resulting from increased glucose uptake as exercise training provides a strong stimulus for mitochondrial biogenesis that increases GLUT4 concentration. Contraction of the skeletal muscle stimulates the cell to translocate GLUT4 receptors to the surface, thus facilitating muscle glucose transport and therefore decreasing the need for insulin secretion (Earnest, 2008). In the PDBE rat, insulin concentration is the highest and the fact that is considerably higher than in the three groups, can possibly be explained as a compensatory increase in insulin secretion during the first stage in T2DM.

Mean \pm SD plasma adiponectin concentrations ($\mu\text{mol.l}^{-1}$) revealed a significant difference between the groups ($p=0.0111$) and a positive correlation with insulin concentrations ($r=0.747$; $p<0.05$). This confirms previous findings (Weyer *et al.*, 2001) that plasma levels of adiponectin are significantly lower in insulin resistant state of T2DM and a major modulator of insulin action.

5.8 Conclusion

It can therefore be concluded that following the exercise programme, hypoinsulinaemia ($p < 0.05$) with elevated 3hFBG, plasma fructosamine and plasma BDNF ($p > 0.05$), but unchanged plasma adiponectin ($p > 0.05$) were recorded in the DBE group ($n=6$), while hyperinsulinaemia was recorded in the PDBE rat with reduced 3hFBG and plasma fructosamine and elevated plasma adiponectin and BDNF levels.

The data from this study therefore suggest that endurance exercise, together with fructose withdrawal from the diet, is not effective in reversing diabetic status, but may be effective in improving glycaemic status in the pre-diabetic stage confirming previous findings by Hwang *et al.* (2011).

In case of PDBE rat, 3hr FBG, plasma fructosamine was less than DBE rats, plasma insulin concentrations was greater than NC and an increase in both plasma BDNF and adiponectin concentrations provides evidence of improved glycaemic control and suggests that endurance exercise is not effective in reversing diabetic status, but may contribute towards improving glycaemic status during the pre-diabetic stage.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

From the results of these two pilot studies it can be concluded that when, as was the case of the study on humans, the condition is chronic and has existed for years resulting in peripheral sensory damage, exercise alone is unlikely to reverse the condition. This was also confirmed in the DBE group of the rodent study in which pancreatic damage had been induced via injection of STZ.

The possibility of the exercise programme resulting in an improvement in reflex nervous function which is independent of glucose control, in humans with T2DM that is accompanied by DPN, does however require further verification.

In the rodent work it was found that in the PDE rat which presented with a higher blood insulin concentration, the exercise programme together with fructose withdrawal, however appeared to have a positive effect on glucose regulation. This was confirmed by the elevated fructosamine, adiponectin and BDNF concentrations in this rat and requires confirmation in further randomised controlled studies in which the effect of exercise alone *vs.* restriction of dietary intake of CHO alone, is examined on progression of a pre-diabetic condition.

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APPENDIX A1



16 May 2013

Professor E Futre
c/o Ms. Stirk
Department of Human Physiology
Westville Campus
University of KwaZulu-Natal

PROTOCOL: The effects of an 8 week endurance exercise program on glucose control and peripheral sensory neuropathy in type 2 diabetic participants. REF: BFC055/13.

The Biomedical Research Ethics Committee (BREC) has considered the abovementioned application.

The study was provisionally approved by a quorate meeting of BREC on 12 March 2013 pending appropriate responses to queries raised. Your responses dated 16 May 2013 to queries raised on 16 May 2013 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval and may begin as from 16 May 2013.

This approval is valid for one year from 16 May 2013. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2004), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at <http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx>. BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

Professor D Wassenaar (Chair)
Biomedical Research Ethics Committee
Westville Campus, Govan Mbeki Building

Postal Address: Private Bag X54001, Durban, 4000, South Africa

Telephone: +27 (0)31 260 2384 Facsimile: +27 (0)31 260 4409 Email: brec@ukzn.ac.za

Website: <http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx>

Founding Campuses: Edgewood Howard College Medical School Pietermaritzburg Westville



The following Committee members were present at the meeting that took place on 12 March 2013:

Prof D Wassenaar	Chair
Prof V Rambiritch	Pharmacology
Prof R Bhimma	Paediatric & Child Health
Dr R Govender	Family Medicine
Dr U Govind	Private Pract. - Gen. Practitioner
Dr Z Khumalo	KZN Health (External) General Medicine
Dr NR Maharaj	Obstetrics & Gynaecology
Dr K Naidoo	Family Medicine
Dr S Paruk	Psychiatry
Prof DJ Pudifin	Medicine
Dr S Singh	Dentistry
Prof J Tsoka-Gwegweni	Public Health-Medicine
Dr A Sathar	External -Medicine

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely



PROFESSOR D R WASSENAAR
Chair: Biomedical Research Ethics Committee

APPENDIX A2



UNIVERSITY OF
KWAZULU-NATAL

INYUVESI
YAKWAZULU-NATALI

**Research Office
Animal Ethics Research Committee**

Govan Mbeki Centre, Westville Campus,
University Road, Chiltern Hills, Westville, 3629, South Africa
Telephone 27 (031) 260-2273/35 Fax (031) 260-2384
Email: animalethics@ukzn.ac.za

5 June 2013

Reference: 102/13/Animal

Prof. E Peters-Futre
School of Laboratory Medicine
and Medical Sciences
University of KwaZulu-Natal
WESTVILLE Campus

Dear Prof. Peters-Futre

Ethical Approval of Research Projects on Animals

I have pleasure in informing you that the Animal Ethics Sub-committee of the University Ethics Committee has granted ethical approval for 2013 on the following project:

“The relationship between changes in GLYCAEMIC control and systemic BDNF concentrations in Exercising Type 2 Diabetic rats.”

Yours sincerely

**Professor Theresa HT Coetzer
Chairperson: Animal Ethics Sub-committee**

Cc Registrar – Prof. J Meyerowitz
Research Office – Dr N Singh
Head of School – Prof. W Daniels
BRU, Dr S Singh



Founding Campuses:

-  Edgewood
-  Howard College
-  Medical School
-  Pietermaritzburg
-  Westville



APPENDIX B

DIVISION OF HUMAN PHYSIOLOGY COLLEGE OF HEALTH SCIENCES

PARTICIPANT INFORMATION FORM

Ms. Louise Stirk and Professor Edith Peters-Futre from the Division of Human Physiology in the School of Laboratory Medicine and Medical Sciences of the College of Health Sciences at the University of KwaZulu-Natal would like to conduct the following research project:

THE EFFECTS OF AN 8-WEEK ENDURANCE EXERCISE PROGRAM ON GLUCOSE CONTROL AND PERIPHERAL SENSORY NEUROPATHY IN PARTICIPANTS WITH TYPE 2 DIABETES

You are being invited to consider participating in this study.

Background to the study:

Diabetic peripheral neuropathy (DPN) is a common complaint amongst diabetic patients and is a great cause for concern as it can lead to other serious complications such as pedal ulcers and possible gangrene and amputation (Diabetic Care, 2012). Although there is much evidence confirming the beneficial effects of different exercise modalities on glucose control in the Type 2 diabetic patient, only one research report focusing on the benefits of exercise on DPN has been completed to date. This reported beneficial effects with significant improvements in sensory function following a low-impact and strength training exercise intervention (Gruney, 2011).

We are however not sure about what causes this improvement in nervous function and reduced pain in their feet after participating in an exercise program and whether endurance exercise will have the same effect. It has been shown that certain brain derived factors are also released by skeletal muscle during endurance exercise and that these factors lower blood glucose concentration, improve insulin sensitivity and increase resting energy expenditure (Nakagawa *et al.*, 2000; Cuppinin *et al.*, 2007; Krabbe *et al.*, 2007). We therefore would like to determine whether an endurance exercise program results in a lessening of the signs and symptoms of DPN

- i. an improvement in glucose control
- ii. raised factors released by the brain and contracting skeletal muscle which have been associated with glucose control

Who is eligible to participate in this study?

Type 2 diabetic participants between 34 and 48 years old who

- have diagnosed DPN
- no other chronic illness
- exercise less than an hour a week
- are willing to participate in all aspects of the trial, which includes regular exercise intervention, completing questionnaires, provision of small blood samples before and after the intervention and clinical tests (neurological testing)
- reside in the Ethekewini municipality and surrounding areas

and do not...

- possess any ailment or chronic illness that will impede participation in the 8-week exercise program (e.g. pedal ulcer, Cardiac Autonomic Neuropathy, rheumatoid arthritis)
- make regular or chronic use of medication for improved peripheral sensory neuropathy

What will be expected from volunteers in this study?

You will be asked to:

1. present yourself for baseline clinical measurements and assessments prior to the onset of program
2. attend all the scheduled spinning classes i.e. 3 times a week for 8 weeks
3. maintain your usual diet and training status throughout the study
4. provide a small (4ml) venous blood sample before and after the exercise intervention
5. present yourself for a post-intervention clinical measurements and assessments after completion of the 8-week program

Outline of tests

On 2 occasions (i.e. pre- and post-assessment) you will be required to present yourselves to the Exercise Physiology Laboratory, Department of Human Physiology, School of Laboratory Medicine and Medical Sciences, Westville Campus, UKZN.

During the pre-assessment, you are to complete a general baseline questionnaire to assess your; i) general health status, ii) lifestyle and iii) physical activity and whether you are able to exercise without causing exercise-induced complications that are prevalent in diabetic patients. Your peripheral sensory neuropathy status will be assessed by a qualified podiatrist (Louise Stirk B. Tech Pod (SA)) in addition to the details provided already after an assessment of your vital signs by a physician. This will include a questionnaire to determine the degree of sensory peripheral neuropathy symptoms experienced. The signs of sensory peripheral neuropathy will be quantitatively assessed using a 10g monofilament, 128Hz tuning fork, Neurotip, Tip Therm, patella hammer and a piece of cotton wool. Lastly a physician will draw a venous blood to provide a sample for testing HbA1c and BDNF concentrations.

The exercise intervention i.e. a 30 minute spinning class will be completed three times a week for 8 weeks under the supervision of a qualified spinning instructor. This will be completed on 20 “spinning bikes” in an environmental controlled exercise laboratory in the PRIME Performance Fitness Centre at the MOSES Mabida Stadium. Participants will wear a Polar heart rate monitor with telemetric links to a central recorder enabling the instructor, a specialist in Exercise Physiology, to ensure that the heart rate of participants is kept within a range of 60-80% of their previously calculated maximum heart rate. At all times, a medical doctor, specialized in Sports Medicine, will be on standby to manage any adverse event or possible complications that may arise.

The post-assessment will be concluded after the exercise intervention has been completed. The level of peripheral sensory neuropathy will be determined as done in the pre-assessment. Another venous blood sample will be drawn by the physician to test HbA1c and BDNF. The samples will be handled and tested as previously mentioned for the pre-assessment.

How can you benefit from participation in this study?

Following the study, you will be given the results of each of the clinical and laboratory tests.

By participating you will establish whether the effects of an 8-week endurance exercise program improves peripheral sensory neuropathy in type 2 diabetics and the possible association with HbA1c levels and BDNF concentrations. You will be the first to be notified of the results of this study.

Will you be exposed to adverse effects of the study?

A minimal risk factor is involved in any physical test and all necessary precautions will be taken to ensure safe conditions.

There is a very slight risk of complications from venipuncture (taking of blood), mainly infection at the site of puncture or inflammation (swelling) of the vein used.

In the unlikely event of a complication occurring, Dr. Craig Roberts will be present at all of the assessments and taking of the blood samples. He will ensure that procedures are performed according to the same standards that you would experience in a hospital environment.

Medical insurance is available in case of the event of an adverse effect.

Can you withdraw from the study?

As your participation is entirely voluntary, you may withdraw from the study at any time without penalty.

Will your individual results remain confidential?

Yes. The records identifying the participants will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. The study is for degree purposes, however, the results of the study may be published. In all cases your identity will remain confidential.

Financial compensation

Any out-of-pocket expenses which you may incur as a result of your participation in this study (e.g. traveling expenses) will also be reimbursed by the research team.

Transport costs to the exercise laboratory /Moses Mabida will be paid at R3,16/km

Further queries

Should you have any queries or wish to obtain further details regarding this study, please do not hesitate to contact us.

Ms. Louise Stirk – 082 839 9203

Prof. Edith Peters-Futre - (031) 260 4237 (W); 0737597974

Contact details of REC administrator and chair – for reporting of complaints / problems.

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION

University of KwaZulu-Natal

Research Office, Westville Campus

Govan Mbeki Building

Private Bag X 54001, Durban, 4000

KwaZulu-Natal, SOUTH AFRICA

Tel: 27 31 2604769 - Fax: 27 31 2604609

Email: BREC@ukzn.ac.za

APPENDIX C



DIVISION OF HUMAN PHYSIOLOGY

COLLEGE OF HEALTH SCIENCES

THE EFFECTS OF AN 8-WEEK ENDURANCE EXERCISE PROGRAM ON GLUCOSE CONTROL AND PERIPHERAL SENSORY NEUROPATHY TYPE 2 DIABETIC PARTICIPANTS

PARTICIPANT CONSENT FORM

I, hereby agree to participate in a research study to be performed by Ms. Louise Stirk and Professor Edith Peters-Futre in the Division of Human Physiology in the College of Health Sciences of the University of KwaZulu-Natal. I have been informed about the study by the principal investigator, Ms. Louise Stirk and understand my participation is at my own risk.

I understand that the basic procedures to be carried out are to include:

1. Completion of a 30-minute spinning class three times a week for 8 weeks.
2. Completion of a brief medical and general health questionnaire before the exercise intervention and provision of 2ml blood samples before and after the exercise intervention.

The details of these procedures have been explained to me in full. I am aware that a certain level of discomfort may occur when the blood is taken and that this procedure may be accompanied by certain medical risks including infection and inflammation of the vein.

I understand that this study will form part of the Masters degree of Ms. Louise Stirk and that the results may be published.

I understand that participation is entirely voluntary and that I may withdraw from the study at any time.

I may contact the principal investigator of the project, Ms. Louise Stirk at 082 839 9203 at any time if I have questions about the research or if I am injured as a result of the research.

Signature of Participant

Date

Signature of Witness

Date (Where applicable)

APPENDIX D



DIVISION OF HUMAN PHYSIOLOGY COLLEGE OF HEALTH SCIENCES

THE EFFECTS OF AN 8-WEEK ENDURANCE EXERCISE PROGRAM ON GLUCOSE CONTROL AND PERIPHERAL SENSORY NEUROPATHY IN TYPE 2 DIABETIC PARTICIPANTS

BASELINE QUESTIONNAIRE

1. Participant code..... 2. Date of Birth.....

PART A: GENERAL HEALTH STATUS

1. Are you currently in good health? (i.e. No illness within the last 3 months)

Yes No If no, please specify

.....
.....

2. Do you suffer from any of the following? Please tick.

Heavy metal poisoning	yes <input type="checkbox"/>	no <input type="checkbox"/>
Vitamin B12 deficiency	yes <input type="checkbox"/>	no <input type="checkbox"/>
Renal disease	yes <input type="checkbox"/>	no <input type="checkbox"/>
Chronic inflammatory demyelinating neuropathy	yes <input type="checkbox"/>	no <input type="checkbox"/>
Inherited neuropathies	yes <input type="checkbox"/>	no <input type="checkbox"/>
Vasculitis	yes <input type="checkbox"/>	no <input type="checkbox"/>

3. Do you suffer from any chronic medical conditions that have been diagnosed more than 3 months ago? E.g. high blood pressure, high cholesterol.

Yes No If yes, please specify

.....
.....

4. Please list any medication that you are currently taking. This includes over the counter medication, vitamins or homeopathic remedies

.....
.....

With regard to your feet,

		YES	NO
1	Has a doctor diagnosed you with neuropathy?		
2.	Do you have burning pain?		
3.	Are they sensitive to the touch?		
4.	Do you have a prickling/ pins and needles feeling in your feet?		
5.	Can you tell from hot and cold water with your feet?		
6.	Have you had an ulcer on your feet?		
7.	Are your feet numb?		
8.	Are your symptoms worse at night?		
9.	Are you able to sense your feet when walking?		
10.	Do your legs hurt when walking?		

PART C: PHYSICAL ACTIVITY QUESTIONNAIRE

		YES	NO
1.	Do you feel pain in your chest at rest, during your daily activities of living or when you do physical activity?		
2.	Do you lose balance because of dizziness or have you lost consciousness in the last 12 months? Please answer NO if your dizziness was associated with over breathing (including during vigorous exercise)		
3.	Do you have a bone or joint problem that could be made worse by becoming more physically active? Please answer NO if you had a joint problem in the past, but it does not limit your current ability to be physically active. For example knee, ankle, shoulder or other		
4.	Has your doctor ever said that you should only do medically supervised physical activity?		
5.	How many hours in the day are you physically active?		
6.	Of those hours that you are physically active, how many are structured exercise?		
7.	What structured exercise do you do?		

Adapted from: The Physical Activity Readiness Questionnaire for Everyone. (PAR-Q+ CSEP approved. 2011)

Thank you for your participation!



APPENDIX E

DIVISION OF HUMAN PHYSIOLOGY COLLEGE OF HEALTH SCIENCES

THE EFFECTS OF AN 8-WEEK ENDURANCE EXERCISE PROGRAM ON GLUCOSE CONTROL AND PERIPHERAL SENSORY NEUROPATHY IN TYPE 2 DIABETIC PARTICIPANTS

POST TRIAL QUESTIONNAIRE

2. Participant code..... 2. Date of Birth.....

PART A: GENERAL HEALTH STATUS:

5. Have you been in good health? (i.e. No illness within the last 8 weeks)

Yes No If no, please specify

.....

6. Please list any medication that you took. This includes over the counter medication, vitamins or homeopathic remedies

.....

7. With regard to your feet,

		YES	NO	Not applicable	Comment
1.	Has the burning pain improved?				
2.	Has sensitivity to touch improved?				
3.	Has the prickling/ pins& needles feeling in your feet improved?				
4.	Has your perception of hot and cold water on your feet improved?				
5.	Has the numbness in your feet improved?				
6.	Are the symptoms at night better?				
7.	Are you able to sense your feet better when walking?				
8.	Has the leg pain when walking improve?				
9.	Any additional comments re sensory changes in your feet?				

Adapted from: Michigan Neuropathy Screening Instrument (Lawrence A *et al.* Screening for Diabetic Neuropathy. Diabetic Microvascular Complications Today. 17-19. 2004)

PART B: LIFESTYLE

Sleep Pattern:

Number of hours per night?.....

Have you suffered from insomnia during the 8 weeks? Yes No

Alcohol intake:

Did you consume alcohol? Yes No

If yes, please specify number of drinks per week

Beer (340ml).....

Wine (250 ml).....

Spirits (tots).....

Caffeine Intake:

Mark the appropriate intake with an X

Coffee: None 1-3 cups per day >3 cups per day

Tea: None 1-3 cups per day >3 cups per day

Stress levels:

How would you rate your overall stress level during the 8 weeks of the trial? (1- coping well; 10- not coping at all). Please elaborate

.....

Diet:

How would you describe your regular dietary intake during the 8 weeks?

Please indicate with an X

Prudent: Low refined carbohydrate, transfat & saturated fat, red meat content & high fruit & vegetable content	
High carbohydrate; Low fat	
High fat; Low carbohydrates	
High protein, high fat	

Were you cautious about your intake of refined sugars and candies? Yes No

Did you supplement with Vitamin B12? Yes No

Did you use any other dietary supplements? If so, please specify

.....

PART C: PHYSICAL ACTIVITY QUESTIONNAIRE

		YES	NO
8.	Do you feel pain in your chest at rest, during your daily activities of living or when you do physical activity?		
9.	Do you lose balance because of dizziness or have you lost consciousness in the last 12 months? Please answer NO if your dizziness was associated with over breathing (including during vigorous exercise)		
10.	Do you have a bone or joint problem that could be made worse by becoming more physically active? Please answer NO if you had a joint problem in the past, but it does not limit your current ability to be physically active. For example knee, ankle, shoulder or other		
11.	Has your doctor ever said that you should only do medically supervised physical activity?		
12.	How many hours in the day were you physically active?		
13.	Of those hours that you are physically active, how many were structured exercise other than the studies exercise intervention?		

14.	What structured exercise did you do in addition to the spinning programme?	
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Adapted from: The Physical Activity Readiness Questionnaire for Everyone. (PAR-Q+ CSEP approved. 2011)

PART D: FINAL GENERAL QUESTIONS

Has anything else that you feel the researchers should be aware of, changed within the last 8 weeks?

.....

.....

.....

How did you feel about completing the exercise program?

Did you enjoy it?.....

How was the intensity? too easy appropriate challenging very challenging

Describe any negative or positive reactions to the regular exercise which you would like the researchers to be aware of.....

.....

.....

Thank you for your participation in this trial!