UGANDA MARTYRS' UNIVERSITY
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BETA CELL FUNCTION, INSULIN RESISTANCE AND GLYCEMIC CONTROL IN TYPE 2 DIABETES MELLITUS

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2012 / M181 / 11001

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF

THE DEGREE OF MASTERS OF MEDICINE IN EMERGENCY MEDICINE

OF UGANDA MARTYRS' UNIVERSITY

AUGUST 2015

DEDICATION

This work is dedicated to my teachers, who have guided me along my journey of learning but most of all to my mother – the greatest and most patient teacher I have had.

ACKNOWLEDGEMENT

I would like to express my gratitude to my Supervisors, Dr. Bahendeka K. Silver and Dr. Mwebaze M. Raymond, for their invaluable guidance and support during all the stages of developing the proposal, doing the research and writing the book. Thank you for critically reviewing the manuscript and for all the corrections.

I am thankful to the following people for guiding me through proposal development and reading my work: Dr. Atuhe David Martin, Dr. Mwaka Amos Deogratius, Dr. Ingabire Prossy and Dr. Namisi Charles.

I am very grateful to Mr. Ggita Jonathan for the support and facilitation.

I am very grateful to the Nurse – In Charge Department of Medicine and Adult / Type 2 Diabetes Mellitus Clinic, Nsambya Hospital: Sr. Sanyu, for her guidance, tolerance and help during recruitment of study participants.

I acknowledge and thank all the patients who accepted to participate in this study. I acknowledge the support from the management of St. Francis Hospital – Nsambya, especially the Department of Medicine – Nsambya Hospital.

I am very grateful to MBN laboratories: Dr. Bwanga Freddie, Ms. Najjingo Irene and Ms. Nakakuyu Rose for their invaluable laboratory work and assistance with preparation and analysis of the blood samples.

I am thankful to Dr. Nakabuye Betty and Mr. Walakila Andrew for their insight and help during data analysis.

I am thankful to my colleague and classmate, Dr. Laker Annet for her friendship and encouragement.

I am indebted to my parents, the late Eng. Francis Xavier Byaruhanga K. and Mrs. Passy N. Kawamara for all they taught me.

I am most grateful to God - the Almighty for his amazing and abundant grace.

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LIST OF ABBREVIATIONS AND ACRONYMS

%S	Percent insulin sensitivity
ß	pancreatic Beta cell
A	Pancreatic cell antibody
ADA	American Diabetes Association
ADAG	The A1C – Derived Average Glucose trial
AIDS	Aquired Immune Deficiency Syndrome
BMI	Body Mass Index
CAD	Coronary Artery Disease
C- peptide	Connecting peptide
CI	Confidence Interval
DCCT	Diabetes Complications and Control Trial
NGSP	National Glycohemoglobin Standardization Program
DKA	Diabetic Ketoacidosis
DM	Diabetes Mellitus
FPG	Fasting plasma glucose
FPI	Fasting plaama insulin
GAD	glutamic acid decarboxylase
IDF	International Diabetes Federation
KPD	Ketosis Prone Diabetes
LADA	Latent autoimmune diabetes in adults
HbA1c	Glycated haemoglobin
HDL	High density lipoprotein
HOMA	Homeostasis model assessment
ICA	Islet Cell Antibodies
IR	Insulin resistance
IS	Insulin sensitivity
MKPGS	Mother Kevin Post – Graduate Medical School
MODY	Mature Onset Diabetes of the Young
OHG	Oral hypoglycemic agents
OR	Odds ratio
r	relative ratio
SUR	Sulphonyl urea
SPSS	Statistical Package for social Sciences
UKPDS	United Kingdom Prospective Diabetes Study

OPERATIONAL DEFINITIONS

Type 2 Diabetes mellitus: a multi - factorial metabolic disorder characterized by chronic hyperglycemia, insulin resistance, and a relative insulin secretion defect. (Surampudi et al., 2009). In this study, a type 2 DM patient was an adult with diabetes having been detected after the age of 30 years.

Optimal glycemic control: glycated haemoglobin levels < 7.0 %. (Magaji and Johnston, 2011, Ametican Diabetes Association, 2013)

Sub optimal glycemic control: glycated haemoglobin levels > 7.0 %. (Magaji and Johnston, 2011, American Diabetes Association, 2013)

Beta cell dysfunction: an abnormal alteration in function and / or decline in islet beta cell reserve reflected by a significant decline (>5%) in insulin production as traced by serum C – peptide levels < 1 ng/ml (0.33 nmol/l) or (Maldonado M et al., 2005, Maldonado MR et al., 2005, Sobngwi et al., 2002)

Homeostasis Model Assessment (HOMA): a mathematical method for assessing β-cell function and insulin resistance (IR) from basal (fasting) glucose and insulin or C-peptide concentrations. (Wallace et al., 2004)

Insulin resistance (HOMA 2 IR): a subnormal biological response to normal insulin concentrations or a state in which a given insulin concentration is associated with a subnormal glucose uptake response; HOMA2 IR > 2.5 (Moller and Flier, 1991, Singh Y et al., 2013)

Insulin sensitivity (HOMA2 %S): is a function of glucose metabolism driven by the action of insulin. (Wallace et al., 2004)

Beta cell dysfunction (HOMA2 % B × HOMA2 %S): a measure of pancreatic β-cell activity; (HOMA2 % B × HOMA2 %S) < 1.0 (Albereda and Rodriguez – Espinosa, 2000, Wallace et al.,2004)

Ketosis prone type 2 Diabetes Mellitus: a group of atypical diabetes syndromes characterized by severe beta cell dysfunction (manifested by presentation with DKA or unprovoked ketosis) and a variable clinical course. (Balasubramanyam et al., 2006)

ABSTRACT

Diabetes Mellitus (DM) patients may be categorized into two major groups: type 1 - and type 2 diabetes. Beta cell failure and insulin resistance is the hall mark of type 2 diabetes. Knowledge on the pathophysiology of diabetes improves clinical management. There is scanty data on beta cell function and insulin resistance in type 2 diabetes patients in Uganda. This study investigated beta cell function, insulin resistance and glycaemic control in type 2 diabetes patients attending St. Francis Hospital, Nsambya, Kampala

Study objective: To describe beta cell function, insulin resistance and glycemic control among type 2 DM patients at Nsambya hospital, Kampala Uganda.

Methods: A cross sectional study was conducted among out – patients at Nsambya hospital, diagnosed with DM at ages above 30 years, on oral hypoglycemic agents and of less than 10 years DM duration. Venous blood samples were drawn and the patients' fasting blood glucose, fasting serum C – peptide and HbA1c levels were determined. Their respective insulin resistance (HOMA2 IR), insulin sensitivity (HOMA2 %S) and beta cell function (HOMA2% B×HOMA2 %S) were estimated using the HOMA2 evaluation tool. Statistical analysis was done using SPSS version 20.

Results: Eighty one study participants were selected from the type 2 DM Clinic at Nsambya hospital. The mean beta cell function was 0.397 $\pm$ 0.052 (Standard Error of Mean (SEM)). The proportion of beta cell dysfunction (HOMA2% B×HOMA2 %S < 1.0) was 94.4 %. The mean Insulin Resistance was 3.3 $\pm$ 0.4 (SEM). The proportion of insulin resistance (HOMA2 IR > 2.5) was 35.8 % (29/81). Twenty seven percent of study participants had severe insulin resistance (HOMA2 IR > 3.0). Sixty eight percent (55/81) of study participants had suboptimal glycemic control (HbA1c > 7.0 %). There were no correlations of statistical significance between participants' baseline characteristics with beta cell function nor insulin resistance.

Conclusions: Beta cell dysfunction was a more predominant factor (94.4%) than insulin resistance (35.8 %) among type 2 DM patients at Nsambya hospital. The proportion of suboptimal glycemic control was high (68%). There were no correlations between patients' baseline characteristics with beta cell dysfunction nor insulin resistance.

Recommendations:

1. It is recommended that case control and / or prospective cohort studies with larger type 2 DM patient numbers would provide a better understanding of the evolution of beta cell function and insulin resistance as a means of accounting for differences in individual physiology and the multi – factorial nature of dependence of glycemic control in type 2 DM.

CHAPTER 1
1.0 Introduction and Background

Type 2 DM disease burden: Diabetes Mellitus (DM) is a non – communicable disease that is categorized into two major groups: type 1 - or type 2 diabetes. (International Diabetes Federation, 2013) Type 2 DM comprises 90 % of DM worldwide. (Shaw et al., 2009) In 2010, the prevalences of probable diabetes (glucose > 11.0 mmol/l) and probable hyperglycemia (7.0 – 11.0 mmol/l) in Uganda were estimated to be 0.4 and 2.9% respectively. (Maher et al., 2010) The Uganda Bureau Of Statistics estimated 1.5 (5%) of the 30 million Ugandan population to be Diabetic. That number represented a 15 fold increase in DM morbidity within a decade when compared with a 98,000 estimate of diabetic Ugandans in the year 2000. (Uganda Bureau Of Statistics, 2010) This increase was attributed to lifestyle factors and quality of life which implied the increase to be largely among the type 2 DM category. (Busingye, 2010) A deficiency was also identified regarding diagnosis, patient education and monitoring. (Nyanzi et al., 2014)

Differentiation between Type 1 and Type 2 DM: It is difficult to distinguish between type 1 and atypical forms of type 2 DM. An exact distinction between the two types can be made by demonstrating pancreatic Islet cell antibodies (ICA), anti - glutamic acid decarboxylase (GAD), insulin or tyrosine phosphatase IA-2 antibodies with which, a patient should be categorized as type 1 as DM. (American Diabetes Association, 2008)

Clinical importance of Beta cell function: Increasing attention is being focused on adult patients who do not fit the typical characteristics of autoimmune type 1 DM and yet present with diabetic ketoacidosis (DKA). (Pietropaolo et al., 2000) These atypical forms of DM have been described with terms such as ‘Ketosis prone diabetes, Mature Onset Diabetes of the Young (MODY), type 1.5 diabetes and latent autoimmune diabetes in adults (LADA). The use of such terms suggests the concept that beta cell dysfunction is likely to be the primary defect in the pathophysiology of DM, regardless of "type". (Naik and Palmaer, 2003, Umpierrez et al., 2006)

Identification of Beta cell dysfunction: Beta cell dysfunction or decline in beta cell reserve can be identified with the aid of serum fasting or glucagon - stimulated C-peptide level as laboratory characteristic markers. (Maldonado M et al., 2005, Maldonado MR et al., 2005) Fasting serum C - peptide levels less than 1.0 ng/ml (0.33 nmol/l) have a sensitivity and specificity of 92% and may reliably be used to identify beta cell dysfunction in type 2 DM. (Sobngwi et al., 2002)

Insulin resistance and Type 2 DM: The metabolic syndrome, defined by hypertension, high serum low-density-lipoprotein (LDL) and low serum high-density-lipoprotein (HDL) cholesterol concentrations has been documented in type 2 DM. The dyslipidemia, increased free fatty acids, inflammatory cytokines from fat and oxidative factors result into the cardiovascular complications observed among type 2 DM patients. (DeFronzo and Ferrannini, 1991)

Homeostasis Assessment Model (HOMA): The HOMA model is a mathematical tool that is used to determine one's insulin resistance, insulin sensitivity and beta cell function basing on one's fasting serum C – petide level, fasting blood glucose (FBG) and / or measured serum insulin levels. (Wallace et al., 2004)

The HOMA – IR evaluation tool for estimating Insulin resistance, and variants of it such as the "HOMA β cell" for estimating beta cell function, have been demonstrated to be simple, reliable and clinically applicable. The model is also comparable to the hyperinsulinemic euglycemic glucose clamp which is the gold standard tool for insulin resistance identification. (r = 0.88) (Wallace et al., 2004)

As a result, the HOMA model has found use in large epidemiological studies to assess the natural history of - , the longitudinal changes in beta cell function and insulin resistance and their effects on treatment outcomes in type 2 DM. Examples of such studies include the United Kingdom Prospective Diabetes Study (UKPDS) and Mexico Diabetes study. (Wallace et al., 2004)

Beta Cell function, Insulin resistance and glycemic control: With emphasis on beta cell functional reserve and severity of insulin resistance, it is possible to appropriately predict and adjust for oral hypoglycemic agent regimens or insulin use to achieve early steady state glycemic control. (Grundy, 2007, Holman et al., 2008)

1.1 Problem statement

In 2010, it was estimated that 285 million people worldwide suffered from DM with type 2 DM comprising 90% of the disease burden. By 2011, the IDF estimated that about 366 million people worldwide had DM. Eighty percent of these people lived in the low and middle income countries. By 2013, the cumulative incidence of DM had been shown to be 4% at 1 year and 21% at nine years, which implied a double the global diabetes morbidity by the year 2030 as compared to the 366 million global DM morbidity estimate in 2011.

In the Framingham Heart Study, the presence of DM alone, doubled the age-adjusted risk for cardiovascular disease in men and tripled it in women. (Kannel and McGee, 1979) The IDF in 2012 estimated that four million deaths in the 20 – 79 age group were attributable to DM related cardiovascular / renal complications, accounting for 7 % of global mortality as a result of long term suboptimal glycemic control.

In Cameroon, suboptimal glycemic control was reported to be directly related to significant beta cell decline at rates as high as 18 - 20%. (Mauvais-Jarvis et al., 2004)

In Uganda at Nsambya Hospital, a retrospective study to assess glycemic and blood pressure control revealed that optimal glycemic control of FPG < 7.2 mmol/l was noted in only 42.8% of the 250 sampled out patient records. (Kibirige et al., 2014)

In routine diabetic clinics in Uganda where the pathologies underlying type 2 DM still remain largely speculative, there is hardly any data into the contribution of beta cell dysfunction and insulin resistance to suboptimal glycemic control.

1.2 Study justification

It is possible to adequately predict steady state optimal glycemic control while on oral hypoglycemic agents and identify insulin dependence and/or resistance early. This could lower macrovascular risk by improving the likelihood of achieving early steady state optimal glycemic control among type 2 DM patients.

Suboptimal glycemic control and eventual insulin requirement have been observed among type 2 DM patients initiated on oral hypoglycemic (OHG) therapy. It was postulated that the evaluation of beta cell function and insulin resistance among these patients could partly provide an explanation for these observations.

1.3 Research objectives

1.3.1 General objective

- To describe beta cell function, insulin resistance and glycemic control in type 2 DM patients at Nsambya hospital.

1.3.2 Specific Objectives

1. To determine the proportion of beta cell function in type 2 DM patients at Nsambya hospital.

2. To determine the proportion and severity of insulin resistance in type 2 DM patients at Nsambya hospital.

3. To determine the proportion of sub - optimal glycemic control in type 2 DM patients at Nsambya hospital.

4. To determine the correlations between type 2 DM patient baseline characteristics with beta cell function and insulin resistance.

1.3.3 Research questions

1. What is the proportion of beta cell function in type 2 DM patients at Nsambya hospital?

2. What is the proportion and severity of insulin resistance in type 2 DM patients at Nsambya hospital?

3. What is the proportion of sub optimal glycemic control in type 2 DM patients at Nsambya hospital?

4. What are the correlations between type 2 DM patient baseline characteristics with beta cell function and insulin resistance?

CHAPTER 2
2.0 Literature review
2.1 Introduction

2.1.1 Type 2 Diabetes mellitus

Type 2 DM is characterized by hyperglycemia, insulin resistance, and a relative impairment in insulin secretion. It is a common disorder with a prevalence that rises with increasing degrees of obesity. (Harris, 1989) The importance of impaired insulin release and insulin resistance in the pathogenesis of type 2 diabetes has been evaluated in several studies. (Chen et al., 1995, Weyer et al., 1999)

Insulin resistance is a strong predictor of type 2 DM in that the majority of patients have a genetic risk for type 2 DM by way of early insulin resistance. (Beck-Nielsen and Groop, 1994) In susceptible subjects, insulin resistance becomes more severe with increasing age and weight thereby unmasking a concurrent defect in insulin secretion. (Kahn and Lecture, 1994) The resulting hyperglycemia leads to a sustained second phase insulin secreation and in the long run, a “pancreatic cell burn out”. (Brunzell et al., 1976)

Also of note is the emerging knowledge of pancreatic islet cell auto antibodies even in adult onset DM, which explains why normoglycemia is often not achieved with oral hypoglycaemic drugs in these patients. (Shaisho, 2014) These atypical type 2 DM patients with islet auto - antibodies have been termed as "latent autoimmune diabetes in adults" (LADA), "type 1.5 diabetes" and "slowly progressing type 1 diabetes". (Naik and Palmaer, 2003) The identified ambiguity of characteristics has given rise to the term “syndromes of ketosis prone diabetes” (Pietropaolo et al., 2000)

The importance of glycemic control in minimizing complications related to diabetes has been proven in type 1 DM. (Diabetes Complications and Control Trial, 1993) Strict glycemic control in patients with type 2 DM results in a similar reduction in risk of microvascular disease and a 25% reduction of macrovascular complications. (Nathan et al., 2008, United Kingdom Prospective Diabetes Study 33, 1998)

2.1.2 Long term glycemic control

Newly formed red blood cells are released into circulation with minimum glucose attached to them. During circulation they are exposed to varying levels of blood glucose to which their cell membranes are freely permeable. The glucose enters the red cells and becomes irreversibly attached to the hemoglobin molecules at a rate dependent on the average prevailing blood glucose. (Nathan et al., 2008)

The glucose – attached / "glycated" hemoglobin provides a more accurate measure of the average prevailing blood glucose in comparison to the use of Random and / or Fasting blood glucose (RBG or FBG) which vary depending on the physiological state of the patient. (Nathan et al., 2007) It is for this reason and it's validation in large clinical trials (e,g DCCT, ADAG, NGSP) that glycated hemoglobin (HbA1c) has found wide spread use as marker or estimate for glycemic levels. (Rohlfing et al., 2002)

2.2 Pathophysiology

2.2.1 Beta cell function

Glucose is taken up by the pancreatic beta cells via glucose transporters (GLUT2) whose expression increases with chronic exposure to high glucose concentrations. (Yasuda et al., 1992) It is then phosphorylated to glucose-6-phosphate by an islet-specific glucokinase that acts as a glucose sensor for the beta cells. (Matschinsky et al., 1993)

Mutations in this glucokinase enzyme lead to one of the forms of maturity-onset diabetes of the young (MODY2).(Froguel P, 1993;) Glucokinase-deficient islets however still respond to sulfonylureas as may be seen in poor glycaemic control in some type 2 diabetes mellitus on OHGs. (Terauchi et al., 1995)

Rapid increases in blood glucose concentration cause rapid bursts of insulin secretion that peak within three to five minutes and subside within 10 minutes ("first-phase" insulin release) in normal individuals. (Ferrannini and Pilo, 1979, McCulloch et al., 1993)

When the blood glucose levels remain high, the resulting rise in insulin secretion becomes sustained ("second-phase" insulin release). A decrease followed by an absence of first-phase insulin secretion in response to intravenous glucose is an early feature of beta-cell dysfunction in patients with type 2 DM. (Brunzell et al., 1976)

The C- peptide is a short 31-amino-acid protein that connects insulin's A-chain to its B-chain in the proinsulin molecule but is cleaved off to give insulin. (Clark, 1999) It has a half life of 30 minutes in comparison to insulin's 3 – 4 minutes and hence can be used as a reliable correlate to insulin concentrations. (Shapiro et al., 1988)

Beta cell dysfunction or decline in beta cell reserve can be diagnosed with the aid of serum fasting or glucagon - stimulated C-peptide levels. Fasting serum c-peptide < 1 ng/ml (0.33 nmol/l) has a shared sensitivity and specificity of 92%. (Sobngwi et al., 2002)

2.2.2 Insulin secretion

Insulin is the primary hormone responsible for lowering elevated blood glucose levels to within normal range. It is a peptide hormone composed of 51 amino acids that is synthesized, packaged, and secreted in pancreatic beta cells found in about one million islets of Langerhans that vary in size from 50 to 300 micrometers in diameter. (Goodner et al., 1977)

Insulin is synthesized as pre - proinsulin in the ribosomes of the rough endoplasmic reticulum. Preproinsulin is then cleaved to proinsulin, which is transported to the Golgi apparatus where it is packaged into secretory granules located close to the cell membrane. Proinsulin is then cleaved into equimolar amounts of insulin and C-peptide in the secretory granules. (Goodner et al., 1977)

Basal (unstimulated) insulin secretion is pulsatile, with a periodicity of 9 to 14 minutes. Loss of pulsatile secretion has been shown to be one of the earliest signs of beta-cell dysfunction in patients destined to have features of type 1 diabetes. (Bingley et al., 1992)

2.2.3 Insulin resistance

Substances secreted by fat cells or adipocytes ("adipokines" e.g leptin, adiponectin, tumor necrosis factor alpha, and resistin) are related to insulin resistance and impaired beta cell growth and function. Leptin secreted in proportion to adipocyte mass, signals the hypothalamus about the quantity of stored fat but also has biologic actions in peripheral tissues, such as the pancreas. (Niswender and Magnuson, 2007) The presence of leptin signaling was associated with impaired glucose tolerance in a pancreas – specific leptin knock out (KO) mouse model study. (Morioka et al., 2007)

Adiponectin, an adipocyte-derived hormone, is associated with improved lipid profiles, better glycemic control and reduced diabetes related inflammation. It’s deficiency plays a role in the development of insulin resistance. (Kadowaki et al., 2006)

With Insulin resistance, the associated hyperinsulinemia (high blood insulin levels), hyperglycemia, and adipocyte cytokines (adipokines) cause vascular endothelial dysfunction, an abnormal lipid profile, hypertension, and vascular inflammation, all of which promote the development of atherosclerotic cardiovascular disease. (DeFronzo and Ferrannini, 1991)

2.2.4 Evaluation of Insulin resistance

Several models and criteria have been used to identify insulin resistance in type 2 DM. The gold standard is the hyperinsulinemic euglycemic glucose clamp (HIEG). However it's application is tedious due to the necessity of several insulin infusions and 3 hour venous insulin sampling. (Wallace et al., 2004)

Obesity (increased body mass index, BMI) and/or abdominal obesity (increased waist circumference), increased blood pressure, increased fasting blood glucose and triglyceride levels, and low HDL have also been used to identify the Metabolic syndrome and insulin resistance as it's function. (Barb and Mantzoros, 2003)

The 2001 National Cholesterol Education Program/ (Adult Treatment Panel) ATP III criteria has also found wide spread use in clinically identifying insulin resistance. However, the ATP III criteria have a sensitivity of identifying features of the metabolic syndrome / Insulin resistance of 52%. (Mc Laughlin et al., 2003) Other surrogate markets of the metabolic syndrome i.e serum triglycerides of more than 130mg/dl and serum triglyceride to HDL ratios more than 3.0, have sensitivities of 67% and 64% and specificities of 64% and 68% respectively. (Mc Laughlin et al., 2003) These methods identify fewer individuals with features of insulin resistance as compared to the HOMA IR evaluation which has a sensitivity of > 70% and comparable specificity of > 60 %. (Singh Y et al., 2013)

Other studies among African populations have supported the superiority of the HOMA and quantitative insulin sensitivity check index (QUICKI) indices over other traditional methods of identifying insulin resistance. (Huguette et al., 2010)

The HOMA – IR model is a reliable evaluation tool for identifying insulin resistance with variants of it such as the "HOMA β cell" having comparable estimation power to the hyperinsulinemic euglycemic glucose (HIEG) clamp. (r = 0.88, $p < 0.0001$) (Wallace et al., 2004)
It's a mathematical approximation of insulin resistance, summarized as;

HOMA-IR = (glucose × insulin)/22.5 (Singh B and Saxena, 2010)

Where;

- Insulin concentration is reported in μU/L and glucose in mmol/L.

- The constant of 22.5 is a normalizing factor; i.e, the product of normal fasting plasma insulin of 5 μU/L, and normal fasting plasma glucose of 4.5 mmol/L typical of a "normal" healthy individual = 22.5.

HOMA2 – IR values of greater than 2.5, carry the highest sensitivity and specificity of determining insulin resistance and correlate well with the interaction between β -cell function reserves and the corresponding Insulin resistance. (Huguette et al., 2010, Singh Y et al., 2013)

2.2.5 Evaluation of the severity of Insulin resistance

The closest correlation of insulin resistance to an actual outcome as a measure of severity has been to Coronary Artery Disease (CAD) in a South African study. In that study, a HOMA IR value of > 3.0 was associated with risk of – or actual manifestation of CAD among type 2 DM. ($P < 0.02$) (Ntintyyane, 2010) The same measure of the severity of Insulin resistance was adopted in this study.

2.3 Effects of Metformin and Sulphonylureas on blood glucose control

Metformin belongs to the class of biguanides. It is effective only in the presence of insulin whose action it improves. (Bailey, 1992) It does this by increasing insulin-mediated glucose use in peripheral tissues (such as muscle and liver), especially after meals. (Bailey and Turner, 1996) It also lowers serum free fatty acid concentrations, the production of glucose from the liver and promotes weight loss. (Wu et al., 1990) As a result blood glucose levels improve. The pancreas is hence relieved of the necessity to produce high insulin concentrations as a result of high blood glucose. (Stumvoll et al., 1995) Metformin lowers fasting blood glucose levels by 20 % and HbA1C by 1.5 %. This effect is similar to that achieved by sulfonylurea medications. (United Kingdom Prospective Diabetes Study 13, 1995) It is for these reasons that metformin is the first choice OHG for type 2 DM at the time of DM diagnosis. (Nathan et al., 2006, Nathan et al., 2009)

Sulphonylureas act via sulfonylurea (SUR1) receptors at which they increase entry of calcium into the pancreatic beta cells hence stimulating more insulin secretion. (Hermann et al., 1994) The result is increased responsiveness of beta cells to both glucose and non-glucose secretagogues e.g amino acids. (Aguilar-Bryan et al., 1995) Sulfonylureas also have extra pancreatic effects, one of which is to increase tissue sensitivity to insulin, but the clinical importance of these effects is minimal. Suphonylureas lower blood glucose concentrations by 20 % and HbA1C by 1 – 2 %. (Bressler and Johnson, 1997)

3.0 Methods

3.1 Study design

This was a cross sectional descriptive study.

3.2 Study Setting

The study site was the Adults' / Type 2 Diabetes Mellitus clinic in St. Francis Hospital, Nsambya. The hospital is located on Nsambya hill, Makindye division, in Kampala city, Uganda. It has a bed capacity of 361 beds. The clinic had a registered 2,218 patients and an estimate of 1,982 actively visiting patients. An average 144 DM patients visit the clinic per month. The clinic runs every Monday morning from 8.00a.m to 2.00p.m. It is run by a team of physicians, senior house officers and nurses. The hospital also offers in- patient treatment of DM related cases. (Nsambya-Hospital-Annual-report, 2011)

3.3 Population

3.3.1 Target population All adults registered at Nsambya hospital diagnosed with type 2 DM at an age of 30 years or above, on oral hypoglycemic agents (OHGs) and within a 10 years' duration since diagnosis.

3.3.2 Accessible population All adults within a 10 years' duration of type 2 DM diagnosis, aged 30 years and above, on OHGs, who attended the Nsambya Hospital adults' diabetes mellitus clinic during the study duration from January 2015 to March 2015.

3.3.3 Study unit An adult with type 2 diabetes mellitus who fulfilled the inclusion criteria for the study.

3.4 Selection Criteria

3.4. 1 Inclusion Criteria

- Diabetic patients diagnosed as type 2 DM at an age > 30 years, not exceeding 10 years of type 2 DM duration, on OHGs and with fasting blood glucose (FBG) not exceeding 15.0 mmol/l. The patients also had none of the exclusion criteria listed below.

3.4.2 Exclusion criteria

- Type 2 diabetes mellitus patients with known co – morbidities of:
    - Established renal failure / uremia, chronic liver failure
    - Exogenous insulin therapy, beta blockers
    - Septicemia – sepsis
    - Acromegaly
    - Ambulatory steroid medications

3.5 Sample size estimation and Sampling procedure

3.5.1 Sample size estimation

The primary aim was to determine the proportion of beta cell function and insulin resistance, using fasting / basal C – peptide levels, among type 2 DM patients at Nsambya hospital, Kampala – Uganda.
The sample size was estimated using the Keish Leslie formula. (Eng, 2002)

$$N = \frac{4 z_{crit}^{2} p(1-p)}{D^2}$$

where;
N = The desired sample size.
4 = design effect otherwise synonymed as ***'deff'.***
$\mathbf{Z_{crit}}$ = Standard normal deviation at 95% CI (1.96).
D = Desired total width of the confidence interval = 0.1.
p = average estimated proportion of significant C-peptide decline (5%) in type 2 DM patients in 10 years' duration. (Niskanen et al., 1994)

A design effect: *'deff'* = **4,** was taken since the study population was to be clustered into 4 groups during analysis. A sample size of 73 patients was calculated. Due to unpredictable occurrences i.e errors in sample collection, processing and storage techniques, a 10% loss of this sample size was anticipated. A sample size of **80** participants was calculated and considered to account for the anticipated loss.

3.5.2 Sampling procedure

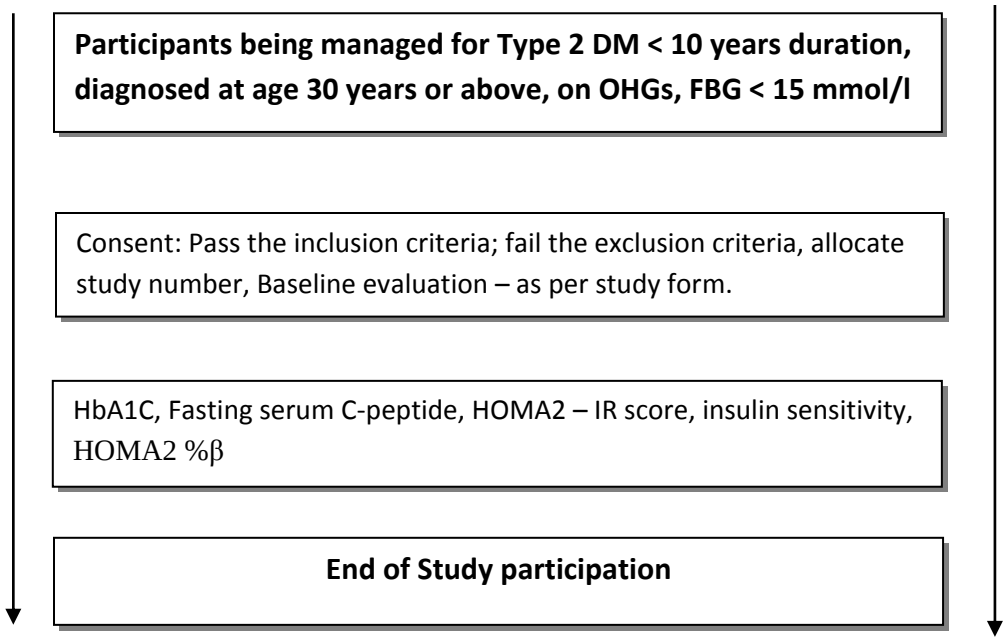
Suitable patients were identified from the St. Francis Hospital adults' / Type 2 DM clinic with the aid of two research assistants. Opportunistic sampling was employed. Eligible patients that satisfied the inclusion criteria but had none of the exclusion were provided with all the necessary information about the study. They were then consented using the consent form **(Appendix 1)**.

The enrolled participants were assigned a random unassigned study number by the Principal Investigator (PI). If an earlier recruited participant declined continued participation in the study, then that participant was withdrawn and their study number re-assigned at any subsequent recruitment.

3.6 Data Collection

The participants' data was collected using semi structured patient study forms. **(Appendix 2).**

Figure 1: Patient flow chart



3.6.1 Assessments, measurements and variables

Upon recruitment, the information obtained included:
Age, sex, age at diagnosis of type 2 DM, (above 30 years), duration since diagnosis of type 2 DM (10 years or less), date and time of last meal (duration of at least 6 to 8 hours prior), family history of DM, Body Mass Index (BMI) and ambulatory oral hypoglycemeic agents.

The participants were generally examined for features of edema, ascites, acromegaly, jaundice, conjuctival pallor, truncal obesity, moon facies and brachial blood pressure (exceeding 160/110 mmHg) to try to eliminate any of the conditions listed under the exclusion criteria.
Other prior laboratory investigations such as a complete blood count (CBC), renal function tests (RFTs) and liver function tests (LFTs) were assessed as availed, to ensure the participants did not have any of the exclusion criteria as per protocol.

3.6.2 Assessment of Fasting Blood Glucose

The Index or middle finder (digiti intermedius) of the study participant was secured and it's palmar surface swabbed thrice with 70% alcohol soaked swab. The palmar surface of the tip to the finger was then pricked with a sterile lancet. The finger was then swabbed again with a sterile 70% alcohol soaked swab at the point of the prick and then gently massaged to encourage bleeding for a drop of blood. A pre – assembled glucose meter with a glucose strip was then brought into contact with the bleed as appropriate that a reading could be analyzed and read off the machine for a fasting blood glucose level.

3.6.3 Collection of blood samples

Having ascertained that the patient had undergone a 6 to 8 hours' fast, blood samples were drawn under aseptic technique by phlebotomy from each participant from the anterior cubital fossa or dorsum of the hand, both for HbA1C and C – Peptide assay. From each study subject, approximately eight millimeters were drawn using a sterile disposable syringe and emptied into a sterile blood K+EDTA specimen bottle to, enhance C- peptide stability for an approximate 24 hours at 4 degrees celcius even without the addition of preservative agents such as 0.1 % Sodium azide. (McDonald et al., 2012) Each specimen bottle was labeled with the participant's respective study number. The samples were then placed immediately into a cool box (at temperature; 4 – 6 degrees celcius)

3.6.4 Analysis of HbA1c

HbA1c estimates were done using BIO-RAD Micromat II – COBAS Roche Integra 800 biokits (Catalogue Number: 720 - 17 Ref; 68059501: Primus Diagnostics, Kansas, USA). The kits used an affinity chromatography method that measures the percent glycated hemoglobin in the sample. Blood from each K+EDTA specimen bottle was added to the first tube which initiated a series of incubation steps that ended in an immune – turbidmetry analysis. Each result was recorded and entered to it's corresponding study number. The ranges provided as normal by the laboratory were 4 - 5.6%, with 5.7 - 6.4% as pre – diabetic and more than 6.5 % as high.

3.6.5 Preparation for C – peptide assays

The C- peptide samples were centrifuged at 1500rpm for 3 – 5 minutes and the serum separated off for storage within 4 hours of sample collection. The samples were stored at target -70 degrees celcius for a period of about 1 week per batch, each in a cryoprecipitate specimen bottle labeled with the matching study number to the corresponding K+EDTA specimen bottle until the C – peptide assays were to be done.

3.6.6 Analysis of C – peptide assays

The C – peptide concentrations were analyzed by a Solid phase Enzyme Linked Immunosorbent assay (ELISA) method (Catalogue Number; 1293 – 15: Diagnostic Automation Inc. California, USA). The sensitivity of this method could detect titers as low as 0.020ng/ml and had a specificity of 100%. The specimen were thawed only on the day of analysis and then assayed in duplicates alongside the calibrator and controls for quality assurance purposes.
Reference ranges provided as normal by the laboratory ranged from 0.6 – 1.9ng/ml. However for the purposes of this study, fasting serum c-peptide levels < 1 ng/ml (0.33 nmol/l) were considered diagnositic of β cell dysfunction.

3.6.7 Deriving fasting serum insulin

Insulin and C-peptide are co-secreted from the pancreatic beta cells on an equimolar basis with a C-peptide to insulin molar ratio that approximates 1.0. Hence, serum C – peptide levels can be used to estimate serum insulin concentrations. (Horwitz et al., 1975, Polonsky et al., 1984)

Using the HOMA equations:

Fasting insulin = [fasting serum C-peptide (ng/ml) × 0.33 × 1000] / 7.175 (Singh B and Saxena, 2010)
Where:

- 0.33 is the conversion factor for C- peptide from ng/ml to nmol/l
- 7.175 is the converting factor for Insulin from mcIU/mL to pmol/L

The value of fasting insulin from the above equation is then fed into the equation:

HOMA-IR = (FPG × fasting insulin)/22.5 (Singh B and Saxena, 2010)

Where:

- HOMA IR is the estimated insulin resistance.
- Fasting Insulin is reported in μU/L and Fasting Plasma Glucose (FPG) in mmol/L.
- 22.5 is a normalizing factor; i.e, the product of normal fasting plasma insulin of 5 μU/L, and normal FPG of 4.5 mmol/L in a normal healthy individual.

Beta cell functional reserve (HOMA %β) can be estimated using the equation:

HOMA %β = (20 × fasting plasma insulin (μU/mL)/FPG (mmol) -3.5 (Haffner et al., 1997)

However, the above equations give best estimates only under steady state conditions at plasma glucose concentrations of 4mmol/l. At such states, the above equations reflect the balance between hepatic glucose output and basal insulin which is maintained by a feedback loop between the liver and pancreatic beta cells. The HOMA2 model corrects for the reduction of peripheral glucose-stimulated glucose uptake, increase in insulin secretion and renal glucose losses which occur in hyperglycemic states. (Wallace et al., 2004)

The HOMA2 computed model calculator (version 2.2.2) was hence used in this study to estimate the percentage beta cell function (HOMA2 %B), insulin sensitivity (HOMA2 %S) and insulin resistance (HOMA2 – IR).

The expression of beta cell function as percentages calculated as HOMA2 %B would be erratic in that for any one individual, the percentage secretion of insulin is inversely proportional to one's insulin sensitivity. (Wallace et al., 2004)

- i.e; for a thin individual with an insulin sensitivity (HOMA2 %S) of 200 %, a basal insulin secretion percentage (HOMA2 %B) of 50 % is still optimal in that the high insulin sensitivity at 200 %, explains the physiological observation of insulin secretion at 50 %. This insulin secretion at 50% could otherwise be erratically interpreted as suboptimal if considered in isolation.

The physiologic hyperbolic relationship between beta cell function and Insulin sensitivity is represented by the equation:

HOMA2 % B = K / HOMA2 %S

Where:

- K – is a constant; the "Disposition index", that represents the hyperbolic relationship between HOMA2 % B and Insulin sensitivity (HOMA2 %S)

K = (HOMA2 % B × HOMA2 %S)

Using the above example;

Where:

- HOMA2 % B = 50 % = 0.5 and HOMA2 % S = 200 % = 2.0

K = (0.5 × 2.0) = 1.0 which approximates $r^2 = 0.802$ ($P < 0.001$), which approximates 1.10; the highest expected value of correlation. (Albereda and Rodriguez – Espinosa, 2000)

Beta cell function is reported with respect to Insulin sensitivity. Hence, the product of HOMA2 % B and Insulin sensitivity (HOMA2 %S) greater than or equal to (> / =) 1.0 was considered as not sub optimal beta cell function. (Albereda and Rodriguez – Espinosa, 2000)

HOMA2 –IR values of greater than 2.5 were considered representative of insulin resistance in this study. (Singh Y et al., 2013)

HOMA2 - IR values greater than 3.0 were considered representative of severe insulin resistance in this study. (Ntyintyyane, 2010)

3.7 Data management and analysis

3.7.1 Data management

The raw data collected was organized, and cleaned. It was then entered into Statistical Package for Social Sciences (SPSS) version 20 software and coded by the principal investigator.

3.7.2 Data analysis

The distributions of HOMA2 %B, HOMA2 %S and HOMA2 IR followed normal distribution curves upon descriptive analysis. Their means compared with those of baseline characteristics showed linear ties of statistical significance (P - Values < 0.05). It is for these reasons that their values were not logarithmically transformed. Their means were calculated simply and not geometrically transformed.

The means, standard errors of means (SEM) of HOMA2% B, HOMA2 %S, HOMA2 –IR and (HOMA2% B×HOMA2 %S) scores were determined.
Categorical data was summarized and their means, standard errors of means (SEM), confidence intervals (CI) at 95% were determined. Line and scatter graphs of correlations between means of HOMA2 %B, HOMA2 –IR and HbA1c were plotted. Continuous variables were summarized using means, standard errors of means (SEM), medians and scatter graphs where due.

Correlations between variables were determined by linear regression and bivariate analysis as due. Odds ratios for likelihood of associations between reference groups were determined using binary logistic analysis.

Correlations of statistical significance (P – value < 0.05) were tested for reproducibility using linear regression analysis with a simple bootstrap method (to 1000 bootstrap samples).

3.7.3 Quality control

The following precautions were considered:

- The questionnaires and forms were pre-tested before commencement of the study.
- The investigator double checked all entities of data values entered.
- The investigator was continuously monitored by the Supervising Principal Investigator and regular progress and identified setbacks solved by solutions counter –checked by the supervising principal investigator, with due notification of the ethics review board of any proposed changes.

3.8 Ethical consideration

3.8.1 Institutional approval

Permission to conduct the study was sought from the Uganda Martyrs' University – Nkozi/Nsambya Institutional Review Board and Ethics Committee. The study was conducted wirh ethical standards according to the Helsinki Declaration on study of Human subjects.

3.8.2 Informed consent

Informed consent was obtained by signature or thumbprint on the consent form, from the study participants.

CHAPTER 4: RESULTS

4.1 Study profile

Between January 2015 and March 2015, 90 patients diagnosed with type 2 DM at an age of 30 years or above, were approached. Seven patients declined to consent for participation in the study. Eighty three of them were enrolled. Eighty one participants' blood samples were analyzed for HbA1c levels and fasting serum C-peptide assays. One blood sample was not analyzed when the it's corresponding participant rescinded her consent for continued participation in the study and the other due to a laboratory error at preparation level for C – peptide assays.

Thirty eight percent of the 81 study participants had Fasting Blood Glucose (FBG) exceeding 10 mmol/l and 68 % had HbA1c levels greater than 7.0 %.

Thirty percent of the study subjects had fasting serum C- peptide levels below 0.6 ng/ml, which was the laboratory lower limit of the normal reference range (0.6 -1.9 ng/ml).

Forty four percent study participants had fasting serum C – peptide levels less than 1.0 ng/ml suggestive of B –cell dysfunction. Only 5.6 % had optimal beta cell function (HOMA2 % B × HOMA2 %S > 1.0).

Insulin resistance (HOMA2 IR > 2.5) was identified in 35.8% of study participants. Twenty seven percent of study participants had severe insulin resistance with HOMA2 IR scores greater than 3.0.

4.2 Description of study participants

The study population consisted of females comprising 56% (45/81). The age range of participants was 30 – 88 years, with 55 years as the average age at diagnosis of type 2 DM. The most frequent age range of DM diagnosis was the 40 – 49 age range. Fifty three percent (43/81) had a positive family history of DM while 7.4 % (6/81) were unsure of their families' DM history. The mean body mass index (BMI) was 26.7 kg/m2. Obese participants (BMI > 30.0 kg/m2) comprised 18.5 % of the study group. All study participants were on metformin medication from their time of diagnosis. Fifty four percent (44/81) of the participants were also on sulphonylurea (SUR) medication. The average duration of type 2 DM in this study population was 4 years, with 65% (53/81) of participants lying within the 0 – 5 years range of DM duration. **(Table 1)**

Table 1: Baseline characteristics of 81 study participants at the Adults / Type 2 DM clinic - Nsambya Hospital

Variables/characteristics		
Gender	**Female (%)**	**45 (56)**
	Male (%)	**36 (44)**
Age (Years)	Mean	59
	Median (range)	58 (30 – 88)
Age at diagnosis (Years)	Mean	55
	30 -39 (%)	9 (11)
	40 -49 (%)	23 (28)
	50 -59 (%)	22 (27)
	60 -69 (%)	15 (19)
	70 – 79 (%)	11 (14)
	80 + (%)	1 (1)
Family history of DM	No family h/o DM (%)	32 (37.6)
	Positive family h/o DM (%)	43 (53)
	Not sure / not ascertained (%)	6 (7.4)
BMI (kg/m2)	< 18.5 kg/m2 (%)	1 (1.2)
	18.5 – 24.9 kg/m2 (%)	24 (29.6)
	25.0 – 29.9 kg/m2 (%)	41 (50.6)
	30.0 – 34.9 kg/m2 (%)	13 (16.0)
	35.0 – 40.0 kg/m2 (%)	2 (2.5)
	40.1 + kg/m2 (%)	0 (0)
Metformin use	Not on metformin (%)	0 (0)
	On metformin (%)	81 (100)
Sulphonyl urea (SUR) use	Not on SURs (%)	37 (46)
	On SURs (%)	44 (54)
Duration of DM & on OHGs (Years)	Mean	4
	0 – 5 (%)	53 (65)
	6 – 10 (%)	28 (35)

4.3 HbA1c levels among 81 study participants at the Adults / Type 2 DM clinic, Nsambya Hospital

The mean ± Standard Error of Mean (SEM) of HbA1c levels in the participants was 8.02 ± 0.2 %, with a range of 3.7 to 15.8 %. Sixty eight percent of HbA1c levels were above 7.0 %.

Table 2: Correlations between baseline characteristics with HbA1c levels among 81 study participants at the Adults / Type 2 DM clinic, Nsambya Hospital

Variable	Mean (SEM)	P – value
Gender		
Female	7.8 (0.3)	0.431
Male	8.3 (0.4)	
Family history of DM		
No family h/o DM	7.5 (0.3)	0.065
Positive family h/o DM	8.1 (0.3)	
Not sure / not ascertained	9.8 (2.0)	
	Correlation co – efficient (95 % Confidence interval	
Age	- 0.02 (- 0.34, - 0.32)	0.865
Age at diagnosis	- 0.01 (- 0.36, 0.31)	0.894
Disease duration	- 0.13 (- 0.52, 0.19)	0.396
BMI (kg/m2)	-0.023 (-0.256, 0.204)	0.841

There were no correlations of statistical significance between HbA1c with any of the participants' baseline characteristics.

Table 3: Correlations between SUR use and DM duration with HbA1c levels among 81 study participants at the Adults / Type 2 DM clinic - Nsambya Hospital

Duration of DM (Years)	N (%)	Mean (SEM) HbA1c	P – Value
0 - 5 years	53 (65%)	8.3 (0.3)	0.090
6 – 10 years	28 (35%)	7.4 (0.3)	
SUR use			
Not on SURs	37 (46%)	8.4 (0.4)	0.176
On SURs	44 (54%)	7.2 (0.2)	

4.3.1 Evaluation of correlation between SUR use with HbA1c levels among 81 study participants at the Adults / Type 2 DM clinic - Nsambya Hospital

Using binary logistic correlations, considering:

HbA1c > 7.0 % = 1

HbA1c < 7.0 % = 0

The study group on SURs was less likely to be associated with HbA1c > 7.0 % (Odds ratio = 0.734) than the group not on SURs (Odds ratio = 0.762) although the difference in likelihoods was not of statistical significance (P – value = 0.953).

The above result also was similar to that determined by a Fishers' exact test comparison between SUR groups and HbA1c groups. There was no difference between the groups that were on or off SUR use when compared to HbA1c < 7.0 % and Hba1c > 7.0 % groups. (P – value = 1.000)

4.4 Fasting serum C – peptide levels among 81 study participants at the Adults / Type 2 DM clinic - Nsambya Hospital

The mean $\pm$ SEM of fasting serum C – peptide was 1.459 $\pm$ 0.161 ng/ml. Thirty percent and 44% of the study participants had C – peptide levels below 0.6 and 1.0 ng/ml respectively. Fasting serum C- peptide levels in this study population ranged from 0.032 to 7.987 ng/ml. (Laboratory normal range: 0.6 - 1.9 ng/ml)

4.4.1 Correlations between baseline characteristics and fasting serum C- peptide among 81 study participants at the Adults / Type 2 DM clinic - Nsambya Hospital

Linear regression and comparisons of means were done to determine correlations between HbA1c and baseline characteristics.

Table 4: Correlations between Fasting serum C – peptide levels and baseline characteristics among 81 study participants at the Adults / Type 2 DM clinic, Nsambya Hospital

Variable	Mean Fasting C- peptide levels (95% confidence interval)	P – values
Gender		
Female	**[ref]**	
Male	0.043 (- 0.693, 0.611)	0.888
Age	-0.006 (- 0.028, 0.14)	0.617
Age at diagnosis	-0.307 (- 0.290, 0.150)	0.591
BMI (kg/m2)	0.006 (-0.191, 0.233)	0.995
	Mean (SEM)	
Family history of DM		
No family h/o DM	1.567 (0.280)	0.681
Positive family h/o DM	1.443 (0.218)	
Not sure / not ascertained	0.997 (0.331)	
SURs use		
On SUR use	1.471 (0.248)	0.947
Not on SUR use	1.449 (0.214)	
Duration of DM		
0 – 5 yrs	1.481 (0.193)	0.851
6 – 10 yrs	1. 416 (0.296)	

There was no correlation of statistical significance between fasting serum C - peptide levels and any of the participants' baseline characteristics.

There were no correlations between fasting C- peptide serum concentrations and HbA1c levels did not demonstrate any significant association. Every 1.0 increase in HbA1c corresponded to decrease in fasting serum C – peptide of – 0.137 ng/ml (P – value = 0.428)

4.5 Insulin sensitivity and Insulin resistance among 81 study participants at the Adults / Type 2 DM clinic, Nsambya Hospital

The mean $\pm$ SEM of Insulin sensitivity (HOMA2 %S) was 85.2 $\pm$ 6.5 %, with a range of 13.3 to 236.6 % and a median of 67.5 %.

The mean $\pm$ SEM of Insulin Resistance (HOMA2 IR) was 3.3 $\pm$ 0.4, with a range of 0.4 – 16.3 and a median of 1.6. The proportion of insulin resistance (HOMA IR > 2.5) was 35.8 %. Twenty seven percent of the participants in this study population had HOMA2 - IR values greater than 3.0.

Table 5: Correlations of insulin sensitivity and baseline characteristics among 81 study participants at the Adults / Type 2 DM clinic, Nsambya Hospital

Variable	Mean HOMA2 %S (95 % Confidence Interval)	P = Values
Age in years	-0.378 (- 1.510 , 0.841)	0.489
Duration of DM in years	-0.691 (- 3.446, 4.442)	0.738
BMI (kg/m2)	-0.086 (-0.335, 0.185)	0.527
Gender		
Female	**[ref]**	
Male	25.232	**0.053**
	Mean Insulin resistance (%S), (SEM)	**P – Value**
Family history of DM		
No family h/o DM	85.5 (11.1)	0.997
Positive family h/o DM	85.2 (8.7)	
Not sure / not ascertained	83.0 (21.4)	
Age of Diagnosis in years	153.6 (35.1)	**0.018**
30 – 39	69.1 (7.1)	
40 - 49	63.9 (14.1)	
50 -59	90.8 (8.6)	
60 – 69	83.0 (12.8)	
70 - 79	35.5	
80 +		

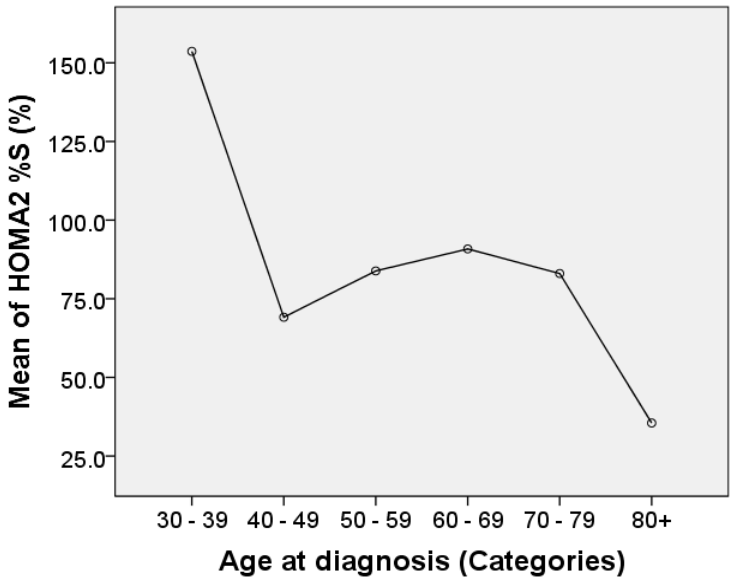
The correlations; mean HOMA2 %S of males in reference to females + 25.232 % (P – value = 0.053) and comparisons of means of HOMA2 %S between age of diagnosis groups (P –value = 0.018), were then subjected to a simple linear regression bootstrap analysis (1000 bootstrap samples).

Linear regression analysis with a simple bootstrap analysis did not reveal any statistically significant correlations between insulin resistance and gender + 23.746 % (41.275, 137.290) 95% CI: P – value = 0.062) in reference to females nor age of diagnosis groups (- 4.733 % (- 14.472, 6.221) 95% CI: P - value = 0.367).

4.5.1 Correlations of insulin resistance (HOMA2 IR) with baseline characteristics among 81 study participants at the Adults / Type 2 DM clinic, Nsambya Hospital

There were no correlations of significance between Insulin resistance (HOMA2 – IR) and study participants' ages (P – value = 0.519), duration of DM (P – value = 0.414), age at diagnosis (P – value = 0.572), gender (P – value = 0.143) nor family history of DM (P –value = 0.289).

Figure 2: Means plots of Mean insulin sensitivity versus ages at diagnosis of 81 study participants at the Adults / Type 2 DM clinic, Nsambya Hospital



4.6 Beta cell function (HOMA2% B×HOMA2 %S) of 81 study participants at the Adults / Type 2 DM clinic, Nsambya Hospital

The estimated beta cell function for each patient was expressed in relation with the corresponding insulin sensitivity (HOMA2% B×HOMA2 %S). The cut off representative of optimal beta cell functional reserve was greater than or equal to (> / =) 1.0.

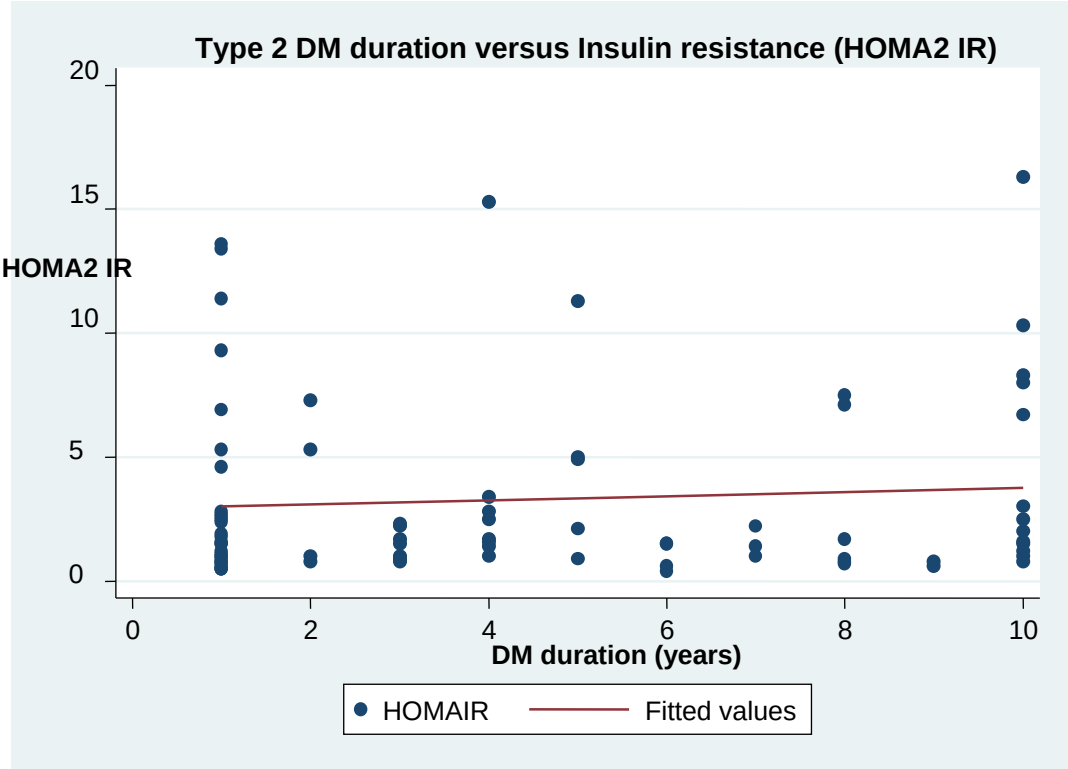
The mean ± Standard Error of Mean (SEM) of (HOMA2% B×HOMA2 %S) was 0.397 ± 0.052, with a range of 0.008 to 2.445. 94.4% of the study population had sub optimal beta cell function (HOMA2% B×HOMA2 %S levels < 1.0).

Table 6: Correlations between (HOMA2 %B×HOMA2 %S) with baseline characteristics among 81 study participants at the Adults / Type 2 DM clinic, Nsambya Hospital

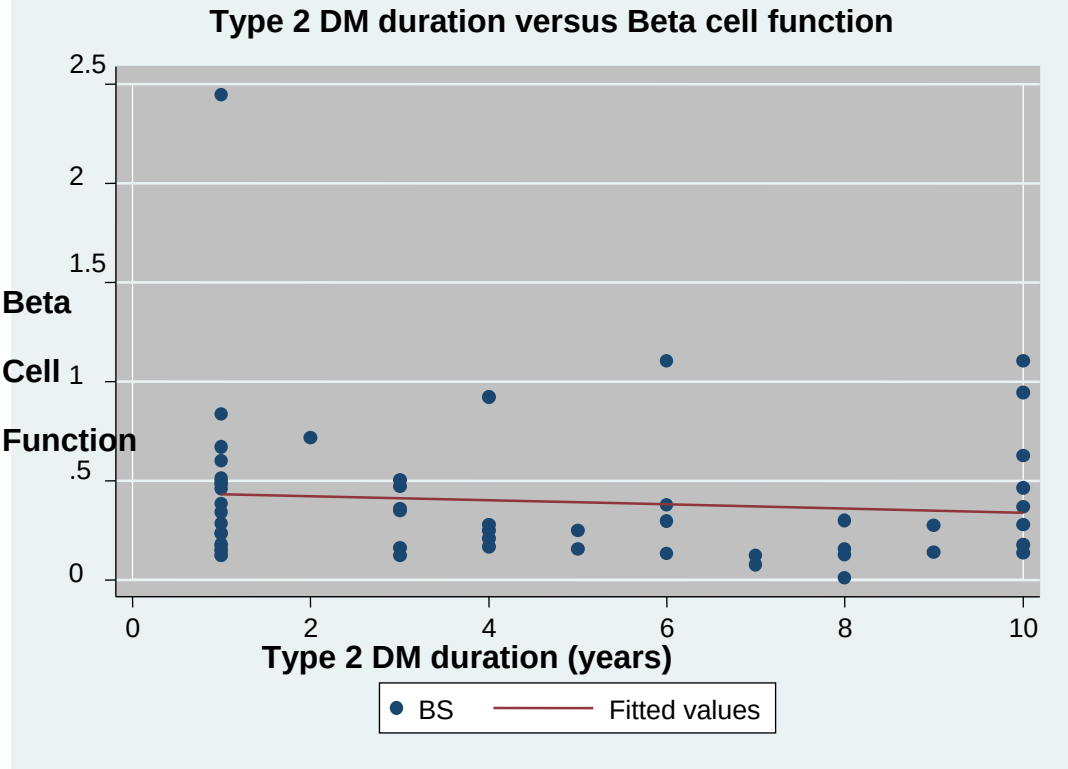
Variable	Mean (HOMA 2%B×HOMA2 %S) (95% Confidence interval)	P value
Age	- 0.006 (- 0.120, 1.329)	0.801
Gender / sex		
Female	**[ref]**	
Male	0.055 (- 0.057, 0.059)	0.618
Age at diagnosis (years)	0.004 (- 0.061, 0.054)	0.884
Duration in years	- 0.010 (-0.044, 0.020)	0.543
BMI (kg/m2)	0.116 (- 0.186, 0.386)	0.405
	Mean HOMA2 %B×HOMA2 %S (SEM)	**P – value**
Family history of DM		
No family h/o DM	0.440 (0.068)	0.734
Positive family h/o DM	0.375 (0.082)	
Not sure /not ascertained	0.281 (0.139)	
SUR use		
Not on SUR use	0.355 (0.039)	0.499
On SUR use	0.428 (0.087)	
Duration of DM in years		
0 – 5 years	0.419 (0.071)	0.590
6 – 10 years	0.360 (0.074)	

There were no correlations of statistical significance between beta cell functional reserve and any of the participants' baseline characteristics.

Figure 3: Scatter Plots of Correlations between Beta Cell function, Insulin Resistance and DM duration



The above scatter graph demonstrated increase in insulin resistance with increase in type 2 DM duration within the study group.



The above scatter graph demonstrated the gradual decline in beta cell function with ongoing type 2 DM duration. The line of best fit within the observations also showed that beta cell dysfunction occurred early at values below 0.5 at an approximate one year of type 2 DM duration.

CHAPTER 5
5.1 DISCUSSION

This was a cross sectional study that was conducted between January 2015 and March 2015 among adult patients above 30 years of age attending the adults / Type 2 DM clinic at St. Francis Hospital, Nsambya. The aims of the study were to describe of beta cell function, Insulin resistance using the HOMA model, and suboptimal glycemic control among type 2 DM patients at Nsambya hospital.

5.1.1 Fasting C – peptide serum concentrations

Whereas 30% of participants had fasting serum C – peptide levels less than 0.6 ng/ml (laboratory reference range; 0.6 – 1.0 ng /ml), 44% of the study participants had low fasting C – peptide serum concentrations (cut off value: < 1.0 ng/ml) which were indicative of beta cell dysfunction.

However the beta cell function – IS index **(**HOMA2% B×HOMA2 %S) identified 94.4 % of study participants as beta cell dysfunctional. In a similar study in Italy that assessed beta cell dysfunction in type 2 DM as estimated by HOMA2 %B, the proportion of beta cell dysfunction ranged between 81 % (using serum C - peptide samples taken in fasting state) to 92 % (using serum C – peptide samples taken in non – fasting state) (P – value < 0.001). (Balzano et al., 2014)

In ideal situations, a peak response to an intravenous glucagon stimulated challenge is preffered method of C - peptide assessment. A fasting serum C – peptide level is analyzed for first, a second serum C – peptide sample is drawn 5 – 10 minutes after a 1 mg infusion of glucagon. The C – peptide level analyzed from the second blood draw is interpreted as the pancreatic beta cell response to the glucagon challenge in consideration of the individuals basal (fasting) C – peptide secretion. A response of more than 1.5ng/ml is considered optimal for normal beta cell function. In less than ideal situations, several C – peptide serum blood draws are taken at least 8 hours apart, each approximately 3 – 4 hours after a meal. The average of the values obtained is considered as the basal serum C – peptide level. (Maldonado et al., 2003)

The above mentioned techniques serve to cater for the differences in individual physiology on basal insulin secretion and differences of basal serum C – peptide within a single individual at different points in time. In this study, single sample fasting serum C – peptide blood draws were taken hence the above mentioned factors were not accounted for. This may explain the discrepancy between proportions estimated using the beta cell function – IS index and fasting C – peptide levels, in this study.

It may be concluded that in consideration of the differences between beta cell dysfunction proportions detected by single sample fasting serum C – peptide and the beta cell function – IS index, the former is not a reliable marker for beta cell function. However, this conclusion may need to be validated by assessing both methods against actual basal serum insulin titers.

Fasting C – peptide levels were not significantly correlated to age, gender, family history of DM, age at diagnosis, duration of type 2 DM or BMI. In this study population, fasting C – peptide levels had no correlations with sulphonylurea use. This observation could be taken to suggest a lack of substantial beta cell functional reserves for the sulphonylureas to stimulate significant insulin secretion.

There was no correlation between fasting serum C –peptide levels and HbA1c (P – value = 0.428). A non – correlation between declining serum C – peptide levels and glycemic control has been identified and documented before in a 10 year prospective study among type 2 DM patients. (Niskanen et al., 1994)

5.1.2 HbA1c and glycemic control

Thirty two percent of participants had HbA1c levels less than 7.0% representative of optimal glycemic control. The percentage in this study population was higher than the observations made by Kibirige et. al. in the same clinic in 2014 for which optimal glycemic control by the same standards was 20.8%. (Kibirige et al., 2014)

There were no correlations between HbA1c levels with age, age at diagnosis, gender, BMI or family history of DM. There were also no correlations between HbA1c levels with duration of type 2 DM or sulphonylurea use.

The mean HbA1c level was higher in the ‘0 – 5 years’ than in the ‘6 – 10 years’ duration groups at; 8.3 % and 7.4% respectively: (P- value = 0.090). It would be anticipated that the reverse would be true. Beta cell function would be expected to decline farther alongside increasing insulin resistance with increasing type 2 DM duration resulting in increasing HbA1c levels. (Ramlo-Halsted and Edelman 2000)

The observation in our study could also be due to the influence of differing drug adherence levels, differences in individual physiology, differing diet and differing levels of exercise or physical activity between study subjects. These factors were not accounted for during this cross - sectional study.

It is possible to conclude that the multi factorial influences on glycemic control in type 2 DM may be difficult to assess for in any single study. That aside, some factors like differences in individual physiology may be better accounted and assessed for in prospective cohort studies

5.1.3 Evaluation of effects of Metformin & Sulphonylurea agents on glycemic control

All patients on oral hypoglycemic agents in the adult / type 2 DM clinic at Nsambya hospital were on metformin as recommended by ADA guidelines. (American Diabetes Association, 2013) Fourty four (54%) of study participants were also on sulphonylurea medications.

The mean HbA1c value was higher in the "Not on SUR" group than in the "On SUR" group, although not significantly. (P – value = 0 .172) The sulphonylurea ("SUR') groups were virtually the same in relation to the "HbA1c > 7.0 %" and the "HbA1c < 7.0 %" groups. (P – value = 1.000) This implied that sulphonylurea use did not seem to have an effect on long term glycemic control in participants in this study. Type 2 DM patients on insulin secretagogues such as suphonylureas are expected have better glycemic controls (lower HbA1c values) than those not on such drugs. (Turner et al., 2010)

In the UKPDS 26 study, a prospective cohort study to assess issues of effective therapy, sulphonylureas were noted to fail at rates that can be correlated to individual type of Aß phenotype at presentation and also varied on the sulphonylurea selected. In that same study sulphonylurea failure as an oral hypoglycemic agent was strongly linked to beta cell function decline even in type 2 DM ($P < 0.0001$). The failure rate of sulphonylurea was 7% per year. At 11 years of type 2 DM duration, up to 50% had failed on sulphonylurea use and required insulin supplementation. (Mathews and Cull, 1998)

The finding of similar trends in this study could suggest the presence of such individuals that need Aß phenotyping for proper classification and management

5.1.4 Insulin resistance and Beta cell function

In this study, the proportion of Insulin resistance (HOMA2 IR > 2.5) was 35.8 %. In a Nigerian study, using a HOMA2 IR value > 2.0, the prevalence of insulin resistance was 40 % in type 2 DM patients. (Bakari and Onyemelukwe, 2005) The proportion of insulin resistance in this study, was comparable to that identified in the study by Bakari and Omenyelukwe. Twenty seven percent of the participants in this study group had HOMA2 IR values greater than 3.0. HOMA2 IR values of greater than 3.0 indicate severe insulin resistance. (Ntyintyyane, 2010)

The proportion of beta cell dysfunction in this study was 94.4%. This suggested the predominance of beta cell dysfunction over insulin resistance in terms of proportional distribution.

In a publication from Cincinatti USA, Ramlo-Halsted and Edelman described the natural history of type 2 DM and it's progression from mild to "Insulin requiring" diabetes. It was stated how while insulin resistance was the main underlying pathology in type 2 DM, it was beta cell function that determined the onset of type 2 DM as well it's progression. (Ramlo-Halsted and Edelman, 2000)

In another study by Marlon E. Cerf describing the interplay between beta cell function and insulin resistance in regard to the causation of type 2 DM, it is explained how beta cell dysfunction 'supercedes' insulin resistance in inducing diabetes and how preservation of beta cell function and insulin signaling in glucose up - taking tissues maintains normal glucose homeostasis. (Cerf, 2013)

The findings in this study though not descriptive of the evolution of type 2 DM pathophysiology, also suggest a more common occurrence of beta cell dysfunction than insulin resistance among these patients.

5.1.6 Importance of beta cell function in Type 2 Diabetes Mellitus

Beta cell dysfunction was the more predominating factor than insulin resistance among Type 2 DM patients at Nsambya hospital.

Increasing attention is being paid to beta cell function as the main influential factor in type 2 DM. Work to support this notion include the animal model research into a Fox01gene- deficient mechanism that explains β-cell dedifferentiation into other hormone producing cell forms rather than the widely accepted apoptosis hypothesis. This has led to prepositions of salvaging dedifferentiated β-cells by re-differentiating them back into functional β-cells. This hypothesis has provided a glimmer of hope and insight into maintenance of β-cell function in type 2 DM. (Talchai et al., 2012)

There are also guidelines and protocols that support the use of short term insulin therapy in symptomatic hyperglycemia not only for glycemic control but to reverse short-term glucotoxicity and lipotoxicity hence promoting midterm β-cell preservation. This method has been termed as "early insulinization". (Itamar Raz and Mosenzon, 2013) This farther emphasizes the influence of beta cell function on designing of DM treatment protocols.

5.2 Study Limitations

The principal investigator was constrained in terms of time and finances and as such only a cross sectional design to this study was feasible. A multi centre, case control and / or prospective cohort study, with larger participant numbers would have provided more information as to the varying nature of the evolution of type 2 DM in our setting. This would provide better informed standpoints from which guidance on patient care can be derived.

This study could not be granted a mandate to standardize participants' oral hypoglycemic therapy as part of it's design. The observations made regarding glycemic control and oral hypoglycemic agents are therefore not conclusive, as it was necessary to standardize the drug regimens so as to make more valid conclusions.

CHAPTER 6

6.1 Conclusions

1. In this study, beta cell dysfunction was a more predominant factor (94.4 %) than insulin resistance (35.8%) among type 2 DM patients at Nsambya hospital, Kampala Uganda.

2. The proportion of suboptimal glycemic control was high (68 %).

3. There were no correlations between patient baseline characteristics with beta cell dysfunction nor insulin resistance.

1.2 Recommendations

1. It is recommend that case control and / or prospective cohort studies with larger type 2 DM patient numbers would provide a better understanding of the evolution of beta cell function and insulin resistance as a means of accounting for differences in individual physiology and the multi – factorial nature of dependence of glycemic control in type 2 DM.

7.0 Conflict of Interest statement

This study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix I; BETA CELL FUNTION AND INSULIN RESISTANCE IN TYPE 2 DIABETES MELLITUS

Consent form;

I …… (The participant / legally responsible next of kin) have been explained to - and understood the intent or purpose, likely investigations, possible benefits and pitfalls pertaining to this study, in which I willingly and knowingly, without any duress consent / consent my patient to participate.

I also understand and have been afforded the freedom to inquire about my / the patient's care, purpose of investigations – whether necessary or not for my care, to withdraw from the research process at any time I so wish and to bind the researcher: **Dr. Karatunga Pascal (0792603416)** to the process of my care / patient's care as due.

…… Study number; ..……

Mobile phone No.; ……

(Consenter's Name, signature or thumb print)

Olukussa;

Nze ……nyinyonyondwa netegela ekigeddelelwa n'omulamwa, okunonyeleza, okuganyulwa ne o'bowonvu ebi kwatagana no'kusoma o'kwokunonyerezza kuno kwe ne'gaseko nga sitisatisibwa oba o'kusindikirizibwa.

Ntegezedwa era n'emanyisibwa eddembe lyange o'kubuzza ku bikwatagana ku kulabirirwa kwange oba o'kwomulwadde wange, o'kubuzza emigasso eri o'kulabiribwa kwange oba o'kwomulwadde wange, emigasso gye nonyerezebwa zonna e'zinaba zikolebwa, o'kulekelawo o'kwetaba mukunonyerezebwa kuno ne o'buvunanyizibwa obwomusawo oba omunonyereza omukulu **Dr. Karatunga Pascal (0792603416)** mu kulabiribwa kwange oba o'kwomulwadde wange.

……. E'namba; ……..

E.namba ye esimu: ……

(A'manya, omukono oba e'kinkumu)

Appendix 2: BETA CELL FUNTION AND INSULIN RESISTANCE IN TYPE DIABETES MELLITUS.

Study form

Patient study number; …….. Age; ……. … Sex; ……..

Time of last meal; ……. Time of samples collection; ……

Level of education; ……

Occupation; ……

IP / DM clinic number: …….

Family history of Diabetes (tick); Yes; ☐ No; ☐

Approximated age of diagnosis of DM (>30) ...……

Date of - / time period with a known diagnosis of type 2 DM; ……

Current Anti – DM medications (Drug & it's dose);

1)…….. 2) …….

3) …… 4) …….

……

History of hormone (Estrogen) replacement or treatment(Yes / No); ……..

Duration of use of the above medications (In years)……....

Examination;

Edema; Yes; ☐ No; ☐

Jaundice; Yes; ☐ No; ☐

Conjuctival pallor; Yes; ☐ No; ☐

Truncal obesity; Yes; ☐ No; ☐

Moon fascies; Yes; ☐ No; ☐

Ascites, Yes; ☐ No; ☐

Brachial blood pressure (exceeding 160/110 mmHg): Yes ☐ No ☐

History of steroid use / features of Cushing's syndrome (Yes / No); ……

Body weight (kg); ……. Height (m2); …….

BME (kg/m2); ……..

Laboratory indices;

Urea; …….. Creatinine; **..**…… Date of RFT test; ……..

Liver function tests; …….. Date of LFT test; ……...

Last HbA1c ……. Date of last HbA1c ……

Current HbA1c …….

Fasting plasma glucose; …….

Fasting serum C – peptide titres; …….

Fasting serum Insulin = [fasting serum C-peptide (ng/ml) × 0.33 × 1000] / 7.175 = ……

HOMA2 – IR estimates = (glucose × insulin)/22.5 **= ……..**

HOMA 2 %β = (20 × fasting plasma insulin (μU/mL)/FPG (mmol) -3.5 = ……