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The relevance of glycosylated haemoglobin in screening for non-insulin dependent diabetes mellitus in a black South African population.

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Opsomming

Agtergrond

As gevolg van bevolkingsgroei, veroudering, verstedeliking, verhoogde voorkoms van vetsug en fisiese onaktiwiteit het diabetes mellitus (DM) een van die belangrikste en mees algemene chroniese siektes geword. Bepaling van geglikosileerde hemoglobien (HbA1c) word tans oraloor gebruik om glukemiese beheer te moniteer as hoeksteen van diabetiese sorg. Dit mag ook ‘n bruikbare siftingshulpmiddel wees vir nie-insulienafhanklike DM, ook bekend as tipe 2 DM (T2DM). Verhoogde HbA1c kan met langtermynrisiko van kardiovaskulêre komplikasies verbind word.

Doel

Die doel van die studie was om te bepaal of HbA1c as betroubare siftingshulpmiddel vir vroeë opsporing van T2DM in ‘n Afrika-bevolking gebruik kan word.

Metodes

Hierdie studie was ‘n dwarssnitstudie en was deel van die Suid-Afrikaanse, Noordwes-provinsie (SA-NWP) been van die 12-jaar Prospektiewe Stedelike en Landelike Epidemiologiese (PURE) studie. Basislyndata is van Maart tot Desember 2005 versamel. ‘n Totaal van 2010 vrywilligers van ewekansigverkose huishoudings is gewerf. Gegewens in verband met sosio-demografiese kenmerke, fisiese aktiwiteit, dieetinnames, bloeddruk en antropometrie is ingewin. HbA1c, vastende plasmaglukose (VPG), lewerensieme en MIV-status is bepaal. Etiese goedkeuring vir die PURE studie is in Julie 2004 verkry. Orale glukosetoleransietoetse (OGGT) is ook gedoen vir ‘n sub-groep van 465 persone. Die Statistiese Konsultasiedienste van die Noordwes-Universiteit is genader om die data met SPSS 17.0 en STATISTICA 9.0 te analiseer.

Resultate

Die HbA1c-waardes in die diabetiese VPG-groepe was 7.46% vir mans en 8.08% vir vroue. HbA1c-waardes het betekenisvol progressief gestyg van die normale VPG-groepe na die groepe met versteurde VPG en die diabetiese VPG-groepe vir beide mans en vroue. Geen betekenisvolle verhogings in HbA1c is gevind tussen die OGTT-groepe nie [normale 2-uur plasmaglukose (PG), versteurde 2-uur PG en diabetiese 2-uur PG]. Totale cholesterol, trigliseriede, liggaamsmassa-indeks en VPG het betekenisvol verhoog en hoë-digtheid lipoproteïencholesterol betekenisvol verlaag met ‘n verhoging in HbA1c-waardes in mans en vroue. Verder het sistoliese bloeddruk betekenisvol verhoog in vroue met verhoogde HbA1c. Dus is ‘n vermeerdering in die aantal risikofaktore gevind met ‘n verhoging in HbA1c. Wanneer HbA1c en VPG saam gebruik is, is die

risiko van ontwikkeling van T2DM in 43 persone in die populasie as ‘n geheel gevind. Wanneer die risiko vir die ontwikkeling van T2DM egter oorweeg is deur OGGT, VPG en HbA1c afsonderlik te gebruik, is slegs een persoon deur al die metodes geïdentifiseer as ‘n risiko vir die ontwikkeling van diabetes.

Bespreking en gevolgtrekking

‘n Verhoging in HbA1c en VPG is geassosieer met ‘n verhoging in risikofaktore en dus met metaboliese sindroom (MS). MS word geassosieer met ‘n verhoogde risiko vir die ontwikkeling van T2DM en die gevolgtrekking kan dus gemaak word dat HbA1c bruikbaar was in die opsporing van individue in hierdie populasie met verhoogde risiko vir die ontwikkeling van T2DM. Die gebruik van VPG en HbA1c in kombinasie is as ‘n beter siftingsmeganisme beskou as wanneer HbA1c alleen gebruik is. Ander faktore as die wat in hierdie studie gemeet is kon die oorsaak gewees het van die onverwagte resultate wat verkry is in die deelnemers met versteurde OGGT.

Sleutelwoorde

HbA1c; Tipe 2 diabetes mellitus; siftingshulpmiddel; diagnose.

Summary

Background

Due to population growth, aging, urbanisation, increasing prevalence of obesity and physical inactivity, diabetes mellitus (DM) has become one of the most important and prevalent chronic diseases. Glycated haemoglobin A1c (HbA1c) assessment is currently being used all over to monitor glycaemic control as a cornerstone of diabetes care. It might also be a useful screening tool for non-insulin dependent DM, also known as type 2 DM (T2DM). Elevated HbA1c can be linked with long-term risk of cardiovascular complications.

Aim

The aim of the study was to determine whether HbA1c can be used as reliable screening tool for early detection of T2DM in an African population.

Methods

This study was a cross-sectional study and was part of the South African, North-West Province (SA-NWP) leg of the 12-year Prospective Urban and Rural Epidemiological (PURE) study. Baseline data was collected from March to December 2005. A total of 2010 volunteers were recruited from randomly selected households. Data was collected on socio-demographic characteristics, physical activity, dietary intakes, blood pressure and anthropometry. HbA1c, fasting plasma glucose (FPG), liver enzymes and HIV status were determined. Ethical approval for the PURE study was obtained in July 2004. Oral glucose tolerance tests (OGTT) were also done for a sub-group of 465 subjects. The Statistical Consultation Services of the North-West University were consulted to analyse data with SPSS 17.0 and STATISTICA 9.0.

Results

The HbA1c values within the diabetic FPG groups were 7.46% for men and 8.08% for women. HbA1c values increased significantly progressively from the normal FPG groups to the groups with impaired FPG and the diabetic FPG groups for both men and women. No significant increases were found in HbA1c between the OGTT groups (normal 2 hour plasma glucose (PG), impaired 2-hour PG and diabetic 2-hour PG). Total cholesterol, triglycerides, body mass index and FPG increased significantly and high-density lipoprotein cholesterol decreased significantly with an increase in HbA1c values in men and women. In addition, systolic blood pressure increased significantly in women with increased HbA1c. Thus, with an increase in HbA1c, an increase in the number of risk factors was observed. When using HbA1c and FPG in combination, 43 subjects of the whole

population were detected with having a risk of developing T2DM. However, when considering the commonality of subjects identified to be diabetic or at risk by the OGTT, FPG and HbA1c individually, only one subject was identified by all the methods as having diabetes or being at risk to develop diabetes.

Discussion and conclusions

An increase in HbA1c and FPG was associated with an increase in risk factors and therefore with metabolic syndrome (MS). MS is associated with an increased risk of developing T2DM and therefore it can be concluded that HbA1c was useful for detecting in this population individuals at increased risk of developing T2DM. The use of FPG and HbA1c in combination was considered a better screening tool when compared to HbA1c alone. Factors other than what were measured in this study might be the cause of the unexpected results obtained in the participants with impaired OGTT.

Keywords

HbA1c; Type 2 diabetes mellitus; screening; diagnosing.

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List of abbreviations

AACE	American Association of Clinical Endocrinologists
ADA	American Diabetes Association
AIDS	Acquired immune deficiency syndrome
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AST	Aspartate aminotransferase
ATPIII	Adult treatment panel III
BG	Blood glucose
BMI	Body mass index
BP	Blood pressure
CAD	Coronary artery disease
CD4	Cluster of differentiation
CHD	Coronary heart disease
CHO	Carbohydrates
CI	Confidence interval
CRP	C-reactive protein
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DCCT	Diabetes Control and Complication Trials
DKA	Diabetic ketoacidosis
DM	Diabetes mellitus
DOH	Department of Health
E	Energy
ECG	Electrocardiogram
EDTA	ethylenediamine tetra acetic acid
ESRD	End-stage renal disease
EUR	Euro
FBG	Fasting blood glucose

FG	Fasting glucose
FH	Family history
FPG	Fasting plasma glucose
GAD	Glutamate decarboxylase
GT	Glucose tolerance
GGT	gamma-glutamyl transpeptidase
HbA1c	Glycated haemoglobin A1c
HC	Hip circumference
HDL-C	High-density lipoprotein cholesterol
HHQ	Household questionnaire
HIV	Human immunodeficiency virus
HPLC	High performance liquid chromatography
ID	Identity
IDF	International Diabetes Federation
IFG	Impaired fasting glucose
IGF-1	Insulin-like growth factor-1
IGT	Impaired glucose tolerance
IHD	Ischaemic heart disease
IR	Insulin resistance
IV	Intravenous
LADA	Latent autoimmune diabetes of the adult
LDH	Lactate dehydrogenase
LDL	Low-density lipoprotein
LDL-C	Low-density lipoprotein cholesterol
LFT	Liver function tests
MI	Miocardial infarction
MODY	Maturity-onset diabetes of the young
MRC	Medical Research Council
MS	Metabolic syndrome
MUFA	Mono-unsaturated fatty acids
N	Number of subjects

NCEP/ATPIII	National Cholesterol Education Program/Adult Treatment Panel
NCD	Non-communicable disease
NFG	Normal fasting glucose
NIDDM	Non-insulin dependent diabetes mellitus
NW	North-West
NWP	North-West Province
NWU	North-West University
OGTT	Oral glucose tolerance test
PAI-1	Plasminogen activator inhibitor-1
PCOS	Polycystic ovary syndrome
PG	Plasma glucose
PRIMER	Profiles of Resistance to Insulin in Multiple Ethnicities and Regions
PUFA	Poly-unsaturated fatty acids
PURE	Prospective Urban and Rural Epidemiological study
Q	Quartiles
QFFQ	Quantitative food frequency questionnaire
RF	Risk factor
ROC	Receiver operator characteristic
SA	South Africa
SBP	Systolic blood pressure
SPSS	Statistical package for social sciences
SSA	Statistics South Africa
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TB	Tuberculosis
TC	Total cholesterol
TE	Total energy

TG	Triglycerides
UK	United Kingdom
USA	United States of America
WC	Waist circumference
WHO	World Health Organization

CHAPTER 1 :

Introduction and motivation

1.1. Background to the study

In South Africa (SA), the black population is larger than any other population sub-group. However, it is considered to be the most impoverished of all groups (Bourne *et al.,* 2002). These authors pointed out that even though the majority of the black South African population resides in non-urban areas (56.7%), the urban proportion (43.3%) is steadily increasing with many of the residents living in informal housing on the fringes of cities. It is mainly the African population experiencing rapid urbanisation and nutrition transition (Vorster *et al.,* 2007). At present, SA is suffering from a triple burden of disease which is described as a combination of poverty-related infectious diseases, lifestyle-related non-communicable diseases (NCD) such as diabetes mellitus (DM) and cardiovascular disease (CVD), and violence-related trauma (Bourne *et al.,* 2002).

DM is a chronic condition that requires continuous medical attention as well as patient education in order to prevent acute complications and to reduce risk of long-term complications (American Diabetes Association or ADA, 2008). It is considered worldwide to be one of the most important and prevalent chronic diseases (Roriz-Filho *et al.,* 2008). According to Balkau *et al.* (2003), DM (more generally glucose intolerance) is described as a group of metabolic disturbances characterised by hyperglycaemia resulting from defects in insulin secretion, insulin action or both. DM is also described as being a potent risk factor for CVD and these complications account for the excess morbidity, mortality and cost of care that is associated with DM (Pratley, 2007). DM represents both a critical public health challenge and an important target for prevention efforts (Pratley, 2007).

Permutt *et al.* (2005) explained that the sudden increase in non-insulin dependant diabetes mellitus (NIDDM), also known as type 2 DM (T2DM) in the last few years is not only due to genetic factors, but also due to the increase in obesity amongst individuals. According to a study done by Schutte *et al.* (2005), in Potchefstroom, SA, where women were divided into lean, overweight and obese groups, the women in the obese group had mean fasting plasma glucose (FPG) levels of 5.48 mmol/L, therefore putting them at increased risk of developing DM. Currently this phenomenon is being documented in Africa, where the incidence of DM is increasing with urbanisation (Permutt *et al.,* 2005) and age (Mollentze, 2010). In a study done in Soweto by Ntyintyane *et al.* (2009) 20% of the subjects included were newly diagnosed with DM by means of an oral glucose tolerance test (OGTT), while 30% were diagnosed with impaired glucose tolerance (IGT). Previously undiagnosed DM and IGT were therefore found to be common abnormalities in that study

population (Ntyintyane *et al.,* 2009). Mollentze and Koning (2007) stated that the total number of individuals with diabetes in the Free State was reported to be 46 676, while a similar number of individuals were unaware of the fact that they have DM. A study from Cape Town by Levitt *et al.* (1999) reported the prevalence of T2DM to be 7.1% (5.8% in men and 8.1% in women) and the prevalence of IGT was 8.0%; 6.5% in men and 9.2% in women. The number of adults diagnosed with DM will have almost doubled worldwide from 177 million in 2000 to 370 million by 2030 (World Health Organisation or WHO, 2003). Therefore, the prevalence of DM is increasing worldwide, with the greatest increase occurring in developing countries (Haque *et al.,* 2005).

1.2. Motivation for the study

Mollentze and Koning (2007) proposed a need to initiate a screening programme in SA to find and treat undiagnosed DM, due to the increase in the prevalence of T2DM (Permutt *et al.,* 2005). Finding an early, rapid, cost effective and trustworthy screening tool is an urgent necessity and it is important to identify individuals who are at increased risk of developing DM in order to prevent and delay its development (Sato *et al.,* 2009; Permutt *et al.,* 2005). DM is associated with premature morbidity and mortality, therefore, it should never be considered to be a "mild" condition (Holt, 2004).

At present there is no consensus on which is the most appropriate screening tool for early detection of T2DM. In the literature the debate is continuing on whether to use FPG or glycated haemoglobin A1c (HbA1c) besides the "gold standard" of the OGTT. Saudek *et al.* (2008) remarked that there is no evidence to suggest that FPG is superior to HbA1c for the detection of T2DM using the OGTT as the reference standard. In fact, HbA1c seems to have a slightly higher specificity than FPG for detecting diabetes, although its sensitivity is slightly lower (Saudek *et al.,* 2008). Bennett *et al.* (2007) and Nakagami *et al.* (2007) declared that HbA1c and FPG are equally effective when screening for the early detection of T2DM, but neither HbA1c nor FPG is effective in detecting IGT. Bennett *et al.* (2007) asserted that standardisation of HbA1c measurements is needed worldwide in order to compare results across laboratories. Motta *et al.* (2009) suggested that measuring HbA1c could be a useful tool in screening the risk of DM. However, according to these authors, the use of HbA1c in the diagnosis of DM is still under debate.

The risk of developing coronary heart disease (CHD) or stroke begins within the normal range of HbA1c (Adams *et al*., 2009). In the study undertaken by Sigal (2005) it was found that 72% of the

excess CVD risk attributable to higher HbA1c levels occurred in patients with HbA1c concentrations of 5.0-6.9%. Therefore, it has been suggested that the cut-off point for a normal HbA1c level should be revised downwards as has been done for cholesterol and blood pressure (BP). The suggested HbA1c cut-off point for the risk of future CHD events was reported as ≥4.6% (Selvin *et al.*, 2005). In the study done by Adams *et al.* (2009) an increase of 1% in HbA1c was associated with a 1.5-fold increase in the probability of a cardiovascular event, compared to a 1.2-fold increase in a study done by Khaw *et al.* (2004). HbA1c levels were associated with higher levels of several inflammatory markers, including C-reactive protein (CRP), erythrocyte sedimentation rate and white blood cell count (Gustavsson & Agardh, 2004).

By making use of a receiver operator characteristic (ROC) curve analysis (in a combination of studies in which the subjects had the potential of being diabetic), it was determined that an HbA1c level of 5.8% yielded the highest combination of sensitivity (86%) and specificity (92%); therefore it was concluded that an HbA1c of 5.8% would be an appropriate cut-off point for T2DM risk (Saudek *et al.*, 2008). In their study it was determined that an HbA1c value of 6.5% or higher should be accepted as a criterion for diagnosing DM. They point out that there is a series of practical considerations that favour the use of HbA1c in screening as well as diagnosing DM. They also consider the advantages of the use of HbA1c when compared with the glucose assay.

Limited data exists on the use of HbA1c as a screening tool in black South Africans, therefore, this study will focus on HbA1c and its applicability as a screening tool for the early identification of T2DM in a black population from the North-West Province (NWP) of SA

1.3. Aim and objectives

1.3.1. Overall aim

The overall aim of this study was to determine whether HbA1c can be used as a screening tool for the early detection of T2DM in an African population.

1.3.2. Objectives

To study the consistency of HbA1c levels for early detection of T2DM in a black South African cohort by making use of different diagnosing methods and criteria:

1. To categorise the participants in the study into (i) normal blood glucose (BG); (ii) impaired BG; (iii) diabetic according to the criteria applicable to FPG
2. To categorise the participants in the study into (i) normal BG; (ii) impaired BG; (iii) diabetic according to the criteria applicable to HbA1c
3. To fit above results with the diagnosis made by using OGTT
4. To determine the most consistent method to screen for early detection of T2DM in this population.

1.4. Positioning of this study in the larger PURE study

The PURE study is a multi-national 12 year Prospective Urban and Rural Epidemiological study which investigates health transition in urban and rural subjects. The SA leg of the study is conducted in the NWP. The baseline data was collected during 2005. The results reported here are from the baseline data of the PURE study, reported as cross- sectional data.

1.5. Ethical approval

The Ethics Committee of the North-West University (NWU), SA, approved the overall study (Ethics number: 04M10**)** in July, 2004**.** In order to do the OGTT, additional ethical approval was obtained (Ethics number: 02M08**).** All participants were assured of confidentiality and anonymity of all the results and they gave written informed consent.

1.6. Structure of the dissertation

- Chapter 1 →Introduction, aim of the study and motivation for the study are given.
- Chapter 2 →A literature review is done concerning T2DM, the prevalence, cost, pathophysiology, risk factors and physiology thereof. Furthermore, the different screening methods for T2DM are discussed, with the main focus on HbA1c, FPG and OGTT.
- Chapter 3 →Materials and methods used in this investigation as well as a description of the population are presented.
- Chapter 4 →The results of the study are reported.

- Chapter 5→Finally, after the discussion, a recommendation on the use of HbA1c for early detection of T2DM in an African population is proposed, based on the conclusions reached in this study.

1.7. Declaration of student

The contribution of the researcher in the PURE study:

Although I did not partake in the data collection of the PURE baseline in 2005, I got the written consent of the PURE team to use the baseline data for this study (see Addendum 1).

As mentioned before, the PURE study is a prospective study and I did the HbA1c analysis as well as the anthropometry of all the participants in the five year follow up during 2010. I did the data management and analysis for this study in consultation with Dr S. Ellis from the Statistical Consultation Services of the NWU.

I also declare that I am aware of the plagiarism policy of the NWU and that I am obliged to that.

Signed on this _____ day of _____

_____ _____

Student Supervisor

CHAPTER 2 :

Literature review

2.1. Introduction

At present, SA is suffering from a triple burden of disease which is described as a combination of poverty-related infectious diseases, lifestyle-related NCD, and violence-related trauma (Bourne *et al.*, 2002). DM is considered to be one of the most important and prevalent chronic diseases (Roriz-Filho *et al.*, 2008). Mollentze and Levitt (2006) describe DM as being a diverse group of metabolic disorders with different clinical characteristics united by hyperglycaemia. DM is categorised into four groups: type 1 DM (T1DM), T2DM, gestational DM and other specific types of DM that occur due to specific causes (ADA, 2008). In this study, the main focus is on T2DM. DM is a chronic illness that requires continuous medical care and patient education in order to prevent acute complications and reduce the risk of long-term complications (ADA, 2008). Votey and Peters (2007) state that long-term medical attention is needed in order to limit the development of its devastating complications and to manage these complications when they occur. According to Balkau *et al.* (2003), DM (more generally glucose intolerance) is described as a group of metabolic disturbances characterised by hyperglycaemia resulting from insulin secretion, insulin action or both (Balkau *et al.*, 2003).

DM represents both a critical public health challenge and an important target for prevention efforts (Pratley, 2007). T1DM (insulin dependent) and T2DM are the two main types of DM (Holt, 2004). DM care is considered to be complex and requires that many issues (besides glycaemic control) be addressed (ADA, 2008). Not only is the detection of DM itself crucial, but early identification of persons at risk for DM is integral to the implementation of effective preventative strategies (Grant *et al.*, 2004). Levitt *et al.* (1999) stated that the growing burden of DM directs more attention towards primary prevention. The utility of DM screening depends on the evidence that early treatment adds benefit over treatment at the time of symptomatic diagnosis, most likely in the form of added years of a complication free life (Edelman *et al.*, 2004).

2.1.1. T1DM

Autoimmune destruction of the β-cells of the pancreatic islets causes T1DM (Holt, 2004; Mollentze & Levitt, 2006). Farmer (2010) points out that individuals with T1DM lack the normal homeostatic mechanism to control blood glucose levels. Although the aetiology of T1DM is poorly understood, it is likely that an environmental factor triggers an autoimmune process in an at-risk individual (Holt, 2004). T1DM is characterised by the marked and progressive inability of the pancreas to

secrete insulin due to the autoimmune destruction of the β-cells and this disease can occur at any age (Votey & Peters, 2007). T1DM usually occurs in children, and with a fairly rapid onset; yet newer antibody tests have allowed for the identification of more people with the new-onset adult form of T1DM known as latent autoimmune diabetes of the adult or LADA (Votey & Peters, 2007). The unique characteristic of a patient diagnosed with T1DM is that if his or her insulin is withdrawn, ketosis and eventually ketoacidosis develop; therefore these patients depend on exogenous insulin (Votey & Peters, 2007).

T1DM is usually diagnosed during childhood, adolescence or early adulthood (Votey & Peters, 2007). Older adults may also develop T1DM and the disease is increasingly being recognised through the measurement of islet-glutamate decarboxylase (GAD) antibodies (Votey & Peters, 2007). Lamb (2009) explains that incidence rates increase with age until mid-puberty, then decline after puberty, but T1DM can develop at any age. Even though it is very unusual that T1DM occur in the first year of life, it must be considered in any infant or toddler, because these children have the greatest risk of mortality if the diagnosis is delayed (Lamb, 2009). These children might have the following symptoms: severe monilial diaper rash, unexplained malaise, poor weight gain and weight loss, increased thirst and vomiting, and dehydration with a constantly wet diaper (Lamb, 2009).

2.1.2. Non-insulin dependent diabetes mellitus (NIDDM)

NIDDM, also known as T2DM (the term which will be used in this document), is caused by both impaired insulin secretion and resistance to the action of insulin (Holt, 2004; Farmer, 2010). Holt (2004) describes T2DM as being a heterogeneous disorder resulting from an interaction between a genetic predisposition towards the disorder and certain high-risk environmental factors. According to the ADA (2008), this type of DM is often not diagnosed until complications appear and more or less one-third of all people with DM may be undiagnosed. The occurrence of T2DM increases with age and most cases are usually diagnosed after the age of 40 years (Holt, 2004). Holt (2004) reported that the rates in rural communities such as those of China and Chile are less than 1%. The regional and ethnic differences in the occurrence do not only reflect differences in the environment, but also differences in genetic susceptibility (Holt, 2004). T2DM was considered to be mild in the past and not associated with the same spectrum of complications as T1DM (Nathan, 2002). Longer survival of patients with this type of DM and the development of the disease at a progressively earlier age in numerous populations have caused an increase in the risk of developing the duration-

dependent complications and currently contributes to more cases of adult-onset vision loss, renal failure and amputation when compared with any other disease (Nathan, 2002).

T2DM is associated with increased cardiovascular as well as all-cause mortality due to accelerated atherosclerosis (Wat *et al.*, 2008). They mention that it has been reported that having a known pre-diabetic state increases the risk of developing CVD and cerebrovascular diseases among non-Chinese subjects. Pratley (2007) emphasises that T2DM is considered to be an epidemic in most developed and many developing countries.

Votey and Peters (2007) remind us that T2DM was once known as adult-onset DM. However, because of the epidemic of obesity and inactivity in children, it now occurs at younger and younger ages. In some countries, 20% or more of new patients with diabetes in childhood and adolescence present with T2DM – a change that is associated with increased rates of obesity globally (Lamb, 2009). Even though it is usually diagnosed in patients older than 40 years of age, it has been diagnosed in children as young as 2 years of age who have a family history (FH) of DM.

Lamb (2009) remarks that most patients diagnosed with T2DM have insulin resistance (IR) and their β-cells do not have the ability to overcome this resistance. Votey and Peters (2007) characterise T2DM by peripheral insulin resistance with an insulin secretory defect that varies in severity. In order for T2DM to develop, both defects must be present. According to the study done by Votey and Peters (2007), all overweight individuals presented with IR, but only those with an inability to increase β-cell production of insulin will develop DM. Before normal glucose tolerance (GT) progresses to abnormal GT, postprandial glucose levels must first increase and eventually, as inhibition of hepatic gluconeogenesis declines, fasting hyperglycaemia develops (Votey & Peters, 2007).

Of all the patients that develop T2DM, about 90% are obese (Votey & Peters, 2007). These patients maintain the ability to secrete some endogenous insulin, therefore, those taking insulin generally do not develop diabetic ketoacidosis (Votey & Peters, 2007). It is considered that these patients often require insulin, but they do not depend on it (Votey & Peters, 2007). These authors hold the opinion that patients with T2DM do not require treatment with oral antidiabetic medications or insulin if they lose weight or decrease unhealthy eating habits when first diagnosed.

Complications associated with DM are hypoglycaemia and hyperglycaemia, increased risk of infections, microvascular complications, neuropathic complications and macrovascular disease (Votey & Peters, 2007). DM causes blindness in adults and can also result in non-traumatic lower-extremity amputation and end-stage renal disease or ESRD (Votey & Peters, 2007). Patients that present with T2DM are at an increased risk of CVD (Pratley, 2007). The mortality, morbidity and high cost associated with this disease, makes it an important global public health challenge and target for prevention (Pratley, 2007).

2.1.3. Prevalence of DM

Estimated prevalence rates are based on demographic changes with the conservative assumption that other risk factor levels such as obesity and physical inactivity remain constant in developed countries or are accounted for by urbanisation in less developed countries (Wild *et al.*, 2004).

In the United States of America (USA), people with DM were estimated to account for 7% or approximately 20.8 million people in 2005 (Votey & Peters, 2007). Approximately 14.6 million of these people have a diagnosis of DM, and DM is undiagnosed in 6.2 million of these people (Votey & Peters, 2007). Approximately 10% of these people have T1DM and the rest are diagnosed with T2DM (Votey & Peters, 2007). The prevalence of DM rises from 12% in people between the ages of 65-70 to 15% in people over the age of 80 (Wild *et al.*, 2004). According to the WHO (2003), the number of adults diagnosed with DM will have almost doubled worldwide from 177 million in 2000 to 370 million by 2030. According to the International Diabetes Federation (IDF) (2010) the prevalence of DM is expected to rise from 12% in 2010 to 23.9% in 2030, therefore a 98% increase in the prevalence of DM in Africa.

There are approximately 800,000 new cases of diabetes each year in the USA, of which almost all are T2DM (Nathan, 2002). Although T1DM is often inherited, only 12-15% of T1DM occurs in families (Holt, 2004). T2DM accounts for approximately 90% of all cases of DM (Holt, 2004). Figure 2.1 represents the prevalence of diabetes in SA according to ADA + WHO, ADA and WHO, criteria, respectively (Levitt *et al.*, 2000). According to the DOH (2003), the prevalence of DM in the North-West Province was reported as 1.5% for men and 1.8% for women.

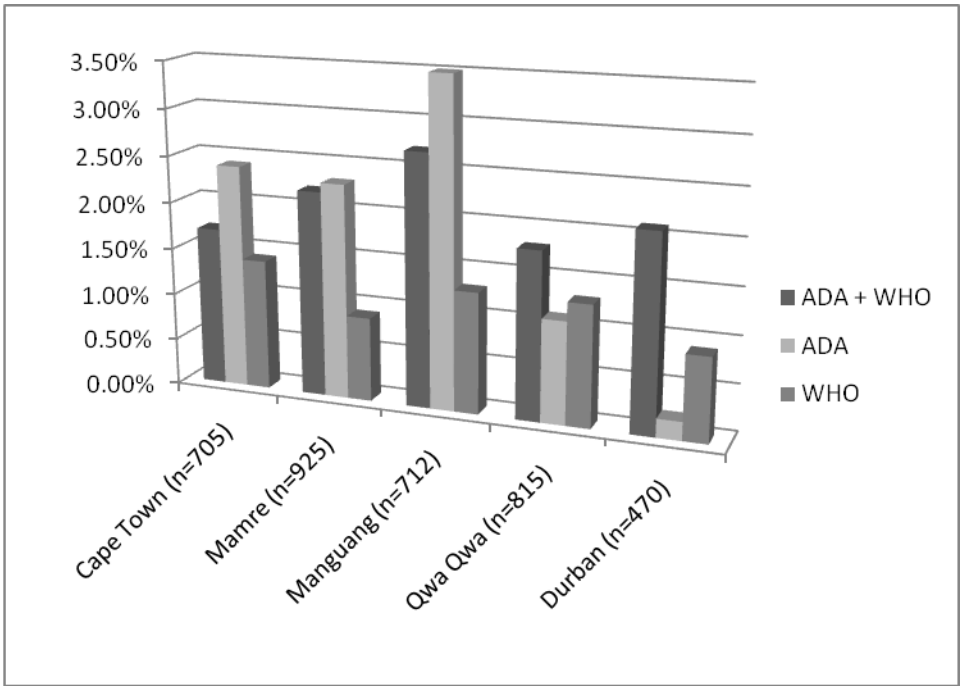

Fig 2.1: Prevalence of DM in South Africa (Levitt *et al.*, 2000).

2.1.4. Costs of DM

DM is also believed to be an excessively expensive disease. In 2002 the per capita cost of health care in the USA was $13 243 for people diagnosed with DM when compared with those without DM which was $2 560 (Votey & Peters, 2007). Perlitz (2009) reported that the total cost of DM worldwide was EUR 166 billion in 2007. The annual expenditure per patient adds up to EUR 2000 per year (Perlitz, 2009).

2.1.5. North-West Province (NWP)

The NWP is situated in the central north of SA and is completely landlocked, bordering Botswana in the north, the Limpopo and Gauteng provinces in the east, the Free State Province in the south and the Northern Cape in the west (Bradshaw *et al.*, 2004*)*. The Province encloses 116 320 km² and constitutes 9.5% of the land area of the country (SSA, 2003). The average population density in 2000 was 32 people per square kilometre (Bradshaw *et al.*, 2004). It was estimated that 3 669 349 people live in the North-West (NW), of which 3 358 450 (91.5%) were black Africans, 56 959 (1.6%) coloureds, 9 906 (0.3%) Indians or Asians and 244 035 (6.7%) whites (Bradshaw *et al.*,

2004). The NWP accommodated slightly more women (50.3%) than men (49.7%) (Bradshaw *et al.,* 2004).

Twenty percent of the population had no formal school education and 44% of those in the age group 15 to 64 were unemployed (Bradshaw *et al.,* 2004). Almost 69% of all the households lived in formal dwellings, whereas 22% lived in informal dwellings and 5% in traditional structures (Bradshaw *et al.,* 2004*).* It was also stated that 3.7 people shared a household (Bradshaw *et al.,* 2004). Bradshaw *et al.* (2004) reported that the majority of the residents (86%) had access to piped water (either in their dwelling, on site or from a communal tap) and one in ten households did not have access to a toilet facility, while 37% had a refuse removal service once a week or more (Bradshaw *et al.,* 2004). It was also stated that electricity was the main source of energy for cooking in 45% of households, wood in 18% and paraffin in 32% (Bradshaw *et al.,* 2004). Bradshaw *et al.* (2004) established in 2002 that 70% of the households had a radio, 54% had a television, 50% had a refrigerator, 14% had a telephone and 28% of the households had a cell phone.

2.2. Physiology of T2DM

Stumvoll *et al.* (2005) state that insulin is the key hormone for regulating BG and generally, normoglycaemia is maintained by balanced interplay between insulin action and insulin secretion. When fasting BG (FBG) levels increase due to glycogen conversion or the intake of carbohydrate (CHO)-containing food, insulin is released and homeostasis is restored through hepatic conversion of glucose to glycogen and uptake of glucose into muscle and fat cells (Farmer, 2010). On the contrary, if BG levels drop too low due to exercise or a lack of food, glucagon is released and causes hepatic conversion of glycogen to glucose. Stumvoll *et al.* (2005) explain that when insulin is secreted by the pancreas, glucose output by the liver is normally reduced, glucose uptake by the skeletal muscle is enhanced and fatty acid release from the fat tissue is suppressed.

These various factors contribute to the pathogenesis of T2DM and affect both insulin secretion and insulin action (Stumvoll *et al.,* 2005). When insulin secretion is decreased, insulin signalling in its target tissue is reduced. The action of insulin in each of the major target tissues is affected by the IR pathway and leads to increased circulating fatty acids and the hyperglycaemia associated with DM (Stumvoll *et al.,* 2005). The raised concentration of glucose and fatty acids in the bloodstream will in turn feed back to worsen both insulin secretion and IR (Sarumpudi *et al.,* 2009). Figure 2.1,

adapted from Stumvoll *et al.,* 2005 and Sarumpudi *et al.,* 2009 represents the abnormalities in T2DM that contribute to hyperglycaemia. In this figure it is shown that glucose output by the liver normally reduces insulin secretion from the pancreas, enhances glucose uptake by skeletal muscle and suppresses fatty acid release from fat tissue. This figure also indicates that decreased insulin secretion reduces insulin signalling in its target tissues. IR also affects the action of insulin in each of the major target tissues and this leads to increased circulating fatty acids and the hyperglycaemia of DM. Raised concentrations of glucose and fatty acids in the bloodstream will feed back and worsen insulin secretion and IR (Stumvoll *et al.,* 2005; Sarumpudi *et al.,* 2009).

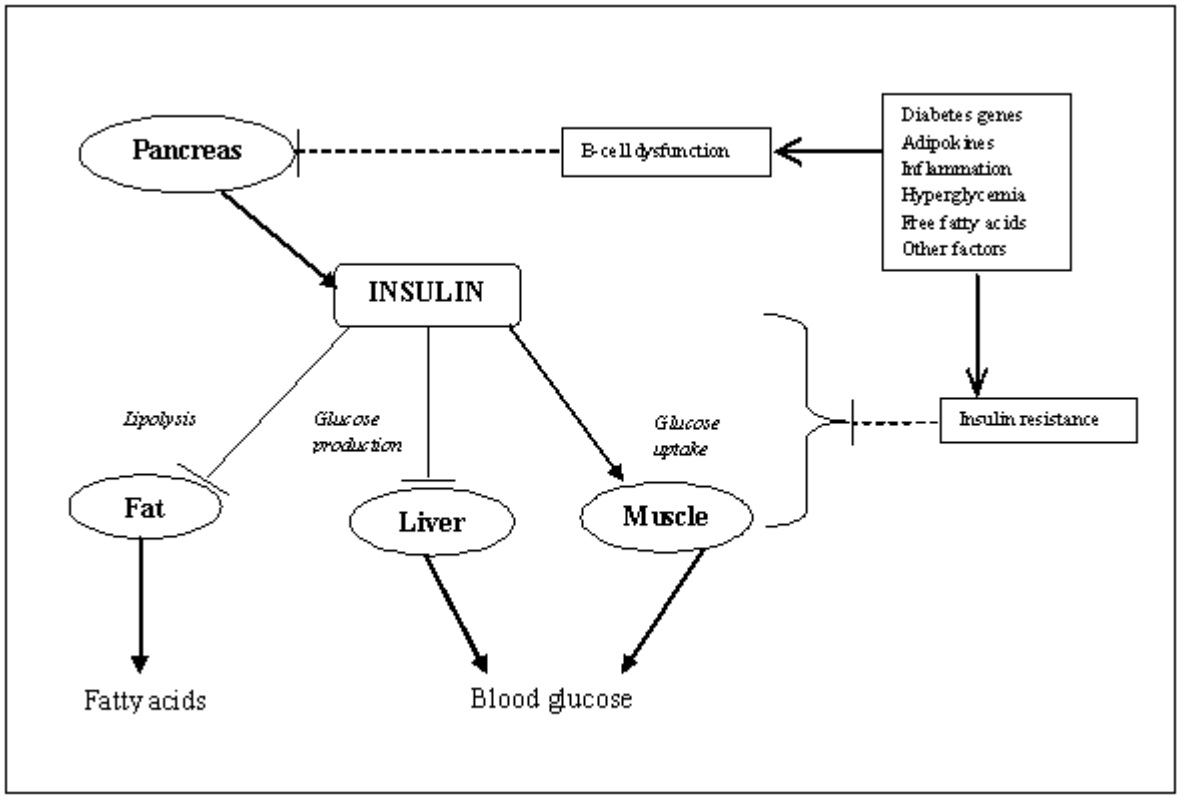

Fig 2.1: Abnormalities in T2DM contributing to hyperglycaemia [adapted from Stumvoll *et al.* (2005) and Sarumpudi *et al.* (2009)].

2.3. Pathophysiology of T2DM

According to Sarumpudi *et al.* (2009), T2DM is considered to be a multifactorial metabolic disorder with an initial clinical manifestation of elevated BG levels. It is characterised by chronic hyperglycaemia, IR and relative insulin defects. The progression to T2DM from normal GT to IGT

and finally T2DM occurs in stages (Sarumpudi *et al*., 2009). Figure 2.2 represents the pathogenesis of DM.

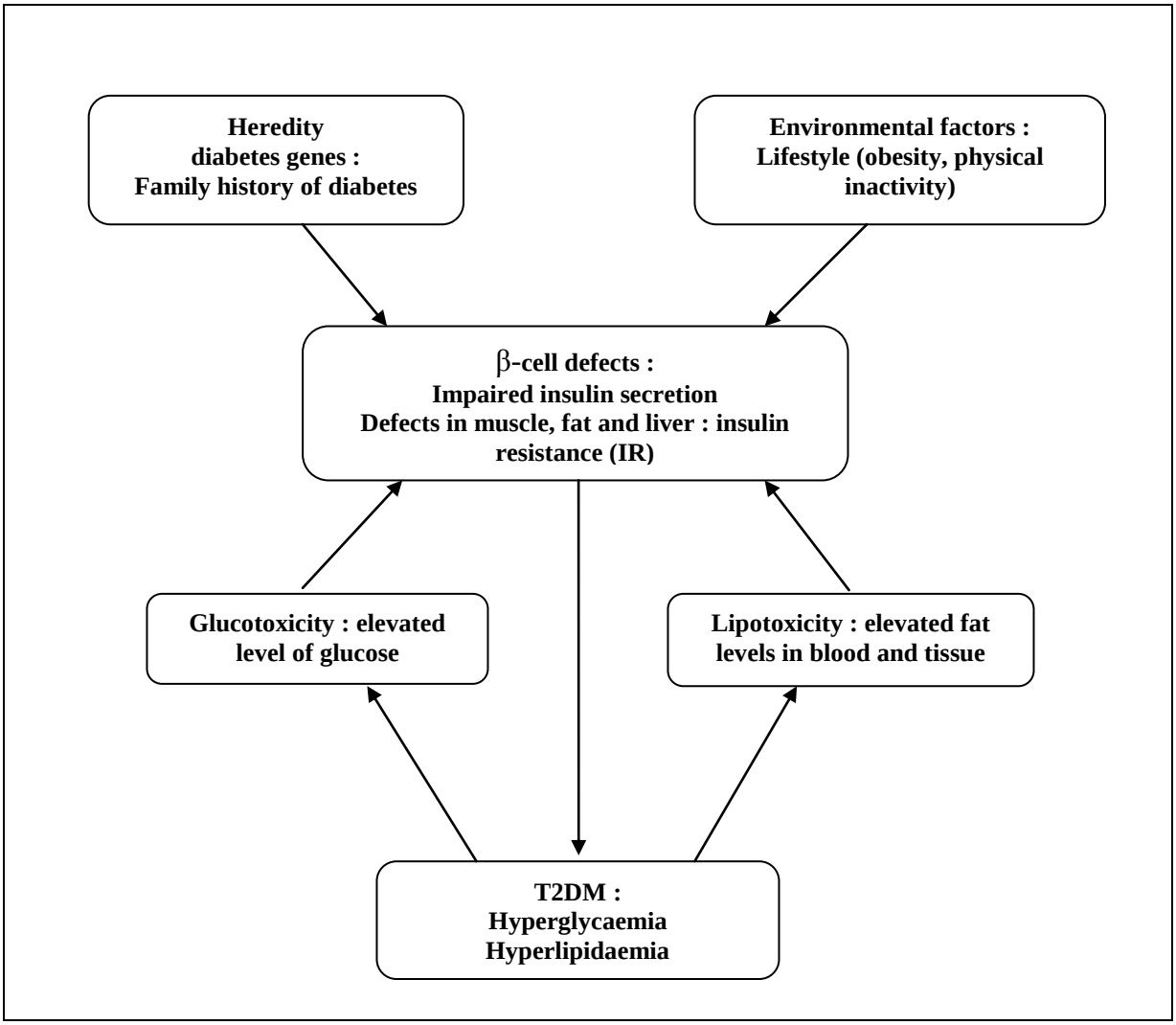

Figure 2.2: Proposed pathogenesis of DM (Sarumpudi *et al*., 2009)

2.3.1. Insulin resistance (IR)

IR is described as being a subnormal biological response to a given concentration of insulin (Sarumpudi *et al.*, 2009). Insulin binds and acts mainly through the insulin receptor and also by means of the insulin-like growth factor-1 (IGF-1) receptor (Olatunbosun & Dagogo-Jack, 2008). The cellular action of insulin involves a wide selection of effects on postreceptor signalling pathways within the target cells (Olatunbosun & Dagogo-Jack, 2008). When insulin binds to its receptor, the ß-subunit of the insulin receptor (tyrosine kinase) is activated, the kinase activity

autophosphorylates the receptor and mediates the multiple actions of insulin (Olatunbosun & Dagogo-Jack, 2008). Obesity, which is considered to be the most common cause of IR, is associated with a decreased number of receptors as well as postreceptor failure to activate the tyrosine kinase (Olatunbosun & Dagogo-Jack, 2008). Adipose cells play an important role in the development of IR (Sarumpudi *et al.,* 2009). Even though adiposity and IR are related, they are not necessarily the same and each may make an independent and different contribution to an increased risk for CVD (Olatunbosun & Dagogo-Jack, 2008).

Holt (2004) mentions that the importance of insulin action on other aspects of the intermediary metabolism, which includes lipid and protein metabolism, has been reported. Olatunbosun and Dagogo-Jack (2008) describe IR as a state in which a given concentration of insulin produces a less-than-expected biological effect and has also randomly been defined as the requirement of 200 or more units of insulin per day to manage glycaemic control and also to prevent ketosis. Furthermore, IR has a broad clinical spectrum which includes obesity, glucose intolerance, DM and metabolic syndrome (MS) and an extreme insulin-resistant state. These authors also mention an association between these disorders and various endocrine, metabolic and genetic conditions and suggest that there might also be an association with immunological diseases which might exhibit distinct phenotypic characteristics. The MS (also known as either syndrome X or the dysmetabolic syndrome), which is a state of IR, has drawn great attention because of its public health importance (Olatunbosun & Dagogo-Jack, 2008). Diagnostic criteria have been developed in an effort to clinically identify patients with IR (Olatunbosun & Dagogo-Jack, 2008).

IR plays a major role in the development of MS, which may include any or all of the following: hyperinsulinaemia, T2DM or glucose intolerance, central obesity, hypertension, dyslipidaemia (including high triglyceride (TG) levels), low high density lipoprotein cholesterol (HDL-C) levels and small, dense low density lipoprotein (LDL) particles as well as hypercoagulability characterised by increased plasminogen activator inhibitor-1 (PAI-1) levels (Olatunbosun & Dagogo-Jack, 2008).

2.3.2. Metabolic syndrome (MS)

The MS is often confused with pre-DM (Votey & Peters, 2007). MS (due to IR) may occur in patients with overtly normal GT, pre-DM or DM (Votey & Peters, 2009). The MS is characterised by central obesity, then dyslipidaemia and hypertension (Votey & Peters, 2007).

The definition of the MS has been adapted, with central obesity being added as a core feature (Holt, 2004). Central obesity is considered to be fundamental to the origin of MS (Maison *et al.,* 2001) and affects just under a quarter of American adults (Ford *et al.,* 2002). MS is known to be a significant risk factor for T2DM and CVD (Holt, 2004). Non-diabetic subjects who present with MS are at high risk of developing DM (Alberti *et al.,* 2006). As stated by Holt (2004), the major problem with the concept of MS is the lack of exact diagnostic criteria or an easy measure of IR. Alberti *et al.* (2006) point out that the IDF has created a new definition for the MS represented in Table 2.1. The new IDF definition is different from the Adult Treatment Panel (ATP) III definition in the sense that evidence of central obesity is required in order to diagnose MS (Alberti *et al.,* 2006). Central obesity is highly correlated with IR (Alberti *et al.,* 2006). European cut-offs for waist circumference or WC (central obesity for males ≥94 cm and for females ≥80 cm) will be used in Sub-Saharan Africans until more specific data is available for the African population (Alberti *et al.,* 2006). The criteria for MS are outlined in Table 2.1. These criteria have facilitated epidemiological research and are improving recognition of individuals at risk of developing DM and CVD (Holt, 2004).

2.3.3. Cardiovascular disease (CVD)

Sigal (2005) points out that DM is considered to be a major risk factor for CVD and unlike hypertension, smoking and dyslipidaemia, it is becoming more prevalent over time. These complications account for the excess morbidity, mortality and cost care that is associated with DM (Pratley, 2007). In a study done by Nielson *et al.* (2006) in which non-diabetic patients were studied to determine whether elevations in BG may be associated with increased risk for coronary artery disease (CAD) it was determined that subjects with higher baseline BG levels in the absence of DM run a significantly higher risk of developing CAD when compared with subjects with lower baseline BG.

2.3.4. Acquired organ dysfunction

Acquired defects refer to the additional defects in glucose homeostasis that take place as the diabetic metabolic environment develops, i.e. beta-cell dysfunction (Leahy, 2005). Early in the disease it is less important what therapy is used and more important that the therapy used be effective in getting BG values as close to normal as possible; therefore maximising the reversal effects (Leahy, 2005).

According to the ADA (2004), chronic hyperglycaemia of DM is often associated with long-term damage and the failure of various organs such as the eyes, kidneys, nerves, heart and blood vessels. There are several pathogenic processes that are involved in the development of DM, such as the destruction of the beta-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action (ADA, 2004).

2.3.5. Microvascular complications

The microvascular complications of DM include retinopathy, nephropathy and neuropathy (Kilpatrick, 2000; Kilpatrick, 2008). Votey and Peters (2007) mention that 25% of individuals that present with T2DM have retinopathy, 9% have neuropathy and 8% have nephropathy at the time of diagnosis. Kilpatrick (2008) states that the patients diagnosed with DM that develop these complications constitute a large percentage of people who develop renal failure, blindness and/or require limb amputation. For most patients diagnosed with DM, there is a greater fear of experiencing an acute complication such as hypoglycaemia than of the possible increased risk of developing long-term small-vessel complications through having chronically high HbA1c values (Kilpatrick, 2000).

2.3.6. Macrovascular complications

As stated by Kilpatrick (2000), even though diabetic microvascular complications are the cause of a large proportion of the excess morbidity and mortality associated with DM, the main pathological outcome remains the effects of macrovascular complications, such as CHD. HbA1c appears to give an indication of macrovascular risk in patients diagnosed with DM and in some way it might indicate the excess risk of coronary events associated with the disease (Kilpatrick, 2000).

Table 2.1: Diagnostic criteria for the MS

NCEP/ATP III (Grundy *et al.*, 2004).	WHO, 1999	AACE (Grundy *et al.*, 2004).	IDF (Alberti *et al.*, 2006)
➢ **WC: men :102cm; women: 88cm**	➢ T2 DM	➢ BMI ≥ 25 kg/m²	➢ WC; men ≥ 94 cm; women ≥ 80 cm
➢ **Fasting TG ≥ 1.7 mmol/L**	➢ IFG: 5.6-6.9 mmol/L	➢ Fasting TG ≥ 1.7 mmol/L	➢ Fasting TG ≥ 1.7 mmol/L
➢ **Blood pressure (BP): ≥ 130/85 mm Hg**	➢ IGT	➢ BP ≥ 130/85 mm Hg	➢ BP ≥130/85 mm Hg
➢ **HDL-C: men: 1.0 mmol/L; women: 1.3 mmol/L**	➢ Glucose uptake levels < lowest quartile for the specific ethnic population, under hyper-insulinaemic, euglycaemic conditions if the FG level is normal	➢ HDL-C: men ≤ 1.0 mmol/L; women ≤ 1.3 mmol/L	➢ HDL-C men: < 1.0 mmol/L; women < 1.3 mmol/L
➢ **FG ≥ 6.1 mmol/L**	➢ Criteria must also include: use of antihypertensives or SBP ≥140 mm Hg, DBP ≥90 mm Hg or both; TG ≥1.7 mmol/L; HDL-C ≤ 0.9 mmol/L (men), ≤ 1.0 mmol/L (women); BMI >30kg/m², waist/hip ratio > 0.9 (men), > 0.85 (women); urinary albumin excretion ≥20 ug/min or albumin/ creatinine ratio ≥ 30ug/min	➢ FG 6.1-7 mmolL	➢ Fasting hyperglycaemia: glucose level ≥5.6 mmol/L or Previous diagnosis of diabetes of impaired glucose tolerance
		➢ Glucose > 7.8 mmol/L after administration of 75g glucose	
		➢ Additional risk factors : FH of T2DM; hypertension; CHD; PCOS; sedentary lifestyle; advanced age; ethnic groups at high risk for T2DM / CHD	

MS=metabolic syndrome; WC = waist circumference; TG = triglyceride; FG = fasting glucose; BP = blood pressure; IGT = impaired glucose tolerance; DM=diabetes mellitus; SBP=systolic blood pressure; DBP=diastolic blood pressure ; HDL-C= high-density lipoprotein cholesterol; FH = Family history; CHD = Coronary heart disease; PCOS = Polycystic ovary syndrome; NCEP/ATP III = National Cholesterol Education Program/ Adult Treatment Panel; WHO = World Health Organization; AACE = American Association of Clinical Endocrinologists; IDF = International Diabetes Federation; T2DM = type 2 diabetes mellitus.

2.3.7. Hypoglycaemia

The most unpleasant and feared complication of DM is probably hypoglycaemia (Lamb, 2009). Children hate the symptoms of this disorder and the loss of personal control it may cause (Lamb, 2009). Gluconeogenesis and glycogenolysis are inhibited by insulin, while glucose uptake is stimulated (Lamb, 2009). In individuals that do not have DM, insulin production by the pancreatic islet cells is suppressed when BG levels fall below 4.6 mmol/L (Lamb, 2009). When injecting insulin in a treated diabetic child who has not eaten sufficient amounts of CHO, the BG levels progressively decrease (Lamb, 2009). Glucose is fuel for the brain, so when glucose levels drop below 3.2 mmol/L, counter-regulatory hormones (glucagon, cortisol and epinephrine) are released and symptoms of hypoglycaemia develop. The symptoms associated with this condition are sweatiness, shaking, confusion, behavioural changes, and eventually a coma when BG levels decrease to 1.7-2.2 mmol/L (Lamb, 2009). The glucose value at which symptoms develop is different in every individual and depends in part on the duration of DM, frequency of hypoglycaemic episodes, rate of fall of glycaemia and overall control (Lamb, 2009).

2.3.8. Hyperglycaemia

In a healthy individual, blood glucose levels usually do not rise above 9 mmol/L (Lamb, 2009). In a child diagnosed with DM, the BG levels increase if insulin is insufficient to a given glucose load and when BG levels exceed 10 mmol/L, the renal threshold for glucose reabsorption is exceeded, causing glycosuria with the typical symptoms of polyuria and polydipsia (Lamb, 2009).

2.3.9. Diabetic ketoacidosis (DKA)

This condition is much less common than hypoglycaemia, but it is far more serious and creates life-threatening medical emergencies (Lamb, 2009). Ketosis does not occur when insulin is present. When insulin is absent, severe hyperglycaemia, dehydration and ketone production contribute to the development of diabetic ketoacidosis (Lamb, 2009). Mallare *et al.* (2003) explain that the reason for DM management is to prevent DKA. A history of polyuria and polydipsia can be elicited retrospectively in all patients that present with DKA, therefore, these classical symptoms are often missed by the patients' family, the doctors caring for them or both (Mallare *et al.*, 2003). According to Mallare *et al.* (2003), awareness among the public of early symptoms of diabetes needs to be increased to reduce the frequency and severity of DKA.

2.4. Factors contributing to the prevalence of T2DM

Due to population growth, aging, urbanisation and the increasing prevalence of obesity and physical inactivity ("Western" lifestyle) (Nathan, 2002), there appears to be an increase in the number of individuals with DM (Wild *et al.*, 2004). The prevalence of T2DM is higher in Hispanics, native Americans and Asian/Pacific Islanders than in non-Hispanic whites (Votey & Peters, 2009). The incidence of T2DM is essentially equal in women and men in all populations (Votey & Peters, 2009). Strong predictors of DM are non-modifiable characteristics such as age and ethnicity, but the stronger predictors include obesity, which should become a major target for the prevention of DM (Ledergerber *et al.*, 2007).

The prevalence of T2DM increases with age (Waugh *et al.*, 2007) and it is becoming increasingly more common because people are living longer than was the case in the past (Votey & Peters, 2007). Even though it occurs more commonly in adults aged 40 years and older, the prevalence of this disease in adolescents and young adults is increasing more rapidly when compared with other age groups (Votey & Peters, 2007).

Many individuals with T2DM are reported to be asymptomatic and the disorder is usually undiagnosed for many years (Votey & Peters, 2007). It is stated by these authors that the typical patient with newly onset T2DM has had DM for at least 4-7 years before having been diagnosed.

In the study undertaken by Crandall *et al.* (2009) it was established that there is an association between moderate alcohol intake and decreased insulin secretion, which was independent of insulin sensitivity. In individuals with high alcohol intake there was a decrease in weight, therefore this might be an explanation for an association between alcohol intake and low DM risk (Crandall *et al.*, 2009). Ledergerber *et al.* (2007) argue that individuals infected with human immunodeficiency virus (HIV) may be at increased risk for developing T2DM due to viral co-infections and adverse effects of treatment.

2.4.1 Genetic predisposition

Leahy (2005) emphasises that T2DM is a renowned genetic disease due to the fact that it occurs in families and that there are ethnic populations that are at high risk of developing this disorder. It is almost certain that the genetic basis for DM is more complex than other common metabolic diseases

(Leahy, 2005). It is also assumed that there will be many susceptibility genes for T2DM with a huge amount of variability in different families and ethnic groups. It is unknown whether there will be a common form of DM that is only due to one or a few susceptibility genes that account for a sizeable percentage of people that are affected (Leahy, 2005).

The heritability of T2DM is estimated to account for 40-80% of total disease susceptibility and is greater than that for T1DM (Holt, 2004). DM is a polygenic disorder and no single major locus can explain its inheritance (Holt, 2004). Many candidate genes appear to be involved in controlling insulin secretion and action and these are all expected to play a part in the development of the disease (Holt, 2004). Leahy (2005) explains that medical care will move towards the genetic testing of individuals diagnosed with DM, followed by providing them with the most effective proven therapy for that genetic form of the disease. Family members of diagnosed individuals will undergo genetic testing while they are still glucose tolerant to conclude whether they carry a genetic predisposition (Leahy, 2005). If this is the case, specific treatments will be developed in order to prevent the disease, based on their proven efficiency for each genetic defect.

If many generations within the same family develop T2DM before the age of 25 years, it is likely that they are affected by maturity-onset diabetes of the young (MODY) which is a monogenic form of DM (Votey & Peters, 2007). Several types exist and some of the genes responsible can be detected through commercially available assays (Votey & Peters, 2007).

2.4.2 Environmental factors

The DM genotype causes only a predisposition to glucose intolerance (Leahy, 2005). Environmental factors (some factors obvious in how they act, others less so) determine whether or not one develops the diabetic phenotype (Leahy, 2005). Obesity and physical inactivity are the greatest environmental risk factors for DM (Holt, 2004). Obesity has largely been responsible for the increase in DM and it is estimated that up to 80% of newly diagnosed DM can be attributed to obesity (Lean, 2000). The average body mass index (BMI) for people with T2DM in the United Kingdom (UK) and in the USA is 30 kg/m² (Jonsson, 2002). Sixty-seven percent of people with T2DM have a BMI of more than 27 kg/m² and 46% have a BMI of more than 30 kg/m² (National Task Force on the Prevention and Treatment of Obesity, 2000). Holt (2004) found that people that exercise for more than 20 min per week had a 46% lower risk of developing DM when compared

with subjects that exercise for less than 20 min per week. Even though some of the differences can be explained by differences in adiposity, those in the most active quintile still had a 26% reduction in the risk of developing DM after adjusting for BMI (Holt, 2004). Many associations have been determined between watching television, high energy diets and physical inactivity, i.e. the modern lifestyle, and T2DM; therefore it is no surprise that there is an explosion in the incidence of DM worldwide (Leahy, 2005). These predisposing factors share an ability to negatively impact the glucose homeostasis system through worsening IR or impairing ß-cell function (Leahy, 2005). When these factors are superimposed onto a genetically compromised glucose homeostasis system, the risk of progressing to hyperglycaemia is increased (Leahy, 2005). The incidence of DM in China and India has rapidly increased as people have moved from the country to the city (Leahy, 2005). There is a 0.1-0.2% incidence of DM for rural farmers in China as opposed to more than 5% for city dwellers (Leahy, 2005). By manipulating lifestyle, an opportunity to reverse the DM trend is provided (Leahy, 2005). Genetic make-up cannot be changed, but environmental factors can be altered (Leahy, 2005). Intrauterine environment is also considered an important factor in the development of DM (Holt, 2004). Currently there are 48 reports linking poor foetal growth with impaired glucose metabolism in later life (Newsome *et al.*, 2003).

2.5. DM and CHO intakes

In a four-month intervention study done by Yancy *et al.* (2005) it was stated that a low CHO, ketogenic diet results in significant improvement of glycaemia as measured by means of FPG and HbA1c in patients with T2DM. The participants in this study also experienced a reduction in body weight, WC and body fat percentage. Nevertheless, these changes were moderate and did not predict the change in HbA1c (Yancy *et al.*, 2005). In the study done by Meyer *et al.* (2000) no evidence was found for an effect of total carbohydrate intake on DM risk and this was consistent with results of previous cohort studies. Nielson and Joensson (2006) report that there is a reduction in fasting and postprandial glucose and HbA1c in patients with T2DM with a reduction in CHO intake. However, low CHO diets are not recommended for the management of DM (Sheard *et al.*, 2004), since foods that contain CHO are important sources of many nutrients such as water soluble vitamins, minerals and fibre (Institute of Medicine, 2002).

Consumption of a high CHO diet is not associated with higher risk of developing DM, but consumption of high glycaemic foods may increase the risk of developing IR (Kennedy *et al.*,

2005). Low-glycaemic index diets lower HbA1c values when compared to high-glycaemic index diets (Sheard *et al.,* 2004). Kennedy *et al.* (2005) state that foods that are high in fibre and whole grain foods may be protective.

Anderson *et al.* (2004) point out that a CHO intake of 50-60% is recommended by most diabetes authorities, where whole grains, vegetables, fruits and dry beans are emphasised. It is strongly recommended that diabetic individuals achieve and maintain weight with a BMI <25kg/m^2 (Anderson *et al.,* 2004). Drago *et al.* (2008) state that the management of DM is dependent upon the control of glucose in the blood stream and the aim is to keep glucose levels as close to normal as possible; therefore eating the same amount of CHO daily can help BG levels remain on target.

Montonen *et al.* (2007) suggest that higher intakes of combined fructose and glucose, and sugar-sweetened beverages may increase the risk of developing T2DM and a fructose intake of 0-90 g/d has a beneficial effect on HbA1c (Livesey & Taylor, 2008). Fructose intakes correlate closely with the DM rate worldwide (Johnson *et al.,* 2009). Johnson *et al.* (2009) explain that fructose does not accurately stimulate insulin and thus causes weight gain. Excessive fructose intake (>50 g/d) may be one of the underlying aetiologies of MS and T2DM and induces features of MS in humans (Johnson *et al.,* 2009). Moderate amounts of sugar may be incorporated into a healthy diet (Janket *et al.,* 2003).

2.6. Liver enzymes and T2DM

According to Hart *et al.* (2010) alcohol consumption is related to an increased risk of developing liver disease. Harris (2005) stated that elevated transaminases are frequently found in individuals with T2DM. He explained that the reasons for elevated liver function tests (LFTs) are ascribed to the following mechanisms : the liver helps with maintenance of normal BG concentration in the fasting and postprandial states and loss of insulin effect on the liver leads to glycogenolysis and increase in hepatic glucose production (Harris, 2005). Abnormalities of TG storage and lipolysis in the liver are considered early manifestations of conditions characterised by IR and are detectable earlier than fasting hyperglycaemia (Harris, 2005). Individuals who present with T2DM have a higher incidence of LFT abnormalities when compared to individuals who do not have DM (Harris, 2005). According to Harris (2005), underlying IR is often reflected by mild chronic elevations of transaminases. He also states that GGT (gamma-glutamyl transpeptidase) is a nonspecific marker

known to rise in patients with T2DM. GGT has been proposed as another marker of IR, due to the fact that it increases in DM and increases with the increase in BMI (Harris, 2005). An increase in ALT (alanine transaminase) concentrations is associated with a decline in hepatic insulin sensitivity and the risk of T2DM (Harris, 2005). It was therefore concluded by Harris (2005) and Tohidi *et al.* (2008) that higher ALT is a risk factor for the development of T2DM and also indicates a potential role of increased hepatic gluconeogenesis and/or inflammation in the pathogenesis of T2DM. It was stated by West *et al.* (2006) that the ALT is 3-4 times higher in patients who present with either T1DM or T2DM when compared to the general population. It was shown that elevated serum GGT and ALT levels, even in normal range, are considered better predictors of DM than the known risk factors (Doi *et al.,* 2007). Doi *et al.* (2007) support the hypothesis that the liver is extremely important in the pathogenesis of T2DM.

2.7. Diagnosis of T2DM

The three methods for diagnosing DM are by analysing fasting glucose (FG) levels, HbA1c levels or the use of the OGTT (ADA, 2008). Table 2.3 represents the diagnostic criteria for T2DM and IGT.

2.7.1. Fasting plasma glucose (FPG)

According to Norman (2010) an elevated blood sugar level after an overnight fast is used to diagnose DM. Cox and Edelman (2009) describe the FPG test as a glucose measurement obtained after an overnight fast (usually 8 hours of fasting). It is used as a diagnostic and screening tool, because of the fact that it is easy, inexpensive and relatively risk free. A person has DM when the FPG level is above 7.8 mmol/L on at least two occasions (Norman, 2010). The FPG level of a normal person is 3.9-6.1 mmol/L (Norman, 2010). The advantages and disadvantages of FPG are indicated in Table 2.3.

2.7.2. Oral glucose tolerance test (OGTT)

According to Cox and Edelman (2009), the OGTT is currently considered to be the gold standard for diagnosis of DM, probably due to its longstanding use. The OGTT is not recommended for clinical use, but may be useful for further evaluation of patients in whom DM is suspected but in whom normal FPG levels or impaired FPG levels are found (ADA, 2008). The 75 g OGTT is considered to be more sensitive and modestly more specific than the FPG test for the diagnosis of DM, but it is poorly reproducible and it is difficult to perform this test in practice, hence, due to the ease of use,

acceptability to patients, and lower cost, the FPG test is considered the preferred diagnostic test (ADA, 2008). The advantages and disadvantages of OGTT are outlined in Table 2.3.

2.7.2.1. OGTT: process (Norman, 2010)

When doing an OGTT, the individual needs to be in a fasting state. An initial blood sample is drawn to determine the FPG level, and thereafter the individual is given a "glucola" beverage, containing a large amount of sugar (75 g of glucose or 100 g glucose for pregnant women). The individual then has his/her blood tested again in 30 minutes, 1 hour, 2 hours and 3 hours after the beverage has been ingested. For best results, the individual needs to be in good health, should be normally active and should not be taking any medicine that could affect the BG. The morning the test is conducted, the individual should not drink tea, coffee or water or smoke, and during the test the individual should lie or sit quietly.

When an individual does not have DM, the BG will rise following drinking the glucose, but will then return to normal very rapidly, because insulin is produced in response to the glucose and insulin has a normal effect of lowering BG levels. When the individual does indeed have DM, insulin is either not produced, or it is produced but the cells of the body do not respond to it; therefore the BG level will rise higher than the normal level and will come down to a normal level much slower. Table 2.2 represents the diagnostic plasma and BG levels for DM.

Table 2.2: Diagnosis of DM

Measurement time	Glucose concentration measured in mmol/L			
	Plasma		Whole blood	
	Venous	Capillary	Venous	Capillary
BG (fasting)	≥7.0	≥7.0	≥6.1	≥6.1
BG (2h after glucose load)	≥11.1	≥12.2	≥10.0	≥11.1

BG=blood glucose. Farmer, 2010

2.7.3. Glycosylated haemoglobin A1c (HbA1c)

HbA1c is also called glycosylated haemoglobin A1c and is formed through the non-enzymatic binding of the circulating glucose to haemoglobin (Farmer, 2010). This process is known as glycation (Kilpatrick, 2000; Gomero *et al.,* 2008). Human haemoglobin undergoes glycation from a

reaction between the β-chain of haemoglobin A0 and glucose to form HbA1c (Amadori reaction) (Farmer, 2010). Similar reactions in the α- and β-chains of haemoglobin result in the formation of other compounds and these can be measured as the total HbA1c (Farmer, 2010).

According to the ADA (2007) and Farmer (2010), the glycated haemoglobin value is used to determine the degree of glycaemic control in an individual and to make decisions regarding therapy in patients diagnosed with DM. The concentration of glycated haemoglobin predicts the progression of microvascular complications in patients with DM (Sacks, 2007). Peterson *et al.* (1998) state that HbA1c is a stable minor haemoglobin variant that is separated by charge and is composed mainly but variably of glycohaemoglobin. Increased levels of glucose in the blood contribute to more binding to haemoglobin and result in higher levels of HbA1c (Gomero, 2008). HbA1c measures average glucose concentration over the previous 6-8 weeks (Farmer, 2010). It is stated by Farmer (2010) that BG concentration over the previous months determines approximately 50% variance in HbA1c (25% by the concentration over 1-2 months and 25% by the concentration from 2-4 months).

Schnedl *et al.* (2000) and Sacks (2007) remind us that measuring HbA1c in patients with DM is an established procedure for evaluating long-term control of DM. Farmer (2010) suggests that HbA1c levels are directly related to the risk of developing DM complications. As stated by Schnedl *et al.* (2000), the HbA1c values in patients without haemoglobinopathies are highly sensitive measuring tools for reflecting elevations of BG. A 1% deviation in HbA1c results from a change of 1.4-1.9 mmol/L in the average BG concentration (Schnedl *et al.,* 2000). The ion-exchange high performance liquid chromatography (HPLC) methods for determining HbA1c generally indicate the presence of variant haemoglobin, but lack the resolution that is required to differentiate haemoglobin variants (Schnedl *et al.,* 2000). Peterson *et al.* (1998) point out that the use of glycohaemoglobin measurements as an indicator of long-term glycaemic control is increasing. However, the quantification of glycohaemoglobins (HbA1c chromatographic fraction) is in the process of being standardised (Peterson *et al.,* 1998). According to the ADA (2007), it is recommended that HbA1c be measured at least twice a year in persons diagnosed with DM.

2.7.3.1. Advantages of using HbA1c for early diagnosis of T2DM

According to Saudek *et al.* (2008), there are a series of practical considerations that favour the use of HbA1c in screening for diabetes as well as the diagnoses of DM. There are some advantages to the use of HbA1c when compared with the glucose assay:

a. HbA1c values provide an overview of the average BG levels over a period of 10 weeks or so (Expert Committee on the Diagnosis and Classification of Diabetes, 2002).
b. HbA1c has a more stable chemical moiety when compared with plasma glucose (PG) measurement (Kilpatrick & Winocour, 2010).
c. For measuring HbA1c the patient does not have to be in a fasting state; therefore it is more convenient and easier for the patient, who will no longer need to undergo a fasting OGTT (Saudek *et al.,* 2008; Expert Committee on the Diagnosis and Classification of Diabetes, 2002).
d. HbA1c levels are tightly correlated with the risk of developing retinopathy, nephropathy and neuropathy (Saudek *et al.,* 2008).
e. HbA1c values are not affected by short-term lifestyle changes, e.g. dieting and increased exercise (Saudek *et al.,* 2008).

The major disadvantage of the measurement of HbA1c is the cost, therefore, not everyone in the community can afford it (Marshall, 2010). However, even though HbA1c is considered to be more costly than glucose, the overall cost as part of a screening or diagnostic pathway may not be more costly (Kilpatric & Winocour, 2010). Motta *et al.* (2009) and Edelman *et al.* (2004) argue that there are additional benefits to the use of HbA1c for predicting expensive clinical complications (high BP, high cholesterol). As stated by Farmer (2010), there are a few conditions that can affect the measurement of HbA1c, e.g. iron deficiency anaemia, haemoglobinopathies, polycythaemia, blood transfusion, haemolysis (haemolytic anaemia), uraemia caused by renal failure and high levels of vitamin C. Table 2.3 represents a summary of the diagnostic criteria and the advantages and disadvantages of the three diagnostic tools.

2.8 HbA1c as a screening tool for T2DM

2.8.1. HbA1c *vs* mean BG

As stated by Kilpatrick (2008), it took a further decade before clinical studies started to appear, which suggested that the increased proportion of HbA1c in patients diagnosed with DM could be

used as a reliable index of glycaemic control. He also remarked that there was a rapid acceptance of glycated haemoglobin as a useful tool for assessing prior glycaemic control of patients diagnosed with T1DM and T2DM. An individual with a mean PG of approximately 10 mmol/L could have an HbA1c of between 6% and 11% and this has noticeable implications when using only HbA1c to set glycaemic targets (Kilpatrick, 2008). The single linear relationship between mean PG and HbA1c has recently been called into question for the reason that it appears different in intensively treated patients when compared with conventionally treated patients of Diabetes Control and Complication Trials or DCCTs (Kilpatrick, 2008). The mean PG level was 1.2 mmol/L lower at 7% HbA1c in intensively treated patients when compared with conventionally treated patients, with the difference becoming 4.6 mmol/L at 11% HbA1c (Kilpatrick, 2008). Therefore the relationship between mean plasma glucose and HbA1c could differ depending on the glycaemic control (Kilpatrick, 2008).

2.8.2. Effect of glucose variability on HbA1c

Until recently there was very little evidence to indicate that two patients with different levels of glucose variability, but the same mean PG, would have similar HbA1c values (Kilpatrick, 2008). Two recent studies (one using DCCT data and the other using data from the UK Prospective Diabetes study) have indicated that glucose instability seems not to have much influence on HbA1c results and that the mean glucose appears to be the main determinant (Derr *et al.*, 2003; McCarter *et al.*, 2006).

2.8.3. HbA1c as a screening test for T2DM

According to Kilpatrick (2000), there is a considerable interest in extending the use of HbA1c measurement as a screening tool as well as for the monitoring of DM. The idea of using HbA1c is likely to be more of a physiological assessment of glucose intolerance than the artificial conditions of the OGTT and certain authors believe that this is the best diagnostic tool for DM (Kilpatrick, 2000; Rohlfing *et al.*, 2000; Perry *et al.*, 2001; Droumaguet *et al.*, 2006; Nakagami *et al.*, 2007; Ginde *et al.*, 2008; Borg *et al.*, 2010; Mostafa *et al.*, 2010; Nakagami *et al.*, 2010). According to consensus statement by Saudek *et al.* (2008), there is no evidence to suggest that FPG is superior to HbA1c for the detection of T2DM, using the OGTT as the reference standard. In fact, HbA1c seems to have a slightly higher specificity than FPG for detecting DM, although its sensitivity is slightly lower (Saudek *et al.*, 2008).

Even though it is speculated that glycation is the underlying reason for complications, one has to be sure that the glycation of haemoglobin indicates an accurate reflection of glycation in small vessels (Kilpatrick, 2000). Guidance from the USA recommends against the use of HbA1c as a diagnostic tool for DM (ADA, 1999), but the European recommendation finds a role for the test only if confirmatory glucose testing is also performed (European Diabetes Policy group, 1999). The use of HbA1c for early detection of T2DM has not yet been confirmed in the African population and will therefore be investigated in this study.

By making use of a ROC analysis it was determined that an HbA1c level of 5.8% yielded the highest combination of sensitivity (86%) and specificity (92%), therefore, it was concluded that HbA1c of 5.8% would be an appropriate cut-off point (Saudek *et al.,* 2008). The study of Saudek *et al.* (2008), however determined that an HbA1c value of 6.5% or greater should be accepted as a criterion for diagnosing DM (Table 2.1). Despite the limitations, HbA1c seems to be destined to continue to be the most valuable glycaemic risk marker (Kilpatrick, 2000).

Bennett and Dharmage (2007) state that standardisation of HbA1c measurements is needed worldwide in order to compare results across libraries. Whether to use HbA1c in the diagnosis of DM is, however, still under debate (Motta *et al.,* 2009). Nakagami *et al.* (2007) have concluded that the use of HbA1c as a screening tool for T2DM seems to be as effective as measuring FPG. Edelman *et al.* (2004) state that an elevated HbA1c level might motivate individuals to adjust their lifestyle or might persuade physicians to devote more effort to lifestyle recommendations for individuals with traditional risk factors.

Table 2.3: Diagnostic criteria, advantages and disadvantages of the different diagnostic tools for DM

Test	Advantages	Disadvantages	IFG (mmol/L)	IGT (mmol/L)	DM (mmol/L)
FPG	➢ Single PG level ➢ Correlated with presence of complications, such as retinopathy ➢ Inexpensive, easy, risk free ➢ Widely available	➢ Requires fasting at least 8 hours ➢ Processing blood sample must be prompt (<2 hour after collecting blood) or results may be falsely low ➢ Confirmed with a second test to avoid false results ➢ Affected by short-term lifestyle changes	5.5-6.9	Not defined	**≥7**
OGTT	➢ Most sensitive test for IGT ➢ Early marker of impaired glucose homeostasis. ➢ Sensitive indicator of risk of developing DM.	➢ 8-hour fasting before testing ➢ Performed in the morning ➢ Commitment of nursing staff ➢ Length of test itself – time consuming ➢ Inconvenient to patients ➢ Necessity of an additional office visit ➢ Impractical in clinical setting ➢ Lower reproducibility than other diagnostic tests ➢ Influenced by numerous medications ➢ Expensive	Not defined	7.8-11.1	**≥11.1**
HbA1c	➢ Gold standard for measuring glucose control ➢ Easy to obtain ➢ No fasting required ➢ Point-of-care testing available ➢ HbA1c values give an overview of the average BG levels over the preceding 2 to 3 months ➢ Earlier detection of DM – could have major public health impact ➢ HbA1c levels are tightly correlated with the risk of developing retinopathy, nephropathy, neuropathy ➢ HbA1c not affected by short-term lifestyle changes, e.g. dieting, increased exercise. ➢ Possibility of using HbA1c in diagnosis of T2DM	➢ Potential for non-glycaemic causes of error ➢ Insensitive for IGT ➢ Expensive, therefore unaffordable in many parts of the world ➢ Not available in many parts of the world	Not defined	Not defined	HbA1c value of ≥6.5% is used for diagnosis of T2DM.

FPG=fasting plasma glucose; PG=plasma glucose; OGTT=oral glucose tolerance test; IGT=impaired glucose tolerance; DM=diabetes mellitus; HbA1c=glycated haemoglobin; BG=blood glucose; T2DM=type 2 DM; IFG=impaired fasting glucose. Expert Committee on the Diagnosis and Classification of Diabetes, 2002; Saudek *et al*,, 2008; Borsh-Jonson & Calagiuri, 2009; Cox & Edelman, 2009; Marshall, 2010; Sacks, 2011.

2.8.4 The effects of HbA1c and BG on CVD risk markers

According to Menon *et al.* (2005), DM is an established CVD risk factor. However, it is indicated through a growing amount of literature that the relationship between glucose levels and CVD may extend below the threshold value that is currently defined as DM. Kato *et al.* (2004) showed that increased plasma levels of HbA1c are significantly correlated with the occurrence of coronary artery disease, further providing evidence that glucose intolerance plays a role in the disease process. The risk of developing CHD or stroke begins within the normal range of HbA1c (Adams *et al*., 2009). According to Khaw *et al.* (2004), HbA1c significantly predicts all-cause mortality, coronary and CVD, even below the threshold value commonly accepted for diagnosing DM. This prediction is independent of age and classic risk factors. It is unclear from the studies that reported an association of HbA1c or impaired glucose metabolism with elevated CHD morbidity and mortality over longer follow-up times, whether the elevated risk is due to the development of DM or baseline HbA1c levels (Barr *et al.*, 2007). The conversion to DM is not the predominant factor in determining the increased risk of CVD associated with elevated HbA1c levels (Barr *et al.,* 2007). The study by Sigal (2005) established that 72% of the excess CVD risk that was attributable to higher HbA1c levels occurred in patients with HbA1c concentrations of 5.0-6.9%; therefore, it has been suggested that the cut-off point for a normal HbA1c level should be revised downward as has been done for cholesterol and BP. The HbA1c cut-off point for the risk of future CHD events was reported as ≥4.6% (Selvin *et al.,* 2005). In the study done by Adams *et al.* (2009) an increase of 1% in HbA1c was associated with a 1.5-fold increase in cardiovascular events, compared with a 1.2-fold increase in a study done by Khaw *et al.* (2004). A 1% increase in HbA1c is associated with a 20% to 30% increase in mortality or probability of cardiovascular events (Khaw *et al*., 2004). Khaw *et al.* (2004) found that a reduction of 0.1% in HbA1c levels in an entire population without DM has the potential to reduce the total mortality by up to 6%. Adams *et al.* (2009) determined that the increased risk for CHD or stroke was higher in men than in women, adjusted for age, and that this risk occurred with HbA1c levels lower than 6%. The prevalence of elevated predicted CVD risk was reported to be low in women, therefore it is suggested that impaired glucose metabolism may be playing a significant role (Adams *et al*., 2009). The relationship between HbA1c and incident events remains, even after adjusting for other CVD risk factors (Adams *et al*., 2009). Individuals with DM have an increased risk for developing macrovascular disease such as CAD and stroke (Haffner *et al.,* 1998).

Gustavsson and Agardh (2004) reported that markers of inflammation were higher in patients with DM when compared with those that do not have DM. In their study all markers of inflammation, except plasma albumin, were associated with HbA1c in non-diabetic patients. In diabetic patients there were no correlations between HbA1c and inflammatory markers. It was confirmed in their study that patients who present with macrovascular disease have elevated levels of markers of inflammation, but new information on the relationship between these markers and HbA1c within the normal range has been added, indicating an early association between the degree of glycaemia, inflammation and atherosclerosis prior to the development of DM. HbA1c levels were associated with higher levels of several inflammatory markers, including CRP, erythrocyte sedimentation rate and white blood cell count (Gustavsson & Agardh, 2004).

It is still uncertain whether HbA1c is related to macrovascular disease independent of other risk factors in people without DM (Adams *et al*., 2009). It was determined that increased HbA1c is related to incident macrovascular disease over a relatively short follow-up period in both men and women without DM and who do not develop DM after adjusting for other major risk factors (Adams *et al*., 2009). There is the possibility that the effects of increased HbA1c may be mediated by a clustering of hypertension, dyslipidaemia, hyperglycaemia and smoking *(*Adams *et al*., 2009*)*. According to Pradhan *et al.* (2007), elevated HbA1c can firmly be linked with the long-term risk of microvascular complications, and HbA1c assessment is currently being used all over for monitoring effective glycaemic control as a cornerstone of DM care. Strong associations were discovered between asymptomatic glycaemic exposure as quantified by HbA1c and incident DM (Pradhan *et al.*, 2007).

Individuals that present with DM have an increased risk for premature disability and death associated with vascular, renal, retinal and neuropathic complications (Khaw *et al*., 2004). The magnitude of the association between HbA1c and mortality and cardiovascular events is larger than for other risk factors (Khaw *et al*., 2004). It might be timely to consider the inclusion of HbA1c in the cardiovascular risk table in order to base therapeutic decisions on to better characterise absolute CVD risk (Khaw *et al*., 2004). Grant *et al.* (2004) support the use of HbA1c for screening purposes of DM and CVD. Results in the study performed by Grant *et al.* (2004) suggest that HbA1c is a better screening test for DM and CVD than random glucose. Grant *et al.* (2004) also suggest that HbA1c may be valuable for detecting the MS. The results in the study done by Grant *et al.* (2004)

also suggest that an HbA1c value of 6% may be a crucial point at which aggressive treatment of LDL cholesterol (LDL-C), BP and lifestyle modifications or metformin therapy should begin, rather than wait for HbA1c levels to rise to higher levels that are traditionally associated with DM.

A strong association was observed between HbA1c, fasting insulin and IR, independent of the subjects' age and gender (Dilley *et al*., 2007). An association was also determined between HDL-C and HbA1c when adjustments were made for age and sex (Dilley *et al.*, 2007). However, it disappeared when adjustment for TG were made (Dilley *et al.*, 2007). An association was determined between various CVD risk factors (age, WC, TG, LDL-C and fasting insulin) and HbA1c by means of multiple regression analysis (Dilley *et al*., 2007). Glycation of proteins increased with age and this supports the association of age with HbA1c (Dilley *et al*., 2007). Due to the strong association of WC, FG and TG with HbA1c, and the fact that the prevalence of MS increases when HbA1c increases, it is suggested that HbA1c be used as a diagnostic criterion for the MS, instead of FG, particularly when it is difficult to obtain a fasting sample (Dilley *et al*., 2007).

Table 2.4: Comparing previous studies done on HbA1c in the diagnosis of T2DM

STUDY	N	STUDY DESIGN	INCLUSION/EXCLUSION CRITERIA	POPULATION	MEASUREMENTS	RESULTS
Rohlfing *et al.*, 2000	6559	Cross-sectional	Excluded if : • previously diagnosed DM. Included if : • Non-Hispanic whites and blacks • Mexican Americans	Non-institutionalised civilian USA population	• FPG • HbA1c	HbA1c sensitivity = 83.4%; specificity = 84.4% for detecting undiagnosed DM at a cut-off of 1SD (5.6%) HbA1c sensitivity = 63.2%; specificity = 97.4% at 2 SD (6.1%)
Perry *et al.*, 2001	244	Prospective double-blind randomised	Excluded if : • age <24 yrs, • pregnant, • cancer treatment, • HIV or TB, • MI, coronary bypass, coronary angioplasty past 6 months, con-gestive heart failure, 3rd-degree atrioventricular heart block, uncontrolled hypertension, • fasting TG >6.8 mmol/L.	Indiana University, Indianapolis, Indiana and Washington University, St Louis, Missouri.	• OGTT • HbA1c • FPG	24% of subjects with FPG levels 5.5-6.0 mmol/L had OGTT-diagnosed DM 50% of subjects with FPG 6.1-6.9 mmol/L had OGTT-diagnosed DM. In those with OGTT-diagnosed DM and FPG between 5.5-8.0 mmol/L, detec-tion of elevated HbA1c (>6.1%): substantial improve-ment in diagnostic sensitivity over FPG threshold of 7.0 mmol/L. Concordant FPG ≥7.0 mmol/L in only 19% with T2 DM.
Grant *et al.*, 2004	704	Community-based	-	New York	• History of DM • BMI • BP • lipid profile • random glucose • HbA1c	32% of subjects had HbA1c >6%; 11.4% had HbA1c >7%. When excluding known diabetes, 24% had HbA1c >6%; 3.4% had HbA1c >7%. HbA1c strongly correlated with TC, TG, LDL-C, SBP, BMI, age in all cases, correlation coefficients higher with HbA1c

						than with random glucose. Significantly higher CVD risk factors in those with HbA1c >6%. 6% may be threshold for MS.
Droumague t *et al.*, 2006	1383 men 1437 women	Epidemiological	Excluded if : • known DM, • unknown glucose status • missing data Included if : • 30- 65 yrs	France	• Information: lifestyle, medi-cation, personal & FH • weight, height, WC, BP, bA1c.	HbA1c 5.9%: sensitivity= 64% ; specificity =77%; positive predictive value of 44%. DM risk increases with increase in HbA1c ($p<0.001$)
Nakagami *et al.*, 2007	1904	Population-based	Included if : • diagnosed with DM but excluded from the analyses of screening properties • 35-89 yrs.	Japan	• HbA1c • FPG • BMI • TC, HDL-C, TG	Areas under the ROC similar for HbA1c and FPG. HbA1c of 5.6%: sensitivity=56.6%; specificity=95.1%
Ginde *et al.*, 2008 **NHANES**	6723	Weighted cross-sectional analysis	Included if : • ≥18 yrs, no prior physician-diagnosed DM. • African Americans & Hispanics	USA	• HbA1c. • Questionnaires to determine previously diagnosed DM	HbA1c strongly correlated with undiagnosed DM. Threshold value ≥6.1% identified patients requiring confirmatory FPG; HbA1c ≤5.4% identified patients for whom DM could be reliably excluded and intermediate HbA1c (5.5-6.0%) may exclude DM in moderate, but not groups that have high risk.
Motta *et al.*, 2009	2796	Epidemiological longitudinal	Included if : • diagnosed for the first time • not received any antidiabetic therapy • have not used any pharmaca that influences BG • 65-84 yrs	Italy	• FPG • HbA1c	NFG group=2.35% among those who had HbA1c<7.02 and 2.99% of those who had>7.02%). IFG group the same analysis gave 14.14% of those with normal HbA1c, increasing progressively in parallel with increase in HbA1c above 7.02%, reaching 19.59%.

Tekumit *et al.*, 2010	166	-	Excluded if : • acute coronary syndrome • MI • undergone invasive cardiologic intervention or any type of surgical or invasive medical intervention Included if : • underwent elective isolated on-pump surgery	Istanbul, Turkey	• HbA1c • FPG • OGTT in all discharged patients without known DM	60% subjects without known DM diagnosed as DM or pre-DM with OGTT. Combined HbA1c and FPG= higher sensitivity (94.1%) and specificity (84.4%).
Nakagami *et al.*, 2010	2154	Population-based study	Included if : • 35-89 yrs	Japan	• FPG • lipids • 75 g OGTT in subjects without treatment of DM • HbA1c • WC • BP • smoking and alcohol habits.	Cut-offs for FPG, 2-h PG & HbA1c reported as 5.36 mmol/L, 7.52 mmol/L &5.1% respectively. HbA1c=same sensitivity and specificity as for FPG 5.56 mmol/L. Specificity higher in 2-h PG 7.80 mmol/L, 2nd highest in HbA1c 5.3%, 3rd highest in FPG 5.56 mmol/L, lower in HbA1c 5.2%. Significantly more cases of DM from subjects with base line IGT & HbA1c 5.2%, compared with baseline FPG 5.56 mmol/L or HbA1c 5.3%
Borg *et al.*, 2010	6258	Population based primary CVD prevention study. Cross-sectional	Excluded if : • missing OGTT and HbA1c measurements • known DM. Included if : • 30-60 yrs	Denmark	• OGTT, • FPG • HbA1c • serum cholesterol • urine albumin/ creatinine ratio, • BP, WC	According to WHO criteria (OGTT), 259 subjects (4.1%) had undiagnosed DM, while 412 (6.6%) had undiagnosed DM according to HbA1c criteria. 42.2% had HbA1c ≥6.5%. HbA1c better at distinguishing high and low predicted risk of

						IHD. Difference between HbA1c and fasting and 2-h PG not statis-tically significant.
Christensen *et al.*, 2010 **Inter99 study**	23094 (white, black and Asian)	Brief report	Excluded if : • missing OGTT and HbA1c measurements • known DM • not of Danish nationality	Denmark, UK, Australia, Greenland, Kenya, India.	• OGTT • FPG • HbA1c • Serum cholesterol • urine albumin/ creatinine ratio • BP, WC	DM prevalence lower with HbA1c diagnostic criteria in 4 of 6 studies. The probability of HbA1c ≥6.5% among OGTT-diagnosed case subjects ranged widely (17.0-78.0%) by study centre
Mostafa *et al.*, 2010 **ADDITION STAR**	8696	Multi-ethnic cohort	Excluded if : • T2DM • terminal illness • pregnant or lactating Included if : • 40-75 yrs	Multi-ethnic population in Leicester and Leicestershire, UK	• HbA1c, • OGTT • WC, W/H ratio, BMI, • BP, lipid profile, albumin/ creatinine ratio	OGTT – 291 newly diagnosed T2 DM HbA1c≥6.5% - 502 subjects. OGTT and HbA1c ≥6.5% - 198 subjects.
Kramer *et al.*, 2010 **Rancho Bernardo Study**	2107	Cross-sectional	Included if: • no known type DM • no anaemia • OGTT between 1984 and 1987.	USA	• OGTT • HbA1c • FPG	Sensitivity/specificity A1c cut point of 6.5% was 44/79%. A1c cut point of 6.5% , 85% of participants were classified as being non-diabetic of whom 34% were normoglycaemic. Limited A1c sensitivity may result in delayed diagnosis of T2DM.
Carson *et al.*, 2010 **NHANES**	6890	Cross-sectional	Included if : • >20 yrs • no self-reported history of DM • fasted ≥9 hours	USA	• FPG • HbA1c	2.3% had undiagnosed DM using A1c >6.5%. 3.6% had undiagnosed DM using FPG. 1.8% of adults had A1c >6.5% and FPG ≥7 mmol/L.
Cavognolli *et al.*, 2010	498	Study of diagnostic accuracy	Included if : • Subjects had increased risk of developing DM/ having DM.	Brazil	• OGTT • HbA1c • FPG	Subjects identified by glucose-based method = 115(23.1%) and identified by HbA1c =56 (11.6%).

			Excluded if : • Subjects had any condition known to interfere with HbA1c			Cut-point of ≥6.5% not enough to diagnose DM.
Van't Riet *et al.*, 2010 **New Hoorn Study**	2753	Population-based	Included if: • 40-65 yrs	Netherlands	• FPG • OGTT • HbA1c	Correlations: • Total population FPG & HbA1c = 0.46 OGTT & HbA1c = 0.33 • Subjects with known DM FPG & HbA1c = 0.71 OGTT & HbA1c = 0.79 HbA1c: sensitivity = 72%; specificity = 91%.

MI= myocardial infarction, N=subjects; HbA1c=glycated haemoglobin; OGTT=oral glucose tolerance test; WC=waist circumference; W/H ratio=waist/hip ratio; BMI=body mass index; BP=blood pressure; TB=tuberculosis; DM=diabetes mellitus; FPG=fasting plasma glucose; TG=triglycerides; PG=plasma glucose; ROC=receiver operator curve; TC=total cholesterol; LDL-C=low-density lipoprotein cholesterol; CVD=cardiovascular disease; NFG=normal fasting glucose; IHD= ischaemic heart disease.

It becomes clear from the detail reported in Table 2.4 that HbA1c is the preferred screening tool for undiagnosed DM and should therefore be used as a screening tool (Rohlfing *et al.,* 2000; Perry *et al.,* 2001; Droumaguet *et al.,* 2006; Nakagami *et al.,* 2007; Ginde *et al.,* 2008; Borg *et al.,* 2010; Mostafa *et al.,* 2010; Nakagami *et al.,* 2010). However, it has not yet been proved that HbA1c can be used for diagnosis of DM (Motta *et al.,* 2009). By measuring HbA1c, the sensitivity of screening in high-risk individuals is improved. HbA1c is a good tool regarding detection of undiagnosed DM. It has also been stated that HbA1c and FPG in combination may be useful to preoperatively identify patients with CAD and those with unknown DM (Tekumit *et al.,* 2010; Manley *et al.,* 2009). Validation of the use of HbA1c and FPG is required (Manley *et al.,* 2009).

2.9. Conclusion

Due to the increase in the prevalence of T2DM there is need for an effective screening tool (Permutt *et al.,* 2005). There is an association between DM and premature mortality and morbidity, therefore, this condition should not be considered a "mild" condition (Holt, 2004; Colagiuri & Davies, 2009). Early detection of T2DM is beneficial to the individual in the sense that not only will hyperglycaemia be treated, but also commonly observed accompanying abnormalities of risk for CVDs (Colagiuri & Davies, 2009). Early detection of DM is also beneficial to the health system, because the cost of detecting undiagnosed DM through opportunistic screening is low, whereas diagnosis of DM is much more expensive due to factors such as hospitalisation and medication (Colagiuri & Davies, 2009).

Figure 2.3 represents the mortality profile for men and women from the NWP. In this figure it is clearly shown that mortality from NCD is very high and therefore it is important to find a screening tool for early detection of DM in this population.

Early detection and standard therapy could reduce all-cause mortality by 3.5% and CVD mortality by 7.1%, whereas early detection and intensive therapy could reduce all-cause and CVD mortality by 5.9% and 8.6% respectively (Colagiuri & Davies, 2009). Identifying individuals with IGT and IFG provides the opportunity to implement interventions in order to decrease the risk of developing T2DM (Colagiuri & Davies, 2009).

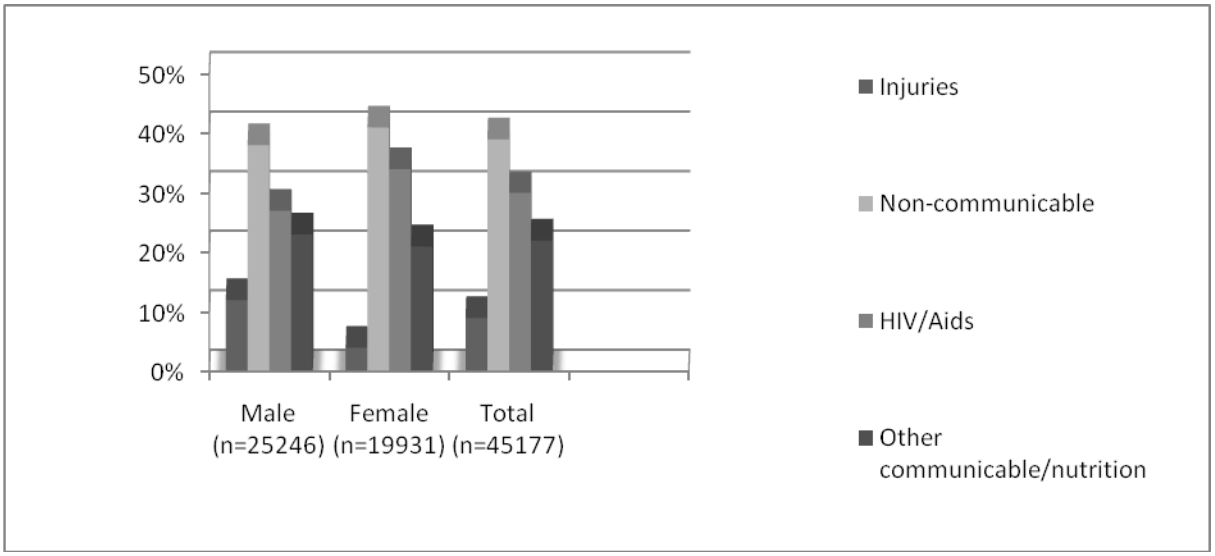

Figure 2.3: Mortality profile of men and women from the NWP, 2000 (Bradshaw *et al.*, 2004)

Motta *et al.* (2009) suggest that HbA1c could be useful for screening T2DM, but using it as a diagnostic tool is still under debate. It is also stated that using HbA1c seems to be as effective as measuring FPG in detecting T2DM (Nakagami *et al.*, 2007). Saudek *et al.* (2008) point out that there is a series of practical considerations that favour the use of HbA1c in screening as well as diagnosing DM (refer to 5.3.1 Advantages of HbA1c). The major disadvantage in the use of HbA1c is cost (Marshall, 2010), however, there are additional benefits for using HbA1c for prediction of expensive clinical complications; hence it is rendered a cost-effective choice (Motta *et al.*, 2009). Marshall (2010) suggests that further work is needed to improve the understanding of HbA1c and to overcome the difficulties associated with the measurement thereof. Except for the results of Ginde *et al.* (2008) in the NHANES study which showed a strong correlation between HbA1c and undiagnosed DM in African Americans. Limited data exists on the use of HbA1c as a screening tool in an African population and therefore the focus of this study will be on the applicability of HbA1c as a screening tool for early detection of T2DM in an African population from the NWP of SA.

The methods applied in the study will be described in the next chapter.

CHAPTER 3 :

Methods

3.1. Study design

This cross-sectional study used the data of the baseline of the SA leg of the 12-year PURE study conducted in the NWP. The PURE study is a multi-national study which investigates health transition during the industrialisation process. The baseline data for PURE-SA-NWP study was collected from March-December 2005.

3.2. Setting

A rural community (A), identified 450 km west of Potchefstroom on the highway to Botswana, and a deep rural community (B), 35 km east of A and only accessible by gravel road, were included. Both communities still function under tribal law. The urban communities (C and D) were chosen close to the University in Potchefstroom. Community C was selected from the established part of the township adjacent to Potchefstroom and D was selected from the informal settlements surrounding community C. The selection of these communities was purely aimed at stability regarding migration as the PURE study is planned over a 12 year period.

3.3. Inclusion criteria

3.3.1 Communities

The main selection criterion was that there should be migration stability within the chosen rural and urban communities.

3.3.2. Participants

The subjects had to be 35 years or older with no reported chronic diseases of lifestyle, tuberculosis (TB) or known HIV, with no mental retardation ("apparently healthy").

3.4. Selection of subjects

A household census of the number of people, their ages and health profiles was done in 6 000 households (a representative sample of 1 500 households in each community), starting with a randomly selected address in each community. The head of the household signed an informed consent form to complete the household questionnaire (HHQ, see addendum 2) and if a person refused or was not at home, the next house was taken and a non-complier questionnaire was filled out. All the possible subjects, who adhered to the inclusion criteria, were identified on the HHQ. These subjects (n ±3 000) were visited at home and after having given voluntary and informed

consent, an extensive questionnaire was completed regarding their physical and psychological health, family history on health, socio-economic background, lifestyle practices and support system. They were invited by the research team that visited the urban sites during August-October 2005 (6 weeks) and the rural sites in October-November 2005 (6 weeks) to collect blood samples and do measurements. The total number of volunteers that participated in the final selection process during these times was 1 004 from urban and 1 006 from rural sites.

3.5. Ethical considerations and organisational procedures

Permission to conduct the study in the communities mentioned above with advice on recruitment procedures was obtained from the NW Department of Health (DOH), tribal chiefs, community leaders, employers and mayors of towns. The Ethics Committee of the NWU, Potchefstroom, SA, approved the PURE study (Ethics number: 04M10) and to do an OGTT in a sub-sample (Ethics number: 02M08). Prior to the study, the subjects were informed of the objectives and procedures of the study. The subjects were asked to be in a fasted state for approximately 8-10 hours prior to sample collection. Trained Setswana-speaking fieldworkers assisted and were available to provide information in the language the participants preferred. All participants were assured of confidentiality and anonymity of all results. They signed an informed consent form on site after the procedures were again explained to them in their language of choice. The subjects received a subject number and all data collected were blinded. For the purpose of referral, the study leader was the only person who was able to match subject number to name. All the results are kept in a locked room in locked cabinets and will be stored for at least five years after the study is completed. Prior to the study, an agreement was reached for services with clinics and hospitals serving these communities from which the subjects were recruited, and those subjects newly identified with HIV, abnormal blood pressure, lung dysfunction, suspected TB, abnormal electrocardiogram (ECG) and diabetes were referred to these clinics and hospitals accompanied with a standardised referral letter without compromising their health status.

3.6. Fieldworkers

Sixteen Setswana speaking fieldworkers who completed their secondary school education were selected from communities involved (eight living in the urban communities and eight living in the rural communities), with the help and support of the local DOH and community leaders. They were extensively trained as interviewees in research methodology. They were enlisted in a health care

programme and were educated in HIV knowledge and management. The training of the fieldworkers also covered ethical issues regarding issues such as respect and the importance of confidentiality.

3.7. Measurements (see addendum 3)

3.7.1. Blood pressure (BP)

Blood pressure was measured by making use of the OMRON HEM-757:

Measurement was done twice on the subjects' right arm. In order to do this measurement the subjects needed to be rested and calm for ≥5 minutes. They should not have been smoking, exercising or have eaten in the foregoing 30 minutes and the subjects should also not have been climbing stairs in the last 30 minutes prior to measurement. The subject was seated upright and relaxed with his/her right arm supported at heart level. The measurement was taken using the brachial artery. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) as well as the heart rate were recorded.

3.7.2. Anthropometric assessment

3.7.2.1. Waist circumference (WC)

For the measurement to be taken, the subjects needed to stand erect with abdomen relaxed and arms at their sides. The measurement was taken over minimal clothing/unclothed abdomen at the smallest diameter between the costal margin and the iliac crest. Furthermore, it was taken to the nearest 0.1 cm at the end of normal expiration by making use of a non-stretchable standard tape measure attached to a spring balance exerting a force of 750 g. The tape measure was kept horizontal.

3.7.2.2. Hip circumference (HC)

The measurement was taken over minimal clothing/unclothed abdomen at the level of the greater trochanter (widest diameter around the buttocks). To see the level of maximum extension of the buttocks, the measurer needed to squat by the subjects' side. The measurement was taken to the nearest 0.1 cm at the end of normal expiration by making use of a non-stretchable standard tape measure attached to a spring balance exerting a force of 750 g. The tape measure was kept horizontal.

3.7.2.3. Weight

The subjects were measured in minimal/no clothing. They were barefoot with their arms hanging freely at their sides. It was ensured that the scale was zeroed before the weight was taken and was stabilised on a horizontal cement or wood floor. Weighing scales (Precision Health Scale, A & D Company, Japan) were calibrated prior to measurements.

3.7.2.4. Height

The subjects were measured against a convenient, flat wall on a stable horizontal level such as a cement or wooden floor. They were barefoot with their arms hanging freely at their sides, heels of the feet together with the medial (inner) border of the feet angles at 60 degrees, the shoulder blades, buttocks and heels in contact with the measuring wall and the head in the Frankfort plain (represented by a line between the margin of the orbit of the eye and the tragion). The height was recorded to the closest 0.1 cm after the subject had inhaled fully and the erect position was maintained without altering the load on the heels. In this position, a mark was made on the wall and recorded with a measuring tape.

3.8. Blood samples (see addendum 3)

Blood was drawn by registered nursing sisters from the ante-cubital vein in the subject's right arm by making use of a sterile disposable needle. For the collection of plasma, the blood collection tubes (ethylenediamine tetra acetic acid or EDTA, citrate and fluoride tubes) were filled to prescribed capacity. This ensured optimal blood: anticoagulant ratios. Excessive use of tourniquets was avoided as this might lead to haemoconcentration and inaccuracies in analytical results. The tubes were gently inverted five times to ensure that the contents of the tubes were mixed thoroughly. Samples were labelled and immediately placed in ice boxes. A new sterile transfer pipette was used to aliquot each individual's collected sample into labelled cyro tubes for analysis to follow. In the urban areas, the plasma was immediately stored at -70°C once it had been centrifuged and separated. In the rural areas it was stored at -20°C for a maximum of 5 days after which it was stored at -70°C for future use. For collecting serum, blood was allowed to clot at room temperature for 30 minutes and centrifuged at 2 000 g for 15 minutes at 10°C. The collected serum was subsequently transferred to cyro tubes and stored in the same way as described for plasma samples until it was analysed. Blood was also centrifuged to prepare plasma.

3.8.1. HIV testing (see addendum 3)

The subjects received pre-test counselling in groups of ten before blood sample collection, as well as post-test counselling during an individual feed-back session for those participants who opted to know the outcome of the test by trained counsellors. All subjects that gave informed consent for HIV testing were additionally given an option to know the outcome of the analysis. Everybody wanted to know their status.

A rapid test was done in accordance with the national DOH of SA's protocol of 2005. The test was done by making use of the First Response Rapid HIV Card test (PMC Medical, India). If the individual tested positive, the test was repeated with the Pareeshak card test (BHAT Bio-tech India) card test. If the individuals tested positive, they were referred for a confirmation cluster of differentiation 4 (CD4) test at the nearest clinic.

3.8.2. Fasting plasma glucose (FPG)

In order for the FPG to be measured, the subjects needed to be in a fasting state (at least 8 hours with no food or beverages, including water). The SYNCRON®System was used to determine FPG on fluoride plasma. The appropriate sample and reagents were automatically proportioned into cuvettes by the system. The ratio used was one part of the sample to 100 parts of the reagent. The change was monitored at 340 nanometres and the change in absorbance was directly proportional to the concentration of glucose reagent in the sample and was used to calculate and express glucose concentration.

3.8.3. Oral glucose tolerance test (OGTT)

This was done by the Profiles of Resistance to Insulin in Multiple Ethnicities and Regions (PRIMER) team joining the PURE study under the leadership of Prof A Olckers. In the urban area, the metabolic unit at the NWU, Nutrition Department, was used for this procedure, and in the rural area, tents with beds were used. A medical doctor was on site for the whole procedure and inserted an intravenous canule in the right arm of each subject. The subjects were asked to lie still on a bed. A sub-group was selected for the OGTT.

3.8.3.1.Participants

Only a sub-sample of 465 (173 men and 292 women) subjects were included in this procedure. The first 12 volunteers per day, who were definitely fasted and gave informed consent were included for the OGTT. Individuals were only included for OGTT testing if they were in a fasting state for at least 10 hours and if they were not clinically stressed (harbouring an acute infection). Subjects were not allowed to follow the procedure if the finger prick glucose level was indicative of outspoken DM.

3.8.3.2. Procedure

Prior to initiating the OGTT, blood samples were collected from all the participants, including a capillary sample after the finger prick and a plasma sample followed by intravenous (IV) catheterisation. Plasma samples were collected at 30, 60, 90 and 120 minutes post-prandial in order to measure glucose levels. Capillary and plasma blood glucose levels were measured by making use of the MediSens OptiumTM sensor glucose machine and MediSens OptiumTM plus blood glucose strips (Abbott Diabetes Care, Alameda, California). The BG is measured when the sample is applied to the electrode and the BG reacts with the chemicals on the electrode. A small electrical current that is equivalent in size to the amount of glucose in the blood is produced by the reaction. The generated current is measured by the machine and the BG concentration is derived in the sample, subsequently displaying the measurement within 20 seconds of application.

After the finger prick, a fasting glucose measurement was taken, followed by IV catheterisation and the subsequent ingestion of 75 g of glucose dissolved in 300 ml of water.

3.8.4. HbA1c

HbA1c was determined by making use of the D-10 Haemoglobin testing system (BIO-RAD) on site according to the following procedure:

- Rack numbers were numbered 1 through 10.
- Sample vial adapters were required for pre-diluted whole-blood samples, controls, calibrators and primers. Samples were identified by the D-10 based on the barcode labels affixed to the adapters. The sample vial adapters were aligned with the magnet facing towards the back of the rack.

- The test tubes were aligned in the rack, barcode facing the back of the rack. Barcode labels were placed to be clearly visible between the slots in the racks.
- The sample rack was inserted through the rack door.
- The D-10 grasped the rack and was moved into position for automatic barcode scanning. The barcode information was then loaded into the RUN screen work list under sample identity (ID).
- After ensuring the correctness of sample details by the researcher, the system started analysing the samples.

3.8.5. Serum lipids

The serum lipids were analysed by making use of the Konelab analysers. The samples were stored for 2 days at 20-25ºC, for 7 days at 4-8ºC and for years at -20ºC. TG, total cholesterol (TC) and HDL-C were used in this study.

TG are hydrolysed to glycerol and fatty acids by lipase. Glycerol is then phosphorylated to glycerol-3-phosphate, which is then oxidised to hydroxyacetone phosphate and hydrogen peroxide. Hydrogen peroxide reacts to 4-aminoantipyrine and 4-chlorophenol and forms a quinoneimine dye. The absorbance of this formed colour is measured at 510 nm.

TC was analysed by making use of oxidase-peroxidase and phenol aminoantipyrine reagents of Thermo Clinical Lab Systems on the Konelab chemistry analyser.

HDL-C was analysed by means of homogeneous enzymatic colorimetric test reagents of Thermo Clinical Lab Systems. HDL-C was determined enzymatically by cholesterol oxidase coupled with polyethylene glycol on the Konelab chemistry analyser.

3.9. Questionnaires

3.9.1. Diet questionnaire (see addendum 4)

A culture-sensitive quantitative food frequency questionnaire (QFFQ), developed and tested for reproducibility (MacIntyre *et al.*, 2000a), and validated for use in the population of the NWP (MacIntyre *et al.*, 2000b), was used to obtain dietary intakes of participants. This questionnaire was completed by the fieldworkers for all the participants and indicates the type of food consumed,

preparation of the food, how much was consumed at a time and how many times a day/week/month it was consumed. Participants estimated portion sizes using a food portion photograph book which was developed and tested for reproducibility and validity in the African population in the NWP (Venter *et al.*, 2000) as well as appropriate utensils, containers and examples of specific foods. The photo books contain the foods regularly consumed in this population group; therefore the participants were able to relate actual foods and portion sizes (Venter *et al.*, 2000). The portion sizes were reported by means of household measures and converted to weight using standard tables. Food intake was then coded by making use of the food codes of the South African food composition database of the South African Medical Research Council (MRC) (Langenhoven *et al.*, 1991) and was expressed as the average amounts consumed per day. The FoodFinder 3 Dietary analysis programme of the MRC was used for the dietary analysis.

3.9.2. Adult questionnaire (see addendum 5)

Fieldworkers completed adult questionnaires for all participants. These questionnaires include demographic information, information regarding alcohol and tobacco use, the use of medication and it also reported on the health status of the participant and people in his/her household. This questionnaire also measures psychological wellbeing and the participant's attitude towards HIV/acquired immune deficiency syndrome (AIDS). The fieldworkers spoke and understood Tswana and were therefore able to communicate with the participants in a way they could both (fieldworker and participant) understand. Interviews were conducted face to face with each participant. These questionnaires are used throughout all countries participating in the PURE study and are specifically adapted to suite each country. The variables used from this questionnaire were the following: history of DM, history of tobacco use and history of alcohol use.

3.10. Statistical analysis

The data was analysed by making use of the SPSS (Statistical Package for Social Sciences, version 17.0) package and STATISTICA 9.0 in consultation with the Statistical Consultation Services from the NWU. Standard descriptive statistics were used to calculate the mean and confidence intervals of variables. Variables were expressed as mean [95% confidence interval (CI)], and categorical data were expressed as frequencies and percentages. Data was analysed for normal distribution to ensure that correct statistical tests were used. Non-parametric analysis was done. General linear model was used to determine differences in HbA1c, FPG and OGTT groups. General linear models were

also utilised to determine differences in risk factors in different HbA1c quartiles. Frequency and cross tabulation were used to indicate clustering of risk factors in order to determine how many risk factors were present in subjects per different HbA1c groups, FPG groups and OGTT groups. Urbanisation and HIV status were seen as possible confounders in this study (refer to Chapter 4, paragraph 4.2.2 and 4.2.3) and therefore were adjusted for.

The results will be presented in the next chapter and interpreted in Chapter 5.

CHAPTER 4 :
Results

4.1. Introduction

The baseline data of the PURE study (2005) was used to investigate the possibility of HbA1c being a better or more trustworthy screening tool for T2DM than FPG in this population. To investigate this, the OGTT was used as a "gold standard" to define DM. The OGTT was done on a sub-sample of 465 out of the total 2010 PURE participants (see Chapter 3, paragraph 3.8.3).

4.2. Population characteristics

Table 4.1 represents demographic information regarding level of urbanisation, smoking habits, alcohol intake, HIV infection status and FH of DM. The aim of this table is to give a descriptive overview of the population and habits of the participants in this population.

Table 4.1: Descriptive characteristics of risk factors for the development of T2DM

RFs for T2DM	Men (n=749) n (% of entire population)	Women (n=1261) n (% of entire population)
Rural	348 (17.3%)	658 (32.7%)
Urban	401 (20%)	603 (30%)
Smoking habits		
Not answered	4 (0.2%)	6 (0.3%)
Formerly used tobacco products	50 (2.5%)	27 (1.3%)
Currently using tobacco products	447 (22.2%)	594 (29.6%)
Never used tobacco products	248 (12.3%)	633 (31.5%)
Alcohol intake habit		
Drinkers	467 (23.2%)	405 (20.2%)
Non-drinkers	259 (12.9%)	818 (40.7%)
Alcohol intake (g/day) (mean/95%CI)	18.97 (16.95-21.00)	7.63 (6.52-8.74)
HIV infection		
Infected	116 (5.8%)	210 (10.5%)
Non-infected	630 (31.3%)	1038(51.6%)
Do not know status	2 (0.1%)	12 (0.6%)
FH of DM		
Diabetic father (reported by participant)		
Not answered	21 (1%)	42 (2.1%)
Yes	10 (0.5%)	26 (1.3%)
No	497 (24.7%)	869 (43.2%)
Unknown	218 (11%)	327(16.3%)
Diabetic mother (reported by participant)		
Not answered	24 (1.2%)	39 (1.9%)
Yes	26 (1.3%)	52 (2.6%)
No	534 (26.6%)	944 (47%)
Unknown	162 (8.1%)	229/(11.4%)
Diabetic sibling (reported by participant)		
Not answered	84 (4.2%)	170 (8.5%)
Yes	16 (0.8%)	48 (2.4%)
No	590(29.4%)	969 (48.2%)
Unknown	56 (2.8%)	76 (3.8%)

RFs=risk factors; T2DM=type 2 diabetes mellitus; n = number of subjects; FH=family history; DM=diabetes mellitus.
%=indicates percentage of entire population

It is indicated in Table 4.1 that there were more women than men included in this study. The mean age for the women in this study was 49.08 and the mean age for the men was 49.76. It can be seen from this table that men consumed more alcohol than women. Considering that the men in this population were less than the women, it is clear that more men smoked when compared with the women. HIV testing with appropriate pre- and post-counseling was done on all the subjects, of which 326 (16.2%) subjects were HIV infected, 1 668 (83.1%) were non-infected and in 14 (0.7%) individuals it was not possible to determine HIV status due to the fact that the blood drawn from the subject was either not enough or the subject might have refused to be tested (Table 4.1). Out of 749 men, 116 were HIV infected and out of 1 261 women, 210 were HIV infected (Table 4.1).

4.2.1. Macronutrient and alcohol intakes

The macronutrient intakes of this population were examined in order to determine the effect on risk for developing T2DM. Table 4.2 represents recommended macronutrient intakes for this population.

Table 4.2: Recommended macronutrient and alcohol intakes

Macronutrient	% intake of TE
Carbohydrate	50-65% of TE
added sugar	<10% of TE
Proteins	10-20% of TE
Animal	<10% of TE
Plant	>10% of TE
Fat	<30% of TE
Saturated	<10% of TE
MUFA	>10% of TE
PUFA	<10% of TE
Alcohol	
Men	<30g/day
Women	<15g/day

TE= total energy intakes, MUFA = monounsaturated fatty acids,
PUFA = polyunsaturated fatty acids
Recommended macronutrient and alcohol intakes as defined by Vorster & Nell, 2001; Wolmarans & Oosthuizen, 2001 and Whitney & Rolfes, 2008.

Table 4.3 represents descriptive characteristics of the macronutrient intake compared between men and women.

Table 4.3: Descriptive characteristics of macronutrient and alcohol intakes compared between men and women (mean; 95% CI)

Variable	Men (mean/95%CI)	Women (mean/95%CI)	P-value
Total energy intakes (kJ)	8332.3 (8049.1-8615.6)	7691.7 (7484.0-7899.3)	**0.00**
CHO (g)	299.7 (289.7- 309.7)	263.8 (257.3-270.3)	**0.00**
	(61%)	(58%)	
Added sugar **(g)**	44.3 (41.9-46.7)	45.0 (43.2-46.7)	0.652
	(9.0%)	(9.9%)	
Fat (g)	51.2 (48.8-53.5)	49.3 (47.5- 51.1)	0.219
	(23%)	(24%)	
Saturated **(g)**	12.3 (11.6-13.0)	12.1 (11.6-12.6)	0.565
	(5.6%)	(5.9%)	
MUFA **(g)**	13.8 (13.1-14.6)	13.3 (12.7-13.8)	0.248
	(6.3%)	(6.6%)	
PUFA **(g)**	15.2 (14.5-15.9)	15.3 (14.5-15.8)	0.991
	(6.9%)	(7.5%)	
Protein (g)	60.5 (58.2-62.8)	52.1 (50.6-53.5)	**0.000**
	(12.3%)	(11.5%)	
Animal **(g)**	24.2 (22.8- 25.6)	21.7 (20.8-22.5)	**0.001**
	(5.0%)	(4.8%)	
Plant **(g)**	34.8 (33.5- 36.0)	29.1 (28.4-29.9)	**0.000**
	(7.1%)	(6.4%)	
Alcohol intake (g/day)	19.0 (17.0-21.0)	7.6 (6.5-8.7)	**0.00**

CHO=carbohydrates; MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids

It can be seen from this table that the CHO intakes reported were significantly (P<0.001) lower for women when compared with men. Total protein, animal protein and plant protein intakes were significantly lower in women when compared with men (P<0.001, P=0.001 and P=0.000, respectively). It can also be seen from Table 4.3 that most of the energy ingested by men and women were from CHO and not from fats and proteins. From this table it is also evident that macronutrient intakes were within recommended ranges (Table 4.2) as identified by Vorster and Nell (2001), Wolmarans and Oosthuizen (2001) and Whitney and Rolfes (2008).

4.2.2. Participant characteristics per level of urbanisation

The fact that one of the PURE study's aims were to track health changes with urbanisation, differences between urban and rural participants were expected and stratified for in the larger PURE study. In this study we "pooled" the data and therefore urbanisation could be regarded as a possible confounder also in the baseline data of the PURE study, which is the data used in this study. To investigate this possibility a comparison of RFs for T2DM in rural and urban areas were made as seen in Table 4.4.

Table 4.4: Characteristics and RFs for DM between rural and urban participants (Mean; 95% CI)

Variable	Men			Women		
	Rural (n=324)	Urban (n=333)		Rural (n=599)	Urban (n=483)	
	Mean (95%CI)	Mean (95%CI)	P-value	Mean (95%CI)	Mean (95%CI)	P-value
BMI (kg/m^2)	20.8 (20.3-21.2)	20.9 (20.4-21.3)	0.716	25.8 (25.3-26.4)	27.8 (27.2-28.4)	**0.00**
BP (mm/Hg) SBP	132.6 (130.0-135.2)	137.4 (134.9-140.0)	**0.009**	127.9 (126.0-129.8)	136.7 (134.6-138.8)	**0.00**
DBP	85.1 (83.5-86.7)	87.8 (86.2-89.4)	**0.017**	86.5 (85.4-87.6)	89.4 (88.2-90.7)	**0.001**
Serum lipids (mmol/L) TC	4.8 (4.6-4.9)	4.9 (4.7-5.0)	0.217	5.1 (5.0-5.2)	5.2 (5.1-5.3)	0.218
TG	1.2 (1.1-1.3)	1.2 (1.2-1.3)	0.203	1.3 (1.2-1.3)	1.5 (1.4-1.5)	**0.00**
HDL-C	1.5 (1.5-1.6)	1.6 (1.6-1.7)	0.103	1.5 (1.5-1.6)	1.5 (1.4-1.5)	0.264
HbA1c (%)	5.5 (5.5-5.6)	5.5 (5.4-5.6)	0.769	5.7 (5.6-5.8)	5.8 (5.7-5.9)	0.064
FPG (mmol/L)	5.4 (5.3-5.6)	5.3 (5.2-5.5)	0.377	5.7 (5.5-5.8)	5.7 (5.5-5.8)	0.917

BMI=body mass index; BP=blood pressure; SBP=systolic blood pressure; DBP=diastolic blood pressure; TC=total cholesterol; TG=triglycerides, HDL-C=high density lipoprotein cholesterol; HbA1c=glycated haemoglobin A1c and FPG=fasting plasma glucose.

- Influence of urbanisation

A significantly higher BMI and BP (SBP and DBP) were observed in urban areas in women (P<0.00). BP [SBP (P=0.009) and DBP (P=0.017)] was also significantly higher in men from urban areas when compared with those from rural areas. Significantly higher TG levels were observed in women from urban areas when compared with women from rural areas (P<0.00). No other differences in risk factors were observed between these groups. The differences in risk factors may be due to the types of food ingested in rural and urban areas, financial differences and/or BMIs.

4.2.3. Participant characteristics between HIV infected and non-infected

Another possible confounder in this study was HIV infection. To investigate this possibility a comparison of RFs for T2DM in HIV infected and non-infected subjects were made as seen in Table 4.5.

Table 4.5: Characteristics and RFs for DM between HIV infected and non-infected participants (Mean; 95% CI)

Variable	Men			Women		
	HIV infected (n=107)	**HIV non-infected (n=547)**		**HIV infected (N=186)**	**HIV non-infected (n=891)**	
	Mean (95%CI)	**Mean (95%CI)**	**P-value**	**Mean (95%CI)**	**Mean (95%CI)**	**P-value**
BMI ($kg/m^{2)}$	20.0 (19.3-20.8)	21.0 (20.6-21.3)	**0.028**	24.4 (23.4-25.4)	27.2 (26.7-27.7)	**0.00**
BP (mm/Hg)						
SBP	126.1 (121.6-130.6)	136.8 (134.8-138.8)	**0.00**	122.7 (119.3-126.1)	133.7 (132.2-135.3)	**0.00**
DBP	82.6 (79.9-85.4)	87.1 (86.0- 88.4)	**0.004**	84.5 (82.5-86.6	88.5 (87.6-89.4)	**0.001**
Serum lipids (mmol/L)						
TC	4.2 (4.0- 4.5)	4.9 (4.8-5.0)	**0.00**	4.5 (4.3- 4.7)	5.3 (5.2-5.4)	**0.00**
TG	1.2 (1.1-1.4)	1.2 (1.1-1.3)	0.950	1.3 (1.2-1.4)	1.4 (1.3-1.4)	0.302
HDL-C	1.3 (1.2-1.4)	1.6 (1.6-1.7)	**0.00**	1.2 (1.1-1.3)	1.5 (1.5-1.6)	**0.00**
HbA1c (%)	5.5 (5.3-5.6)	5.5 (5.5-5.6)	0.422	5.6 (5.4-5.7)	5.8 (5.7-5.8)	**0.011**
FPG (mmol/L)	5.2 (5.0-5.5)	5.4 (5.3-5.5)	0.233	5.4 (5.2-5.7)	5.7 (5.6-5.8)	**0.023**

BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; TC=total cholesterol; TG=triglycerides, HDL-C=high density lipoprotein cholesterol; FPG=fasting plasma glucose and HbA1c=glycated haemoglobin A1c.

- Influence of HIV infection

Previous studies stated that HIV threatens to intersect with obesity-related T2DM (Samaras, 2009). Patients with treated HIV infections have an increased risk of developing T2DM (Samaras, 2009). The presence of HIV has been proven to perturbate metabolism of carbohydrates and lipids in the intestinal cells (Lutz *et al.*, 1997). According to Hardy *et al.* (2001), lipid and glucose homeostasis abnormalities and gonadal abnormalities have been noted in patients with HIV, whether or not they were taking anti-retroviral drugs. Therefore, HIV is considered a confounding factor in this study. As indicated by the P-values in both Table 4.4 and Table 4.5, there was a significant difference between risk factors for the development of T2 DM if urban and rural and HIV infected and HIV non-infected subjects were compared, therefore, HIV status and urbanisation were adjusted for.

In the next section, dietary intakes will be examined in OGTT-, FPG- and HbA1c groups in order to determine the effect it had on the development of T2DM in this population.

4.3. Dietary intake

Dietary intakes were examined in the different OGTT groups which were based on the 2 hour BG level of the OGTT. Table 4.6 represents dietary intakes in OGTT groups of men.

Table 4.6: Macronutrient and alcohol intakes between OGTT groups of men (Mean; 95% CI) (Adjusted for HIV and urbanisation)

Macronutrient	**<7.8 mmol/L (Normal) (n=136)**	**7.8-11.1 mmol/L (IGT) (n=28)**	**>11.1 mmol/L (DM) (n=9)**
Total energy intakes (kJ)	9034.4 (8421.7-9647.11)	9565.2 (7964.7-11165.6)	11738.7 (8603.4-14874.1)
CHO(g) **%E**	315.9 (293. 3-338.5) **(59%)**	273.3 (216.6-330.0) **(49%)**	314.9 (171.1-458.6) **(46%)**
Sugar (g) **%E**	43.9 (37.6-50.0) **(8.3%)**	34.4 (25.1-43.8) **(6.1%)**	23.9 (9.0-38.8) **(3.5%)**
Fat (g) **%E**	50.8 (45.4-56.1) **(21%)**	50.2 (34.7-65.8) **(20%)**	37.1 (18.7-55.5) **(12%)**
Saturated (g) **%E**	11.6 (10.2-13.0) **(4.9%)**	12.3 (8.0-16.6) **(4.9%)**	8.3 (3.3-13.3) **(2.7%)**
MUFA (g) **%E**	13.3 (11.7-14.9) **(5.6%)**	13.4 (8.4-18.3) **(5.3%)**	9.8 (4.5-15.2) **(3.2%)**
PUFA (g) **%E**	15.3 (13.7-17.0) **(6.4%)**	15.3 (10.6-20.0) **(6.1%)**	11.2 (6.3-16.1) **(3.6%)**
Protein (g) **%E**	60.0 (54.9- 64.9) **(11.3%)**	56.2 (43.0- 69.3) **(10%)**	63.2 (32.1-94.3) **(9.2%)**
Animal (g) **%E**	21.9 (19.1-24.8) **(4.1%)**	24.3 (17.4-31.3) **(4.3%)**	18.8 (8.0-29.6) **(2.7%)**
Plant (g) **%E**	36.2 (33.3-39.0) **(6.8%)**	31.1 (24.6- 37.5) **(5.5%)**	43.8 (22.0- 65.5) **(6.3%)**
Alcohol (g/day)	21.3 (16.09-26.42)	16.6 (5.04-28.06)	47.9 (7.7- 88.1)

IGT=impaired glucose tolerances; DM= diabetes mellitus; CHO=carbohydrates; MUFA=monounsaturated fatty acids; PUFA=polyunsaturated fatty acids; n=numbers; **%E=** percentage of total energy intakes

There were no significant differences in total CHO, fat and protein intakes between these groups for men after adjustment for HIV and urbanisation. An increase was observed in total energy intake with an increase in OGGT for the men, however, these results were not significant after adjustment for HIV status and urbanisation. Table 4.7 represents the nutrient intakes in the different OGTT groups of women.

Table 4.7: Macronutrient and alcohol intakes between OGTT groups of women (Mean; 95%CI) (Adjusted for HIV and urbanisation)

Macronutrient	**<7.8mmol/L (Normal) (n=155)**	**7.8-11.1 (IGT) (n=130)**	**>11.1 (DM) (n=7)**
Total energy intakes (kJ)	7226.9 (6623.1-7830.7)	7450.7 (6821.3-8080.2)	8248.9 (5149.6- 11348.2)
CHO(g)	262.9 (242.8-283.1)	271.0 (252.2- 289.9)	248.1 (168.4- 327.8)
%E	**(62%)**	**(62%)**	**(51%)**
Sugar (g)	43.0 (38.3-47.7)	44.5 (38.9- 50.2)	21.8 (5.0- 38.5)
%E	**(10.1%)**	**(10.2%)**	**(4.5%)**
Fat (g)	45.7 (40.6-50.8)	48.0 (42.3- 53.6)	34.1 (23.2- 45.0)
%E	**(24.0%)**	**(24.5%)**	**(15.7%)**
Saturated (g)	10.6 (9.4-11.9)	11.9 (10.3- 13.6)	6.7 (3.2- 10.1)
%E	**(5.6%)**	**(6.1%)**	**(3.1%)**
MUFA (g)	11.8 (10.3-13.3)	12.7 (10.9- 14.5)	7.2 (3.9- 10.5)
%E	**(6.2%)**	**(6.5%)**	**(3.3%)**
PUFA (g)	14.1 (12.5-15.8)	14.8 (12.8- 16.7)	11.9 (7.3- 16.5)
%E	**(7.4%)**	**(7.5%)**	**(5.5%)**
Protein (g)	49.3 (44.9- 53.7)	52.5 (48.3- 56.8)	41.0 (27.1- 55.0)
%E	**(11.6%)**	**(12.0%)**	**(8.5%)**
Animal (g)	19.2 (16.8- 21.7)	20.9 (18.3- 23.6)	12.0 (4.6- 19.4)
%E	**(4.5%)**	**(4.8%)**	**(2.5%)**
Plant (g)	29.1 (26.7- 31.4)	30.2 (27.9- 32.5)	28.9 (17.7- 40.1)
%E	**(6.8%)**	**(6.9%)**	**(6.0%)**
Alcohol (g/day)	6.5 (3.8- 9.1)	8.3 (4.4- 12.2)	9.8 (10.1- 29.7)

IGT=impaired glucose tolerances; DM= diabetes mellitus; CHO=carbohydrates; MUFA=monounsaturated fatty acids: PUFA=polyunsaturated fatty acids; n=numbers; **%E=** percentage of total energy intakes

From Table 4.7 it can be seen that there were no significant differences in total energy intakes, CHO, fat and protein intakes between these groups of women after adjustment for HIV and urbanisation. It is important to mention that the mean alcohol intakes in the diabetic groups (according to OGGT) were higher when compared to the normal and IGT groups, though not statistically significantly so due to large personal variation within groups as indicated by the 95% CI (Tables 4.5 and 4.6). Mean alcohol consumption in the diabetic groups was within expected ranges for women (15 g/d) and higher than the expected ranges for men i.e. 30 g/d (Whitney & Rolfes, 2008). The total population was divided into three FPG groups: normal FPG, IGT and DM (according to Norman, 2010). Table 4.8 represents nutrient intakes in different FPG groups of men.

Table 4.8: Macronutrient intakes and alcohol intakes between FPG groups of men (Mean; 95% CI) (Adjusted for HIV and urbanisation)

Risk factors	**<5.5 mmol/L (n=595) (normal FPG)**	**5.5.-7 mmol/L (n=91) (IGT)**	**>7 mmol/L (n=15) (DM)**
Total energy intakes (kJ)	8425.6 (8097.3- 8753.8)	7470.9 (6857.9- 8083.9)	12360.0 (10118.7- 14601.5)
CHO(g)	300.2 (288.9-311.5)	292.6 (265.5-319.7)	292.5 (223.4-361.6)
%E	**(61%)**	**(67%)**	**(40%)**
Sugar (g)	44.1 (41.4-46.9)	43.8 (37.6-50.1)	46.4 (27.4-65.5)
%E	**(8.9%)**	**(10.0%)**	**(6.4%)**
Fat (g)	50.0 (47.5-52.5)	53.7 (46.5- 61.0)	62.4 (44.2-80.6)
%E	**(23%)**	**(27%)**	**(19%)**
Saturated (g)	11.9 (11.2-12.6)	13.2 (11.2-15.2)	16.1 (11.0-21.1)
%E	**(5.4%)**	**(6.7%)**	**(4.9%)[ab]**
MUFA (g)	13.4 (12.6-14.2)	15.2 (12.9- 17.5)	18.0 (11.7-24.2)
%E	**(6.0%)**	**(7.7%)**	**(5.5%)**
PUFA (g)	14.8 (14.1-15.5)	15.9 (13.5-18.2)	18.8 (13.2-24.5)
%E	**(6.7%)**	**(8.1%)**	**(5.8%)**
Protein (g)	59.9 (57.4-62.4)	61.9 (55.4-68.4)	61.9 (46.6-77.2)
%E	**(12.1%)**	**(14%)**	**(8.5%)**
Animal (g)	23.5 (22.8-24.2)	25.7 (21.6-29.8)	29.3 (19.7-38.9)
%E	**(4.7%)**	**(5.9%)**	**(4.0%)**
Plant (g)	34.9 (33.5-36.3)	34.2 (30.8-37.6)	30.3 (22.9-37.7)
%E	**(7.0%)**	**(7.8%)**	**(4.2%)**
Alcohol (g/day)	19.1 (16.7-21.4)	21.2 (15.7-26.7)	13.2 (5.2-21.2)

Superscript letters indicate significant differences between groups, (P<0.05): [a]vs normal group;[b] vs IGT group, [c]vs DM group). P-value derived from ANOVA.
IGT=impaired glucose tolerances; DM= diabetes mellitus; CHO=carbohydrates; MUFA=monounsaturated fatty acids; PUFA=polyunsaturated fatty acids; n=numbers. **%E=** percentage of total energy intakes

In Table 4.8 it is illustrated that saturated fatty acid intakes were higher in subjects with DM when compared with IGT and normal FPG. An increase in total energy was observed with an increase in FPG. This result was not significant after adjustment for HIV and urbanisation. No other differences were observed between groups. Table 4.9 represents nutrient intakes in different FPG groups of women. Alcohol intakes in the different FPG groups (men and women) did not follow the same pattern as in the OGGT groups. In fact, alcohol consumption tended to be lower with increasing FPG values.

Table 4.9: Macronutrient and alcohol intakes between different FPG groups of women (Mean; 95% CI) (Adjusted for HIV and urbanisation)

Risk factors	<5.5 mmol/L (n=941) (normal FPG)	5.5.-7.0 mmol/L (n=154) (IGT)	>7.0 mmol/L (n=50) (DM)
Total energy intakes (kJ)	7515.4 (7273.6- 7757.9)	8282.1 (7608.4- 8955.7)	8460.2 (7543.1- 9377.3)[a]
CHO (g)	262.1 (254.4-269.8)	256.9 (241.4- 272.3)	249.1 (217.6-280.7)
%E	**(59%)**	**(53%)**	**(50%)**
Sugar (g)	44.0 (42.0-46.0)	42.6 (38.2-47.1)	41.0 (34.5-47.5)
%E	**(9.9%)**	**(8.7%)**	**(8.2%)[a]**
Fat (g)	47.8 (45.6- 49.9)	49.1 (45.2- 53.0)	47.7 (41.3- 54.1)
%E	**(24%)**	**(23%)**	**(21%)[a]**
Saturated (g)	11.5 (10.9-12.1)	12.3 (11.1-13.4)	12.2 (10.3- 14.2)
%E	**(5.8%)**	**(5.6%)**	**(5.5%)[a]**
MUFA (g)	12.7 (12.0-13.4)	13.4 (12.1-14.7)	13.5 (11.4-15.6)
%E	**(6.4%)**	**(6.1%)**	**(6.1%)[a]**
PUFA (g)	14.9 (14.1- 15.6)	14.9 (13.7-16.2)	14.0 (12.1-15.9)
%E	**(7.5%)**	**(6.9%)**	**(6.3%)[a]**
Protein (g)	50.7 (48.9- 52.4)	52.3 (48.9- 55.7)	49.9 (44.4- 55.3)
%E	**(11.5%)**	**(10.7%)**	**(10%)[a]**
Animal (g)	20.4 (19.4- 21.5)	22.3 (20.2- 24.3)	20.9 (18.0- 23.9)
%E	**(4.6%)**	**(4.6%)**	**(4.2%)[ab]**
Plant (g)	29.0 (28.1-29.9)	28.8 (26.9- 30.8)	27.5 (23.7-31.4)
%E	**(6.6%)**	**(5.9%)**	**(5.5%)**
Alcohol (g/day)	6.9 (5.8-8.1)	11.6 (7.8-15.4)	4.2 (1.4-6.9)[a]

Superscript letters indicate significant differences between groups (P<0.05): [a]vs normal group;[b] vs IGT group, [c]vs DM group. P-value derived from ANOVA.
IGT=impaired glucose tolerances; DM= diabetes mellitus; CHO=carbohydrates; MUFA=monounsaturated fatty acids; PUFA=polyunsaturated fatty acids; n=numbers; **%**E= percentage of total energy intakes

Total energy intakes were significantly higher in the diabetic FPG group, when compared with subjects with normal FPG. When comparing macronutrients in the FPG groups for women (Table 4.9), lower sugar intakes were observed in subjects from the DM group when compared with subjects from the normal FPG group. Lower total fat intakes were observed in women from the DM group when compared with the normal FPG group. However, saturated fatty acid intake and MUFA intakes were higher in women from the DM group and PUFA intakes were lower in the DM group when compared with the normal FPG group. Furthermore, lower total protein and animal protein intakes were observed in the DM group when compared with other groups. The alcohol intakes were higher in the IGT group when compared to the normal and diabetic groups for men and women (Tables 4.8 and 4.9). Alcohol intakes for women in the DM FPG group were significantly lower when compared to the normal FPG group. These results, however, were not statistically significant. In this study, dietary intakes were also examined between HbA1c quartiles. Table 4.10 represents nutrient intakes in different HbA1c groups of men. HbA1c quartiles were divided into quartiles in

order to determine whether there was a difference in macronutrient intake with an increase in HbA1c values.

Table 4.10: Macronutrient intake between HbA1c quartiles of men (Mean; 95% CI) (Adjusted for HIV and urbanisation)

Variables	**Q1 (<5.2%) (n=216)**	**Q2 (5.2-5.5%) (n=128)**	**Q3 (5.5-5.7%) (n=152)**	**Q4 (>5.7%) (n=230)**
Total energy intakes (kJ)	8550.7 (8004.3-9097.1)	8310.1 (7649.6- 8970.6)	8742.3 (8086.6- 9397.9)	8805.5 (8236.2- 9374.8)
CHO(g)	291.4 **(58%)**	288.5 **(59%)**	314.9 **(61%)**	293.7 **(57%)**
%E	(270.5- 312.2)	(271.3- 316.2)	(288.9- 340.9)	(271.3- 316.2)
Sugar (g)	42.0 **(8.4%)**	42.8 **(8.7%)**	42.6 **(8.3%)**	47.0 **(9%)**
%E	(36.9- 47.2)	(37.7- 47.8)	(36.2- 49.4)	(41.5- 52.6)
Fat (g)	48.0 **(21%)**	49.1 **(23%)**	53.0 **(23%)**[ab]	48.8 **(21%)**
%E	(43.2- 52.7)	(44.5- 53.8)	(47.1- 58.9)	(43.7- 53.9)
Saturated (g)	11.8 **(5.3%)**	11.6 **(5.3%)**	12.1 **(5.3%)**[ab]	12.2 **(5.3%)**
%E	(10.5- 13.1)	(10.3- 12.9)	(10.5- 13.7)	(10.8- 13.6)
MUFA (g)	12.8 **(5.7%)**	13.1 **(6.0%)**	14.3 **(6.2%)**[ab]	13.3 **(5.7%)**
%E	(11.3- 14.3)	(11.6- 14.6)	(12.4- 16.2)	(11.7- 15.0)
PUFA (g)	14.1 **(6.3%)**	14.9 **(6.8%)**	16.1 **(7.0%)**[a]	14.2 **(6.1%)**
%E	(12.7- 15.5)	(13.5- 16.3)	(14.3- 17.9)	(12.6- 15.7)
Protein (g)	57.6 **(11%)**	58.8 **(12.%)**	63.3 **(12%)**	58.3 (**11.3%)**
%E	(52.9- 62.3)	(54.2- 63.4)	(57.5- 69.2)	(53.3- 63.4)
Animal (g)	22.0 **(4.4%)**	24.0 **(4.9%)**	23.9 **(5%)**	23.9 **(4.6%)**
%E	(19.2- 24.8)	(21.2- 26.7)	(20.4- 27.4)	(20.9- 26.9)
Plant (g)	34.3 **(6.8%)**	33.3 **(6.8%)**	37.5 **(7%)**[b]	33.0 **(6.4%)**
%E	(31.8- 36.9)	(30.8- 35.9)	(34.3- 40.7)	(30.3- 35.8)
Alcohol (g/day)	20.4 (16.1- 24.7)	18.7 (14.5- 22.9)	21.4 (16.1- 26.8)	17.5 (12.8- 220.1)

Superscript letters indicate significant differences between quartiles (P<0.05).: [a]vs quartile 1;[b] vs quartile 2, [c] vs quartile 3 [d]vs quartile 4 P-value derived from ANOVA.
IGT=impaired glucose tolerances; DM= diabetes mellitus; CHO=carbohydrates; MUFA=monounsaturated fatty acids; PUFA=polyunsaturated fatty acids; n=numbers; **%E=** percentage of total energy intakes

When investigating the macronutrient intakes in the HbA1c quartiles for men, significant increases in total fat, saturated fat, MUFA and PUFA were observed in the 3rd quartile. No other differences in macronutrient intakes were seen between quartiles of men. Table 4.11 represents nutrient intakes in different HbA1c groups of women.

Table 4.11: Comparing macronutrient intakes between HbA1c quartiles of women (Mean; 95% CI)(Adjusted for HIV and urbanisation)

Variables	Q1 (<5.3%) (n=350)	Q2 (5.3-5.6%) (n=192)	Q3 (5.6-5.9%) (n=316)	Q4 (>5.9%) (n=331)
Total energy intakes (kJ)	7872.4 (7459.9- 8284.9)	7576.1 (7046.1- 8106. 1)	7169.2 (6770.2- 7568.3)	7169.2[ab] (6835.6- 7502.8)
CHO(g)	266.2 **(57%)**	255.1 **(57%)**	247.4 **(59%)**	253.2 **(59%)**
%E	(253.2- 279.2)	(241.3- 268.9)	(233.3- 261.5)	(238.2- 268.3)
Sugar (g)	43.7 **(9.4%)**	42.0 **(9.4%)**	40.2 **(9.5%)**	46.8 (**11%)**
%E	(40.2- 47.2)	(38.3- 45.7)	(36.4- 44.0)	(42.8- 50.9)
Fat (g)	47.6 **(23%)**	44.8 **(22.5%)**	45.2 **(24%)**	47.0 **(25%)**
%E	(44.2- 51.1)	(41.1- 48.1)	(41.4- 49.0)	(43.0- 51.0)
Saturated (g)	11.5 **(5.5%)**	11.1 **(5.5%)**	11.0 (**5.8%)**	11.5 **(6.1%)**
%E	(10.5- 12.5)	(10.0- 12.1)	(10.0- 12.1)	(10.4- 12.7)
MUFA (g)	12.8 **(6.2%)**	11.8 **(5.9%)**	12.0 **(6.3%)**	12.8 **(6.8%)**
%E	(11.7- 13.9)	(10.6- 12.9)	(10.8- 13.2)	(11.5- 14.1)
PUFA (g)	14.7 (**7.1%)**	13.6 **(6.8%)**	13.9 (**7.4%)**	14.4 **(7.6%)**
%E	(13.6- 15.9)	(12.3- 14.8)	(12.6- 15.1)	(13.1- 15.7)
Protein (g)	51.9 (**11.2%)**	48.4 (**11%)**	48.0 (**11.4%)**	50.0 (**11.8%)**[a]
%E	(49.0- 54.8)	(45.3- 51.5)	(44.9- 51.2)	(46.6- 53.3)
Animal (g)	20.5 (**4.4%)**	19.3 (**4.3%)**	19.7 **(4.7%)**	21.4 **(5.1%)**
%E	(18.8- 22.3)	(17.4- 21.2)	(17.8- 21.6)	(19.3- 23.4)
Plant (g)	29.7 (**6.4%)**	28.0 **(6.3%)**	27.5 **(6.5%)**	27.2 (**6.4%)** [ab]
%E	(28.1- 31.2)	(26.4- 29.7)	(25.9- 29.2)	(25.4- 28.9)
Alcohol (g/day)	12.1 (9.9- 14.3)	7.3 (4.9- 9.6)	4.8 (2.9- 7.2)	3.5 (1.0- 6.1)

Superscript letters indicate significant differences between quartiles, (P<0.05): [a]vs quartile 1;[b] vs quartile 2, [c] vs quartile 3 [d]vs quartile 4. P-value derived from ANOVA.
IGT=impaired glucose tolerances; DM= diabetes mellitus; CHO=carbohydrates; MUFA=monounsaturated fatty acids; PUFA=polyunsaturated fatty acids; n=numbers; **%E=** percentage of total energy intakes

From Table 4.11 it can be seen that there was a significant decrease in mean energy intakes with an increase in HbA1c value for women. Total protein intake was significantly lower in the 4th quartile when compared with the 1st quartile. A significant decrease in plant protein was observed between HbA1c quartiles for women. Lower alcohol intakes were also observed with an increase in HbA1c levels for men and women (Tables 4.10 and 4.11).

4.4. Risk factors (RFs) for T2DM

In this section the RFs will firstly be reviewed and therefore the health profile of the participants in this study described. Then the risk factors will be compared in different OGTT groups, FPG groups and HbA1c groups in order to determine which one is the best tool to use in screening for T2DM. Measured risk factors for T2DM for men and women from this population are represented in Table 4.12.

Table 4.12: Rfs for DM and metabolic syndrome compared for men and women (mean; 95% CI)

RFs for T2DM	Healthy limits*	Men (Mean (95% CI))	Women (Mean (95% CI))	P-value
Plasma lipids :				
TC (mmol/L)	< 4.5	4.81 (4.71 - 4.9)	5.15 (5.07 - 5.23)	**0.00**
TG (mmol/L)	< 1.7	1.20 (1.15 - 1.26)	1.34 (1.301 - 1.39)	**0.00**
HDL-C (mmol/L)	> 1.04 men > 1.29 women	1.58 (1.5 - 1.6)	1.49 (1.449 - 1.52)	**0.00**
Anthropometry :				
WC (cm)	< 80 women < 94 men	76.20 (75.49 - 76.90)	81.78 (80.99 - 82.57)	**0.00**
HC (cm)	---	86.98 (86.39 - 87.57)	102.16 (101.27 - 103.05)	**0.00**
Height (m)	---	1.68 (1.67 - 1.68)	1.57 (1.56 - 1.57)	**0.00**
Weight (kg)	---	58.47 (57.58 - 59.36)	66.18 (65.13 - 67.23)	**0.00**
BMI (kg/m^2)	< 25	20.78 (20.48 - 21.07)	26.91 (26.49 - 27.32)	**0.00**
FPG (mmol/L)	< 6.1	5.37 (5.25 - 5.49)	5.67 (5.58 - 5.76)	**0.01**
HbA1c **(%)**	< 6.0	5.53 (5.46 - 5.59)	5.72 (5.67 - 5.78)	**0.00**
BP :				
SBP (mm/Hg)	< 130	135.04 (133.20 - 136.89)	131.83 (130.39 - 133.27)	**0.01**
DBP (mm/Hg)	< 85	86.44 (85.34 - 87.55)	87.80 (86.95 - 88.66)	**0.04**
Serum liver enzymes				
AST (IU/L)	10-40 men 7-30 women	42.93 (40.26-45.60)	32.85 (31.03-34.68)	**0.00**
ALP (IU/L)	45-115 men 30-100 women	140.55 (130.24-150.85)	130.29 (127.37-133.21)	**0.02**
ALT (IU/L)	10-40 men 9-25 women	25.79 (24.21-27.36)	19.73 (18.87- 20.59)	**0.00**
LDH (IU/L)	≤270	244.04 (235.91-252.17)	252.83 (246.62- 259.04)	0.09
GGT (IU/L)	≤65 men ≤45 women	125.33 (107.75-142.92)	80.86 (71.29-90.44)	**0.00**

*Recommended ranges as defined by Alberti *et al.,* (2006); www.aidsinfonet.org.
RFs=risk factors; T2DM=type 2 diabetes mellitus; TC = total cholesterol; TG = triglycerides; HDL-C = high density lipoprotein cholesterol; WC = waist circumference; HC = hip circumference; BMI = body mass index; FPG=fasting plasma glucose; HbA1c=glycated haemoglobin A1c; SBP = systolic blood pressure; DBP = diastolic blood pressure; AST = aspartate transaminase; ALP = alkaline phosphatase; ALT = alanine transaminase; LDH = lactate dehydrogenase; GGT = gamma-glutamyl transferase; 95%CI = 95% confidence interval.

Table 4.12 indicates the measured RFs for MS as defined by the IDF (Alberti *et al.*, 2006). The clinical cut-points for serum TC, TG and HDL-C for women and the HDL-C for men are as indicated in Table 4.11 and as outlined by the IDF in 2006. The mean serum TC levels for men and

women were reported as 4.8 mmol/L for men and 5.15 mmol/L for women. Serum TG levels for men and women were within normal ranges (1.2 mmol/L for men and 1.35 mmol/L for women) and HDL-C levels were also considered to be within normal ranges for both men (1.58 mmol/L) and women (1.49 mmol/L). BP in these subjects were high for both men and women (135.05 mm Hg/86.44 mm Hg for men and 131.83 mm Hg/87.80 mm Hg for women) according to the MS criteria as defined by Alberti *et al.* (2006). The mean BMI for men was within normal weight ranges (20.81 kg/m^2), however, the mean BMI value for women (26.71 kg/m^2) indicated overweight, thus most of the women in this population are considered to be overweight. The mean HbA1c levels were normal (5.53% for men and 5.72% for women). The mean WC for the women were slightly higher than the recommended WC (according to the IDF), but within normal ranges for the men. European cut-points were used in this study, because of the fact that cut-points have not yet been established for the African population (Alberti *et al.*, 2006). When comparing mean serum liver enzymes between men and women, it was clear that women had significantly lower aspartate aminotransferase (AST), alkaline phosphatase (ALP), ALT and GGT levels when compared with men. Lactate dehydrogenase (LDH) levels, however, were higher in women compared with men, therefore the women were at an increased risk of developing liver disease. The mean serum ALP and GGT levels were higher than the normal values for both men and women.

4.4.1. Screening tools

In this section, the effectiveness FPG and HbA1c as screening tools for early detection of T2DM will be compared. The presence of RFs in the FPG groups and HbA1c groups will be compared against results from the OGTT. Firstly, risk factors will be investigated within three OGTT-defined groups which were divided into normal glucose, IGT and T2DM (OGTT groups), thereafter, the same will be done for FPG groups divided into normal, IGT and T2DM (FPG groups) and HbA1c divided into normal, risk for DM and T2DM (HbA1c groups).

4.4.1.1. OGTT as a "gold standard" for diagnosing T2DM

In this section, we will investigate RFs for T2DM in the different OGTT groups as defined by Farmer in 2010, for men and women. The BG cut-points for defining the three different OGTT risk groups for T2DM were based on the 2 hour BG level of the OGTT. Table 4.13 represents the RF for T2DM in the different OGTT groups of men.

Table 4.13: RFs for T2DM in different OGTT groups of men (Adjusted for HIV and urbanisation) (Mean; 95% CI)

RFs for T2DM	Healthy limits*	<7.8 mmol/L Normal (n=136)	7.8-11.1 mmol/L IGT (n=28)	>11.1 mmol/L DM (n=9)
Age (years)	---	42.04 (41.10- 42.90)	40.89 (39.37-42.41)	44.11 (40.15-48.08)
Blood pressure (mm/Hg)				
SBP	< 130 mm/Hg	124.18 (121.37-127.00)	125.39 (117.96-132.83)	134.33 (117.24-151.42)
DBP	< 85 mm/Hg	81.27 (79.10-83.44)	83.14 (77.55-88.74)	90.33 (76.87-103.80)
Lipids (mmol/L)				
TC	< 4.5 mmol/L	4.69 (4.46; 4.92)	4.66 (4.13; 5.19)	4.82 (3.51; 6.13)
TG	< 1.7 mmol/L	1.05 (0.97; 1.13)	1.11 (0.71; 1.51)	0.87 (0.67; 1.06)
HDL-C	> 1.04 mmol/L	1.55 (1.44; 1.67)	1.63 (1.29; 1.97)	2.00 (1.22; 2.78
Anthropometry				
WC (cm)	< 94 cm men	74.12 (72.92-75.33)	73.09 (70.70-75.49)	74.37 (69.13-79.61)
HC (cm)	---	85.64 (84.51- 86.78)	84.13 (81.44- 86.81)	84.44 (78.95- 89.94)
Waist/hip ratio	---	0.87 (0.86-0.88)	0.87 (0.85-0.89)	0.88 (0.84-0.92)
Height (m)	---	1.69 (1.67-1.70)	1.63 (1.61-1.66)[a]	1.73 (1.68-1.79)[b]
Weight (kg)	---	57.13 (55.46-58.81)	53.16 (50.55-55.76)	56.98 (51.47- 62.48)
Weight/height ratio	---	33.90 (32.95-34.84)	32.65 (30.84- 34.45)	32.86 (30.03- 35.68)
BMI ($kg/m^{2)}$	< 25 kg/m^2	20.14 (19.58-20.69)	20.09 (18.72- 21.46)	18.97 (17.39- 20.56)
HbA1c (%)	< 6.0%	5.41 (5.34-5.49)	5.48 (5.30- 5.70)	5.71 (5.14-6.38)
FPG (mmol/L)	< 6.1mmol/L	4.74 (4.61- 4.86)	4.88 (4.49-5.27)	4.39 (3.95-4.83)

*Recommended ranges as defined by Alberti *et al.*, (2006).
Superscript letters indicate significant differences between groups (P<0.05).: [a]vs normal group ;[b]vs IGT group. and [c]vs DM group. P-value derived from ANOVA. RF=risk factors; T2DM=type 2 diabetes mellitus; DM=diabetes mellitus; IGT=impaired glucose tolerance; SBP=systolic blood pressure; DBP=diastolic blood pressure; TC=total cholesterol; TG=triglycerides; HDL-C=high density lipoprotein cholesterol; WC= waist circumference; HC=hip circumference; BMI= body mass index; HbA1c=glycated heamoglobin A1c; FPG=fasting plasma glucose.

In Table 4.13 it can be seen that there were no significant differences in RFs between the OGTT groups. Table 4.14 represents the RFs for T2DM in the different OGTT groups of women.

Table 4.14: RFs for T2DM in different OGTT groups of women (Adjusted for HIV and urbanisation)(Mean; 95% CI)

RFs for T2DM	Healthy limits*	<7.8 mmol/L Normal (n=155)	7.8-11.1mmol/L IGT (n=130)	>11.1mmol/L DM (n=7)
Age (years)	---	41.43 (40.62- 42.24)	43.13 (42.30- 43.96)	40.14 (35.44- 44.84)
Blood pressure (mm/Hg)				
SBP	< 130 mm/Hg	118.95 (115.78- 122.12)	122.34 (119.22- 125.46)	124.29 (107.34- 141.24)
DBP	< 85 mm/Hg	82.44 (80.22- 84.67)	84.23 (81.88- 86.59)	86.71 (71.56- 101.87)
Lipids (mmol/L)				
TC	< 4.5 mmol/L	4.81 (4.59- 5.03)	5.16 (4.89- 5.42)	5.31 (4.30- 6.33)
TG	< 1.7 mmol/L	1.09 (0.99- 1.19)	1.13 (1.04- 1.23)	0.91 (0.67- 1.15)
HDL-C	> 1.29 mmol/L	1.39 (1.30- 1.48)	1.46 (1.35- 1.57)	1.59 (0.82- 2.36)
Anthropometry				
WC (cm)	< 80 cm women	78.25 (76.26- 80.24)	82.81 (80.48- 85.14)[a]	84.34 (60.26- 108.42)
HC (cm)	---	100.96 (98.75- 103.16)	103.58 (100.92- 106.24)	102.38 (78.64- 126.11)
Waist/hip ratio	---	0.78 (0.77- 0.79)	0.80 (0.79- 0.81)[a]	0.82 (0.76- 0.87)
Height (m)	---	1.58 (1.57- 1.59)	1.58 (1.56- 1.59)	1.58 (1.54- 1.63)
Weight (kg)	---	64.17 (61.47- 66.87)	68.27 (64.93- 71.60)	69.25 (40.15- 98.35)
Weight/height ratio	---	40.56 (38.92- 42.20)	43.36 (41.25- 45.48)	43.99 (24.89- 63.09)
BMI ($kg/m^{2)}$	< 25 kg/m^2	25.69 (24.66- 26.71)	27.61 (26.21- 29.01)	27.97 (15.36- 40.58)
HbA1c (%)	<6.0%	5.48 (5.42- 5.53)	5.61 (5.53- 5.68)[a]	5.19 (4.94- 5.43)
FPG (mmol/L)	<6.1mmol/L	4.74 (4.64- 4.83)	4.99 (4.86- 5.12)[a]	4.67 (4.12- 5.22)

*Recommended ranges as defined by Alberti *et al.*, (2006).
Superscript letters indicate significant differences between groups (P<0.05).: [a]vs normal group ;[b]vs IGT group. and [c]vs DM group. P-value derived from ANOVA. RFs=risk factors; T2DM=type 2 diabetes mellitus DM=diabetes mellitus; IGT=impaired glucose tolerance; SBP=systolic blood pressure; DBP=diastolic blood pressure; TC=total cholesterol; TG=triglycerides; HDL-C=high density lipoprotein cholesterol; WC= Waist circumference; HC=Hip circumference; BMI= body mass index; HbA1c=glycated heamoglobin A1c; FPG=fasting plasma glucose

In Table 4.14 the following results can be observed: WC, waist/hip ratios, HbA1c and FPG values were significantly higher in the IGT group when compared with the normal group.

Table 4.15 represents the liver enzymes in the OGTT groups of men. The aim of this table is to determine whether the subjects who were identified as diabetics already presented with the risk of developing liver disease.

Table 4.15: Liver enzymes in OGTT groups of men (Mean; 95% CI)

Liver enzymes	**<7.8 mmol/L (Normal) (n=136)**	**7.8-11.1 mmol/L (IGT) (n=28)**	**>11.1 mmol/L (DM) (n=9)**
ALT (10-55U/L)*	25.27 (22.41; 28.12)	31.18 (15.98; 46.38)	43.82 (31.23; 56.41)
ALP (45-115U/L)*	134.57 (123.28; 145.86)	167.58 (123.16; 212.01)	165.0 (119.78; 210.21)
AST (10-40IU/L)*	46.25 (40.93; 51.58)	59.53 (34.91; 84.16)	70.77 (32.46; 109.08)
LDH (≤270U/L)*	294.08 (265.22; 322.95)	304.38 (212.07; 396.68)	275.94 (206.63; 345.26)
GGT (≤65U/L)*	109.47 (83.92; 13.02)	153.14 (71.95; 234.33)	**291.35[a] (123.04; 459.66)**

*Normal values according to www.aidsinfonet.org.
Superscript letters indicate significant differences between groups (P<0.05).: [a]vs normal group;[b]vs IGT group, [c]vs IGT group, P-value derived from ANOVA. DM=diabetes mellitus; IGT=impaired glucose tolerance; AST = aspartate aminotransferase; ALP = alkaline phosphatase; ALT = alanine transaminase; LDH = lactate dehydrogenase; GGT = gamma-glutamyl transferase.
Values highlighted in red=values higher than recommended values

From Table 4.15 it can be seen that serum GGT levels were significantly higher in the diabetic group when compared to the other groups. It can also be seen that the serum ALP, AST, LDH and GGT levels were high in all three groups, indicating a risk of liver damage (Harris, 2005). Table 4.16 represents liver enzymes in different OGTT groups of women.

Table 4.16: Liver enzymes in OGTT groups of women (Mean; 95% CI)

Liver enzymes	<7.8 mmol/L (Normal) (n=155)	7.8-11.1 mmol/L (IGT) (n=130)	>11.1 mmol/L (DM) (n=7)
ALT (7-30U/L)*	18.12 (15.83; 20.41)	19.21 (16.87; 21.54)	21.83 (9.82; 33.84)
ALP (30-100IU/L)*	115.10 (107.64; 122.56)	128.18 (119.48; 136.87)	176.28 (93.98; 258.58)
AST (9-25U/L)*	33.24 (28.92; 37.55)	33.17 (28.44; 37.90)	53.18 (13.08; 93.27)
LDH (≤270U/L)*	275.60 (253.93; 297.27)	300.80 (273.15; 328.45)	297.70 (203.17; 392.22)
GGT (≤45U/L)*	62.90 (44.06; 81.73)	74.77 (55.94; 93.59)	234.25[ab] (-131.04; 599.53)

*Normal values according to www.aidsinfonet.org.
Superscript letters indicate significant differences between groups (P<0.05).: [a]vs normal group;[b]vs IGT group, [c]vs IGT group, P-value derived from ANOVA. DM=diabetes mellitus; IGT=impaired glucose tolerance; AST = aspartate aminotransferase; ALP = alkaline phosphatase; ALT = alanine transaminase; LDH = lactate dehydrogenase; GGT = gamma-glutamyl transferase
Values highlighted in red=values higher than recommended values

From Table 4.16 it is evident that there was an increase in GGT between subjects with T2DM and subjects who have IGT or normoglycaemic subjects. ALP, AST, LDH and GGT values for the women were also above normal values in all three groups, indicating a high possibility of risk for liver disease (Harris, 2005).

4.4.1.2. FPG as screening tool for T2DM

Risk factors in FPG groups will be investigated in this section. Table 4.17 summarises the RFs for men in the different FPG groups defined by Norman (2010).

Table 4.17: Comparing RFs for T2DM between FPG groups of men (Adjusted for HIV and urbanisation)(Mean; 95% CI)

RFs for T2DM	Healthy limits*	<5.5mmol/L Normal (n=595)	5.5.-7.0 mmol/L IGT (n=91)	>7.0 mmol/L DM (n=15)
Age (years)		49.45 (48.59-50.31)	51.56 (49.73-53.38)	51.53 (45.68-57.37)
Blood pressure (mm/Hg)				
SBP	< 130 mm/Hg	133.8 (131.90-135.70)	141.55 (136.82-146.28)	140.37 (77.25-203.49)
DBP	< 85 mm/Hg	85.99 (84.78- 87.20)	89.42 (86.55-92.29)	91.05 (50.11-131.99)
Lipids (mmol/L)				
TC	< 4.5 mmol/L	4.7 (4.59-4.81)	4.95 (4.71-5.19)	5.79 (5.16-6.42)
TG	< 1.7 mmol/L	1.2 (1.13-1.27)	1.50 (1.29-1.71)[a]	1.79 (1.36-2.22)[a]
HDL-C	> 1.04 mmol/L	1.59 (1.54-1.64)	1.53 (1.29-1.77)	1.34 (1.14-1.54)
Anthropometry				
WC (cm)	< 94 cm men	75.3 (74.58-76.02)	79.03 (77.02-81.04)[a]	87.59 (81.96-93.22)[ab]
HC (cm)	---	86.2 (85.60-86.80)	90.04 (88.03-92.05)[a]	93.09 (80.11-106.07)[ab]
Waist/Hip ratio	---	0.87 (0.87-0.87)	0.88 (0.87- 0.89)	0.94 (0.91-0.97)[ab]
Height (m)	---	1.67 (1.66-1.68)	1.68 (1.67-1.69)	1.66 (1.64-1.68)
Weight (kg)	---	57.3 (56.40-58.20)	62.83 (60.30- 65.36)[a]	66.35 (59.34-73.36)[a]
Weight/height ratio	---	34.2 (33.69-34.71)	37.28 (35.81-38.75)[a]	39.90 (36.04- 43.76)[a]
BMI ($kg/m^{2)}$	< 25 kg/m^2	20.42 (20.11-20.73)	22.16 (16.64-27.68)[a]	24.02 (21.87-26.17)[ab]
HbA1c (%)	<6.0%	5.43 (5.38-5.48)	5.59 (5.49-5.69)	7.46 (6.21- 8.71)[ab]
FPG (mmol/L)	<6.1 mmol/L	4.49 (4.44-4.54)	5.90 (0.00-22.85)[a]	9.73 (6.85-12.61)[ab]

.*Recommended ranges as defined by Alberti *et al.*, (2006).
Superscript letters indicate significant differences between groups (P<0.05).: [a]vs normal group, [b]vs IGT group, [c]vs normal group, P-value derived from ANOVA.
RFs=risk factors; T2DM=type 2 diabetes mellitus; DM=diabetes mellitus; IGT=impaired glucose tolerance; SBP=systolic blood pressure; DBP=diastolic blood pressure; TC=total cholesterol; TG=triglycerides; HDL-C=high density lipoprotein cholesterol; WC= waist circumference; HC=hip circumference; BMI= body mass index; HbA1c=glycated haemoglobin A1c; FPG=fasting plasma glucose.

Mean serum TG levels in men indicated an increase as the FPG values became more similar to that of a diabetic person. When investigating the anthropometric data for men (Table 4.17) between these groups, it was clear that WC, HC, waist/hip ratio, weight, weight/height ratio and BMI indicated increases, which were associated with central obesity and therefore MS and CVD (Alberti *et al.*, 2006). HbA1c values for men were significantly higher in the second (IGT) and third

(diabetic) group when compared with the first (normal) group. Table 4.18 represents RFs for T2DM between different FPG groups of women.

Table 4.18: Comparing RFs for T2DM between FPG groups of women adjusted for HIV status and level of urbanisation (Mean; 95% CI)

RFs for T2DM	Healthy limits*	< 5.5 mmol/L Normal FPG (n=941)	5.5-7.0 mmol/L IGT (n=154)	>7.0 mmol/L DM (n=50)
Age (years)		48.37 (47.70-49.04)	49.69 (48.26-51.12)	53.66 (50.76-56.56)
Blood pressure (mm/Hg)				
SBP	< 130 mm/Hg	130.67 (129.09-132.25)	135.06 (131.50- 138.62)	141.55 (136.81-146.29)
DBP	< 85 mm/Hg	87.29 (86.35-88.23)	90.55 (88.49-92.61)	93.09 (89.96- 96.22)
Lipids (mmol/L)				
TC	< 4.5 mmol/L	5.06 (4.97-5.15)	5.44 (5.24- 5.64)[a]	5.40 (5.06-5.74)[a]
TG	< 1.7 mmol/L	1.27 (1.22-1.32)	1.61 (1.49-1.73)[a]	1.71 (1.50-1.92)
HDL-C	> 1.29 mmol/L	1.51 (1.47-1.55)	1.42 (1.33-1.51)	1.37 (1.22-1.52)
Anthropometry				
WC (cm)	< 80 cm women	79.94 (79.06-80.82)	86.74 (84.47- 89.01)[a]	89.91 (87.01- 92.81)[a]
HC (cm)		100.54 (99.54-101.54)	104.53 (102.09-106.97)[a]	108.32 (104.74-111.90)[a]
Waist/hip ratio		0.80 (0.80- 0.80)	0.83 (0.82-0.84)[a]	0.83 (0.81-0.85)[a]
Height (m)		1.57 (1.57- 1.57)	1.56 (1.55- 1.57)	1.58 (1.56-1.60)
Weight (kg)		64.14 (62.99- 65.29)	70.85 (67.83-73.87)[a]	75.07 (71.19-78.95)[a]
Weight/height ratio		40.87 (40.16-41.58)	45.28 (43.40- 47.16)[a]	47.68 (45.23- 50.13)[a]
BMI (kg/m^2)	< 25 kg/m^2	26.09 (25.64- 26.54)	28.97 (27.79- 30.15)[a]	30.33 (28.71-31.95)[a]
HbA1c (%)	<6.0%	5.55 (5.52-5.58)	5.87 (5.75- 5.99)[a]	8.08 (7.33-8.83)[ab]
FPG (mmol/L)	<6.1mmol/L	4.55 (4.51-4.59)	6.0 (5.95- 6.05)[a]	9.94 (8.87- 11.01)[ab]

.*Recommended ranges as defined by Alberti *et al.*, (2006).
Superscript letters indicate significant differences between groups (P<0.05).: [a]vs normal group, [b]vs IGT group, [c]vs normal group, P-value derived from ANOVA.
RFs=risk factors; T2DM=type 2 diabetes mellitus; DM=diabetes mellitus; IGT=impaired glucose tolerance; SBP=systolic blood pressure; DBP=diastolic blood pressure; TC=total cholesterol; TG=triglycerides; HDL-C=high density lipoprotein cholesterol; WC= waist circumference; HC=hip circumference; BMI= body mass index; HbA1c=glycated haemoglobin A1c; FPG=fasting plasma glucose.

Significant increases were also observed in mean serum TC and TG between these groups of women. When looking at anthropometric data, it was clear that there were increases in WC, HC,

waist/hip ratio, weight, weight/height ratio and BMI with increased FPG. The HbA1c values also differed significantly between these three groups. Table 4.19 represents the liver enzymes in the OGTT groups of men.

Table 4.19: Liver enzymes in different the FPG groups of men

Liver enzymes	**<5.5mmol/L Normal (n=595)**	**5.5.-7.0 mmol/L IGT (n=91)**	**>7.0 mmol/L DM (n=15)**
ALT (10-55U/L)*	24.94 (23.22; 26.66)	29.28 (24.82; 33.75)	**37.73 (28.34; 47.13)[a]**
ALP (45-115U/L)*	135.31 (130.42; 140.21)	134.87 (122.18; 147.56)	143.09 (116.42; 169.77)
AST (10-40IU/L)*	43.16 (40.06; 46.26)	43.01 (34.97; 51.04)	49.41 (32.51; 66.30)
LDH (≤270U/L)*	242.64 (233.55; 251.73)	264.45 (240.88; 288.02)	243.22 (193.67; 292.78)
GGT (≤65U/L)*	121.97 (101.32; 142.63)	134.26 (80.72; 187.81)	202.73 (90.143; 315.32)

*Normal values according to www.aidsinfonet.org.
Superscript letters indicate significant differences, (P<0.05): [a]vs normal group;[b]vs IGT group, [c]vs IGT group, P-value derived from ANOVA. DM=diabetes mellitus; IGT=impaired glucose tolerance; AST = aspartate aminotransferase; ALP = alkaline phosphatase; ALT = alanine transaminase; LDH = lactate dehydrogenase; GGT = gamma-glutamyl transferase
Values highlighted in red=values higher than recommended values

Table 4.19 indicates that there was a significant increase in ALT in the group with T2DM compared with the group with normal FPG. It was also indicated that ALP, AST and GGT were higher than the recommended ranges in all three groups, therefore indicating a risk of developing liver disease. Table 4.20 represents liver enzymes in the different FPG groups of women.

Table 4.20: Liver enzymes in different the FPG groups of women

Liver enzymes	**< 5.5 mmol/L Normal FPG (n=941)**	**5.5-7.0 mmol/L IGT (n=154)**	**>7.0 mmol/L DM (n=50)**
ALT (7-30U/L)*	19.15 (18.17; 20.12)	22.60 (20.12; 25.07)	22.06 (18.13; 25.98)
ALP (30-100U/L)*	129.22 (125.86; 132.57)	**130.43 (121.88; 138.99)[a]**	138.41 (124.83; 151.99)
AST (9-25IU/L)*	32.97 (30.77; 35.117)	36.30 (30.68; 41.93)	31.47 (22.55; 40.40)
LDH (≤270U/L)*	255.91 (248.36; 263.46)	247.59 (228.33; 266.85)	244.36 (213.79; 274.93)
GGT (≤45U/L)*	72.75 (64.57; 80.92)	**95.78 (74.93; 116.63)[a]**	78.38 (45.28; 111.48)

*Normal values according to www.aidsinfonet.org.
Superscript letters indicate significant differences between groups (P<0.05).: [a]vs normal group;[b]vs IGT group, [c]vs IGT group, P-value derived from ANOVA. DM=diabetes mellitus, IGT=impaired glucose tolerance, AST = aspartate aminotransferase; ALP = alkaline phosphatase; ALT = alanine transaminase; LDH = lactate dehydrogenase; GGT = gamma-glutamyl transferase.
Values highlighted in red=values higher than recommended values

From Table 4.20 it can be seen that there were significant differences in ALP and GGT between groups with normal FPG and groups with IGT. GGT, AST and LDH in the diabetic group were lower when compared to other groups. It was also indicated that ALP, AST and GGT were higher than the recommended ranges in all three groups, once again indicating a risk of liver disease.

4.4.1.3. HbA1c as a screening tool for T2DM

In order to determine whether HbA1c can be used for screening in this population, RFs for MS and T2DM were investigated within HbA1c quartiles in Tables 4.21 and 4.22. HbA1c was divided into quartiles in order to determine whether there were differences in RFs with an increase in HbA1c values.

Table 4.21: Mean (95% CI) characteristics and RFs for T2DM of men across HbA1c quartiles (Adjusted for HIV and urbanisation)

RFs for T2DM	Healthy limits*	Q1 (n = 173) <5.2%	Q2 (n = 117) 5.2-5.5%	Q3 (n = 129) 5.5-5.7%	Q4 (n = 198) >5.7%
Plasma lipids (mmol/L)					
TC	< 4.5 mmol/L	4.56 (4.34; 4.77)	4.68 (4.48; 4.88)	5.04[ab] (4.83; 5.26)	4.93[a] (4.75; 5.10)
TG	< 1.7 mmol/L	1.16 (1.03; 1.29)	1.06 (0.94; 1.18)	1.22 (1.09; 1.35)	1.33[ab] (1.22; 1.44)
HDL-C	> 1.04 mmol/L	1.66 (1.553; 1.767)	1.66 (1.55; 1.76)	1.59 (1.48; 1.69)	1.46[abc] (1.37; 1.55)
Anthropometry					
BMI (kg/m²)	<25kg/m^2	19.94 (19.28; 20.60)	20.55 (19.93; 21.17)	20.69 (20.02; 21.36)	21.70[ab] (21.15; 22.24)
WC(cm)	<94cm	74.78 (73.33- 76.23)	75.37 (73.95- 76.80)	77.02 (75.22- 78.83)	79.17[ab] (77.62- 80.73)
FPG	<6.1mmol/L	5.12 (4.9; 5.3)	5.25 (5.05; 5.45)	5.35 (5.14; 5.57)	5.64[ab] (5.46; 5.81)
Blood pressure (mm/Hg)					
SBP	<130mm Hg	133.83 (129.90; 137.76)	135.59 (131.90; 139.29)	133.01 (129.06; 136.97)	136.79 (133.56; 140.03)
DBP	<85mm Hg	86.64 (84.23; 89.04)	86.68 (84.42; 88.93)	85.30 (82.88; 87.72)	86.90 (84.92; 88.88)

*Recommended ranges as defined by Alberti *et al.*, (2006).
Superscript letters indicate significant differences between quartiles (P<0.05), : [a] vs quartile 1;[b] vs quartile 2; [c] vs quartile 3 (p<0.05) adjusted for HIV status and urbanisation. P-value derived from ANOVA test.
RF=risk factors; T2DM=type 2 diabetes mellitus; CI=confidence interval; Q=quartiles; n= number of subjects; TC= total cholesterol; TG= triglycerides; HDL-C= high density lipoprotein cholesterol; BMI=body mass index; WC=waist circumference; FPG=fasting plasma glucose; SBP=systolic blood pressure; DBP=diastolic blood pressure.

In Table 4.21 RFs for DM are compared in different HbA1c quartiles of men in order to see whether RFs increase with an increase in HbA1c. From this table it is clear that there were significant increases in mean serum TC and serum TG levels, WC and BMI and a decrease in mean serum HDL-C levels with an increase in HbA1c levels after adjusting for level of urbanisation and HIV status. The FPG value also increased with an increase in HbA1c. The FPG value in the 4th quartile (>5.7%) was reported as 5.64 mmol/L. There were no significant differences in SBP and DBP between these quartiles. Table 4.22 represents RFs in HbA1c quartiles of women.

Table 4.22: Mean (95% CI) characteristics and RFs for T2DM of women across HbA1c quartiles (Adjusted for HIV and urbanisation)

RFs for T2DM	**Healthy limits***	**Q1 (n = 288) <5.3%**	**Q2 (n = 159) 5.3-5.6%**	**Q3 (n = 263) 5.6-5.9%**	**Q4 (n = 281) >5.9%**
Plasma lipids (mmol/L)					
TC	< 4.5 mmol/L	4.99 (4.82; 5.17)	5.17 (5.0; 5.34)	5.16 (5.0;5.32)	5.26 (5.1; 5.41)
TG	< 1.7 mmol/L	1.21 (1.12; 1.30)	1.26 (1.17; 1.35)	1.32^{a} (1.24; 1.40)	1.55^{abc} (1.47; 1.63)
HDL-C	> 1.29 mmol/L	1.66 (1.58; 1.74)	1.55 (1.48; 1.63)	1.45^{a} (1.38; 1.52)	1.33^{abc} (1.26; 1.40)
Anthropometry					
BMI (kg/m²)	<25 kg/m^2	24.09 (23.21; 24.97)	25.88^{a} (24.85; 26.55)	26.88^{ab} (26.09; 27.68)	29.44^{abc} (28.65; 30.22)
WC (cm)	<80 cm	76.37 (74.86- 77.88)	79.52^{a} (77.91- 81.13)	81.64^{a} (80.0- 83.28)	89.52^{abc} (87.77- 91.26)
FPG	<6.1mmol/L	5.32 (5.11; 5.53)	5.40 (5.20; 5.60)	5.56 (5.37; 5.74)	6.29^{abc} (6.10; 6.47)
Blood pressure (mm/Hg)					
SBP	<130mm/Hg	129.82 (126.74; 132.90)	130.07 (127.10; 133.04)	130.68 (127.91; 133.45)	136.83^{abc} (133.28; 138.73)
DBP	<85mm/Hg	87.75 (85.93; 89.57)	87.04 (85.29; 88.79)	87.21 (85.57; 88.84)	89.07 (87.46; 90.68)

*Recommended ranges as defined by Alberti *et al*., (2006).
Superscript letters indicate significant differences between quartiles (P<0.05).: ^{a} vs quartile 1;^{b} vs quartile 2; ^{c}vs quartile 3 (p<0.05) adjusted for HIV status and urbanisation. P-value derived from ANOVA test.
RF=risk factors; CI= confidence interval; Q=quartiles; n= number of subjects; TC= total cholesterol; TG= triglycerides; HDL-C= high density lipoprotein cholesterol; BMI=body mass index; WC=waist circumference; FPG=fasting plasma glucose; SBP=systolic blood pressure; DBP=diastolic blood pressure.

In Table 4.22 the RFs for MS and T2DM were compared in the same way as for the men. After adjusting for HIV status and level of urbanisation statistically significant increases were found for mean serum TG, BMI, FPG and SBP with an increase in HbA1c. Significant decreases were observed for mean serum HDL-C with an increase in HbA1c. There was a significant increase in mean FPG with an increase in mean HbA1c for the women in this population. The FPG in the 4th

quartile (>5.9%) was reported as 6.29 mmol/L. Table 4.23 represents liver enzymes in the different FPG groups of men.

Table 4.23: Liver enzymes in different HbA1c quartiles of men

Liver enzymes	Q1 (n = 173) <5.2%	Q2 (n = 117) 5.2-5.5%	Q3 (n = 129) 5.5-5.7%	Q4 (n = 198) >5.7%
ALT (10-55U/L)*	28.75 (25.76; 31.74)	24.57 (20.89; 28.25)	**23.71 (20.26; 27.16) [a]**	25.48 (22.68; 28.27)
ALP (45-115U/L)*	137.27 (128.78; 145.75)	135.44 (125.0; 145.89)	132.25 (122.46; 142.04)	136.67 (128.75; 144.60)
AST (10-40IU/L)*	50.67 (45.36; 55.99)	44.71 (38.17; 51.25)	**39.34 (33.21; 45.47) [a]**	**38.41 (33.45; 43.38)[a]**
LDH (≤270U/L)*	252.93 (237.20; 268.67)	231.33 (211.97; 250.69)	245.12 (226.97; 263.27)	246.75 (232.06; 261.44)
GGT (≤65U/L)*	176.61 (141.1; 212.12)	**119.18 (75.48; 162.88) [a]**	**99.38 (58.42; 140.34) [a]**	**103.36 (70.21; 136.51) [a]**

*Normal values according to www.aidsinfonet.org.
Superscript letters indicate significant differences in quartiles (P<0.05).: [a] vs quartile 1;[b] vs quartile 2; [c] vs quartile 3. P-value derived from ANOVA. AST = aspartate aminotransferase; ALP = alkaline phosphatase; ALT = alanine transaminase; LDH = lactate dehydrogenase; GGT = gamma-glutamyl transferase.
Values highlighted in red=values higher than recommended values

Table 4.23 indicates that GGT was significantly lower in the 2nd, 3rd and 4th quartile when compared with the 1st quartile. ALP and GGT levels were above recommended ranges. Table 4.24 represents liver enzymes in the different HbA1c groups of women.

Table 4.24: Liver enzymes in different HbA1c quartiles of women

Liver enzymes	Q1 (n = 288) <5.3%	Q2 (n = 159) 5.3-5.6%	Q3 (n = 263) 5.6-5.9%	Q4 (n = 281) >5.9%
ALT (7-30U/L)*	20.85 (19.21; 22.49)	20.22 (18.04; 22.40)	**18.43 [a] (16.73; 20.12)**	19.60 (17.94; 21.25)
ALP (30-100U/L)*	132.08 (126.44; 137.73)	127.95 (120.44; 135.45)	124.72 (118.87; 130.56)	**133.55 [c] (127.85; 139.45)**
AST (9-25IU/L)*	39.57 (35.89; 43.25)	36.41 (31.52; 41.30)	**30.87 [a] (27.07; 34.68)**	**27.50 [ab] (23.79; 31.21)**
LDH (≤270U/L)*	253.53 (240.80; 266.26)	245.80 (228.89; 262.72)	259.94 (246.78; 273.11)	253.70 (240.86; 266.53)
GGT (≤45U/L)*	107.18 (93.45; 120.91)	**74.84 [a] (56.59; 93.10)**	**60.16 [a] (45.95; 74.37)**	**61.21 [a] (47.36; 75.06)**

*Normal values according to www.aidsinfonet.org.
Superscript letters indicate significant differences between quartiles (P<0.05).: [a] vs quartile 1;[b] vs quartile 2; [c] vs quartile 3. P-value derived from ANOVA. AST = aspartate aminotransferase; ALP = alkaline phosphatase; ALT = alanine transaminase; LDH = lactate dehydrogenase; GGT = gamma- glutamyl transferase.
Values highlighted in red=values higher than recommended values

From Table 4.24 it can be seen that GGT levels decreased with an increase in HbA1c levels. ALT levels in the 3rd quartile were significantly lower when compared with the 1st quartile. A significant

decrease in AST was also associated with an increase in HbA1c values. ALP levels were significantly higher in the 4th quartile when compared with the 3rd quartile. ALP, GGT and AST levels were higher than recommended ranges in all four quartiles, therefore indicating a risk of liver disease.

4.5. Which screening tool to use?

In the next section, the aim was to determine how many subjects were identified as diabetic or IGT by the three screening methods and what the percentage of overlap of subjects identified as normal, IGT and diabetic was by each method previously discussed i.e. OGTT, FPG and HbA1c were. Figure 4.1 represents the prevalence of possible cases of DM as identified by the three screening methods. (OGTT=>11.1 mmol/L; FPG=>7.0mmol/L and HbA1c >6.5%).

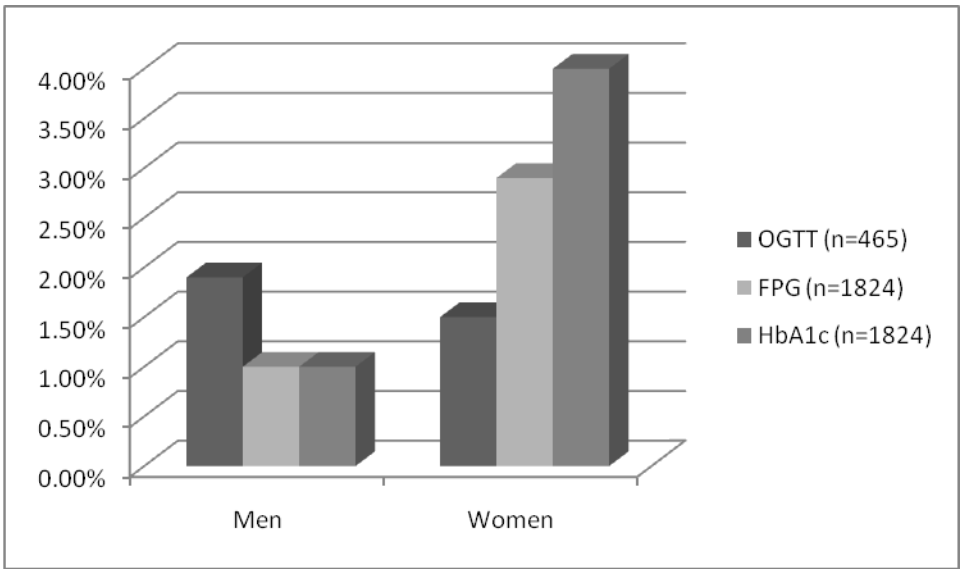

Fig 4.1: Prevalence of possible cases of DM

In Figure 4.1 the prevalence of possible cases of DM for men is shown, namely 1.9%, 1% and 1% according to the OGTT, FPG and HbA1c results, respectively, and the prevalence of possible cases of DM for women was 1.5%, 2.9% and 4% for OGTT, FPG and HbA1c, respectively. Sixteen (3.4%) subjects were identified as possibly having diabetes according to OGTT, 72 (3.9%) according to FPG and 92 (5%) according to HbA1c. Figure 4.2 represents the prevalence of IGT as diagnosed by the three screening methods (OGTT=7.8-11.1 mmol/L; FPG=5.5-7.0mmol/L and HbA1c =6%).

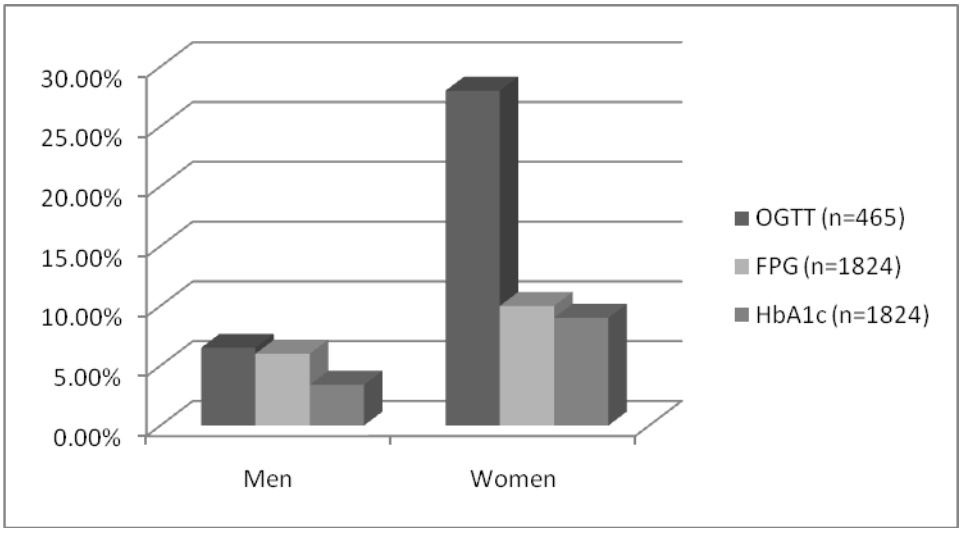

Fig 4.2: Prevalence of IGT

In Figure 4.2 it is shown that the prevalence of IGT for men was 6.5%, 6% and 3.4% according to the OGTT, FPG and HbA1c results, respectively and the prevalence of IGT for women was 28%, 10% and 9% for OGTT, FPG and HbA1c, respectively. One hundred and sixty three (35%) subjects were identified as having IGT according to OGTT, 293 (16%) according to FPG and 225 (12.3%) according to HbA1c. Figure 4.3 represents the men identified as possible diabetics by different screening methods.

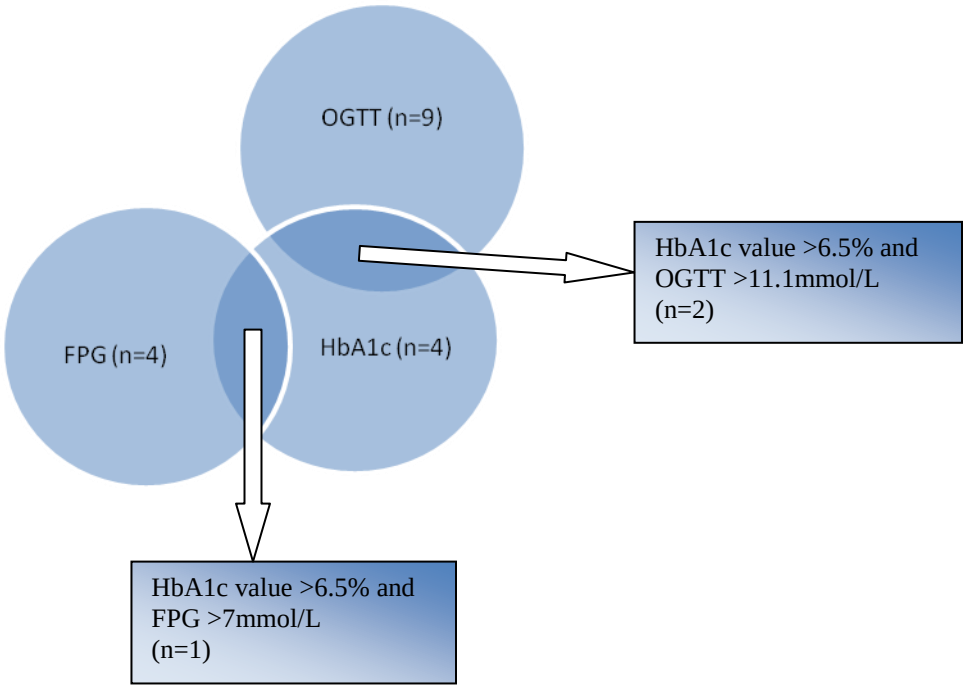

Figure 4.3: Men identified as possible diabetics by different screening methods
(see Addendum 6, Tables 1, 2 and 3)

From Figure 4.3 it can be seen that male subjects identified as possible diabetics by OGTT, FPG and HbA1c were nine, four and four respectively. There were two subjects identified as diabetics according to the OGTT who had an HbA1c value >6.5%. Only one subject in the OGTT group had a FPG >7mmol/L and HbA1c >6.5% and none of the men identified as being diabetic according to the OGTT had a FPG higher than 7.0 mmol/L. Figure 4.4 represents the women identified as possible diabetics by the different screening methods.

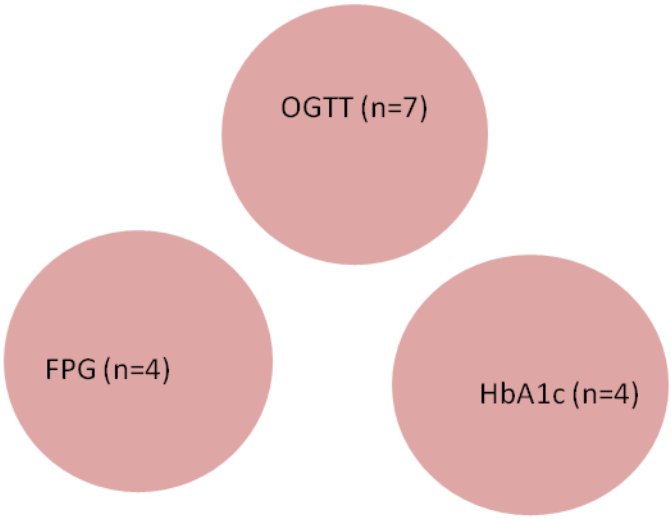

Figure 4.4: Women identified as possible diabetics by different screening methods
(see Addendum 6, Table 1, 2 and 3)

Figure 4.4 indicates that none of the women in the OGTT group had HbA1c levels higher than 6.5% and FPG levels higher than 7 mmol/L. Figure 4.5 represents the number of men detected with having a risk of having T2DM by making use of FPG and HbA1c in combination (in the total group).

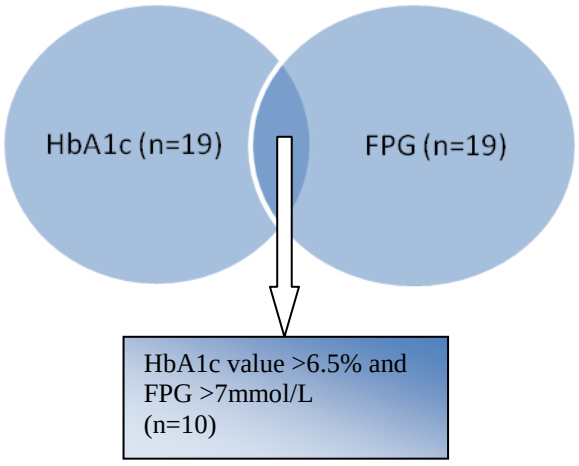

Figure 4.5: HbA1c vs FPG (men) in total group (see Addendum 6, Table 4)

Figure 4.5 indicates that ten of the men had FPG values >7mmol/L and HbA1c levels >6.5%. In Figure 4.6 the aim was to determine how many women could be detected having a risk of having T2DM by making use of FPG and HbA1c in combination (in the total group).

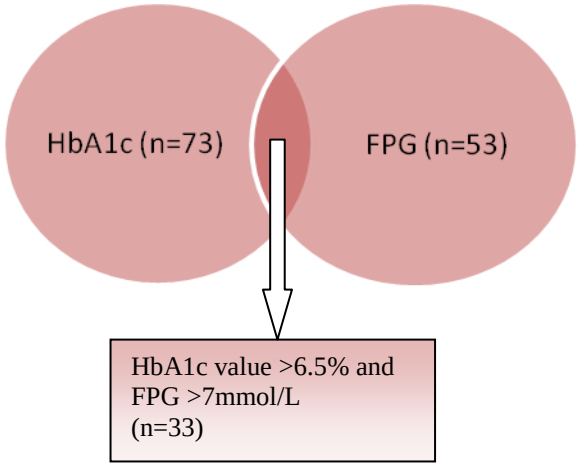

Figure 4.6: HbA1c vs FPG (women) in total group (see Addendum 6, Table 4)

In Figure 4.6 it is indicated that 33 of the women had FPG values >7.0 mmol/L and HbA1c >6.5%.

4.6. Clustering of RFs

In Table 4.25 subjects with MS were identified by making use of the IDF criteria and HbA1c values were compared between groups who had MS and groups who did not have MS.

Table 4.25: Identifying subjects with MS and comparing HbA1c values (Mean; 95%CI)

	WC0RF0 N=312 Mean (95%CI)	**WC1RF0 N=71 Mean (95%CI)**	**WC1RF1 N=259 Mean (95%CI)**	**WC1RF2 N=208 Mean (95%CI)**	**W1RF3+ N=148 Mean (95%CI)**
HbA1c (%)	5.40 (5.35; 5.44)	5.64 (5.49; 5.78)	5.67 (5.60; 5.75)	5.89 (5.73; 6.06)	6.65 (6.33; 6.97)

WC=waist circumference; RF=risk factor; N=number of subjects; WC0=normal waist circumference; WC1=Waist circumference > 94cm for men and >80cm for women; RF0=no risk factors; RF1=one risk factor; RF2=two risk factors; RF3+=more than 3 risk factors.

In Table 4.25 subjects were divided into MS groups, indicating how many of the subjects presented with MS according to the IDF criteria (Alberti *et al.*, 2006). It was determined that 356 subjects

already presented with MS. The mean HbA1c value for the subjects that had high WC and two other RFs, was reported as 5.89% and the mean HbA1c value for the subjects that presented with high WC and three or more risk factors was 6.65%.

The main findings from this study were that the TC, TG, BMI and FPG increased significantly and HDL-C decreased significantly with an increase in HbA1c values in men and women. In addition, SBP increased significantly in women. Thus, with an increase in HbA1c, an increase in the number of RFs was observed. HbA1c values increased significantly progressively from the normal FPG groups to groups with IFG and diabetic FPG groups for both men and women. No significant increases were reported in HbA1c between the OGTT groups. The above mentioned results will be discussed in Chapter 5.

CHAPTER 5 :

Conclusion and recommendations

5.1. Introduction

Early detection of DM is beneficial to individuals as well as to the health system (Colagiuri & Davies, 2009), therefore it is important to find an effective screening tool for the early detection of T2DM. Early detection and standard therapy could reduce all cause and CVD mortality by 3.5% and 7.1%, respectively, whereas early detection and intensive therapy could reduce all cause mortality by 5.9% and CVD mortality by 8.6% (Colagiuri & Davies, 2009). Early detection of subjects with IGT and IFG provides the opportunity to implement interventions in order to decrease the risk of developing T2DM in future life (Colagiuri & Davies, 2009). In this study we explored the use of two possible screening tools, namely FPG and HbA1c and fitted their outcomes against the OGTT, which is currently considered to be the "gold standard" for diagnosis of diabetes. In this study the aim was to investigate the possibility of HbA1c being a better or more stable screening tool for T2DM than FPG in this population.

5.2. Description of the total population (men=749; women=1261)

5.2.1. Dietary intakes

The macronutrient intakes of the subjects in this study were within expected ranges as defined by Vorster and Nell (2001). CHO and protein intakes of the women were significantly lower when compared to the men (Table 4.3). It was determined that men tended to consume more alcohol than women (Table 4.1). Pisa *et al.* (2010) reported that the mean alcohol intakes for men and women from the NWP were 29.9 g/d and 23.3 g/d respectively. These authors also concluded that more men tended to consume alcohol compared to the women (two thirds of men vs one third of women). Results in this study were consistent with the findings in the study done by Pisa *et al*. (2010). Setlalentoa *et al.* (2010) state that alcohol plays a major role in the lives of South Africans in the sense that it does not only have direct and indirect effects on health and nutrition, but also plays a role in the social and economic aspects of the community. Availability in terms of location, time and affordability is one of the causes of alcohol abuse and misuse in these areas (Setlalentoa *et al.,* 2010).

5.2.2. Liver enzymes

ALP and GGT levels were higher than the recommended values for men and women, indicating that most of the men and women from this study might have had liver abnormalities (Aragon & Younossi, 2010). The women from this study had significantly lower AST, ALP, ALT and GGT

levels, when compared with the men (Table 4.12). This finding could be ascribed to the fact that men ingested more alcohol than women, therefore had higher liver enzymes (Table 4.1). The values used in this study, were for individuals from the US and need to be adapted for the African population

5.2.3. Risk factors (RFs)s for MS

RFs for MS were compared between men and women (Table 4.12). The reason for looking at these RFs was that MS increases the risk of developing T2DM. From the results of this study it can be concluded that the subjects had a mean age of 49 years, thus this was an older cohort (Table 4.1) and therefore in this study, elevated RFs for T2DM were expected. (Votey & Peters, 2007). Serum TC levels (Table 4.12) were considered high for men (4.81mmol/L) and women (5.15mmol/L). TG and HDL-C levels were within normal ranges for men and women. Normal HDL-C and TG levels are associated with normal FPG (Quin *et al.,* 2010) levels and these are reflected in the men and women from this study. The women in this study were considered overweight when considering the mean BMI (Table 4.1).

5.2.4. Family history (FH) of T2DM

Leahy (2005) stated that T2DM is a renowned genetic disease due to the fact that it occurs in families and that there are ethnic populations that are at higher risk of developing this disorder. When looking at the FH of DM, it was determined that 3.4% of the subjects had fathers who had diabetes, 7.6% had mothers who had DM and 5.9% had a sibling with DM (Table 4.1). It is important to mention that underreporting might have occurred due to the fact that 1.2-2.1% did not answer and 8.1%-16.3% mentioned unknown and therefore, the total percentage could be much higher.

5.3. Dietary intakes and the development of T2DM

According to Xu *et al.* (2007), a number of factors, including diet, may affect glyceamic control, therefore the macronutrient intakes of this population were examined in order to determine the effect on risk for developing T2DM. The macronutrient ranges of the subjects in this study agreed with those outlined by Vorster *et al.* (1999) i.e. diets followed in rural areas are low in fat, low in sugar, present with low variety, monotonous and consist mainly of staple foods, whereas the affluent Western diet is high in fat, high in sugar, high in animal protein, low in complex CHO and is diverse

and varied. No differences were seen in macronutrient intakes in OGTT groups for men and women. However, with an increase in OGGT, FPG, and HbA1c there were increases in mean total energy intakes for men and women, except for the women in Q4 of the HbA1c group, even though these increases were not significant after adjustment for HIV and urbanisation. High fat intakes were accompanied by increases in FPG values for men and women. It was also determined that macronutrient intakes did not have an effect on HbA1c. From the results it can be concluded that dietary intakes may have an effect on the development of T2DM, considering that the higher energy intakes have an effect on BMI and other RF.

5.4 Screening tools

According to OGTT results in Chapter 4, 3.4% (1.9% of the men and 1.5% of the women) of subjects were identified as having diabetes. According to the DOH (2003), the prevalence of DM in the NWP was 1.5% for men and 1.8% for women. Compared to Figure 2.1, the prevalence of DM (using WHO and ADA criteria) reported by Levitt *et al.* (2000), was 1,7% in Cape Town, 2.2% in Mamre, 2.7% in Manguang, 1.8% in Qwa Qwa and 2.1% in Durban. In order to compare screening tools, a sub-sample of 465 subjects were divided into three OGTT groups, as defined by Farmer (2010). BG cut-points were based on the 2-hour BG levels of the OGTT. Thereafter, the whole population was divided into three FPG groups, as defined by Norman (2010) as well as HbA1c quartiles. RFs were compared between these groups in order to determine which screening method is the best for detecting undiagnosed T2DM in this population. These results are explained in the next section of this chapter.

5.4.1 OGTT as "gold standard" for the diagnosis of T2DM

OGTT was used in a sub-sample of 465 out of 2010 to screen subjects with T2DM in the study population. According to Cox and Edelman (2009), the OGTT is considered to be the "gold standard" for diagnosis of DM, due to its longstanding use. Out of 465 subjects in this subgroup, 3.4% presented with DM. When comparing RFs for T2DM in the different OGTT groups, where we expected to see an increase in TG and a decrease in HDL-C thus resulting in IGT and an increase in BMI and WC, resulting in increased IR and FPG, no significant differences in RFs between these groups were seen, indicating that OGTT is not the best screening method for this population [Table 4.13(men) and Table 4.14(women)]. From this study it was seen that OGTT did not seem to be effective or sensitive for early detection of T2DM in this population. This could however be due to

the fact that the groups were small and under-powered, therefore no differences were observed between these groups.

Due to high alcohol intakes, high liver enzymes were observed, which increase these subjects' risk of developing liver abnormalities. According to Harris (2005), individuals with T2DM have a higher incidence of LFT abnormalities when compared to subjects who do not have T2DM. GGT is also known to rise in subjects with T2DM (Harris, 2002). These results were consistent with the findings in this study. According to O'Keefe *et al.* (2007), light to moderate alcohol consumption is associated with a reduction in the prevalence and incidence of DM, whereas high alcohol intakes increase the risk of CHD and all-cause mortality, therefore DM in this study might have been alcohol induced, accordingly, alcohol consumption might have played a role in the development of T2DM in this population. In depth investigation is needed in order to confirm this.

5.4.2. FPG as possible screening tool for T2DM

FPG was explored as a diagnostic and screening tool, because of the fact that it is easy, inexpensive and relatively risk free (Norman, 2010). From this study it was seen that a higher FPG value (≥7mmol/L) is associated with an increase in DM RFs (BMI, blood lipids, anthropometric measurements and HbA1c values). Diagnosis of the MS starts with IFG and therefore FPG is useful in detecting subjects with MS who are at increased risk of developing T2DM. FPG can be used as a screening tool for T2DM in this population.

5.4.3. HbA1c as possible screening tool for T2DM

According to Saudek *et al.* (2008), there are a series of practical considerations that favour the use of the more expensive HbA1c test (than FPG) in screening for DM. Measuring HbA1c in patients with DM is an established procedure for evaluating long-term control of DM (Schnedl *et al.*, 2000; Sacks, 2007). According to Nakagami *et al.* (2007), the use of HbA1c as a screening tool for T2DM seems to be as efficient as measuring FPG. Khaw *et al.* (2004) state that HbA1c significantly predicts all-cause mortality, coronary and CVD even below the threshold value commonly accepted for diagnosing DM. However, the use of HbA1c as diagnostic tool for T2DM is still under debate (Motta *et al*, 2009), therefore, in this study we aimed to determine whether HbA1c is a more effective test than FPG in screening for undiagnosed DM in this African population.

According to Saudek *et al.* (2008), an HbA1c value of 6.5% or greater should be accepted as a criterion for diagnosing DM. The HbA1c values for subjects in the diabetic FPG group are values expected for diabetic subjects (7.46% for men and 8.08% for women). Thus, higher HbA1c values are associated with higher FPG values and therefore associated with the development of T2DM. It was also seen in this study that an increase in HbA1c levels is associated with an increase in DM RFs (TG, HDL-C, BMI and FPG), therefore indicating that HbA1c can be used to identify subjects who have MS and are therefore at an increased risk of developing T2DM. These results were consistent with the findings in the studies done by Rohlfing *et al.* (2000); Perry *et al.* (2001); Drougamaguet *et al.* (2006); Nakagami *et al.*(2007); Ginde *et al.* (2008); Borg *et al.* (2010); Mostafa *et al.* (2010) and Nakagami *et al.* (2010), which also found HbA1c to be the preferred* screening tool for detecting undiagnosed DM. All these studies were done in developed countries whilst the results of the study reported here are, as far as we know, the first in a developing country.

5.5. Combining screening tools

Tekumit *et al.* (2009) and Manley *et al.* (2009) also found that using HbA1c and FPG in combination is useful for detecting individuals who pose a risk of developing DM. In this study we found that when using FPG, HbA1c and OGTT in combination as detection criteria for T2DM in the sub-sample of 465 subjects, none of the subjects were determined to have T2DM, whereas one diabetic subject was detected when using FPG and HbA1c in combination and two subjects were detected when using OGTT and HbA1c in combination. However, five men and 26 women were determined to have IGT when using OGTT and FPG in combination; three men and 22 women were detected as having IGT when using OGTT and HbA1c in combination and eight women were detected as having IGT when using FPG and HbA1c in combination in the OGTT group, therefore OGTT and FPG in combination detected more individuals with an increased risk of IGT and therefore an increased risk of developing T2DM (see Addendum 6, Tables 1, 2 and 3). However, when using FPG and HbA1c in the whole population, 43 subjects were detected with a risk of developing DM. Accordingly, we can say that FPG and HbA1c in combination is a better screening method for detecting subjects who are at an increased risk of developing T2DM when compared to only using HbA1c or FPG. These results were consistent with the results from previous studies (Tekumit *et al.*, 2009; Manley *et al.*, 2009).

5.6. Clustering of risk factors

When making use of the IDF criteria for MS (Alberti *et al.*, 2006), 356 subjects were diagnosed as having MS. Therefore, these subjects are at an increased risk of developing T2DM or might already have T2DM, due to the fact that MS is a RFs for the development of T2DM (Holt, 2004). The HbA1c value in the subjects who had high WC and another two MS RFs was 5.89% and the HbA1c value for the subjects with high WC and more than three RFs was 6.65%. It was also seen that HbA1c increased in subjects with MS when compared with subjects who do not have MS. A high HbA1c value is associated with MS, therefore associated with the risk of developing T2DM.

5.7. Conclusion and recommendations

In conclusion, total energy intakes might have had an effect on the development of T2DM in this population, therefore, dietary adjustments should be made in order to prevent the development of obesity and T2DM. HbA1c and FPG in combination can be used to detect subjects with an increased risk of developing T2DM. HbA1c and FPG, however, have not been proven to be the best diagnostic tools for DM in this population. By detecting subjects with a risk of developing T2DM, the development of T2DM could be prevented with early treatment interventions.

5.8. Limitations of this study

According to the literature, bias can occur at any stage of the research process. It can arise due to measurement error, errors in reporting, flaws in the study design (sampling) or prejudice in reporting findings (Nelson *et al.*, 2004). "Selection bias" and "information bias" are the two major types of bias that can affect the validity of a study (Margetts & Nelson, 1998). In the PURE study, bias may have occurred in the selection of the subjects. Even though stratified sampling techniques were used to recruit the subjects, participation was still based on volunteerism.

Some of the subjects regarded taking something to drink or a small snack or meal as having not eaten and therefore not all the subjects might have been in an 8-10 hour fasted state. To overcome this, the fieldworkers were carefully instructed to explain to the participants what is meant by a "fasted state" and at enrolment of the participants the time of the last consumption of the last drink was documented. All the subjects allocated to the OGTT group were "truly" fasted, as verified by their FBG measurements. One of the advantages of the HbA1c measurement is that the participant does not have to be in a fasting state.

Although 2010 subjects participated in the PURE study, only 1 739 subjects had complete data sets according to Tables 4.4. When comparing dietary intakes, only 1 731 subjects had complete data sets. Only 1 746 subjects had complete data sets according to Tables 4.8 and 4.9 and only 1925 subjects had complete data sets according to Tables 4.10 and 4.11. When comparing RFs in FPG groups only 1746 subjects had complete data sets and when comparing RFs in HbA1c quartiles, only 1 608 subjects had complete data sets. Furthermore, only a sub-sample of the PURE individuals underwent an OGTT and therefore, only 465 subjects had complete data sets according to Tables 4.6, 4.7, 4.13 and 4.14. To overcome this all, statistics performed were suitable for nonparametric data. Furthermore, in this study, there were only 16 subjects diagnosed with DM according to the OGTT (considered "gold standard"), therefore the sample may not have had sufficient power.

5.9 Future research

Further research is needed in larger groups in order to determine whether HbA1c can be used for diagnosis of T2DM. ROC analysis should be done in future studies in order to determine the cut-point for HbA1c in this population, for in this study it could not be done, due to the small sample size of diabetic subjects. Furthermore, analysis of HbA1c has been simplified by a small portable instrument, similar to a glucometer, produced by Bayer Health Care, namely A1CNow, which requires a small (5 uL) blood sample, is easy to use and, according to the manufacturers, laboratory accurate. Verification of this claim should be made in a future study before the instrument can be used in field studies.

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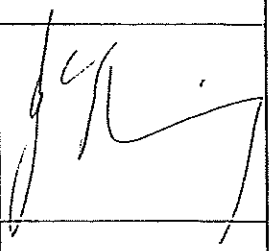
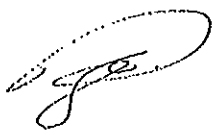
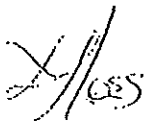
Baseline data gathering

Data	Researcher responsible for this data	E-mail address	Signature
Blood samples Lab work OGTT	Prof Johann Jerling	Johann.Jerling@nwu.ac.za	
Data entry- and manager All questionnaires	Prof Annamarie Kruger	Annamarie.Kruger@nwu.ac.za	
Blood pressure	Prof Alta Schutte	Alta.Schutte@nwu.ac.za	
Anthropometry	Dr Hanlie Moss	Hanlie.Moss@nwu.ac.za	

PURE/South Africa

To be completed by a knowledgable household member

We are very grateful to you for your participation in this study. All information given by you will be held in strict confidence, and will be used for the purpose of this study only after removing any personal identifying information.

Household Questionnaire

INSTRUCTIONS

Please answer EACH question by marking an X in ONE BOX on each line: (unless otherwise instructed)

[X]

OR

By writing number(s) in the spaces provided:

[1] [8]

OR

By specifying the answer on the line(s) provided

Feb 16, 2005

Household ID

☐☐ Centre # ☐☐☐ Community # ☐☐☐ Household #

Subject Initials ☐☐☐ F M L

Today's date: ☐☐☐☐ year ☐☐ month ☐☐ day

Type of House

1. **Type of roof on the main house** (check one only)

☐ Thatch ☐ Tiles ☐ Reinforced concrete ☐ Slate ☐ Fibrocement sheets/carton sheets

☐ Galvanized iron sheets ☐ Asbestos sheets ☐ Other _____

2 a) **Does the house have electricity?** ☐ No ☐ Yes

b) **Primary fuel used for cooking** (check one only)

☐ Kerosene ☐ Charcoal ☐ Coal ☐ Gas ☐ Wood ☐ Agriculture/crop

☐ Electricity ☐ Animal dung ☐ Shrub/grass ☐ Other _____

c) **Primary heating source during the cold/rainy season** (check one only)

☐ Coal open fire ☐ Wood open fire ☐ Gas furnace ☐ Portable heater

☐ None ☐ Electricity ☐ Other _____

Water Facilities

3. **Primary drinking water source** (check one only)

☐ Household well ☐ Community well ☐ Bore well ☐ Hand pump ☐ Collected rain water

☐ Artificial tank ☐ Natural lake ☐ River ☐ Piped water ☐ Bottled/packaged water

Labour and Time Saving Devices

4. **Does the household own any of the following?** (check all that apply)

☐ Moped/motorbike ☐ Car/jeep → # owned ☐ ☐ Other four-wheeler/tractor ☐ Bicycle

☐ Livestock cart ☐ Computer → # owned ☐ ☐ Washing machine ☐ Kitchen mixer

☐ Refrigerator ☐ TV → # owned ☐ ☐ Stereo/transistor/radio ☐ Telephone

Household ID

☐☐ Centre # ☐☐☐ Community # ☐☐☐ Household #

Subject Initials ☐☐☐ F M L

Household Income

5 a) **Current average monthly household income:** _____

b) **How much money is spent in one month on food for the entire household?** _____

6 a) **Does the household own any cultivable land?** ☐ No ☐ Yes → **(answer 6b, c)**

b) **How much cultivable land does the household own?:** ☐☐☐☐.☐ ☐ acres ☐ hectares ☐ Sq meters

8. **Name of Interviewer:** *(please print)* _____ *First Initial* _____ *Last Name* **Interviewer Code:** ☐☐☐

YUNIBESITI YA BOKONE-BOPHIRIMA
NORTH WEST UNIVERSITY
NOORDWES UNIVERSITEIT

POTCHEFSTROOM CAMPUS: FACULTY OF HEALTH SCIENCES

PURE-SA Project (Prospective Urban and Rural Epidemiology)

CHECK LIST

NAME……NUMBER……

DATE:……

WHAT DID YOU DRINK THIS MORNING:……

WHAT DID YOU EAT THIS MORNING……

STATION	INSTRUCTIONS	SIGNATURE
1. recruitment	1 HIV Counselling 2 Informed consent	M Watson…… A Kruger……..
2. Blood sample	OGGT : Y N Time of sample……. Problems experienced Y N Name the problem……..	Signature: ……..
3. Spirometry	Y N	
4. Hand grip	Y N	
5. Anthropometry	Weight:…….. Height……..	
6. ECG	Y N	
7. BP	Y N	
8. Urine sample	Y N	
9. Physical activity	Questionnaire Y N	
10. Referral letter	Y N	
11. check out		A Kruger……

PURE Quantitative Food Frequency Questionnaire

Subject ID

Centre #	Community #	Household #	Subject #
☐☐	☐☐☐	☐☐☐	☐☐

Subject Initials

F	M	L
☐	☐	☐

Today's date: ☐☐☐☐ (year) ☐☐ (month) ☐☐ (day)

1. Name: _____ _____

2. Not applicable in South Africa

3. National identity # or equivalent _____ **N/A** ☐

4. DOB: ☐☐☐☐ ☐☐ ☐☐ **OR** Age ☐☐ years

5. Sex: ☐ Female ☐ Male

Please think carefully about the food and drink you have consumed during the ***past month*** (four weeks). I will go through a list of foods and drinks with you and I would like you to tell me:

- If you eat the food
- How the food is prepared
- How much of the food you eat at a time
- How many times a day you eat it and if you do not eat it everyday, how many times a week or a month you eat it.

To help you to describe the amount of a food you eat, I will show you pictures of different amounts of the food. Please say which picture is the closest to the amount you eat, or if it is smaller, between the sizes or bigger than the pictures.

There are no right or wrong answers.

Everything you tell me is confidential. Only your subject number appears on the form.

Is there anything you want to ask now?

Are you willing to go on with the questions?

FOOD FREQUENCY QUESTIONNAIRE

INSTRUCTIONS: Circle the subject's answer. Fill in the amount and times eaten in the appropriate columns.

I shall now ask you about the type and the amount of food you have been eating in the last few months. Please tell if you eat the food, how much you eat and how often you eat it. We shall start with maize meal porridge.

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
PORRIDGE AND BREAKFAST CEREALS AND OTHER STARCH								
Maize-meal porridge	Stiff (pap)						3400	
Maize-meal porridge	Soft (slappap)						3399	
Maize-meal porridge	Crumbly (phutu)						3401	
Ting								
Mabella	Stiff						3437	
Mabella	Soft							
Oats							3239	
Other cooked porridge	Type: _____							
Breakfast cereals	Brand name of cereals at home now: _____ _____ _____ _____							
Do you pour milk on your porridge or cereal?		Yes 1		No 2				
If yes, what type of milk (whole fresh, sour, 1%, fat free, milk blend, etc) _____								
If yes, how much milk								

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Do you put sugar on your porridge or cereal?		Yes 1	No 2					
If yes, how much sugar							3989	
							3989	
							3989	
Samp	Bought Self ground						3250	
Samp and beans	Give ratio of samp:beans						3402 (1:1)	
Samp and peanuts	Give ratio of samp:peanuts						3250 (samp)	
Rice	White						3247	
	Brown						3315	
	Maize Rice						3250	
Pasta	Macaroni Spaghetti Other specify: _____ _____						3262	
Pizza	Home made: Specify topping _____ _____						3353 (base+ch)	
	Bought: Specify topping _____ _____						3353 (base+ch)	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		

You are being very helpful. Can I now ask you about meat?

CHICKEN, MEAT, FISH

How many times do you eat meat (beef, mutton, pork, chicken, fish) per week? _____

Chicken (codes with skin)	Boiled						2926	
	Fried: in batter/crumbs Eg Kentucky						3018	
	Fried: Not coated							
	Bought: Chicken Licken						2925	
	Bought: Nando's							
	Roasted / Grilled						2925	
	Other: _____							

Do you eat chicken skin? Always 1 Sometimes 2 Never 3

Chicken bones stew								
Chicken feet							2997	
Chicken offal								
Red meat	How do you like meat? With fat Fat trimmed							
Red meat	Fried							
	Stewed							
	Mince with tomato and onion						2987	
	Other:							
Beef Offal	Intestines: boiled nothing added						3003	
	Stewed with vegetables							
	Liver						2920	
	Kidney						2923	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Other: Specify _____ _____							
Goat meat	Boiled						4281	
	Stewed with vegetables							
	Grilled / Roasted						4281	
What type of vegetables is usually put into meat stews? _____								
Wors / Sausage							2931	
Bacon							2906	
Cold meats	Polony						2919	
	Ham						2967	
	Vienna						2936	
	Other: Specify _____ _____ _____							
Canned meat	Bully beef							
	Other: Specify _____ _____							
Meat pie	Beef						2939	
	Steak and kidney						2957	
	Cornish						2953	
	Chicken						2954	
	Other							
Hamburger	Bought							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Dried beans/peas/lentils	Soup						3145	
	Salad							
Soya products eg. Toppers	Brands at home now: _____ _____						3196 (Toppers)	
Pilchards in tomato/chilli/brine	Whole						3102	
	Mashed with fried onion							
Fried fish	With batter/crumbs							
	Without batter/crumbs							
Other canned fish	Tuna						3056 (oil)	
	Pickled fish							
	Other: Specify _____							
Fish cakes	Bought: Fried						3080	
	Home made with potato						3098	
Fish fingers	Bought						3081	
Eggs	Boiled/poached						2867	
	Scrambled: milk + fat							
	Fried: Fat							
Now we come to vegetables and fruit **VEGETABLES AND FRUIT**								
Cabbage	How do you cook cabbage?							
	Boiled, nothing added						3756	
	Boiled with potato and onion and fat							
	Fried, nothing added Fried in							
	Boiled, then fried with potato, onion							
	Other:							
	Don't know							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Spinach/morogo/ beetroot leaves other green leafy	How do you cook spinach?							
	Boiled, nothing added						3913	
	Boiled with fat added Type of fat							
	With onion, tomato, potato							
	With peanuts							
	Other:							
	Don't know							
Tomato and onion gravy	Home made with fat Type of fat							
	Without fat						3925	
	Canned						4192	
Pumpkin (yellow)	How do you cook pumpkin?							
	Boiled, nothing added						4164	
	Cooked in fat and sugar Fat							
	Boiled, little sugar and fat Fat							
	Other							
	Don't know							
Carrots	How do you cook carrots?							
	Boiled, nothing added						3757	
	Boiled, sugar and fat Fat							
	With potato and onion: Fat							
	Raw, salad						3709	
	Chakalaka							
	Other							
	Don't know							
Mealies/ Sweet corn	How do you eat mealies?							
	On cob – fat added Fat							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	On cob – no fat added						3725	
	Creamed sweet corn / canned						3726	
	Whole kernel/canned						3942	
Beetroot	Salad						3699	
	Boiled, nothing added						3698	
Potatoes	How do you cook potatoes?							
	Boiled/baked with skin						4155	
	Boiled/baked without skin						3737	
	Mashed							
	Roasted Fat							
	French fries (chips)						3740	
Sweet potatoes	How do you cook sweet potatoes?							
	Boiled/baked with skin						3748	
	Boiled/baked without skin						3903	
	Mashed							
	Other: _____							
	Don't know							
Salad vegetables	Mixed salad: tomato, lettuce and cucumber						3921	
	Raw tomato						3750	
	Other salad vegetables: _____ _____							
Other vegetables, specify + preparation	_____ _____ _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Do you like fruit?		Yes 1	No 2					
Apples							3592	
Pears							3582	
Oranges							3560	
Naartjie							3558	
Grapes							3550	
Peaches	Fresh						3565	
	Canned						3567	
Apricots	Fresh						3534	
	Canned						3535	
Mangoes							3556	
Guavas	Fresh						3551	
	Canned						3553	
Avocado							3656	
Wild fruit/berries	Specify type: _____							
Dried fruit	Types: _____							
Other fruit	_____ _____							
If subject eats canned fruit: Do you have custard with the canned fruit?		Yes 1	No 2					
Custard	Home made: Milk							
	Commercial eg Ultramel						2716	
BREAD AND BREAD SPREADS								
Bread / Bread rolls	White						3210	
	Brown						3211	
	Whole wheat						3212	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Do you spread anything on the bread?			Always 1	Sometimes 2	Never 3			
Margarine	What brand do you have at home now?							
	Don't know _____							
Peanut butter							3485	
Jam/syrup/honey							3985	
Marmite / Fray bentos / Oxo							4058	
Fish/meat paste							3109	
Cheese	Type: _____ _____ _____							
Achaar								
Other spreads	Specify: _____ _____							
Dumpling								
Vetkoek	White flour						3257	
	Whole wheat flour						3324	
Provita, crackers, etc							3235	
Mayonnaise / salad dressing	Mayonnaise						3488	
	Other: Specify _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
DRINKS								
Tea	English (normal)						4038	
	Rooibos						4054	
Coffee							4037	
Sugar/cup tea or coffee	Tea:						3989	
	Coffee:						3989	
Milk/cup tea or coffee	What type of milk do you use in tea and coffee?							
	Fresh/long life: whole/full						2718	
	Fresh/long life: 2%/low fat						2772	
	Fresh/long life: fat free						2775	
	Whole milk powder Brand: _____						2721 (powder)	
	Low fat milk powder Brand: _____						2825 (powder)	
	Skimmed milk powder Brand: _____						2825 (powder)	
	Milk blend Brand: _____						2770 (powder)	
	Whitener: type _____ _____							
	Condensed milk						2714	
	Evaporated milk						2715	
	None							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Milk as such	What type of milk do you drink milk as such?							
	Fresh/long life: whole/full						2718	
	Fresh/long life: 2%/low fat						2772	
	Fresh/long life: fat free						2775	
	Condensed milk						2714	
	Sour/maas						2787	
	Other: _____ _____							
Milk drinks	Nestle: _____							
	Milo: _____							
	Flavoured milk: _____							
	Other:							
Yoghurt	Drinking yoghurt						2756	
	Thick yoghurt						2734	
	Low fat sweetened with fruit						2732	
Squash	Sweet O						4027	
	Six O							
	Oros/Lecol – with sugar						3982	
	- artificially sweetener						3990	
	KoolAid						4027	
	Other: _____ _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Fruit juice	Fresh/Liquifruit/Ceres						2866	
	Tropica (Dairy –fruit juice mix)						2791	
	Other: _____ _____ _____							
Fizzy drinks Coke, fanta, etc	Sweetened						3981	
	Diet							
Maueu/Motogo							4056	
Home brew								
Tlokwe							4039	
Beer							4031	
Spirits							4035	
Wine red							4033	
Wine White							4033	
Other specify	_____ _____ _____							
SNACKS AND SWEETS								
Potato crisps							3417	
Peanuts	Raw						4285	
	Roasted						3458	
Cheese curls, Niknaks, etc							3267	
Raisins							3552	
Peanuts and raisins								

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Chocolates	Name: _____ _____ _____							
Candies	Sugus, gums, hard sweets, etc						4000	
Sweets	Toffees, fudge, caramels						3991	
Biscuits/cookies	Type: _____ _____ _____							
Cakes and tarts	Type: _____ _____ _____							
Scones								
Rusks	Type: _____ _____							
Savouries	Sausage rolls						2939	
	Samoosas: Meat filling						3355	
	Samoosas: Vegetable filling						3414	
	Biscuits eg bacon kips							
	Other specify: _____ _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Jelly							3983	
Baked pudding	Type: _____							
Instant pudding	Milk type: _____							
Ice cream							3483	
Sorbet							3491	
Other specify	_____ _____ _____							
SAUCES, GRAVIES AND CONDIMENTS								
Tomato sauce / Worcester sauce							3139	
Chutney							3168	
Pickles							3866	
Packet soups							3165	
Other:	_____ _____							
WILD BIRDS, ANIMALS OR INCECTS (hunted in rural areas or on farms)								
Wild fruit								

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
MISCELLANEOUS: Please mention any other foods used more than once/two times a week which we have talked about:								
INDIGENOUS/TRADITIONAL FOODS/PLANTS/ANIMALS Please tell me if you use any indigenous plants OR other indigenous foods like mopani worms, locusts ect to eat								
Specify								

Thank you very much for your cooperation and patience.

Good-bye!

PURE Salt intake questionnaire

Subject ID Initials

Centre # | Community # | Household # | Subject #

Subject

F M L

Today's date: year month day

1. Name: _____

_____

2. Not applicable in South Africa

3. National identity # or equivalent _____ ☐
N/A

4. DOB: ☐☐☐☐ ☐☐ ☐☐ **OR** **Age** ☐☐ **years**

5. Sex: ☐ Female ☐ Male

NUTRITIONAL AND LIFESTYLE HABITS The following questions are about your dietary and life-style habits. All your answers will be strictly confidential							***Office use***	
During the **PAST 7 days (1 week)** did you eat any of the following? IF YES, ASK HOW OFTEN (*if no, circle never*) [DO NOT PROMPT THE ANSWER OPTIONS BELOW]								
Food item	***NEVER***	NOT EVERY DAY		EVERY DAY				
		1-3 times per **week**	4-6 times per **week**	1 time a **day**	2 times a **day**	3+ times a **day**		
White bread/ white bread rolls	0	1	2	3	4	5		4
Brown/wholewheat bread/ Rolls	0	1	2	3	4	5		
Breakfast Cereal (processed)	0	1	2	3	4	5		
Breakfast Cereal (weetbix, muesli)	0	1	2	3	4	5		
Crackers (ProVita etc)	0	1	2	3	4	5		
Cookies, biscuits,rusks	0	1	2	3	4	5		
Cake/scone/ muffin/ puddings/pancake/fruit pie/koeksister	0	1	2	3	4	5		
Roti/ samoosa/springroll/doughnut	0	1	2	3	4	5		
Pizza	0	1	2	3	4	5		
Pasta/noodle dishes with cheese sauces (macaroni cheese, lasagne, noodle salad etc.)	0	1	2	3	4	5		
Popcorn	0	1	2	3	4	5		

NUTRITIONAL AND LIFESTYLE HABITS

The following questions are about your dietary and life-style habits. All your answers will be strictly confidential

Office use

During the **PAST 7 days (1 week)** did you eat any of the following? IF YES, ASK HOW OFTEN
(*if no, circle never*) [DO NOT PROMPT THE ANSWER OPTIONS BELOW]

Food item	***NEVER***	NOT EVERY DAY		EVERY DAY			
		1-3 times per **week**	4-6 times per **week**	1 time a **day**	2 times a **day**	3+ times a **day**	
Crisps (Simba and Niknaks etc.)	0	1	2	3	4	5	
Sausage (wors)	0	1	2	3	4	5	
Polony/salami/bacon/salami/pork suasages (processed meat, cooked, smoked and canned)	0	1	2	3	4	5	
Meat or chicken pies/sausage rolls	0	1	2	3	4	5	
Chicken - battered (KFC etc). and chicken burger only	0	1	2	3	4	5	
Meat and meat dishes (steaks, minced meat, cottage pie, mince, meatballs, stew, bobotie, etc.)	0	1	2	3	4	5	
Gravy, made with stock or gravy powder	0	1	2	3	4	5	
Biltong/dry wors/bokkems	0	1	2	3	4	5	
Milk (all types, also dairy fruit juice, malted milk, milk shakes)	0	1	2	3	4	5	
Maas	0	1	2	3	4	5	
Cheese	0	1	2	3	4	5	
Yoghurt	0	1	2	3	4	5	
Eggs	0	1	2	3	4	5	
Tinned fish (pilchards/tuna, etc.)	0	1	2	3	4	5	
Other fish and seafood	0	1	2	3	4	5	
Potato chips/french fries and potato salad	0	1	2	3	4	5	
Canned vegetables, incl. Baked beans, tomato paste, sweetcorn, etc.	0	1	2	3	4	5	
Soup (all types)	0	1	2	3	4	5	
Salad dressing/mayonnaise	0	1	2	3	4	5	
Ice cream (all types)	0	1	2	3	4	5	
Margarines, all types, also butter	0	1	2	3	4	5	
Chutney / atchar/chakalaka / Worcester sauce	0	1	2	3	4	5	
Savoury sauces (mushroom, monkey gland, white,cheese)	0	1	2	3	4	5	
Tomato sauce	0	1	2	3	4	5	
Salt	0	1	2	3	4	5	
Aromat / Fondor /mustard	0	1	2	3	4	5	
Peanuts	0	1	2	3	4	5	
Peanut butter	0	1	2	3	4	5	
Marmite/Bovril	0	1	2	3	4	5	
Chocolate sweets and sauce	0	1	2	3	4	5	
Beer and cider	0	1	2	3	4	5	

20

PURE 24-hour recall dietary intake

Subject ID

Centre #	Community #	Household #	Subject #

Subject Initials

F	M	L

Today's date: ____ ____ ____ (year) ____ (month) ____ (day)

1. Name: _____ _____

2. Not applicable in South Africa

3. National identity # or equivalent _____ **N/A** ☐

4. DOB: ____ ____ ____ **OR** **Age** ____ **years**

5. Sex: ☐ Female ☐ Male

6. What day was yesterday? (tick correct one)

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday

7. Would you describe the food that you ate yesterday as typical of your usual food intake?

Yes	1	No	2

Greetings!

Thank you for giving up your time to participate in this study. I hope you are enjoying it so far. Here we want to find out what people living in this are eat and drink. This information is important to know as it will tell us if people are eating enough and if they are healthy.

There are no right or wrong answers.

Everything you tell me is confidential. Only your subject number appears on the form.

Is there anything you want to ask now?

Are you willing to go on with the questions?

I want to first ask you a few general questions about your food intake, the preparation of food and the type of food that you use in your home.

Instruction
Circle the subject's answer.

8. What type of pot do you usually use to prepare food in? (may answer more than one)

Iron pot ..... ☐ 1

Stainless steel pot ..... ☐ 2

Aluminium pot ..... ☐ 3

Glass ware ..... ☐ 4

Other (specify) ..... ☐ 5

9. Do you eat maize meal porridge? Yes 1 No 2

If YES, what type do you have at home now?

Brand name: _____

Don't know: _____ 2

Grind self: _____ 3

If brand name is given, do you usually use this brand? Yes 1 No 2 Don't know 3

Where do you get your maize meal from? (may answer more than one)

Shop ..... ☐ 1

Employer ..... ☐ 2

Harvest and grind self ..... ☐ 3

Other (specify) ..... ☐ 4

Don't know ..... ☐ 5

10. Do you eat fat/margarine or use it in the preparation of food? Yes 1 No 2

If YES, what type do you have at home now?

Brand name: _____

Don't know: _____ 2

If brand name is given, do you usually use this brand? Yes 1 No 2 Don't know 3

11. Do you use oil in the preparation of food? Yes 1 No 2

If YES, what type do you have at home now?

Brand name: _____

Don't know: _____ 2

If brand name is given, do you usually use this brand? Yes 1 No 2 Don't know 3

What type of oil do you buy for deep frying?

Brand name: _____

Do you use the same oil more than once? Yes 1 No 2

If yes, how many times will you use the same oil? _____

12. What type of salt do you use?

Give brand names _____

Do you add salt to food while it is being cooked?

Always	Sometimes	Never	Don't know
1	2	3	4

Do you add salt to your food after it has been cooked?

Always	Sometimes	Never
1	2	3

Do you like salty foods eg salted peanuts, crisps, chips, fritos, biltong, dried sausage, etc

Very much	Like it	Not at all
1	2	3

13. Do you use any of the following:

	Name of product	Amount per day
Vitamins/vitamins and minerals		
Tonics		
Health foods		
Body building preparations		
Dietary fibre supplement		
Other: Specify _____ _____ _____		

I want to find out about everything you ate or drank yesterday, including water or food you pick from the veld. Please tell me everything you ate from the time you woke up yesterday up to the time you went to sleep. I will also ask you where you ate the food and how much you ate.

To help you to describe the amount of a food you eat, I will show you pictures and examples of different amounts of the food. Please say which picture or example is the closest to the amount you eat, or if it is smaller, between the sizes or bigger than the pictures.

Time	Place	Description of food and preparation method	Amount	Amount in gram	Code (offic use)

PURE/South Africa

We are very grateful to you for your participation in this study. All information given by you will be held in strict confidence, and will be used for the purpose of this study only after removing any personal identifying information.

Adult Questionnaire

INSTRUCTIONS

Please answer EACH question by marking an X in ONE BOX on each line: (unless otherwise instructed)

X

OR

By writing number(s) in the spaces provided:

1	8

OR

By specifying the answer on the line(s) provided

April 28, 2005

Adult Questionnaire

Subject Initials- F= first letter of first name
M= first letter of middle name
L= first letter of last name

3. National I.D#

If not applicable please mark the N/A box

Ethnicity Codes

01 **-** South Asian (India, Sri Lanka, Pakistan, Bangladesh)

02 **-** Chinese (China, Hong Kong, Taiwan)

03 - Japanese

04 - Malays

05 **-** Other Asian (Korea, Malaysia, Papua New Guinea, Thailand, Philippines, Indonesia, Nepal,Vietnam, Cambodia, Laos, Myanmar/Burma, Bhutan, Singapore)

06 **-** Persian

07 **-** Arab

08 **-** Black African

09 **-** Coloured African (Subsaharan African only)

10 **-** European

11 **-** Native North/South American or Australian Aborigine

12 **-** Latin American (Latino)

13 **-** Bantu/Semi Bantu

14 **-** Hemitic/Semi Hemitic

15 **-** Nilotic/Hausa

16 **-** Pygmie

17 **-** Swahili

18 **-** Other (any other ethnoracial group not listed above)

Subject ID

☐☐ ☐☐☐ ☐☐☐ ☐☐
Centre # *Community#* *Household #* *Subject #*

Subject Initials ☐☐☐
F M L

Today's date: ☐☐☐☐ ☐☐ ☐☐
year *month* *day*

1. Name: _____ _____
Given name *Surname*

2. Not applicable in South Africa

3. National identity # or equivalent: _____ **N/A** ☐

4. DOB: ☐☐☐☐ ☐☐ ☐☐ **OR** **Age** ☐☐ yrs
year *month* *day*

5. Sex: ☐ Female ☐ Male

6. Marital status: (check one only)

☐ Never married ☐ Currently married ☐ Common law/Living with partner
☐ Widowed ☐ Separated ☐ Divorced

7. Ethnicity: ☐☐ → (Please refer to facing page for codes)

8. Caste/Tribe: _____

9. What level of formal education have you <u>completed</u>? (check highest level only)**:**

☐ None
☐ Primary
☐ Secondary/highschool/higher secondary
☐ Trade School
☐ College/University
☐ Unknown

Adult Questionnaire

11. Occupation

Group 1: Legislators, senior officials and managers

Legislators and senior officials
Corporate managers
General managers
Businessman

Group 2: Professionals

Physical, mathematical and engineering science professionals
Life science and health professionals
Teaching professionals
Other professionals

Group 3: Technicians and associate professionals

Physical, mathematical and engineering-science associate professionals/technicians
Life science and health associate professionals/technicians
Teaching associate professionals/technicians
Other associate professionals/technicians

Group 4: Clerks

Clerks
Customer service clerks

Group 5: Service workers and shop and market sales workers

Personal and protective services workers
Models, salespersons and demonstrators

Group 6: Skilled agricultural and fishery workers

Market-oriented skilled agricultural and fishery workers
Subsistence agricultural and fishery workers

Group 7: Craft and related trade workers

Extraction and building trade workers
Metal, machinery and related trades workers
Precision, handicraft, printing and related trades workers
Other craft and related trades workers

Group 8: Plant and machine operators and assemblers

Stationary plant and related operators
Machine operators and assemblers
Drivers and mobile plant operators

Group 9: Elementary occupations

Sales and services elementary occupations
Agricultural, fishery and related labourers
Labourers in mining, construction, manufacturing and transport

Group 10: Armed forces

Armed forces

Group 11: Homemaker

Housewife/Househusband

Subject ID

☐☐ ☐☐☐ ☐☐☐ ☐☐

Centre # *Community#* *Household #* *Subject #*

Subject Initials ☐☐☐

F M L

10. Not applicable in South Africa

11a) Not applicable in South Africa

b) Please indicate which group best describes your main occupation.
(Please refer to facing page for definitions of groups and instruction manual for detailed definitions)

☐ **Group 1** ☐ **Group 2** ☐ **Group 3** ☐ **Group 4** ☐ **Group 5**
☐ **Group 6** ☐ **Group 7** ☐ **Group 8** ☐ **Group 9** ☐ **Group 10** ☐ **Group 11**

c) Not applicable in South Africa

d) What is your main source of income? _____

If occupation is group 11 (homemaker) go to question 13

12. Are you currently employed?

☐ No → **(answer 12a - 12b)** ☐ Yes → **Go to #13**

a) Are you retired/stopped work from your primary occupation due to old age? ☐ No ☐ Yes

b) Have you stopped working due to illness? ☐ No ☐ Yes

Subject ID

Centre #	Community#	Household #	Subject #

Subject Initials F M L

13. CURRENT DISABILITY:

		No	Yes
a)	Do you have any problems using your fingers to grasp or handle?	☐	☐
b)	Do you have any trouble walking about?	☐	☐
c)	Do you have any trouble bending down and picking up an object from the floor?	☐	☐
d)	Do you require a walking stick cane/walker to move about?	☐	☐
e)	Do you have any trouble reading or seeing the individual grains of rice/corn on your plate? (with glasses worn)	☐	☐
f)	Do you have trouble seeing a person from across the room? (12 feet/3.5 meters) (with glasses worn)	☐	☐
g)	Do you have trouble speaking and being understood?	☐	☐
h)	Do you have any trouble hearing what is said in a normal conversation?	☐	☐

Subject Medical History

14. Have you experienced any of the following in the last six months?

	No	Yes		No	Yes
a) Chest pain or tightness with usual activity	☐	☐	**i)** Vomiting	☐	☐
If Yes, → does the pain spread to the back, neck or inner border of arm	☐	☐	**j)** Loss of appetite	☐	☐
b) Breathlessness with usual activity	☐	☐	**k)** Painful or bleeding teeth/gums	☐	☐
c) Cough for at least 2 weeks	☐	☐	**l)** Jaundice	☐	☐
d) Any sputum while coughing	☐	☐	**m)** Burning while passing urine	☐	☐
e) Blood in sputum	☐	☐	**n)** Swelling of feet	☐	☐
f) Wheezing or whistling in the chest	☐	☐	**o)** Swelling of face	☐	☐
g) Early morning cough with chest tightness	☐	☐	**p)** Blood in urine	☐	☐
h) Loose stools/diarrhea for at least 3 days	☐	☐	**q)** Involuntary weight loss of > 3kg	☐	☐

15. Not applicable in South Africa

16 a) Do you use glasses/spectacles/contact lenses at present? No ☐ Yes ☐

b) Do you use a hearing aid? No ☐ Yes ☐

Adult Questionnaire

Cancer Sites

1= Mouth
2= Esophagus
3= Stomach
4= Small intestine
5= Large intestine including rectum
6= Pancreas
7= Liver
8= Lung
9= Breast
10= Cervical/uterine/ovarian
11= Prostate
12= Head and neck
13= Other, specify

Subject ID

☐☐ ☐☐☐ ☐☐☐ ☐☐
Centre # *Community#* *Household #* *Subject #*

Subject Initials ☐☐☐ *F M L*

17. Have you ever been diagnosed with any of the following?(check all that apply)

	No	Yes	#of yrs since diagnosis
a) Diabetes	☐	☐	☐☐
b) Hypertension/ high blood pressure	☐	☐	☐☐
c) Stroke	☐	☐	☐☐
d) Angina/heart attack/ Coronary artery disease	☐	☐	☐☐
e) Heart failure	☐	☐	☐☐
f) Other heart disease	☐	☐	☐☐
h) Hepatitis/Jaundice	☐	☐	☐☐
g) Cancer	☐	☐	☐☐

Please refer to facing page for cancer sites ☐☐ *site*

_____ *other, specify*

	No	Yes	#of yrs since diagnosis
i) COPD	☐	☐	☐☐
j) Asthma	☐	☐	☐☐
k) Tuberculosis	☐	☐	☐☐
l) Malaria	☐	☐	☐☐
m) Chagas	☐	☐	☐☐
n) HIV/AIDS	☐	☐	☐☐

Not answered ☐

18. Have you been taking any medications regularly (ie. at least once per week) in the last month? ☐ No → **go to 19** ☐ Yes

a) If yes, for what conditions:

	No	Yes
Blood pressure	☐	☐
Cholesterol lowering drugs	☐	☐
Stroke	☐	☐
Diabetes	☐	☐
Asthma	☐	☐
Chinese medicine	☐	☐
Others	☐	☐ → If Yes, specify _____
Unknown	☐	☐

Adult Questionnaire

18b) If name of medication is unknown, please list as unknown.

Subject ID

Centre #	Community#	Household #	Subject #
☐☐	☐☐☐	☐☐☐	☐☐

Subject Initials ☐☐☐ (*F M L*)

18b) List all the medications you are <u>currently</u> consuming at least once a week for the last month?

i) _____ ii) _____

iii) _____ iv) _____

v) _____ vi) _____

vii) _____ viii) _____

<u>Men go to question #23</u>

<u>For Women Only (Questions 19 - 22)</u>

19. Are you currently pregnant ? ☐ No ☐ Yes → **Go to #21**

20. Do you still have periods? ☐ No → **(answer 20a)** ☐ Yes → **Go to #21**

a) How many years since you stopped menstruating? ☐☐ years

21. Have you ever used an oral/ injectable contraceptive? ☐ No ☐ Yes

22a) How many live children have you given birth to? ☐☐ Boys ☐☐ Girls

b) Did you breast feed any of your children? ☐ No ☐ Yes

Adult Questionnaire

23. Accidents and Injuries

Location of Injury

1= Factory/industrial place
2= Office
3= Agriculture field/farm
4= Home
5= Road
6= Sport/game e.g. track, court, field, etc.
7= Public building
8= Mine/quarry
9= Construction site e.g. building, road-works, etc.
10 = Other

Type of Injury

1= Burns
2= Scalds
3= Fractures
4= Muscle and ligament sprains/tears
5= Cuts and lacerations
6= Bruises and abrasions
7= Suffocation
8= Head injury (where person did not lose consciousness)
9= Head injury (where person lost consciousness for some time)

Subject ID

Centre #	Community#	Household #	Subject #

Subject Initials F M L

23. During the past 12 months, have you had any injuries that were serious enough to limit your normal activities? (check all that apply)

☐ No → **Go to #24** ☐ Yes → **(answer 23a - 23s)**

If yes, please provide details:

Please refer to facing page for Location and Type Codes

Cause of injury			Location	Type	Absence from work or usual activities (Days)
a) Motor vechicle accident (as a passenger)	☐ No	☐ Yes →			
b) Motor vehicle accident (as a pedestrian)	☐ No	☐ Yes →			
c) Struck by an object	☐ No	☐ Yes →			
d) Explosion	☐ No	☐ Yes →			
e) Natural/environmental factors (gales/cyclones/lightning, etc.)	☐ No	☐ Yes →			
f) Suffocation	☐ No	☐ Yes →			
g) Poisoning	☐ No	☐ Yes →			
h) Snake/scorpion bite	☐ No	☐ Yes →			
i) Fall	☐ No	☐ Yes →			
j) Fire/flames, resultant fumes	☐ No	☐ Yes →			
k) Physical assault (gun, kidnapping, etc.)/violent crime	☐ No	☐ Yes →			
l) Domestic violence (beaten by a family member)	☐ No	☐ Yes →			
m) Drowning/submersion	☐ No	☐ Yes →			
n) Hot or corrosive liquids/floods/substances	☐ No	☐ Yes →			
o) Crush injuries (boulders, building materials, etc.)	☐ No	☐ Yes →			
p) Accident caused by machinery	☐ No	☐ Yes →			
q) Attempted suicide	☐ No	☐ Yes →			
r) Armed conflict	☐ No	☐ Yes →			
s) Other(specify) _____	☐ No	☐ Yes →			

Adult Questionnaire

Location of Fractures

1= Hip/pelvis
2= Thigh
3= Leg
4= Forearm
5= Wrist
6= Hand/finger
7= Vertebrae (back)
8= Other

Fractures: In situations where subjects are in a cast and cannot differentiate between ligament tear or fracture, include as fracture only if doctor confirmed it as a broken bone

25c) Tobacco: Regular use is defined as consuming at least one tobacco product per day.

Duration of use:

For those that have consumed tobacco for <1 year, please enter “0”

Subject ID

☐☐ *Centre #* ☐☐☐ *Community#* ☐☐☐ *Household #* ☐☐ *Subject #*

Subject Initials ☐☐☐ *F M L*

24. Have you ever fractured a bone? ☐ No (go to #25) ☐ Yes (if yes, answer a),b) and c)

a) Number of fractures ☐☐

b) Years since last fracture ☐☐ (yrs)

c) Bone (s) broken in the most recent fracture(if more than 3, list most severe sites) (location) If other, specify

☐ → _____

☐ → _____

☐ → _____

Please refer to facing page for fracture locations

Tobacco

25. Which best describes your history of tobacco use?

a) ☐ Formerly used tobacco products ☐ Currently use tobacco products ☐ **Never used tobacco products** → **Go to #26**

b) At what age did you start? ☐☐ yrs

c) Have you ever regularly used any of the following tobacco products? (check all that apply)

	Average amount/day		Past users only: Duration (years)	When Stopped (years ago)	If less than 1 yr (months ago)
(i) Cigarettes (all kinds)	☐☐☐	number	☐☐	☐☐	☐☐
(ii) Beedies	☐☐☐	number	☐☐	☐☐	☐☐
(iii) Cigars	☐☐☐	number	☐☐	☐☐	☐☐
(iv) Pipes	☐☐☐	number	☐☐	☐☐	☐☐
(v) Sheesha/water pipe Hookah	☐☐☐	# of times	☐☐	☐☐	☐☐
(vi) Chewing tobacco	☐☐☐	# of times	☐☐	☐☐	☐☐
(vii) Snuff	☐☐☐	# of times	☐☐	☐☐	☐☐
(x) Other _____ *Specify*	☐☐☐		☐☐	☐☐	☐☐

Subject ID

☐☐ ☐☐☐ ☐☐☐ ☐☐

Centre # *Community#* *Household #* *Subject #*

Subject Initials ☐☐☐

F M L

Question 26 to be answered by non-smokers and former smokers only

26. During the past 12 months, have you been regularly (at least once per week) exposed to other people's tobacco smoke?
("Exposed" is defined as a minimum of 5 consecutive minutes, during which you inhale other people's smoke.)

☐ No → **Go to #27** ☐ Yes → Please answer questions **26a**

a) Over the past 12 months, what has been your typical exposure to other peoples smoke?
("Exposed" is defined as a minimum of 5 consecutive minutes, during which you inhale other peoples smoke)

Select ONE only

☐ 1-2 times/week ☐ 3-6 times/week ☐ at least once a day ☐ 2-3 times/day ☐ 4 or more times/day

27. Not applicable in South Africa

Adult Questionnaire

28c) Alcoholic Beverage: Regular use is defined as at least once a month**.**

Subject ID

Centre #	Community#	Household #	Subject #
☐☐	☐☐☐	☐☐☐	☐☐

Subject Initials ☐☐☐ (*F M L*)

28. Which best describes your history of alcohol use?

a) ☐ Formerly used alcohol products ☐ Currently use alcohol products ☐ **Never used alcohol products** → **Go to #29**

b) At what age did you start? ☐☐ yrs

c) What forms of alcohol have you regularly used? (check all that apply)

Form of Alcohol	Approx. size of one "drink"	Frequency: Daily	Frequency: Weekly	Frequency: Monthly	Average # of drinks	Duration (years)	Past users only When Stopped (years ago)
(i) Spirits(rum,whisky, gin,vodka etc)	30ml	☐	☐	☐	☐☐	☐☐	☐☐
(ii) Wine	125ml	☐	☐	☐	☐☐	☐☐	☐☐
(vi) Beer	375ml	☐	☐	☐	☐☐	☐☐	☐☐
(vii) Country liquor/arrack/ sugar cane spirit	30ml	☐	☐	☐	☐☐	☐☐	☐☐

d) **At least once a month, do you consume >5 alcoholic drinks/day?** ☐ No → **Go to #29** ☐ Yes

If yes,(i,ii)

i) How many times per month do you consume >5 alcoholic drinks in a day? ☐☐

ii) What is the average number of drinks that you consume each time? ☐☐

29 a) During your longest or nocturnal sleep period, what time do you normally go to bed? ☐☐ : ☐☐ (00:00-23:59)

b) During your longest or nocturnal sleep period, what time do you normally wake up? ☐☐ : ☐☐ (00:00-23:59)

c) Do you usually take naps/siestas? ☐ No ☐ Yes → *Total nap duration* ☐☐☐ mins

33. **<u>Civic organization</u>:** are defined as non-profit, voluntary organization societies, self help groups and clubs.

<u>Religious organization</u>: are defined as different types of formal and informal groups set up on a religious basis.

Subject ID

☐☐ ☐☐☐ ☐☐☐ ☐☐

Centre # *Community#* *Household #* *Subject #*

Subject Initials ☐☐☐

F M L

30. Are you a member of any of the following:

How often do you participate in the activities of this group?

			Per Month	**OR**	**Per Year**
(i) Self help group, Co-operative, Social club, Sports club,	☐ No	☐ Yes →	☐☐		☐☐
(ii) Religious Group (e.g: church group, etc.)	☐ No	☐ Yes →	☐☐		☐☐
(iii) Other _____ *Specify*	☐ No	☐ Yes →	☐☐		☐☐

31. Please answer the following: (choose only one option for each)

	Strongly Disagree	**Somewhat Disagree**	**Somewhat Agree**	**Strongly Agree**
(i) People are generally honest and want to help others.	☐	☐	☐	☐
(ii) If I do nice things for someone, I can anticipate that they will respect me and treat me just as well as I treat them.	☐	☐	☐	☐

32a) The television, radio, newspaper or magazine advertisements help me decide to buy the type of: (choose only one option for each)

					Not Applicable
(i) Cooking oil	☐	☐	☐	☐	☐
(ii) Flour	☐	☐	☐	☐	☐
(iii) Rice/ Maize meal	☐	☐	☐	☐	☐

b) The television, radio, newspaper or magazine advertisements influence whether I buy: (choose only one option for each)

(i) Soft drinks	☐	☐	☐	☐	☐
(ii) Snacks	☐	☐	☐	☐	☐
(iii) Cigarettes	☐	☐	☐	☐	☐
(iv) Alcohol	☐	☐	☐	☐	☐

33. In a difficult situation, whose help can you count on from?(Please see facing page for definitions)

(i) Civic organizations: specify _____

☐ none ☐ little ☐ moderate/average ☐ a great deal

(ii) Religious organizations: specify _____

☐ none ☐ little ☐ moderate/average ☐ a great deal

Subject ID

Centre #	Community#	Household #	Subject #
☐☐	☐☐☐	☐☐☐	☐☐

Subject Initials ☐☐☐ (*F M L*)

34. Have you experienced any of the following events during the last 12 months?

	No response	No	Yes	
(i) Loss of job	☐	☐	☐	
(ii) Retirement	☐	☐	☐	
(iii) Loss of crop/business failure	☐	☐	☐	
(iv) Household break in	☐	☐	☐	
(v) Marital separation/divorce	☐	☐	☐	
(vi) Other major intra-family conflict	☐	☐	☐	→ Please specify _____
(vii) Major personal injury or illness	☐	☐	☐	
(viii) Violence	☐	☐	☐	
(ix) Armed conflict/war	☐	☐	☐	
(x) Death of a spouse	☐	☐	☐	
(xi) Death/major illness of another close family member	☐	☐	☐	
(xii) Other major stress	☐	☐	☐	→ Please specify _____
(xiii) Wedding of family member	☐	☐	☐	
(xiv) New job	☐	☐	☐	
(xv) Birth in the family	☐	☐	☐	
(xvi) Seperation from family	☐	☐	☐	
(xvii) Unavailability of food/ food insecurity	☐	☐	☐	

Subject ID

Centre #	Community#	Household #	Subject #
☐☐	☐☐☐	☐☐☐	☐☐

Subject Initials ☐☐☐ *F M L*

35. Please answer the following: (Choose only one option for each)

For the following question, stress is defined as feeling irritable or filled with anxiety, or as having sleeping difficulties as a result of conditions at work or at home.

	No response	Never Experienced Stress	Some Period of Stress	Several Periods of Stress	Permanent Stress
a) How often have you felt stress at work in the last 12 months? (Mark here if not applicable: i.e. no longer working ☐)	☐	☐	☐	☐	☐
b) How often have you felt stress at home in the last 12 months?	☐	☐	☐	☐	☐

36. What level of financial stress have you felt in the last 12 months?

☐ No response ☐ Little/none ☐ Moderate ☐ High/severe

37. During the past twelve months, was there ever a time when you felt sad, blue, or depressed for two weeks or more in a row?

☐ No ☐ Yes ⟶ **If yes, during those times, did you:**

	No response	No	Yes
a) Lose interest in most things like hobbies, work or activities that usually give you pleasure?	☐	☐	☐
b) Feel tired or low on energy?	☐	☐	☐
c) Gain or lose weight?	☐	☐	☐
d) Have more trouble falling asleep than you usually do?	☐	☐	☐
e) Have more trouble concentrating than usual?	☐	☐	☐
f) Think a lot about death (either your own, someone else's, or death in general)	☐	☐	☐
g) Feel down on yourself, no good or worthless?	☐	☐	☐

Subject ID

Centre #	Community#	Household #	Subject #
☐☐	☐☐☐	☐☐☐	☐☐

Subject Initials ☐☐☐ (*F M L*)

38. Please answer the following: (Choose only one option for each)

	Strongly Disagree	Somewhat Disagree	Somewhat Agree	Strongly Agree
a) I can do most of my regular shopping (food, household necessities, etc.) at stores within easy walking distance (less than 15 minutes) of my home.	☐	☐	☐	☐
b) Walking or bicycling in my neighbourhood is difficult because of the speed and/or amount of traffic.	☐	☐	☐	☐
c) My neighbourhood is generally free from pollution (litter, air pollution and noise pollution).	☐	☐	☐	☐
d) My neighbourhood streets are well lit at night.	☐	☐	☐	☐
e) I can see other people when I am walking in my neighbourhood.	☐	☐	☐	☐
f) I can speak to other people when I am walking in my neighbourhood.	☐	☐	☐	☐
g) There is a high crime rate in my neighbourhood.	☐	☐	☐	☐
h) There is a problem with unattended dogs in my neighbourhood.	☐	☐	☐	☐

Subject ID

Centre #	Community#	Household #	Subject #
☐☐	☐☐☐	☐☐☐	☐☐

Subject Initials ☐☐☐ (*F M L*)

38a) Please answer the following: (Please check all that apply)

i) Has your household been a victim of the following crime(s) in the last 12 months?

	No	Yes
1. Armed robbery	☐	☐
2. Violent attacks	☐	☐
3. Murder	☐	☐
4. Vehicle hijacking	☐	☐
5. House breaking	☐	☐
6. Theft	☐	☐
7. Rape	☐	☐
8. Women abuse eg. (beat,swear-words,sexual) please specify _____	☐	☐
9. Child abuse eg. (burn,swear-words,rejection) please specify _____	☐	☐
10. Child sexual abuse	☐	☐
11. Other, please specify _____	☐	☐

ii) Do you think that crime in your area has increased in the past 5 years? ☐ No ☐ Yes

if yes, which of the following crime(s)?

- ☐ Armed robbery
- ☐ Violent attacks
- ☐ Murder
- ☐ Vehicle hijacking
- ☐ House breaking
- ☐ Theft
- ☐ Rape
- ☐ Women abuse
- ☐ Child abuse
- ☐ Child sexual abuse
- ☐ Other, please specify _____

Subject ID

Centre #	Community#	Household #	Subject #
☐☐	☐☐☐	☐☐☐	☐☐

Subject Initials ☐☐☐ (*F M L*)

38b) Questions on HIV:

i) Do you know people who have HIV/AIDS? ☐ No ☐ Yes

if yes, which of these people: (please mark all that apply)

- ☐ Your children
- ☐ Your grandchildren
- ☐ Your spouse
- ☐ Your family members
- ☐ Your friends
- ☐ People in the community

ii) What would you consider the mean age of the people who are ill/have died of HIV/AIDS?

- ☐ Younger than 10 years ☐ Between 11-20 years ☐ Between 21-30 years
- ☐ Between 31-40 years ☐ Between 41-50 years ☐ Over 50 years

iii) If someone in your household is HIV positive, who is the primary caregiver?

- ☐ Spouse
- ☐ Parents
- ☐ Family member
- ☐ Child.children
- ☐ Friends
- ☐ Volunteer

38c) Do you care for any orphans in your family? ☐ No ☐ Yes

Adult Questionnaire

40b) Health History:

Cancer Sites

1= Mouth
2= Esophagus
3= Stomach
4= Small intestine
5= Large intestine including rectum
6= Pancreas
7= Liver
8= Lung
9= Breast
10= Cervical/uterine/ovarian
11= Prostate
12= Head and neck
13= Other, specify

Subject ID

Centre # | Community# | Household # | Subject #

Subject Initials F M L

39. How long would it take you to get from your house to the nearest facility <u>if you walked</u>?

	Minutes	Don't know		Minutes	Don't know
i) grocery/convenience store			iv) video store		
ii) bank			v) non-fast food restaurant		
iii) post office			vi) fast food restaurant		

40a) Total number of siblings

b) Health History: Complete for all parents and siblings, alive or dead

	Father			Mother			Siblings			# of siblings with the condition
	Unknown	No	Yes	Unknown	No	Yes	Unknown	No	Yes	If yes →
Diabetes										
Coronary Heart Disease										
High Blood Pressure										
Stroke										
Cancer (Please refer to facing page for cancer sites)										
if Yes, indicate site										
	Other, Specify			Other, Specify			Other, Specify			

Adult Questionnaire

If subject refuses to provide any of the measures, enter a value of “0” into each of the boxes for that question

For more detailed instructions please refer to the instruction manual

Subject ID

Centre # | Community# | Household # | Subject #

Subject Initials F M L

41. Physical Measurements

Sitting

a) Right arm blood pressure

#1 [ ][ ][ ] *Systolic* [ ][ ][ ] *Diastolic* **mmHg**

#2 [ ][ ][ ] *Systolic* [ ][ ][ ] *Diastolic* **mmHg**

b) Heart Rate

#1 [ ][ ][ ] **beats/min**

#2 [ ][ ][ ] **beats/min**

c) Waist

#1 [ ][ ][ ].[ ] **cm** → ☐ minimal/no clothing ☐ full clothing

#2 [ ][ ][ ].[ ] **cm**

d) Weight [ ][ ][ ].[ ] **kg** → ☐ minimal/no clothing ☐ full clothing

e) Hip

#1 [ ][ ][ ].[ ] **cm** → ☐ minimal/no clothing ☐ full clothing

#2 [ ][ ][ ].[ ] **cm**

f) Height [ ][ ][ ].[ ] **cm** (without shoes)

42a) Circumference of mid upper right arm: [ ][ ].[ ] **cm**

b) Circumference of right calf: [ ][ ].[ ] **cm**

c) Head Circumference: [ ][ ][ ].[ ] **cm**

d) Upper flexed arm circumference [ ][ ].[ ] **cm**

43a) Right arm triceps skinfold:

#1 [ ][ ][ ].[ ] **mm**

#2 [ ][ ][ ].[ ] **mm**

#3 [ ][ ][ ].[ ] **mm**

b) Right calf skinfold:

#1 [ ][ ][ ].[ ] **mm**

#2 [ ][ ][ ].[ ] **mm**

#3 [ ][ ][ ].[ ] **mm**

Subject ID

Centre #	Community#	Household #	Subject #

Subject Initials F M L

c) Biceps skinfold

#1 ___.___ mm
#2 ___.___ mm
#3 ___.___ mm

d) Subscapular skinfold

#1 ___.___ mm
#2 ___.___ mm
#3 ___.___ mm

e) Supra spinal skinfolds

#1 ___.___ mm
#2 ___.___ mm
#3 ___.___ mm

44 a) Humerous breadth ___.___ cm

b) Femur breadth ___.___ cm

45. Grip Strength (Maximal contraction):

a) Non-dominant hand:
#1 ___ kg.
#2 ___ kg.
#3 ___ kg.

b) Dominant hand:
#1 ___ kg.
#2 ___ kg.
#3 ___ kg.

Adult Questionnaire

If subject refuses to provide any of the measures, enter a value of “0” into each of the boxes for that question

For more detailed instructions please refer to the instruction manual

46. Spirometry:

American Thoracic Society criteria for acceptable spirograms:
Spirograms are acceptable if they are free from:

1. Cough during exhalation
2. Early termination or cut-off
3. Variable effort
4. Leaks
5. Obstructed mouth piece

Subject ID

☐☐ ☐☐☐ ☐☐☐ ☐☐

Centre # *Community#* *Household #* *Subject #*

Subject Initials ☐☐☐ *F M L*

46. Spirometry:

a) FEV1 (Litre): **#1** ☐.☐☐ **#2** ☐.☐☐ **#3** ☐.☐☐

b) Does FEV1 obtained meet ATS criteria?

☐ No → (answer (i) to (iii) ☐ Yes → Go to **c)**

Reasons for not meeting the ATS criteria: (check all that apply)

i) Cough ☐

ii) Values not within 0.2L of each other ☐

iii) Less than 3 values ☐

c) FVC (Litre): **#1** ☐.☐☐ **#2** ☐.☐☐ **#3** ☐.☐☐

d) Does FVC obtained meet ATS criteria?

☐ No → (answer (i) to (iii) ☐ Yes → Go to **e)**

Reasons for not meeting the ATS criteria: (check all that apply)

i) Cough ☐

ii) Values not within 0.2L of each other ☐

iii) Less than 3 values ☐

e) PEFR (Litre/min): **#1** ☐☐☐ **#2** ☐☐☐ **#3** ☐☐☐

f) Does PEFR obtained meet ATS criteria?

☐ No → (answer (i) to (ii) ☐ Yes → Go to **Q#47**

Reasons for not meeting the ATS criteria: (check all that apply)

i) Cough ☐

ii) Less than 3 values ☐

Subject ID

Centre #	Community#	Household #	Subject #

Subject Initials F M L

47. **Not applicable in South Africa**

48. **ECG obtained?** No ☐ → **Go to #49** Yes ☐

a) 2 0 ☐☐ (year) ☐☐ (month) ☐☐ (day) →

Place ECG :File Label Here

b) **Please print ECG label #:** ☐☐☐☐☐☐

49 a) **Blood sample obtained?** No ☐ → **Go to #50** Yes ☐

b) ☐ Fasting sample ☐ Non-fasting sample

c) 2 0 ☐☐ (year) ☐☐ (month) ☐☐ (day) **Time** ☐☐ : ☐☐ (00:00-23:59) → **Hours since any food/beverage consumed (excluding water)** ☐☐

d) **Please print Blood label #:** ☐☐☐☐☐☐

Place Blood label here

50 a) **Urine sample obtained?** No ☐ → **Go to #51** Yes ☐

b) ☐ Fasting sample ☐ Non-fasting sample

c) **Please print Urine label #:** ☐☐☐☐☐☐

Place Urine label here

51. **Name of Interviewer:** ***(please print)*** _____ *First Initial* _____ *Last Name* **Interviewer Code:** ☐☐☐

ADDENDUM 2

Table 1. Fasting plasma glucose vs OGTT (men and women) in the 465 subjects

	Level of diagnosis towards NIDDM	**OGTT**		
Men (n=173)	**FPG**	**OGTT (n=134) Normal** (7.8 mmol/L)	**OGTT (n=30) IGT 2** (7.8-11.1 mmol/L)	**OGTT (n=9) Diabetes 3** (≥11.1 mmol/L)
Normal	**(n=149)** (≤5.5 mmol/L)	78.5% (n=117)	15.4% (n=23)	6.0% (n=9)
Impaired	**2 (n=20)** (5.5-7 mmol/L)	75% (n=15)	25% (n=5)	0% (n=0)
Diabetes	**3 (n=4)** (≥7 mmol/L)	50% (n=2)	50% (n=2)	0% (n=0)
Women (n=287)	**FPG**	**OGTT (n=148) Normal** (7.8 mmol/L)	**OGTT (n=132) IGT** (7.8-11.1 mmol/L)	**OGTT (n=7) Diabetes** (≥11.1 mmol/L)
Normal	**(n=243)** (≤5.5 mmol/L)	54.7% (n=133)	42.8% (n=104)	2.5% (n=6)
Impaired	**(n=42)** (5.5-7 mmol/L)	36% (n=15)	62% (n=26)	2% (n=1)
Diabetes	**(n=2)** (≥7 mmol/L)	0% (n=0)	100% (n=2)	0% (n=0)

OGTT=oral glucose tolerance test, n=subjects, FPG=fasting plasma glucose

Table 2. HbA1c vs OGTT (men and women) in the 465 subjects

	Level of diagnosis towards NIDDM	**OGTT**		
Men (n=173)	**HbA1c**	**OGTT (n=134) Normal** (7.8mmol/L)	**OGTT (n=30) Impaired** (7.8-11.1mmol/L)	**OGTT (n=9) Diabetes** (≥11.1mmol/L)
Normal	**(n=158)** (<6.0%)	79.7% (n=126)	16.5% (n=26)	3.8% (n=6)
Impaired	**(n=11)** (6.0-6.5%)	63.6% (n=7)	27.3% (n=3)	9.1% (n=1)
Diabetes	**(n=4)** (>6.5%)	25% (n=1)	25% (n=1)	50% (n=2)
Women (n=290)	**HbA1c**	**OGTT (n=150) Normal** (7.8mmol/L)	**OGTT (n=133) Impaired** (7.8-11.1mmol/L)	**OGTT (n=7) Diabetes** (≥11.1mmol/L)
Normal	**(n=253)** (<6.0%)	54.9% (n=139)	42.3% (n=107)	2.8% (n=7)
Impaired	**(n=33)** (6.0-6.5%)	33.3% (n=11)	66.7% (n=22)	0% (n=0)
Diabetes	**(n=4)** (>6.5%)	0% (n=0)	100% (n=4)	0% (n=0)

OGTT=oral glucose tolerance test, n=subjects, HbA1c=glycated heamoglobin A1c

Table 3. Fasting plasma glucose vs HbA1c (men and women) in 465 subjects

	Level of diagnosis towards NIDDM	HbA1c		
Men (n=173)	**FPG**	**HbA1c (n=149) Normal** (<6.0%)	**HbA1c (n=20) Impaired** (6.0-6.5%)	**HbA1c (n=4) Diabetes** (>6.5%)
Normal	**(n=158)** (≤5.5mmol/L)	91.3% (n=136)	100% (n=20)	50% (n=2)
Impaired	**(n=11)** (5.5-7mmol/L)	6.7% (n=10)	0% (n=0)	25% (n=1)
Diabetes	**(n=4)** (≥7mmol/L)	2% (n=3)	0% (n=0)	25% (n=1)
Women (n=287)	**FPG**	**HbA1c (n=243) Normal** (<6.0%)	**HbA1c (n=42) Impaired** (6.0-6.5%)	**HbA1c (n=2) Diabetes** (>6.5%)
Normal	**(n=250)** (≤5.5mmol/L)	89% (n=216)	78.6% (n=33)	50% (n=1)
Impaired	**(n=33)** (5.5-7mmol/L)	9.9% (n=24)	19% (n=8)	50% (n=1)
Diabetes	**(n=4)** (≥7mmol/L)	1.1% (n=3)	2.4% (n=1)	0% (n=0)

HbA1c=glycated heamoglobin A1c, n=subjects, FPG=fasting plasma glucose

Table 4. Fasting plasma glucose vs HbA1c (men and women) in total group

	Level of diagnosis towards NIDDM	HbA1c		
Men (n=697)	**FPG**	**HbA1c (n=616) Normal** (<6.0%)	**HbA1c (n=62) Impaired** (6.0-6.5%)	**HbA1c (n=19) Diabetes** (>6.5%)
Normal	**(n=569)** (≤5.5mmol/L)	90.3% (n=514)	8.6% (n=49)	1.1% (n=6)
Impaired	**(n=109)** (5.5-7mmol/L)	87% (n=95)	10% (n=11)	3% (n=3)
Diabetes	**(n=19)** (≥7mmol/L)	36.8% (n=7)	10.5% (n=2)	52.6% (n=10)
Women (n=1127)	**FPG**	**HbA1c (n=891) Normal** (<6.0%)	**HbA1c (n=163) Impaired** (6.0-6.5%)	**HbA1c (n=73) Diabetes** (>6.5%)
Normal	**(n=890)** (≤5.5mmol/L)	84.4% (n=751)	13.1% (n=117)	2.5% (n=22)
Impaired	**(n=184)** (5.5-7mmol/L)	69% (n=127)	21% (n=39)	10% (n=18)
Diabetes	**(n=53)** (≥7mmol/L)	24.5% (n=13)	13.2% (n=7)	62.3% (n=33)

HbA1c=glycated heamoglobin A1c, n=subjects, FPG=fasting plasma glucose