PPARγ gene polymorphisms in black South African females with Type 2 Diabetes Mellitus

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DECLARATIONS

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LIST OF ABBREVIATIONS

2-h PG	Two-hour plasma glucose
β-cells	Beta-cells
α-cells	Glucagon cells
%	Percentage
ACE I/D	Angiotensin-I converting enzyme
ADA	American Diabetes Association
Ala	Alanine
ATP	Adenosine triphosphate
BC	Before Christ
BMD	Areal bone mineral density
BMI	Body mass index
BMC	Bone mineral content
C	Cytosine
CDKAL1	CDK5 regulatory subunit associated protein 1-like 1
CDKN2A/B	Cyclin-dependent kinase inhibitor 2A/B
cDNA	Complementary DNA
CEO	Chief Executive Officer
C-peptide	Connecting peptide
DBD	DNA binding domain
DCCT	Diabetes control and complications trial
d-cells	Somatostatin cells
DEXA	Dual-energy X-ray absorptiometry

DKA	Diabetic ketoacidosis
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
FDA	Food and Drug Administration
FPG	Fasting plasma glucose test
FTO	Fat mass and obesity associated gene
EXT2	Exostosin glycosyltransferase 2
g	Gram
G	Guanine
GDM	Gestational diabetes mellitus
GI	Glycaemic index
GL	Glycaemic load
GIP	Glucose-dependent insulinotropic peptide
GLP	Glucagon-like peptide
GLUT	Glucose transporters
HbA1c	Glycosylated haemoglobin
HDL	High-density lipoproteins
HHEX	Haematopoietically expressed homeobox protein
Kg	Kilogram
IDF	International Diabetes Federation
IEM	Inborn errors of metabolism
IGF2BP2	Insulin-like growth factor 2 MRNA binding protein 2

IRS1	Insulin receptor substrate 1
JEMDSA	Journal of Endocrinology, Metabolism and Diabetes of South Africa
KCNJ11	Potassium inwardly-rectifying channel, subfamily J, member 11
LBD	Ligand binding domain
LDL	Low-density lipoproteins
LMI	Liggaamsmassaindeks
MCAD	Medium-chain acyl-coenzyme A dehydrogenase
Mg	Milligram
MLH1	MutL Homolog 1 gene
mmHG	Millimeter of mercury
mmol/l	Millimole per litre
MNT	Medical nutrition therapy
mRNA	Messenger RNA
mtDNA	Mitochondrial DNA
MTHFR	Methylenetetrahydrofolate reductase
MTNR1B	Melatonin receptor 1B
NHLS	National Health Laboratory Service
NHP	Neutral protamine Hagedorn
NNIA	Nestlé Nutrition Institute Africa
NPDR	Non-proliferative diabetic retinopathy
PCR	Polymerase chain reaction
pp-cells	Pancreatic polypeptide cells
PPARs	Peroxisome proliferator-activated receptors

PPARγ	Peroxisome proliferator-activated receptor – gamma / peroxisoom Proliferator-geaktiveerde-reseptor – gamma geen
PPARα	Peroxisome proliferator-activated receptor – alpha
PPARβ	Peroxisome proliferator-activated receptor – beta
Pro	Proline
RNA	Ribonucleic acid
RPG	Random plasma glucose test
RXR	Retinoid X receptor
SAJCN	South African Journal of Clinical Nutrition
SANHANES	South African National Health and Nutrition Examination Survey
SLC30A8	Solute carrier family 30 (zinc transporter), Member 8
SNP	Single nucleotide polymorphism
TCF7L2	Transcription factor 7-like 2 (T-cell specific, HMG-box)
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus / Tipe 2 diabetes mellitus
TNF-α	Tumour necrosis factor – Alpha
tRNA	Transfer RNA
VLDL	Very low density lipoproteins
WHO	World Health Organisation

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND AND MOTIVATION

Diabetes mellitus (DM) is a metabolic disorder with various etiologies, caused by defects in insulin secretion, insulin action or both. Diabetes is characterised by chronic hyperglycaemia and impaired carbohydrate, fat and protein metabolism (Amod *et al.*, 2012:S5; Guyton & Hall, 2006:972). Insulin, a hormone produced by the beta-cells of the pancreas, is necessary for the use and storage of body fuels (Amod *et al.*, 2012:S5; Franz, 2012:679). Insulin plays an important role in controlling organic metabolism and is increased during the absorptive state and decreased during the post-absorptive state (Widmaier *et al.*, 2006:620). Individuals with diabetes do not produce enough or respond inappropriately to insulin. Without adequate insulin, hyperglycaemia occurs, which can lead to serious health complications and premature death. Individuals with diabetes can control the disease and lower the risks of complications: nutritional therapy as well as medical management are vital to diabetes care and management (Amod *et al.*, 2012:S5; Franz, 2012:679).

Two types of DM are generally distinguished, namely Type 1 diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM). Gestational diabetes mellitus (GDM) can be diagnosed when hyperglycaemia occurs with first recognition or onset during pregnancy. Other specific types of diabetes include genetically defined forms of diabetes, diseases of the pancreas, drug- or chemical-induced diabetes, and diabetes resulting from surgery, infections, and other illnesses. These conditions are relatively uncommon, however (Amod *et al.*, 2012:S6).

The prevalence of diabetes worldwide among adults older than 20 years of age was estimated to be more than 171 million in the year 2000. This figure was already 11% higher than the previous estimate of 154 million in 1995 (King *et al*., 1998:1415). If the age-specific prevalence remains constant, based on demographic changes, the number of people with diabetes in the world is predicted to double between 2000 and 2030 (Wild *et al.*, 2004:1050). The International Diabetes Federation (IDF) Atlas shows that there were 366 million diabetes sufferers worldwide in 2011 and in 2013 there were 382 million adults aged between 40 and 59 with diabetes, 80% of them

living in low and middle income countries (Guariguata *et al.*, 2013:13). This number is set to reach 471 million in the year 2035 (Guariguata *et al.*, 2013:7). There is a gender difference in global diabetes prevalence, with 14 million more men than women living with diabetes (198 million men versus 184 million women) (Guariguata *et al.*, 2013:34). With T2DM currently accounting for more than 90% of all diabetes cases, diabetes may certainly be considered a major public health concern (Amod *et al.*, 2012:S4).

The IDF has shown that there are 19.8 million people living with diabetes in Africa, and the estimate for 2035 is 41.4 million; thus an increase of 109% (Guariguata *et al.*, 2013:15). In South Africa, according to the sixth edition of the IDF in 2013, 2.6 million (7-9%) adults aged between 20 and 79 have diabetes, although an age-adjusted prevalence of up to 13% was described in urban populations as early as 1994 (Guariguata *et al.*, 2013:36,56; Amod *et al.*, 2012:S4). Due to the increased prevalence of non-communicable diseases in South Africa, the South African National Health and Nutrition Examination Survey (SANHANES) was conducted to obtain a better understanding of the prevalence of such diseases and the associated risk factors among South Africans, and to use this information for the development of effective health policies, health programmes and services. The results indicate that diabetes is prevalent in 9.5% of South Africans, with no significant difference in gender distribution (Shisana *et al.*, 2013:91), although SANHANES indicated females had a significantly higher rate than males for high blood sugar (11% and 7.9%) (Shisana *et al.*, 2013:92). In the black African population 8.2% was diagnosed with diabetes (Shisana *et al.*, 2013:93). The urban settings in South Africa had the highest percentage of individuals with diabetes (11.3%), whereas the rural informal settlements had the second highest percentage of individuals with diabetes (9.2%) (Shisana *et al.*, 2013:92). Rural formal and urben informal settlements had the third least and least percentage of diagnoses diabetics respectively (Shisana *et al.*, 2013:93.) The SANHANES reports that the prevalence of diabetes increases with advancing age, reaching a peak at the ages of 45 to 64 years, and that the prevalence of diabetes is also the highest in rural informal (11.9%) and then in urban formal (11.3%) residents (Shisana *et al.*, 2013:94). The prevalence of overweight and obesity in South Africans indicated that the females was significantly more overweight (24.8%) and obese (39.2%) than the males (20.1% and 10.6%)

respectively (Shisana *et al.*, 2013:136). Black African females had the highest prevalence of obesity compared to other ethnicities (39.9%) (Shisana *et al.*, 2013:140). A study done in Cape Town in the black African areas found a prevalence of 12.1% individuals diagnosed with diabetes (Peer *et al*., 2012:3). The resuls also indicated that the prevalence of diabetes was higher in females than in males, reaching a peak at the ages of 65 to 74 years old (Peer *et al*., 2012:3). In these black Africans, 80% of all the individuals with diabetes was overweight and obese (Peer *et al*., 2012:6). Urbanisation and unhealthy lifestyles are contributing to the increase in the prevalence of obesity and T2DM, as seen in the 2003 Demographic and Health Survey, which indicates that 50% of women and 30% of men in South Africa are overweight or obese (Department of Health *et al.*, 2007:24). This data offers an update on the growing public health burden of diabetes in South Africa and also across the world.

The human and economic cost of the diabetes epidemic is extensive. In 30 – 85% of cases of T2DM, the disease remains undiagnosed until, at the time of diagnosis, 20% of the patients have already developed complications. The treatment of complications adds to the economic cost, which could have been prevented with earlier detection and diagnosis (Amod *et al.*, 2012:S4). In developing countries, mortality due to communicable diseases and also infant and maternal mortality rates are decreasing; however the increased prevalence of diabetes will inevitably result in a rising proportion of deaths from cardiovascular disease, as well as the increased prevalence of diabetes-related complications. The only way that this diabetes epidemic can be addressed is with a global plan (Wild *et al.*, 2004:1051).

With the high prevalence of T2DM in the world, as well as in South Africa, it has become important for researchers to understand the etiology of the disease better and to develop prevention strategies. Even though genetic make-up sets the stage for disease, environmental factors like nutrition and other lifestyle choices determine the risk for its development. This can be demonstrated by comparing a Pima Indian population living in northern Mexico who are lean, with Pima Indians living in the south west, who have a high prevalence of obesity and T2DM (DeBusk, 2012:145).

Increasingly, genetic research is able to explain the relation between genetic variations and dysfunction and disease (DeBusk, 2012:145). If it is understood that

diseases are genetically based but environmentally influenced, intervention and prevention may be the targets that require the focus. Nutrition therapy based on genetic composition is therefore expected to feature more significantly as the basis in the prevention and management of chronic, diet- and lifestyle-related diseases (DeBusk, 2012:145).

To understand the effect of nutrition on genetic expression, it is essential to realise that nutrients and other bioactive food components can serve as ligands. Ligands are molecules that bind to specific nucleotide sequences within a gene's regulatory region. This binding causes a change in gene expression through the regulation of transcription (DeBusk, 2012:154). Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear hormone receptor family (Blaschke *et al.*, 2006:30). As members of the nuclear receptor family, PPARs act by controlling networks of target genes. This subfamily of nuclear receptors can be activated by dietary fatty acids and their metabolic derivatives in the body, and therefore serve as lipid sensors, which when activated can redirect metabolism. There are three isoforms of PPARs, encoded by separate genes: *PPAR-gamma* (*PPARγ*), *PPAR-alpha* (*PPARα*), and *PPAR-beta* (*PPARβ*). They have different patterns of distribution and share 60 to 80% homology in their ligand- and DNA-binding domains, exhibiting distinct patterns of expression and overlapping but distinct biological activities (Blaschke *et al.*, 2006:30; Forman *et al.*, 1997:4312). Therefore, each of these PPARs carries out distinctive functions in the regulation of the energy metabolism (Blaschke *et al.*, 2006:30; Evans *et al.*, 2004:357).

In this study, the focus will be on the Pro12Ala polymorphism of the *PPARγ* gene. Various studies worldwide link the presence of the Pro12Ala polymorphism of the *PPARy* gene with T2DM. A narrative review by Stumvoll and Häring (2002:2345) concludes that *PPARy* is a master regulator between nutrients, susceptibility to obesity, control of peptides released from adipocytes, and insulin sensitivity. The alanine allele of the Pro12Ala polymorphism in the isoform of *PPARy2* is associated with a 25% reduced risk for T2DM in Caucasians, whereas the high risk Pro-allele is present in more than 75% of the population. This lowering effect when the Ala allele is present is mediated by increased insulin sensitivity, which may be due to more efficient suppression of free fatty acids released from fat tissue, where the *PPARy* is

expressed. The underlying mechanism for the Pro12Ala polymorphism is a moderate reduction of the ligand-independent activity of *PPARy.* In a study on Caucasians with diabetes, it was shown that adipose tissue is the major site of expression of the *PPARγ*2 isoform which may have important consequences related to obesity, insulin resistance, and T2DM (Yen *et al.*, 1997:273).

1.2 PROBLEM STATEMENT

T2DM is a global problem, with an increase in prevalence also in South Africa. Environmental and lifestyle causes of T2DM have been researched and described widely. The role of genes, on the other hand, including the Pro12Ala polymorphism of the *PPARγ* gene, has not been studied in a black female South African population where obesity and foetal origin of disease could be important contributing factors.

1.3 AIM AND OBJECTIVES

The main aim of this study is to determine and describe the prevalence of Pro12Ala polymorphisms of the *PPARγ* gene in black female individuals with T2DM in Bloemfontein, South Africa, as a possible measure for early detection of T2DM.

To reach the aim, the following objectives are set:

- To determine the presence of *PPARγ* Pro12Ala polymorphisms in the study population;
- To describe the association between Pro12Ala *PPARγ* gene polymorphisms and body adiposity in the study population;
- To describe the association between Pro12Ala *PPARγ* gene polymorphisms and blood glucose control (measured as HbA1c levels) in the study population.

1.4 STRUCTURE OF THIS DISSERTATION

This dissertation is divided into seven chapters. Chapter 1 includes the introduction and motivation for the study. The problem statement, aim and objectives of the study are stated, and the structure of this dissertation is described.

Chapter 2 is a literature review discussing the history of diabetes mellitus, the classification and epidemiology of diabetes mellitus, the physiology of insulin, etiology and pathophysiology of diabetes mellitus, diagnosis and screening, signs and symptoms, glucose control, management of diabetes, and complications. It also discusses nutrigenomic influences on diabetes mellitus, genetics and diseases, inheritance, and the *PPAR* gamma (*PPARy*) polymorphism and its association with diabetes.

Chapter 3, the methodology chapter, describes the type of study and population sample and the inclusion and exclusion criteria. The data collection and procedures are explained in this chapter. Techniques used for data collection and statistical analysis are also described in Chapter 3. Ethical issues taken into consideration during this study are also explained.

The three objectives of this study are reported on separately, each in its own chapter: Chapters 4, 5 and 6. These three chapters are written in the article format as approved by the University of the Free State. The articles are written according to the author's instructions for the specific journal to which it is submitted. In Chapter 4, the article discusses the prevalence of *PPARy* Pro12Ala polymorphisms in the study population; Chapter 5 focuses on body adiposity; and Chapter 6 explains and expands upon blood glucose control (measured as HbA1c levels). In each of the articles the methods, results and discussion of the results are presented. The data is interpreted by comparing it to other studies within the scope of the topic. Each article includes a conclusion and recommendations.

Chapter 7 provides an overview of the conclusions and recommendations of the study as a whole. The research significance and limitations of the research study are also described in this chapter, and recommendations for future studies are provided.

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CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

Diabetes mellitus (DM) is characterised by elevated blood glucose concentrations, which result from defects in insulin secretion, insulin action or both. Insulin can be defined as a hormone which is produced by the beta-cells (β-cells) of the pancreas and is needed for the use or storage of body fuels, which include carbohydrate, protein and fat (Franz, 2012:676).

Diseases are genetically based, but environmentally influenced; therefore intervention and prevention must be the focus of diabetes care. Nutrition therapy, modified to an individual's unique genetic make-up, is therefore expected to feature more and more significantly in the prevention and management of chronic, diet- and lifestyle-related diseases (DeBusk, 2012:145).

This chapter will discuss the history of DM, the different types of DM, the etiology, pathophysiology, clinical signs and symptoms, as well as options for treatment and prevention. The genetic focus will be on the *PPARγ* gene polymorphism and its association with type 2 diabetes mellitus (T2DM).

2.2 THE HISTORY OF DIABETES MELLITUS

Egyptians referred to diabetes mellitus as early as 1500 before Christ (BC), pointing out in the Eben Papyrus that polyuria is a symptom of "sugar disease". An Egyptian physician, Hesy-Ra, recorded this on papyrus between 500 and 1500 BC. About 1000 years later, Susruta of India described a clinical diagnosis for diabetes. Two Greek scientists, Galen and Celsus, thereafter gave a description of the symptoms of diabetes and Galen incorrectly concluded that diabetes was a disease of the kidneys (King & Rubin, 2003:1091). The name diabetes was introduced by Aretaeus of Cappadocia who also gave the first understandable and comprehensive description of diabetes; he was a physician of the late Hellenistic period (Laios *et al.,* 2012:109; King & Rubin, 2003:1091).

In Greek diabetes means “siphon”, and Aretaeus referred to diabetes as “...a remarkable disorder, and not very common to man. It consists of a moist and cold wasting of the flesh and limbs into urine, from a cause similar to that of dropsy; the secretion passes in the usual way, the kidney and the bladder. The individuals never cease making water, but discharge is as incessant as a sluice to let off. This disease is chronic in character, and is slowly engendered, though the patient does not survive long when it is completely established for the marasmus produced is rapid and death is speedy” (Widmaier *et al.*, 2006:628; King & Rubin, 2003:1091). Aretaeus wrote a very thorough manuscript of which the full text was divided into three parts. The first part described the signs and symptoms, giving the etiology and emphasising the role of the kidney and bladder. In the second part the patient’s symptoms were analysed in detail according to the stages of disease progression; important new information was also presented which was absent from other physicians’ medical texts. The third part provided more novel particulars and insights like the correlation of diabetes with other diseases, leading to the conclusion that the onset of disease is manifested by a series of events occurring in an organism (Laios *et al.,* 2012:112).

Diabetes was considered to be a constitutional disease in ancient times. It was characterised by the light colour of the urine which had a sweet odour and it was proposed that one would taste the sugar if it was tasted (King & Rubin, 2003:1091). Due to the sweetness of the urine in individuals with diabetes, the word mellitus, which is the Latin word for honey, was added to the term diabetes (Widmaier *et al*., 2006:628; King & Rubin, 2003:1091).

During the 16th century, Paracelsus described diabetes as a general disorder, although there was still uncertainty about the cause of this disease. In 1675 Thomas Willis, a British physician, was the first European to taste the urine of a patient with diabetes, finding that it was sweet. He was the first to recommend a diet high in carbohydrates and low in calories with milk, water, bread and barley. In 1697, he changed his recommendation to a diet consisting mainly of meat, high in fat and high in protein, but low in carbohydrates. Still, very little was known about the disease (King & Rubin, 2003:1092).

In the 17th century Dr Matthew Dobson further confirmed the findings of Thomas Willis, and also confirmed that urine was not only sweet, but that it also had a crystalline characteristic when boiled. He also found that sugar was present not only in the urine, but also in the blood of individuals with diabetes. In 1788 Thomas Cawley, a researcher, described the association between the pancreas and diabetes (King & Rubin, 2003:1092). In 1870, during the Franco-Prussian War, a French physician noted a reduction in glycosuria when the portion of food given to the soldiers was altered (King & Rubin, 2003:1092).

By the 19th century individuals presenting with glycosuria were diagnosed with diabetes mellitus. An important breakthrough in the history of diabetes occurred early in the 19th century, when Claude Bernard hypothesised that glycogen was stored in the liver and that the liver secreted a substance that was sugary into the blood – it was this overproduction of glucose that he considered caused diabetes mellitus (King & Rubin, 2003:1094). In 1869, a medical student named Paul Langerhans discovered islet cells in the pancreas. He died in 1888, without having explained them or discovering their importance. In 1889 two Germans, Joseph von Meiring, a pharmacist, and Oscar Minkowski, a diabetologist, discovered that when the pancreas is removed from the body, diabetes develops. This then led Gustave Laguesse, a French doctor, to discover in 1893 that the islet cells were involved in a role other than secretion to aid digestion, and named them the islets of Langerhans (King & Rubin, 2003:1094). After this historic breakthrough about the islets of Langerhans, Moses Barron, while doing an autopsy on a man with diabetes, discovered that the islets of Langerhans were damaged. He realised that this must be the cause of diabetes, and found that the substance from these cells was treatment for diabetes. In 1910 Sir Edward Albert Sharpey-Schafer, a physiologist, named this substance insulin, the Latin word for island. Thereafter Frederick Banting and Charles Best, in collaboration with John Macleod at the University of Toronto, tried to isolate and extract insulin from healthy dogs and inject it into diabetic dogs, a procedure which proved to be flawed (King & Rubin, 2003:1094). After collaborating with James Collip, a biochemist, who extracted a pure form of insulin from the pancreas of cattle, Banting and Best finally won the acclaim (King & Rubin, 2003:1095). In 1922 insulin was first isolated from the pancreas by Banting and Best. This changed the outlook for an individual with diabetes from certain death to a

nearly normal lifestyle (Guyton & Hall, 2006:961). Leonard Thompson was the first person to receive Banting and Best's insulin. Thompson lived very well, gaining weight and strength. In 1923 Banting and Macleod received the Nobel Prize in physiology/medicine for discovering insulin. Banting shared his prize with Best and Macleod shared his prize with Collip (King & Rubin, 2003:1095).

2.3 CLASSIFICATION OF DIABETES MELLITUS

Diabetes is mainly classified into two types, although other types are also identified. The two major types are Type 1 and Type 2 diabetes mellitus (T1DM and T2DM) (Amod *et al.*, 2012:S6; Guyton & Hall, 2006:972). Other types of diabetes include gestational diabetes mellitus (GDM), which refers to hyperglycaemia where first recognition or onset is identified during pregnancy, and also genetically defined forms of diabetes, diseases of the pancreas, drug- or chemical-induced diabetes, and diabetes caused by surgery, infections, and other illnesses/diseases. These other types however are quite rare (Amod *et al.*, 2012:S6).

In T1DM, the primary defect, pancreatic β-cell destruction, usually leads to an absolute insulin deficiency, resulting in hyperglycaemia, polyuria and polydipsia, weight loss, dehydration, electrolyte disturbance and ketoacidosis (Amod *et al.*, 2012:S5; Franz, 2012:676; Widmaier *et al.*, 2006:628). A healthy pancreas is capable of secreting much more insulin than what is normally needed; therefore there can be an extensive asymptomatic period of months leading to years, during which β-cells are undergoing gradual destruction before the clinical onset of diabetes (Franz, 2012:677; Widmaier *et al*., 2006:628). Of all diagnosed diabetes cases, only 5 to 10% are T1DM. Individuals with T1DM depend on exogenous insulin to prevent ketoacidosis and death. Even though T1DM may occur at any age, the majority of cases are diagnosed in individuals younger than the age of 30 years, with a peak incidence at ages 10 to 12 years in girls and 12 to 14 years in boys (Franz, 2012:676; Guyton & Hall, 2006:972). T1DM presents in two forms: immune-mediated diabetes mellitus and idiopathic diabetes mellitus. Immune-mediated diabetes mellitus is caused by an autoimmune destruction of the β-cells of the pancreas, the only cells in the body that produce the hormone insulin, which

regulates blood glucose levels. Idiopathic diabetes mellitus refers to forms of the disease that have an unknown etiology (Franz, 2012:676). Therefore, diabetes resulting from an autoimmune process and diabetes for which the etiology of β-cell destruction is unknown, are both classified as T1DM (Amod *et al.*, 2012:S6).

This study will focus on T2DM. T2DM is the most common type of diabetes that may account for 90-95% of all diagnosed cases of diabetes (Amod *et al.*, 2012:S6; Guyton & Hall, 2006:974). T2DM is dominated by disorders of insulin action (insulin resistance). Cellular sensitivity to insulin is lower than normal, with insulin deficiency relative to a prevailing secretory defect (Amod *et al.*, 2012:S6; Widmaier *et al*., 2006:628). Insulin resistance in peripheral tissues is a high-risk factor and nearly always precedes the development of T2DM (Ostegard *et al.*, 2005:99). T2DM risk factors include genetic and environmental factors. Genetic and environmental risk factors shown to influence the incidence of T2DM includes foetal or intrauterine malnutrition, a family history of diabetes, older age, obesity, intra-abdominal obesity, physical inactivity, a prior history of gestational diabetes, pre-diabetes and ethnicity (Franz, 2012:678; Beck-Nielsen *et al*., 2003:463). Adiposity and obesity are important risks factors for T2DM; small amounts of weight loss are associated with improved glycaemic control in individuals with pre-diabetes (Franz, 2012:679; Widmaier *et al.*, 2006:629). Obesity, in individuals with a genetic predisposition, seems to be the main contributing factor to the development of T2DM. Another possibility is that a high risk genetic predisposition leads independently to obesity and insulin resistance, which increases the risk for T2DM (Franz, 2012:679). The pathogenesis of T2DM is therefore recognised as multifaceted, involving both lifestyle and genetic predisposition (Ostegard *et al*., 2005:99).

Some individuals with T2DM may experience the classic symptoms of uncontrolled diabetes, whereas others will not experience any symptoms, and usually they do not develop ketoacidosis. Although initially individuals with T2DM do not require exogenous insulin for survival, with time and with the loss of β-cell secretion function, more individuals with T2DM will eventually require exogenous insulin for adequate blood glucose control (Franz, 2012:679).

2.4 NORMAL PHYSIOLOGY OF INSULIN SECRETION AND INSULIN ACTION

The energy requirements of humans are met mainly by glucose and fats (Dawson, 2010:943; Yu *et al*., 2005:311). Energy is produced from endogenous glycogen stores in the muscles and liver or manufactured from substrates like amino acids and lactate. Glucose is supplied to the bloodstream from the gastrointestinal tract and liver. The plasma membranes of cells are permeable to glucose, and the diffusion of glucose into cells is controlled by glucose transporters (GLUT 1-4) specific to each tissue. The glucose transporters of endothelial cells of the brain and erythrocytes (GLUT 1) do not need activation with the hormone insulin. The heart, adipose, and skeletal muscle cells have insulin receptors on the cell membrane that bind to insulin and activate glucose transporters (GLUT 4), increasing glucose transport immediately in threefold. Activated glucose transporters translocate to the cell membrane and facilitate the diffusion of glucose (Dawson, 2010:943; Widmaier *et al.*, 2006:622; Yu *et al*., 2005:314).

Insulin has multiple and various metabolic and vascular effects on the body and is an important controller of organic metabolism (Katsilambros *et al.,* 2006:43; Widmaier *et al*., 2006:620). Insulin regulates glucose metabolism by promoting glucose uptake in insulin-sensitive tissues, muscle cells and adipose tissue, and by inhibiting hepatic glucose production by inhibiting glycogenolysis and gluconeogenesis and promoting glycogen synthesis in the liver (Widmaier *et al*., 2006:620; Yu *et al*., 2005:317-318).

Insulin, a peptide hormone, is composed of two chains, alpha (α) and beta (β), with a total of 51 amino-acid residues with 30 chains in the α chain and 21 in the β chain, and is secreted by the islets of Langerhans (Katsilambros *et al.,* 2006:50; Widmaier *et al*., 2006:620). Insulin is produced in the β-cells of the pancreas. β-cells are specialised cells inside the special cellular aggregates in the pancreas known as the islets of Langerhans (Dawson, 2010:943; Katsilambros *et al.,* 2006:50; Widmaier *et al*., 2006:620; Yu *et al*., 2005:313). β-cells have a variety of cellular receptors for different peptides, hormones and neurotransmitters that can affect insulin secretion. Even insulin affects glucose-dependent secretion by the β-cells, through special insulin receptors on the surface of the β-cells (Katsilambros *et al.,* 2006:44). The islets of Langherhans also contain other types of cells that produce a variety of hormones, such as glucagon (α-cells), somatostatin (d-cells) and pancreatic

polypeptides (PP cells), which communicate with each other through a neurovascular network of arterioles and autonomous nerves. Primarily, insulin is composed of pre-pro-insulin in the ribosomes of the rough endoplasmic reticulum, to be rapidly converted to pro-insulin, which is a mixture of insulin and C-peptide, after the splitting of a small part from the molecule. Pro-insulin is then transferred to the Golgi apparatus of the cell, where it is stored in special secretory granules and stays in this form inside the cytoplasm of the cell until a stimulus for secretion is applied to the granules (Dawson, 2010:943; Katsilambros *et al.,* 2006:50; Yu *et al*., 2005:312). Pro-insulin is then split into equi-molar amounts of insulin and connecting peptide (C-peptide) and is excreted from the cell, with only small amounts of pro-insulin normally secreted. The pancreas produces and secretes insulin continuously in a pulsatile way over 24 hours, approximately every 9 to 14 minutes (Guyton & Hall, 2006:962; Katsilambros *et al.*, 2006:50). The basal secretion is intended to regulate hepatic glucose production, which includes glycogenolysis and gluconeogenesis, which in the case of insulin shortage remains unconstrained, and is the main cause of fasting hyperglycaemia in diabetes (Katsilambros *et al.*, 2006:50).

The basic stimulus for insulin secretion, however, is plasma glucose concentration after a meal (Widmaier *et al.*, 2006:623). The β-cells are able to determine plasma glucose concentration continuously and adapt insulin secretion accordingly. This combination of plasma glucose concentration with insulin secretion is attained through the ability of glucose, with the help of special glucose-transporters (GLUT2), to enter the β-cell freely and then to oxidise itself in the mitochondria and produce energy in the form of adenosine triphosphate (ATP). Special potassium channels on the cell-surface close as a result of the increased intracellular concentration of ATP, which leads to depolarisation of the cell membrane and opening of special calcium channels in the cell membrane. Due to the entry of calcium into the cell, the intracellular calcium concentration increases and causes exocytosis of the vesicles with the stored insulin (Dawson, 2010:943; Katsilambros *et al.*, 2006:51; Yu *et al.*, 2005:314).

As already mentioned, insulin is very important for the initial reduction or inhibition of glycogenolysis and gluconeogenesis and for promotion of glycogen synthesis in the liver. Insulin secretion from the pancreas occurs directly into the portal vein and

therefore is transferred initially to the liver, after a meal. It is estimated that about 25% to 50% percent of an oral glucose load is taken up by the liver and stored as glycogen, while the remainder is distributed mostly between the muscle (80% to 85%) and adipose tissue (10% to 25%) (Katsilambros *et al.,* 2006:44). Apart from glucose, which is the most significant stimulus for insulin secretion, an increase in circulating levels of amino acids, free fatty acids and the gastrointestinal hormones, glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptides or glucose-dependent insulinotropic peptides (GIP) also promote insulin secretion. These substances notify the pancreas about the presence of glucose into circulation after a meal and promote both production and secretion of insulin (Dawson, 2010:943; Widmaier *et al.*, 2006:624; Katsilambros *et al.,* 2006:51). In contrast, an increase of other factors, such as catecholamines, cortisol, growth hormone, leptin and tumour necrosis factor-a, decreases insulin secretion (Dawson, 2010:943; Katsilambros *et al.*, 2006:52; Widmaier *et al.*, 2006:624; Yu *et al.*, 2005:318). Since it was found that GLP-1 is decreased in T2DM, interest in this substance or analogues of it for therapeutic use in diabetes has increased (Katsilambros *et al.*, 2006:52; Yu *et al.*, 2005:315). Recent reports suggested that GLP-1 has an associated risk with pancreatitis, however this is not clear yet. GLP-1 suppresses glucagon secretion and slows gastric motility, it is not associated with hypoglycaemia, but causes a high frequency of gastrointestinal disturbances (Nathan *et al.*,2009:197).

Insulin secretion from the β-cell occurs after a meal in two phases: there is a quick (5 to 6 minutes) first phase, intending to suppress hepatic glucose production, and a more prolonged second phase of lower intensity, promoting entry of plasma glucose into insulin-sensitive cells, primarily the muscle cells and adipocytes. Insulin secretion in the first phase is derived from insulin stored in vesicles found near or in contact with the β-cell membrane, while the second phase insulin comes from newly synthesised insulin or insulin stored in vesicles deeper in the cytoplasm (Katsilambros *et al.*, 2006:52; Yu *et al.*, 2005:317). The first phase of insulin release is the first to be disrupted during the early phase of diabetes. The quantity of pro-insulin secreted from the pancreas increases in diabetes by up to 30 to 40%, which suggests that there is possibly a disturbance either in the secretion or in the process of insulin production inside the β-cell (Katsilambros *et al.*, 2006:52; Yu *et al.*, 2005:318).

The effect of insulin on lipid metabolism is also very significant. Insulin stimulates lipid synthesis via an increase of endothelial lipoprotein lipase activity, promotes lipogenesis, inhibits lipolysis in adipose tissue and inhibits very-low-density lipoprotein (VLDL) synthesis in the liver. Insulin also stimulates protein synthesis and the transfer of amino acids into muscle and liver cells. The effect of all these actions of insulin after a meal is a reduction in the plasma levels of glucose, triglycerides and free fatty acids (Dawson, 2010:944; Katsilambros *et al.*, 2006:44; Yu *et al.*, 2005:318).

2.5 PATHOPHYSIOLOGY OF TYPE 2 DIABETES MELLITUS

T2DM, a heterogeneous syndrome, has a multifaceted interaction of genetic and environmental factors, affecting multiple phenotypic manifestations in the body (Katsilambros *et al.*, 2006:43; Yu *et al.*, 2005:318). T2DM is usually characterised by resistance to insulin action and insufficient secretion of insulin from the β-cells of the pancreas in response to a rise in plasma glucose concentration. Both of these are necessary for the development of the T2DM (Franz, 2012:679; Katsilambros *et al.,* 2006:43; Widmaier *et al*., 2006:629).

2.5.1 Insulin resistance and insufficient secretion

Insulin resistance is the incapability of insulin of performing its usual biological effect on circulating plasma glucose levels that are effective in normal subjects, causing decreased tissue sensitivity or responsiveness to insulin (Franz, 2012:679; Katsilambros *et al.*, 2006:45; Widmaier *et al*., 2006:629). The most important and measurable physiological effect of insulin is on glucose, consequently the effects of insulin on carbohydrate metabolism (Yu *et al*., 2005:318). Insulin resistance is expressed as an insufficient uptake of glucose by the muscles and adipose tissue and the inability of insulin to suppress hepatic glucose production (Franz, 2012:279; Katsilambros *et al.*, 2006:45).

T2DM development can be classified into three stages, namely: normal glucose tolerance, impaired glucose tolerance, and clinical manifestation of diabetes (Katsilambros *et al.*, 2006:46). The first evidence of insulin resistance is found in the target tissues, mainly muscle, liver, and adipose cells (Franz, 2012:679). It is for this reason that plasma insulin levels are usually increased long before the development of diabetes (Katsilambros *et al.*, 2006:46). In most cases the pancreatic β-cells are unable to continue to produce sufficient insulin, hyperglycaemia presents and diabetes is diagnosed (Guyton & Hall, 2006:975). It is for this reason that insulin levels are deficient relative to elevated glucose levels before hyperglycaemia develops (Franz, 2012: 679).

Hyperglycaemia first occurs by an elevation of postprandial blood glucose, which is caused by insulin resistance at cellular level. This is followed by an increase in fasting glucose concentrations. As insulin secretion decreases, hepatic glucose production increases, which causes an increase in pre-prandial blood glucose levels. The insulin response can also not suppress the α-cell glucagon secretion, which results in glucagon hypersecretion and increased hepatic glucose production (Franz, 2012: 679).

Insulin resistance is also seen at adipocyte level, resulting in lipolysis and an increase in circulating free fatty acids (Yu *et al.*, 2005:318). Intra-abdominal obesity, which refers to an excess accumulation of visceral fat around and inside abdominal organs, causes increased free fatty acids to the liver, leading to an increase in insulin resistance. Increases in fatty acids also cause a reduction in insulin sensitivity at cellular level, impair pancreatic insulin secretion, and enhance hepatic glucose production. All of these contribute to the development of T2DM (Franz, 2012:679).

Insulin resistance always precedes β-cell failure; with the result that hyperinsulinaemia is observed long before impaired glucose tolerance is apparent, which at first led to the belief that impaired insulin secretion develops later, secondary to peripheral insulin resistance. However, many obese, non-diabetic individuals, with insulin resistance, never develop diabetes or impaired glucose tolerance, indicating that the pancreases of these individuals are able to secrete enough insulin to conquer peripheral resistance. Insulin resistance therefore is not sufficient by itself to lead to dysfunction, insufficient secretion of the β-cell, and later

to diabetes. For this reason, it can be concluded that for development of DM, both of these pathophysiologic disturbances, which act simultaneously and independently, on peripheral tissues and the pancreas are necessary (Katsilambros *et al.*, 2006:46).

Kahn (2003:3) emphasises that β-cell function is a major determinant of oral glucose tolerance in subjects with normal and reduced glucose tolerance and that in all populations the progression from normal to impaired glucose tolerance and subsequently to T2DM is associated with declining insulin sensitivity and β-cell function. The genetic and molecular basis for these reductions in insulin sensitivity and β-cell function are not fully understood but it seems that body-fat distribution and especially intra-abdominal fat are important determinants of insulin resistance, while reduction in β-cell mass contribute to β-cell dysfunction (Kahn, 2003:3).

2.6 ETIOLOGY OF DIABETES

All diseases are linked to information coded in genes – directly or indirectly, which also applies to diabetes (DeBusk, 2012:146). Foetal programming and the thrifty gene hypothesis, resulting from poor intrauterine growth and low birth weight as etiology for T2DM, is also explained by Hales and Barker (2001:7). Family history, aging, behavioural and lifestyle risk factors, including dietary risk factors and physical inactivity, all play a role in the etiology of diabetes and will be discussed (Amod *et al.*, 2012:S18; Sheen, 2005:32; Wild *et al.*, 2004:1050; North *et al.*, 2003:1447; Hu *et al.*, 2001:793).

2.6.1 Genetic influences and family history

Various disease conditions are directly connected to the genetic make-up of an individual, which influences health (DeBusk, 2012:148). Diet- and lifestyle-related disorders that result from these interactions, are the focus of nutritional genomics, which includes nutrigenetics and nutrigenomics (DeBusk, 2012:148).

Research on genetic factors that underlie insulin resistance indicates that heredity explains an extensive proportion of the variability of the factors and emphasises the

fact that it should receive attention in prevention strategies for relatives of people with insulin resistance (North *et al.*, 2003:1447). A study by Van't Riet *et al.* (2010:766-767) over 20 years and including 73,227 women, shows that having a family member with T2DM is a very strong risk factor for T2DM and a higher body mass index (BMI). The association between having a family history of DM and the incidence of T2DM is very strong with a first-degree family member, with a similar risk associated with having a paternal or maternal history of diabetes (Van't Riet *et al.*, 2010:766-767). A Chinese study (Lee *et al.,* 2001:649) shows that the age of a person at the onset of T2DM is influenced by family history and metabolic factors. It can therefore be concluded that family history and heredity of T2DM is an important risk factor for the development of T2DM.

2.6.2 Foetal programming and the thrifty gene hypothesis

The basic principle of the thrifty gene hypothesis is that poor foetal nutrition drives foetal programming (Hales & Barker, 2001:7). Poor maternal nutrition which results in poor foetal nutrition is the cause for poor development of pancreatic β-cell mass and islet function, linking poor early nutrition to T2DM later in life (Hales & Barker, 2001:7).

Foetal nutrition seems to set the mechanisms for foetal nutritional thrift, resulting in a differential impact on the growth of different organs, with the protection of brain growth. This growth altering to adapt to the inadequate foetal environment permanently changes organ structures and also the way the body functions (Hales & Barker, 2001:7).

Hales and Barker (2001:15) explain that foetal malnutrition not only impacts on protection of important organs, but also leads to metabolic adaption to ensure postnatal survival. Therefore the thrifty phenotype is not only thrifty with respect to antenatal life, but also with regard to the use of nutritional resources in the postnatal environment. The poorly nourished mother provides the foetus with a prediction of the nutritional environment into which it will be born, setting processes in action which lead to a postnatal metabolism adapted to survival under poor nutritional conditions. These adaptations become harmful when the postnatal environment

differs from the mother's "predictions", with an oversupply of nutrients (Hales & Barker, 2001:15).

2.6.3 Intrauterine growth retardation and low birth weight

Various studies have explained the association between poor intrauterine growth and low birth weight with T2DM. In a French study intrauterine growth retardation was associated with a decreased insulin-stimulated glucose uptake, although no evidence for major β-cell function impairment could be found (Jaquet *et al.*, 2000:1406). In a study on twins, investigating genetic versus the environmental causes of the development of T2DM, it was found that both play a role in the development of diabetes, but that environmental factors, like intrauterine malnutrition, seemed to be more important. It was shown that T2DM does not develop before environmental factors, such as intrauterine malnutrition, are added to the genetic predisposition (Beck-Nielsen *et al.*, 2003:461). In a study by Pulizzi *et al.* (2009:825) the interaction between birth weight and common gene variants and the risk of developing T2DM were investigated. Results from this study indicate that low birth weight might affect the strength of the association of some common gene variants with T2DM (Pulizzi *et al.*, 2009:828). These evidence-based studies therefore conclude that intrauterine growth retardation and low birth weight do play a role in the development of T2DM.

2.6.4 Age

Even in the unlikely event of the prevalence of obesity remaining static until 2030, it is anticipated that the number of people with diabetes will more than double as a consequence of population aging and urbanisation. With the increase in prevalence of obesity across the world and the role obesity plays as a risk factor for diabetes, the increase in the number of cases of diabetes may be significantly higher (Wild *et al*., 2004:1050).

Based on the knowledge that elderly people are more glucose intolerant and insulin resistant than younger people, and that many will develop T2DM, Sheen (2005:28)

conducted a study to investigate whether this decrease in function is due to biological aging or due to environmental or lifestyle variables. The conclusion from the study was that the development of glucose intolerance is a well-established part of the human aging process. The deterioration in glucose metabolism is ascribed to diminished sensitivity to insulin and its target tissues and to lowered pancreatic *β*-cell function, explaining the higher risk for glucose intolerance and T2DM with aging. Lifestyle modifications, including weight loss and physical activity, however, show health benefits and can improve insulin sensitivity and prevent T2DM with aging (Sheen, 2005:32). Research by Ihm *et al*. (2007:S154) shows that with increasing age the insulin secretory function of islets in response to glucose deteriorates and expression of insulin synthesis/secreted-related genes decreases.

Aging therefore contributes to the development of T2DM by increasing the risk for glucose intolerance and decreased beta cell function.

2.6.5 Behavioural and lifestyle-related risk factors

In a large cohort, Hu *et al*. (2001:793) showed that a combination of lifestyle related factors, including maintaining a body mass index of 25kg/m^2 or lower, eating a diet high in fibre and polyunsaturated fat and low in saturated and trans fat and glycaemic load, exercising regularly, and consuming alcohol moderately was associated with a 90% lower occurrence of T2DM.

2.6.5.1 Obesity

Obesity is described by Lois and Kumar (2009:38) as an imbalance between energy expenditure and intake, resulting in excess energy storage in the form of fat. The link between obesity and diabetes can be seen by the parallel increase in these two conditions. Both of these metabolic disorders have defects with regard to insulin action (Lois & Kumar, 2009:38).

Obesity leads to some degree of insulin resistance in individuals, with or without diabetes, specifically in muscle and adipose tissue cells (Widmaier *et al.,* 2006:629).

Insulin resistance is associated with obesity, especially when adipose tissue is centrally distributed. The link between increased adiposity and insulin resistance is still being investigated, and evidence suggests that adipose tissue-released cytokines like adiponectin, resistin, leptin and tumour necrosis factor-α (TNF α) may be contributing factors that change insulin secretion and insulin action (Sokkar *et al.*, 2009:79). The theory is that too much adipose tissue overproduces a messenger that results in down-regulation of insulin-responsive glucose transporters or blocks insulin action (Widmaier *et al.*, 2006:629). Insulin resistance in obese individuals leads to failure of the pancreatic β-cells, which is suggested as the pathogenic mechanism of obesity leading to diabetes (Lois & Kumar, 2009:38).

Hu *et al.* (2001:793) indicate in their study that the number of cases of diabetes that can be prevented by diet and exercise is greater among women with a normal body weight than obese women. A study by Oguma *et al.* (2005:950) indicates that any weight gain, even in those with a healthy weight, increases the risk for T2DM Hossain *et al.* (2007:215), which also confirms that changes in lifestyle that lead to weight loss, reduce the incidence of diabetes and hypertension.

2.6.5.2 Fat intake

Weigensberg *et al.* (2005:1633), in a study on children aged 6.5 to 14 years, found that a fat intake higher than the recommended intake was associated with lower insulin sensitivity, independent of body fat and gender.

Salmerón *et al.* (2001:1025) found that total fatty acid, saturated fatty acid and monounsaturated fatty acid intake are not associated with T2DM, but that trans-fatty acid intake increases the risk for T2DM and polyunsaturated fatty acid intake decreases the risk for T2DM.

Micha and Mozaffarian *et al*. (2010:898) conducted a review on the association between saturated fat and cardiometabolic risk factors, coronary heart disease, stroke, and diabetes. In this review no difference in markers of glucose-insulin homeostasis were found in healthy individuals when comparing intakes of saturated fatty acids with monounsaturated fatty acids, polyunsaturated fatty acids or

carbohydrates. In individuals predisposed to insulin resistance or having DM it was found in two large trials that saturated fatty acids intake worsened the glucose-insulin homeostasis in comparison to monounsaturated fatty acids or carbohydrates. In short term studies some researchers suggest an association of saturated fatty acids with DM. In long term studies, however, researchers found no association between saturated fatty acid consumption and the onset of diabetes (Micha & Mozaffarian, 2010:898).

2.6.5.3 Physical inactivity

Physical inactivity increases the risk of diabetes, independent of obesity, and should form part of the prevention and treatment plan for individuals at risk of or with T2DM. Physical activity improves insulin sensitivity, reduces cardiovascular risk factors, assists with weight control, and improves well-being. Moderate to high levels of physical activity and cardio-respiratory fitness are associated with an extensive decrease in morbidity and mortality in individuals with T2DM (Amod *et al.*, 2012:S18). Hu *et al.* (2003:1785) examined the relationship between sedentary behaviour, risk for obesity and T2DM, concluding that sedentary behaviour is directly associated with obesity and T2DM risk, and that even low to moderate activity, such as standing or walking around at home and a 30 minute of brisk walking per day, will significantly lower these risks (Hu *et al.*, 2003:1791).

Blood glucose control improves with physical activity, due to decreased insulin resistance and increased insulin sensitivity, which cause an increased peripheral use of glucose during and after activity, independent of any effect on body weight. Exercise also increases the effects of counter-regulatory proteins like GLUT 4 which will translocate to the cell membrane and facilitate the diffusion of glucose, increasing glucose transport, which reduces the hepatic glucose output, contributing to improved glucose control (Franz, 2012:688; Dawson, 2010:943; Widmaier *et al.*, 2006:622; Yu *et al.*, 2005:314). It can therefore be concluded from research evidence, that physical inactivity increases the risk for T2DM.

2.6.5.4 Alcohol consumption

Mozaffarian *et al.* (2009:804) examined lifestyle factors over a period of ten years in four communities in the United States. The results associated alcohol intake independently with diabetes incidence among adults. Contrary to this finding, a cardiovascular health survey found that light to moderate alcohol intake, up to six drinks per week, was associated with a lower incidence of diabetes in the elderly (Djoussé *et al.*, 2007:1758). Other researchers also support moderate alcohol consumption to decrease the risk for T2DM in men and women (Boggs *et al.*, 2010:960; Imamura *et al.*, 2009:43; Baliunas *et al.*, 2009:2131). Joosten *et al.* (2010:1777) support this by reporting that moderate alcohol consumption is associated with a 40% lower risk for T2DM when compared to no alcohol consumption. Therefore, it can be concluded from the majority of studies that a low to moderate consumption of alcohol decreases the risk for T2DM.

2.7 SCREENING AND DIAGNOSIS OF DIABETES MELLITUS

The tests used for screening and diagnoses of diabetes are the same. Screening for diabetes is only recommended in a health care setting (Amod *et al.*, 2012:S9). In a health care setting, screening with a portable blood glucose meter is the most practical (Woolthuis *et al.*, 2007:201). Diabetes is usually identified when there is clinical symptoms of diabetes during random screening, opportunistic screening or targeted screening; then it also expands further to individuals with clinical features of diabetes who undergo testing because of suspicion, and also the individuals with classic symptoms or metabolic decompensation (Amod *et al.*, 2012:S9).

In the next section of this literature review, diagnostic criteria and benchmarks for the diagnosis of diabetes will be discussed. Four methods are mostly used to diagnose diabetes, namely the random plasma glucose (RPG) test, the glycosylated haemoglobin (HbA1c) test, the fasting plasma glucose (FPG) test and the two-hour plasma glucose (2-h PG) test. For the first two tests no fasting is needed and the test can be taken any time of the day. For the other two tests, fasting is necessary to ensure accurate interpretation of results (Amod *et al.*, 2012:S7; Codario, 2005:18).

2.7.1 Random plasma glucose (RPG)

Random refers to any time during the day, without any regard to when a meal was last eaten. This test diagnoses diabetes at a plasma glucose value of higher than 11 mmol/l, if classic symptoms of diabetes or a hyperglycaemic crisis are present. Classic symptoms of hyperglycaemia include polyuria, polydipsia and weight loss. A hyperglycaemic crisis refers to diabetic ketoacidosis or hyperosmolar non-ketotic hyperglycaemia (Amod *et al.*, 2012:S7; Codario, 2005:18).

2.7.2 Glycosylated haemoglobin (HbA1c)

When haemoglobin and other proteins are exposed to glucose, glucose attaches to the protein in a slow, non-enzymatic, and concentration-dependent manner. Glycosylated haemoglobin (HbA1c) is a standardised and reliable measure of chronic glucose levels and reflects a weighted average of plasma glucose concentrations over time (Franz, 2012:6810). HbA1c reflects the average blood glucose levels for the preceding 2 – 3 months, and does not reflect recent changes in glucose levels (Litchford, 2012:203). HbA1c is expressed as percentage of total haemoglobin. An HbA1c above 6.5% indicates diabetes (Litchford, 2012:203), with the World Health Organization (WHO) and the American Diabetes Association (ADA) both recommending that an HbA1c concentration of 6.5% or higher can be used to diagnose diabetes (World Health Organisation, 2011; Amod *et al.*, 2012:S4; ADA, 2010). Gomez-Perez *et al.* (2010:307) also recommends HbA1c as a marker for glycemic control should be used as a prognostic tool for future complications.

2.7.3 Fasting plasma glucose (FPG)

Fasting is defined as no energy intake for at least eight hours (Amod *et al.*, 2012:S7). The Fasting plasma glucose (FPG) test measures plasma glucose levels and will indicate impaired fasting glucose levels at a value between 6.1 - 6.9 mmol/l and diabetes at > 7 mmol/l (Amod *et al.*, 2012:S7; Guyton & Hall, 2006:975; Codario, 2005:18).

2.7.4 Two-hour plasma glucose during oral glucose tolerance test (2-h PG)

For the 2-hour plasma glucose oral glucose tolerance test (2-h PG), the WHO prescribes using a glucose load containing the equivalent of 75g anhydrous glucose dissolved in 250 ml water ingested over five minutes. This test indicates a normal fasting glucose with plasma glucose < 7.8 mmol/l, impaired glucose tolerance between 7.8 and 11.0 mmol/l, and diabetes at > 11 mmol/l (Amod *et al.*, 2012:S7; Codario, 2005:18).

A single abnormal test is adequate to confirm the diagnosis of diabetes in symptomatic individuals with hyperglycaemia or unequivocal hyperglycaemia; however hyperglycaemia in conditions of acute infective, traumatic, cardiovascular or other stressors, should not be considered as diagnostic of diabetes until confirmed afterwards (Amod *et al.*, 2012:S8; Guyton & Hall, 2006:975).

Diagnosis in asymptomatic individuals or uncertain hyperglycaemia should not be based on a single abnormal test result (Amod *et al.*, 2012:S8). It is advised that a random plasma glucose test or HbA1c be done, and if the result is abnormal, the same test be repeated on another day (Amod *et al.*, 2012:S9).

2.8 CLINICAL SIGNS AND SYMPTOMS

Diabetes may present with symptoms such as thirst, polyuria, blurring of vision and weight loss (Amod *et al.*, 2012:S5). Dehydration triggers compensatory responses, including the release of antidiuretic hormone and triggering of the thirst mechanism. Due to diuresis, antidiuretic hormone is ineffective in decreasing water loss through kidneys, resulting in polydipsia (Casey, 2011:19). When the amount of glucose that is filtered by the kidneys overwhelms reabsorption mechanisms, polyuria occurs (Casey, 2011:19). Most of these manifestations are the result of the presence of excess glucose. Osmotic changes in fluid levels in the eyes may cause blurred vision, with long term damage causing retinopathy. Due to the symptoms often being very slight, a person with T2DM may present very late with diabetic complications already present (Casey, 2011:19). The most severe clinical sign of T2DM is

ketoacidosis or non-ketotic hyperosmolar state, which may lead to stupor, coma, and, in the absence of treatment, death (Amod *et al.*, 2012:S5).

2.9 COMPLICATIONS OF DIABETES MELLITUS

Prevention of microvascular and macrovascular complications requires maintaining optimal blood glucose levels and reaching target HbA1c levels (Garg & Ulrich, 2006:117). When target HbA1c levels and optimal blood glucose levels are not achieved and maintained, diabetic complications result. Two types of diabetic complications are distinguished: acute and chronic complications.

2.9.1 Acute complications of diabetes mellitus

The most common acute complications related to diabetes are hypoglycaemia and hyperglycaemia, with or without diabetic ketoacidosis.

2.9.1.1 Hypoglycaemia

Hypoglycaemia, also referred to as low plasma glucose concentration, is a general side effect of insulin therapy. Blood glucose levels of 3.8 mmol/l or lower should be treated immediately (Franz, 2012:702; Widmaier *et al*., 2006:630). General causes of hypoglycaemia include inappropriate medication dosages; excessive and intensive insulin dosages; improper timing of insulin injection in relation to food intake; inadequate or delayed food intake including snacks and meals; unplanned, excessive or prolonged duration of physical activity; and alcohol intake without food (Franz, 2012:702; Widmaier *et al*., 2006:630).

Autonomic symptoms are usually the first signs of mild hypoglycaemia. Other symptoms include adrenergic symptoms, like shakiness, sweating, palpitations, anxiety, nervousness and hunger. Neuroglycopenic symptoms can also occur, resulting from an insufficient supply of glucose to the brain. The first signs of neuroglycopenia include a slowdown in performance, poor concentration and

difficulty with reading, headache and dizziness. As the blood glucose level decreases, frank mental confusion and disorientation occur, and slurred or rambling speech, irrational or unusual behaviours, extreme fatigue, lethargy, seizures and unconsciousness can present (Franz, 2012:702; Widmaier *et al*., 2006:630).

2.9.1.2 Hyperglycaemia and diabetic ketoacidosis

Hyperglycaemia usually results from alterations in dietary intake, inactivity, inadequate use of anti-diabetic medication, or any combination of these factors (Dawson, 2010 952). Fasting hyperglycaemia occurs frequently in individuals with diabetes. The amount of insulin needed to normalise blood glucose during the night is less in the predawn period (1:00 to 3:00) than in the dawn period (4:00 to 8:00). This increased insulin need at dawn often causes a rise in fasting blood glucose levels, referred to as the dawn phenomenon. It results when insulin levels are inadequate between predawn and dawn or when hepatic glucose output overnight becomes excessive, as often seen in T2DM (Franz, 2012:703). Hypoglycaemia followed by hyperglycaemia in response is called the Somogyi effect. The Somogyi effect occurs with hypoglycaemia after secretion of counteracting regulatory hormones and is mostly caused by excessive exogenous insulin doses. Hepatic glucose production is then stimulated which raises blood glucose levels (Franz, 2012:704).

Diabetic ketoacidosis (DKA) is a life-threatening complication of DM, which is reversible. DKA is characterised by elevated blood glucose levels (greater than 13.8 mmo/l, but less than 33.3 mmol/l) and the presence of ketones in the blood and urine (Franz, 2012:703). Insulin deficiency is combined with counter-regulatory hormone excess in DKA (Thripathi & Srivastava, 2006:137). DKA always refers to insufficient insulin for glucose use. Glucogeogenesis, glycogenolysis, and ketone body formation in the liver is promoted by the decreased ratio of insulin to glucagon; it also increases fatty acids and amino-acid delivery from fat and muscle to the liver (Thripathi & Srivastava, 2006:137). As a result, the body will rely on fat for energy and ketones will form (Franz, 2012:703). Ketosis will result from an increase in fatty acid release from adipocytes due to increased lipolysis (Thripathi & Srivastava,

2006:137). Acidosis results from increased production and decreased use of acetoacetic acid and 3-*beta*-hydroxybutyric acid from fatty acids. These ketones will then be present in the urine (Franz, 2012:70). Symptoms of DKA include polyuria, polydipsia, hyperventilation, dehydration, the fruity odour of ketones, and fatigue. Coma and death can be the result if DKA is left untreated (Franz, 2012:703).

2.9.2 Long-term complications of diabetes mellitus

Long-term complications of DM include macrovascular complications, microvascular complications and diabetic neuropathy (Amod *et al*., 2012:S60; Dawson, 2010:953).

2.9.2.1 Macrovascular disease

Macrovascular complications refer to diseases of large blood vessels providing circulation to the brain, heart and extremities. These complications include cardiovascular disease and stroke, as well as peripheral vascular disease (Dawson, 2010:953). Diabetes is an independent risk factor for coronary artery disease. Risk factors for coronary artery disease such as dyslipidaemia, hypertension and impaired fibrinolysis, are nevertheless present in uncontrolled diabetics and improve with improved blood glucose control. These risk factors may be linked to the presence of the compensatory hyperinsulinemia of T2DM (Dawson, 2010:953).

2.9.2.1.1 Dyslipidaemia

The prevalence of cardiovascular disease in individuals with T2DM is two to three times higher in men and three to five times higher in women, compared to people without diabetes (Amod *et al*., 2012:S56). Seventy percent of mortality in individuals with T2DM is due to atherosclerosis (Amod *et al.,* 2012:S57).

The prevalence of increased cholesterol levels is 28 – 34% in individuals with T2DM. In almost 5 – 14% of individuals with T2DM, triglycerides levels are elevated. Low high-density lipoprotein (HDL) levels are often seen in individuals with T2DM.

Atherogenicity is further increased in individuals with T2DM, because low-density lipoprotein (LDL) particles are denser (Franz, 2012:704).

2.9.2.1.2 Hypertension

Individuals with diabetes have lipid abnormalitites as already explained. This causes damage to the arteries and results in atherosclerosis due to the smaller and dencer LDL particles. Atherosclerosis then causes the high blood pressure (Franz, 2012:704). Hypertension is common in individuals with T2DM. To reduce the risk of macrovascular and microvascular disease, aggressive treatment of hypertension in T2DM is needed (Franz, 2012:704). The target blood pressure for individuals with T2DM is between 120/70 mmHg and 140/80 mmHg (Amod *et al.,* 2012:S60).

2.9.2.2 Microvascular disease

Microvascular complications refer to damage to the small blood vessels and include nephropathy and retinopathy (Amod *et al.,* 2012:S60; Dawson, 2010:953). Hyperglycaemia has been shown to disrupt platelet function and growth of the basement membrane. Thickening of capillary basement membranes has been shown to decrease with improved glycaemic control. Other risk factors for microvascular disease include hypertension and smoking (Dawson, 2010:953).

2.9.2.2.1 Nephropathy

The initial clinical evidence of nephropathy is the appearance of abnormal urinary albumin levels, referred to as micro-albuminuria. In the absence of intervention, progression to overt nephropathy or clinical albuminuria occurs over a period of years. Screening for micro-albuminuria should be done in individuals with T2DM at diagnosis and during pregnancy (Franz, 2012:705; ADA, 2004:S79). The characteristic lesion of diabetic nephropathy is glomerulosclerosis or thickening and hardening of the basement membrane of capillaries in the glomeruli. Filtration, an

essential kidney function, occurs in the glomerulus. The first stage of diabetic nephropathy is an increase in the glomerular flow rate, or the rate of blood flow through the glomerulus. An increased flow rate leads to hyper-filtration in the glomerulus, or a rise in the rate at which blood is filtered. The mechanisms leading to the increase in glomerular flow rate are unclear but are evidently related to poor glycaemic control. As hyper-filtration progresses, the glomeruli become damaged, leading to blockage and leaking of the capillaries, leading to micro-albuminuria. As micro-albuminuria advances, the glomerular filtration rate drops and renal failure develops. Hypertension is an important contributing factor to diabetic nephropathy (Dawson, 2010:953; ADA, 2004:S79).

The risk and progression of nephropathy can be decreased by glucose and blood pressure control. Both glycaemic control and aggressive antihypertensive treatment can decrease the development of microalbuminuria and overt nephropathy (Franz, 2012:705; ADA, 2004:S80).

2.9.2.2.2 Retinopathy

Diabetic retinopathy has three stages. In the early stage of non-proliferative diabetic retinopathy (NPDR) it presents with micro-aneurysms, pouch-like dilations of the terminal capillary, lesions that include cotton-wool spots, and the formation of new blood vessels. NPDR results from the metabolic need of the retina for oxygen and other nutrients supplied by the blood (Franz, 2012:705; Fong *et al*., 2004:S84). With progression of the disease to the middle stages of moderate, severe and very severe NPDR, gradual loss of the retinal microvasculature occurs, causing retinal ischemia. The final stage is proliferative diabetic retinopathy: the most vision-threatening stage of diabetic retinopathy, referring to the onset of ischemia-induced new vessel proliferation at the optic disk or somewhere else in the retina. These new ischemia-induced vessels are fragile and prone to bleeding, resulting in vitreous haemorrhage. Gradually neovascularisation has a tendency to undergo fibrosis and contraction, resulting in retinal traction, retinal tears, vitreous haemorrhage, and retinal detachment (Franz, 2012:705; Fong *et al*., 2004:S84). Diabetic macular oedema occurs, which involves thickening of the central portion of the retina, and glaucoma,

in which fibrous scar tissue increases intraocular pressure (Franz, 2012:705; Fong *et al*., 2004:S84).

Both glycaemic control and blood pressure control can reduce or delay the risk of diabetic retinopathy (Fong *et al.*, 2004:S86).

2.9.2.3 Neuropathy

The third complication is diabetic neuropathy (Amod *et al.*, 2012:S60; Dawson, 2010:953). Damage to the nervous system also results from chronic high blood glucose levels (Franz, 2012:706). Neuropathic complications are divided into autonomic dysfunction and sensory dysfunction (Dawson, 2010:953). Peripheral/sensory neuropathy affects the nerves that control sensation in the feet and hands, leading to carpal tunnel syndrome and lack of sensation in the feet (Franz, 2012:706; Dawson, 2010:953). Autonomic neuropathy affects nerve function controlling different organ systems, causing gastrointestinal disturbances, bladder dysfunction, tachycardia, sexual dysfunction, postural hypotension and decreased responsiveness to cardiac nerve impulses, leading to painless or silent ischemic heart disease (Franz, 2012:706; Dawson, 2010:953).

Glycaemic control will improve nerve function and decrease perceived pain. Hyperglycaemia, hypertriglyceridemia, obesity, smoking and hypertension are modifiable vascular risk factors for the development of neuropathy (Dawson, 2010:954). Treatment of hyperglycaemia reduces the risk and slows progression of diabetic neuropathy, but it will not reverse neural loss (Franz, 2012:706).

Nutrition therapy, exercise and pharmacologic treatment play a major role in reducing the risk for chronic complications for individuals living with diabetes (Dawson, 2010:953).

2.10 MANAGEMENT OF DIABETES

Improved glycaemic control reduces the risk for macro- and microvascular as well as neuropathic complications in individuals with diabetes (Franz, 2012:693; Benhalima *et al.*, 2010:206). HbA1c provides the best index of overall diabetes or blood glucose control (Franz, 2012:693). HbA1c should be determined at least biannually in individuals with stable glycaemic control, and quarterly in individuals with poor glycaemic control (Franz, 2012:694).

Results from the Diabetes Control and Complications Trial (DCCT) showed the importance of nutrition therapy in glycaemic control and its association with complications in individuals with diabetes (Diabetes Control and Complications Trial, 2005:7). Results from this trial showed that an intensive treatment protocol reduces the prevalence of cardiovascular complications more than conventional therapy does (Diabetes Control and Complications Trial, 2005:7).

2.10.1 Medical nutrition therapy

Medical nutrition therapy (MNT) is very important in the prevention and management of T2DM. Healthy lifestyle changes should be made as a therapeutic intervention to manage T2DM. These changes include making better food choices and increasing physical activity. Dietary recommendations for individuals with diabetes should be made by a registered dietician with the necessary skills and knowledge to facilitate practical implementation (Franz, 2012:683). The medical nutrition therapy for T2DM will be discussed subsequently.

2.10.1.1 Carbohydrate intake

A carbohydrate intake of 45 to 60% of the total daily energy requirement is recommended (Amod *et al.*, 2012:S15). An intake of 25 to 30g of soluble and insoluble fibre should be consumed daily due to its beneficial effect on glycaemic control, insulin levels and plasma lipids (Amod *et al.*, 2012:S16; Franz, 2008:777).

The rate of glucose released into the bloodstream from carbohydrate digestion and absorption and the ability of insulin to clear glucose from circulation, determines blood glucose levels (Franz, 2012:685). Optimum glycaemic control can be achieved by monitoring carbohydrate intake (Amod *et al.*, 2012:S15).

An important strategy to achieve glycaemic control is by monitoring the total amount of carbohydrates consumed (Franz, 2012:685). According to Franz (2012:685), when individuals choose from a variety of starches and sugars, the glycaemic response is the same if the total amount of carbohydrates is similar.

2.10.1.2 Glycaemic index and glycaemic load

Glycaemic index (GI) refers to the physiological effects of carbohydrate intake on blood glucose levels. GI measures the relative area under the postprandial glucose curve of 50g of digestible carbohydrates compared with 50g of a standard food such as white bread or any other glucose. The index does not determine how fast blood glucose levels increase. The peak glucose response for individual foods and meals, either high or low glycaemic index, occurs at the same time (Franz, 2012:685). The glycaemic load (GL) is calculated by multiplying the GI by the amount of carbohydrates in the food and then totalling the values for all foods in a meal (Franz, 2012:685).

A study by Thomas and Elliot (2010:801) shows that a low-GI diet significantly improves diabetes control and decreases HbA1c values. A low-GI diet should therefore form part of the long-term management of diabetes. Jenkins *et al.* (2008:2752) show that a low-GI diet in individuals with T2DM results in lower HbA1c values than a high fibre diet.

2.10.1.3 Sweeteners

Elimination of sucrose from the diet based on its rapid digestion and absorption is not justified. Regardless of whether the source of carbohydrates is from starch or sucrose, the total amount of carbohydrates eaten at a meal is the primary determinant of postprandial glucose levels and the glycaemic effect cannot be predicted based on the structure (Franz, 2012:685). It is however still widely recommended that individuals with T2DM should be modest in their consumption of sucrose. A sucrose intake of 10 to 35% of total energy does not show a negative effect on glycaemic response, and an intake of 5% or less is now the new recommendation (WHO, 2014:Online; Amod *et al.*, 2012:S16; Franz, 2012:686). Shulze *et al*. (2004:933) however report that consumption of one or more sugar-sweetened soft drinks per day increases the risk for T2DM. Malik *et al*. (2010:2481) also show a clear association between the intake of sugar-sweetened beverages and T2DM, with a 26% greater risk of developing T2DM in individuals with a high intake, compared to those with a low intake. A low intake of sugar-sweetened beverages was defined as less than one serving per month and high intake as more than one or two servings per day.

Fructose provides the same amount of energy per gram, as other carbohydrates do. Fructose has a lower glycaemic response than sucrose and other sugars, but large amounts (15 to 20% of total energy) show negative effects on plasma lipid levels (Franz, 2012:686). Stanhope *et al*. (2009:1329) conducted a study to see the effect of fructose on insulin sensitivity and found that fructose consumption at 25% of total energy requirements decreased insulin sensitivity. The recommended intake of fructose is less than 60g per day (Amod *et al.*, 2012:S16).

Montonen *et al*. (2007:1452) investigated the role of different sugars on the development of T2DM. A high intake of fructose, glucose, and sweetened beverages was linked with an increased risk for T2DM; this risk was not linked with a higher sucrose intake.

Low energy sweeteners include the sugar alcohols, sorbitol, mannitol, xylitol, isomalt, lactitol, erythritol and hydrogenated starch hydrolysates, as well as the sweetener tagatose. These low energy sweeteners produce a very low glycaemic response,

contain only 8.4 kilojoules/g and are all approved by the Food and Drug Administration (FDA), with a daily recommended intake of less than 10g. Non-nutritive sweeteners, also approved by the FDA, include saccharin, aspartame, neotame, acesulfame potassium, and sucralose, with an acceptable daily intake of 50mg/kg body weight per day (Amod *et al.*, 2012:S16; Franz, 2012:686). Other sweeteners and natural sweeteners can contain energy and carbohydrates, and should therefore be used with caution (Franz, 2012:686).

There is currently controversy due to new studies presented on low energy artificial sweeteners. A recent study by Suez *et al*. (2014:5) shows that non-caloric artificial sweeteners enhance the development of glucose intolerance via induction of compositional and functional changes to the intestinal microbiota. Pepino *et al*. (2013:2534) also conducted a study on non-nutritive sweetener ingestion and its metabolic effects on humans. The results show that sucralose affected the glycaemic and hormonal responses in obese individuals who do not have a regular intake of artificial sweeteners (Pepino *et al*., 2013:2534). These studies show the need for further research and therefore, until proven otherwise, the recommendation remains the same for artificial sweeteners.

2.10.1.4 Protein

A protein intake of 15 to 20% of total energy is recommended for individuals living with DM (Amod *et al.*, 2012:S16). According to Franz (2012:686) the amount of protein typically consumed (within the recommendation of 15 to 20% of total energy) by individuals with T2DM has minimal acute effects on glycaemic response, lipids and hormones, and no long-term effects on insulin requirements. Non-essential amino acids undergo gluconeogenesis, but in well-controlled individuals with diabetes, the glucose produced does not appear in the general circulation.

Protein delays gastric emptying and in doing so decreases the glycaemic response. This should not be confused with the absorption rate of carbohydrates. Protein does not slow the absorption of carbohydrates; therefore adding protein to a meal to treat hypoglycaemia does not necessarily prevent subsequent hypoglycaemia (Franz, 2012:686; Karamanlis *et al*., 2007:1366). Moghaddam *et al*. (2006:2509)

investigated the effect of protein and fat on glycaemic response and found that protein has an effect on the glycaemic index, by lowering the glycaemic response in humans. This effect was also described by Kirpitch and Maryniuk (2011:156), who indicated that adding protein or fat to a high-GI food will cause the GI of that food to decrease. A study by Karamanlis *et al*. (2007:1366) evaluated the mechanisms by which protein lowers the blood glucose response to oral glucose. The results of this study indicate that by adding protein to oral glucose, postprandial blood glucose concentrations are lowered acutely by decreasing gastric emptying (Karamanlis *et al*., 2007:1367). Short-term studies with a low numbers of subjects with diabetes participating, showed that high protein diets (higher than 20% of total energy intake) may improve glucose and insulin levels, reduce appetite, and improve satiety; but these diets are difficult to follow and the long-term effects on regulation of energy intake, satiety and weight loss have not been studied adequately (Franz, 2012:687). Ang *et al*. (2012:1698) suggest that the type of protein modulates insulin response and action differently.

The effects of protein on glycaemic response are still found to be minimal, causing the protein intake recommendation to remain unchanged (Franz, 2012:686).

2.10.1.5 Fat

Individuals with diabetes have an increased risk for cardiovascular disease; therefore the next priority, after achieving glycaemic control, should be cardio-protective nutrition interventions (Franz, 2012:687). Total fat intake should be less than 35% of the total energy intake, with saturated fat intake less than 7% and poly-unsaturated fatty acids restricted to less than 10% of total energy intake. Trans-fat intake should be minimised. Mono-unsaturated fats should be consumed from plant and fish sources and polyunsaturated fatty acids, especially omega-3 fatty acids, are recommended, with two to three servings of fish per week also being suggested (Amod *et al.,* 2012:S16; Franz, 2012:687).

2.10.1.6 Salt

Sodium intake should be restricted to less than 2300 mg per day (5.8 g salt), which may help to lower blood pressure. Restricting sodium intake to improve blood pressure levels in individuals with T2DM forms part of the effort to implement healthy lifestyle changes. The main source of sodium is salt. Packaged, tinned and processed food is usually high in sodium and intake should be limited (Amod *et al.*, 2012:S16).

2.10.1.7 Alcohol

The same alcohol recommendations that apply to the general public, apply to individuals with diabetes. Moderate intake of alcohol is advised and is defined as one to two drinks per day or 15 to 30g of alcohol (Amod *et al.*, 2012:S16; Franz, 2012:687).

Small amounts of alcohol ingested with food have very little, if any, effect on glucose and insulin levels. Alcohol should be considered an addition to the regular food intake and meal plan for individuals with T2DM. Ingestion of light to moderate amounts of alcohol does not raise blood pressure, whereas excessive, chronic ingestion of alcohol (more than two drinks per day or more than 30 g of alcohol per day) does raise blood pressure and may be a risk factor for T2DM. Consuming alcohol without eating can cause problems, as this can result in alcohol-induced hypoglycaemia. Alcohol does not require insulin to be metabolised. The consumption of large amounts of alcohol on a regular basis can contribute to hyperglycaemia due to alcohol being metabolised by the liver. If excess amounts of alcohol are consumed it will compromise the liver, which will impair its ability to regulate blood glucose levels (Amod *et al.*, 2012:S16; Franz, 2012:687; Whitney & Rolfes, 2005:242).

2.10.2 Medical management

T2DM is a progressive disease, and nutrition therapy alone is sometimes not adequate to maintain HbA1c levels at 6.5% or less. With disease progression the pancreas may fail to secrete enough insulin to maintain adequate glucose control, creating the need for medical management to support nutrition therapy (Franz, 2012:683; Casey, 2011:20). The goal of medical management is to achieve and maintain glycaemic control and to alter interventions, including the use of insulin, when therapeutic goals are not met. Individuals with diabetes, who do not produce enough endogenous insulin, need additional insulin (Franz, 2012:689). Medical management for T2DM includes insulin and non-insulin therapy which will be discussed.

2.10.2.1 Insulin therapy

Insulin may be needed to restore glycaemia to near normal in individuals with T2DM (Franz, 2012:689). A study by Ryan *et al*. (2004:1032) shows that in individuals with newly diagnosed T2DM and elevated fasting blood glucose levels, immediate intensive insulin therapy can successfully lay the foundation for lasting, good glycaemic control. To prevent macrovascular and microvascular complications, optimal blood glucose levels and achievement of HbA1c target levels is required (Garg & Ulrich, 2006:117). Many individuals with T2DM do not achieve 24-hour euglycemia and therefore require intensive insulin regimens (Garg & Ulrich, 2006:117). Synthetic insulin differs from human insulin in amino acid sequence, but binds to insulin receptors in the same way and therefore functions similarly to human insulin, and is called analogue insulin (Amod *et al.*, 2012:S34; Franz, 2012:691).

Insulin therapy usually starts with long-acting or premixed insulin at bedtime or before dinner to control fasting blood glucose levels. Many individuals with T2DM need a more physiological insulin regime at bedtime or at dinner to achieve control. If large insulin doses are needed, oral medications like insulin sensitisers are usually combined with the insulin regime (Franz, 2012:691). Insulin therapy is characterised according to three characteristics: onset of the action, peak action and effect duration (Franz, 2012:692).

2.10.2.1.1 Rapid-acting insulin

Rapid-acting insulin includes Lispro (Humalog), Aspart (Novorapid) and Glulisine (Apidra). Rapid-acting insulin is used as bolus insulin, which implies use at mealtimes. Rapid-acting insulin has an onset of action within 15 minutes, peak action in 30 to 90 minutes, and effect duration of 3 to 5 hours (Amod *et al.*, 2012:S34; Franz, 2012:691). The benefits of rapid-acting insulin include rapid absorption, little variability in absorption, that it can be injected immediately before food intake, that it limits the postprandial glucose increase effectively and the lowers risk for nocturnal hypoglycaemia (Davies & Srinivasan, 2006:73).

2.10.2.1.2 Regular insulin

Regular insulin such as Insuman, Actrapid or Humulin R should be administered 30 to 60 minutes before a meal, due to the slower onset of action. Regular or short acting insulin has an onset of action within 15 to 60 minutes after injection and duration of action between 5 to 8 hours. (Franz, 2012:691; Davies & Srinivasan, 2006:73).

2.10.2.1.3 Intermediate-acting insulin

The only intermediate-acting insulin available is Neutral protamine Hagedorn (NHP) or Isophane, examples are Humulin N or Protophane. It should be administered 2 hours before a meal due to the onset of action being 2 to 4 hours after injection, with a peak effect between 6 and 10 hours and action duration of 10 to 18 hours (Franz, 2012:691; Amod *et al.,* 2012:S34; Davies & Srinivasan, 2006:73).

2.10.2.1.4 Long-acting insulin

Long acting insulin includes Glargine (Lantus) and Determir (Levemir). Glargine results in a relatively constant delivery without peaks over 24 hours, due to its slow dissolution at the injection site. Due to its acidic pH, it cannot be mixed with any other insulin in the same syringe before injection. Glargine can be given before any meal, but it is important that it is given consistently. It is usually given at bedtime. Determir is quickly absorbed from the subcutaneous tissue but then binds to albumin in the bloodstream, which results in a prolonged action time of 16 to 24 hours. For this reason it needs to be administered twice daily (Amod *et al.,* 2012:S34; Franz, 2012:691). A study by Wiesli *et al*. (2009:1818) compares the effects of different long-acting insulin preparations on glucose levels in individuals with T2DM. They concluded that all of the long-acting insulin preparations given at bedtime result in a fasting glucose of <7 mmol/l in the morning, without hypoglycaemia when participants continued to fast during the day (Wiesli *et al*., 2009:1818).

2.10.2.1.5 Premixed insulin

Premixed insulin consists of various mixtures, e.g. 70% NPH and 30% regular; 75% Lispro proamine and 25% Lispro; 50% Lipro protamine and 50% Lispro; and 70% Protamine and 30% Aspart (Franz, 2012:692). The onset time of action for premixed insulin is 5 to 15 minutes and the duration of action is 10 to 16 hours, with a dual peak (Amod *et al.,* 2012:S34). It is essential to use premixed insulin with meals and to ensure consistent carbohydrate intake to prevent hypoglycaemia (Franz, 2012:692). All oral anti-diabetic drugs must be stopped when starting the use of premixed insulin, except for Metformin, due to risks of weight gain and hypoglycaemia (Amod *et al.,* 2012:S34; Holman *et al*., 2007:1729). A study on dual therapy by Holman *et al*. (2007:1729) excluded Thiazolidinediones due to concerns of cardiovascular disease, heart failure, and increased fracture rates in women.

2.10.2.1.6 Insulin regimes

Insulin therapy is developed to mimic normal insulin action. Normal insulin action involves a rapid increase in insulin concentrations after a meal, following increased plasma glucose levels, peak concentrations in 30 to 60 minutes and a return to basal concentrations within 2 to 3 hours. To imitate this, rapid-acting insulin is administered before meals with the quantities adjusted based on the amount of carbohydrates in the meal (Franz, 2012:692). The decision on the amount of mealtime insulin to inject will be guided by an established insulin-to-carbohydrate ration for each individual. The basal or background insulin (long-acting insulin) dose is the amount of insulin necessary in the post-absorptive state to manage endogenous glucose output, primarily from the liver. Timing and type of insulin regimes should be based on a person's eating and exercise habits and blood glucose concentrations (Franz, 2012:693).

Research by Fonseca (2006:51) shows that basal insulin, both intermediate and long-acting, plays a very important role in the management of T2DM. For insulin-naïve individuals with T2DM who control poorly on oral drug therapy, the addition of a single basal insulin dose at bedtime will significantly lower HbA1c levels, with very low risk of hypoglycaemia (Fonseca, 2006:58). The addition of basal insulin (Glargine or Determir) results in less weight gain and long-acting insulin may reduce variability in fasting blood glucose values in patients. Long-acting insulin has been associated with lower rates of hypoglycaemia, which can have a positive effect on glycaemic control during daytime (Fonseca, 2006:59).

The use of insulin pumps is becoming more common amongst individuals with T2DM. Insulin pumps provide basal rapid-acting or short-acting insulin, pumped continuously, 24 hours a day by a mechanical device in micro amounts through a subcutaneous catheter. Both Lispro and Aspart can be used in insulin pumps. Boluses of insulin are calculated and programmed to be added before meals. Pump therapy requires commitment and a minimum of four blood glucose tests daily are recommended as well as keeping food and blood glucose records (Franz, 2012:693).

2.10.2.2 Non-insulin therapies

Glucose-lowering medications (non-insulin therapies) work at various levels to target the different pathways in the pathogenesis of T2DM. This includes addressing insulin resistance at cellular level, incretin defects, endogenous insulin deficiency, elevated levels of glucagon and excessive hepatic glucose release (Franz, 2012:689).

2.10.2.2.1 Biguanides

Metformin (Glucophage) and other biguanides (phenformin and buformin) were developed in 1950 and were used to treat symptoms characteristic of diabetes in early times. As the other biguanides caused lactic acidosis, metformin is the only biguanide which is still used and commercially available (Amod *et al.*, 2012:S23; Davies & Srinivasan, 2006:71). Metformin suppresses hepatic glucose production by activating adenosine monophosphate kinase. Hypoglycaemia does not occur with the use of metformin and small weight losses are common when therapy starts (Franz, 2012:689). Gastrointestinal disturbances are the most common side effect and lactic acidosis occurs rarely and mostly with inappropriate use (Amod *et al.,* 2012:S23; Franz, 2012:689; Davies & Srinivasan, 2006:71). Taking metformin with food in the lowest dose (500mg, twice a day for a week) and then gradually increasing it to maximum doses will minimise the gastrointestinal side-effects (Franz, 2012:689; Davies & Srinivasan, 2006:71).

2.10.2.2.2 Sulfonylureas

Sulfonylureas have also been used since the 1950s (Amod *et al.,* 2012:S25; Franz, 2012:691). Sulfonylureas are insulin secretagogues that bind to the sulphonylurea receptor of the pancreatic β-cell, promoting closure of ATP-dependent potassium channels and stimulating insulin secretion from the β-cells of the pancreas (Amod *et al.*, 2012:S25; Franz, 2012:691; Davies & Srinivasan, 2006:71). Sulfonylureas are effective in lowering fasting plasma glucose and reducing HbA1c levels by 1.5–2.0% (Davies & Srinivasan, 2006:71). Adverse effects include weight gain and the risk of

hypoglycaemia. An advantage is its cost-effectiveness (Amod *et al.*, 2012:S25; Franz, 2012:691). Examples of Sulfonylureas used in South Africa are Glucotrol, Amaryl and Glimepiride.

2.10.2.2.3 Thiazolidinediones

Thiazolidinediones (Avandia or Actos) improve peripheral insulin sensitivity, by enhancing fatty acid uptake and adipokine release (Amod *et al.*, 2012:S29; Franz, 2012:691). Thiazolidinediones enhance the effects of endogenous insulin on target organs. Thiazolidinediones are agonists of the peroxisome proliferator-activated receptor *γ* (PPAR*γ*) that is present in liver, skeletal muscle and adipose tissue (Davies & Srinivasan, 2006:71). Thiazolidinediones also have a beneficial effect on lipid levels and do not cause hypoglycaemia. By reducing insulin resistance peripherally, glitazones improve HbA1c by up to 1.5 %, and pioglitazone particularly increases HDL cholesterol and reduces triglyceride levels (Davies & Srinivasan, 2006:71). Detrimental effects include weight gain; and the most common side-effect is fluid retention (Amod *et al.*, 2012:S29; Franz, 2012:691; Davies & Srinivasan, 2006:71).

2.10.2.2.4 Glucagon-like peptide-1 agonist

Incretins are hormones produced by the gastrointestinal tract and are released during absorption. When meals include protein and fat, the intestinal mucosa release glucagon-like peptide-1 (GLP-I), which stimulates insulin synthesis and releases insulin. GLP-1 decreases glucagon secretion, slows gastric emptying time and promotes satiety, by decreasing appetite (Franz, 2012:691; Lysen & Israel, 2012:466). Glucagon-like peptide-1 agonists have several of the same glucose-lowering effects as the body's naturally occurring incretin, GLP-1. GLP-1 enhances glucose-dependent insulin secretion and suppresses postprandial glucagon secretion. There are two GLP-1 agonists that are commercially available (Franz, 2012:691), namely exenatide (Byetta) and liraglutide (Victoza). Weight loss and a

reduction in HbA1c are associated with the use of GLP-1 agonists (Franz, 2012:691).

2.10.2.2.5 Alpha glucosidase inhibitors

Acarbose (Glucobay) and miglitol (Glyset) are alpha-glucosidase inhibitors, which work in the brush border of the small intestine to inhibit enzymes that digest carbohydrates (Amod *et al.*, 2012:S26; Franz, 2012:691). These inhibitors constrain the enzymes that hydrolyse oligosaccharides and polysaccharides in the brush border intestinal cells, thereby preventing or delaying glucose absorption; hence lowering postprandial glucose levels (Davies & Srinivasan, 2006:71; Amod *et al.*, 2012:S26; Franz, 2012:691). When used alone, neither hypoglycaemia nor weight gain are experienced. Flatulence, diarrhoea, cramping and abdominal pain may however be experienced, due to the delay in digestion of carbohydrates (Amod *et al.*, 2012:S26; Franz, 2012:691). .

2.10.2.2.6 Glinides

There are two glinides: Repaglinide (Novonorm) and Nateglinide (Starlix). Nateglinide only works when glucose is present and is a less potent secretagogue. Glinides stimulate insulin secretion from β-cells and should be given before meals to decrease postprandial glucose secretions and to lower the risk of hypoglycaemia. Glinides have a shorter metabolic half-life compared to sulfonylureas. A side-effect of glinides, similar to sulfonylureas, is the risk for weight gain (Franz, 2012:691).

2.10.2.2.7 Amylin agonists

Pramlintide (Symlin) is a synthetic analogue of the β-cell hormone, amylin. Amyl agonists slow gastric emptying and inhibit glucagon production, which decreases mealtime hepatic glucose release and prevents postprandial hyperglycaemia. Amyl agonists should be injected before meals (Franz, 2012:691).

2.10.2.2.8 Dipeptidyl peptidase 4 inhibitors

The two dipeptidyl peptidase 4 inhibitors, Sitaglipton (Januvia) and Saxaglipin (Onglyzia), degrade the effects of GLP-1 and glucose-dependent insulinotropic peptides, which are the main intestinal stimulants for insulin secretion. When dipeptidyl peptidase 4 inhibitors are used as monotherapy, they are well tolerated, do not affect weight and do not cause hypoglycaemia (Franz, 2012:691).

2.11 GENETICS AND CHRONIC DISEASE

Chronic diseases such as T2DM result from a complex interaction of genetic and environmental factors. The role of genetic factors in the pathogenesis of various diseases is quickly emerging with the progression of new research. These progressions can lead to preventative and therapeutic interventions for complex diseases, modified according to an individual's genetic profile (Janssens & Van Duijn, 2008:166; Yang *et al.*, 2005:1135). When changes in DNA structure are identified, the abnormal protein can also be identified, and the resulting phenotype defined. In cases of chronic diseases, multiple genes, with multiple variations, contribute in small ways to the overall chronic condition, instead of a single variant having a dramatic effect (DeBusk, 2012:156). A person might have gene variants that predispose them to a particular chronic disease, but the disease may or may not develop due to environmental conditions (DeBusk, 2012:156; Yang *et al.*, 2005:1135). The high degree of variability in individual responses to nutrition therapy in a population with genetic variability is therefore not surprising. Changes in genes, diet and lifestyle can have a severe effect and cause disease outright, but most gene variations affect the extent of response and only increase susceptibility. Many variants are responsive to diet and other lifestyle parameters, providing the opportunity to minimise the effect through better lifestyle choices (DeBusk, 2012:156; Buchanan *et al*., 2006:570).

2.12 DISEASE INHERITANCE

2.12.1 Multifactorial or complex traits

Traits are transmitted from one generation to the next (Malats & Calafell, 2003:481; Korf, 2006:3) and can be described as a single-gene or a polygenic trait. A polygenic trait is controlled by the effect of more than one gene. Both single-gene and polygenic traits can be multifactorial, which refers to the degree of influence by the environment. Pure polygenic traits are not influenced by the environment and are very rare (Lewis, 2003:132; Malats & Calafell, 2003:481).

Multifactorial traits are not limited to diseases or illnesses but can affect height, skin, colour, body weight, behavioural conditions and tendencies. Another term for multifactorial traits is complex traits, referring to the difficulty in predicting expression of the genes because of the combination of gene and environmental interactions. With polygenic multifactorial traits, each gene presents a degree of susceptibility, but the contribution of these genes is not automatically equal (Lewis, 2003:132).

The combined action of many genes produces a continuously changing phenotype in a polygenic trait. Multifactorial traits are constantly changing if it is polygenic. It is the multi-gene component of the trait that adds to the continuous variation of the phenotype (Lewis, 2003:132). Individual genes that present polygenic traits all contribute to a phenotype in an additive way, without being recessive or dominant to each other, whereas single-gene traits are more discrete, giving an “all or none” phenotype, expressed as normal versus affected (Lewis, 2003:132).

Bateman (2006:78) defines multifactorial inheritance as a genetic predisposition to a disease with the manifestations or expression of the disease being influenced by environmental factors in either the intrauterine or postnatal period. It can therefore be concluded that genes and the environment are two forces that interact to shape many of our characteristics and risk for disease.

2.12.2 Inheritance patterns

Each cell nucleus contains a complete set of genetic material, which is called the genome. In humans this is divided into 22 pairs of chromosomes (DeBusk, 2012:149; Bateman, 2006:82). Humans are diploid organisms and inherit a complete set of genes carried on 22 chromosomes and an X or Y chromosome, from each parent. The alleles – the two individual copies of a gene – on homologous chromosomes segregate at meiosis and new combinations are paired together during fertilisation. The specific alleles at a locus compose the genotype. The physical characteristic that results from action of the alleles is the phenotype (Korf, 2006:3).

Because of the large burden of disease and predisposition to disease, the identification of genes associated with complex diseases is a high priority (Yang *et al*., 2005:1133). As mentioned, most diseases in humans result from an interaction between multiple genetic variants and environmental factors. Yang *et al*. (2005:1135) show that variants of more than 20 susceptible genes may cause more than 50% of common complex diseases. Considering this, it can be shown that by intervening with risk factors such as weight, cholesterol levels or blood pressure, the pathophysiological pathways seem to be interruptible, without regard for the genes or the environmental factors that led to the development of the risk factors (Buchanan *et al*., 2006:570). There are three ways for traits to transmit from one generation to the next: Mendelian inheritance, mitochondrial inheritance and epigenetic inheritance, which will be discussed.

2.12.2.1 Mendelian inheritance

Mendelian inheritance follows a pattern of inheritance according to the rules of Mendel. Mendelian traits are determined by just one genetic locus with a complete penetrance, so the phenotype always occurs when the corresponding genotype is present. Mendelian inheritance can be dominant, recessive or sex linked (Malats & Calafell, 2003:481; Korf, 2006:3).

Five classic modes of Mendelian inheritance exist, namely: autosomal dominant, autosomal recessive, X-linked dominant, X-linked recessive, and Y-linked. Each gene in an individual is there in two copies, or alleles, one on each chromosome. When both are the same, the individual is homozygous, and if they differ, the individual is referred to as heterozygous (Korf, 2006:3; DeBusk, 2012:149). When a trait is expressed in a heterozygous individual that has one common allele and one variant allele, it is referred to as dominance or recessiveness. If a trait is expressed when only a single copy of a variant allele is present, the allele is dominant; when the allele does not dominate the genotype when only a single copy is present it is called recessive (Korf, 2006:3; DeBusk, 2012:149).

In dominant inheritance one copy of an abnormal gene is sufficient to cause disease. The abnormal gene is inherited from a parent who also has the disorder and it will be present in every generation in the family (Malats & Calafell, 2003:481). Recessive inheritance is a type of inheritance in which two abnormal copies of the gene must be present for the individual to be affected (Malats & Calafell, 2003:481). Each parent contributes one abnormal copy of the gene to the child who has the disorder. Heterozygous individuals are called carriers of the disorder because they have one normal and one abnormal copy of the gene, although they do not show any disorder symptoms (Malats & Calafell, 2003:481).

Sex-linked inheritance is followed by the traits caused by genes located on the Y or X chromosomes (Malats & Calafell, 2003:481). X-linked disorders can be dominant or recessive. When the abnormal gene that is responsible for a recessive disorder is located on the X chromosome only males are usually affected because they do not have a second, normal, copy of the gene – these males are called hemizygous (Malats & Calafell, 2003:481).

Penetrance refers to the probability that a particular genotype will be expressed in the phenotype (Malats & Calafell, 2003:481). Penetrance reflects the inability of a genetic variation to impair function and cause disease, unless the individual is exposed to specific environmental triggers, such as diet and lifestyle factors. Therefore even when a pedigree suggests that a dominant gene is present and a specific trait should be expressed, the trait or disease may not be expressed in a measureable form (DeBusk, 2012:149). A penetrance of 100% means the

associated phenotype always occurs when the corresponding genotype is present. If only 30% of those carrying a particular allele exhibit a phenotype, the penetrance is 30% (Malats & Calafell, 2003:481).

Epistasis refers to interaction between different alleles on different genes (Malats & Calafell, 2003:480). The effects of a given gene on a biological trait are masked or enhanced by one or more other genes (Moore, 2005:13). This can occur at the same step or at various stages of the same biological pathways (Malats & Calafell, 2003:480). According to Moore (2003:81), several studies indicate that epistasis is a common component of the genetic architecture of common human disease and those complex interactions are more important than the independent main effects of any one susceptibility gene.

2.12.2.2 Mitochondrial inheritance

The mitochondrion is an organelle that contains its own genome. Mitochondrial DNA (mtDNA) is a small circular DNA and encodes 13 proteins. Each of these proteins forms part of one of the subunits of the mitochondrial electron transport chain. The mitochondrial genome also encodes a unique set of transfer RNAs (tRNA), as well as two ribosomal RNAs. Mutations throughout the intronless genes on the mtDNA can cause disease, which is manifested by disturbances in energy metabolism (DeBusk, 2012:150; McCandless & Cassidy, 2006:9).

Mitochondrial inheritance does not follow the rules of Mendelian inheritance (McCandless & Cassidy, 2006:9), but appears in a matrilineal pattern. This means that the disorder can be seen in males or females, but can only be transmitted from an affected female to her children (McCandless & Cassidy, 2006:16). Gametogenesis is the process where the ovum accumulates a large number of mitochondria – each containing multiple copies of the mitochondrial genome. In every ovum there may be a variety of mutations, because the nucleotide sequence of these mitochondrial genomes is not identical and is not present in every copy of the mtDNA. None of the mitochondria are delivered into the fertilised egg because the sperm compartmentalises its mitochondria to the motor unit of the tail. Therefore

the mother supplies all of the mitochondria into the fertilised egg which explains the inheritance pattern (McCandless & Cassidy, 2006:16).

2.12.2.3 Epigenetic inheritance

Epigenetic inheritance refers to a heritable change in gene expression that is not caused by changes in the DNA sequence, but by modifying gene expression (Slatkin, 2009:849; Bird, 2007:398; Ganten & Ruckpaul, 2006:620; Richards, 2006:396). Epigenetics is based on DNA methylation, acetylation, histone alteration in chromatin structure during the cell life or the life of the organism (Ganten & Ruckpaul, 2006:620). Unless inherited epigenetic changes are more common than mutations or have more distinct effects on disease risk, it will be hard for inherited epigenetic modifications to account for the missing heritability of the complex disease (Slatkin, 2009:849). There are primarily three mechanisms involved: histone modification, DNA modification and RNA interference (DeBusk, 2012:150; Jaenish & Bird, 2003:245).

Proteins associated with DNA are called histones. DNA is wrapped around these histones which creates nucleosomes. When the DNA is condensed, it is not available for transcription into mRNA and consequently protein formation. The relaxing or condensation of DNA is controlled by attaching and removing acetyl groups to histone proteins. DNA can also be modified by the covalent attachment and removal of functional groups, such as methyl groups to cytosine nucleotides. Methylation silences gene expression and demethylation promotes gene expression (DeBusk, 2012:150; Bird, 2007:398; Richards, 2006:395).

RNA interference is a post-transcriptional mechanism where short pieces of single-stranded RNA attach to DNA or messenger RNA (mRNA) and interfere with gene expression by the prevention of translation of the gene into its encoded protein. Attaching to DNA results in silencing of whole regions of chromosomes, also referred to as epigenetic gene silencing. This is the basis for X-inactivation in mammalian females in which one of the two X-chromosomes is silenced. In this way the quantity of information contributed by the X-chromosome is equalised between females and males, after some time resulting in only a single X-chromosome (DeBusk, 2012:150).

DNA methylation, an epigenetic mark, is involved in control mechanisms of various biological processes. The methyl groups are symmetrically positioned on two complementary DNA strands and the methyl groups represent a clonally inheritable feature of the DNA (Razin, 2005:1). Methyl patterns are established by embryogenesis and the patterns are maintained for many cell generations by the maintenance methyltransferase. These patterns are interpreted by proteins that interact with DNA, depending on the state of methylation. Therefore DNA methylation patterns fulfil their tasks by guiding specific proteins to target sites on DNA (Razin, 2005:1).

Diet has been shown to influence at least one epigenetic mechanism, which is DNA methylation of which the effects can be inherited. Diet and other possible environmental factors may have a transgenerational effect through their influence on epigenetic markings which affects gene expression without changing the DNA sequence (DeBusk, 2012:151). Switzeny *et al*. (2012:1) studied the impact of an antioxidant, folate and vitamin rich diet on the epigenetic pattern on the *MutL Homolog1 (MLH1)* gene in individuals with T2DM. Oxidative stress may lead to increased levels of unrepaired cellular DNA damage. The *MLH1* gene is one important gene in the mismatched repair complex that acts as proofreading complex to maintain genomic integrity. The conclusion from this study was that the vitamin and antioxidant rich diet affected the methylation of this *MLH1* gene. The higher methylation might have been a result of the reactive oxygen species scavenging antioxidant rich diet, leading to lower DNA demethylation enzyme. Individuals with T2DM benefited from dietary intervention, involving epigenetic DNA repair mechanisms (Switzeny *et al*., 2012:7). As already mentioned, DNA methylation is critical for genomic structure and function and is dependent on adequate folate status (Shelnutt *et al*., 2004:554). Research by Shelnutt *et al*. (2004:555) on women 20 to 30 years of age evaluated the effect of folate enzyme polymorphism known as the *methylenetetrahydrofolate reductase (MTHFR)* polymporphism, on changes in DNA methylation in these young women consuming a low folate diet. The study results indicated that an inadequate intake of folate may affect DNA methylation and that women with homozygous genotype for the *MTHFR* polymorphism will benefit more from folate supplementation (Shelnutt *et al*., 2004:559). From these studies it

can be concluded that diet can influence DNA methylation and therefore gene expression and that the effects can be inherited.

2.12.3 Inheritance and disease

Alterations to the genetic material have the potential to change one or more proteins that may be essential to the operation of the cells, tissues, and organs of the body, whether to the chromosomal DNA, mitochondrial DNA or a single nucleotide. Genetic material can be altered at several levels resulting from fatal to unnoticed mutations (DeBusk, 2012:151).

2.12.3.1 Disease at chromosomal level

Chromosomal abnormalities include the loss or duplications of larger regions of DNA and change the functions of more than one gene (Bateman, 2006:78). Chromosomal disorders are identified by means of visualisation of the chromosomes in the form of a karyotype (Bateman, 2006:79). During gametogenesis, chromosomal abnormalities originate, caused by non-junction or anaphase lag. Failure of separation of homologous chromosomes may take place in the first division; in the second, failure of chromatid separation of the duplicated chromosomes may take place. These cases will result in complementary gametes with 24 and 22 chromosomes, if they are fertilised by a normal gamete (23 chromosomes); the zygote will have 47 chromosomes, one being present in triplicate, also known as trisomy. The other zygote would have 45 chromosomes with one missing, also known as monosomy (Bateman, 2006:83).

Chromosomal abnormalities at birth usually lead to death at an early age. The individuals with chromosomal abnormalities who do survive have complicated lives. Some chromosomal abnormalities influence the diets and lifestyles of these individuals. Examples of chromosomal abnormalities are: Trisomy 18 (Edward Syndrome), an autosomal trisomy where there is intrauterine growth retardation and feeding difficulties; Down Syndrome; and Prader-Willi Syndrome, a microdeletion

syndrome, presenting with clinical obesity and failure to thrive (Bateman, 2006:84; Kruisselbrink *et al*., 2006:1).

2.12.3.2 Disease at mitochondrial level

Mitochondrial DNA is inherited through the maternal germline. Mitochondrial diseases due to defects in the mitochondrial encoded DNA will show only maternal inheritance (Bateman, 2006:78; Kruisselbrink *et al*., 2006:83; Korf, 2006:4). Mostly mitochondrial dysfunction is due to changes in nuclear genes and inherited in an autosomal recessive pattern. Changes in mitochondrial DNA are typically degenerative and primarily affect tissues with an elevated demand for oxidative phosphorylation, because the mitochondria generate energy for cellular processes by producing adenosine triphosphate (ATP) through oxidative phosphorylation (Kruisselbrink *et al*., 2006:83; McKenzie *et al*., 2004:589). True mitochondrial disorders include abnormalities of mitochondrial energy metabolism only, particularly oxidative phosphorylation, fatty acid oxidation, ketogenesis, and ketolysis (Patay, 2005:546).

Mitochondrial diseases are complex multisystem disorders, as almost all tissue depends on oxidative metabolism (Montoya *et al*., 2009:52). Mitochondrial disease includes a variety of neurologic, ophthalmologic, cardiac, endocrine, gastrointestinal, and pulmonary manifestations. Examples of mitochondrial diseases are: mitochondrial encephalomyopathy; lactic acidosis and stroke-like episodes; myoclonic epilepsy with ragged-red fibres; Leber's hereditary optic neuropathy; and Kearns-Sayre syndrome (Montoya *et al*., 2009:48; Kruisselbrink *et al*., 2006:83; Patay, 2005:546). Mitochondrial diseases can therefore be defined as a group of disorders originating from a deficient synthesis of ATP, by mutations in mtDNA (maternally inherited) (Montoya *et al*., 2009:52). Single nucleotide polymorphisms (SNP) in mitochondrial DNA have also been significantly associated with T2DM (Schrauwen-Hinderling *et al*., 2007:118; Petersen *et al*., 2004:668; Kelley *et al*., 2002:2949).

2.12.3.3 Disease at molecular level

Most of the disease conditions or genetic defects associated with nutritional genomics involve changes at molecular level. Changes to DNA typically involve a single nucleotide change or several nucleotides within a single gene through substitutions, additions or deletions, causing an alteration in one or more amino acids of the protein product (Bateman, 2006:78; Lopez-Bigas *et al*., 2005:269). Numerous inherited diseases are associated with point mutations or SNPs that are of functional importance to the organism that generally occur within an exon or exon-intron boundary or in the regulatory sequence (Bateman, 2006:78).

2.12.3.3.1 Nutrigenetic diseases

The basis of Mendelian inheritance patterns are mutations of single genes, including autosomal dominant, autosomal recessive and X-linked (Bateman, 2006:78). Autosomal recessive disorders are more common and include metabolic disorders of amino acid, carbohydrate, and lipid metabolism (Gillbert-Barness & Debich-Spicer, 2005:416). An example of an autosomal inherited disorder is maturity-onset diabetes in young children, where inheritance of one copy of the mutant gene is enough to cause this autosomal inherited disorder in a child. Other examples are inborn errors of metabolism (IEM) and include familial hypercholesterolemia, and polycystic kidney disease (Ganten & Ruckpaul, 2006:118).

Galactosemia is an autosomal recessive disorder of carbohydrate metabolism. Galactosemia refers to the deficiency of galactose-1 phosphate uridyltransferase, galactokinase, and UDP-galactose 4'epimerase (Edelmann *et al*., 2007:101). This deficiency causes galactose, galactose-1 phosphate and galactitiol accumulation in muscles. If a newborn with galactosemia is fed lactose, vomiting, diarrhoea, hyperbilirubinemia, hepatospleganomaly, renal tubular dysfunction, liver failure and cataracts develop. Galactose is present in the blood of an affected newborn infant (Gillbert-Barness & Debich-Spicer, 2005:415). Prenatal diagnosis of galactosemia can provide opportunity for immediate dietary restriction (Edelmann *et al*., 2007:101). Fructose intolerance is another example of autosomal recessive IEM of the

carbohydrate metabolism, where fructose is prevented from being converted to glucose (DeBusk, 2012:151; Gillbert-Barness & Debich-Spicer, 2005:415).

Phenylketonuria is another example of IEM of amino acid metabolism and is inherited as an autosomal recessive trait. It is attributable to the deficiency of the enzyme phenylalanine hydroxylase, resulting in an inability to convert the amino acid phenylalanine to tyrosine. Untreated infants can become mentally retarded and develop seizures, eczema and have a mousy odour to the urine (Edelmann *et al*., 2007:97; Gillbert-Barness & Debich-Spicer, 2005:427). With early diagnosis and dietary intervention, mental and growth retardation can be prevented (Edelmann *et al*., 2007:98).

An example of an autosomal recessive disorder of lipid metabolism is the deficiency of medium-chain acyl-coenzyme A dehydrogenase (MCAD), which prevents medium-chain fatty acids from being oxidised to provide energy during periods of fasting (DeBusk, 2012:151; Edelmann *et al*., 2007:103). Complications of the disease include hepatomegaly, acute liver disease, and brain damage (Edelmann *et al*., 2007:103). MCAD deficiency can be treated with changes in diet to avoid fasting and saturated fats, and ingestion of carbohydrates prior to bed time (Edelmann *et al*., 2007:103). Most of the disease conditions or genetic defects involve changes at molecular level, and nutritional intervention plays an integral role in disease onset and outcome.

Nutrigenetics refers to how a person's particular genetic variations affect function; whereas nutrigenomics can be defined as how food and genes interact and the consequences of this interaction (DeBusk, 2012:154; Palou, 2007:5). Nutrigenomics will help to provide optimal dietary intervention approaches to restore and improve metabolic homeostasis, health and well-being and to prevent diet-related diseases (Palou, 2007:5).

Nutrigenomic research is helping to clarify disease pathogenesis and the influence of bioactive components in food. Diagnostic tests and assessment of disease susceptibility, together with genetic testing and family history analysis will come from these advances, which will allow health care professionals to predict individuals who are at risk for a certain disorder. Optimal nutrition can diminish the harmful effects of

many genetic influences that predispose a person to a disease and it can also offer effective approaches for disease prevention. With widespread knowledge on genetic makeup and knowledge to make lifestyle choices that support the specific genotype, individuals will have the opportunity to live to their full genetic potential throughout a healthy, active life (DeBusk, 2012:145). Nielsen and El-Sohemy (2012:565) compared the effects of giving genotype-based dietary advice with giving general recommendations. They concluded that people understand genotype-based dietary advice better and that it had a better effect than general dietary recommendations (Nielsen & El-Sohemy, 2012:565).

2.13 NUTRIGENOMIC INFLUENCES ON TYPE 2 DIABETES MELLITUS

The pathogenesis of T2DM is multifaceted, involving both lifestyle and genetic predisposition (Ostegard *et al.*, 2005:99). T2DM is an example of a complex genetic trait resulting from the contribution of more than one gene as well as environmental influences (Hansen & Pedersen, 2005:128). There have been many genes associated with T2DM. The genes researched that have mostly been associated with T2DM are *peroxisome proliferator-activated receptor-gamma* (*PPARy)* polymorphism, *Transcription Factor 7-Like 2 (TCF7L2)* polymorphism, *angiotensin-I converting enzyme (ACE I/D)* polymorphism, methylenetetrahydrofolatereductase enzyme *(MTHFR) C667T* polymorphism, *Potassium channel, inwardly rectifying subfamily J, member 11* (*KCNJ11) (rs5219)* polymorphism, *Cyclin-Dependent Inase Inhibitor 2A* (*CDKN2A/B) (rs10811661)* polymorphism, *Insulin-like Growth Factor 2 mRNA Binding Protein 2* (*IGF2BP2) (rs4402960)* gene, *Hematopoietically Expressed Homeobox* (*HHEX) (rs7923837)* polymorphism, *Exostosin Glycosyltransferase 2* (*EXT2) (rs1113132)* polymorphism and *Solute Carrier Family 30* (*SLC30A8) (rs13266634)* polymorphism (Al-Rubeaan *et al*., 2013:175; McCarthy, 2010:2345; Chandak *et al.*, 2007:67). The *Fat Mass-and Obesity-Associated* (*FTO)* gene, *CDK5 Regulatory Subunit Associated Protein 1-Like 1* (*CDKAL1), Melatonin Receptor 1B (MTNR1B)* and *Insulin Receptor Substrate-1* (*IRS1)* genes have also been associated with T2DM. Most of these genes are involved in energy metabolism and insulin action (Al-Rubeaan *et al*., 2013:175; McCarthy, 2010:2345; Pascoe *et al*., 2007:3104).

Of the above mentioned genes and their association with T2DM, the *PPARy* polymorphism was identified for this study. The *PPARy* polymorphism has been widely researched, as seen in the literature. There has however been controversy over the *PPARy* gene polymorphism and its association with T2DM. The association with T2DM differs among various population groups with different ancestry. For this reason the focus of this research study will be on the *PPARy* Pro12Ala polymorphism in a specific population group, where this polymorphism has not yet been researched.

2.13.1 *PPARy* gene function

Ligands are molecules that bind to specific nucleotide sequences within a gene's regulatory region. These bindings cause an alteration in gene expression through the regulation of gene transcription (DeBusk, 2012:154; Desvergne *et al*., 2006:498; Muller & Kersten, 2003:315). Nutrients and other bioactive components in food can serve as ligands and influence gene expression. Some bioactive molecules can penetrate membrane barriers and interact directly with the DNA – depending on their size and lipid solubility, while others may not be able to cross the cell membrane and will rather interact with a receptor on the cell surface and set into motion the cascade of signal transduction events that results in a transcription factor being activated (DeBusk, 2012:155; Desvergne *et al*., 2006:498; Muller & Kersten, 2003:315). An example of ligand activated receptors are the peroxisome proliferator-activated receptors (PPARs) which belong to the nuclear hormone receptor family that act by controlling networks of target genes (Blaschke *et al.,* 2006:30).

This subfamily of nuclear receptors can be activated by dietary fatty acids and their metabolic derivatives in the body, and therefore serve as lipid sensors which, when activated, can markedly redirect metabolism. There are three isoforms, encoded by separate genes: *PPAR-gamma (PPARγ), PPAR-alpha (PPARα), and PPAR-beta (PPARβ)*. Their pattern of distribution differs but they share 60 to 80% homology in their ligand- and DNA-binding domains, and exhibit distinct patterns of expression and overlapping and distinct biological activities (Blaschke *et al.,* 2006:30; Forman *et al.,* 1997:4312). Therefore, each of these PPAR receptors carries out a unique

function in the regulation of energy metabolism (Blaschke *et al.,* 2006:30; Evans *et al.,* 2004:357). *PPARγ* is a nuclear receptor that is an important regulator of adipocyte differentiation, as well as lipid metabolism and insulin sensitivity. Evidence shows that *PPARγ* is a master regulator in the formation of fat cells and the fat cells' ability to function normally (Evans *et al.,* 2004:357). Thus, *PPARγ* is a promising candidate gene to combat disorders, including obesity and T2DM mellitus (Yen *et al*., 1997:271).

Two protein isoforms of *PPARγ* have been identified, namely *PPARγ1* and *PPARγ2*. *PPARγ2* produces a protein that contains an additional NH2 terminal region, composed of 30 amino acids (Blaschke *et al.,* 2006:30; Desvergne & Wahli *et al.,* 1999:651). *PPARγ2* is encoded in seven exons, the common six exons and a specific *PPARγ2* exon (exon B) which encodes an additional 30 N-terminal amino acids (Beamer *et al.,* 1997:758). PPARγ1 is widely expressed, whereas *PPARγ2* expression is primarily limited to adipose tissue (Desvergne & Wahli *et al.,* 1999:651). Human *PPARγ* protein is formed out of four domains. The most important are the DNA binding domain (DBD) and ligand binding domain (LBD). The DBD can regulate transcription of target genes by forming heterodimers with the retinoid X receptor (RXR) which is a regular DNA binding partner to many nuclear receptors of the steroid/thyroid receptor superfamily, and binding to specific PPAR response elements in the promoter region of target genes (Desvergne & Wahli *et al.,* 1999:650; Chu *et al.,* 1995:11596).

PPARγ2 plays an important role in glucose homeostasis and is the molecular target for a class of insulin-sensitising drugs, called thiazolidinediones, which are *PPARγ2* ligands and widely used in the treatment of T2DM (Evans *et al.,* 2004:357). The expression and secretion of adiponectin, a hormone exclusively produced by the adipocyte that increases insulin sensitivity, is increased in the presence of *PPARγ2* agonists. It therefore seems that adipose tissue is the primary target of *PPARγ2* ligands, resulting in an improvement in insulin sensitivity in liver and muscle (Evans *et al.,* 2004:358). For this reason *PPARγ2* is a promising candidate gene for several human disorders and complex phenotypes, like obesity and T2DM.

2.13.2 *PPARy* gene polymorphism

The *PPARy* polymorphism that has been mostly associated with T2DM is the Pro12Ala polymorphism. The polymorphism involves a common cytosine to guanine single nucleotide polymorphism in *PPARγ2* exon B (CCA-to-GCA missense mutation). This variation results in a Proline to Alanine substitution at codon 12, which has been found to modulate the transcriptional activity of the gene (Masugi *et al.,* 2000:180). The Pro12Ala polymorphism has been widely investigated in relation to various disorders including T2DM, insulin sensitivity, and obesity. Numerous studies on the *PPARy* Pro12Ala polymorphism have indicated an association with T2DM (Lamri *et al.*, 2012:223; Sokkar *et al*., 2009:85; Ghoussaini *et al*., 2005:7; Yamauchi *et al*., 2001:1012; Masugi *et al*., 2000:180). A recent study on an Iranian population indicates this association and found the Pro12Ala *PPARy* polymorphism is likely to be related to the development of T2DM (Mohammadi-Asl *et al*., 2013:1332). A very recent study done in South Africa in Cape Town on a population of mixed ancestry by Vergotine *et al*. (2014:5) also indicates an association between T2DM or insulin resistance and the *PPARy* Pro12Ala polymorphism (Vergotine *et al*., 2014:6). The Ala allele, in contrast, has been found to be associated with a protective effect against the development of diabetes (Huguenin & Rosa, 2010:496; Honka *et al*., 2009:545; Scacchi *et al*., 2007:639, Yamauchi *et al*., 2001:1012). See Table 2.1 with summary of data from studies conducted to investigate the association of *PPARy* Pro12Ala polymorphism with Type 2 diabetes mellitus (T2DM) and obesity.

Numerous studies support an association between the *PPARy* Pro12Ala polymorphism gene and T2DM, expressing an increasing need to study the genetic structure of people where changes in environmental factors, including dietary intake, could interfere with their genes and endanger their health. Nutrigenomics can contribute in designing optimised dietary intervention approaches to restore and improve metabolic homeostasis, improve health and well-being and prevent diet-related diseases.

Table 2.1. Summary of data from studies conducted to investigate the association of *peroxisome proliferator-activated receptor-gamma (PPARy)* Pro12Ala polymorphism with Type 2 diabetes mellitus (T2DM) and obesity.

Study reference	Study Design	Population	Sample size	*PPARy* associated with T2DM	*PPARy* associated with obesity
Gonzales *et al*., 2014	Observational Study	Spain (Caucasians)	298 (77 with T2DM) (221 nondiabetic)	No	Not researched
Pattanayak *et al*., 2014	Unknown	West Bengal (Eastern) India	400 (200 with T2DM) (200 nondiabetic)	No	Not researched
Majumdar *et al*., 2014	Unknown	Guwahati (Eastern) India	50 (50 with T2DM)	No	Not researched
Vergotine *et al*., 2014	Cohort Study	South Africa (mixed ancestry)	787 (212 with T2DM) (575 nondiabetic)	Yes	Not researched
Ye *et al*., 2014	Unknown	China	453 (198 with T2DM) (225 nondiabetic)	No	Not researched
Mohammadi-Asl *et al*., 2013	Unknown	Iran	160 (80 with T2DM) (80 nondiabetic)	Yes	Not researched
Shahrjerdi *et al*., 2013	Unknown	Western India	50 (25 with T2DM) (25 nondiabetic)	Yes	Not researched
Lamri *et al*., 2012	Cohort Study	France	4676 (1030 with T2DM) (3646 nondiabetic)	Yes	Yes
Chandrase-karan *et al*., 2012	Unknown	South India	140 (72 with T2DM) (68 nondiabetic)	Yes	Not researched
Ding *et al*., 2012	Cohort Study	China	820 (820 normal)	Not researched	Yes
Ali *et al*., 2009	Unknown	Tunis	675 (387 obese) (288 normal)	No	Yes
Sokkar *et al*., 2009	Unknown	Egypt	54 (15 nondiabetic) (15 obese nondiabetic) (24 with T2DM)	Yes	Yes
Sanghera *et al*., 2008	Unknown	Northern India	918 (532 with T2DM) (386 nondiabetic)	Yes	Not researched
Montagnana *et al*., 2008	Unknown	Sweden	4787 (4787 nondiabetic)	No	No
Badii *et al*., 2007	Case-controlled Study	Qatari	850 (400 with T2DM) (400 nondiabeticl)	No	No
Tonjes *et al*., 2006	Meta-Analysis	Caucasians (meta-analysis)	32000 (32000 nondiabetic)	No	No
Ghoussaini *et al*., 2005	Case-controlled Study	France (Caucasians)	3250 (2126 with T2DM) (1124 nondiabetic)	Yes	Yes

Tavares *et al*., 2005	Unknown	Brazil	377 (207 with T2DM) (170 nondiabetic)	Yes	Not researched
Tanko *et al*., 2005	Unknown	Denmark	1501 (1501 normal)	Not researched	Yes
Bouassida *et al*., 2005	Case-controlled Study	Tunis	488 (242 with T2DM) (246 nondiabetic)	No	No
Tai *et al*., 2004	Cross-Sectional Study	Asia	3938 (3938 normal)	No	No
Kim *et al*., 2004	Unknown	Korea	1051 (1051 normal)	Not researched	Yes
Robitaille *et al*., 2003	Cohort Study	Canada (Caucasians)	720 (720 nondiabetic)	Yes	Yes
Frederikson *et al*., 2002	Unknown	Denmark	2245 (2245 nondiabetic)	No	No

2.14 CONCLUSION

Diabetes is a challenging, chronic condition, with increasing prevalence. The increased prevalence of diabetes, high blood glucose levels and obesity in females in South Africa, especially black African females is a growing problem, increasing their risks for diabetes-related complications and death (Shisana *et al.*, 2013:92,93,140; Garg & Ulrich, 2006:117; Wild *et al.*, 2004:1051). Medical nutrition therapy is important in preventing diabetes, managing existing diabetes and preventing or reducing the rate of complications.

T2DM results from a complex interaction of genetic and environmental factors. The role of genetic factors in the pathogenesis of various diseases is becoming more evident; and in this study the possible role of the *PPARγ* polymorphism in T2DM was investigated in a black African population where T2DM and obesity is an increasing problem. This population group and polymorphism was investigated, as various studies relates the *PPARγ* polymorphism, T2DM and obesity in various ethnicities, but not in the black African population, where these risk factors are present and increasing. These advances can lead to preventative and therapeutic interventions modified for individuals based on their genetic profiles. If no association is found between the *PPARγ* polymorphism and T2DM in this study population it can be expected that this SNP is possibly not relevant to the South African black population and narrows down the SNPs with T2DM influence in the study population.

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CHAPTER 3: METHODOLOGY

3.1 INTRODUCTION

This chapter describes the study design, population, sample selection, study procedures and techniques, as well as the operational definitions, validity and reliability of measures and limitations of the study. Research procedures and methods used in statistical analysis of the data are included. Furthermore, ethical approval for the study and the timeframes and planning for implementation of this study are presented.

3.2 STUDY DESIGN

An observational, descriptive study was performed at the diabetes clinics of Pelonomi Tertiary Hospital and Universitas Academic Hospital in Bloemfontein, South Africa.

3.3 POPULATION

The study population consisted of black females attending the outpatient diabetes clinics at Pelonomi and Universitas Hospitals in Bloemfontein, Mangaung, Free State, South Africa. These clinics serve an area which includes Bloemfontein, Botshabelo and Thaba 'Nchu. Patients are seen on Wednesdays at Universitas Hospital and Thursdays at Pelonomi Hospital. The clinics accommodate 15 to 25 patients (males and females) per week and patients visit these clinics on a six-monthly follow-up basis. The languages spoken are Sesotho, Afrikaans, English, isiXhosa and Setswana. The Mangaung area covers more than 6263 km^2 and the population includes 850 000 people (SALGA, 2010:Online; Shall, no date:Online).

3.4 SAMPLE

This study included a convenience sample of 72 female participants diagnosed with Type 2 Diabetes Mellitus (T2DM). The sample size of 72 was chosen on the basis of funds available for the genomic test, which unfortunately implied that the statistical tests would have relatively low power. Participants were recruited from the diabetes clinics at Pelonomi Tertiary Hospital and Universitas Academic Hospital. In other research studies, investigating the *PPARy* polymorphism in participants with T2DM, the number of participants included varied between 54 and 958 (Sokkar *et al.*, 2009:79; Scacchi *et al*., 2007:633).

From April 2013 until November 2013, for seven consecutive months, patients with T2DM and who met the inclusion criteria, were screened by the researcher at the diabetes clinics of Universitas Academic Hospital and Pelonomi Tertiary Hospital. Participants who met the inclusion criteria were invited to participate in the study. Informed consent was obtained from participants in the language of their choice and with the use of a translator after they agreed to participate. In participants recruited from Universitas Hospital, anthropometry, blood samples and the dual-energy X-ray absorptiometry (DXA) scan could be done immediately, but participants recruited at Pelonomi Tertiary Hospital had to come back to Pelonomi Tertiary Hospital on the Friday to be transported to Universitas Academic Hospital for the anthropometry, DXA scan and blood samples. Transport to and from Universitas Academic Hospital was arranged for participants, and a snack provided.

The following inclusion and exclusion criteria were used, in order to exclude possible confounders:

3.4.1 Inclusion criteria

The sample included black, female participants:

- previously diagnosed with T2DM by a medical doctor; or
- with a glycosylated haemoglobin (HbA1c) > 6.5%;
- who were 60 years and younger;
- weighed less than 136 kg (DXA scan maximum weight);
- with a height less than 196 cm (the DXA scan table length).

3.4.2 Exclusion criteria

Participants were excluded from the study if they:

- were diagnosed with Type 1 diabetes mellitus;
- were male;
- were pregnant; or
- were younger than 18 years old.

3.5 PROCEDURES AND DATA COLLECTED

Figure 1 is a schematic representation of the manner in which the study was conducted. Approval for this study was obtained by the researcher from the Chief Executive Officers (CEOs) of Universitas Academic Hospital and Pelonomi Tertiary Hospital (Addendum 1), as well as the Ethics Committee of the Faculty of Health Sciences, University of the Free State (reference number ECUFS 162/2012) (Addendum 2). A pilot study that included four participants were conducted to test procedures and documentation. As no major changes were made, these participants were included in the main study. Black female individuals with T2DM, 60 years and younger, were invited to participate in this study. Informed consent (Addendum 3) in the language of choice (Sesotho, English or Afrikaans) was obtained before inclusion in the study with the help of a translator. Participants were transported to Universitas Hospital if they were recruited from Pelonomi Hospital, where blood samples were taken, and weight, height and body composition measured. The researcher obtained anthropometric measurements and body composition was measured by a registered technologist using DXA. Blood samples were drawn by a registered professional nurse for genetic testing after body composition was measured. All participants received a snack after bloods were drawn. The latest HbA1c levels were obtained from the patient file. All of the measurements and information were captured on the data form (Addendum 9). With the consent of participants, blood samples will be stored for a maximum of 15 years and used for future genetic studies by the Department of Haematology and Cell Biology. Genetic testing and genotype determination for this study was done at the University of the Free State, Department

of Haematology and Cell Biology. The genetic results, anthropometric data and body composition are described in this study. Results from this study will be disseminated by means of scientific publications in peer reviewed journals and a report will be made available to the Department of Health and Pelonomi and Universitas hospitals. Feedback on anthropometric measures was provided to participants, but no feedback on genetic research was given as scientific interpretation and clinical application is not relevant.

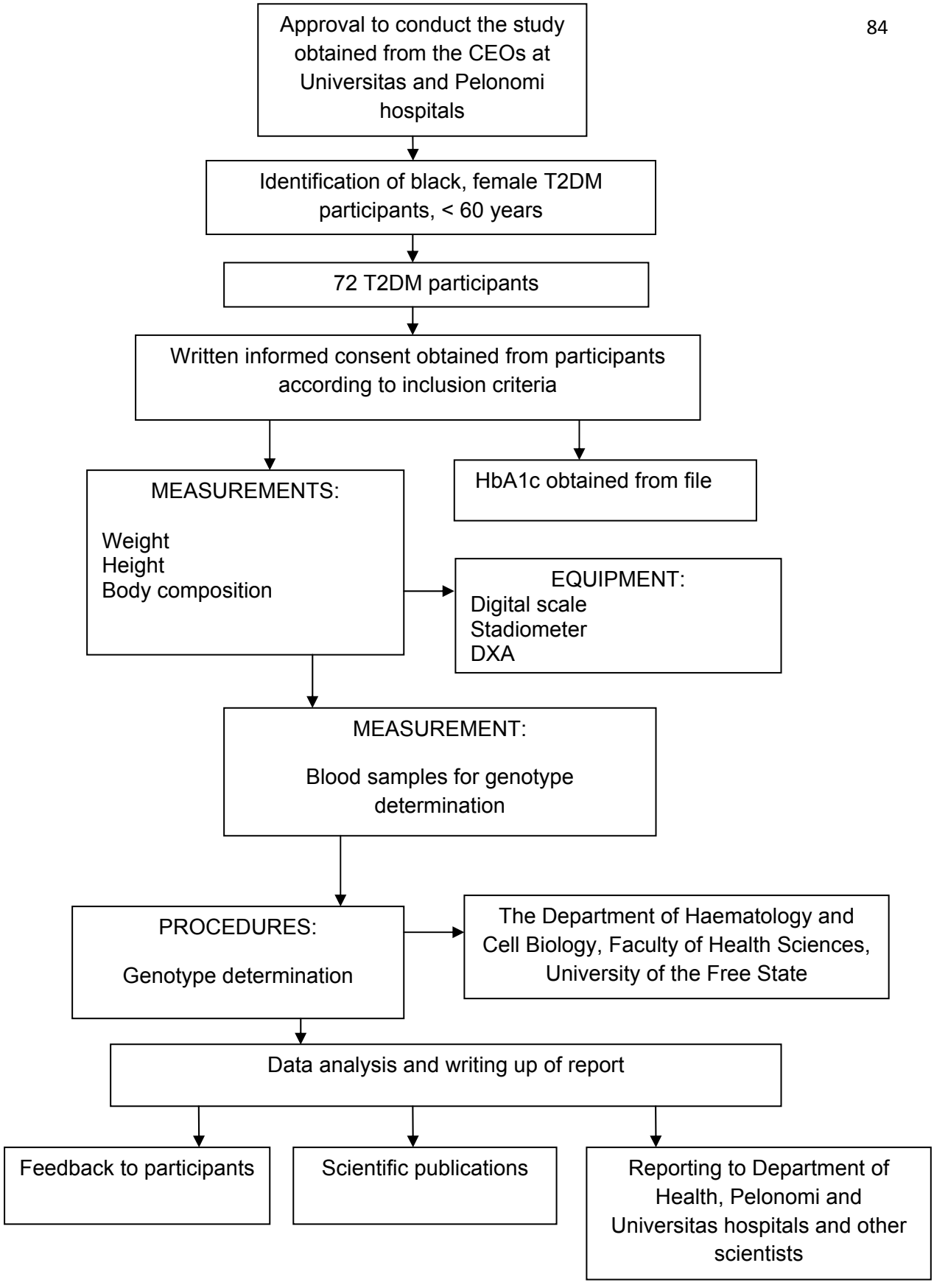

Figure 3.1. Flow chart of the study procedures.

3.6 OPERATIONAL DEFINITIONS

3.6.1 Pro12Ala polymorphisms

The *PPARy* gene (ENSG00000132170) is a nuclear receptor that acts by regulating gene expression of several other genes involved in adipocyte differentiation (Al-Rubeaan *et al*., 2013:168; Evans *et al.,* 2004:357). The *PPARγ2* Pro12Ala polymorphism (dbSNP accession no. rs 1801282) is located on chromosome 3p25, and is a cytosine to guanine single nucleotide polymorphism (SNP) in exon B (ENSE00003683648) (CCA-to-GCA missense mutation). This variation results in a Proline to Alanine substitution at codon 12, which has been found to modulate the transcriptional activity of the gene (Masugi *et al.,* 2000:180).

3.6.2 Body composition

Body composition measurements include bone mineral content (BMC) measured in grams, areal bone mineral density (BMD) measured in gram/cm^2, fat mass (g), lean mass including BMC (g) and percentage fat, calculated as fat mass divided by total mass x 100 (Kelly *et al.,* 2009:2). Body composition used for the purpose of this study was total fat mass and percentage fat. Reference values for body composition are indicated in Table 3.1.

Table 3.1. Standard values for body composition (Laquatra, 2000:561).

Males		**Females**	
Total fat • Storage fat • Essential fat	10% - 25% 10% - 25% 3%	Total fat • Storage fat • Essential fat	18% - 30% 18% - 30% 12%
Muscle	44.8%	Muscle	38%
Bone	14.9%	Bone	12%
Remainder	16.3% - 32.3%	Remainder	15% - 29%

3.6.3 Total body adiposity

Total body adiposity refers to the combination of essential and storage fats; expressed as a percentage of total body weight, that is associated with optimal health. Essential fat is needed for normal physiological functioning and is stored in small amounts, whereas storage fat refers to the energy reserve, primarily as triglycerides in adipose tissue. Storage fat accumulates under the skin and around the internal organs having a protecting function; it is more expendable and capable of wide variation (Lysen & Israel, 2012:463). A body fat mass percentage of 25% or more in males and 30% or more in females indicates obesity, which is associated with increased metabolic and health risks (Lysen & Israel, 2012:463; Kelly *et al.,* 2009:2; Mahan & Escott-Stump, 2000:561).

3.6.4 Body mass index

Body mass index (BMI) evaluates an individual's weight in relation to height and is calculated as kg/m^2. In this study, participants were classified as obese (BMI >30 kg/m^2) and non-obese (BMI <30 kg/m^2) (Hammond & Litchford, 2012:166). BMI categories are indicated in Table 3.2. BMI however does not provide specific information about body fat mass, and may misclassify individuals with a high muscle mass as being overweight or obese, especially individuals with a short stature (Völgyi *et al*., 2008:700). In this study, DXA was used to describe specific body fat percentage.

Table 3.2. Body mass index classification (Lysen & Israel, 2012:470).

BMI	Classification
< 18.5	Underweight
18.5 – 24.9	Normal
25 – 29.9	Overweight
30 - 34.9	Obese class 1
35 - 39.9	Obese class 2
> 40	Obese class 3

3.6.5 Glycosylated haemoglobin (HbA1c)

HbA1c refers to glycosylated haemoglobin. The red blood cells contain a protein called haemoglobin, which transports oxygen from the lungs to all the cells in the body. This protein binds with sugars such as glucose and as blood glucose levels increase, more haemoglobin gets glycosylated (American Diabetes Association:Online). Glycosylated haemoglobin does not reflect recent changes in glucose levels and is therefore a valuable reflection of the average blood glucose levels for the preceding 2 to 3 months. Glycosylated haemoglobin is expressed as % total haemoglobin and an HbA1c above 6.5% is used to diagnose diabetes mellitus (Litchford, 2012:203).

3.7 TECHNIQUES

Techniques used in this study for the collection of the information included genotyping; anthropometry, weight and height measurements; body composition which was taken with a DXA scanner; and biochemical assays for HbA1c.

3.7.1 Genotyping

Peripheral blood samples were drawn by a registered professional nurse under supervision of the medical specialist attending to the diabetes clinic of Universitas Academic and Pelonomi Tertiary Hospitals, for genotype determination. Genotype determination was performed by the Department of Haematology and Cell Biology, Faculty of Health Sciences from the University of the Free State with the assistance of the researcher. After collection, genomic DNA was isolated from 3 ml peripheral blood collected in ethylenediaminetetraacetic acid (EDTA) tubes, using the Wizard® Genomic DNA Purification Kit (Promega). The DNA isolation yield was quantified using Nanodrop (ThermoFisher) spectrophotometry technology. DNA isolation was repeated for samples that yielded a DNA concentration below 10ng/ul. Real-time PCR was performed using an optimised Taqman® Assay according to standard

recommendations for the LC 480 Real Time PCR system (Roche). Real Time PCR was performed using the Roche LC 480 instrument. Reaction mixture contained Template DNA [100ng], Primers [400nM] and LC480 probe master mix (Roche, Germany). All reactions were performed according to the following cycling conditions starting with 95°C for 5 min then 35 repeats of a two step cycling between 95°C for 10 seconds and 60°C for 45 seconds. Taqman assays are probe-based which require the use of fluorogenic probes to detect specific PCR products as accumulation occur during the PCR cycle. A probe consisting out of a single stranded DNA molecule with a reporter dye on the 5' end and a quenching dye on the 3' end is required to perform a Taqman-based assay (Heid *et al*., 1996). While the probe is still intact, the fluorescence emitted from the reporter gets absorbed by the quenching dye, thus no emission of light will occur. Emission of light can only occur if the probe attaches to a specific DNA target sequence in order for DNA polymerase to cleave the probe by its 5'-3' exonuclease activity (Applied Biosystems, 2005). After cleavage, the quencher will be unable to absorb the fluorescence from the reporter molecule. No light emission will occur because the quencher and reporter will no longer be in close enough proximity (Heid *et al*., 1996).

3.7.2 Anthropometry

Anthropometry includes obtaining physical measurements of an individual to relate them to standards that reflect the growth and development of that person. These measurements are mostly used to indicate over- or under-nutrition (Hammond & Litchford, 2012:165; WHO, 2008:3-3-9). Anthropometrical measurements that were measured in this study included weight, height (to calculate BMI) and body composition.

3.7.2.1 *Weight*

Actual body weight is the body weight measurement obtained at the time of examination and can be influenced by changes in fluid status (Hammond & Litchford, 2012:166). The scale used was a Nagata digital scale (serial no. 92502). The model

number is KW-302-RL 15 and it was manufactured in Taiwan. Its capacity is 300 kg and it auto-calibrates (Nagata Scale Co.Ltd:Online). Body weight was measured by the researcher at Universitas hospital with this calibrated electronic scale for accuracy. The scale was placed on a flat and firm surface, and was never moved or shifted. The scale was zeroed before each participant's weight was taken, and the researcher also made sure the display showed 0.0 before the participants stepped onto the scale. The participants were weighed in minimal clothing, and without shoes. When the participants stepped onto the scale platform it was ensured that the weight was evenly distributed: the participants had to stand in the centre of the scale platform, hands at their sides and looking straight ahead. Each participant's weight was measured three times to ensure accuracy and recorded to the nearest 0.1 kg, if the weight differed the mean was taken of all three measurements (De Bruyne *et al.,* 2012:391; WHO, 2008:3-3-9; NHANES, 2007:3).

3.7.2.2 *Height*

The direct measurement of height requires a stadiometer and the participant must be able to stand straight up or recline flat (Hammond & Litchford, 2012:165). The stadiometer used was a fixed stadiometer at Universitas Hospital, to ensure reliability. The headboard of the stadiometer could tighten when at the correct position, to ensure that it did not move while the measurement was taken. Height was measured without shoes or any headgear. Participants were asked to stand with their feet together with their weight evenly distributed and heels straight and against the measuring board. The participants were asked to stand up straight, looking ahead, without tipping the head up or down. The top of the ear and outer corner of the eye was in a line parallel to the floor (Frankfort plane). Participants were asked to breathe in and stand tall, then the headboard of the stadiometer was lowered, until it rested firmly on the top of the head with sufficient pressure to compress the hair (De Bruyne *et al.,* 2012:390; WHO, 2008:3-3-8; NHANES, 2007:8). Height was read and recorded in centimetres to the nearest 0.1 centimetre. The height was measured three times, and if the height differed the mean height was taken from all three measurements (De Bruyne *et al.,* 2012:390).

3.7.3 Body composition

Body composition is determined in order to provide a description of overall health and to indicate health risk. In individuals of similar height, differences in skeletal size and the proportion of lean body mass can contribute to body weight variations (Hammond & Litchford, 2012:167). One of the most accurate methods to describe body composition is by means of dual-energy X-ray absorptiometry (DXA). Two X-ray beams with different energy levels are used to determine absorption in DXA and provide an indication of body composition. The quantity of energy loss depends on the type of tissue through which the beam passes; and the result can be used to measure mineral, fat, and lean tissue compartments (Hammond & Litchford, 2012:171). The DXA scanner used in this study was a Discovery QDR Hologic model. This DXA model is a third generation QDR densitometer, manufactured by Hologic, Incorporated (Hologic:Online).

To ensure accuracy of the results of the DXA measurement, it was required of the participants to lie still while the machine emitted low levels of radiation (Hammond & Litchford, 2012:171). All jewellery and other personal items containing metal were removed, to prevent interference with the DXA. Participants who weighed more than 136 kg or who were taller than 196 cm were not considered for the study, as these are the DXA scan table weight and height limits (Kelly *et al.,* 2009:1). The measurements were taken by skilled technologists, trained to operate and take measurements with the DXA scanner. The DXA scanner was at Universitas Hospital at the Endocrinology Clinic in a room specifically designed to do DXA scans. In the room there was a small area where the participants could undress and remove all items not allowed during a DXA scan. The scan time periods varied from 20 to 45 minutes, depending on the participants' body composition and level of cooperation.

3.7.4 Biochemical assays

HbA1c analyses are routinely performed during each six monthly visit of participants to the diabetes clinics, and the latest results from the participant's file were used. The HbA1c of all the patients were analysed at the NHLS Laboratories, an accredited laboratory facility situated at Pelonomi Tertiary and Universitas Academic

Hospitals. HbA1c is a reflection of the average blood glucose levels for the preceding 2 – 3 months, and does not reflect recent changes in glucose levels. HbA1c is expressed as % total haemoglobin. An HbA1c above 6.5% is used to diagnose diabetes mellitus (Litchford, 2012:203).

3.8 VALIDITY AND RELIABILITY

3.8.1 Validity of measurements

The validity of a measurement instrument refers to the extent to which the instrument measures what it is intended to measure (Leedy & Ormrod, 2010:28). In terms of genotyping, validity was assured by testing for the *PPARy* gene polymorphism related to T2DM as described in literature. T2DM is a multifactorial trait influenced by more than one gene with multiple variants that interact with environmental factors. Limited data is available on the prevalence of this polymorphism in the study population.

Validity of anthropometric measurements was assured by using a calibrated electronic scale, which is recommended for direct weight measurements (De Bruyne *et al.,* 2012:391). Height was measured with a stadiometer, which is a standard method for height measurement (De Bruyne *et al.,* 2012:390). The measurement of body composition was done using DXA. DXA is regarded as one of the most appropriate methods for analyses of body adiposity (Elder *et al.*, 2012:53).

Validity of the HbA1c values was assured by the fact that HbA1c is a true reflection of the average blood glucose levels for the preceding 2 to 3 months; and does not reflect recent changes in glucose levels (Litchford, 2012:203). The HbA1c was measured at an accredited NHLS Laboratory at Pelonomi Tertiary and Universitas Academic Hospitals.

3.8.2 Reliability of measurements

Reliability refers to the steadiness with which a measuring instrument yields a certain outcome when the entity being measured has not changed. Reliability also refers to measuring accurately only when it can be measured consistently (Leedy & Ormrod, 2010:29). Reliability of genetic testing was assured by following standardised collection of samples, sample storage and validated laboratory techniques with the inclusion of controls for each genotyping assay. Real-time PCR reagents were used according to the manufacturers' standard recommendations for the Roche LC480 Real Time analysis system (Roche). All assays were performed with non-template controls and positive controls for each allele. The allele-specific controls used in each genotyping run were sequenced beforehand or synthetically manufactured to ensure validity. Furthermore, duplicate reactions were performed for all samples and controls.

Reliability of anthropometry was assured by measuring weight and height three times, and using the mean to calculate BMI (De Bruyne *et al.,* 2012:390). The measuring instruments were standardised and the researcher, a registered dietician, took these measurements (Leedy & Ormrod, 2010:93).

In terms of the HbA1c, reliability was assured due to it being done in an accredited NHLS Laboratory. As soon as the blood was drawn for the HbA1c it went to the laboratories immediately as the laboratories are situated at Pelonomi Tertiary and Universitas Academic Hospitals.

Reliability of this study also is considered a strength, as measurements taken can typically be measured with accuracy when measured by a skilled researcher using standardised measuring tools. Results from the study did not rely on data obtained from participants, but were all directly measured by the researcher or obtained from the patient files.

3.9 IMPLEMENTATION OF STUDY (TIME FRAME AND PLANNING OF DATES)

The study was conducted according to the following time schedule:

Table 3.3. Time schedule.

18 June – 3 September 2012	Protocol planning and contracting with study leaders
3 September 2012	Distributing protocol to Evaluation Committee
10 September 2012	Evaluation Committee meeting
25 September 2012	Hand protocol in to Ethics Committee
18 October 2012	Ethical approval granted
1 February 2013 – 30 March 2013	Protocol amendments and pilot study
1 April 2013 – 30 November 2013	Pilot Study, Sample selection, validation and genotyping optimisation
1 December 2013 – 30 June 2014	Genotype determination
1 July 2014 – 20 October 2014	Data analysis
24 October 2014	Notice of handing in dissertation

3.10 PRACTICAL IMPLEMENTATION AND LIMITATIONS OF THE STUDY

The study required participants recruited from the Pelonomi diabetes clinic on Thursdays to be transported to Universitas Hospital for DXA scans, anthropometry and blood samples on Fridays. Although transport was arranged free of charge for participants from Pelonomi Tertiary Hospital to Universitas Academic Hospital, ten participants who agreed to participate in the study and signed the informed consent form did not return on the Friday to be transported to Universitas Academic Hospital for the measurements. Two participants were admitted to the hospital due to high blood pressure or uncontrolled blood glucose levels; and eight of the participants lived far from the hospital and did not show up. No more than four participants could be recruited per day for the study, because of the time needed for each DXA scan, which placed a restriction with the number of participants that could be included per week. On Wednesdays at the Universitas diabetes clinic, participants were recruited for the study and the DXA scan was done on the same day, but there were also restrictions on the number of participants per day, because of limited time available for DXA scans. One of the individuals recruited for the study had a prosthetic arm and leg, resulting in the situation that the DXA scan could not be performed and this volunteer could therefore not be included in the study.

The practical limitations of the study and the same patients that returned to the clinic resulted in the small group of participants that could be included in the study. The genetic tests were also expensive, which further limited the study size and unfortunately also gave the statistical tests relatively low power.

3.11 STATISTICAL ANALYSIS

The data analysis for this study was done using SAS/STAT software, Version 9.3 of the SAS system for Windows, Copyright © 2010 SAS Institute Inc. Statistical analysis of data from this study was reported as means with standard deviations and frequencies. Pearson correlations were used to correlate different variables, with an r-value equal or less than 0.3 indicating a weak correlation. Statistical significance was set at p-values equal or less than 0.05. The small sample size (constrained by the cost of testing) unfortunately resulted in low power for the Hardy-Weinberg

calculations (where power is equivalent to the likelihood of a non-significant chi-square value, cf. Rohlfs & Weir, 2008:1611).

3.12 ETHICAL CONSIDERATIONS

Ethical approval for this study was obtained from the Ethics Committee of the Faculty of Health Sciences, University of the Free State before onset of the study (Ethics reference number ECUFS 162/2012) (Addendum 2).

Approval and permission to conduct the study was obtained from the CEOs of the institutions involved (Addendum 1). Written informed consent (Addendum 3) was obtained from all participants. Participants were provided with an information letter in which the procedures were explained in simple and understandable terms in the language of their choice (Sesotho, English or Afrikaans). The risks associated with drawing of blood (physical discomfort, potential risks for infection or bruising) were included in the informed consent. Consent was also requested to store and use the participant's DNA sample for genetic research. Participants were also informed that the results of this study may be published. In the case of Pelonomi Tertiary Hospital, participants were transported to the DXA machine and back to the clinic free of charge. Participation in the study was voluntary and participants were free to withdraw from the study at any time. Participants in this study received R100 remuneration to cover their travel expenses to the hospital. Confidentiality was maintained during all stages of the research by ensuring that no names were disclosed, or written down in the data analysis spreadsheet. No names were used during data analysis and in the discussion of results, only numbers were used. All participants received a snack after bloods had been drawn.

3.13 CONCLUSION

The methodology for conducting this study has been described in this chapter. The study sample and the selection of the sample were discussed, and the procedures and data that were collected during this study have been outlined. Techniques that were used have been explained, as well as the methods used for statistical analysis. Attention was given to actions taken to ensure validity and reliability, as well as the limitations of the study. Practical implementation of the study was also discussed, as well as ethical considerations.

3.14 REFERENCES

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CHAPTER 4: PRESENCE OF THE *PPARY* PRO12ALA POLYMORPHISM IN BLACK FEMALES WITH TYPE 2 DIABETES MELLITUS ATTENDING DIABETES CLINICS IN BLOEMFONTEIN

The aim of this publication is to report on the findings of the first aim of the research project by describing the presence of the *PPARy* Pro12Ala polymorphism in black females with Type 2 Diabetes Mellitus (T2DM). This article was submitted to the Journal of Endocrinology, Metabolism and Diabetes of South Africa (JEMDSA) and is prepared according to the authors instructions for JEMDSA (Addendum 4).

Title

PPARγ Pro12Ala polymorphism in black females with type 2 diabetes mellitus attending diabetes clinics in Bloemfontein, South Africa: a descriptive study

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Abstract

Objective: To determine the presence of the *PPARγ* Pro12Ala polymorphism in black females with type 2 diabetes mellitus (T2DM) attending diabetes clinics in Bloemfontein.

Design: An observational, descriptive study was performed.

Setting and subjects: Seventy-two black females with T2DM, between 20 and 60 years of age, attending diabetes clinics at Pelonomi- and Universitas Hospitals in Bloemfontein, Mangaung District in the Free State Province, were included in the study.

Outcome measures: Blood samples were obtained for genotyping to investigate the presence of the *PPARγ* Pro12Ala polymorphism in the study population. Body weight, height and body composition were measured to describe adiposity. Descriptive statistics were used to analyse data.

Results: Of the 72 participants, 71 (98.6%) carried the Pro/Pro allele, while one heterozygotic participant carried the Pro/Ala allele. The participants' mean body mass index (BMI) was 36.4 kg/m^2 (SD ± 7.8) and their mean total fat percentage was 42% (SD ± 6.2).

Conclusions: No homozygotic *PPARγ* 12Ala individuals were found and only one patient was heterozygotic in this population, implicating that the Pro12Ala polymorphism does not contribute towards the development of T2DM in this black, female population. The *PPARγ* Pro12Ala single nucleotide polymorphism (SNP) would therefore not be a suitable biomarker for early risk prediction of T2DM in this population.

Keywords: type 2 diabetes mellitus; T2DM; prevention; nutrigenomics; *PPARγ* Pro12Ala polymorphism; nutrition

Introduction

Type 2 diabetes mellitus (T2DM) is the most common and prevalent type among all diabetes cases, and an increase of 55% until 2035 has been estimated.[1] In 2011, 366 million individuals lived with diabetes globally, rising to 382 million individuals (between 40 and 59 years) in 2013. A projected increase in T2DM to 471 million individuals by 2035 is expected. In Africa, 19.8 million individuals are living with diabetes mellitus, and in 2013, 2.6 million (7–9%) adults in South Africa suffered from diabetes.[1] According to the South African National Health and Nutrition Examination Survey, 9.5% of individuals aged 15 years and older in South Africa have T2DM.[2] The global increase in the incidence of diabetes mellitus, in particular T2DM, necessitates action, especially in the form of prevention strategies.

Genetic make-up sets the stage for disease, while environmental factors such as nutrition and lifestyle choices determine the risk for the development of disease. Nutrigenomics may therefore be the key to developing prevention strategies through nutritional intervention.[3,4] Nutrigenomics can be described as genome-wide influences of nutrition on the transcriptome, proteome and metabolome of cells, tissues, or organisms at a specific time i.e. how nutrients and bioactive components affect gene expression.[5] Nutrients and bioactive compounds can optimise and preserve physiological processes at cellular, tissue, organ and whole body level to prevent development of disease[5] by acting as dietary signals, detected by the cellular sensor systems that influence gene and protein expression and metabolite production.[6] Nutrition therapy based on genetic composition is expected to feature with increasing significance in the prevention and management of chronic, diet- and lifestyle-related diseases.[3]

Several genes have been associated with an increased risk for T2DM, specifically the *PPARγ* Pro12Ala polymorphism which is a widely studied gene, with controversy in the outcome in various populations.[7] The peroxisome proliferator-activated receptors (PPARs) belong to the nuclear hormone receptor family, which act by controlling networks of target genes. They are ligand-activated receptors, activated by nutrients and subsequently redirect metabolism.[8] *PPARγ* is a nuclear receptor that is an important regulator of adipocyte differentiation, as well as lipid metabolism (adipogenesis and lipogenesis) and insulin sensitivity. Evidence shows that *PPARγ* is a master regulator in the formation of fat cells and fat cells' ability to function normally.[9] Two protein isoforms of *PPARγ*, namely *PPARγ*1 and *PPARγ*2, have been identified. *PPARγ2* expression is primarily restricted to adipose tissue and plays a significant

role in glucose homeostasis and the expression and secretion of adiponectin, a hormone exclusively produced by the adipocyte that increases insulin sensitivity.[9] This specific gene function renders the *PPARγ2* gene an ideal candidate for early risk prediction of T2DM.

The *PPARγ* polymorphism that has mostly been associated with T2DM is the Pro12Ala single nucleotide polymorphism (SNP).[10,12,13,14,15,16,17] It is a cytosine to guanine change in *PPARγ2* exon B (CCA-to-GCA missense mutation), which has been found to modulate/reduce the transcriptional activity of the gene.[10]

In the Coronary Artery Risk Development in Young Adults (CARDIA) study,[11] the association of *PPARγ* Pro12Ala and insulin sensitivity and adiposity were among the variables determined over a period of 15 years in African Americans and Caucasians. The prevalence of the Pro12Ala polymorphism was 1% in African Americans and 24% in white Americans. The protective Ala allele, associated with reduced risk for T2DM, was therefore more prevalent in white Americans, with a very low prevalence in the African American population, as supported by a significant difference shown between the genotype frequency distribution in the two ethnic groups. In both ethnicities, the Ala allele was significantly associated with decreased risk for insulin resistance. Associations between the *PPARγ* Pro12Ala polymorphism and body adiposity and insulin sensitivity were found in both ethnicities, but these associations were in opposite directions among African Americans and whites. In the white population, significant associations between the Pro12Ala polymorphism and adiposity were found, while in the black population, a significant association between the Pro12Ala polymorphism and measures of insulin sensitivity occurred. These results highlighted the important role of body mass index (BMI) and ethnicity in influencing the relationship between *PPARγ* gene variation, adiposity and insulin resistance.[11]

No genetic data on the prevalence of the *PPARγ* polymorphism in a black South African population with T2DM is available. This study investigated the presence of the *PPARγ* Pro12Ala polymorphism among black females with T2DM, in order to determine whether dietary and lifestyle interventions affecting the expression of this gene could provide the basis for an effective early prevention strategy.

Methods

Study design and setting

This descriptive study was conducted between May and November 2013 and included black female adults with T2DM attending the outpatient diabetes clinics at Pelonomi Regional Hospital or Universitas Tertiary Hospital in Bloemfontein, South Africa. These clinics mainly serve the Bloemfontein, Botshabelo, and Thaba 'Nchu areas and accommodate 15 to 25 patients per week, who return six-monthly for follow-up visits.

Study population, sampling and samples

A convenience sample of 72 female participants was recruited on Wednesdays and Thursdays at Pelonomi and Universitas Hospitals' clinics. Black, adult, female participants, previously diagnosed with T2DM by a medical doctor or with a glycated haemoglobin (HbA1c) > 6.5%, between the ages of 18 years and 60 years, weighing less than the Dual-Energy X-ray Absorptiometry (DXA) scan table weight limit of 136 kg, with a height less than the DXA scan table length of 196 cm, were eligible for inclusion in the study. Participants were excluded from the study if they were diagnosed with type 1 diabetes mellitus, were male or pregnant, or unable to give consent. Recruitment continued during clinic hours for seven consecutive months.

Data collection

Participants complying with the inclusion criteria were invited to participate in this study. Informed consent in the language of choice (Sesotho, English or Afrikaans) was obtained from the participants. Participants recruited at Pelonomi Hospital were transported to Universitas Hospital where weight and height were measured and body composition determined by means of DXA. Blood samples were collected and participants received a snack afterwards.

DNA was isolated from 3 ml peripheral blood collected in EDTA tubes, using the Wizard® Genomic DNA Purification Kit (Promega, Wisconsin, USA). Real-time PCR was performed using an optimised Taqman® Assay according to standard recommendations for the LC 480

Real Time PCR system (Roche, Mannheim, Germany). The analysis of the *PPARγ* Pro12Ala (rs1801282) polymorphisms was performed through RealTime PCR allelic discrimination.

Statistical analysis

The data analysis for this study was performed using SAS/STAT software, Version 9.3 of the SAS system for Windows (© 2010 SAS Institute Inc.). Data are reported by means of descriptive statistics using means with standard deviations and frequencies.

Ethical considerations

Approval for this study was obtained from the Chief Executive Officers (CEO) of Universitas Academic and Pelonomi Regional Hospitals, as well as the Ethics Committee of the Faculty of Health Sciences, University of the Free State (reference number ECUFS 162/2012). Written informed consent was obtained from all participants.

Results

The age of participants ranged between 25 and 60 years, with a mean age of 50.4 (± 7.3) years. As indicated in Table 4.1, the age distribution showed a strong tendency towards the older categories, as expected in a population presenting with T2DM.

Table 4.1 further shows 34.7% of the participants had a BMI indicative of class 3 obesity. Only 5.6% had a normal healthy BMI. The mean BMI was 36.4 kg/m^2 (SD ± 7.8). Results from the DXA scans indicated that the participants had a mean total body fat percentage of 42% (SD ± 6.2).

Seventy-one (98.6%) of the 72 participants carried the homozygotic Pro/Pro genotype as indicated in Table 4.1. Conformity of the *PPARγ* genotype distributions to the Hardy-Weinberg law was assessed and equilibrium was found.

Table 4.1. Age distribution, body mass index (BMI) and *PPARγ* genotype frequencies in black female participants with T2DM (N=72).

Variable	Frequency	Percentage
Age group (years)		
20–29	1	1.39
30–39	4	5.56
40–49	24	33.36
50–60	43	59.75
BMI category (kg/m^2)		
Normal (18.5–24.9)	4	5.6
Overweight (25–29.9)	11	15.3
Obese class 1 (30–34.9)	21	29.2
Obese class 2 (35–39.9)	11	15.3
Obese class 3 (> 40)	25	34.7
PPARγ genotype		
Pro/Pro (C/C)	71	98.6
Pro12Ala (C/G)	1	1.4
Ala/Ala (G/G)	0	0

Discussion

Research has confirmed that the *PPARγ* Pro12Ala polymorphism is related to T2DM, although the presence of this polymorphism was not found in this South African population of black females with T2DM. Studies where the *PPARγ* Pro12Ala polymorphism was found in individuals with T2DM was in Egypt,[12] a French population with obese subjects,[13] a South Indian population,[14] an Iranian population,[15] a community of Khatri Sikhs in Northern India,[16] and also in a Western Indian population.[17]

The absence of the homozygotic *PPARγ* Pro12Ala polymorphism and the very low prevalence of the heterozygotic polymorphism in this mainly obese, black, female group with T2DM was also found in other studies. Populations that also did not present with a significant association included Caucasians from Spain,[18] individuals from the Eastern region of India,[19] Qatari population,[20] Tunisians,[21] a Chinese population,[22] and an Asian population.[23]

The BMI and total body fat were very high in this black, female population when compared to the recommended total body fat percentage of 18 to 30% in females and recommended BMI.[24]

Conclusion

This study was the first to investigate the presence of the *PPARγ* Pro12Ala polymorphism in a black South African population with T2DM. The presence of the homozygotic *PPARγ* G/G genotype was not determined in this black, urban population of female type 2 diabetics in Bloemfontein in the Free State Province, South Africa. The BMI and total body fat percentage in this population was very high, which strongly correlate with the risk and incidence of T2DM. Even though the results obtained from this study represent a small subset of the population and is limited by the absence of a non-diabetic control group, the allele frequency of the SNP suggests that it may not be a significant predictor of T2DM in the black South African population. It is very likely that the G/G genotype is rare in the population. Vergotine et al.[25] (2014:5) found that the G allele was present in only 5.3% of 704 South African individuals with mixed ancestry. The *PPARγ* Pro/Ala polymorphism can therefore not be regarded as a directly contributing factor to the development of T2DM in this population and is not a suitable genetic marker for early risk prediction of T2DM.

Limitations

Limitations acknowledged in this study are the lack of a non-diabetic control group and the relative small sample size, resulting from budgetary constraints and the fact that participants re-entered the clinic cycle after a six month period.

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Conflict of interest

The authors have no conflict of interest to declare.

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CHAPTER 5: THE ASSOCIATION OF THE *PPARY* PRO12ALA POLYMORPHISM AND BODY ADIPOSITY IN BLACK FEMALES WITH TYPE 2 DIABETES MELLITUS ATTENDING DIABETES CLINICS IN BLOEMFONTEIN: A DESCRIPTIVE STUDY

According to the objectives of the study, this article was planned to describe the association between Pro12Ala *PPARγ* gene polymorphisms and body adiposity in the study population. The genetic results from this study however revealed that 71 (98.6%) of the 72 participants, carried the Pro/Pro allele and only one heterozygote participant carried the Pro/Ala allele. There was therefore no homozygote Ala genotype present to associate with body adiposity in this population with type 2 diabetes mellitus (T2DM). In order to present and report this section of the data in a practical way for possible publication, the title therefore was changed. This article was submitted to the Diabetes Care Journal (Diabetes Care) and is presented according to the author's instruction for Diabetes Care Journal (Addendum 5).

Title

Body adiposity in black females with type 2 diabetes mellitus attending diabetes clinics in Bloemfontein, South Africa

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Abstract

Objectives: To describe body adiposity in black females with type 2 diabetes mellitus attending diabetes clinics in Bloemfontein, in the Free State Province, South Africa.

Research Design and Methods: A descriptive research design was used. A convenience sample of 72 females between the ages of 20 and 60 years old, with type 2 diabetes, were included in this study from out-patient diabetes clinics at Pelonomi Tertiary and Universitas Academic Hospitals in Bloemfontein, Free State Province, South Africa. Weight and height measures and body composition as measured by dual-energy X-ray absorptiometry (DXA) was done. Data were analysed using descriptive statistics.

Results: The mean body mass index (BMI) of this study population was 36.4 kg/m^2 (SD ± 7.8) and mean total body fat percentage was 42% (SD ± 6.2). A very strong significant correlation between BMI, total fat and trunk fat was found.

Conclusion: The prevalence of obesity in this black female population with type 2 diabetes is very high. The high BMI and high total body fat percentage supports the strong association between obesity and risk/incidence of type 2 diabetes. Dietary education and interventions aiming to prevent and decrease the presence of obesity in these individuals is important in order to decrease insulin resistance and the development of complications. Limitations acknowledged in this study are the lack of a non-diabetic control group and the relative small sample size, resulting from budgetary constraints and the fact that participants re-entered the clinic cycle after a six month period.

Keywords: type 2 diabetes mellitus; body adiposity; black population; DXA; BMI

Introduction

In 2013, approximately 382 million individuals worldwide, aged between 40 and 59, were accounted for living with diabetes mellitus, with negligible difference between genders (1,2). In Africa, an estimated 19.8 million people are living with diabetes (1), and in South Africa in 2013, 2.6 million (7–9%) adults aged between 20–79 years had diabetes (1,3). With type 2 diabetes mellitus, which currently accounts for more than 90% of all diabetes cases (3), type 2 diabetes is considered a global public health concern.

Central adiposity and overall obesity has been shown to be a strong risk factor for type 2 diabetes (4), with both body mass index (BMI) and central/abdominal obesity showing a strong significant association with the incidence of type 2 diabetes. These two features are regarded as predictors for the development of diabetes mellitus (5,6). Although central obesity is regarded as a better risk indicator of type 2 diabetes than BMI, both are considered important risk predictors for type 2 diabetes (7). In a study by Rana et al. (8), it was shown that obesity contributed to the development of type 2 diabetes and that women with a BMI of 40 kg/m^2 had a much higher risk for the development of type 2 diabetes than women with a BMI of 21 kg/m^2. Meisinger et al. (9) reported that both overall and abdominal adiposity play an important role in the development of type 2 diabetes.

Ethnic variation in BMI and anthropometric indices of general and abdominal obesity exist within groups, and increases in any of the adiposity indices are associated with an increased risk for diabetes mellitus and cardiovascular disease. A large variation in risk for diabetes mellitus or cardiovascular diseases between different ethnic groups has been described (10). When comparing obesity rates in the United States of America and the United Kingdom, much higher obesity rates are found in African and Hispanic Americans, especially African American women, than in white Americans. The rates of obesity were lower in white British individuals than in white American residents, indicating significant differences between different ethnic groups and populations (11). In different ethnic backgrounds, it was also shown that visceral adiposity accumulation or body fat distribution differs. When comparing total body fat and abdominal adipose tissue between individuals from Aboriginal, Chinese, South Asian or European origin, body fat distribution was found to differ according to ethnicity (12).

This study describes body adiposity in black females with type 2 diabetes, in Bloemfontein, South Africa.

Research design and methods

Study design

A descriptive research design was used.

Study population

Black, female patients between the ages of 20 and 60 years old with type 2 diabetes, attending the diabetes clinics of Pelonomi Tertiary and Universitas Academic Hospitals in Bloemfontein, were recruited for this study. These clinics serve the catchment areas of Bloemfontein, Botshabelo and Thaba 'Ncu in the Free State Province, South Africa.

Selection of participants

Participants were recruited on Wednesdays at the Universitas clinic and Thursdays at the Pelonomi clinic, between May and November 2013. Participants who met the following inclusion criteria were invited to participate in the study: black ethnicity, female, previously diagnosed with type 2 diabetes by a medical doctor or presenting with a glycosylated haemoglobin (HbA1c) > 6.5% (48 mmol/mol) as determined from their files, 60 years and younger, weighing less than the dual-energy X-ray absorptiometry (DXA) scan table weight limit of 136 kg, and with a height less than the DXA scan table length of 196 cm (13). Participants were excluded from the study if they were diagnosed with type 1 diabetes mellitus, were male, pregnant, or younger than 18 years old. A convenience sample of 72 female participants, diagnosed with type 2 diabetes, was selected

Data collection

The study formed part of a larger study and although data collection was done together, the results are reported separately. Black female individuals with type 2 diabetes complying with the inclusion criteria were invited to participate in this study. Informed consent in the language of choice (Sesotho, English or Afrikaans) was obtained before inclusion in the study. Patients willing to participate in the study were transported to Universitas Hospital where data were collected. Weight and height were measured and body composition

determined by means of DXA. Participants received a snack after bloods for the larger study were drawn.

Anthropometry

Weight was measured using a Nagata calibrated electronic scale (Nagata Scale Co. Ltd, Tainan City, Taiwan; serial number 92502) model number KW-302-RL 15. Its capacity is 300 kg and the scale auto calibrates (14).

Participants were weighed in minimal clothing without shoes. Weight was evenly distributed, with participants standing in the center of the scale platform, hands at their sides and looking straight ahead. Weight was recorded to the nearest 0.1 kg according to international standards (15–17). Height was measured with a calibrated, wall-mounted stadiometer. Participants were measured without shoes and hair gear, standing with their feet together, weight evenly distributed and heels against the measuring board, with knees straight. Participants were asked to breathe in and stand tall in the Frankfort plane to be measured (15–17). Height was recorded to the nearest 0.1 centimeter (15).

Body composition

DXA was used to measure body composition on a Hologic Discovery QDR model (Bedford, Hologic Incorporated) (18). Participants were required to lie still and remove all jewelry and other personal items containing metal to prevent interference with the DXA. Measurements were taken by skilled, registered technologists trained to operate and take measurements with the DXA scanner in the presence of the researcher (19).

Statistical analysis

The data analysis for this study was done using SAS/STAT software, Version 9.3 of the SAS system for Windows, (© 2010 SAS Institute Inc.). Data are reported as medians, means with standard deviations, and frequencies. Pearson correlations were used to correlate variables, with an r-value ≤ 0.3 indicating a weak correlation. Statistical significance was set at p-values ≤ 0.05.

Ethical considerations

Approval for this study was obtained from the Chief Executive Officers (CEO) of Universitas and Pelonomi Hospitals, as well as the Ethics Committee of the Faculty of Health Sciences, University of the Free State (reference number ECUFS 162/2012). Informed consent was obtained from all participants.

Results

The total number of females that participated in the study was 72. The majority of females were between the ages of 50 and 60 years (59.8%), with a mean age of 50.4 years (range 25–60 years).

Table 5.1 provides a summary of the basic anthropometrical data. Weight and height were measured to calculate BMI. The mean BMI of the group is 36.4 kg/m^2 (SD ± 7.8). Table 5.2 shows the distribution of participants in the different BMI categories. More than a third (34.7%) of the participants' BMI was classified as obese class 3.

Table 5.3 summarizes the minimum, maximum, mean and standard deviation of body fat percentage as measured by DXA. Body composition was determined separately for the left- and right leg, left- and right arm, head and trunk. The mean total body fat percentage was 42%. A strong, significant correlation between total body fat and trunk fat (r=0.94660), and BMI and trunk fat (r=0.9372), as well as total body fat and BMI (r=0.96452) was found. We also found a significant correlation between BMI and total trunk fat percentage (r=0.82636), and BMI and total body fat percentage (r=0.84066).

Discussion

The purpose of this study was to describe body adiposity in black females with type 2 diabetes attending diabetes clinics in Bloemfontein. Results from this study showed a very high prevalence (94.4%) of overweight or obesity in this population. The mean BMI was 36.4 kg/m^2, which is classified as obese class 3. It has been recommended that a healthy BMI should be between 18.5 kg/m^2 and 24.9 kg/m^2 (20). Mean total body fat was 42%, whereas in healthy females the recommendation is 18% to 30% (21). BMI and total body fat in this population were therefore much higher than the recommended cut-off points for optimal health.

Total body fat and trunk fat were, as expected, significantly associated with BMI, supporting the use of BMI as a cost-effective indicator of body adiposity, also in this population. The high prevalence of overweight and obesity in this population has also been found by other studies. Strotmeyer et al. (22) reported that individuals with diabetes had a higher BMI, fat mass, and abdominal visceral fat in both men and women. Gallagher et al. (23) indicated from their research that individuals with type 2 diabetes had more visceral adipose tissue and intermuscular adipose tissue, and less subcutaneous adipose tissue than healthy individuals. The strong association between obesity, specifically abdominal fat, and type 2 diabetes is also supported by findings from other studies (24–27).

Conclusion

This study showed a very high incidence of obesity with high levels of body fat in a black female population with type 2 diabetes in Bloemfontein, South Africa. These results support various studies showing a strong association between obesity and body adiposity, and the incidence of type 2 diabetes. Findings from this study raise concern about the very high incidence of obesity and its health implications, and emphasize the importance of dietary information and implementation of strategies to prevent and decrease body adiposity and obesity to prevent diseases of lifestyle and ensure optimal health.

Author contributions

This article is based on research done by I.v.N (University of the Free State), under supervision of R.L and G.M (University of the Free State). J.R (University of the Free State) was responsible for the statistical analysis of data

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I van Niekerk researched data and wrote the manuscript. R Lategan reviewed and edited the manuscript. G Marx reviewed and edited the manuscript. J Raubenheimer was the biostatistician who did the data analysis. The guarantor of the article is Inge van Niekerk, who takes full responsibility for the work as a whole, including the study design, access to data, and the decision to submit and publish the manuscript.

Conflict of interest

The authors have no conflict of interest to declare.

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Table 5.1. Summary of anthropometric data of black females with type 2 diabetes mellitus (N=72).

Anthropometric variable	Minimum	Maximum	Mean (SD)
Weight (kg)	52.3	130.6	89.7 (± 20.9)
Height (cm)	141.5	172.0	156.8 (± 5.9)
BMI (kg/m^2)	21.6	52.2	36.4 (± 7.8)

Table 5.2. Distribution of black female participants with type 2 diabetes mellitus with regard to body mass index (BMI) category (N=72).

BMI category (kg/m^2)	Frequency	Percentage
Normal (18.5–24.9)	4	5.6
Overweight (25–29.9)	11	15.3
Obese class 1 (30–34.9)	21	29.2
Obese class 2 (35–39.9)	11	15.3
Obese class 3 (> 40)	25	34.7

Table 5.3. Body fat distribution of black female participants with type 2 diabetes mellitus (N=72).

Body area	Minimum (%)	Maximum (%)	Mean (%)	Standard deviation (SD)
Left arm	27.0	68.9	51.6	± 9.0
Right arm	32.3	66.9	51.2	± 9.1
Trunk	25.1	52.8	41.3	± 6.7
Left leg	19.8	55.2	42.5	± 7.4
Right leg	17.8	57.1	43.2	± 7.9
Head	17.6	22.8	19.4	± 1.1
Total fat	26.2	52.9	42.0	± 6.2

CHAPTER 6: THE ASSOCIATION BETWEEN THE PREVALENCE OF THE PRO12ALA PPARY GENE POLYMORPHISM AND BLOOD GLUCOSE CONTROL (MEASURED AS HBA1C) IN BLACK FEMALES WITH TYPE 2 DIABETES MELLITUS ATTENDING DIABETES CLINICS IN BLOEMFONTEIN

According to the objectives of the study, this article was planned to describe the association between Pro12Ala *PPARγ* gene polymorphisms and blood glucose control (measured as HbA1c levels) in the study population. The genetic results from this study however revealed that 71 (98.6%) of the 72 participants, carried the Pro/Pro allele and only one heterozygote participant carried the Pro/Ala allele. There was therefore no homozygote Ala genotype present to associate with body adiposity in this population with T2DM. In order to present and report this section of the data for publication, the title was adapted. This article was submitted to the Diabetes Research and Clinical Practice Journal and is presented according to the author's instruction for Diabetes Research and Clinical Practice (Addendum 6).

Title

Body adiposity is a poor predictor of blood glucose control in black females with type 2 diabetes mellitus attending diabetes clinics in Bloemfontein, South Africa

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Conflict of interest statement

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Abstract

Aims: To describe body adiposity as a predictor of blood glucose control in black females with type 2 diabetes mellitus (T2DM) attending diabetes clinics in Bloemfontein, Free State Province, South Africa.

Methods: A descriptive study was performed. A convenience sample of 72 females between the 20 and 60 years old, attending diabetes clinics at Pelonomi Tertiary Hospital and Universitas Academic Hospital in Bloemfontein, were included in the study. Glycosylated haemoglobin (HbA1c) values were captured from clinic records, which are routinely measured at six-monthly clinic visits. Analysis of HbA1c was done by the local accredited National Health Laboratory Services (NHLS). Weight and height measures and body composition as measured by dual-energy X-ray absorptiometry (DXA) was done.

Results: The mean HbA1c was 8.4% (SD ± 2.2), with a minimum of 5.3% and a maximum of 13.8%. The mean BMI of the study population was 36.4 kg/m^2 (SD ± 7.6). No significant correlation was found between blood glucose control and BMI (r -0.00591; p=0.9607), or blood glucose control and body fat percentage (r -0.06648; p=0.5790).

Conclusions: Blood glucose control in this black female population with T2DM was poor. BMI and body adiposity were not associated with blood glucose control as measured by HbA1c, and blood glucose control was also not associated with age in this population. Poor blood glucose control as reflected by high HbA1c levels put this population with T2DM at a high risk for developing complications. Dietary counselling, optimal management and self-management are essential to improve blood glucose control and prevent complications and early mortality.

Keywords: type 2 diabetes mellitus; blood glucose control; glycosylated haemoglobin; HbA1c; diabetic complications

Introduction

Poor glycemic control in individuals with type 2 diabetes mellitus (T2DM) has been associated with an increase in microvascular and macrovascular complications [1,2]. Despite the benefits of maintaining optimal blood glucose control, poor control remains a problem in a substantial number of individuals with diabetes, thus increasing the risk for long-term complications. In Africa, Asia, Europe and South America, the prevalence of macrovascular complications reportedly occurs in 27% of individuals with T2DM, while between 37% and 89% of individuals present with microvascular complications [1]. Prevention of acute and chronic complications requires optimal blood glucose control and achievement of target glycosylated haemoglobin (HbA1c) levels [1,2].

Diabetes mellitus is one of the most challenging chronic diseases with an increasing prevalence worldwide. In 2013, there were 382 million diabetic patients globally, with an estimate of 471 million by 2035 [3]. In South Africa in 2013, 2.6 million adults between 20 and 79 years of age had diabetes [3,4]. T2DM, which is associated with resistance to insulin, currently accounts for more than 90% of all diabetic cases. Diabetes mellitus can therefore be considered as a major public health concern [4].

HbA1c levels are an indication of long-term blood glucose control [4,5]. Haemoglobin becomes glycosylated when glucose is non-enzymatically attached to one of its terminal amino acids. The amount of HbA1c formed is proportional to the amount of glucose in plasma, reflecting the mean blood glucose levels for the previous 100 to 120 days [6]. It does not reflect recent changes in glucose levels. HbA1c is expressed as % of total haemoglobin [7]. An HbA1c concentration of 6.5% or higher is used to diagnose diabetes [4,5]. HbA1c is also recommended as a marker for glycemic control as a prognostic tool for future complications of diabetes mellitus [8].

This study describes blood glucose control in these individuals with T2DM, and investigated body adiposity as a predictor of blood glucose control in black females with T2DM attending diabetes clinics in Bloemfontein, Free State Province, South Africa.

Methods

Sample selection and population

A convenience sample of 72 female participants was recruited from diabetes clinics at Pelonomi Tertiary and Universitas Academic Hospitals. Black, adult, female participants between the ages of 20 and 60 years with T2DM, weighing less than the dual-energy X-ray absorptiometry (DXA) scan table weight limit of 136 kg, with a height of less than the DXA scan table length of 196 cm [9], were eligible for inclusion in the study. Participants were excluded from the study if they were diagnosed with type 1 diabetes mellitus, were male or pregnant, or unable to give consent. Recruitment continued during clinic hours for seven consecutive months from April 2013 to November 2013.

Body mass index (BMI) calculation and body fat percentage measurement

BMI evaluates an individual's weight in relation to height and is calculated as kg/m^2. In this study, participants were classified as obese (BMI > 30 kg/m^2), overweight (BMI 25–29.9 kg/m^2) and normal weight (BMI 18.5–24.9 kg/m^2) [10,11].

DXA was used to describe specific body fat percentage. A body fat mass percentage of 25% or more in males and 30% or more in females indicates obesity, which is associated with increased metabolic and health risks [9,11,12].

Data collection

The investigation formed part of a larger study and although data collection was done simultaneously, the results are reported separately. Black female individuals with T2DM complying with the inclusion criteria were invited to participate in this study. Informed consent in the language of choice (Sesotho, English or Afrikaans) was obtained before inclusion in the study. Participants willing to participate in the study were transported to Universitas Academic Hospital where data was collected. Weight and height were measured to calculate BMI and body composition determined by means of DXA. Blood samples were collected and participants received a snack after blood was drawn. HbA1c levels were

obtained from the patients' files, which were analysed by the National Health Laboratory Services (NHLS) at Pelonomi Tertiary and Universitas Academic Hospitals.

Biochemical analysis

HbA1c analyses are done routinely during each six-monthly visit to the diabetes clinics. The latest results from the participants' files were used. The analysis was done at an accredited NHLS laboratory at Pelonomi Tertiary Hospital and Universitas Academic Hospital.

Statistical analysis

The data analysis for this study was done using SAS/STAT software, Version 9.3 of the SAS system for Windows, © 2010 SAS Institute Inc. Data from this study are reported as medians, means with standard deviations and frequencies. Pearson correlations were used to correlate variables, with an r-value less than or equal to 0.3 indicating a weak correlation. Statistical significance was set at p-values equal or less than 0.05.

Ethical considerations

Approval for the study was obtained from the Chief Executive Officer (CEO) of Universitas Academic and Pelonomi Tertiary Hospitals, respectively, and the Ethics Committee of the Faculty of Health Sciences, University of the Free State (reference number ECUFS 162/2012). Informed consent was obtained from all the participants.

Results

Seventy-two individuals participated in this study. The majority of participants (59.8%) were between 50 and 60 years of age, with a minimum age of 25 years, a maximum age of 60 years and mean age of 50.47 years (SD ± 7.3). The minimum body mass index (BMI) was 21.5 kg/m^2 and the maximum was 52.2 kg/m^2, with a mean BMI of 36.4 kg/m^2 (SD ± 7.8). The BMI distribution for this group of participants is shown in Table 6.1.

Table 6.1. Distribution of T2DM participants (n=72) with regard to body mass index (BMI).

BMI category	Frequency	Percentage
Normal (18.5–24.9 kg/m^2)	4	5.6
Overweight (25–29.9 kg/m^2)	11	15.3
Obese class 1 (30–34.9 kg/m^2)	21	29.2
Obese class 2 (35–39.9 kg/m^2)	11	15.3
Obese class 3 (> 40 kg/m^2)	25	34.7

The participants' mean total body fat was 42%, whereas the recommendation for optimal health in females is 18% to 30% [13]. Table 6.2 summarises the findings on body fat distribution.

Table 6.2. Body fat distribution among T2DM participants (n=72).

Body area	Fat %			± SD
	Minimum	Maximum	Mean	
Left arm	27.0	68.9	51.6	9.2
Right arm	32.3	66.9	51.2	9.1
Trunk	25.1	52.8	41.3	6.7
Left leg	19.8	55.2	42.5	7.4
Right leg	17.8	57.1	43.2	7.8
Head	17.6	22.8	19.4	1.1
Total fat	26.2	52.9	42.0	6.2

The mean HbA1c of participants in this study was 8.4 % (SD ± 2.2), with a minimum of 5.3% and a maximum of 13.8%. The participants' mean HbA1c levels according to BMI category are indicated in Table 6.3.

Table 6.3. Mean HbA1c of T2DM participants (n=72) according to BMI category.

BMI category	Mean HbA1c (%) (± SD)
Normal (18.5–24.9 kg/m^2)	7.8 (± 3.0)
Overweight (25–29.9 kg/m^2)	8.5 (± 3.6)
Obese class 1 (30–34.9 kg/m^2)	8.2 (± 2.1)
Obese class 2 (35–39.9 kg/m^2)	9.7 (± 2.4)
Obese class 3 (> 40 kg/m^2)	8.2 (± 1.5)

Only 15 (20.8%) participants had a BMI of less than 30 kg/m^2 (the limit for overweight but not obese), only three (4.2%) had a body fat percentage below 30% (the upper recommended limit), and only 18 (25.0%) had an HbA1c of less than or equal to the recommended 6.5%. A comparison of these factors is shown in Figure 6.1. Only five individuals had a BMI below 30 kg/m^2 and an HbA1c below 6.5%, although all five of these individuals had body fat percentages above 30%. Three of the individuals who had body fat percentages below 30% had HbA1c levels above 6.5%, with one (12.3%) being almost double the recommended level.

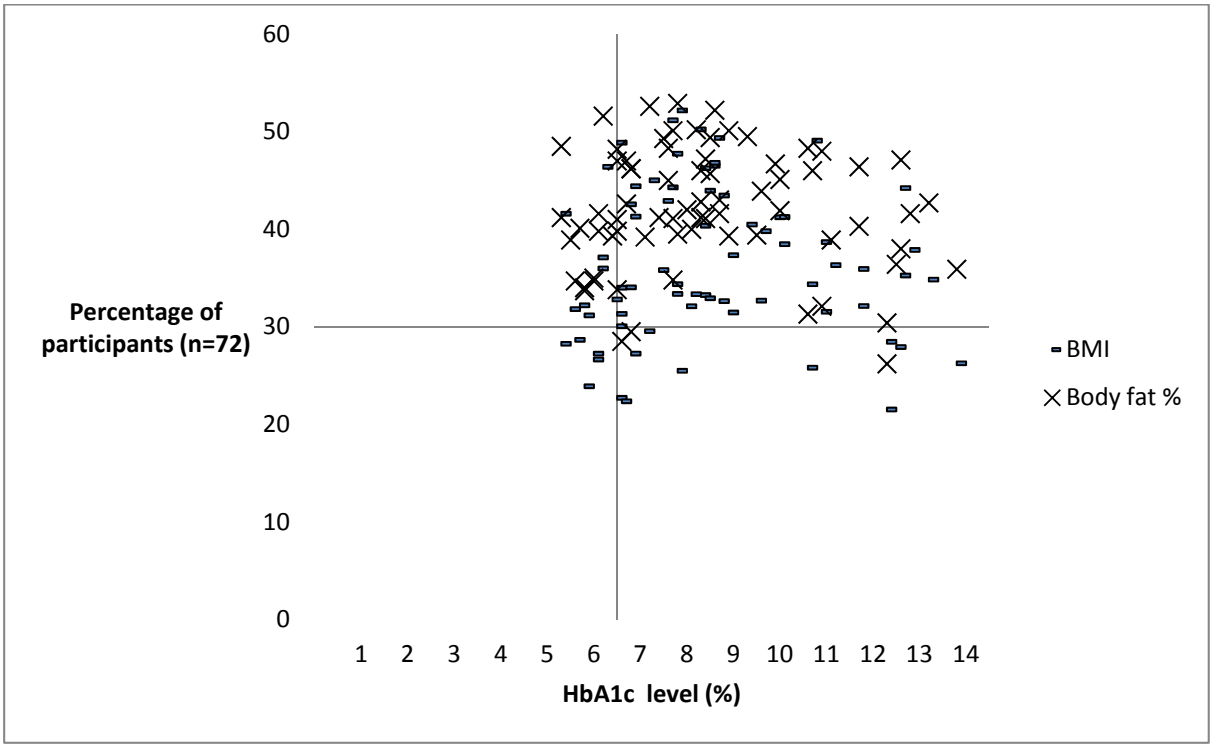

Figure 6.1. Body mass index (BMI) and body fat percentage compared to HbA1c.

Discussion

More than one third (34.7%) of the study population was classified as obese class 3, while the mean total body fat of 42% was much higher than the total body fat of 18–30% recommended for healthy females [9,11,12]. The mean BMI of 36.4 kg/m^2 also exceeded the normal BMI recommendation of 18.5 kg/m^2 to 24.9 kg/m^2 [11,13]. The mean HbA1c of 8.4% was higher than the recommended level of 6.5% or less [4,5]. Poor weight and glucose control was therefore evident in these black female individuals with T2DM, despite clinic attendance.

No correlation was found between blood glucose control and BMI (r=-0.00591; p=0.9607) or between blood glucose control and body fat percentage (r=-0.06648; p=0.5790). However, Figure 1 clearly illustrates a problem evident in attempting an analysis of this sort, namely a severe restriction in range. However, even after correction of the correlations for restriction in range [14], the correlations remained very weak, indicating that, in this sample at least, there was no relationship between blood glucose control and either BMI or body adiposity. The extreme obesity and adiposity of the participants in this study could thus not be attributed to, nor be considered as leading to, uncontrolled blood sugar.

The majority of participants (79.2%) were obese according to BMI category and no association between BMI categories and blood glucose control could be found. When dividing participants in a younger and older group (where participants below 40 years of age were regarded as "younger"), no association between age and blood glucose control was found, possibly due to the fact that only 9.2% of participants were between 20 and 40 years, and 90.3% between 40 and 60 years of age.

As expected, no correlation was found between BMI, body adiposity and HbA1c, indicating that BMI did not predict blood glucose control in this population. Some other external variables should thus be considered to be driving the variables measured here, which might be related to diet and/or lifestyle. Age also did not seem to influence glucose control. Contradictory to the results from this study, Koro et al. [15] found that blood glucose control was better in individuals diagnosed with T2DM who had a higher BMI and were older.

HbA1c levels in this black female population with T2DM were high, greatly increasing the risk for diabetes-related complications. Research has shown that with every percentage point drop in HbA1c, the risk of microvascular complications decreases by 40% [16], and that maintaining optimal blood glucose control will decrease the risk for complications and death

[17]. Andersson et al. [18] reported that overweight and obese individuals with T2DM, with high HbA1c concentrations, showed increased cardiovascular and mortality risks. Monami et al. [19] also concluded that blood glucose control was the most prudent approach to prevent risks of cardiovascular complications in individuals with T2DM.

Chao et al. [20] investigated the effects of an educational intervention programme on Taiwanese individuals with T2DM on blood glucose control. The educational intervention included general information on diabetes, dietary suggestions and self-care requirements for T2DM. Their results indicated a significant improvement in blood glucose control [20]. The importance of dietary education and information on diabetes was also supported by results indicating that diabetic control was worse in individuals with T2DM taking medication, when compared to those only on a diet [15]. The results from both of these studies highlight the importance and effectiveness of an educational intervention programme on diabetes–to prevent complications and reduce the risk of mortality.

In conclusion, blood glucose control was found to be very poor in this group of black females with T2DM, with no association with BMI, body adiposity or age. This group are at risk for micro- and macrovascular complications, and focussed dietary education and support is necessary for optimal blood glucose control and prevention of complications and risks for early mortality.

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Conflict of interest

The authors have no conflict of interest to declare.

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CHAPTER 7: CONCLUSIONS AND RECOMMENDATIONS

7.1 INTRODUCTION

The main aim of this study was to determine the presence of the *PPARy* Pro12Ala polymorphism in a black, female population with type 2 diabetes mellitus (T2DM) attending diabetes clinics in Bloemfontein, South Africa, with the objective to determine its association with body adiposity and blood glucose control; and to describe these variables in the study population group. This study was the first in South Africa to investigate the presence of the *PPARy* Pro12Ala polymorphism in a black, female population with T2DM in Bloemfontein, in the Free State.

The results from this study are presented in article format according to the objectives of the study. Each objective and its results are described in a separate article. The first article describes the presence of the *PPARy* Pro12Ala polymorphism in this population. The second article was planned to describe the association of *PPARy* Pro12Ala polymorphism with body adiposity, and the third article the association of the single nucleotide polymorphism (SNP) with blood glucose control. The focus of the second and third articles however had to change to accommodate the results obtained in the genetic study, as the Ala (G/G) homozygote genotype was not present in this population. The Pro/Pro allele was present in 71 of the 72 participants and only one heterozygotic participant carried the Pro/Ala allele. In order to present and report the data in a useful format, the titles of the second and third articles were therefore adapted. As a result, the second article described body adiposity in the population, and the third article described body adiposity in relation to blood glucose control in this black, female population.

7.2 CONCLUSIONS

7.2.1 The presence of *PPARγ* Pro12Ala polymorphisms

The homozygotic *PPARy* Ala/Ala (G/G) genotype was not found in this black, urban population of Bloemfontein in the Free State, South Africa. Even though the results obtained from this study represent a small part of the population and are limited by the absence of a non-diabetic control group, it seems that the Ala/Ala (G/G) genotype is not common in this population and that the Pro/Pro allele was mainly prevalent. Vergotine *et al*. (2014:4) found the same low presence of the Ala (G) allele in only 5.3% of 704 participants in a mixed ancestry South African population, and that the Pro12Ala *PPARy* polymorphism was significantly associated with insulin resistance. From our results the *PPARy* Pro12Ala polymorphism is not considered to be associated with the development of T2DM in this population. It would therefore not be a practical genetic marker for early risk prediction of T2DM. A recent study done by Gonzalez *et al*. (2014:2) found no participant in Spain with the Ala (G) allele. Another recent supporting study in Northern India also found the same results, with the Pro allele being present in the majority, and one Pro/Ala heterozygote individual and absence of the Ala homozygote, confirming the insignificant link found between the Pro12Ala polymorphism and T2DM (Majumdar *et al*., 2014:8).

From this study it can therefore be concluded that the homozygotic *PPARy* Ala allele is not present in the population investigated. Therefore, it cannot be considered as an associating factor for the development of T2DM, thus it is not a genetic marker for early risk prediction of T2DM.

7.2.2 The association between Pro12Ala *PPARγ* gene polymorphisms and body adiposity

The association between the *PPARy* Pro12Ala polymorphism and body adiposity could not be described due to the Ala allele only being present in one individual. Therefore, body adiposity in this population was described for the results to be presented.

The mean BMI and total body fat percentage in this black female population with T2DM was very high; and a study done in Cape Town in the black African areas supports these results by showing in these black Africans, 80% of all the individuals with diabetes was overweight and obese (Peer *et al*., 2012:6). A study by Gomez-Ambrosi *et al*. (2011:1440) also supports these results by showing that the majority of individuals with T2DM were also obese in a white population. Goodpaster *et al*. (2003:374) conducted a study in Tennessee and Pennsylvania, and reports that the mean BMI in white females with T2DM is 30.1 kg/m^2 and body fat is 37.9%, both much lower than the mean BMI and total body fat determined in this black, female population.

As expected, a very strong significant association between BMI, body adiposity and trunk fat was found in this female population with T2DM. This is not unexpected, since obesity is a risk factor for T2DM. Vasquez *et al*. (2007:119) support the strong significant association between BMI, abdominal obesity and incidence of T2DM. This association is also supported by other studies (Bray *et al*., 2009:1217; Wang *et al*., 2005:560; Meisinger *et al*., 2006:488; He *et al*., 2008:1083).

The high incidence of obesity and high body adiposity is concerning and needs to be addressed.

7.2.3 The association between Pro12Ala *PPARγ* gene polymorphisms and blood glucose control, measured as HbA1c levels

The association between the *PPARγ* Pro12Ala polymorphism and blood glucose are not described due to the Ala genotype not being present in homozygote form in this population. Therefore, body adiposity in relation to blood glucose control in this population was described.

Blood glucose control in this study population was poor, increasing their risk for the development of complications. Prevention of microvascular and macrovascular complications requires maintaining optimal blood glucose levels and reaching target HbA1c levels of below 6.5 mmol/l (Garg & Ulrich, 2006:117). In a study done on South Africans of a mixed ancestry, the mean HbA1c in individuals with T2DM was

7.8% (Vergotine *et al*., 2014:3). In this study group the mean HbA1c was 8.4%, 0.633% higher than the blood glucose control in the mixed ancestry group, given that the standard deviations and sample sizes of both studies had a 95% confidence interval of 0.060 to 1.206. Therefore, the HbA1c in the black, female population was significantly higher, indicating poorer blood glucose control in this population. In comparison, a study in Tennessee and Pennsylvania on white females with T2DM determined a mean HbA1c of 7.6% (Goodpaster *et al*., 2003:374), indicating better control. In India the mean HbA1c in individuals with known T2DM was also 8.4%, which also indicates poor blood glucose control, comparing with the South African study population (Nair *et al*., 2011:98). Seen in all studies, including this study, it is clear that blood glucose control in individuals with T2DM is mostly uncontrolled, even though patients are attending diabetes clinics.

No correlation between body adiposity and blood glucose control in this study population were found. BMI, body fat percentage as well as age were all poor indicators of blood glucose control in this study.

7.3 RESEARCH SIGNFICANCE

Obesity and diabetes mellitus is a growing problem in South Africa and also globally. The number of individuals with T2DM is increasing daily. As can be seen from other research (Vergotine *et al*., 2014:3; Nair *et al*., 2011:98; Goodpaster *et al*., 2003:374), as well as results from this study, blood glucose control in individuals with T2DM is poor, even though they are attending diabetes clinics on a six-monthly basis. This increases their risk of complications. Preventative measures must be taken to enable earlier detection and lower the risk of complications. This study investigated genetic risk as a possible measure for early detection. If T2DM can be detected earlier or even prevented, these individuals can avoid complications, and even insulin resistance. The prevalence of the *PPARy* Pro12Ala polymorphism has never been researched in a black, female population group with T2DM in the Free State. Contrary to literature (Sokkar *et al*., 2009:85; Ghoussaini *et al.,* 2005:2; Chandrasekaran *et al.,* 2012:1025; Mohammadi-Asl *et al.,* 2013:1332; Sanghera *et al*., 2008:64; Shahrjerdi *et al*., 2013:233) results from this study show that the

PPARy Ala allele is not a contributing factor in this population, which makes this research significant.

7.4 LIMITATIONS OF THIS STUDY

To improve future studies in this area, the following limitations are acknowledged: the lack of a comparable, non-diabetic control group, and the relatively small sample size. The major reason for the small sample size and for not including a control group was a limited budget and the high cost of genetic analysis as well as the limited number of patients with T2DM attending diabetes clinics and meeting the inclusion criteria. As patients attending the clinic have follow-up visits every six months, the same participants could not be included again and a constraint on the number of participants that could be included was experienced. The inclusion criteria also placed a restriction on the total number of participants as individuals 60 years and younger are mostly working, and often not interested in participating in research studies due to time restrictions. As only black females could participate, the sample size was further restricted.

7.5 RECOMMENDATIONS

The *PPARy* Pro12Ala polymorphism was not found to be a relevant genetic marker for early risk prediction of T2DM in this population. There is a need to carry out an extensive study with a larger population, which would give a wider picture of the presence of this SNP in the black population (Majumdar *et al*., 2014:2). A prospective case-control study design should be used to compare the prevalence of a SNP between a case and control group. Other gene mutations which could possibly be a genetic marker for early risk prediction of T2DM should also be investigated, as there are more than 50 gene-associated SNPs with T2DM (Al-Rubeaan *et al*., 2013:175; McCarthy, 2010:2345; Chandak *et al*., 2007:67; Pascoe *et al*., 2007:3104).

Specialised diabetes education must be provided to each individual diagnosed with T2DM in a structured format and followed up with a focus on dietary adaptions to

help improve control of blood glucose levels and facilitate weight loss. The importance of physical activity, self-care requirements for diabetes, and general information on diabetes should also receive special attention (Chao *et al*., 2014:50; Franz, 2012:683; Koro *et al*., 2004:19; Tuomilehto *et al*., 2001:1348).

In this population, blood glucose control was not associated with BMI, age or body adiposity. Although HbA1c is tested every six months, it should be tested quarterly in individuals with poor glycaemic control, and interpretation of results and focused education should be done in order to facilitate better control to prevent microvascular and macrovascular complications (Chao *et al*., 2014:50; Franz, 2012:683, 694; Koro *et al*., 2004:19).

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KEYWORDS

PPARγ Pro12Ala polymorphism, Type 2 Diabetes Mellitus, Nutrigenomics, Nutrition, Body Adiposity, Duel X-ray Absorptiometry, Blood glucose control (HbA1c), Prevention, South Africa, Diabetic Complications

SUMMARY

Diabetes mellitus is a lifestyle disease, with complications affecting quality of life. It is also increasing in prevalence on a global scale. Type 2 diabetes mellitus (T2DM) accounts for more than 90% of all diabetes cases and is a growing public health concern. Diabetes mellitus is a complex disease, genetically based but environmentally influenced. Nutrition therapy, based on knowledge of genetic composition, can be used in the prevention of a chronic disease like T2DM. One of the genes associated with T2DM which plays a significant role in insulin metabolism is the *peroxisome proliferator-activated receptor-gamma 2* (*PPARγ*) gene. The Pro12Ala polymorphism of the *PPARγ* gene is associated with T2DM in various studies around the world. In this study, the presence of the Pro12Ala allele in a black, female population with T2DM in the Free State, South Africa, was investigated, with the purpose of describing the association between Pro12Ala *PPARγ* gene polymorphisms and body adiposity in the study population and the association between Pro12Ala *PPARγ* gene polymorphisms and blood glucose control (measured as HbA1c levels). A total of 72 black, female participants were included in the study. The data obtained from the study showed that of the 72 participants, 71 had the Pro/Pro (C/C) allele and only one was a heterozygote with the Pro/Ala (C/G) allele. The presence of a homozygotic *PPARγ* Ala (G/G) genotype was not found in this study. In this population body mass index (BMI) and total body fat percentage was very high, factors which are strongly associated with the risk and incidence of T2DM. Blood glucose control was poor, and showed no association with age, BMI or body adiposity. The allele frequency of this SNP is not known in the Black South African population and even though the sample used in this study represents a small subset of the population and is limited by the absence of a non-diabetic control group, it can be derived from the results that it is likely that the Ala/Ala (G/G) genotype is rare in the population. The *PPARγ* Pro/Ala polymorphism can therefore not be regarded as a direct contributing factor to the development of T2DM in this population and can therefore also not be regarded as a suitable genetic marker for early risk prediction of T2DM.

OPSOMMING

Diabetes mellitus is ’n lewenstylsiekte met komplikasies wat lewenskwaliteit affekteer en waarvan die voorkoms wêreldwyd toeneem. Tipe 2 diabetes mellitus (T2DM) is verantwoordelik vir meer as 90% van diabetiese gevalle en is ’n groeiende publieke gesondheidsprobleem. Diabetes mellitus is ’n komplekse siekte met ’n genetiese basis, maar wat deur die omgewing beïnvloed word. Voedingterapie, gebaseer op kennis van genetiese samestelling, kan voorkomend in ’n kroniese siekte, soos T2DM, gebruik word. Een van die gene wat geassosieer word met T2DM en ’n betekenisvolle rol speel in insulienmetabolisme is die *peroxisoom proliferator-geaktiveerde reseptor-gamma 2* (*PPARγ*) geen. Die Pro12Ala polimorfisme van die *PPARγ* geen word met T2DM in verskeie studies in die buiteland geassosieer. In hierdie studie het ons die voorkoms van die Pro12Ala alleel in ’n Swart, vroulike populasie met T2DM in die Vrystaat, Suid-Afrika, ondersoek, om die verband tussen die Pro12Ala *PPARγ* geen polimorfisme en liggaamsvet in hierdie studie populasie te beskryf en die verband tussen die Pro12Ala *PPARγ* geen polimorfisme en bloedglukosebeheer (gemeet as HbA1c vlakke) te bepaal. ’n Totaal van 72 Swart, vroulike deelnemers was by hierdie studie ingesluit. Die data wat verkry is het getoon dat van die 72 deelnemers, 71 die Pro/Pro (C/C) alleel gehad het en slegs een die heterosigotiese Pro/Ala (C/G) alleel. Die homosigotiese *PPARγ* Ala (G/G) genotipe is nie gevind in hierdie studiepopulasiegroep nie. In hierdie populasie was die liggaamsmassaindeks (LMI) en liggaamsvet persentasie besonder hoog, wat ’n sterk verband met die risiko en voorkoms van T2DM toon. Bloedglukosekontrole was baie swak en geen verband met ouderdom, LMI of liggaamsvet is gevind nie. Die alleelfrekwensie van hierdie enkelnukleotied polimorfisme is nie bekend in hierdie Swart Suid-Afrikaanse populasie nie en al was die resultate in hierdie studie uit ’n klein subgroep van die populasie verkry en beperk deur die afwesigheid van ’n nie-diabetiese kontrole groep, kan daar afgelei word dat die Ala/Ala (G/G) genotipe moontlik skaars in hierdie populasie is. Die *PPARγ* Pro/Ala polimorfisme kan daarom nie beskou word as ’n direkte bydraende faktor tot die ontwikkeling van T2DM in hierdie populasie nie, en kan daarom ook nie as ’n geskikte genetiese merker vir vroeë opsporing vir die risiko van T2DM beskou word nie.

LIST OF ADDENDUMS

Addendum 1 Approval from participating institutions

pelonomi hospital

Department of Health
Pelonomi Regional Hospital
FREE STATE PROVINCE

DATE:	07 May 2013		
TO:	Me Inge van Niekerk Dept. of Nutrition and Dietetics CR de wet building UFS	FROM:	Dr Benganga B.A Director: Clinical Services E-mail: BengangaBA@fshealth.gov.za Tel: 051 4051936

SUBJECT: THE ASSOCIATION OF PPARY GENE POLYMORPHISM WITH TYPE 2 DIABETES MELLITUS IN A BLACK SOUTH AFRICAN POPULATION OF MANGAUNG

Pelonomi Regional Hospital **grants you permission** and the following criteria must be met.

[X] The Hospital incurs no cost in the course of your research.

[X] Access to the staff and patients at the Pelonomi Hospital will not interrupt the daily provision of services.

[X] Prior to conducting the research you will liaise with the supervisors of the relevant sections and introduce yourself with permission letter and to make arrangements with them in a manner that is convenient to the sections.

Yours Sincerely

PELONOMI HOSPITAL
Dr Benganga BAF
Head of Clinical Service
03-05-2013
DEPARTMENT OF HEALTH

Benganga B.A
Director: Clinical Services

health
Department of
Health
FREE STATE PROVINCE

18 September 2012

Me. I. van Niekerk
Dietitian
Pelenomi Hospital
072 100 3086

Dear Me. van Niekerk

RESEARCH PROJECT: THE ASSOCIATION OF PPARy GENE POLYMORPHISM WITH TYPE 2 DIABETES MELLITUS IN A BLACK SOUTH AFRICAN POPULATION OF MANGAUNG.

Herewith permission for the mentioned project to be done at Universitas Academic Hospital on the following conditions:

1. The research should not expose the users and the Department to any avoidable harm.
2. Annual progress reports should be submitted and also a research report at the end of the research process.
3. Reporting of Adverse Events related to the research process must be done within 48 hours of discovery.
4. There shall be provision for obtaining informed consent from all patients/staff where appropriate.
5. Briefing sessions should be conducted with all stakeholders prior to commencement and at the end of the study to provide feedback where appropriate.
6. That approval is obtained from the Ethics Committee.

The Chief Executive Officer must be notified if the findings of the project will be published and a research report needs to be sent to the Head Clinical Services as soon as the study is completed.

Yours sincerely

DR NIC R J VAN ZYL
HEAD: CLINICALSERVICES
UNIVERSITAS ACADEMIC HOSPITAL

DR NIC R J VAN ZYL
2012 -08- 10

HEAD: CLINICAL SERVICES: DR NRJ VAN ZYL
Private Bag X20660, Bloemfontein, 9300. Tel. No.: 051-4052866,
Fax: 051-4053500, Room 1077, First Floor, Universitas Academic Hospital
Email: vanzylnr@universitas.fs.gov.za

Addendum 2 **Ethical Approval (ECUFS 162/2012): University of the Free State**

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA
UFS UV

Research Division
Internal Post Box G40
☎(051) 4052812
Fax (051) 4444359

E-mail address: StraussHS@ufs.ac.za

Ms H Strauss

2012-10-19

REC Reference nr 230408-011
IRB nr 00006240

MS IT VAN NIEKERK
C/O DR R LATEGAN
DEPT OF NUTRITION AND DIETETICS
CR DE WET BUILDING
UFS

Dear Ms van Niekerk

ECUFS NR 162/2012
PROJECT TITLE: THE ASSOCIATION OF *PPARγ* GENE POLYMORPHISM WITH TYPE 2 DIABETES MELLITUS IN A BLACK SOUTH AFRICAN POPULATION OF MANGAUNG.

- You are hereby kindly informed that the Ethics Committee approved the above project at the meeting held on 18 October 2012.
- Committee guidance documents: Declaration of Helsinki, ICH, GCP and MRC Guidelines on Bio Medical Research. Clinical Trial Guidelines 2000 Department of Health RSA; Ethics in Health Research: Principles Structure and Processes Department of Health RSA 2004; Guidelines for Good Practice in the Conduct of Clinical Trials with Human Participants in South Africa, Second Edition (2006); the Constitution of the Ethics Committee of the Faculty of Health Sciences and the Guidelines of the SA Medicines Control Council as well as Laws and Regulations with regard to the Control of Medicines.
- Any amendment, extension or other modifications to the protocol must be submitted to the Ethics Committee for approval.
- The Committee must be informed of any serious adverse event and/or termination of the study.
- A progress report should be submitted within one year of approval of long term studies and a final report at completion of both short term and long term studies.
- Kindly refer to the ECUFS reference number in correspondence to the Ethics Committee secretariat.

Yours faithfully

.....
PROF WJ STEINBERG
FOR CHAIR: ETHICS COMMITTEE

Cc Dr R Lategan

Addendum 3 Informed Consent Forms

Informed Consent 1

I, Inge van Niekerk, am a student at the University of the Free State at the Department of Nutrition and Dietetics. I am conducting a study to evaluate if there is a genetic cause of Type 2 diabetes mellitus (sugar disease) in the black population, for the purpose of obtaining a higher degree. To do this study, human blood is needed to assess genes inside the blood (genes are what you inherit from your mother and father – therefore it will be in your blood). In the study, weight, height and body composition will also be needed.

You are invited to participate in this study by volunteering to give a small amount of your blood, and allowing me to measure your weight, height and body fat composition with a DXA machine. If you agree, we would also like to keep your blood for further genetic tests to do future research.

All blood will be drawn by a qualified professional nurse at the diabetes clinic. Three small tubes of blood (15ml) will be drawn from a vein in your arm. Your weight and height will be taken by me, a qualified dietician, trained to take these measurements, your blood pressure will be taken by a professional nurse, and your body composition will be taken with a DXA machine, and will be done by technologists. This involves exposure to a low level of X-ray radiation that will not harm or hurt you. You will also be transported to where this machine is at Universitas Hospital and transported back to the clinic if needed.

During the blood drawing process, you may experience discomfort and bruising at the site where the blood is drawn. For the DXA machine, you will be required to lie down very still for a few minutes.

You will not benefit financially from participating in this study, and there will be no costs to you. You will receive a light snack after blood has been drawn. Participating in this study will not have direct bearing on your normal medical treatment. You can request the results of the body composition measurements, but no feedback can be given on genetic testing because the role of this gene on the population is still unknown. By participating in this study, you will make a contribution to help find if a gene generally linked to type 2 diabetes in black South Africans and thereby assist to help treat Type 2 diabetes mellitus in the future.

The researcher will keep records of all the genetic information, weight, height, and body composition in a secure database. Only the researchers will know the identity of the study participants, because the blood will be marked with a number and not names.

The results of this study will be published once the study is completed. Should you feel that your participation in this study has been detrimental to you, please contact me at 0721003086.

Your signature on this form means that you understand the information given to you and that - you are volunteering to participate in this study. It also means that we may use your blood for other genetic studies after this study is completed. You can withdraw from this study at any time. If you are unhappy to have your blood stored for future research – it will be disposed of at the end of the study. If you have any sensitivity on how your blood should be disposed of please indicate how, when the blood is taken. These will be recorded and taken into account at the time of disposal. Your routine medical treatment will not be compromised in any way.

_____ _____

Signature of Participant Signature of researcher

Contact information for Inge van Niekerk:

Email: ingekahts@yahoo.com .

Contact number: 0721003086

Contact information for Ethical Committee if there are any ethical concerns:

Contact number: 0514052812

Informed Consent 2 - INFORMATION DOCUMENT FOR GENETIC RESEARCH

We are planning a research project on the genetic cause of type 2 diabetes mellitus and request your permission to draw your blood (10ml) and to use your DNA (blood) for further laboratory tests.

Genes are what you inherit from your parents. They are found in every part of your body and therefore they will be present in blood.

The findings of this study will not have direct bearing on your management, but may eventually benefit others in terms of prevention or treatment of the diabetes.

You are free to refuse consent and you do not have to give reasons for doing so.

Privacy and Confidentiality

The following arrangements have been made to ensure privacy and confidentiality of your genetic information:

- Your blood sample will be marked with a code and not your name. Researchers will therefore be able to identify the sample, but not technicians working with the sample.

Results of research

It is not intended to provide feedback because the association of the gene is still not clear.

If research generates information about you which may be of relevance to the health of other family members, your consent will be sought before offering to disclose such information to the family members concerned.

Family members

Information about family members, in addition to that provided by you, is not required for the research.

Your material and information will not be released for other uses other than research without consent, unless required by law.

Storage

We would like to retain your blood and DNA for possible future research.

The duration of storage will be maximum fifteen years.

If you are unhappy to have your blood stored for future research, your genetic material and information will be disposed of at the end of this study, once the sample storage and record-keeping requirements of good research practice have been met.

Do you have any sensitivity on how your blood should be disposed of? If so, what are they?

_____

_____

These will be recorded and taken into account at the time of disposal.

We can dispose of your genetic material even after the research has started since the samples are stored in an identifiable form.

Voluntary Participation

You do not have to agree to take part in this research and you are free to withdraw from the research at any time. Your routine medical treatment will not be compromised in any way if you do not participate.

----------	----------
Signature of participant:	Signature of researcher
Name :	Name:
Date:	Date

Addendum 4 Author's Instructions for Journal of Endocrinology, Metabolism and Diabetes of South Africa (JEMDSA)

AUTHOR INSTRUCTIONS

JEMDSA 2010 Volume 15 No 3

Manuscript categories

Manuscripts submitted to JEMDSA must be in the form of Original Research, scientific letters, Clinical Review Articles, Critical appraisals of Clinical Trials (CATs), Protocols for Debate, Brief Reports, Case Reports, Correspondence, Clinical Quiz, Opinion or Forum Papers and Editorials. The Journal will consider the publication of National Guidelines, Conference Proceedings, Supplements, Press Releases and Book Reviews.

Original articles: Scholarly work in endocrinology, metabolism and diabetes will be accepted for further review.

- ***Abstracts*** that are longer than 200 words will be truncated by most international indexes and will not be accepted.
- ***Original research papers*** must be 3 500 words or less (excluding references), with up to 6 tables or illustrations.
- ***References*** should preferably be limited to no more than 25.

Technical manuscript preparation

All JEMDSA papers must comply with the Uniform Requirements for Manuscripts Submitted to Biomedical Journal Journals (Ann Intern Med 2000; 133:229-231 [editorial]; http://www.icmje.org, full text).

- ***Font*** All articles must be typed in 12 pt Times New Roman with double spacing.
- ***Small tables and figures*** (1/4–1/2 page) may be included in the manuscript. If tables are large (i.e. 1 page landscape) or if images are large in file size (> 500 KB), they must be uploaded as separate supplementary files (Step 4 in electronic submission process).
- ***Research articles*** should have a structured abstract not exceeding 200 words (50 for short reports) comprising: Objectives, Design, Setting, Subjects,

Outcome measures, Results and Conclusions. Refer to articles in recent issues for guidance on the presentation of headings and subheadings.

- ***Abbreviations:*** These should be spelt out when first used in the text and thereafter used consistently.
- ***References****:* Must be strictly in Vancouver format (Reference numbers in the text must be strictly numerical and by typed in superscript, not in brackets and must be placed after the full stop comma.
- ***Scientific measurements****:* These should be expressed in SI units except: blood pressure should be given in mmHg and haemoglobin values in g/dl. If in doubt, refer to 'uniform requirements' above.
- ***Illustrations:*** Figures consist of all material that cannot be set in type, such as photographs and line drawings.

Addendum 5 Author's Instructions for Diabetes Care Journal (American Diabetes Association, Indianapolis, IN)

AUTHOR INSTRUCTIONS

All manuscripts and correspondence to be submitted electronically to: http://mc.manuscriptcentral.com/diabetescare

Copyright

Diabetes Care publishes only material that has not been published previously and is not under consideration for publication elsewhere, with the exception of an abstract that is less than 400 words in length.

Manuscripts

- All manuscripts must include an ***abstract*** (not exceeding 250 words).
- ***Original articles*** of 4 000 words or less, excluding words in tables, figures, title page, acknowledgments and references.
- ***Original article*** is limited to 4 tables and figures.
- ***References*** should preferably be limited to 40 citations.

Technical Manuscript preparation

- ***Research articles*** should have a structured abstract not exceeding 250 words comprising: Objectives, Research Design and Methods, Results and Conclusions.
- ***Abbreviations*** should be spelt out when first used in the text and thereafter used consistently. HbA1c values should be dually reported as % (mmol/mol).
- ***Scientific measurements*** should be expressed in SI form.
- ***Tables*** should be inserted on a separate page at the end of the document with the table number, title, and legend indicated.
- ***Acknowledgments*** are located after the main text and before the reference list. Acknowledgments should contain the author contributions paragraph, brief statements of assistance, the guarantor's name (persons taking responsibility for the contents of the article), funding/financial support, and reference to prior publication of the study in abstract form, where applicable.
- ***Author Contributions*** Authors are required to include a paragraph in the Acknowledgments section listing each author's role.

- ***References*** should go at the end of the document. In text reference numbers should appear in chronological order in normal type and in parentheses. No footnotes or endnotes may be used. All authors must be listed by first initials and last name in each reference, and provided inclusive page numbers. Journal titles should be abbreviated according to the National Library of Medicine's List of Journals Indexed for Medline; for unlisted journals, please provide complete journal titles. Material in press may be cited, but copies of such material may be requested. Authors are responsible for the accuracy of the references.
- ***Font*** including text, title and author names, should be in 12-point Arial or Times New Roman. Please avoid using boldface font. Text in tables should be no smaller than 10-point font.

Addendum 6 Author's Instructions for Diabetes Research and Clinical Practice Journal (Elsevier Ltd, Oxford, UK)

AUTHOR INSTRUCTIONS

All manuscripts submitted to *Diabetes Research and Clinical Practice* should report original research not previously published or being considered for publication elsewhere, make explicit any conflict of interest, identify sources of funding and generally be of a high ethical standard. Submission of a manuscript to this journal gives the publisher the right to publish that paper if it is accepted. Manuscripts may be edited to improve clarity and expression. Submission of a paper to *Diabetes Research and Clinical Practice* is understood to imply that it has not previously been published and that it is not being considered for publication elsewhere.

Contributors

Each author is required to declare his or her individual contribution to the article: all authors must have materially participated in the research and/or article preparation, so roles for all authors should be described. The statement that all authors have approved the final article should be true and included in the disclosure.

Manuscripts

- ***Original Research Articles*** should be designated either (a) Basic Research (b) Clinical Research or (c) Epidemiology. Divide the manuscript into the following sections: Title Page; Structured Abstract; Introduction; Subjects, Materials and Methods; Results; Discussion; Acknowledgements; References; figures and tables with legends.
- All manuscripts must include an ***abstract*** of 200 words structured according to Aims, Methods, Results, Conclusions and Keywords
- ***Original articles*** of 5 000 words or less, the word limit includes a combined total of five figures or tables with legends, but does not include references and an abstract.
- ***Original article*** is limited to 5 tables and figures with legends.
- ***References*** should preferably be limited to 50 references.

Technical Manuscript preparation

- ***Essential title page information***
    - ***Title.*** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
    - ***Author names and affiliations.*** Where the family name may be ambiguous (e.g., a double name), please indicate this clearly. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
    - ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. **Ensure that phone numbers (with country and area code) are provided in addition to the e-mail address and the complete postal address. Contact details must be kept up to date by the corresponding author.**
    - ***Present/permanent address.*** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.
- ***Language*** Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific
- ***Research articles*** should have a structured abstract not exceeding 200 words and the manuscript comprising: Abstract; Introduction; Subjects, Materials and Methods; Results; Discussion; Acknowledgements; References; figures and tables with legends.
- ***Subdivision - numbered sections*** Divide your article into clearly defined and numbered sections. Subsections should be numbered (then 1.1.1, 1.1.2, ...),

1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

- ***Keywords*** Immediately after the abstract, provide a maximum of 6 keywords, avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.
- ***Acknowledgments*** All contributors who do not meet the criteria for authorship as defined above should be listed in an acknowledgements section. Examples of those who might be acknowledged include a person who provided purely technical help, writing assistance, or a department chair who provided only general support. Authors should disclose whether they had any writing assistance and identify the entity that paid for this assistance.
- ***References***
    - *Citation in text* Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.
    - *Reference links* Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, CrossRef and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles, publication year and pagination may prevent link

creation. When copying references, please be careful as they may already contain errors. Use of the DOI is encouraged.

- *Web references* As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.
- *Reference formatting* There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct. If you do wish to format the references yourself they should be arranged according to the following examples:

- *Reference style*
  - *Text:* Indicate references by number(s) in square brackets in line with the text. The actual authors can be referred to, but the reference number(s) must always be given.
  - *List:* Number the references (numbers in square brackets) in the list in the order in which they appear in the text.
- ***Font*** Make sure you use uniform lettering and sizing of your original artwork. Embed the used fonts if the application provides that option. Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or use fonts that look similar.
- ***Tables*** Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the

use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules.

- ***Figure captions*** Ensure that each illustration has a caption. Supply captions separately, not attached to the figure. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Addendum 7 Cover Letter for Journal of Endocrinology, Metabolism and Diabetes South Africa (JEMDSA)

24 November 2014

The Editor
Journal of Endocrinology, Metabolism and Diabetes South Africa

Dear Sir/ Madam

Submission of manuscript

We hereby wish to submit our article, *PPARγ Pro12Ala polymorphism in black females with type 2 diabetes mellitus attending diabetes clinics in Bloemfontein, South Africa: a descriptive study,* to be considered for publication in *JEMDSA*.

The authors (in correct sequence) are Inge van Niekerk (BSc Dietetics), Ronette Lategan (PhD Dietetics), Gerda Marx (PhD Molecular Genetics) and Jacques Raubenheimer (PhD Research Psychology).

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The authors declare that the article is reporting independent and original research, conducted by the authors as listed. Acknowledgement has been given to individuals that have contributed to any extent to the research and writing of the article. The authors have all contributed to the research and writing of the manuscript.

The article has not been submitted previously, and it is not currently under consideration for publication elsewhere. Ethical approval for this study was obtained from the Ethics Committee of the Faculty of Health Sciences, University of the Free State, Bloemfontein, before onset of the study, with ethics reference number ECUFS 162/2012.

The authors have no conflict of interest to declare. This statement is included in the text after the acknowledgements.

We trust that the manuscript complies with the journal's standards and requirements, and we are looking forward to receive your feedback.

Yours sincerely

Inge van Niekerk

Addendum 8 Professional Editing and Translating Certificate

NANETTE J LÖTTER

PROFESSIONAL EDITING AND TRANSLATING

TO WHOM IT MAY CONCERN

This is to certify that Chapters 1, 2, 3 and 7 of the following dissertation titled: *PPARγ gene polymorphisms in Black South African females with Type 2 Diabetes Mellitus*, by Ms Inge van Niekerk, have been electronically language edited and are of a suitably high standard.

Nanette J Lötter
BA, HED, MA (Linguistics and Translating)
Accredited Professional Translator (South African Translators Institute)
Accredited Professional Editor (South African Translators Institute)

Cell no: 082 202 2422
Email: nanette.lotter@gmail.com

17/12/2014

Addendum 9 Data Form

Appendix 3 – Data Form

INSTRUCTIONS: MARK THE APPROPRIATE BLOCK WITH AN X OR WRITE YOUR ANSWER IN THE SPACE PROVIDED.

FOR OFFICE USE ONLY

1-6

1. Date: ____/____/____

2. Age: _____ year

3. SEX:

Male (1)	Female (2)

d	**d**	**m**	**m**	**y**	**y**

4. T2DM CONFIRMED:

7-12

Yes (1)	No (2)

13-14

5. HbA1c: _____ (>6.5% poor control)

6. WEIGHT: _____ (Kg)

15

7. HEIGHT: _____ (cm)

8. BMI: weight/height^2

_____ (kg/m^2)

16

Obese (1)	Overweight (2)	Healthy (3)

		.	

17-20

9. FAT MASS: _____ (g)

10. LEAN MASS: _____ (g)

			.		

21-26

11. FAT %: Fat mass/total fat mass x 100 = _____ (%)

			.		

27-32

Obese (1)	Healthy (2)

(>25% Females = obese; > 20% Males = Obese)

33-34

12. BONE MINERAL CONTENT:

_____ (g)

13. AREAL BONE MINERAL DENSITY:
_____ (g/cm^2)

35

		.		

36-40

14. CAN BLOOD BE USED FOR FUTURE STUDIES?

Yes (1)	No (2)

		.		

41-45

15.1 Disposal of blood:

Normal (1)	Other (2)

46-47

_____

48

16. BLOOD PRESSURE:

_____

		.		

49-53

		.		

54-58

59

60

			/		

61-66