An in-depth understanding of the clinical, metabolic, and immunologic profile of adult patients with newly diagnosed diabetes in Uganda: the Uganda Dlabetes Phenotype (UDIP) study.

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Statement of own work

I, Dr. Davis Kibirige, confirm that the entire work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed 

Davis Kibirige

Date: 16th September 2022

“ If I have seen further, it is by standing on the shoulders of giants”----------Sir Isaac Newton.

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DEDICATION

This Ph.D. thesis is dedicated to my adorable parents, Mr. Oswald Dilys Kasujja and Mrs. Gertrude Rovies Kasujja.

For inculcating in me the virtues of integrity, patience, persistence, and humility, and for inspiring me to aspire, I am eternally grateful.

Thesis abstract

Background

Despite the growing burden of type 2 diabetes in sub-Saharan Africa (SSA), its aetiopathogenesis has not been robustly investigated. The overarching aim of this thesis was to describe the clinical, metabolic, and immunologic profile of adult Ugandan patients with new-onset diabetes to develop a pragmatic approach to the categorisation of diabetes subgroups.

Methods

Relevant clinical, metabolic, and immunologic data were collected from 568 adult participants with new-onset diabetes. All participants were subjected to an assessment of markers of pancreatic function, glucose metabolism, and three islet autoantibodies. Randomly selected participants were screened for diabetic nephropathy and peripheral arterial disease.

Results

The narrative review showed that diabetes manifests differently in Africans compared to white populations of European descent.

A low prevalence of islet autoantibody positivity (6.4%) was observed and was independently associated with living in a rural area and being initiated on insulin therapy at the time of diagnosis.

Confirmed new-onset type 2 diabetes in lean individuals (negative status for islet autoantibodies and BMI <25 kg/m^2) was noted in approximately a third of participants (32%). This “lean type 2 diabetes phenotype” was associated with minimal insulin resistance, visceral adiposity, metabolic syndrome, and features of pancreatic beta-cell dysfunction predominated.

Peripheral arterial disease and diabetic nephropathy were relatively prevalent. Female sex, urine albumin creatinine ratio, and fasting blood glucose independently predicted peripheral arterial disease while hypertension comorbidity and obesity independently predicted diabetic nephropathy.

Conclusions

This research shows that pancreatic autoimmunity is an uncommon cause of adult-onset diabetes in our study population. We also showed that the lean type 2 diabetes phenotype is relatively common and is associated with reduced pancreatic beta-cell function. The prevalence of peripheral arterial disease and diabetic nephropathy was also relatively high. These study findings have broad implications for the screening, management, and prevention of diabetes in Uganda.

Acknowledgement

I am profoundly grateful to folks who lent a hand one way or the other towards the completion of this Ph.D. While they are countless, the following truly deserve individual mention by name: My esteemed Ph.D. supervisors - Professor Moffat Nyirenda and Professor Liam Smeeth of the Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, UK, and Professor Andrew Hattersley, and Associate Professor Angus Jones of the University of Exeter Medical School, Exeter UK. I am deeply indebted for the tutelage, patience, and unwavering belief in me throughout the entire course of my Ph.D. studies.

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I am also grateful to Associate Professor Silver Bahendeka and Dr. Raymond Mwebaze of the Mother Kevin Postgraduate School, Nkozi University Uganda for nurturing my passion for pursuing a career in Diabetology during the early years of my clinical career at St. Francis Hospital Nsambya, Uganda.

I would like to thank these peer mentors and colleagues - Dr. William Lumu, Dr. Anxious Niwaha, Dr. Wisdom Nakanga, Dr. Jean Claude Katte, Miss Priscilla Balungi, Dr. Richard Sanya, Dr. David Katende, Dr. David Martin Atuhe, Dr. James Apollo Kapisi, Dr. George Patrick Akabwai, Dr. Patrick Kaliika, Dr. Kenneth Luzinda, Dr. Daniel Kiggundu, and Mr. Moses Kabuye, for the moral support and camaraderie, especially during the grim times of the COVID-19 pandemic.

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“For what you have done, I will always praise you in the presence of your faithful people. And I will hope in your name, for your name is good”----------Psalms 52:9.

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List of acronyms

ABI- Ankle brachial index

BIA- Bioimpedance analysis

BMI- Body mass index

BP- Blood pressure

CI - Confidence intervals

CRF- Case report form

DKD – Diabetic kidney disease

DOHaD- Developmental origins of Health and Disease

e-GFR- Estimated glomerular filtration rate

FBG- Fasting blood glucose

GADA- Autoantibody to glutamic acid decarboxylase

HbA1c- Glycated haemoglobin

HC- Hip circumference

HDLC- High-density lipoprotein cholesterol

HT- Hypertension

HOMA2-%B- Homeostatic model assessment beta-cell function

HOMA2-IR- Homeostatic model assessment- insulin resistance

IA-2A- Autoantibody to the protein tyrosine phosphatase

IDF- International Diabetes Federation

IGI - Insulinogenic index

IQR- Inter-quartile range

KDIGO- Kidney Disease: Improving Global Outcomes

KPD- Ketosis prone diabetes

LDLC- Low-density lipoprotein cholesterol

LMIC- Low-and middle-income countries

MRC/UVRI & LSHTM- Medical Research Council/Uganda Virus Research Institute and London School of Hygiene and Tropical Medicine

OGTT- Oral glucose tolerance test

OR - Odds ratio

PAD- Peripheral arterial disease

QUICKI- Quantitative insulin sensitivity check index

RCT- Randomised controlled trial

SSA- Sub-Saharan Africa

TC- Total cholesterol

TGL- Triglycerides

UACR- Urine albumin creatinine ratio

UDIP- Uganda Diabetes Phenotype

WC- Waist circumference

WHO- World Health Organisation

WHR- Waist: hip circumference ratio

WHtR- waist circumference: height ratio

ZnT8-A- Autoantibody to zinc transporter 8

CHAPTER ONE: THESIS DESIGN AND STRUCTURE, STUDENT'S CONTRIBUTION, AND PH.D. ASSOCIATED PUBLICATIONS

This chapter describes the thesis design and structure, the student's contribution, and the publications related to this Ph.D. work.

1.0 Thesis design and structure

1.1 Thesis design

This thesis is part of the Uganda Diabetes Phenotype (UDIP) study which broadly aimed to understand the clinical, metabolic, and immunologic profile of adult patients with newly diagnosed diabetes in Uganda. I served as the principal investigator for this study and oversaw the entire processes of data collection, entry, cleaning, and analysis and drafting of all manuscripts for publication.

This Ph.D. thesis is structured to discuss in detail the data derived from the UDIP study which was a cross-sectional study aimed to comprehensively describe the clinical, metabolic, and immunologic profile of adult Ugandan patients with newly diagnosed diabetes. In addition, the thesis included a narrative review that I performed to understand the manifestation of diabetes in adult African populations, highlighting mainly the distinct diabetes phenotypes, the plausible explanations for the documented differences in diabetes phenotypes between African and white populations of European ancestry, and the related clinical and policy implications of appropriately defining the African diabetes phenotype.

1.2 Thesis structure

This thesis is made up of nine chapters that have been written in a research style format. It includes five key independent research papers which are directly related to the Ph.D. project. Despite each research paper being independent, information in the methods section (study setting, population, inclusion and exclusion criteria, and

research definitions) may be repeated across the different papers. The research papers are presented clearly to correspond with the order of the study objectives. All of these research papers have been written following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

Below is a clear elaboration of the contents of each chapter:

Chapter 1: The thesis design and structure show the general outline of the Ph.D. thesis and explain what is expected in the different chapters.

Chapter 2: The introduction chapter reviews the literature on the epidemiology of diabetes globally, in Africa, and Uganda, the burden of related cardiovascular risk factors like hypertension, overweight and obesity, alcohol consumption, and smoking in Uganda, and the key insights into the described African diabetes phenotypes, the plausible theories to explain the differences in diabetes phenotypes in white populations of European descent and African populations, and the local significance of the UDIP study.

Chapter 3: Summarises the problem statement, study rationale, aim, objectives, hypothesis, and methods.

Chapter 4: It includes the published narrative review that discussed the studies on the clinical and metabolic characteristics of diabetes in adult populations in sub-Saharan Africa, the plausible explanations for the differences in diabetes phenotypes described in white populations of European descent, and African populations, the two atypical diabetes subtypes described in African populations, and the related research gaps in the phenotyping studies performed in sub-Saharan Africa.

Chapter 5: It addresses the first objective of the thesis which is aimed to understand the burden and characteristics associated with islet autoantibody positivity as a marker of islet autoimmunity in adult Ugandan patients with newly diagnosed diabetes.

Chapter 6: It addresses the second objective of the thesis which is aimed to undertake clinical and metabolic characterisation of adult Ugandan patients with confirmed new-onset type 2 diabetes to understand the phenotypic differences between lean and non-lean individuals.

Chapter 7: It addresses the third objective of the thesis which is aimed to determine the burden and correlates of peripheral arterial disease in randomly selected adult Ugandan patients with newly diagnosed diabetes.

Chapter 8: It also addresses part of the third objective of the thesis which is aimed to investigate the burden and correlates of diabetic kidney disease in randomly selected adult Ugandan patients with newly diagnosed diabetes.

Chapter 9: The discussion is a summary of the key findings from the entire Ph.D. work and how these findings relate to existing medical literature, their implication for clinical practice in Uganda and sub-Saharan Africa, the study strengths and limitations, the suggestions for future research work, and the conclusions.

The Appendices consist of the key study documents such as the initial ethical and regulatory approvals, informed consent forms, storage consent forms, and case report forms.

1.3 Student's contribution

Since I joined the MRC/UVRI and LSHTM Uganda Research Unit as a part-time Ph.D. Fellow in August 2017, I played a pivotal role in the setting up and undertaking of the UDIP study.

1.4 Ph.D. associated publications

I. **Narrative review:**

Kibirige D, Lumu W, Jones AG, Smeeth L, Hattersley AT, Nyirenda MJ. Understanding the manifestation of diabetes in sub-Saharan Africa to inform

therapeutic approaches and preventive strategies: a narrative review. Clin Diabetes Endocrinol. 2019; 14; 5:2.

I participated in the drafting of the protocol paper, retrieval of the papers, review of full-text articles for the manuscript, identification of the eligible papers for inclusion in the narrative review, retrieving the study information of interest, drafting of the first and final versions of the manuscript, submitting the manuscript, and responded to the reviewers' comments.

II. Paper on islet autoantibody positivity

Kibirige D, Sekitoleko I, Balungi P, Kyosiimire-Lugemwa J, Lumu W, Jones AG, Hattersley AT, Smeeth L, Nyirenda JM. Islet autoantibody positivity in an adult population with recently diagnosed diabetes in Uganda. PLoS One 2022;17 (5): e0268783.

III. Paper on lean type 2 diabetes phenotype

Kibirige D, Sekitoleko I, Lumu W, Jones AG, Hattersley AT, Smeeth L, Nyirenda MJ. Understanding the pathogenesis of lean non-autoimmune diabetes in an African population with newly diagnosed diabetes. Diabetologia 2022; 65(4):675-683.

IV. Paper on peripheral arterial disease

Kibirige D, Sekitoleko I, Lumu W, Jones AG, Hattersley AT, Smeeth L, Nyirenda MJ. High frequency of severe peripheral arterial disease in adult Ugandan patients with recently diagnosed diabetes. To be submitted to the Journal of Foot and Ankle Research.

V. Paper on diabetic nephropathy

Kibirige D, Sekitoleko I, Kiggundu SD, Kalyesubula R, Lumu W, Jones AG, Hattersley AT, Smeeth L, Nyirenda MJ. Diabetic nephropathy in adult Ugandan patients with new-onset diabetes. To be submitted to BMC Endocrine Disorders.

For research papers 2, 3, 4, and 5, I fully participated in the study design, data collection, management, analysis, and interpretation. I also drafted the first version of the manuscript, incorporated all comments from the supervisors and other co-authors, submitted the final manuscript, and coordinated all the correspondence with the reviewers.

1.5 Courses attended

September 2019: Introductory Course in Epidemiology and Medical Statistics (ICEM), London School of Hygiene and Tropical Medicine, UK.

September 2017: Good Clinical Practice (GCP) training for healthcare practitioners, MRC/UVRI, and LSHTM Uganda Research Unit, Entebbe Uganda.

1.6 Conferences and workshop presentations

June 2022: 27th Uganda Diabetes Association annual scientific conference, Kampala Uganda (oral presentation).

February 2020: 5th East African Diabetes Study Group Scientific Congress, Entebbe Uganda (poster presentation).

December 2018: International Congress of Endocrinology/53rd Society of Endocrinology, Metabolism, and Diabetes of South Africa (SEMDSA) congress, Cape Town South Africa (poster presentation).

February 2018: East Africa Diabetes Study Group conference, Kigali Rwanda (oral poster).

CHAPTER TWO: BACKGROUND

2.0 Introduction

This chapter presents an overview of the global, regional, and local burden of diabetes, a broad description of the African diabetes phenotype, and the local significance of the Uganda Diabetes Phenotype study.

2.1 Burden of diabetes: a global, African, and Ugandan perspective

Globally, the burden of diabetes continues to rapidly increase to epidemic proportions, disproportionately affecting low-and middle-income countries (LMIC). The recent 2021 International Diabetes Federation (IDF) estimates show that about 537 million adults (one in ten adults), aged 20-79 years, are living with diabetes globally. This number is predicted to rise to 643 million adults by 2030 and 784 million adults by 2045. Over four in five adults with diabetes (81%) live in LMIC (1).

In the African region, the estimated number of adults living with diabetes increased from 19 million in 2019 to 24 million (1 in 22 adults) in 2021. According to future estimates, the greatest increase in the number of adults living with diabetes globally is predicted to occur in Africa (a 129% increase to 55 million adults by 2045). About 54% of adults with diabetes in Africa are undiagnosed, which is the highest among the seven IDF sub-regions (1).

This documented increase in the burden of diabetes in Africa exerts a substantial economic burden on the weak healthcare structures that are poorly structured and inadequately financed to manage diabetes and other related non-communicable diseases (2). This also has far-reaching economic implications for people living with diabetes and their immediate and/or extended families.

In Uganda, based on the findings from several community-based studies, the reported prevalence of diabetes ranges between 0.4 and 9% (3-8). This wide disparity in the

reported prevalence may be explained by the differences in study settings (rural vs urban areas), study sample sizes, and study definitions or diagnostic approaches for diabetes. The only representative general population-based study conducted in 2014 among 3689 adults using the World Health Organisation (WHO) STEP-wise methodology reported a prevalence of diabetes and impaired fasting glucose of 1.4% (95% CI 0.9– 1.9%) and 2% (95% CI 1.5–2.5%), respectively. The burden of diabetes was higher in urban areas (2.7%) compared to rural areas (1%), demonstrating an urban-rural gradient. About 50% of the study participants were unaware of their diabetes status. In this study, increasing age, male gender, households with cement or tiled floor finishing as a proxy for high socioeconomic status, and abdominal obesity assessed using waist circumference measurement were associated with increased odds of developing diabetes (8).

The above population-based study was part of the 2014 Uganda national non-communicable diseases (NCD) risk factors STEPs survey which aimed to determine the local prevalence of hypertension, diabetes, dyslipidemia, and related cardiovascular risk factors like smoking, alcohol consumption, overweight, and obesity. In this study, the documented prevalence of hypertension (defined as blood pressure ≥140/90 mmHg or use of antihypertensive therapy), overweight (defined as body mass index or BMI of 25-29.9 kg/m^2), obesity (defined as BMI ≥30 kg/m^2), history of current smoking, and alcohol ingestion was 24.3%, 14.5%, 4.6%, 11%, and 28.9%, respectively (9).

2.2 African diabetes phenotypes: the key insights

Type 2 diabetes is a heterogenous polygenic metabolic condition characterised by multiple underlying pathophysiological defects whose aetiology, manifestation, and progression greatly vary across populations (10, 11). Due to the recognised

environmental factors and genetic diversity, the diabetes phenotype in some African populations may be distinct from c in high-income countries. Clinical studies performed in some adult African populations have shown that patients with type 2 diabetes are younger at diagnosis (which may suggest early disease onset or a generally younger population in the region) and have less adiposity (a lower mean BMI and waist: hip circumference ratio). Pathophysiologically, most African patients with type 2 diabetes exhibit more features of pancreatic beta-cell secretory dysfunction as opposed to insulin resistance. The pancreatic beta-cell secretory dysfunction may manifest either as greater blunting of the acute and delayed phases of insulin secretion in response to an oral or intravenous glucose load, lower mean fasting C-peptide concentrations, insulinogenic index, or disposition index, and isolated postprandial hyperglycaemia (12-17). The predominance of pancreatic beta-cell dysfunction may explain the frequent clinical observations of lower mean BMI and diabetic ketoacidosis (regardless of the type of diabetes) at the time of diagnosis, and the early onset of secondary oral hypoglycaemic agent failure during the course of the condition.

To further highlight the distinctiveness in the manifestation of diabetes in SSA, specific atypical diabetes subtypes like fibro calculous pancreatic diabetes and Ketosis Prone Diabetes (KPD) have been described mainly in patients of African ancestry (18-21). In particular, patients with KPD present with acute severe hyperglycaemia, transient insulin deficiency, and ketoacidosis similar to what is well-described in patients with type 1 diabetes, but distinctively test negative for islet autoantibodies and also lack the HLA genetic association of type 1 diabetes. The acute severe hyperglycaemia at clinical presentation often abates with short-term insulin therapy, and most patients achieve spontaneous and prolonged normoglycaemic remissions or full recovery of

pancreatic secretory function, necessitating discontinuation of prescribed insulin or oral hypoglycaemic agents (22).

These well-described differences in diabetes phenotypes in African and white populations of European descent may broadly be explained by effects of environmental factors like chronic communicable diseases notably HIV infection and tuberculosis (23-25), early-life (in-utero and/or during early childhood) chronic malnutrition and related stressors like congenital infections (as well elaborated in the developmental origins of health and disease or DOHaD theory) (26, 27), and genetic diversity (28-30).

2.3 Local significance of the Uganda Diabetes Phenotype study

There is a growing burden of diabetes in Uganda, largely driven by rapid urbanisation, changes in lifestyle, and an aging population. Despite this growing burden, there are still limited phenotyping studies investigating the clinical, metabolic, and immunological profile of adult patients with new-onset diabetes.

The Uganda DIabetes Phenotype (UDIP) study is the first of its kind to robustly undertake clinical, metabolic, and immunologic characterisation of adult patients with newly diagnosed diabetes in Uganda. The study findings will offer additional insights into the heterogeneity of diabetes phenotypes and the underlying pathophysiology of diabetes in African populations. This will have significant implications for clinical practice and policy. The study also forms the groundwork for a future randomised controlled trial (RCT) to investigate the optimal first-line oral diabetes therapy for adult patients with new-onset type 2 diabetes in Uganda. The findings of this RCT can offer relevant local evidence that will guide appropriate individualised diabetes therapy and the formulation of local diabetes treatment guidelines in Uganda. The blood samples

obtained from these well-phenotyped patients will be archived for future biochemical, epigenetic, and genetic studies.

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CHAPTER THREE: PROBLEM STATEMENT, STUDY RATIONALE, AIM, HYPOTHESIS, OBJECTIVES, AND METHODS

3.1 Introduction

This chapter presents the problem statement, study rationale, aim, hypothesis, objectives, and methods.

3.2 Problem statement

The burden of type 2 diabetes continues to increase exponentially. Optimal individualised management of type 2 diabetes should ideally be based on an in-depth understanding of the underlying pathophysiologic mechanisms and the prevalent diabetes phenotypes in a local population.

There is limited data on the clinical, metabolic, and immunologic characterisation of adult patients with new-onset diabetes in SSA. This limits the formulation of locally relevant diabetes treatment guidelines that should be guided by clinical evidence from phenotyping studies of local populations with type 2 diabetes. The use of inappropriate diabetes treatment regimens ultimately translates to suboptimal glycaemic control and the onset and/or progression of diabetes complications.

3.3 Study rationale

The underlying pathophysiologic mechanisms of diabetes especially in adult patients with new-onset diabetes have not been robustly investigated in SSA. The few published clinical studies have notable flaws like small sample sizes, limited information on the clinical, metabolic, and immunologic characteristics, and most enrolled participants with long-standing diabetes which affects important clinical and metabolic phenotypic features like BMI, pancreatic beta-cell function, and islet autoantibody positivity (their pattern and frequency vary with increasing duration of diabetes).

The UDIP study aimed to provide robust information on the clinical, metabolic, and immunological characterisation of a cohort of adult patients with new-onset diabetes in Uganda to understand the underlying pathophysiological mechanisms of diabetes in this population and, therefore, stimulate robust interventional research in individualised diabetes therapy in our adult population.

3.4 Study aim

This study aimed to describe the clinical, metabolic, and immunologic profile of adult patients with newly diagnosed diabetes in Uganda to develop a pragmatic approach to the categorisation of diabetes subgroups based on simple clinical features.

3.5 Study hypothesis

Several local environmental factors like communicable diseases (notably HIV infection and tuberculosis), and early-life stressors like chronic malnutrition, and genetic diversity can increase the future risk of developing diabetes and also affect or modulate its expression. Because of this, we hypothesised that most of the adult patients with newly diagnosed diabetes in our population will possess unique phenotypic characteristics like low median age and BMI, the predominance of pancreatic beta-cell secretory dysfunction, and a low frequency of islet autoantibody positivity as a marker of islet autoimmunity.

3.6 Study objectives

Objective one: To determine the burden of islet autoantibody positivity as a marker of islet cell autoimmunity and associated clinical and metabolic characteristics in adult patients with newly diagnosed diabetes in Uganda.

Objective two: To undertake a detailed clinical and metabolic characterisation of adult patients with confirmed new-onset type 2 diabetes (after exclusion of those with pancreatic autoimmunity) in Uganda to understand the clinical and metabolic features

of pancreatic beta-cell secretory capacity, insulin resistance, and metabolic syndrome, in distinct emerging phenotypes defined based on BMI.

Objective three: To determine the burden and related correlates of peripheral arterial disease (PAD) and diabetic nephropathy in randomly selected adult patients with newly diagnosed diabetes in Uganda.

3.7 Study methods

3.7.1 Study design, settings, and duration

The UDIP study was a multicentre cross-sectional study that was conducted in seven tertiary public and private not-for-profit mission, or church-founded hospitals located in Central and Southwestern Uganda, serving urban, peri-urban, and rural populations. We selected these two categories of hospitals because they offer healthcare to about 85% of Ugandans. These hospitals run once-weekly outpatient adult and paediatric diabetes clinics. All attending patients are reviewed by clinical officers, medical officers, and Internists. The study was carried out from February 2019 to October 2020.

3.7.2 Study participants and recruitment method

Eligible participants were adult patients aged ≥18 years with a recent diagnosis of diabetes which was defined as a diagnosis of diabetes, regardless of type, made within three preceding months. The diagnosis of diabetes would have been made by the attending clinicians based either on fasting blood glucose (FBG), random blood glucose (RBG), and glycated haemoglobin (HbA1c) measurement at various general outpatient clinics before referral to the specialist diabetes outpatient clinics for continued specialist management. Most clinicians in Uganda diagnose diabetes based on the WHO guidelines for diagnosis of diabetes, which recommend a FBG, RBG, and HbA1c concentration of ≥7 mmol/l (126 mg/dl), ≥11.1 mmol/l (200 mg/dl) with signs

and symptoms suggestive of hyperglycaemia, and ≥6.5% (48 mmol/mol), respectively, to confirm a diagnosis of diabetes (1).

Critically ill adult patients necessitating urgent admission for medical treatment were not immediately recruited into the study at the time of presentation. They were invited to enroll in the study at least two weeks after discharge from the hospital (but still within three months following diagnosis) when they re-attended the outpatient diabetes clinics in a more stable clinical condition. Both treatment naïve and those initiated on glucose-lowering therapy were recruited in the study.

About 85% of the study participants enrolled in the study were diagnosed based on a combination of at least two recommended tests, minimising the risk of misdiagnosis or overdiagnosis. The diagnosis of diabetes mellitus was made based on an abnormal random blood glucose, fasting blood glucose, glycated haemoglobin, urine glucose, and oral glucose tolerance test in 77.9%, 21.7%, 29.9%, 16.7%, and 0.5% of participants, respectively.

Study participants were recruited consecutively at each study site until the desired sample size was attained. About 95% of participants invited to participate in the study consented to be recruited in the study.

Figure 1 below summarises the participant flow and the objectives of the UDIP study.

3.7.3 Eligibility criteria

Inclusion criteria

1. Adult men and women aged ≥ 18 years.
2. Willing to provide written informed consent.
3. Diagnosis of diabetes made within the preceding three months based on WHO guidelines of diagnosis of diabetes.

Exclusion criteria

1. Pregnant women with newly diagnosed diabetes.

3.7.5 Assessment of socio-demographic, clinical, and biophysical characteristics

All study participants were assessed after an overnight fast of ≥8 hours. Using a pre-tested case report form (CRF), the relevant socio-demographic (age at diagnosis, gender, residence, level of education, family history of diabetes, smoking, and alcohol intake status) and clinical characteristics (history of admission at diagnosis, presence of urine or serum ketones at admission, initiated diabetes and ancillary drugs, co-existing medical comorbidities) were collected by the study nurses. Following standardised study procedures, biophysical measurements which included anthropometric measurements (weight, height, waist circumference [WC], hip circumference [HC] for calculation of BMI, waist: hip circumference ratio [WHR], and waist circumference: height ratio [WHtR]) and resting blood pressure (BP) using an automated OMRON® BP machine were performed. Body composition (total body fat and visceral fat levels) was evaluated using bioimpedance analysis (BIA) with an OMRON® BF511 body composition monitor (Omron Healthcare Co. Ltd, Tokyo Japan).

The traditional WHO-defined BMI cut-offs of <25 and ≥25 kg/m^2 were used to classify the participants as lean and non-lean, respectively (2). Participants with hypertension (newly diagnosed or pre-existing) were those with a systolic BP ≥140 mmHg and/or diastolic BP ≥90 mmHg on clinical examination or a self-reported history of pre-existing hypertension either on antihypertensive therapy or treatment naïve (3).

3.7.6 Assessment of metabolic and immunological characteristics

After performing the biophysical measurements, a fasting blood sample was drawn for measurement of FBG, HbA1c, insulin, C-peptide, lipid profile, uric acid, leptin, and the

three commonly measured pancreatic autoantibodies (glutamic acid decarboxylase autoantibodies [GADA], zinc transporter 8 autoantibodies [ZnT8-A] and autoantibody to the protein tyrosine phosphatase [IA-2A]). Following this, all participants were subjected to a 75-gram oral glucose tolerance test (OGTT) and blood samples were drawn again 30 and 120 minutes after glucose ingestion to determine the serum glucose, insulin, and C-peptide concentrations at those two-time points.

Following phlebotomy, all serum and whole blood samples were initially stored at 3°C and room temperature, respectively, and then later transported to the Medical Research Council/Uganda Virus Research Institute and London School of Hygiene and Tropical Medicine (MRC/UVRI & LSHTM) Uganda Research Unit clinical chemistry laboratory where they were aliquoted and then stored at -80°C until laboratory analysis. All collected blood and urine samples were stored in the biorepository section for future analysis.

3.7.7 Laboratory measurements and assessment of markers of pancreatic beta-cell function, insulin resistance and sensitivity, and islet autoantibody positivity

All the laboratory tests were performed at the ISO-certified MRC/UVRI & LSHTM Uganda Research Unit clinical chemistry laboratory within three days of sample collection using electro-chemiluminescence immunoassays manufactured by Roche diagnostics Limited, Germany on a Cobas 6000 C-model SN 14H3-15 machine (Hitachi High Technologies Corporation, Tokyo Japan).

Fasting blood glucose was determined quantitatively from plasma using the hexokinase enzymatic principle, with a limit of detection of 0.24 -40 mmol/L while HbA1c level was determined quantitatively from whole blood by the turbidimetric inhibition immunoassay principle, with a limit of detection of 23 -196 mmol/mol (4.2-20.1%). Insulin and C-peptide concentrations were determined quantitatively from

serum using the immunoassay sandwich principle, with the limit of detection for insulin and C-peptide of 0.2-1000 u/ml and 0.999-4433 pmol/L, respectively. Lipids were measured quantitatively from serum using the enzymatic colorimetric principle, with the limits of detection for triglycerides, total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol of 0.1-10 mmol/L, 0.1-20 mmol/L, 0.1-14 mmol/L, and 0.08-3.88 mmol/L, respectively.

Pancreatic autoantibody testing was undertaken using autoantibody ELISA kits from RSR Limited (Cardiff CF14 5DU, UK) on the Dynex DS2 ELISA Robot (Dynex Technologies, Worthing, UK). The lower detection limit of this islet cell autoantibody assay at +2 standard deviations was 1.3 u/mL with an intra-assay and inter-assay precision %CV of 4.4-7.9% and 3.3-5.8%, respectively, and sensitivity and specificity of 94% and 95.6%, respectively. The islet autoantibody testing process also involved a rigorous external laboratory validation exercise with paired samples measured in duplicate for all participants at the Clinical Chemistry and Immunology laboratories, Royal Devon and Exeter NHS Foundation Trust, Exeter United Kingdom.

The online homeostatic model assessment-2 (HOMA2) calculator by the Diabetes Trial Unit of the University of Oxford, Oxford UK was used to calculate the insulin resistance (HOMA2-IR) and the pancreatic beta-cell function (HOMA2-%B) (4). Pancreatic beta-cell function was also assessed using oral insulinogenic index (IGI) that was calculated using the formula: IGI = difference between the serum insulin concentration at the 30-minute and 0-minute time point/difference between the glucose concentration at the 30-minute and 0-minute time point (5). The quantitative insulin sensitivity check index (QUICKI) was calculated from fasting serum glucose and insulin concentrations using the online QUICKI calculator (6).

Islet autoantibody positivity was confirmed present if GADA, IA-2A, and ZnT8-A levels were >34 U/ml, >58 U/ml, and >67.7 U/ml, respectively. These diagnostic thresholds were obtained after measuring archived serum samples of 600 randomly selected healthy Ugandan adults without diabetes (defined as an HbA1c <5.5% and a random blood glucose <5 mmol/l) that were enrolled in the MRC/UVRI & LSHTM Uganda Research Unit general population cohort. These diagnostic thresholds represented the 97.5th percentile (to give a 97.5% specificity) (Balungi P et al. 2021, Unpublished).

3.7.8 Baseline assessment of the diabetes complications at diagnosis

Randomly selected participants underwent screening for diabetic nephropathy and PAD as the diabetic microvascular and macrovascular complication, respectively. These two were specifically selected as the diabetes complications of choice because of the relatively easy access to the recommended screening methods in our study settings (a point-of-care urine albumin creatinine ratio machine for diabetic nephropathy and a portable automated ankle brachial index measuring device). Treatment modalities for both PAD (oral antiplatelet, anticoagulants, and statins) and diabetic nephropathy (angiotensin converting enzyme inhibitors, angiotensin II receptor blockers, and sodium-glucose cotransporters II inhibitors) are also readily available in clinical care. Lack of access to portable non-mydriatic digital fundus cameras and existing logistical challenges during the period of the COVID-19 pandemic greatly hindered us from screening participants for diabetic retinopathy.

For the screening of diabetic nephropathy, all participants recruited in the study were considered. Regarding the screening for PAD, we selected every 2nd participant recruited in the study at three randomly selected study sites (one public and two private not-for-profit tertiary hospitals).

Following clear instructions from the study nurses, the participants collected a spot mid-stream urine sample in a sterile urine container for the measurement of a spot urine albumin creatinine ratio (UACR) using Siemens Healthcare Clinitek® microalbumin strips and a point-of-care Clinitek® status analyser. A serum creatinine concentration was also determined from the collected fasting blood samples for estimation of the glomerular filtration rate (e-GFR) using the Chronic Kidney Disease Epidemiology (CKD-EPI) formula (7).

The e-GFR and UACR were classified according to the Kidney Disease: Improving Global Outcomes (KDIGO) 2020 Clinical Practice Guideline for Diabetes Management in Chronic Kidney Disease. The e-GFR was categorized as follows: G1: ≥90mL/min/1.73m^2 (normal kidney function), G2: 60–89mL/min/1.73 m^2 (mildly decreased), G3a: 45–59mL/min/1.73 m^2 (mildly to moderately decreased), G3b: 30–44mL/min/1.73 m^2 (moderately to severely decreased), G4: 15–29mL/min/1.73 m^2 (severely decreased), and G5: <15mL/min/1.73m^2 (kidney failure). Albuminuria based on the measured spot UACR was classified as follows: A1: <3mg/mmol (normal to mildly increased), A2: 3–30mg/mmol (moderately increased), and A3: >30mg/mmol (severely increased). Diabetic nephropathy was defined as the presence of a spot UACR ≥3 mg/mmol (A2 and A3), reduced e-GFR of <60 ml/min/1.73 m^2 (G3a-G5), or both (8).

After a minimum of 10 minutes of resting in a supine position, ankle brachial index (ABI) measurement to assess for the presence of PAD was performed in randomly selected participants using an automated MESI® ABI measuring device (MESI® APBI MD). Differently coloured and well-labelled cuffs in appropriate adult sizes were separately placed on each participant's left upper arm and slightly above the left and right ankle joints and the instructions for ABI measurement were closely followed by

the study nurse performing the procedure. The presence of PAD was defined as the presence of an ABI of ≤0.9 in any of the lower limbs while severe PAD was defined as an ABI ≤0.5 in either lower limb. Participants with an ABI of 0.91-0.99, 1.00-1.40, and >1.40 were considered to have borderline ABI, normal ABI, and noncompressible arteries, respectively (9).

3.7.9 Data entry, management, and quality assurance

To ensure good data integrity, the study standard operating procedures were closely followed by the study team. The study was conducted in accordance with the principles of Good Clinical Research Practice. The participant data were collected from the different study sites using CRF. These were checked daily by a trained data manager who also oversaw the quality assurance component of the study for completeness. All complete CRF were then sent to trained data clerks for data entry using the Redcap software. This data was double-entered and verified by the data entrant to identify and rectify any errors.

3.7.10 Statistical analysis plan

A data analysis plan for each of the study objectives was developed before the actual analysis. It clearly outlined the specific study outcomes, definitions, analysis strategy, and statistical analytical tests to be used. For the entire data cleaning and analysis process, I worked closely with a medical statistician. All statistical analyses were done using STATA statistical software version 15 College Station, TX: StataCorp LLC.

a. Statistical analysis plan for the thesis objective one: To determine the burden of islet autoantibody positivity as a marker of islet cell autoimmunity and associated clinical and metabolic characteristics in adult patients with newly diagnosed diabetes in Uganda.

-Study main outcome: Prevalence of islet autoantibody positivity (defined based on diagnostic thresholds derived from a cohort of adult Ugandan individuals without diabetes reflecting 97.5% specificity) based on the three measured islet autoantibodies, i.e. GADA, IA-2A, and ZnT8-A.

-Study definition: Islet autoantibody positivity was confirmed present if GADA, IA-2A, and ZnT8-A levels were >34 U/ml, >58 U/ml, and >67.7 U/ml, respectively.

-Analysis strategy: GADA, IA-2A, and ZnT8-A were measured in all the recruited participants. Based on the diagnostic thresholds, the participants were then classified into islet autoantibody-positive and negative subgroups.

-Statistics analysis tests:

The categorical and continuous variables were expressed as proportions and medians with inter-quartile range (IQR), respectively. Islet autoantibody positivity (combined and individual autoantibodies) was expressed as a frequency. The differences in the socio-demographic, clinical, and metabolic characteristics of participants with and without islet autoantibody positivity were analysed using the x^2 test for categorical data and the Kruskal Wallis test for continuous data.

b. Statistical analysis plan for the thesis objective two: To undertake a detailed clinical and metabolic characterisation of adult patients with confirmed new-onset diabetes in Uganda to compare the phenotypic features between lean and non-lean individuals.

-Study main outcome: Comparison of the sociodemographic, clinical, and metabolic characteristics of lean and non-lean participants with confirmed new-onset type 2 diabetes.

-Study definitions: Lean and non-lean participants were defined as those with BMI of <25 and ≥25 kg/m2, respectively. Participants with confirmed new-onset type 2 diabetes were those who tested negative for islet autoantibodies on assessment.

-Analysis strategy: A phenotype-focused classification approach based on islet autoantibody status was used first to identify the participants' positive and negative for the three measured islet autoantibodies (GADA, IA-2A, and ZnT8-A). Diagnostic thresholds to define islet autoantibody positivity were derived from a cohort of 600 healthy adult individuals without diabetes. These thresholds represented the 97.5th percentile (to give a 97.5% specificity), giving GADA, IA2A, and ZnT8-A positivity cut-offs of >38 U/ml, >58 U/ml, and >67.7 U/ml, respectively.

Participants that were positive for islet autoantibodies and those with missing islet autoantibody data were first excluded and the remaining participants with confirmed type 2 diabetes were then classified as lean and non-lean based on traditional WHO-defined BMI cut-offs of <25 and ≥25 kg/m^2, respectively.

-Statistical analysis tests:

The categorical and continuous variables were expressed as proportions and medians with inter-quartile range (IQR), respectively. The differences in the socio-demographic, clinical, and metabolic characteristics between the lean and non-lean participants were analysed using the x^2 test for categorical data and the Kruskal Wallis test for continuous data because most of the data was not normally distributed. Because several variables were compared between the lean and non-lean participants, the Bonferroni correction (defined as adjusted p-value =set significance α / number of variables tested) was used to adjust the p-value for multiple comparisons (10).

Statistical analysis plan for the thesis objective three: To determine the burden and related correlates of the screened diabetic complications (notably diabetic nephropathy and PAD).

Sub-study 1: Burden and correlates of PAD

-Study main outcome: Burden and correlates of PAD in randomly selected adult Ugandan patients with newly diagnosed diabetes.

-Study definitions: PAD was defined as an ABI ≤0.9 in either lower limb (9).

-Analysis strategy: The ABI was measured in a randomly selected number of participants. Based on the predefined ABI cut-offs, the participants were classified as having confirmed PAD, borderline ABI, normal ABI, and incompressible arteries. The proportions of each subgroup were determined.

-Statistical analysis tests

Proportions and medians with inter-quartile range (IQR) were used to describe the categorical and continuous variables, respectively. The differences in the socio-demographic, clinical, and metabolic characteristics between participants with and without PAD were analysed using the x^2 test for categorical data and the Kruskal Wallis test for continuous data. Multivariate logistic regression was performed to identify the independent correlates of PAD in this study population. A p-value <0.05 with a 95% confidence interval (95% CI) without 1 was considered statistically significant.

Sub study 2: Burden and correlates of diabetic nephropathy

-Study main outcome: Burden and correlates of diabetic nephropathy in randomly selected adult Ugandan patients with newly diagnosed diabetes.

-Study definitions: Diabetic nephropathy was defined as the presence of a spot UACR ≥3 mg/mmol, reduced e-GFR of <60 ml/min/1.73 m^2, or both (8).

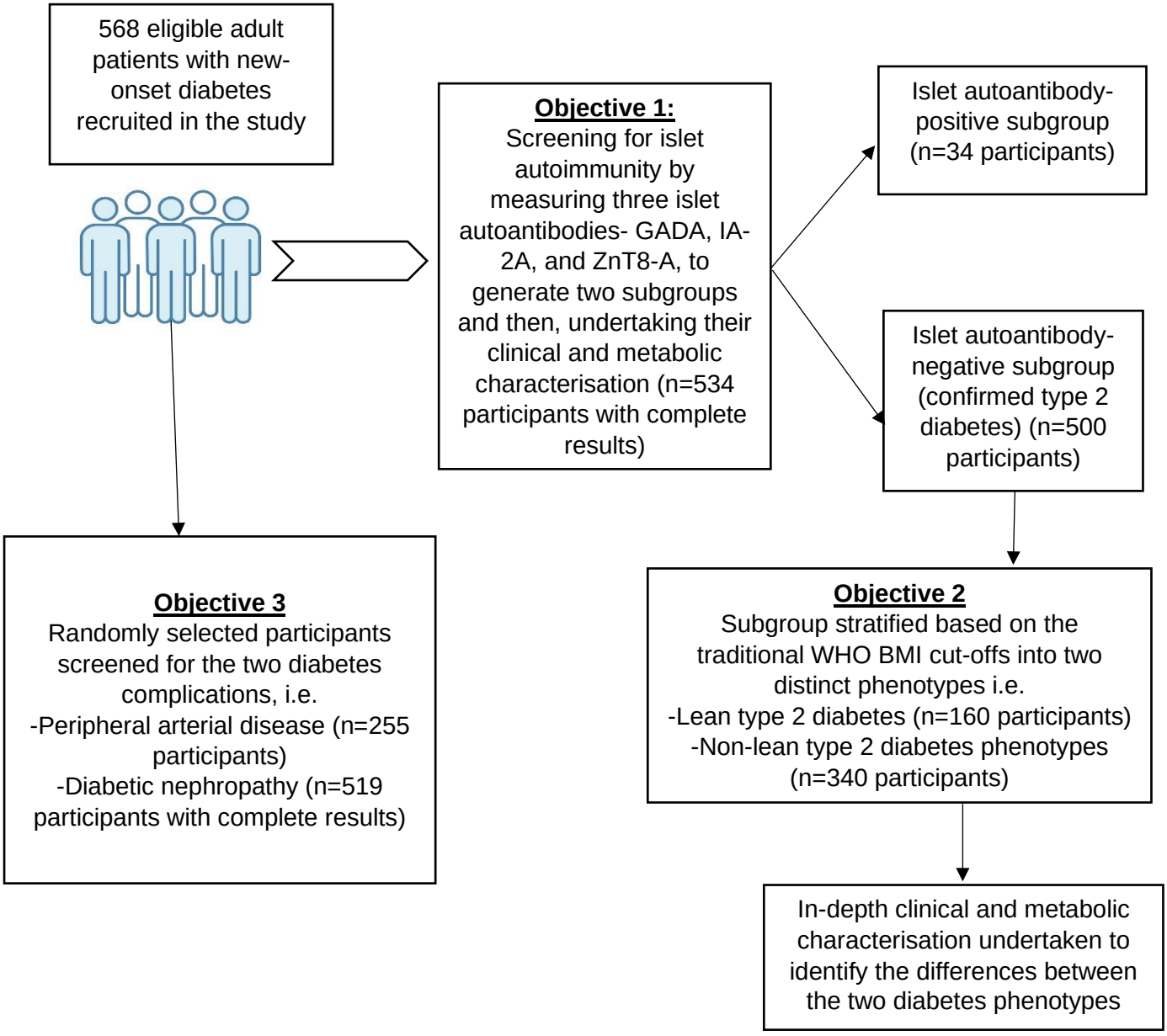
-Analysis strategy: Based on the UACR and e-GFR cut-offs, the participants with and without diabetic nephropathy were identified and proportions of each subgroup were determined.

-Statistical analysis tests: Proportions and medians with inter-quartile range (IQR) were used for categorical and continuous variables, respectively. The differences in the socio-demographic, clinical, and metabolic characteristics between participants with and without diabetic nephropathy were analysed using the x^2 test for categorical data and the Kruskal Wallis test for continuous data. Odds ratios (OR) and their corresponding 95% confidence intervals (CI) were estimated using logistic regression to identify the independent predictors of diabetic nephropathy.

3.7.11 Ethical approval

This study was approved by the Research Ethics Committee of Uganda Virus Research Centre, Entebbe Uganda on 25th May 2018 (GC/127/18/05/650) and the Uganda National Council of Science and Technology on 29th October 2018 (HS 2431). Administrative approval was also obtained from all participating study sites. All enrolled study participants provided written informed consent to participate in the study. The ethical approvals are attached in the appendix.

Figure 1: Summary of the participant flow and objectives of the Uganda Diabetes Phenotype (UDIP) study



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CHAPTER FOUR: NARRATIVE REVIEW ON UNDERSTANDING THE MANIFESTATION OF DIABETES IN SUB-SAHARAN AFRICA

This chapter presents the findings of the narrative review that I wrote summarising the published literature on the manifestation of diabetes in adult African populations.

4.1 Ph.D. research paper 1: Narrative review on understanding the manifestation of diabetes in sub-Saharan Africa

4.2 Summary of key findings

Kibirige D, Lumu W, Jones AG, et al. Understanding the manifestation of diabetes in sub–Saharan Africa to inform therapeutic approaches and preventive strategies: a narrative review. Clinical Diabetes and Endocrinology (2019) 5:2.

In this narrative review, following a literature search performed in January 2018, I reviewed the literature from the few published studies in SSA on the clinical and metabolic characterisation of type 2 diabetes, including the atypical diabetes subtypes, and also highlighted the plausible explanations for the distinct African diabetes phenotypes. On review of the published literature, the lack of studies undertaking robust clinical, metabolic, and immunological characterisation of adult African patients with newly diagnosed diabetes was an evident research gap.

The link to the publication:

https://clindiabetesendo.biomedcentral.com/track/pdf/10.1186/s40842-019-0077-8.pdf

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SECTION A: STUDENT DETAILS

Student	Davis Kibirige
ID No	LSH 1705262
Principal Supervisor	Prof. Moffat Nyirenda
Title	An in-depth understanding of the clinical, metabolic, and immunologic profile of adult patients with newly diagnosed diabetes in Uganda: the Uganda DIabetes Phenotype (UDIP) study

If the Research Paper has previously been published, please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	Clinical Diabetes and Endocrinology
When was the work published?	2019
If the work was published before registration for your research degree, give a brief rationale for its inclusion	N/A
Have you retained the copyright for the work? *	No
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Stage of publication	

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the	I participated in drafting the protocol paper, retrieval of the papers, review of full-text

paper and in the preparation of the paper. (Attach a further sheet if necessary)	articles for the manuscript, identification of the eligible papers for inclusion in the narrative review, retrieving the study information of interest, drafting the first and final version of the manuscript, submitted the manuscript, and responded to the reviewers' comments.

SECTION E

Student's signature	[redacted]
Date	31/05/2022

Primary supervisor's signature	[redacted]
Date	31/05/2022

Kibirige et al. Clinical Diabetes and Endocrinology (2019) 5:2
https://doi.org/10.1186/s40842-019-0077-8

Clinical Diabetes and Endocrinology

REVIEW ARTICLE Open Access

Understanding the manifestation of diabetes in sub Saharan Africa to inform therapeutic approaches and preventive strategies: a narrative review

Davis Kibirige[1,2*], William Lumu[3], Angus G. Jones[4], Liam Smeeth[5], Andrew T. Hattersley[4] and Moffat J. Nyirenda[1,5]

Abstract

Background: Globally, the burden of diabetes mellitus has increased to epidemic proportions. Estimates from the International Diabetes Federation predict that the greatest future increase in the prevalence of diabetes mellitus will occur in Africa.

Methods: This article reviews literature on the manifestation of diabetes in adult patients in sub-Saharan Africa highlighting the distinct phenotypes, plausible explanations for this unique manifestation and the clinical significance of comprehensively defining and understanding the African diabetes phenotype.

Results: There are few studies on the manifestation or phenotype of diabetes in Africa. The limited data available suggests that, compared to the Western world, the majority of patients with diabetes in Africa are young and relatively lean in body size. In addition, hyperglycaemia in most cases is characterised by a significantly blunted acute first phase of insulin secretion in response to an oral or intravenous glucose load and pancreatic beta cell secretory dysfunction, rather than peripheral insulin resistance predominates. Genetic and environmental factors like chronic infections/inflammation, early life malnutrition and epigenetic modifications are thought to contribute to these distinct differences in manifestation.

Conclusions: While published data is limited, there appears to be distinct phenotypes of diabetes in sub-Saharan Africa. Large and more detailed studies are needed especially among newly diagnosed patients to fully characterize diabetes in this region. This will further improve the understanding of manifestation of diabetes and guide the formulation of optimal therapeutic approaches and preventive strategies of the condition on the continent.

Keywords: Diabetes, Manifestation, Diabetes phenotype, Adult patients, Sub-Saharan Africa

Background

Burden of diabetes: Globally and in Africa

Globally, the prevalence of diabetes mellitus (DM) has reached epidemic levels especially in low and middle income countries. According to the 2017 International Diabetes Federation (IDF) estimates, about 425 million adults have DM. This figure is projected to increase to 629 million adults by 2045, which is a 48% increase [1].

Africa is estimated to have 15.9 million adults living with DM which is a regional prevalence of 3.1%. The African continent has the greatest proportion of people with undiagnosed DM and global projections show that it will experience the greatest future increase in the burden of DM of about 156% by 2045 [1].

This growing burden of DM globally and in Africa has also been demonstrated by the pooled analysis of 751 population based studies performed in 146 countries from 1980 to 2014 by the Non-Communicable Diseases Risk Factor Collaboration (NCD-RisC) [2]. The global

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age-standardized diabetes prevalence increased from 4·3% (95% CI 2.4–7.0) in 1980 to 9·0% (95% CI 7.2–11.1) in 2014 in men and from 5% (95% CI 2.9–7.9) to 7.9% (95% CI 6.4–9.7) in women and worldwide, the number of adults with diabetes increased from 108 million in 1980 to 422 million in 2014. North Africa was one of the regions with the highest age standardized diabetes prevalence [2].

According to findings from the NCD-RisC Africa working group that analysed pooled data of 76 surveys (182,000 participants) from 32 countries performed between 1980 and 2014, the age standardized prevalence of DM increased from 3.4% (1.5–6.3) to 8.5% (6.5–10.8) in men, and from 4.1% (2.0–7.5) to 8.9% (6.9–11.2) in women [3]. The burden of DM was mostly higher in the Northern and Southern regions and a positive association was observed between mean body mass index (BMI) and diabetes prevalence in both sexes during that period [3].

The increasing dual burden of non-communicable diseases (NCD) like DM and communicable diseases such as HIV and tuberculosis puts a significant economic strain on the existing resource constrained health systems in sub-Saharan Africa (SSA). It also has huge economic implications for patients and their immediate families. It will therefore be crucial to fully understand how DM manifests in Africa to formulate and implement effective targeted preventive strategies and optimal management to reduce diabetes related morbidity and mortality.

Methods

We searched PubMed, Google scholar, Scopus and African Journal Online databases for any published review articles, case reports and original research articles, regardless of year of publication that reported information about the manifestation of diabetes in adult patients in SSA emphasising mainly the reported distinct phenotypes. References of the identified publications were searched for more research articles to include in this narrative review.

The search terms used were: "manifestation of diabetes" OR "diabetes phenotypes" OR "presentation of diabetes" OR "characteristics of diabetes" OR "atypical diabetes" AND "Africa" OR "sub-Saharan Africa".

We excluded research articles published in languages other than English and whose full texts were not accessible.

A total of 16 original articles, review articles and case reports containing information about the distinct diabetes phenotype in SSA were included in this narrative review [4–19].

Results

Manifestation of diabetes in sub Saharan Africa: Metabolic and immunologic characterization and atypical forms of diabetes

Both type 1 DM (T1DM) and type 2 DM (T2DM) are heterogeneous diseases that are characterized by a constellation of metabolic disorders and vary considerably in clinical presentation and disease progression [20].

A number of reports show that compared to high-income countries, the majority of adult patients diagnosed with DM in sub-Saharan Africa (SSA) are of median age of < 50 years (signifying early disease onset), lean body size (have low or normal BMI) and pancreatic beta cell secretory dysfunction characterised by a significantly blunted acute first phase of insulin secretion in response to an intravenous or oral glucose load predominates rather than peripheral insulin resistance [4–12].

This is in contrast with evidence reported from the Western world where DM appears to develop later in life (> 50 years), is common among overweight or morbidly obese individuals and increased IR, hyperinsulinaemia with progressive pancreatic beta cell secretory dysfunction occurring later in the course of the disease, as the hallmarks of DM [21, 22].

The observed predominant pancreatic beta cell secretory dysfunction might explain why DM in Africa is commonly associated with low BMI, keto-acidosis, rapid decline in fasting plasma C-peptide concentrations and early onset of secondary oral agent failure following diagnosis. The reasons for the above discussed differences in manifestation or diabetes phenotype are unclear, but may reflect genetic diversity and unique environmental factors in Africa.

Currently, there is a paradigm shift in the classification of DM from the definition based on need of insulin therapy to achieve euglycaemia and age of disease onset or diagnosis to a definition based on an in-depth definition of the underlying aetiopathogenetic mechanisms of hyperglycaemia [23]. This underscores the need to comprehensively describe and understand the phenotype of diabetes in SSA. This would help in guiding optimal and individualised management of patients with DM in clinical practice.

Clinical and biochemical characterisation of diabetes in adult diabetic populations in sub-Saharan Africa

There have been very few published studies that have thoroughly investigated the clinical, metabolic and immunologic profile of African patients with DM. Despite this limitation, the available studies performed to demonstrate the distinct diabetes phenotype in SSA offer useful insights into the clinical and biochemical profile particularly with regard to the frequency of insulin resistance, beta cell secretory dysfunction and presence of pancreatic auto-immunity.

In one cross sectional study of 105 adult patients with DM conducted in the Tigray region of Northern Ethiopia (a semi-arid region), the mean age and median BMI was 41 ± 16 years and 20.6 (18.5–23.9) kg/m^2 respectively [5], highlighting a young age at diagnosis and

lean body size. Insulin deficiency expressed as C-peptide negative status on examination was reported in 43% of the patients with 28 and 3.8% of patients positive for glutamic acid decarboxylase antibodies (GADA) and islet antigen 2 antibody (IA-2A) tests respectively. About 38 (36%) patients had immunological and C-peptide characteristics that were not consistent with the classical T1DM and T2DM phenotypes, despite having clinical features similar to patients with T1DM (similar median age at diagnosis, glycated haemoglobin level and BMI). GADA positivity and C-peptide negativity in this sub-group was confirmed in 29 and 71% respectively [5]. This underscores the presence of diabetes phenotypes in SSA that may not fit the conventional classification of DM.

In another case control study that assessed the degree of basal insulin resistance (IR) and insulin secretion (IS) using the homeostatic model assessment (HOMA) among 146 patients with T2DM and 33 healthy controls performed in an urban hospital in Nigeria, IR and reduced IS prevalence among the T2DM patients was 95.5 and 74.7% respectively [7], demonstrating a high dual burden of IR and pancreatic beta cell secretory dysfunction. However, approximately 85% of the patients in this study were obese, which could explain the higher prevalence of IR. The independent predictors of IR in this study were age at diagnosis, waist circumference and duration of DM and those for reduced IS, duration of DM and waist circumference [7]. While the duration of DM in these patients was not reported, the prevalence of beta cell secretory dysfunction reported could probably have arisen as a result of beta cell exhaustion occurring as the disease progresses.

Another similar small study conducted in Nigeria comparing 40 patients with T2DM to 36 healthy controls reported an IR (defined as HOMA1-IR values > 1) prevalence of 87.5% in patients and 27.8% in the controls [8]. When a HOMA1-IR score ≥ 2 was used, IR was prevalent in 40% of the patients and 19.4% of the controls.

Two studies reported from Ghana reported severe pancreatic beta cell secretory dysfunction among a small adult population with DM [9, 10]. Amoah A et al. in their study that compared 15 healthy controls without family history of DM (group 1) with 11 healthy controls with first degree family history of DM (group 2) and 10 patients with T2DM (group 3) found that group 3 had severely blunted acute phases of insulin secretion following an intravenous glucose load as measured by the absolute and incremental area under the curve [10]. The mean acute first phase insulin secreted in the 1st, 2nd and 3rd group was 122 ± 75, 320 ± 11.7 and 7.8 ± 5.7 mU/l x minutes respectively with insulin sensitivity index lowest in the diabetic group [10].

Plausible explanations for recognised distinct manifestation of diabetes in SSA

The African continent harbors the highest genetic heterogeneity. It is also unique because it continues to have a high burden of infectious diseases and other challenges like famine, civil strife and malnutrition. These exposures might modulate the pathogenesis and clinical course of NCD like DM, as summarized in Fig. 1.

There are clearly defined potential causes of the well documented distinct diabetes phenotype in SSA, while the others are less well investigated.

Chronic infections and chronic pro-inflammatory state

Chronic infections notably HIV and tuberculosis (TB) that are highly endemic in SSA provoke a state of chronic inflammation which may play an important role in the pathogenesis of DM and other NCD, with proposed mechanisms including increased oxidative stress, vascular endothelial dysfunction and DNA damage [24]. This inflammatory cascade can lead to premature aging of key homeostatic organs like the pancreas and progressive decline of physiological functions. This eventually results into early onset of cardio-metabolic disorders like DM.

The pro-inflammatory state associated with infections like TB is also linked to increased release of counter regulatory stress hormones like cortisol and epinephrine which cause reactionary hyperglycaemia due to their antagonistic effects to insulin [25, 26]. The consequent reactionary hyperglycaemia can persist after TB treatment. The immune activation associated with HIV infection is also associated with a state of peripheral insulin resistance and reduced insulin secretion, hence increasing the risk of DM and metabolic syndrome at an early age [27].

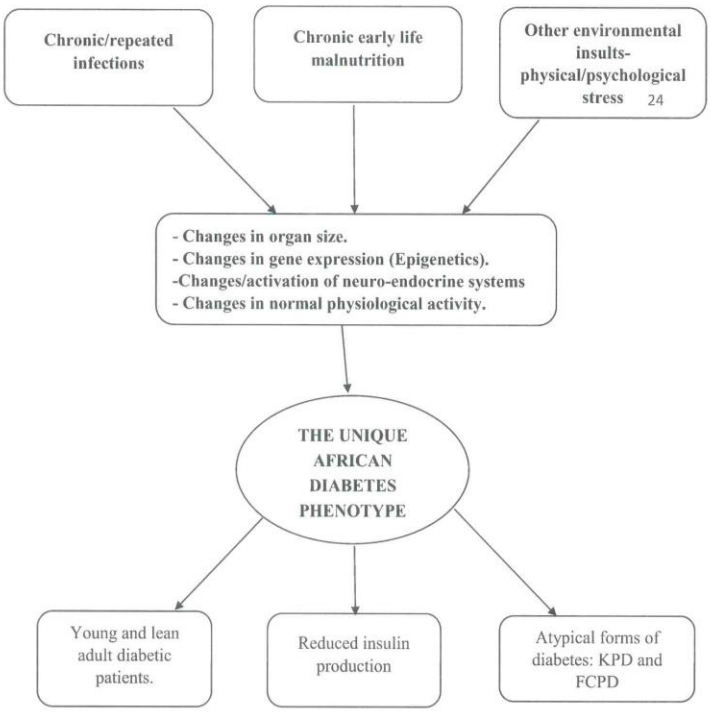
Infection induced hypersensitivity reaction/autoimmunity

Exaggerated immune responses (hypersensitivity reaction) and molecular mimicry as seen with some chronic infections have been implicated in pathogenesis of autoimmune conditions with subsequent organ dysfunction occurring especially in genetically predisposed individuals [28–30]. The underlying mechanisms explaining the hypersensitivity and autoimmunity induced by chronic infections include presence of antigenic mimicry, neo-antigen formation and immune dysregulation of the host [28].

Autoimmune destruction of pancreatic beta cells induced by enteroviruses like coxsackie B virus, rubella virus, rotavirus, flavivirus, herpes virus, rhinovirus and retrovirus have long been implicated as infectious aetiologies of T1DM [29–33].

Direct organ damage by the pathogen

Direct pancreatic beta cell damage with subsequent endocrine dysfunction has also been highlighted as a



KPD: Ketosis Prone Diabetes, FCPD: Fibrocalculous pancreatic diabetes.

Fig. 1 Plausible explanations for the distinct diabetes phenotype in sub Saharan Africa. KPD: Ketosis Prone Diabetes, FCPD: Fibrocalculous pancreatic diabetes

potential mechanism of how infections can cause DM, impaired glucose tolerance at an early age and pancreatic beta cell secretory dysfunction. Tuberculous destruction of the beta and alpha cells of the pancreas, for example is often associated with brittle DM which is frequently characterised by labile blood glucose levels [25]. A close association has also been described between human herpes virus type 8, a virus endemic in the tropics responsible for causing Kaposi sarcoma and an atypical form of DM, ketosis prone diabetes (KPD) [13]. It is thought that it causes endoplasmic reticulum stress and direct destruction of the pancreatic beta cells which results in reduced insulin secretion [13].

Effect of treatment of the infections

The HIV epidemic in SSA, coupled with increased access to antiretroviral therapy (ART) and longer life expectancy of HIV infected patients have been associated with development of multiple metabolic derangements [34] and could also partly explain the distinct phenotypes of diabetes in SSA.

Anti-retroviral therapy notably efavirenz and protease inhibitors have been shown to increase the risk of dysglycaemia [35–37]. Unequivocal evidence shows that these classes of ART cause mitochondrial dysfunction, significant changes in body fat distribution (increased central adiposity and peripheral lipoatrophy) and reduce cellular uptake of glucose by impairing the activity of the glucose transporter-4 [27, 36–39].

Rifampicin, one of the potent anti TB drugs is also associated with a transient hyperglycaemia that may persist during and after treatment because of its effect of augmenting intestinal glucose absorption [25].

Chronic prenatal and postnatal malnutrition (thrifty phenotype hypothesis)

Chronic prenatal and postnatal malnutrition frequently seen in most parts of SSA especially in the rural areas is a recognised precursor for DM and other NCD and could also explain the distinct diabetes phenotypes seen in SSA. The link between perinatal malnutrition during periods of famine and increased odds of developing DM

later in life has been demonstrated in studies reported from Ukraine [40] and Ethiopia [41].

The thrifty phenotype hypothesis which was described several decades ago by Nicholas Hales and David Barker expounds on the link between chronic perinatal malnutrition which manifests as low birth weight and failure to thrive with subsequent development of NCD like T2DM in adulthood [42]. One hypothesis to explain the future development of T2DM in patients with a history of early life malnutrition is possibly impaired development, innervation and function of pancreatic beta cell mass and islet of Langerhans [42, 43]. Fetal malnutrition exacerbates the risk of IR and obesity later in life in cases of reversed (nutrient rich) environment and positive calorie balance due to increased food intake and decreased energy expenditure [44].

This increased susceptibility to NCD like DM due to early life exposure and changes in adult lifestyles due to globalisation is what is essentially seen in SSA.

Vitamin D deficiency which is caused by malnutrition and other factors like chronic infections like TB, HIV and dark skin pigmentation may also explain the increased the odds of developing DM and the unique manifestation in SSA [45]. Some of the integral roles of vitamin D are increasing pancreatic beta cell production of insulin by increasing intra-cellular calcium concentrations, activation of intra cellular endopeptidases that cleave pro-insulin to insulin and by preventing inflammatory damage of pancreatic beta cells [46].

Epigenetic modifications

Findings from both animal studies and recent large scale human epigenome wide associated studies show epigenetics as the common link between genome and environmental factors like chronic malnutrition and the development of DM [47–49]. These could explain the uniqueness in diabetes phenotypes in SSA.

According to the Developmental Origins of Health and Disease fetal origins of adult disease hypothesis, in-utero fetal programming induced by exposures to malnutrition, stress and fetal infections like malaria and toxoplasmosis, rubella, CMV, herpes simplex and syphilis (TORCHES) results in short and long term adaptations which are partly mediated by epigenetic changes. These adaptations are essentially to ensure fetal survival [50].

Epigenetic changes ranging from DNA methylation, histone modification and noncoding RNAs occur during development, are transmitted from cell to cell (mitotic inheritance) or generation to generation (transgenerational epigenetic inheritance) and cause alteration of gene expression, cellular growth, composition and physiology [51].

These epigenetic changes result in simple organ failure (reduction in cellular size and number), alteration in endocrine systems (upregulation of the hypothalamic-pituitary-adrenal axis and changes in secretion and sensitivity to insulin and insulin-like growth factor-1) and changes in expression and regulation of DNA [52]. Epigenetic modifications that result in reduced pancreatic beta cell mass and function coupled with changes in cellular insulin signaling, reduced muscle mass and increased adiposity lead to an increased likelihood of development of DM and could partly explain the unique diabetes phenotype seen in SSA.

Changes or activation of the neuro-endocrine systems

Environmental insults such as maternal infection, stress and malnutrition have been shown to activate the hypothalamus pituitary adrenal axis with resultant increase in the expression of the glucocorticoid receptors, dampening of the hypothalamic negative feedback mechanism and increased production of stress hormones (glucocorticoids) by the adrenal glands [53, 54].

Studies in rat models have demonstrated that high fetal glucocorticoid levels due to stressful states or environmental insults like malnutrition attenuate the expression and activity of the placental enzyme 11β-hydroxysteroid dehydrogenase type-2 that is key in modulating fetal exposure to glucocorticoids [55, 56]. The downregulation of this enzyme subsequently is associated with early onset of glucose intolerance, hypertension and other cardiovascular diseases in adulthood.

Atypical forms of diabetes in Africa: Fibrocalculous pancreatic diabetes and ketosis prone diabetes

The distinctiveness in the diabetes phenotype in SSA as explained by the discussed factors above is further emphasized by the presence of these 2 unique atypical forms of the diabetes i.e. KPD and fibrocalculous pancreatic diabetes (FCPD) which have been described particularly among patients of African ancestry [12, 14–18]. Despite being exclusively described in African populations, we lack population based studies in the region investigating the prevalence of these atypical sub-types.

Ketosis prone diabetes (KPD)

Patients with KPD present with acute severe hyperglycaemia and keto-acidosis but, in contrast to classic T1DM lack pancreatic islet beta cell auto antibodies or a genetic association with HLA [19]. The defects in pancreatic beta cell function and insulin sensitivity at presentation in this condition remarkably improve with insulin therapy, and many patients can discontinue insulin following treatment of the acute episode, with near normoglycaemic remission that may last from months to years [19]. This insulin free period in patients with KPD is similar to the well described "honeymoon period" seen in patients with T2DM [57] and T1DM [58], which is a drug free period with observed sustained optimal

glycaemic control. This clinical observation is explained by the resumption of endogenous insulin production by the pancreatic beta cells after glucotoxicity is resolved following acute intensive insulin therapy [59].

Fibrocalculous pancreatic diabetes (FCPD)

FCPD is one of the unique forms of malnutrition related DM that develops secondary to non-alcoholic chronic calcific pancreatitis and has been widely described in the tropical developing countries in SSA [14, 18]. It is diagnosed mainly among young patients presenting with abdominal pain, radiological confirmation of pancreatic calcification and features of pancreatic exocrine (steatorrhea) and endocrine (severe hyperglycaemia) insufficiency [60]. Patients with FCPD present with early disease onset (< 30 years), an associated male preponderance (70%), are underweight, of a low socio-economic status and have severe hyperglycaemia that requires insulin therapy in low doses to achieve euglycaemia [18, 60]. Diabetic keto-acidosis rarely develops following insulin withdrawal because of partial preservation of pancreatic beta cell function as evidenced by relatively normal C-peptide levels, low glucagon levels and decreased adipose tissue mass [60].

Discussion

The current paradigm shift in the classification of DM underscores the need to understand the underlying pathophysiologic mechanisms of hyperglycaemia because of their potential therapeutic implications [23]. Evidence based management of DM recommends the use of combination therapy based on the defined underlying pathophysiological abnormalities or defects to optimize management [23, 61].

From an African perspective, an in-depth understanding of the underlying pathophysiologic defects and manifestation of DM offers important insights about the optimal drug combinations or prevention strategies for management and prevention of DM in this specific patient population. These insights can also offer a platform for future interventional studies to investigate which pharmacotherapy (monotherapy or in combination) would be optimal in managing hyperglycaemia, preservation of beta cell function or retarding progressive beta cell failure in an African adult population with DM, and may inform lifestyle advice and other strategies to prevent or screen for diabetes.

Most clinicians in SSA manage their adult patients with DM basing on guidelines developed by international diabetes associations like American Diabetes Association [23]. These international guidelines are developed based on evidence from phenotyping studies of Caucasian or mixed ancestry populations, and may therefore not be applicable and need to be cautiously extrapolated to other populations.

An example is the recommendation to use metformin as the first line therapy in the management of DM in adult patients [23]. Metformin is currently the first choice of therapy for T2DM in international guidelines and it is the most commonly used first line therapy in clinical practice in SSA. While there is clear evidence for metformin being the optimal therapy in obese western populations with T2DM, there is no evidence to show this is the optimal first line treatment in African populations who have a completely different phenotype.

Conclusion

To address the paucity of clinical studies describing the diabetic phenotype of native African patients, more large studies comprehensively assessing the clinical and metabolic profile of adult patients especially among newly diagnosed patients with DM are warranted to further improve the understanding of the manifestation of DM in SSA. This will be key in informing the formulation of optimal therapeutic options and targeted preventive strategies for DM that are individualised for the African population.

Abbreviations

BMI: Body mass index; DM: Diabetes mellitus; FCPD: Fibrocalculous pancreatic diabetes; GADA: Glutamic acid decarboxylase antibodies; GC: glucocorticoid; HOMA: Homeostatic model assessment; IA-2A: Islet antigen 2 antibody; IDF: International Diabetes Federation; IR: Insulin resistance; IS: Insulin secretion; KPD: Ketosis prone diabetes; NCD: Non-communicable diseases; NCD-RisC: Non-Communicable Diseases Risk Factor Collaboration; SSA: Sub-Saharan Africa; T1DM: Type 1 DM; T2DM: Type 2 DM; TB: Tuberculosis

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Authors' contributions

DK and MJN conceived the concept of writing this review article. DK performed the literature review and drafted the initial manuscript. WL, AGJ, LS, ATH and MJN appraised the initial manuscript, contributed more literature to it and approved the final manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

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Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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CHAPTER FIVE: ISLET POSITIVITY IN ADULT UGANDAN PATIENTS WITH NEW-ONSET DIABETES

This chapter presents the findings of the first objective of the Ph.D. aimed to determine the frequency of islet autoantibody positivity and its associated clinical and metabolic characteristics in our adult cohort with new-onset diabetes.

5.1 Ph.D. research paper 2: Burden and associated characteristics of islet autoantibody positivity in adult Ugandans with new-onset diabetes

5.2 Summary of key findings

Kibirige D, Sekitoleko I, Balungi AP, et al. Islet autoantibody positivity in an adult population with newly diagnosed diabetes in Uganda. PLoS One 2022;17 (5): e0268783.

In this study, we measured three common islet autoantibodies (GADA, IA-2A, and ZnT8-A) in 534 adult patients with new-onset diabetes to identify the participants with islet autoantibody positivity as a marker of islet autoimmunity and its associated characteristics. We documented a low frequency of islet autoantibody positivity in this study population (6.4%), signifying that pancreatic autoimmunity is an uncommon cause of adult-onset diabetes in Uganda. Living in a rural area and initiation on insulin therapy at the time of diagnosis of diabetes were independent predictors of islet autoantibody positivity.

The link to the publication:

https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0268783

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ID No	LSH 1705262
Principal Supervisor	Prof. Moffat Nyirenda
Title	An in-depth understanding of the clinical, metabolic, and immunologic profile of adult patients with newly diagnosed diabetes in Uganda: the Uganda DIabetes Phenotype (UDIP) study.

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SECTION B – Paper already published

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	and coordinated all the correspondence with the reviewers.

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PLOS ONE

RESEARCH ARTICLE

Islet autoantibody positivity in an adult population with recently diagnosed diabetes in Uganda

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Abstract

Aims

This study aimed to investigate the frequency of islet autoantibody positivity in adult patients with recently diagnosed diabetes in Uganda and its associated characteristics.

Methods

Autoantibodies to glutamic acid decarboxylase-65 (GADA), zinc transporter 8 (ZnT8-A), and tyrosine phosphatase (IA-2A) were measured in 534 adult patients with recently diagnosed diabetes. Islet autoantibody positivity was defined based on diagnostic thresholds derived from a local adult population without diabetes. The socio-demographic, clinical, and metabolic characteristics of islet autoantibody-positive and negative participants were then compared. The differences in these characteristics were analysed using *the* x^2 test for categorical data and the Kruskal Wallis test for continuous data. Multivariate analysis was performed to identify predictors of islet autoantibody positivity.

Results

Thirty four (6.4%) participants were positive for ≥1 islet autoantibody. GADA, IA-2A and ZnT8-A positivity was detected in 17 (3.2%), 10 (1.9%), and 7 (1.3%) participants, respectively. Compared with those negative for islet autoantibodies, participants positive for islet autoantibodies were more likely to live in a rural area (n = 18, 52.9% Vs n = 127, 25.5%, p = 0.005), to be initiated on insulin therapy (n = 19, 55.9% Vs n = 134, 26.8%, p<0.001), to have a lower median waist circumference (90 [80–99] cm Vs 96 [87–104.8], p = 0.04), waist circumference: height ratio (0.55 [0.50–0.63] vs 0.59 [0.53–0.65], p = 0.03), and fasting C-

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peptide concentration (0.9 [0.6–1.8] Vs 1.4 [0.8–2.1] ng/ml, p = 0.01). On multivariate analysis, living in a rural area (odds ratio or OR 3.62, 95%CI 1.68–7.80, p = 0.001) and being initiated on insulin therapy (OR 3.61, 95% CI 1.67–7.83, p = 0.001) were associated with islet autoantibody positivity.

Conclusion

The prevalence of islet autoantibody positivity was relatively low, suggesting that pancreatic autoimmunity is a rare cause of new-onset diabetes in this adult Ugandan population. Living in a rural area and being initiated on insulin therapy were independently associated with islet autoantibody positivity in this study population.

Introduction

Autoimmune diabetes in European and Asian populations occurs throughout life with at least half of it occurring in adults [1–4]. Islet autoantibodies as markers of beta-cell autoimmunity may be detected in adults with clinically presumed new-onset type 2 diabetes. This category of patients is often described as latent autoimmune diabetes in adults (LADA) and possesses phenotypic features that are difficult to distinguish from type 2 diabetes at diagnosis in absence of pancreatic autoantibody testing [5, 6]. It is important to note that a proportion of these LADA cases may represent false-positive test results given the imperfect specificity of the islet autoantibody assays [6–8].

In resource-constrained settings like sub-Saharan Africa (SSA), screening for common islet autoantibodies like antibodies to glutamic acid decarboxylase-65 (GADA), zinc transporter 8 (ZnT8-A), tyrosine phosphatase (IA-2A), and insulin (IAA) is not feasible in routine clinical practice. Diagnosis of type 1 diabetes is usually based on the presence of distinctive clinical features like low body mass index (BMI) and age at diagnosis, ketosis, and other features of insulin deficiency. This poses a major diagnostic challenge because SSA is known to harbour atypical diabetes phenotypes that manifest with similar clinical features, such as the presence of type 2 diabetes in relatively lean individuals, fibro calculous pancreatic diabetes, and ketosis-prone diabetes [9, 10].

The true prevalence and features associated with islet autoantibody positivity in adult patients with clinically presumed new-onset type 2 diabetes in SSA are largely unknown. Most studies have enrolled individuals with long-standing diabetes, which can influence the frequency and pattern of islet autoantibodies detected [11–20]. Indeed, in most cases, only one islet autoantibody (commonly GADA) has been measured, with the potential to underestimate the prevalence of pancreatic autoimmunity. Conversely, in most studies, islet autoantibody positivity has been defined based on manufacturer's cut-offs (instead of the local population) which, in settings with low background autoimmunity, may result in low test specificity and high false-positive rates [6–8].

In addition, few studies in SSA have reported detailed information on the correlation between islet autoantibody positivity and key metabolic characteristics like optimum markers of pancreatic beta-cell function (either oral insulinogenic index, fasting or 120-minute C-peptide concentration or homeostatic model assessment 2- beta-cell function/HOMA2-%B) and insulin resistance or sensitivity (HOMA2-insulin resistance and QUICKI) [11, 12, 15, 18, 20].

To address these gaps, we undertook the Uganda DIabetes Phenotype (UDIP) study in which we measured three common islet autoantibodies (GADA, IA2A, and ZnT-8A) to screen

for pancreatic autoimmunity in patients with recently diagnosed adult-onset diabetes in Uganda. We compared socio-demographic, clinical, and metabolic (including insulin secretion and resistance indices) characteristics between antibody-positive and antibody-negative participants to identify characteristics independently associated with islet autoantibody positivity.

Materials and methods

Study sites and duration

The study participants were recruited from outpatient diabetes clinics of seven tertiary public and private not-for-profit (PNFP) mission or church-founded hospitals located in Central and Southwestern Uganda. These particular hospitals serve urban, semi-urban, and rural populations, and most of the patients self-refer to these hospitals for chronic disease management, with a minority being referred from the lower-tier healthcare centres. All patients aged ≥18 years with new-onset and long-standing diabetes are usually managed and followed up at the outpatient diabetes clinics of these tertiary hospitals. About 85% of Ugandans receive medical treatment from public and PNFP hospitals.

For this study, participants that reside in areas legally designated as cities, towns, and municipalities by the Ministry of Lands, Housing, and Urban Development of the Republic of Uganda were classified as an urban population while those living outside the designated cities, towns, and municipalities were classified as a rural population. All participants living in peri-urban areas (within an arbitrary 10-kilometre radius from a city, town, or municipal) were considered an urban population.

The study was carried out from February 2019 to October 2020.

Study participants

We recruited 534 participants aged ≥18 years with recently diagnosed diabetes (diabetes diagnosed within the preceding three months) of any type from the diabetes outpatient clinics in Uganda. Both treatment naïve and patients on glucose-lowering therapy were included. The diagnosis of diabetes would have been made by clinicians at the different general outpatient clinics based on either fasting blood glucose (FBG) concentration ≥7 mmol/l, random blood glucose concentration ≥11.1 mmol/l with signs and symptoms suggestive of hyperglycaemia, or glycated haemoglobin (HbA1c) concentration ≥6.5% (48 mmol/mol) as recommended by the World Health Organisation guideline on the diagnosis of diabetes [21].

After a diagnosis of diabetes is made, patients are usually then referred to the outpatient diabetes clinics for further management. All recruited study participants were black Africans of Ugandan origin. Pregnant women were excluded. Critically ill patients that required urgent hospitalisation were not immediately recruited at the time of presentation but were eligible if they re-attended the clinic in a stable condition within three months of diagnosis.

Assessment of socio-demographic, clinical, and biophysical characteristics

All study participants were assessed after an overnight fast of ≥8 hours. Using a pre-tested study questionnaire and standardised study procedures, we collected relevant socio-demographic (age at diagnosis, gender, and residence) and clinical data (history of admission at diagnosis, presence of urine or serum ketones at admission, and diabetes therapies initiated at diagnosis), and undertook biophysical measurements including resting blood pressure and anthropometric measurements (weight, height, waist circumference [WC], hip circumference [HC]—for calculation of body mass index [BMI], waist: hip circumference ratio [WHR] and

waist circumference: height ratio [WHtR]). Body composition (total body fat and visceral fat levels) was evaluated using bioimpedance analysis with an OMRON® BF511 body composition monitor.

Assessment of metabolic characteristics and laboratory measurements

A fasting blood sample was collected to measure blood glucose (FBG), HbA1c, insulin, C-peptide, lipid profile, and the three islet autoantibodies (GADA, ZnT8-A, and IA-2A). This was followed by a 75-gram oral glucose tolerance test (OGTT), with blood samples drawn again 30 and 120 minutes after glucose ingestion to determine the serum glucose, insulin, and C-peptide concentrations at those two time-points. The blood samples were collected in serum separating tubes (SST) II and ethylenediamine tetra-acetic acid (EDTA) tubes and immediately kept at 3˚C and room temperature, respectively at each study site before being transported to the clinical chemistry laboratory. Similar temperatures were maintained during transport. On arrival at the clinical chemistry laboratory, the samples were aliquoted and stored at -80˚C.

All these laboratory tests were performed at the ISO-certified clinical chemistry laboratory at Medical Research Council/Uganda Virus Research Institute and London School of Hygiene and Tropical Medicine Uganda Research Unit, Entebbe Uganda within three days of sample collection using electro-chemiluminescence immunoassays manufactured by Roche diagnostics Limited, Germany on a Cobas 6000 C-model SN 14H3-15 machine (Hitachi High Technologies Corporation, Tokyo Japan).

Fasting blood glucose concentration was determined quantitatively from plasma using the hexokinase enzymatic principle, with a limit of detection of 0.24–40 mmol/L while HbA1c concentration was determined quantitatively from whole blood by the turbidimetric inhibition immunoassay principle, with a limit of detection of 23–196 mmol/mol (4.2–20.1%). Insulin and C-peptide concentrations were determined quantitatively from serum using the immunoassay sandwich principle, with the limit of detection for insulin and C-peptide of 0.2–1000 u/ml and 0.999–4433 pmol/L, respectively. Lipids were measured quantitatively from serum using the enzymatic colorimetric principle, with the limits of detection for triglycerides, total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol of 0.1–10 mmol/L, 0.1–20 mmol/L, 0.1–14 mmol/L, and 0.08–3.88 mmol/L, respectively.

Pancreatic autoantibody testing was undertaken using autoantibody ELISA kits from RSR Limited (Cardiff CF14 5DU, UK) on the Dynex DS2 ELISA Robot (Dynex Technologies, Worthing, UK). The lower detection limit of this islet cell autoantibody assay at +2 standard deviations was 1.3 u/mL with an intra-assay and inter-assay precision %CV of 4.4–7.9% and 3.3–5.8%, respectively, and sensitivity and specificity of 94% and 95.6%, respectively [22]. The islet autoantibody testing process also involved a rigorous external laboratory validation exercise with paired samples measured in duplicate for all participants at the Clinical Chemistry and Immunology laboratories, Royal Devon and Exeter NHS Foundation Trust, Exeter United Kingdom.

The online homeostatic model assessment-2 (HOMA2) calculator by the Diabetes Trial Unit of the University of Oxford, Oxford UK was used to calculate the insulin resistance (HOMA2-IR) and the pancreatic beta-cell function (HOMA2-%B) [23]. Pancreatic beta-cell function was also assessed using oral insulinogenic index (IGI) that was calculated using the formula: IGI = difference between the serum insulin concentration at the 30-minute and 0-minute time point/difference between the glucose concentration at the 30-minute and 0-minute time point [24]. The quantitative insulin sensitivity check index (QUICKI) was calculated from fasting serum glucose and insulin concentrations using the online QUICKI calculator [25].

Definition of islet autoantibody positivity and prevalent diabetes subtypes

Islet autoantibody positivity was confirmed present if GADA, IA-2A, and ZnT8-A levels were >34 U/ml, >58 U/ml, and >67.7 U/ml, respectively. These diagnostic thresholds were obtained after measuring the concentrations of the three islet autoantibodies in archived serum samples of 600 randomly selected healthy Ugandan adults without diabetes (defined as HbA1c <5.5% and random blood glucose <5 mmol/l) that were enrolled in the Medical Research Council/Uganda Virus Research Institute and London School of Hygiene and Tropical Medicine Uganda Research Unit general population cohort. The mean concentration of GADA, IA-2A, and ZnT8-A in this control cohort was 13.4 U/ml, 17.2 U/ml, and 13.3 U/ml, respectively, with the autoantibody concentrations ranged from 2.5 to 1229 U/ml for GADA, 2.6 to 971 U/ml for IA-2A, and 4.2 to 720 U/ml for ZNT8A. The diagnostic thresholds representing the 97.5^{th} percentile (to give a 97.5% specificity) were >38 U/ml, >58 U/ml, and >67.7 U/ml for GADA, IA2A, and ZnT8-A, respectively.

Participants who tested positive and negative for the three islet autoantibodies were classified as having presumed autoimmune diabetes and confirmed type 2 diabetes, respectively.

Sample size estimation

Using the Leslie Kish formula (1965. Survey Sampling, New York: John Wiley and Sons, Inc.) of sample size calculation, $n = Z^2Pq/d^2$ where n = sample size, Z = 1.96, the normal value corresponding to the 95% confidence interval, d = 3% as the margin of error and a prevalence (P) of GADA positivity in 235 patients with type 2 diabetes in Nigeria of 14% [19], we estimated a sample size of a minimum of 514 participants.

Statistical analysis

The categorical and continuous variables describing the study participants were expressed as proportions and medians with inter-quartile range (IQR), respectively. The prevalence of positivity for the islet autoantibodies was expressed as frequencies. To assess the socio-demographic, clinical, anthropometric, and metabolic characteristics of participants associated with islet autoantibody positivity, we used the x^2 test for categorical variable comparisons and the Kruskal Wallis test to compare medians for continuous data between the two groups (islet autoantibody-positive and negative participants).

Based on the x^2 and Kruskal Wallis tests, variables that were found to be associated with the islet antibody positivity were then fitted in univariable and multivariable logistic regression models to assess the effect of each of the variables on the main outcome. Logistic regression was used to estimate the effect of each of the potential predictor variables on the main outcome. Univariable logistic regression were fitted to estimate the crude effect of each predictor variable on the outcome. Variables found to have an effect on the outcome were then added to the multivariable logistic regression model one at a time to estimate the effect of each of the predictor variables in the presence of other variables. A new model including all potential predictors identified when investigated one at a time was then fitted. The model was tested for goodness of fit using the Hosmer-Lemeshow test specifying 10 groups.

Odds ratios and 95% confidence intervals from the different models were then obtained and reported. All analyses were done using STATA statistical software version 15 College Station, TX: StataCorp LLC. A p-value <0.05 was considered statistically significant.

Ethical approval

This study was approved by the Research Ethics Committee of Uganda Virus Research Centre, Entebbe Uganda on 25th May 2018 (GC/127/18/05/650) and the Uganda National Council of Science and Technology on 29th October 2018 (HS 2431). Administrative approval was also obtained from all participating study sites. All enrolled study participants provided written informed consent to participate in the study. For participants who could not read and write, a thumbprint was used to express informed consent in addition to written informed consent offered by an impartial witness representing the illiterate participant.

Results

Baseline characteristics of all study participants

The socio-demographic, clinical, anthropometric, and metabolic characteristics of all study participants are summarised in Table 1.

The median (IQR) age at diagnosis, BMI, HbA1c and fasting C-peptide for all the participants was 48 (39–57) years, 27.3 (23.5–31.3) kg/m^2, 10.4 (7.7–12.5) % (90 [61–113] mmol/mol), and 1.4 (0.8–2.7) ng/ml, respectively. 56% of all study participants were females and about 81% (n = 432) of the participants were enrolled in the study within two months of diagnosis. Approximately 30% of participants had a history of the presence of ketones in serum or urine at the time of admission. Insulin therapy was initiated immediately following diagnosis in about 29% of participants.

Prevalence of islet autoantibody positivity

Thirty four participants (6.4%) were positive for at least one of the three islet autoantibodies. GADA, IA-2A, and ZnT8-A were positive in 17 (3.2%), 10 (1.9%), and 7 (1.3%) patients, respectively. Of the 34 participants that were positive for islet autoantibodies, only four (11.8%) were positive for >1 autoantibody, all of whom had GADA and ZnT8-A. Fig 1- [Pattern of islet autoantibody positivity].

Socio-demographic, clinical, anthropometric, and metabolic characterisation of participants with islet autoantibody positivity

The socio-demographic, clinical, anthropometric, and metabolic characteristics of participants with and without islet autoantibody positivity are summarised in Table 1.

Participants who were positive for islet autoantibodies were more likely to live in a rural area (n = 18, 52.9% vs n = 127, 25.5%, p = 0.005) and to have a lower median WC (90 [80–99] cm vs 96 [87–104.8], p = 0.04) and WHtR (0.55 [0.50–0.63] vs 0.59 [0.53–0.65], p = 0.03), compared to those who were negative. In addition, a greater proportion of participants with positive islet autoantibodies were immediately started on insulin therapy following diagnosis (n = 19, 55.9% vs n = 134, 26.8%, p<0.001). No differences in age at diagnosis were noted between the two groups.

Differences in BMI and body composition measures between those with and without islet autoantibody positivity were not statistically significant (BMI-24.9 [21.1–30.4] vs 27.4 [23.6–31.4] kg/m^2, p = 0.06, total body fat- 31.9 [20.1–39.4] vs 36.4 [26.5–45.4] %, p = 0.24, and visceral fat level- 8 [5–12] vs 9 [7–12], p = 0.18).

Participants who had islet autoantibodies had significantly lower median fasting C-peptide (0.9 [0.6–1.8] vs 1.4 [0.8–2.1] ng/ml, p = 0.02) and 30-minute fasting C-peptide levels (1.4 [0.9–2.8] vs 2.1 [1.1–3.3], p = 0.03). No statistically significant differences were observed with other measures of pancreatic beta-cell function (HOMA2-%B (44.9 [20.2–70.4] vs 43.1 [20.7–77.6], p = 0.90) and oral

Table 1. Socio-demographic, anthropometric, and metabolic characteristics of all study participants and participants with and without islet autoantibody positivity separately.

Characteristic	All study participants (n = 534)	Patients with islet autoantibody positivity (n = 34)	Patients without islet autoantibody positivity (n = 500)	P value
Socio-demographic and clinical				
Age at diagnosis, years*	48 (39–57)	48 (39–57)	48 (39–58)	1.00
Gender§				
Male	236 (44.2)	19 (55.9)	217 (43.4)	0.16
Female	298 (55.8)	15 (44.1)	283 (56.6)	
Residence§				
Urban	386 (72.4)	16 (47.1)	370 (74.2)	0.005
Rural	145 (27.2)	18 (52.9)	127 (25.5)	
History of admission at diagnosis§	220 (41.4)	18 (52.9)	202 (40.6)	0.31
Presence of urine or serum ketones at admission§	77 (30.9)	7 (33.3)	70 (30.7)	0.80
Treatment used§				
Metformin	425 (79.6)	24 (70.6)	401 (80.2)	0.18
Sulfonylureas	199 (37.3)	8 (23.5)	191 (38.2)	0.09
Insulin	153 (28.7)	19 (55.9)	134 (26.8)	<0.001
Systolic blood pressure, mmHg*	126 (115–137)	125 (119–132)	126 (115–137)	1.00
Diastolic blood pressure, mmHg*	84 (77–91)	83 (79–87)	84 (77–91)	0.83
Anthropometry*				
Body mass index, kg/m^2	27.3 (23.5–31.3)	24.9 (21.1–30.4)	27.4 (23.6–31.4)	0.06
WC, cm	95.5 (86.5–104)	90 (80–99)	96 (87–104.8)	0.04
HC, cm	103 (95.5–111)	99 (92–107)	103 (96–111.5)	0.11
WHR	0.92 (0.88–0.96)	0.92 (0.85–0.95)	0.92 (0.88–0.96)	1.00
WHtR	0.59 (0.53–0.65)	0.55 (0.50–0.63)	0.59 (0.53–0.65)	0.03
Total body fat, %	36.3 (26.1–45.1)	31.9 (20.1–39.4)	36.4 (26.5–45.3)	0.24
Visceral fat level	9 (7–12)	8 (5–12)	9 (7–12)	0.18
Metabolic*				
TC, mmol/l	4.0 (3.2–5.0)	3.9 (3.1–4.8)	4.0 (3.3–5.0)	0.50
HDLC, mmol/l	0.9 (0.8–1.2)	0.9 (0.8–1.2)	1.0 (0.7–1.2)	0.79
TGL, mmol/l	1.3 (1.0–1.8)	1.2 (0.8–1.7)	1.3 (1.0–1.8)	0.36
LDLC, mmol/l	2.6 (1.9–3.4)	2.3 (1.9–3.1)	2.6 (1.9–3.4)	0.24
Non-HDLC, mmol/l	3.0 (2.3–3.8)	2.5 (2.1–3.7)	3.0 (2.4–3.8)	0.06
TC/HDLC	4.2 (3.4–5.3)	4.1 (3.4–5.2)	4.2 (3.4–5.3)	0.69
TGL/HDLC	1.4 (1.0–2.2)	1.5 (0.9–2.0)	1.4 (1.0–2.2)	0.84
HbA1c, %	10.4 (7.7–12.5)	11.0 (8.6–12.7)	10.3 (7.7–12.5)	0.46
HbA1c, mmol/mol	90 (61–113)	97 (71–115)	90 (61–113)	0.49
Fasting blood glucose, mmol/l	8.6 (6.3–13.3)	7.7 (6.6–11.3)	8.6 (6.2–13.4)	0.42
Fasting serum insulin, μU/ml	6.0 (3.0–10.5)	6.8 (3.3–10.5)	5.9 (3.0–10.6)	0.48
Fasting serum C-peptide, ng/ml	1.4 (0.8–2.7)	0.9 (0.6–1.8)	1.4 (0.8–2.1)	0.01
30 min blood glucose, mmol/l	13.0 (9.9–18.1)	12.5 (9.9–17)	12.5 (9.9–17.0)	0.71
30 min serum insulin, μU/ml	10.9 (5.4–22.4)	8.4 (4.7–17.0)	11.1 (5.5–22.5)	0.35
30 min C-peptide, ng/ml	2.1 (1.1–3.3)	1.4 (0.9–2.8)	2.1 (1.1–3.3)	0.03
120 min blood glucose, mmol/l	17.4 (12.6–23.3)	19 (15.3–23.0)	17.2 (12.3–23.3)	0.26
120 min serum insulin, μU/ml	13.8 (6.8–27.6)	13.9 (5.8–36.4)	13.7 (6.9–27.1)	0.98
120 min serum C-peptide, ng/ml	2.8 (1.4–4.8)	2.6 (1.1–4.6)	2.8 (1.5–4.8)	0.70

(*Continued*)

Table 1. (Continued)

Characteristic	All study participants (n = 534)	Patients with islet autoantibody positivity (n = 34)	Patients without islet autoantibody positivity (n = 500)	P value
HOMA2-IR	1.22 (0.77–2.03)	1.24 (0.79–2.13)	1.21 (0.77–2.03)	0.90
QUICKI	0.35 (0.31–0.42)	0.34 (0.31–0.42)	0.35 (0.31–0.42)	0.50
HOMA2-%B	43.4 (20.7–77.0)	44.9 (20.2–70.4)	43.1 (20.7–77.6)	0.90
Oral insulinogenic index	1.30 (0.47–3.86)	0.67 (0.31–2.01)	1.31 (0.47–3.86)	0.21

§Results are presented as numbers and proportions

*Results are presented as median (inter-quartile range/IQR)

HbA1c-Glycated haemoglobin, HC-Hip circumference, HDLC-High dense lipoprotein cholesterol, HOMA2-%B-Homeostatic model assessment-beta cell function, HOMA2-IR- Homeostatic model assessment- insulin resistance, LDLC-Low dense lipoprotein cholesterol, TC-Total cholesterol, TGL-Triglycerides, QUICKI-quantitative insulin sensitivity check index, WC-waist circumference, WHR-Waist: hip circumference, WHtR- waist circumference: height ratio.

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IGI (0.67 [0.31–2.01 vs 1.31 [0.47–3.86], p = 0.21) and insulin sensitivity (QUICKI- 0.34 [0.31–0.42] vs 0.35 [0.31–0.42], p = 0.50). Similarly, no significant differences were seen in circulating levels of metabolic markers such as FBG, HbA1c, lipid profile, and HOMA2-IR.

Predictors of islet autoantibody positivity on multivariate analysis

Table 2 summarises the clinical and metabolic characteristics that were independently associated with islet autoantibody positivity.

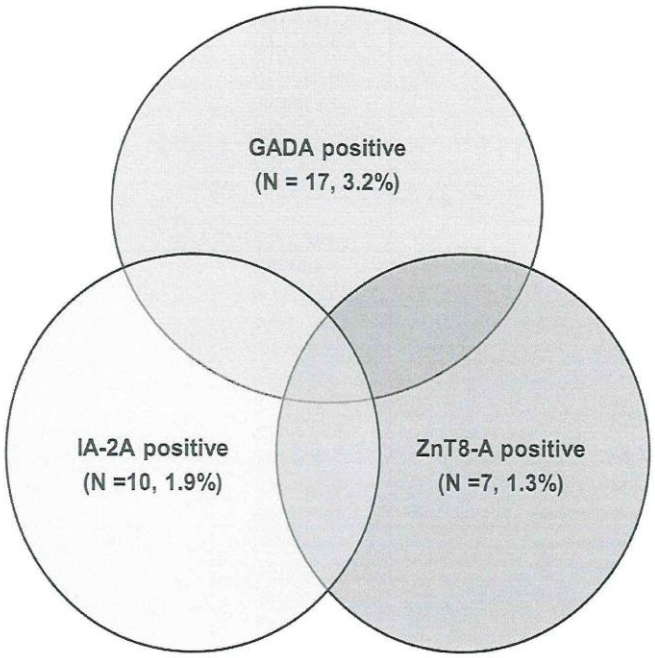

Fig 1. The pattern of islet autoantibody positivity in participants positive for islet autoantibodies (n = 34). GADA-Autoantibody against glutamic acid decarboxylase-65, IA-2A- Antibody against tyrosine phosphatase, ZnT8-A: autoantibody against zinc transporter 8.

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Table 2. Predictors of islet autoantibody positivity on multivariate analysis.

Characteristic	AOR (95% CI)	P-value
Age at diagnosis (years)	0.98 (0.96–1.01)	0.26
Rural residence	3.62 (1.68–7.80)	0.001
Initiation of insulin therapy at diagnosis	3.61 (1.67–7.83)	0.001
Waist circumference (cm)	0.98 (0.96–1.01)	0.23
Fasting C-peptide (ng/ml)	1.01 (0.70–1.46)	0.95

AOR- Adjusted odds ratio, CI- confidence intervals

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On multivariate analysis, living in a rural area (OR 3.62, 95%CI 1.68–7.80, p = 0.001) and being initiated on insulin therapy at the time of diagnosis (OR 3.61, 95% CI 1.67–7.83, p = 0.001) were independently associated with islet autoantibody positivity.

Discussion

To the best of our knowledge, this is the first study in SSA to simultaneously screen for islet autoantibody positivity (defined using local population-derived diagnostic thresholds) based on testing three islet autoantibodies in adult patients with recently diagnosed diabetes using a high performing islet autoantibody assay in the international Islet Autoantibody Standardisation Program with concurrent rigorous external laboratory validation.

Our study shows that islet autoantibody positivity is relatively infrequent in adult patients with recently diagnosed diabetes in Uganda. The prevalence of islet autoantibody positivity in our cohort was close to the expected autoantibody-positive rate in a population without autoimmune diabetes. With the use of 97.5% specificity test thresholds, we would expect 2.5% of those without autoimmune diabetes to test positive for each test [6–8]. This, therefore, is consistent with a very low prevalence of autoimmune diabetes in this study population and suggests that routine testing for islet autoantibodies in adult Ugandan patients with recently diagnosed diabetes would result in many false positives. The rates of false-positive results would further increase if the lower manufacturer's cut-offs were used to define islet autoantibody positivity.

Islet autoantibody positivity is thought to be common in European populations with adult-onset diabetes (4.5–9.7%) with GADA positivity rates being significantly higher than those of IA-2A and ZnT8-A (4.5–11.1% vs 0.2–2.3%, respectively) [26–29]. In one study performed in a selected adult Czech population with LADA, confirmed maturity-onset diabetes of the young, and healthy controls, a high prevalence of ZnT8-A positivity of 23.7% was noted in 59 participants with study-defined LADA. All of these participants were positive for GADA and IA-2A [30].

Lower prevalence rates based on the positivity of either two or all the three islet autoantibodies (GADA, IA-2A, and ZnT8-A) have been reported in other populations, such as in the Middle East (2.8% based on GADA and IA-2A positivity) [31] and Asia (1.5%-8.6% based on GADA, IA-2A, and ZnT8-A positivity) [32–36]. Similar to findings of European population-based studies, GADA positivity has been reported to be more prevalent than IA-2A and ZnT8-A positivity in Asians with phenotypic type 2 diabetes [34–36].

Generally, few studies have screened for islet autoantibody positivity in unselected adult populations with diabetes (where specific types of diabetes are not excluded) using more than one islet autoantibody. Most studies in SSA have screened for only GADA positivity and have reported higher prevalence rates compared to what we observed in our study (3.2%).

Two studies conducted in Kenya and Tanzania in adult patients with apparent type 2 diabetes reported almost similar prevalence of GADA positivity of 5.7% and 5.3%, respectively [11, 12]. Screening for IA-2A positivity in the latter study increased the prevalence of islet autoantibody positivity to 7.3% [12]. Higher prevalence levels of GADA positivity in adult-onset diabetes have also been reported in Madagascar (12%) [15] and in West African populations in Nigeria (10.5–14%) [13, 14, 19] and Ghana (8.9–14.3%) [16, 17].

The reason for this apparent difference in the prevalence of GADA positivity noted in our study and the highlighted studies above is likely to be the varying study definition of GADA positivity. The majority of those studies used manufacturer's cut-off points to define positivity. In contrast, we found that the manufacturer's cut-off point was considerably lower than the local population-derived cut-off, which we used in our study, and using the manufacturer's cut-offs would increase the frequency of false-positive tests, leading to inaccurate estimates of the prevalence of islet autoantibody positivity [6–8]. This need for using appropriate population-defined references ranges to define islet autoantibody positivity was recently also demonstrated in a large study of type 1 diabetes in Ethiopia, where a GADA assay and threshold well-established in European populations had poor specificity, testing positive in 8.5% of those without diabetes [37].

On univariate analysis, islet antibody-positive individuals were more likely to live in a rural area, to be initiated on insulin therapy at diagnosis, and exhibited lower measures of obesity (WC and WHtR) and pancreatic beta-cell function (fasting and postprandial C-peptide). These findings are consistent with what has been observed in participants with islet autoantibody positivity in other populations [6, 26, 27, 38, 39]. In addition to having lower markers of obesity, a finding of lower fasting and postprandial C-peptide concentrations in islet autoantibody-positive participants as shown in our study is an indicator of reduced pancreatic beta-cell function which is linked to progressive autoimmune-mediated damage of the pancreatic beta-cells [40]. A high prevalence of pancreatic beta-cell dysfunction based on a lower fasting C-peptide concentration in participants with islet autoantibody positivity has been widely documented in several similar studies [39, 41–43]. No association between islet autoantibody positivity and insulin resistance was observed in this study.

Living in a rural area and being initiated on insulin therapy at diagnosis were noted to be independently associated with islet autoantibody positivity in this study population on multivariate analysis. The observation of an association between living in a rural area and islet autoantibody positivity is of special interest and is supported by previous studies. For example, in a study in Ghana, islet autoantibody positivity was seen more commonly in rural areas compared to urban areas (14.3% Vs 8.9%) [16]. Furthermore, one of the highest prevalence levels of islet autoantibody positivity in Africa (28%) has been reported from a rural semi-arid famine-prone area in Ethiopia [18]. The underlying mechanisms that increase the likelihood of pancreatic autoimmunity in rural areas are unclear, but chronic malnutrition has been implicated [44, 45].

Initiation on insulin therapy at diagnosis as an independently associated factor of islet autoantibody positivity in this study population signifies pancreatic beta-cell dysfunction which is a predictor of early initiation of insulin therapy in islet autoantibody-positive participants in several studies [26, 27, 41, 42, 46].

In European and Asian population-based studies, patients with adult-onset diabetes and positive for islet autoantibodies are significantly younger at diagnosis and have lower BMI, blood pressure, and a more favourable lipid profile, when compared to those with type 2 diabetes [26–28, 39]. In contrast, data from our study and most of the studies in African patients have not demonstrated these differences between patients with and without islet autoantibody positivity [14, 15, 17, 19]. While this may partly be due to the low numbers of patients positive

for islet autoantibodies, several studies and clinical observations have suggested that Africans appear to develop type 2 diabetes at a young age and lower levels of BMI, which might influence these relationships [10].

Strengths and limitations

This study recruited a cohort of adult patients with recently diagnosed diabetes within three months of diagnosis to minimise the decline in rates of islet autoantibody positivity that occurs with increasing diabetes duration [47, 48]. Screening for islet autoantibody positivity was based on testing three common autoantibodies using diagnostic thresholds derived from an appropriate healthy adult Ugandan population without diabetes, as widely recommended, hence ensuring a high test specificity [6–8]. The study also used one of the highest performing islet autoantibody assays in the international Islet Autoantibody Standardisation Program [49] with extensive validation performed on paired samples in an external laboratory (Royal Devon and Exeter NHS Foundation Trust, Exeter UK), to ensure robust results. The study also assessed an additional number of metabolic characteristics, especially measurements of pancreatic beta-cell function, insulin resistance, and sensitivity.

Limitations of our study include recruitment from only specialist diabetes clinics of the tertiary hospitals which potentially introduces a selection bias and might result in reporting a higher burden of islet autoantibody positivity in patients with adult-onset diabetes, as milder cases may be more likely to be managed without attending a specialist clinic. However, the majority of patients in Uganda are seen in these clinics in tertiary hospitals for the management of chronic diseases, with very limited provision of diabetes care in lower-tier district hospitals. It is also possible that our delayed recruitment of those requiring admission (with recruitment only offered on re-attending the diabetes clinic) could have reduced the prevalence of islet autoantibody positivity in this cohort, leading to an underestimate of the prevalence. We, however, managed to recruit most of these patients following discharge from the hospital at their subsequent clinical review.

Because of the small number of participants with islet autoantibody positivity, the study had limited power to detect differences in some clinical characteristics in this population. Lastly, because we performed multiple tests, we cannot rule out chance findings.

Conclusion

The prevalence of islet autoantibody positivity in this study population of adults with recently diagnosed diabetes in Uganda was relatively low, suggesting that pancreatic autoimmunity is a rare cause of adult-onset diabetes in the Ugandan population. Due to the low rates of islet autoantibody positivity in this study population, routine testing of islet autoantibodies would have limited clinical significance in Uganda and would likely result in many false-positive results, especially if the lower manufacturer's cut-offs are used to define autoantibody positivity. The study finding of an association between living in a rural area and islet autoantibody positivity is of unique interest and warrants further investigation.

Supporting information

S1 Data.
(XLSX)

Acknowledgments

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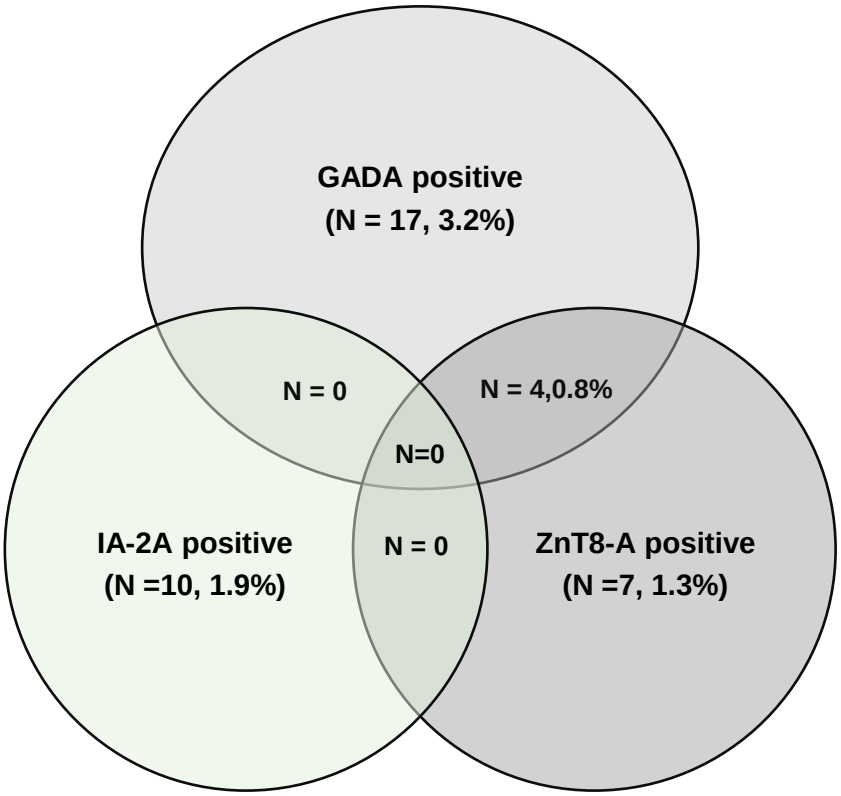
Figure 2: Pattern of islet autoantibody positivity in participants positive for islet autoantibodies



GADA-Autoantibody against glutamic acid decarboxylase-65, IA-2A- Antibody against tyrosine phosphatase, ZnT8-A: autoantibody against zinc transporter 8.

CHAPTER SIX: CHARACTERISATION OF LEAN TYPE 2 DIABETES PHENOTYPE

This chapter presents the findings of the second objective of the Ph.D. aimed to understand the clinical and metabolic characterisation of confirmed lean type 2 diabetes phenotype in our adult cohort with new-onset diabetes.

6.1 Ph.D. research paper 3: Clinical and metabolic characterisation of the lean type 2 diabetes phenotype in adult Ugandans with new-onset diabetes

6.2 Summary of key findings

Kibirige D, Sekitoleko I, Lumu W, Jones AG, Hattersley AT, Smeeth L, Nyirenda MJ. Understanding the pathogenesis of lean non-autoimmune diabetes in an African population with newly diagnosed diabetes. Diabetologia 2022; 65(4):675-683.

In this study, we performed a comprehensive clinical, metabolic, and immunological characterisation of 500 adult Ugandan patients with confirmed new-onset type 2 diabetes to understand the phenotypic differences between lean and non-lean individuals. We reported that, in this study population, approximately one in three adults with confirmed new-onset type 2 diabetes were lean in body size (BMI < 25 kg/m^2). Pathophysiologically, the lean type 2 diabetes phenotype was characterised by the predominance of pancreatic beta-cell dysfunction in addition to less metabolic syndrome, insulin resistance, and visceral adiposity.

The link to the publication:

https://link.springer.com/content/pdf/10.1007/s00125-021-05644-8.pdf

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RESEARCH PAPER COVER SHEET

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SECTION A: STUDENT DETAILS

Student	Davis Kibirige
ID No	LSH 1705262
Principal Supervisor	Prof. Moffat Nyirenda
Title	An in-depth understanding of the clinical, metabolic, and immunologic profile of adult patients with newly diagnosed diabetes in Uganda: the Uganda DIabetes Phenotype (UDIP) study.

If the Research Paper has previously been published, please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	Diabetologia
When was the work published?	2022
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	N/A
Have you retained the copyright for the work? *	No
Was the work subject to academic peer review?	Yes

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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	
Please list the paper's authors in the intended authorship order:	
Stage of publication	

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I fully participated in the study design, data collection, management, analysis, and interpretation. I also drafted the first version of the manuscript, incorporated all comments from the supervisors and other co-authors, submitted the final manuscript, and coordinated all the correspondence with the reviewers.

SECTION E

Student's signature	
Date	31/05/2022

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ARTICLE

Understanding the pathogenesis of lean non-autoimmune diabetes in an African population with newly diagnosed diabetes

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Abstract

Aims/hypothesis Apparent type 2 diabetes is increasingly reported in lean adult individuals in sub-Saharan Africa. However, studies undertaking robust clinical and metabolic characterisation of lean individuals with new-onset type 2 diabetes are limited in this population. This cross-sectional study aimed to perform a detailed clinical and metabolic characterisation of newly diagnosed adult patients with diabetes in Uganda, in order to compare features between lean and non-lean individuals.

Methods Socio-demographic, clinical, biophysical and metabolic (including oral glucose tolerance test) data were collected on 568 adult patients with newly diagnosed diabetes. Participants were screened for islet autoantibodies to exclude those with autoimmune diabetes. The remaining participants (with type 2 diabetes) were then classified as lean (BMI <25 kg/m^2) or non-lean (BMI ≥25 kg/m^2), and their socio-demographic, clinical, biophysical and metabolic characteristics were compared.

Results Thirty-four participants (6.4%) were excluded from analyses because they were positive for pancreatic autoantibodies, and a further 34 participants because they had incomplete data. For the remaining 500 participants, the median (IQR) age, BMI and HbA_{1c} were 48 years (39–58), 27.5 kg/m^2 (23.6–31.4) and 90 mmol/mol (61–113) (10.3% [7.7–12.5]), respectively, with a female predominance (approximately 57%). Of the 500 participants, 160 (32%) and 340 (68%) were lean and non-lean, respectively. Compared with non-lean participants, lean participants were mainly male (60.6% vs 35.3%, p<0.001) and had lower visceral adiposity level (5 [4–7] vs 11 [9–13], p<0.001) and features of the metabolic syndrome (uric acid, 246.5 [205.0–290.6] vs 289 [234–347] μmol/l, p<0.001; leptin, 660.9 [174.5–1993.1] vs 3988.0 [1336.0–6595.0] pg/ml, p<0.001). In addition, they displayed markedly reduced markers of beta cell function (oral insulinogenic index 0.8 [0.3–2.5] vs 1.6 [0.6–4.6] pmol/mmol; 120 min serum C-peptide 0.70 [0.33–1.36] vs 1.02 [0.60–1.66] nmol/l, p<0.001).

Conclusions/interpretation Approximately one-third of participants with incident adult-onset non-autoimmune diabetes had BMI <25 kg/m^2. Diabetes in these lean individuals was more common in men, and predominantly associated with reduced pancreatic secretory function rather than insulin resistance. The underlying pathological mechanisms are unclear, but this is likely to have important management implications.

Keywords Beta cell dysfunction · Lean non-autoimmune diabetes · Newly diagnosed diabetes sub-Saharan Africa · Type 2 diabetes · Uganda

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Research in context

What is already known about this subject?

- Type 2 diabetes is a heterogenous condition, and understanding the distinct phenotypes may improve clinical management by enabling targeted therapy

What is the key question?

- What proportion of individuals that present with type 2 diabetes in Uganda are lean and what are their associated clinical and metabolic features?

What are the new findings?

- Approximately a third of adult patients presenting with type 2 diabetes in this study were lean (BMI <25 kg/m^2)
- Type 2 diabetes in these lean adult individuals is not associated with traditional markers of the metabolic syndrome
- Mechanistically, type 2 diabetes in these lean patients was associated predominantly with reduced pancreatic beta cell function

How might this impact on clinical practice in the foreseeable future?

- The presence of type 2 diabetes in lean individuals and the related predominance of pancreatic beta cell dysfunction questions the effectiveness of traditional approaches of management and prevention of diabetes, such as lifestyle modification and pharmacological therapies like metformin, in this population. Alternative therapies, such as those that improve beta cell secretory function and/or mass, may be required

Abbreviations

GADA	GAD autoantibodies
IA-2A	Autoantibody to the protein tyrosine phosphatase
IGI	Insulinogenic index
WHtR	Waist circumference:height ratio
ZnT8-A	Zinc transporter eight autoantibodies

Introduction

Type 2 diabetes has reached epidemic proportions globally. In sub-Saharan Africa, it remains a major and rapidly growing problem, posing a significant public health challenge and causing a considerable strain on the healthcare systems [1]. Overweight, obesity, rapid urbanisation and changes in lifestyle are implicated in the increasing burden of type 2 diabetes globally [1, 2]. Despite obesity and overweight being well-documented risk factors of type 2 diabetes, there is accumulating evidence, particularly from low- and middle-income countries, that type 2 diabetes can also develop in lean individuals [3–10].

Most of the evidence on type 2 diabetes in lean individuals has originated from South Asian populations, where diabetes is commonly seen in people with normal BMI. Initial studies showed that, although BMI was normal, these individuals typically had increased waist circumferences, WHR, total body and visceral adiposity, and ectopic fat deposition, with a metabolic profile characterised by an atherogenic lipid profile (low HDL-cholesterol, high VLDL-cholesterol and triacylglycerol concentrations) [11–15]. This was suggestive of insulin resistance as the underlying pathogenic mechanism. However, recent evidence has shown that beta cell secretory dysfunction may be the primary pathogenic defect in such individuals [6, 7, 11, 12, 15–19].

In contrast, studies undertaking robust clinical and metabolic characterisation of true new-onset type 2 diabetes (where islet cell autoimmunity has been excluded) in lean individuals in sub-Saharan Africa are few in number. To add to the existing literature, we conducted the Uganda DIabetes Phenotype (UDIP) study to examine the socio-demographic, clinical, biophysical and metabolic characteristics of adult patients with newly diagnosed type 2 diabetes in Uganda, in order to compare the features associated with diabetes in lean and non-lean individuals.

Methods

Study setting and participants This study was conducted in seven tertiary public and private not-for-profit mission or church-founded hospitals in Central and Southwestern Uganda serving urban, peri-urban and rural populations between February 2019 and October 2020. About 85% of the Ugandan population receives medical care from public and private not-for-profit hospitals.

We consecutively recruited adult patients aged ≥18 years with a recent diagnosis of diabetes. The diagnosis of diabetes had been made by clinicians at various general outpatient clinics, based on results for fasting blood glucose, random blood glucose and HbA_{1c} measurement. After diagnosis, patients are referred to the diabetes clinics for further management.

Patients were recruited within 3 months of diagnosis when they attended the outpatient diabetes clinics for routine clinical reviews. Critically ill patients who required urgent hospitalisation for medical treatment were not immediately recruited into the study but were invited to enrol at least 2 weeks after discharge from hospital (but within 3 months of diagnosis) when they re-attended the diabetes clinics in a stable condition. Both treatment-naive patients and those who had commenced glucose-lowering therapy were allowed to participate in the study. Pregnant women were excluded. All recruited study participants were black Africans of Ugandan origin. A total of 568 adult patients with newly diagnosed diabetes were recruited into the study.

Assessment of socio-demographic, clinical and biophysical characteristics All study participants were assessed after an overnight fast of ≥8 h. Relevant socio-demographic variables (age at diagnosis, sex, residence, level of education, family history of diabetes, smoking and alcohol intake status) and clinical data (history of admission at diagnosis, presence of urine or serum ketones at admission, use of diabetes and ancillary drugs, coexisting medical comorbidities) were collected by the research team using a pre-tested case report form. This was followed by biophysical measurements including resting blood pressure and relevant anthropometric measurements (weight, height, waist circumference and hip circumference for calculation of BMI, WHR and waist circumference:height ratio [WHtR]) according to standardised study procedures. Body composition (total body fat and visceral fat levels) was evaluated by bioimpedance analysis using an OMRON BF511 body composition monitor (Omron Healthcare, Tokyo, Japan). Hypertension was defined as systolic BP ≥140 mmHg and/or diastolic BP ≥90 mmHg on clinical examination or a self-reported history of pre-existing hypertension either on antihypertensive therapy or without treatment [2].

Assessment of metabolic characteristics A fasting blood sample was collected for measurement of fasting blood glucose, HbA_{1c}, insulin, C-peptide and lipid profile, uric acid, leptin, and three pancreatic autoantibodies (GAD autoantibodies [GADA], zinc transporter eight autoantibodies [ZnT8-A] and autoantibody to the protein tyrosine phosphatase [IA-2A]). This was followed by a 75 g OGTT, with blood samples drawn again 30 and 120 min after glucose ingestion to determine the serum glucose, insulin and C-peptide concentrations at those two time points.

Laboratory measurements and assessment of markers of pancreatic beta cell function, insulin resistance and sensitivity All the laboratory tests were performed at the ISO-certified clinical chemistry laboratory at Medical Research Council/Uganda Virus Research Institute and London School of Hygiene and Tropical Medicine Uganda Research Unit, Entebbe, Uganda, using electrochemiluminescence immunoassays manufactured by Roche (Germany) using a Cobas 6000 C-model SN 14H3–15 machine (Hitachi High Technologies, Japan). Pancreatic autoantibody testing was performed using autoantibody ELISA kits from RSR (UK) on the Dynex DS2 ELISA robot (Dynex Technologies, UK).

The HOMA2 calculator (Diabetes Trial Unit, University of Oxford, UK) was used to calculate the insulin resistance (HOMA2-IR) and the pancreatic beta cell function (HOMA2-%B) [20]. Insulinogenic index (IGI) as an optimum marker of pancreatic beta cell function was calculated using this formula: IGI = (30 min insulin − 0 min insulin in pmol/l)/(30 min glucose − 0 min glucose in mmol/l) [21]. The quantitative insulin sensitivity check index (QUICKI) to indicate insulin sensitivity was calculated from fasting serum glucose and insulin concentrations using the online QUICKI calculator [22].

Exclusion of patients with pancreatic autoimmunity Pancreatic autoantibody testing was performed in all participants to exclude those with islet autoantibody positivity as a marker of pancreatic autoimmunity. Pancreatic autoantibody positivity was defined as levels of GADA >34 U/ml or IA-2A > 58 U/ml or ZnT8-A > 67.7 U/ml. These thresholds represent the 97.5th percentile for 600 randomly selected healthy Ugandan adults without diabetes enrolled in the Medical Research Council/Uganda Virus Research Institute and London School of Hygiene and Tropical Medicine Uganda Research Unit general population cohort (Balungi et al, unpublished data).

After excluding those with pancreatic autoimmunity, the remaining participants with type 2 diabetes were classified as lean and non-lean based on the traditional BMI cut-offs of <25 and ≥25 kg/m^2, respectively, because there are no Africa- or Uganda-specific BMI cut-offs to define obesity. The socio-demographic, clinical, biophysical and metabolic characteristics of both groups were then compared.

Ethical approval The study received ethical approval from the research ethics committee of the Uganda Virus Research Institute (GC/127/18/05/650) and Uganda National Council of Science and Technology (HS 2431). All participating study sites offered administrative approval prior to initiation of the study. All study participants recruited into the study provided written informed consent.

Statistical analysis The categorical and continuous variables describing all the study participants are expressed as percentages and medians with inter-quartile range (IQR), respectively. The differences in the socio-demographic, clinical and metabolic characteristics between the lean and non-lean participants were analysed using the χ^2 test for categorical data and the Kruskal–Wallis test for continuous data. Because of comparison of multiple variables between the lean and non-lean participants, the Bonferroni correction (adjusted p value = set significance α/number of variables tested) was used [23]. With the 47 variables to be compared between the two groups and the set significance α of 0.05, the adjusted p value to signify statistical significance became 0.001. All analyses were performed using STATA statistical software version 15 (StataCorp, USA). A p value <0.05 was considered statistically significant.

Results

A total of 568 adult patients with newly diagnosed diabetes were recruited. Complete data on islet autoantibody status was available in 534 patients (94%) with newly diagnosed diabetes. Of these, 34 participants (6.4%) were excluded because they were positive for at least one of the three pancreatic autoantibodies. Applying the BMI cut-offs of <25 and ≥25 kg/m^2 in the remaining 500 participants, 160 (32%, 95% CI 27.9–36.3%) and 340 (68%, 95% CI 63.7–72.1%) were lean and non-lean, respectively.

The socio-demographic, clinical, biophysical and metabolic characteristics of all study participants with newly diagnosed diabetes are summarised in Table 1.

Socio-demographic, clinical and biophysical characterisation of the lean and non-lean participants The socio-demographic, clinical, biophysical and metabolic characteristics of the lean and non-lean participants are summarised in Table 1.

Compared with those who were non-lean, lean participants were predominantly male (60.6% vs 35.3%, p<0.001) and were started on insulin therapy at diagnosis (42.5% vs 19.4%, p<0.001). No difference in age at diagnosis was noted between the two subgroups (48 [37–58] vs 48 [40–57] years, p=1.00).

Lean participants also had significantly lower median (IQR) waist circumference (83.0 [77.0–90.0] cm vs 101.6 [95.0–108.0] cm, p<0.001), WHR (0.90 [0.85–0.95] vs 0.93 [0.89–0.97], p<0.001), WHtR (0.51 [0.48–0.55] vs 0.63 [0.58–0.68], p<0.001), total body fat (22.5 [16.1–31.6] % vs 42.0 [32.8–47.9] %, p<0.001) and visceral fat level (5 [4–7] vs 11 [9–13], p<0.001).

Metabolic characterisation of lean and non-lean participants Biochemically, compared with non-lean participants, lean participants had significantly lower median fasting insulin (29.17 [14.58–44.44] pmol/l vs 48.61 [25.69–85.42] pmol/l, p<0.001), fasting C-peptide (0.33 [0.20–0.53] nmol/l vs 0.53 [0.36–0.76] nmol/l, p<0.001), 30 min insulin (52.08 [21.53–100.00] pmol/l vs 95.83 [45.83–178.47] pmol/l, p<0.001), oral IGI (0.8 [0.3–2.5] pmol/mmol vs 1.6 [0.6–4.6] pmol/mmol, p=0.001) and HOMA2-IR (0.89 [0.65–1.58] vs 1.32 [0.84–2.17], p=0.001). The oral IGI, as an optimum marker of beta cell function, and HOMA2-IR of the lean and non-lean participants are summarised as box plots in Figs 1 and 2, respectively.

Lean participants were also more likely to have lower circulating concentrations of biomarkers of the metabolic syndrome such as leptin (660.9 [174.5–1993.1] pg/ml vs 3988.0 [1336.0–6595.0] pg/ml, p<0.001), and uric acid (246.5 [205.0–290.6] μmol/l vs 289.0 [234.0–347.0] μmol/l, p<0.001).

Discussion

In this study of well-characterised adult patients with newly diagnosed type 2 diabetes, being lean in body size (BMI <25 kg/m^2) was relatively common, occurring in approximately a third of participants. These individuals showed biochemical features that are consistent with pancreatic beta cell dysfunction rather than insulin resistance. These findings are in direct contrast with the conventional picture of diabetes in adulthood, consisting of overweight or obesity and insulin resistance, with pancreatic beta cell failure occurring later during the condition.

However, our study findings are in accordance with other data emerging from sub-Saharan Africa that show a relatively high proportion of those with type 2 diabetes are thin. [5, 24–26]. For example, three large population-based studies performed in Uganda [24, 25] and Ethiopia [26] reported that type 2 diabetes in lean individuals accounted for approximately 60% of all cases of type 2 diabetes. These are significantly higher rates than observed in our study, perhaps because more that 70% of the total participants in these surveys had a BMI <25 kg/m^2. The Research on Obesity and Diabetes among African Migrants (RODAM) study, which was performed in native and migrant Ghanaians, also reported high proportions of type 2 diabetes (55.4% and 35.6% in rural and urban native Ghanaians, respectively) in individuals with BMI <25 kg/m^2.

In contrast, a lower frequency of type 2 diabetes in individuals with BMI <25 kg/m^2 has been reported in large-scale studies in people of European extraction or Asian and Hispanic populations, where prevalence rates ranged between 5 and 23.5% [4, 6, 8–10]. More importantly, our study adds to the increasing evidence that type 2 diabetes is a heterogenous disorder whose pathogenesis differs across populations or ethnicities [27].

Table 1 Socio-demographic, clinical, anthropometric and metabolic characteristics of all study participants, and for lean and non-lean participants separately

Characteristic	All study participants (n=500)	Lean participants (n=160)	Non-lean participants (n=340)	p value (lean vs non-lean)
Socio-demographic and clinical data				
Age (years)	48 (39–58)	48 (37–58)	48 (40–57)	1.00
Sex				
Male	217 (43.4)	97 (60.6)	120 (35.3)	<0.001***
Female	283 (56.6)	63 (39.4)	220 (64.7)	
Residence[a]				
Urban	370 (74.1)	119 (74.8)	251 (73.8)	0.77
Rural	127 (25.5)	39 (24.5)	88 (25.9)	
Prior admission at diagnosis[b]	202 (40.6)	81 (50.6)	121 (35.8)	0.003
Presence of urine or serum ketones at admission[c]	70 (30.7)	34 (39.1)	36 (25.5)	0.02
Treatment used[d]				
Diet	18 (3.6)	3 (1.9)	15 (4.4)	0.16
Metformin	401 (80.2)	114 (71.3)	287 (84.4)	0.001***
Sulfonylureas	191 (38.2)	49 (30.6)	142 (41.8)	0.02
Insulin	134 (26.8)	68 (42.5)	66 (19.4)	<0.001***
Self-reported HT comorbidity[e]	171 (35.0)	39 (24.8)	132 (39.9)	0.002
Acanthosis nigricans present	89 (17.8)	17 (10.6)	72 (21.2)	0.004
Systolic BP (mmHg)	126 (115–137)	123 (109–133)	127 (117–139)	0.08
Diastolic BP (mmHg)	84 (77–91)	80 (74–87)	85 (79–93)	<0.001***
HT on clinical examination (SBP≥140 and/or DBP ≥90 mmHg)	178 (35.6)	43 (27.0)	135 (39.8)	0.006
Anthropometric data				
Weight (kg)	72.0 (62.5–82.0)	58.2 (52.2–65.0)	77.2 (71.0–86.6)	<0.001***
Height (cm)	162.0 (156.4–167.2)	163.1 (158.0–168.6)	161.1 (156.0–166.5)	0.06
BMI (kg/m^2)	27.5 (23.6–31.4)	22.2 (20.3–23.5)	30.1 (27.3–33.1)	<0.001***
WC (cm)	96.0 (87.0–104.8)	83.0 (77.0–90.0)	101.6 (95.0–108.0)	<0.001***
HC (cm)	103.0 (96.0–111.5)	93.5 (88.0–98.0)	107.0 (102.0–116.0)	<0.001***
WHR	0.92 (0.88–0.96)	0.90 (0.85–0.95)	0.93 (0.89–0.97)	<0.001***
WHtR	0.59 (0.53–0.65)	0.51 (0.48–0.55)	0.63 (0.58–0.68)	<0.001***
Total body fat (%)	36.4 (26.5–45.3)	22.5 (16.1–31.6)	42.0 (32.8–47.9)	<0.001***
Visceral fat level	9 (7–12)	5 (4–7)	11 (9–13)	<0.001***
Metabolic data				
TC (mmol/l)	4.0 (3.3–5.0)	3.8 (3.1–4.7)	4.2 (3.4–5.0)	0.03
HDLC (mmol/l)	1.0 (0.7–1.2)	1.0 (0.7–1.2)	0.9 (0.8–1.2)	0.21
TGL (mmol/l)	1.3 (1.0–1.8)	1.2 (0.9–1.7)	1.4 (1.1–1.9)	0.02
LDLC (mmol/l)	2.6 (1.9–3.4)	2.4 (1.7–3.3)	2.6 (2.0–3.5)	0.11
Non-HDLC (mmol/l)	3.0 (2.4–3.8)	2.8 (2.2–3.6)	3.1 (2.5–3.9)	0.03
TC/HDLC	4.2 (3.4–5.3)	3.9 (3.3–5.0)	4.4 (3.5–5.4)	0.002
TGL/HDLC	1.4 (1.0–2.2)	1.3 (0.9–2.0)	1.5 (1.0–2.3)	0.05
Uric acid (μmol/l)	273.0 (222.0–335.0)	246.5 (205.0–290.6)	289.0 (234.0–347.0)	<0.001***
Leptin (pg/ml)	2538.3 (606.8–5477.0)	660.9 (174.5–1993.1)	3988.0 (1336.0–6595.0)	<0.001***
HbA_{1c} (mmol/mol)	90 (61–113)	99 (58–121)	86 (61–110)	0.02
HbA_{1c} (%)	10.3 (7.7–12.5)	11.1 (7.4–13.2)	10.0 (7.7–12.2)	0.02
Fasting blood glucose (mmol/l)	8.6 (6.2–13.4)	9.1 (5.8–14.5)	8.4 (6.2–12.8)	0.28
Fasting serum insulin (pmol/l)	40.97 (20.83–73.61)	29.17 (14.58–44.44)	48.61 (25.69–85.42)	<0.001***
Fasting serum C-peptide (nmol/l)	0.46 (0.27–0.70)	0.33 (0.20–0.53)	0.53 (0.36–0.76)	<0.001***
30 min blood glucose (mmol/l)	13.0 (10.0–18.3)	13.5 (9.9–20.0)	12.6 (10.0–17.5)	0.19

Table 1 (continued)

Characteristic	All study participants (n = 500)	Lean participants (n=160)	Non-lean participants (n=340)	p value (lean vs non-lean)
30 min serum insulin (pmol/l)	77.08 (38.19–156.25)	52.08 (21.53–100.00)	95.83 (45.83–178.47)	<0.001***
30 min C-peptide (nmol/l)	0.70 (0.36–1.09)	0.50 (0.23–0.83)	0.80 (0.50–1.23)	<0.001***
120 min blood glucose (mmol/l)	17.2 (12.3–23.3)	18.8 (14.0–25.2)	16.5 (11.8–22.0)	0.02
120 min serum insulin (pmol/l)	95.14 (47.92–188.19)	61.81 (29.86–123.61)	115.28 (56.25–227.08)	<0.001***
120 min serum C-peptide (nmol/l)	0.93 (0.50–1.59)	0.70 (0.33–1.36)	1.02 (0.60–1.66)	<0.001***
HOMA2-IR	1.21 (0.77–2.03)	0.89 (0.65–1.58)	1.32 (0.84–2.17)	0.001***
QUICKI	0.35 (0.31–0.42)	0.37 (0.33–0.50)	0.34 (0.31–0.39)	0.001***
HOMA2-%B	43.1 (20.7–77.6)	33.3 (15.5–75.8)	44.3 (23.4–77.7)	0.11
Oral IGI (pmol/mmol)	1.3 (0.5–3.9)	0.8 (0.3–2.5)	1.6 (0.6–4.6)	0.001***

Data are presented in form of percentages for categorical variables and as median with IQR for continuous variables

[a] One participant (in the lean category) had missing data on residence

[b] Two participants (in the non-lean category) had missing data on prior admission status

[c] Percentages are calculated using the numbers of people with prior history of admission at diagnosis as the denominator; differences are due to missing data

[d] Used as monotherapy or in combination

[e] Three participants in the lean category and nine participants in the non-lean category had missing data on self-reported HT comorbidity

DBP, Diastolic blood pressure; HC, hip circumference; HDLC, HDL-cholesterol; HT, hypertension; LDLC, LDL-cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TGL, triacylglycerol; WC, waist circumference

***p<0.001

South Asians have also been shown to develop type 2 diabetes at lower BMI values than people of European extraction, which has led to use of Asia-specific lower BMI thresholds for defining obesity [28]. Despite low BMI, South Asians have been shown to have a propensity towards central obesity, increased markers of the metabolic syndrome (ectopic fat, atherogenic lipid profile and higher systolic and diastolic pressures) and lower circulating levels of adiponectin [11, 12, 14, 15]. In contrast, anthropometric (waist circumference, WHR, WHtR) and body composition measurements (total body fat and visceral fat levels) in the lean participants in our study did not indicate increased adiposity. In addition, participants in our study had significantly lower prevalence of other features of the metabolic syndrome (such as hyperleptinaemia and hyperuricaemia) and low HOMA2-IR compared with the non-lean participants. These findings suggest that insulin resistance is not the major underlying mechanism for type 2 diabetes in lean patients in Uganda. Instead, pancreatic beta cell secretory dysfunction, as reflected by a lower oral IGI, fasting and 120 min C-peptide levels and greater blunting of both the first and delayed phases of insulin secretion appears to be the predominant primary pathophysiological defect.

Although the presence of central obesity and related features in the South Asian population with lean type 2

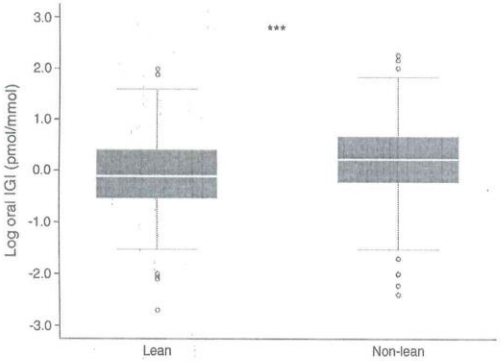

Fig. 1 Comparison of log oral IGI among the lean and non-lean participants. ***p<0.001

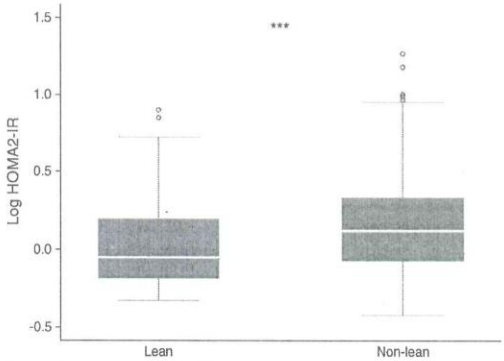

Fig. 2 Comparison of log HOMA-IR between the lean and non-lean participants. ***p<0.001

diabetes led to the speculation that insulin resistance is the driving pathogenic mechanism, more recent studies have established that pancreatic beta cell dysfunction is the primary defect [6, 7, 15, 18, 19]. In this respect, the lean type 2 diabetes phenotype in South Asia may share a common aetiological mechanism with our Ugandan adult population. Differences in manifestation of diabetes may be influenced by other local factors, such as dietary patterns. Phenotypic features similar to those of South Asians, such as increased abdominal visceral fat content relative to abdominal subcutaneous fat, low BMI at diagnosis and pancreatic beta cell dysfunction as the predominant pathogenic defect, have also been described in East Asians with type 2 diabetes [29–31].

The mechanisms that lead to pancreatic beta cell failure in lean patients with type 2 diabetes are not clear. One hypothesis, the Developmental Origins of Health and Disease (DOHaD) concept, suggests that undernutrition during critical windows of development may impair organ development, resulting in a naturally existing small beta cell mass, or may impair beta cell replication or neogenesis or induce metabolic/epigenetic changes that ultimately lead to increased risk of metabolic diseases such as type 2 diabetes later in life [32, 33]. Undernutrition, particularly during pregnancy and in early childhood, remains common in sub-Saharan Africa, and yet coexists with the global obesity epidemic. The combination of adverse early-life influences and a demographic shift towards urbanisation and westernisation may fuel the increased susceptibility to type 2 diabetes in sub-Saharan Africa.

Sub-Saharan Africa is also rich in genetic heterogeneity, which may influence the pathogenesis and clinical course of diabetes in African populations. For example, a recent genome-wide association study of 5231 African patients with type 2 diabetes identified a novel significant locus for type 2 diabetes called *ZRANB3* (encoding zinc finger RANBP2-type containing 3). This gene product, through apoptotic events, leads to reduced pancreatic beta cell mass [34]. The gene encoding transcription factor 7-like 2 (*TCF7L2*), which is known to affect pancreatic secretory function, has also been described in African populations with type 2 diabetes [35].

The relatively high prevalence of type 2 diabetes in lean individuals in sub-Saharan Africa has major implications for screening or diagnosis because age and BMI are widely used to clinically differentiate between type 1 and type 2 diabetes. Each type has a different management approach (one requiring immediate insulin treatment to save life and the other easily managed by lifestyle and/or oral hypoglycaemic therapy). Similarly, age and BMI thresholds are used to decide whom to screen for type 2 diabetes as well as to guide prevention and treatment strategies. The diabetes management approach that involves use of an algorithm that begins with lifestyle intervention or metformin as the first-line agent are based on evidence of benefit in studies of mainly obese or overweight patients of European extraction with predominant insulin resistance in high-income countries [36–38]. It is unclear whether these therapeutic interventions have the same effectiveness in sub-Saharan Africa where many adult patients are relatively young, lean in body size and not insulin-resistant.

Our study had a number of strengths. It had a large sample size and was undertaken across multiple tertiary hospitals recruiting only newly diagnosed patients (within three months of diagnosis). This minimised the potential confounding effects of long-term complications of the disease. We used rigorous study protocols, and screened participants for the presence of three common islet autoantibodies (using local population-derived diagnostic cut-off points for positivity) to exclude those with presumed autoimmune diabetes. We also used one of the highest performing pancreatic autoantibody assays as assessed by the international Islet Autoantibody Standardization Program, with an extensive validation exercise performed on paired samples in an external laboratory (Royal Devon and Exeter NHS Foundation Trust, UK) to ensure robust results.

Despite these strengths, the study had some limitations. Participants were recruited only from tertiary hospitals. This, to an extent, introduces selection bias, which affects the generalisability of study findings to the adult Ugandan population with diabetes. However, it is important to note that these facilities serve the general surrounding population and the majority of patients self-refer to the diabetes clinics for management. In addition, total body fat and visceral fat as markers of body adiposity were assessed using bioimpedance analysis, which is a less sensitive approach and has not been widely validated in adult African populations with type 2 diabetes.

Conclusion Our study has shown that approximately one-third of patients with adult-onset diabetes were lean (BMI <25 kg/m^2) and that, pathophysiologically, features of reduced pancreatic beta cell secretory capacity predominate with little contribution from increased total body and visceral adiposity and insulin resistance.

The mechanisms explaining the observed pancreatic secretory dysfunction need to be rigorously investigated, and additional studies are required to develop individualised therapeutic approaches to improve management of lean patients with type 2 diabetes.

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Data availability The data are available on request from the authors.

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Contribution statement DK participated in the study design, data acquisition, analysis and interpretation, and wrote the initial draft of the manuscript. IS participated in the study design, data analysis and interpretation, and reviewed all the versions of the manuscript. WL participated in the data collection process, data interpretation, and reviewed all the versions of the manuscript. AGJ, ATH, LS and MJN supervised this work, collectively contributed to the research idea, and reviewed all versions of the manuscript. All authors read and approved the final draft of the manuscript. DK is responsible for the integrity of the work as a whole.

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CHAPTER SEVEN: PERIPHERAL ARTERIAL DISEASE IN ADULT UGANDANS WITH NEW-ONSET DIABETES: BURDEN AND CORRELATES

This chapter presents the findings of a sub-study that investigated the burden and predictors of peripheral arterial disease in a randomly selected adult population with new-onset diabetes, as part of the third objective of the Ph.D. aimed to determine the prevalence and correlates of two specific diabetes complications (peripheral arterial disease and diabetic nephropathy) in this cohort.

7.1 Ph.D. research paper 4: Burden and correlates of peripheral arterial disease in adult Ugandan patients with recently diagnosed diabetes

7.2 Summary of key findings

In this study, peripheral arterial disease (PAD) was assessed by measuring the ankle brachial index in 255 randomly selected adult patients with newly diagnosed diabetes receiving diabetes care at three tertiary hospitals. PAD was noted to be present in 17.3% of the participants, with the majority having a severe form of the condition (93.2%). Despite the high frequency of severe PAD, none of the participants had intermittent claudication or gangrene on clinical examination. Female gender, urine albumin creatinine ratio, and fasting blood glucose were noted to independently predict PAD in this study population. No association was observed between PAD and the conventional cardiovascular risk factors like age, smoking, hypertension, and dyslipidaemia.

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Principal Supervisor	Prof. Moffat Nyirenda
Title	An in-depth understanding of the clinical, metabolic, and immunologic profile of adult patients with newly diagnosed diabetes in Uganda: the Uganda DIabetes Phenotype (UDIP) study.

If the Research Paper has previously been published, please complete Section B, if not please move to Section C.

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Stage of publication	Manuscript completed and ready for submission.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I fully participated in the study design, data collection, management, analysis, and interpretation. I also drafted the first version of the manuscript, incorporated all comments from the supervisors and other co-authors, submitted the final manuscript, and coordinated all the correspondences with the reviewers.

SECTION E

Student's signature	[redacted]
Date	31/05/2022

Primary supervisor's signature	[redacted]
Date	31/05/2022

High frequency of severe peripheral arterial disease in adult Ugandan patients with recently diagnosed diabetes

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ABSTRACT

Background

Peripheral arterial disease (PAD) is a major cause of cardiovascular morbidity and mortality in patients with diabetes globally. However, the true burden and predictors of PAD, particularly at the time of diagnosis of diabetes, in sub-Saharan Africa are largely unknown. This study aimed to determine the prevalence and clinical correlates of PAD in a randomly selected adult Ugandan population with new-onset diabetes.

Methods

Two hundred fifty five adult patients with a recent diagnosis of diabetes (<3 months) were recruited from three tertiary hospitals in Uganda. Relevant socio-demographic, clinical, anthropometric, and metabolic data were collected. PAD was assessed by measurement of resting ankle brachial index (ABI) in the lower limbs and was defined as an ABI ≤0.9 in either lower limb. Multivariate logistic regression was performed to identify the independent predictors of PAD.

Results

The median (IQR) age, ABI, and glycated haemoglobin of the study participants were 50 (39-59) years, 1.14 (1.04-1.21), and 10.6 (7.3-12.5) % or 92 (57-113) mmol/mol, respectively. PAD was observed in 44 (17.3%) participants. Nearly all of them (41, 93.2%) had severe PAD (defined as ABI ≤0.5). Predictors of PAD were female gender (odds ratio or OR 3.35 95% CI 1.49-7.52, p=0.003), urine albumin creatinine ratio or UACR (OR 1.09 95% CI 1.03-1.16, p=0.006), and fasting blood glucose or FBG (OR 1.11 95% CI 1.05-1.19, p=0.001). No association was noted with other conventional cardiovascular risk factors like increasing age, obesity, smoking, dyslipidaemia, and hypertension.

Conclusions

PAD was noted to occur in about 1 in every 6 adult patients with recently diagnosed diabetes, with the majority having severe PAD. Female gender, UACR, and FBG were identified as the independent predictors of PAD in this study population.

Keywords: Peripheral arterial disease, adult-onset diabetes, new-onset diabetes, Uganda, sub-Saharan Africa.

INTRODUCTION

Peripheral arterial disease (PAD) is a coronary artery disease equivalent and one of the common cardiovascular causes of morbidity and mortality in patients with diabetes globally [1, 2]. Despite the documented increasing prevalence of PAD in sub-Saharan Africa (SSA), the condition remains largely underdiagnosed in most clinical settings. Routine screening for PAD in patients with diabetes at the time of diagnosis or in routine clinical care remains suboptimal [3]. Measurement of resting ankle brachial index (ABI) is the first noninvasive approach recommended for the assessment of PAD in clinical care [4, 5].

Most studies that have investigated the burden and correlates of PAD in SSA have been performed in adult patients with long-standing type 2 diabetes [6-15]. Few studies have investigated its burden in adult patients with new-onset diabetes. Studies evaluating the burden of diabetes complications in patients with newly diagnosed diabetes in SSA are important because the diagnosis of diabetes is often delayed, and a significant proportion of patients may present with debilitating complications at diagnosis.

This study aimed to determine the burden and clinical correlates of PAD in an adult population with newly diagnosed diabetes receiving outpatient diabetes care from three tertiary public and private hospitals in Uganda.

METHODS

Study design, sites, and participants

This cross-sectional study was conducted in outpatient diabetes clinics of three tertiary private and public hospitals located in Central and Southwestern Uganda from February 2019 to October 2020. Participants aged ≥18 years with a recent diagnosis of diabetes (<3 months) and who had provided written informed consent were consecutively recruited into the study. Pregnant women were excluded from the study. A total of 255 patients were recruited for this study.

Socio-demographic, clinical, anthropometric, and metabolic characterisation

Participants were assessed following an overnight fast of ≥8 hours. Using a pre-tested study questionnaire, relevant socio-demographic and clinical data were collected from each study participant by the study research team. This included age at diagnosis, gender, residence, smoking and alcohol consumption habits, co-existing medical conditions (hypertension and HIV notably), and diabetes and ancillary therapies initiated at diagnosis.

Following standardised study procedures, all study participants were then subjected to biophysical measurements which included resting blood pressure (BP) and anthropometric measurements (weight, height, waist circumference [WC], hip circumference [HC], waist: hip circumference ratio [WHR] and waist circumference: height ratio [WHtR]).

A fasting venous blood sample was then collected for the measurement of blood glucose (FBG), glycated haemoglobin (HbA1c), lipid profile, uric acid, and serum creatinine (for estimation of the estimated glomerular filtration rate or e-GFR). All of these tests were performed using electro-chemiluminescence immunoassays manufactured by Roche diagnostics Limited, Germany on a Cobas 6000 C-model SN

14H3-15 machine (Hitachi High Technologies Corporation, Tokyo Japan). A spot mid-stream urine sample was also obtained for the assessment of urine albumin creatinine ratio (UACR) using the Siemens Healthcare Clinitek® microalbumin reagent test strips and a point-of-care Clinitek® status analyser. All the tests were performed at the ISO-certified clinical chemistry laboratory at the Medical Research Council/Uganda Virus Research Institute and London School of Hygiene and Tropical Medicine Uganda Research Unit, Entebbe Uganda.

Measurement of ABI to assess the presence of PAD

After a 10-minute rest in a still supine position, an ABI measurement to assess the presence of PAD was performed using an automated MESI® ABI measuring device (MESI® APBI MD). Differently coloured and well-labelled cuffs in appropriate adult sizes were separately placed on each participant's left upper arm and slightly above the left and right ankle joints. The manufacturer's instructions were closely followed during the entire process of the ABI measurement.

Study definition of PAD

The presence of PAD was defined as the presence of an ABI of ≤0.9 in any of the lower limbs while severe PAD was defined as an ABI ≤0.5 in either lower limb. Participants with an ABI of 0.91-0.99, 1.00-1.40, and >1.40 were considered to have borderline ABI, normal ABI, and non-compressible arteries, respectively [4].

Statistical analysis

Proportions and medians with inter-quartile range (IQR) were used to describe the categorical and continuous variables, respectively. The differences in the socio-demographic, clinical, anthropometric, and metabolic characteristics between participants with and without PAD were analysed using the x^2 test for categorical data and the Kruskal Wallis test for continuous data. Specific sociodemographic, clinical,

and metabolic characteristics that have been shown to be associated with PAD in literature were added to the logistic regression model to identify the independent predictors of PAD. All analyses were done using STATA statistical software version 15. A p-value <0.05 with a 95% confidence interval (95% CI) without 1 was considered statistically significant.

RESULTS

Baseline characteristics of all study participants

The socio-demographic, clinical, and metabolic characteristics of all the study participants are summarised in table 1.

The median age and ABI of the study participants were 50 (30-59) years and 1.14 (1.04-1.21), respectively with about 52% of the participants being female. A current or past history of smoking and alcohol consumption was reported in about 7% and 34% of participants, respectively. About 33% and 11% of the participants reported co-existing hypertension and HIV infection, respectively. A small proportion of participants were initiated on oral lipid-lowering and antiplatelet therapies at the time of diagnosis by their attending clinicians (19% and 7%, respectively).

Burden of PAD

An ABI of ≤0.9 was documented in 44 study participants, reflecting a prevalence of PAD of 17.3%. Of these 44 participants, 41 (93.2%) had severe PAD. A borderline and normal ABI was recorded in 2.4% and 80%, respectively. Only one (0.4%) participant had noncompressible arteries.

Comparison of socio-demographic, clinical, anthropometric, and metabolic characteristics of participants with and without PAD

Table 1 summarises the socio-demographic, clinical, anthropometric, and metabolic characteristics of the participants with and without PAD.

Compared with those without PAD, participants with PAD were more likely to be female (≈73% vs 47%, p=0.002), to have HIV infection (21% vs 9%, p=0.02), a higher median UACR (3.41 [1.14-6.82] vs 2.27 [1.14-3.41] mg/mmol, p=0.002) and FBG (11.0 [7.6-16.5] vs 8.3 [6.1-11.9] mmol/l, p=0.001), and a lower median e-GFR (108.1 [90.8-130.1 vs 120.5 [106.4-133.4] ml/min/1.73m^2, p=0.004). No association was noted between PAD and other conventional cardiovascular risk factors like age at diagnosis (p=0.17), current or history of smoking (p=0.26), alcohol ingestion (p=0.15), presence of hypertension comorbidity (p=0.11), systolic BP level (p=0.14), measures of increased adiposity (BMI [p=0.24] and WC [p=0.87]), and dyslipidaemia on bivariate analysis. Despite the high frequency of participants with severe PAD, none had intermittent claudication or gangrene on clinical assessment.

On multivariate analysis, PAD in this study population was independently associated with female gender (odds ratio or OR 3.35 95% CI 1.49-7.52, p=0.003), FBG (OR 1.11 95% CI 1.05-1.19, p=0.001), and UACR (OR 1.09 95% CI 1.03-1.16, p=0.006).

Table 2 summaries the independent predictors of PAD.

DISCUSSION

In this study population, we report PAD to occur in about one in every six adult patients with newly diagnosed diabetes, with most having severe PAD. Female gender, UACR, and FBG were identified as the independent predictors of PAD.

The prevalence of PAD in adult patients with diabetes in SSA has been reported in cross-sectional studies to range from 8.3% to 61.7% [6-15]. The reasons for this striking variation in the documented burden of PAD in SSA are unclear but may relate to genetic heterogeneity and population differences in the distribution of other cardiovascular risk factors such as smoking, obesity, dyslipidaemia, as well as methodological differences. In particular, the duration of diabetes plays an important

role in the manifestation of PAD. Our findings are closest to those of studies from Ghana [8] and Tanzania [9], where rates of 18.6% and 20.7%, respectively were observed. However, these were studies performed in patients with long-standing diabetes, rather than in patients with newly diagnosed diabetes. Because of this, the findings are, therefore, not directly comparable.

In general, the prevalence of PAD in African populations is significantly higher than that reported in white and South Asian populations, where rates of 6.6 to 10.4% have been observed [16-21]. In addition, PAD in these populations is strongly associated with common cardiovascular risk factors, including increased age, smoking, obesity, dyslipidaemia, and insulin resistance. While some of these risk factors, such as the presence of microalbuminuria and evidence of poor glycaemic control (high fasting glucose and HbA1c) were higher in our study participants with PAD, we did not observe an association with several other conventional cardiovascular risk factors like increasing age, smoking, hypertension, dyslipidaemia, markers of adiposity (BMI, WC, WHR, and WHtR), and hyperuricaemia. In addition, despite the high prevalence of HIV co-infection comorbidity in patients with PAD (20.5%), no association was observed with PAD at multivariate analysis. The reasons for this discrepancy are unknown but may represent the existence of novel emerging risk factors or genetic predisposition in our local population and probably could also be related to the insufficient power of the study.

Microalbuminuria has also been reported to be associated with PAD in the Asian and white populations [22-24]. In addition to being a predictor of kidney damage, microalbuminuria is also a well-documented predictor of subclinical vascular damage and increased cardiovascular morbidity and mortality in the general population and specific patient populations like patients with diabetes and hypertension [25-27].

Our observation of female preponderance in PAD risk is in accord with other previous studies in the region and internationally. For example, a study of 229 adult patients with long-standing diabetes in Uganda showed that being female increased the odds of having PAD by almost 2-fold [10]. Similarly, large studies in white populations of European descent have also documented an increased burden of PAD in females compared to male participants. Some of the postulated reasons for increased PAD among women include older age, higher BMI, blood pressure, lipid, and inflammation indices [28, 29].

Study limitations

Despite the findings, our study had some limitations. Participants were recruited from a limited number of tertiary hospitals; therefore, these findings cannot be directly extrapolated to the general Ugandan population with newly diagnosed diabetes. Being cross-sectional in design, the study does not provide evidence about the temporal relationship between PAD and diabetes.

The study also had an insufficient sample size or limited power, and this could explain the observed lack of association between the conventional cardiovascular risk factors and PAD in this study population.

CONCLUSION

PAD was noted to occur in about one in every six adult patients with recently diagnosed diabetes, with the majority having severe PAD. Female gender, UACR, and FBG were identified as the independent predictors. The absence of association of PAD with the conventional cardiovascular risk factors indicates that PAD in our adult population with new-onset diabetes might be influenced by novel cardiovascular risk factors which need to be robustly investigated. In addition, the absence of intermittent

claudication and gangrene on clinical assessment in patients with severe PAD is intriguing and the reasons for this observation warrant further investigation.

List of abbreviations

ABI-ankle brachial index, BMI-body mass index, BP-blood pressure, CVD-cardiovascular disease, e-GFR-estimated glomerular filtration rate, FBG-fasting blood glucose, HbA1c-glycated haemoglobin, HC-hip circumference, PAD-peripheral arterial disease, SSA-sub-Saharan Africa, UACR-urine albumin creatinine ratio, WC-waist circumference, WHR-waist: hip circumference ratio, WHtR-waist circumference: height ratio.

Declarations

Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of Uganda Virus Research Centre, Entebbe Uganda (GC/127/18/05/650) and the Uganda National Council of Science and Technology (HS 2431). Administrative approval was also obtained from all participating study sites. All enrolled study participants provided written informed consent to participate in the study.

Consent for publication

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

DK oversaw the entire data collection process and wrote the initial draft of the manuscript. IS performed the statistical analysis and reviewed all the versions of the manuscript. WL contributed to the discussion and reviewed all the versions of the manuscript. AGJ, ATH, LS, and MJN supervised this work, collectively contributed to the research idea, and reviewed all the versions of the manuscript. All the authors read and approved the final draft of the manuscript.

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Table 1. Baseline sociodemographic, clinical, anthropometric, and metabolic characteristics of all study participants, and those with and without PAD separately

Characteristics	All study participants (n=255)	PAD present (n=44, 17.3%)	PAD absent (n=211, 82.7%)	P-value
Socio-demographic and clinical				
Age, years	50 (39-59)	55 (44-63)	49 (38-58)	0.17
Gender Males	123 (48.2)	12 (27.3)	111 (52.6)	0.002
Females	132 (51.8)	32 (72.7)	100 (47.4)	
Residence Urban	210 (82.4)	31 (70.5)	179 (84.8)	0.05
Rural	44 (17.3)	13 (29.6)	31 (14.7)	
Smoking status Yes	6 (2.4)	2 (4.6)	4 (1.9)	0.26
Quit	11 (4.3)	3 (6.8)	8 (3.8)	
Alcohol consumption				
Yes	57 (22.4)	5 (11.4)	52 (24.8)	0.15
Quit	29 (11.5)	6 (13.6)	23 (11.0)	
Self-reported comorbidities				
Hypertension	84 (32.9)	19 (43.2)	65 (30.8)	0.11
HIV infection	27 (10.6)	9 (20.5)	18 (8.5)	0.02
Systolic BP, mmHg	125 (115-135)	121 (109-134)	125 (117-135)	0.14
Diastolic BP, mmHg	84 (77-90)	83 (78-91)	84 (77-90)	0.43
Anthropometry				
BMI, kg/m^2	27.3 (23.6-31.7)	25.9 (22.7-32.9)	27.5 (23.9-31.5)	0.24
BMI <25 kg/m^2	86 (34)	18 (41.9)	68 (32.4)	0.46
25-29.9 kg/m^2	79 (31.2)	11 (25.6)	68 (32.4)	
≥30 kg/m^2	88 (34.8)	14 (32.5)	74 (35.2)	
WC, cm	96 (88-105)	96.5 (88-108)	96 (88-104)	0.87
HC, cm	104 (96.5-111.8)	104.1 (96.0-116.5)	104 (96.5-111.8)	0.97
WC: HC ratio	0.91 (0.87-0.96)	0.90 (0.85-0.95)	0.91 (0.87-0.96)	0.48
WC: height ratio	0.59 (0.53-0.65)	0.59 (0.51-0.69)	0.59 (0.53-0.65)	1.00
Metabolic				
TC, mmol/l	4.1 (3.3-5.2)	4.2 (3.3-4.7)	4.0 (3.3-5.2)	0.73
HDLC, mmol/l	1.0 (0.8-1.2)	1.1 (0.8-1.4)	1.0 (0.8-1.2)	0.11
TGL, mmol/l	1.3 (0.9-1.8)	1.4 (1.1-2.0)	1.2 (0.9-1.8)	0.31
LDLC, mmol/l	2.6 (1.9-3.5)	2.5 (1.8-3.4)	2.6 (1.9-3.5)	0.72
Non-HDLC, mmol/l	3.0 (2.4-3.8)	3.1 (2.5-3.6)	3.0 (2.3-3.8)	0.71
TC/HDLC	4.1 (3.3-5.1)	3.9 (3.2-5.4)	4.1 (3.3-5.1)	0.46
TGL/HDLC	1.4 (0.9-2.1)	1.4 (0.9-2.0)	1.4 (0.9-2.1)	0.96
Uric acid, mmol/l	282.7 (223-346)	268 (217-342)	284 (226-346.4)	0.69

UACR, mg/mmol	2.27 (1.14-3.41)	3.41 (1.14-6.82)	2.27 (1.14-3.41)	0.002
Serum creatinine, µmol/l	65 (54-76)	66 (56-78)	65 (54-76)	0.78
e-GFR, ml/min/1.73m^2	119.6 (102.5-131.9)	108.1 (90.8-130.1)	120.5 (106.4-133.4)	0.004
HbA1c, %	10.6 (7.3-12.5)	10.6 (8.6-13.2)	10.6 (7.2-12.4)	1.00
HbA1c, mmol/mol	92 (57-113)	92 (71-114)	92 (55-112)	1.00
FBG, mmol/l	8.6 (6.5-13.1)	11. 0 (7.6-16.5)	8.3 (6.1-11.9)	0.001

Data is presented in form of percentages for categorical variables and median with IQR for continuous variables

Table 2. Multivariate analysis to identify independent predictors of PAD

Characteristic	**AOR (95%CI)**	**P-value**
Age, years	1.02 (0.99-1.05)	0.11
Female gender	3.35 (1.49-7.52)	0.003
UACR, mg/mmol	1.09 (1.03-1.16)	0.006
Fasting blood glucose, mmol/l	1.11 (1.05-1.19)	0.001

AOR- Adjusted odds ratio, CI- Confidence interval

CHAPTER EIGHT: DIABETIC KIDNEY DISEASE IN ADULT UGANDANS WITH NEW-ONSET DIABETES

This chapter presents the findings of another sub-study that investigated the burden and predictors of diabetic nephropathy in randomly selected adult patients with new-onset diabetes, as part of the third objective of the Ph.D. that aimed to determine the prevalence and correlates of two specific diabetes complications (peripheral arterial disease and diabetic nephropathy) in this cohort.

8.1 Ph.D. research paper 5: Burden and predictors of diabetic kidney disease in an adult Ugandan population with new-onset diabetes

8.2 Summary of key findings

In this study, diabetic kidney disease (DKD) was assessed by measurement of urine albumin creatinine ratio and estimated glomerular filtration rate (e-GFR) in 519 randomly selected adult patients with newly diagnosed diabetes. DKD was confirmed in 33.7% of the participants, of which, only 1.4% had an e-GFR <60 ml/min/1.73 m^2 suggestive of chronic kidney disease. Self-reported hypertension comorbidity and obesity were noted to independently predict DKD in this study population.

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Principal Supervisor	Prof. Moffat Nyirenda
Title	An in-depth understanding of the clinical, metabolic, and immunologic profile of adult patients with newly diagnosed diabetes in Uganda: the Uganda DIabetes Phenotype (UDIP) study.

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Stage of publication	Manuscript completed and ready for submission.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I fully participated in the study design, data collection, management, analysis, and interpretation. I also drafted the first version of the manuscript, incorporated all comments from the supervisors and other co-authors, submitted the final manuscript, and coordinated all the correspondences with the reviewers.

SECTION E

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Primary supervisor's signature	
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Burden and predictors of diabetic kidney disease in an adult Ugandan population with new-onset diabetes

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ABSTRACT

Background

Despite the growing evidence of diabetic kidney disease (DKD) in adult patients with long-standing diabetes in sub-Saharan Africa, data on its burden and correlates in adult African patients with new-onset diabetes are limited. We, therefore, undertook this study to determine the burden and predictors of DKD in an adult population with new-onset diabetes in Uganda.

Methods

We collected data on the relevant sociodemographic, clinical, anthropometric, and metabolic characteristics in 519 participants with newly diagnosed diabetes recruited from seven tertiary hospitals. A spot mid-stream urine sample was collected for determination of the urine albumin creatinine ratio (UACR) using Clinitek® microalbumin strips and a point-of-care Clinitek® status analyser. The estimated glomerular filtration rate (e-GFR) was determined using the Chronic Kidney Disease Epidemiology formula. The presence of DKD was defined as a spot UACR ≥3 mg/mmol with or without an e-GFR <60 ml/min/1.73m^2.

Results

The median (IQR) age, UACR, and e-GFR of the participants were 48 years (39-57), 2.27 mg/mmol (1.14-3.41), and 121.8 ml/min/1.73m^2 (105.4-133.9). UACR ≥3 mg/mmol and e-GFR <60 ml/min/1.73m^2 was noted in 175 (33.7%) and 7 (1.4%) participants, respectively. DKD was documented in 175 participants (33.7%). Compared with those without DKD, participants with DKD were more likely to be ≥50 years of age (53.7% vs 43%, p= 0.02) and to have co-existing hypertension at the time of diagnosis (40.6% vs 30.1%, p=0.02). On multivariate analysis, self-reported hypertension comorbidity (OR 1.76 95% CI 1.24-2.48, p=0.002) and body mass index

(BMI) ≥ 30 kg/m^2 (OR 0.61 95% CI 0.41-0.91, p=0.02) were noted to independently predict DKD.

Conclusion

In this study population, DKD was relatively common and was independently associated with self-reported hypertension comorbidity and BMI ≥ 30 kg/m^2.

Keywords

Diabetic kidney disease, adult patients, new-onset diabetes, Uganda, sub-Saharan Africa.

INTRODUCTION

Diabetic kidney disease (DKD) is a heterogeneous clinical condition characterised by the presence of persistent overt proteinuria (urine albumin creatinine ratio or UACR ≥300 mg/g or 3 mg/mmol) and declining renal function reflected by an estimated glomerular filtration rate (e-GFR) of <60 ml/min/1.73 m^2 (1). Traditionally, the presence of albuminuria has been recognised as the hallmark of DKD (classical albuminuric DKD). However, recent studies have shown that, in addition to the classical albuminuric DKD phenotype, two new nonalbuminuric phenotypes of DKD exist, i.e., nonalbuminuric DKD and progressive renal decline, suggesting that progression of DKD can also occur through a non-albuminuric pathway (2, 3).

An increasing burden of DKD has been reported in adult patients with type 2 diabetes in sub-Saharan Africa (SSA) (4-6). While there is reasonable data on the prevalence of DKD in African patients with long-standing diabetes, data on its burden and predictors in adult patients with newly diagnosed diabetes remains limited.

This study aimed to investigate the prevalence, phenotypes, and predictors of DKD in an adult Ugandan population with newly diagnosed diabetes.

METHODS

Study settings, duration, and participants

This study was performed in seven tertiary hospitals located in Central and Southwestern Uganda from February 2019 to October 2020. These hospitals serve the surrounding urban, peri-urban, and rural populations.

Participants were patients aged ≥18 years with a new diagnosis of diabetes (<3 months from the time of diagnosis). They were recruited from outpatient specialist diabetes clinics. Participants with fever, any acute illness, and pregnancy were excluded. Both treatment naïve and those who had been initiated on any glucose-lowering treatment were eligible to participate. A total of 519 participants were recruited for the study.

Socio-demographic, clinical, anthropometric, and metabolic characterisation

Participants were assessed following an overnight fast (≥8 hours). Using a pre-tested case report form, relevant socio-demographic and clinical data were collected. This included age at diagnosis, gender, smoking habits (current and past), and co-existing hypertension comorbidity. Following standardised study procedures, biophysical measurements which included resting blood pressure and relevant anthropometric measurements (weight, height, waist circumference [WC], hip circumference [HC], body mass index [BMI], and waist: hip circumference ratio [WHR] were then performed.

A fasting blood sample was then collected for the measurement of blood glucose (FBG), glycated haemoglobin (HbA1c), lipid profile, uric acid, a complete blood count (for estimation of the haemoglobin level), serum urea, and serum creatinine (for estimation of the e-GFR) using electro-chemiluminescence immunoassays

manufactured by Roche diagnostics Limited, Germany on a Cobas 6000 C-model SN 14H3-15 machine (Hitachi High Technologies Corporation, Tokyo Japan).

The study participants were then advised on how to collect a spot mid-stream urine sample in a sterile urine container, and UACR was assessed using the Siemens Healthcare Clinitek® microalbumin reagent test strips and a point-of-care Clinitek® status analyser. The Clinitek® status analyser also provided data on urine leucocytes, nitrites, glucose, blood, and pH.

All laboratory tests were performed at the ISO-certified clinical chemistry laboratory at the Medical Research Council/Uganda Virus Research Institute and London School of Hygiene and Tropical Medicine Uganda Research Unit, Entebbe Uganda.

The e-GFR was determined using the Chronic Kidney Disease Epidemiology (CKD-EPI) formula (7). The e-GFR and the UACR were classified according to the Kidney Disease: Improving Global Outcomes (KDIGO) 2020 Clinical Practice Guideline for Diabetes Management in Chronic Kidney Disease. The e-GFR was categorized as follows: G1: ≥90mL/min/1.73m^2 (normal kidney function), G2: 60–89mL/min/1.73 m^2 (mildly decreased), G3a: 45–59mL/min/1.73 m^2 (mildly to moderately decreased), G3b: 30–44mL/min/1.73 m^2 (moderately to severely decreased), G4: 15–29mL/min/1.73 m^2 (severely decreased), and G5: <15mL/min/1.73m^2 (kidney failure). Albuminuria categories based on the UACR were as follows: A1: <3mg/mmol (normal to mildly increased), A2: 3–30mg/mmol (moderately increased), and A3: >30mg/mmol (severely increased) (1).

Definition of diabetic kidney disease and its phenotypes

DKD was defined as a spot UACR ≥3 mg/mmol (A2 and A3) with or without a reduced e-GFR of <60 ml/min/1.73m^2 (G3a-G5). Albuminuric and non-albuminuric DKD was defined as a spot UACR ≥3 mg/mmol (A2 and A3) regardless of the e-GFR and an e-

GFR<60 ml/min/1.73m^2 with UACR <3 mg/mmol (G3a-G5 and A1). No DKD was defined as an e-GFR ≥90 ml/min/1.73m^2 and UACR <3 mg/mmol (G1 and A1) (1).

Statistical analysis

To describe the characteristics of all study participants, we used proportions for the categorical variables and medians with inter-quartile range (IQR) for the continuous variables. Proportions were also used to express those with and without DKD, and the two DKD phenotypes (albuminuric and non-albuminuric DKD).

The differences in the socio-demographic, clinical, anthropometric, and metabolic characteristics of participants with and without DKD were analysed using the x^2 test for categorical data and the Kruskal Wallis test for continuous data. Odds ratios (OR) and their corresponding 95% confidence intervals (CI) were estimated using logistic regression. Specific sociodemographic, clinical, and metabolic characteristics well-known to be associated with DKD were added to the logistic regression model to identify the independent predictors of DKD. All analyses were done using STATA statistical software version 15 College Station, TX: StataCorp LLC.

RESULTS

The characteristics of all study participants and those with and without DKD separately are shown in table 1.

The median (IQR) age, HbA1c, UACR, and e-GFR of the participants was 48 years (39-57), 10.6% (7.8-12.5) or 92 mmol/mol (62-114), 2.27 mg/mmol (1.14-3.41), and 121.8 ml/min/1.73m^2 (105.4-133.9). A UACR of <3 mg/mol (A1 category), 3-30 mg/mmol (A2 category), and >30 mg/mmol (A3 category) were noted in 344 (66.3%) participants, 167 (32.2%) participants, and 8 (1.5%) participants, respectively. An e-GFR of ≥90 ml/min/1.73m^2 (G1 category), 60-90 ml/min/1.73m^2 (G2 category), 45-59 ml/min/1.73m^2 (G3a category), 30-44 ml/min/1.73m^2 (G3b category), and <15

ml/min/1.73m^2 (G5 category) was noted in 461 (88.8%) participants, 51 (9.8%) participants, 4 (0.8%) participants, 2 (0.4%) participants, and 1 (0.2%) participant, respectively. No participant had an e-GFR between 15 and 29 ml/min/1.73m^2.

DKD was observed in 175 participants (33.7%) participants.

Socio-demographic, clinical, and metabolic characterisation of participants with and without DKD

Compared with those without DKD, participants with DKD generally were more likely to be ≥50 years of age (53.7% vs 43%, p= 0.02) and have co-existing hypertension at the time of diagnosis (40.6% vs 30.1%, p=0.02). No significant differences in sex, anthropometric (BMI, WC, and WHR), and metabolic (glycaemic indices-FBG and HbA1c, lipid profile, and uric acid concentrations) characteristics were noted between the two groups.

Independent predictors of DKD

Table 2 shows the multivariate analysis to identify predictors of DKD.

On multivariate analysis, self-reported hypertension comorbidity (OR 1.76 95% CI 1.24-2.48, p=0.002) and BMI ≥ 30 kg/m^2 (OR 0.61 95% CI 0.41-0.91, p=0.02) were noted to be independent predictors of DKD.

DISCUSSION

In this study population, we report that DKD was relatively common, with the majority of participants presenting with the albuminuric phenotype. We also documented that pre-existing hypertension increased the odds of DKD while obesity reduced the risk.

A wide variation in the prevalence of DKD has been reported across African populations (4-6, 8, 9). In one systematic review and meta-analysis of 21 medium- and high-quality studies performed in SSA to document the epidemiology of chronic kidney disease, the prevalence of DKD in the included studies was reported to range between

7.3% and 24%, with proteinuria being used as the main marker of the presence of DKD, in 96% of the studies (6). In another systematic review of 32 studies (two population-based and 30 clinic-based) performed in Africa by Noubiap JJ et al, the prevalence of DKD varied from 11% to 83.7%, with about 63% of the studies diagnosing DKD based on urine protein measurement (4). Another systematic review and meta-analysis that evaluated the burden of DKD and its association with hypertension in 27 studies performed in SSA reported a pooled prevalence of DKD of 35.3% (5), similar to what we documented in our study.

The reasons to explain these significant variations in the prevalence of DKD across the different African populations may be related to differences in diagnostic methods (urine protein and e-GFR measurement) and diagnostic thresholds used, populations studied (long-standing vs newly diagnosed or type 1 vs type 2 diabetes or combined), and study design (population-based vs health facility-based or private vs public health facilities).

In our study, pre-existing hypertension was documented to be associated with increased odds of having DKD. Hypertension is a well-recognised risk factor of DKD, regardless of phenotype, in African (5, 8, 10-12), white (13, 14), and Asian (15, 16) populations. The mechanisms to explain the association between hypertension and DKD are not well-understood but may be related to augmented sodium retention, pro-inflammatory cascade, renin-angiotensin-aldosterone, sympathetic nervous system, endothelial cell dysfunction, and oxidative stress, which ultimately result in glomerular damage (17, 18).

Obesity (BMI ≥30 kg/m^2) was documented to reduce the odds of DKD in our study population, a finding conflicting with what has been observed in most studies

investigating risk factors of DKD (19). This underscores the heterogeneity in the pathogenesis of DKD across populations.

A similar protective effect of obesity towards having DKD was also reported in a retrospective study performed in rural South Africa in 253 adult patients with diabetes. Obesity and severe obesity defined as BMI >27 kg/m^2 and >33 kg/m^2 were present in 63% and 36.5% of the participants, respectively. On multivariate analysis, severe obesity was associated with reduced odds of having microalbuminuria (OR 0.27 95% CI 0.08-0.48, p=0.002) (11). A BMI >25 kg/m^2 was also noted to be associated with a reduced likelihood of chronic kidney disease, regardless of cause, in an urban and peri-urban community-based study performed in Uganda (20).

The reasons explaining the increased risk of DKD in individuals with normal or low BMI as reported in our study have not been investigated in great detail but could partly be explained by the developmental origins of health and disease (DOHaD) theory or developmental programming. Early-life environmental insults (in-utero or early childhood) like chronic infections, pre-eclampsia, and maternal malnutrition induce epigenetic changes that affect gene expression, organ development, and function later in life resulting in several cardiometabolic conditions. Increased susceptibility to hypertension (a common predictor of kidney disease in African patients) and chronic kidney disease often develop due to an existing low nephron number or mass (Brenner hypothesis) (21).

It is important to also note that patients with diabetes can develop kidney diseases due to other aetiologies. Hospital and community-based studies conducted in Uganda have reported hypertension, postinfectious glomerulonephritis, and HIV co-infection as common causes of kidney disease in adult Ugandans (12, 20, 22).

Study limitations

We used a single-spot UACR measurement to determine participants with DKD. This could have led to an over-representation of the burden of DKD in this study population. We were also unable to screen for diabetic retinopathy, as recommended, to correlate the diagnosis of DKD, especially in patients with the albuminuric phenotype.

CONCLUSION

DKD especially the albuminuric phenotype was relatively frequent in these adult Ugandan patients with newly diagnosed diabetes. A positive association was observed between hypertension comorbidity and DKD while obesity had an inverse association. Measurement of UACR as a measure of assessing DKD should be encouraged in clinical care, especially in patients with co-existing hypertension and BMI <30 kg/m^2.

List of abbreviations

BMI-body mass index, CKD-EPI- Chronic Kidney Disease Epidemiology formula, DKD-diabetic kidney disease, e-GFR-estimated glomerular filtration rate, FBG-fasting blood glucose, HbA1c-glycated haemoglobin, HC-hip circumference, KDIGO- Kidney Disease: Improving Global Outcomes, SSA-sub-Saharan Africa, UACR-urine albumin creatinine ratio, WC-waist circumference, WHR-waist: hip circumference ratio.

DECLARATIONS

Ethics approval and consent to participate

The study received ethical approval from the research ethics committee of the Uganda Virus Research Institute (GC/127/18/05/650) and the Uganda National Council of Science and Technology (HS 2431). All participating study sites offered administrative approval before the initiation of the study. All study participants recruited into the study offered written informed consent.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

All the authors report no conflict of interest.

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Authors' contributions

DK oversaw the entire data collection process, and data interpretation, and wrote the initial draft of the manuscript. IS performed the statistical analysis, and data interpretation, and reviewed all the versions of the manuscript. WL contributed to data collection and interpretation and reviewed all the versions of the manuscript. AGJ, ATH, LS, and MJN supervised this work, collectively contributed to the research idea, and reviewed all the versions of the manuscript. All the authors read and approved the final draft of the manuscript.

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Table 1. Socio-demographic, clinical, anthropometric, and metabolic characteristics of all participants, and participants with and without DKD separately

Characteristics	All study participants (n=519)	DKD present (n=175, 33.7%)	No DKD (n=344, 66.3%)	P value
Socio-demographic and clinical				
Age (years)§	48 (39-57)	50 (42-59)	47 (39-56)	0.36
≥ 50 years*	242 (46.6)	94 (53.7)	148 (43)	0.02
Sex*				
Males	227 (43.7)	68 (38.9)	159 (46.2)	0.11
Females	292 (56.3)	107 (61.1)	185 (53.8)	
Smoking habits (current or quit)*	45 (8.7)	12 (6.9)	33 (9.6)	0.57
Self-reported hypertension comorbidity*	174 (33.7)	71 (40.6)	103 (30.1)	0.02
SBP (mmHg)§	125 (115-136)	126 (116-142)	125 (115-133)	0.50
DBP (mmHg)§	84 (77-91)	85 (78-92)	83 (76-90)	0.38
Anthropometric§				
BMI (kg/m^2)	27.4 (23.4-31.4)	26.5 (23-31.3)	27.6 (23.7-31.6)	0.51
BMI <25 kg/m^2	174 (33.7)	66 (37.9)	108 (31.6)	0.34
25-29.9 kg/m^2	164 (31.8)	53 (30.5)	111 (32.5)	
≥30 kg/m^2	178 (34.5)	55 (31.6)	123 (35.9)	
WC (cm)	96 (87-104)	96 (87-104.1)	96 (87.5-104)	0.60
HC (cm)	103.5 (95.5-111.8)	102.5 (94.5-110.5)	104 (96-112)	0.67
WHR	0.92 (0.88-0.96)	0.93 (0.89-0.97)	0.92 (0.87-0.96)	0.63
Metabolic§				
Haemoglobin (g/dl)	14.3 (13.2-15.4)	14.2 (12.8-15.4)	14.3 (13.5-15.4)	0.27
Urea (mmol/l)	3.2 (2.5-4.1)	3.2 (2.5-4.3)	3.1 (2.4-4)	0.20
e-GFR (ml/min/1.73m^2)	121.8 (105.4-133.9)	117.5 (97.9-132.3)	124.6 (109.5-134.6)	0.41
TC (mmol/l)	4 (3.2-5)	4.1 (3.2-5)	4 (3.2-5.0)	0.11
HDLC (mmol/l)	0.9 (0.7-1.2)	1 (0.8-1.3)	0.9 (0.7-1.2)	0.30
TGL (mmol/l)	1.4 (1-1.9)	1.4 (1-1.9)	1.3 (1-1.8)	0.36
LDLC (mmol/l)	2.6 (1.9-3.4)	2.6 (1.8-3.52)	2.6 (2-3.3)	0.53
Non-HDLC (mmol/l)	3 (2.3-3.8)	3.1 (2.3-3.9)	3 (2.3-3.8)	0.43
Uric acid (µmol/l)	270 (217-330.2)	272 (214-335)	268 (217-327)	0.68
HbA1c (%)	10.6 (7.8-12.5)	10.8 (8.6-12.5)	10.4 (7.5-12.6)	0.44
HbA1c (mmol/mol)	92 (62-114)	95 (70-113)	90 (59-114)	0.43
FBG (mmol/l)	8.6 (6.3-13.4)	9.5 (6.5-14)	8.1 (6.2-12.8)	0.42

*Data is presented in form of absolute numbers and percentages §Data is presented as median with IQR.

BMI-Body mass index, e-GFR- Estimated glomerular filtration rate, FBG- Fasting blood glucose, HbA1c-Glycated haemoglobin, HC-Hip circumference, HDLC-High

dense lipoprotein cholesterol, LDLC-Low dense lipoprotein cholesterol, TC-Total cholesterol, TGL-Triglycerides, WC-waist circumference, WHR-Waist: hip circumference.

Table 2. Multivariate analysis to identify predictors of diabetic kidney disease

Characteristic	**AOR (95%CI)**	**P-value**
Age at diagnosis (years)	1.01 (1.00-1.02)	0.19
Sex of participants	1.26 (0.89-1.75)	0.19
Self-reported hypertension comorbidity	1.76 (1.24-2.48)	0.002
BMI <25 kg/m2	1	
25-29.9 kg/m2	0.77 (0.52-1.15)	0.20
≥ 30 kg/m2	0.61 (0.41-0.91)	0.02

AOR- Adjusted odds ratio, CI- Confidence interval, BMI- Body mass index

CHAPTER NINE: KEY FINDINGS FROM THE Ph.D. STUDIES, STUDY STRENGTHS, AND LIMITATIONS, AND IMPLICATIONS FOR PRACTICE, POLICY, AND FUTURE RESEARCH

9.1 Discussion of the thesis

This chapter mainly discusses the key findings from the Ph.D. studies, study strengths, and limitations, and implications for clinical practice, policy, and future research.

This Ph.D. comprised a published narrative review on understanding the manifestation of diabetes in SSA to inform therapeutic approaches and preventive strategies, and a cross-sectional study of 568 adult Ugandan patients with newly diagnosed diabetes.

The cross-sectional study was designed to undertake a detailed clinical, metabolic, and immunologic characterisation of an adult Ugandan population with newly diagnosed diabetes to understand the burden of islet autoantibody positivity (as a marker of pancreatic autoimmunity) and its related characteristics, the phenotypic differences between lean and non-lean individuals with confirmed type 2 diabetes, and the burden and correlates of diabetes complications at diagnosis (peripheral arterial disease and diabetic nephropathy).

We hypothesised that, due to the effect of local environmental exposures like chronic early-life malnutrition, infections, low-grade inflammation, and genetic diversity, the Ugandan adult diabetes phenotype will be distinct from what has been described in white populations of European ancestry in high-income countries. We hypothesised that the majority of the Ugandan adult patients with newly diagnosed diabetes will be younger at diagnosis with lower levels of adiposity (BMI, WHR, visceral adiposity), and pathophysiologically, pancreatic beta-cell secretory dysfunction, as opposed to insulin resistance, would be the predominant pathophysiological defect.

9.2 Main findings and implications for practice and policy

9.2.1 Narrative review

After a detailed literature search performed in January 2018 in PubMed, Google Scholar, Scopus, and African Online Journals, a total of eight cross-sectional studies (1-8), six review articles (9-14), and two case reports (15, 16) with information on the distinct diabetes phenotypes in African populations were included in the narrative review. Of the eight cross-sectional studies, none was performed in adult patients with new-onset diabetes. Most studies recruited a small number of patients and included limited information on clinical and metabolic characteristics.

Studies performed in Ethiopia (1) and Ghana (4, 5) reported a predominance in pancreatic beta-cell dysfunction, manifesting as reduced mean fasting C-peptide concentrations and greater blunting of the acute phase of insulin secretion in response to an oral glucose load following a 75g OGTT. Due to the high local prevalence of obesity, studies performed on adult patients with type 2 diabetes in Nigeria reported a dual predominance of insulin resistance and pancreatic beta-cell dysfunction (2, 3).

The review also highlighted the clinical, metabolic, and immunological features of two unique diabetes subtypes (KPD and fibro calculous pancreatic diabetes) that have been described mainly in patients of African ancestry (6-8, 15, 16), further emphasising the distinctiveness of the diabetes phenotype in African populations.

Due to the paucity of well-powered studies that have undertaken the robust clinical, metabolic, and immunologic characterisation of adult patients with new-onset diabetes in SSA, our cross-sectional study (the Uganda DIabetes Phenotype or UDIP study) of 568 adult participants with newly diagnosed diabetes in Uganda was highly justified.

For this narrative review, we acknowledge the lack of formal assessment of the validity of the included studies using pre-defined standard criteria like the Newcastle-Ottawa scale as a major limitation and this may have introduced some bias.

9.2.2 Cross-sectional study

For the first time in SSA, the UDIP study aimed to comprehensively understand the manifestation of diabetes in a cohort of adult patients with newly diagnosed diabetes in Uganda. We collected data on relevant sociodemographic, clinical, anthropometric, metabolic, and immunologic characteristics from 568 patients (aged ≥18 years) with new-onset diabetes (<3 months from diagnosis) from February 2019 to October 2020.

9.2.2.1 Understanding the burden of islet autoantibody positivity and related characteristics

In our study population, we reported a low frequency of islet autoantibody positivity (6.4%). This suggests that pancreatic autoimmunity is a rare cause of adult-onset diabetes in the Ugandan population and testing for islet autoantibodies in adult patients with new-onset diabetes would have limited clinical impact in Uganda.

Of the three islet autoantibodies, GADA was the most prevalent in this study population (3.2%). Additional screening for IA-2A and ZnT8-A identified 50% more participants with islet autoimmunity. Multiple islet autoantibody positivity (positive for >1 islet autoantibody) to reflect the presence of true autoimmune disease (type 1 diabetes) was very infrequent (<1%).

Islet autoantibody positivity, based on the screened islet autoantibodies, has been documented to be relatively more common in white European populations with prevalence ranging between 4.5% and 9.7% (17-19), but infrequent in the Middle East (2.8% based on GADA and IA-2A positivity) (20) and Asia (1.5%-8.6% based on GADA, IA-2A, and ZnT8-A positivity) (21-25). Similar to the findings of European

population-based studies, GADA positivity has been reported to be more prevalent than IA-2A and ZnT8-A positivity in Asians with phenotypic type 2 diabetes (23-25).

In SSA, few studies have screened for islet autoantibody positivity using more than one islet autoantibody. Most have screened for only GADA positivity and have defined islet autoantibody positivity based on the manufacturer's cut-offs, which are significantly lower than the local population-derived diagnostic thresholds that we used. Using the manufacturer's cut-offs to define islet autoantibody positivity, as opposed to local population-derived cut-offs, is associated with a low test specificity, hence increasing the likelihood of including many false-positive cases and reporting an inaccurate prevalence of islet autoantibody positivity (26-28). In addition, none of these studies was performed in adult patients with new-onset diabetes. This results in the possibility of reporting an imprecise prevalence of islet autoantibody positivity since its prevalence is greatly affected by the duration of diabetes. Because of these notable differences in study methodology, a comparison of our study findings with other studies performed in SSA ought to be done with caution.

Based on the reported GADA positivity rates only, studies performed in two Eastern African adult populations with apparent type 2 diabetes (Kenya and Tanzania) reported almost similar prevalence of GADA positivity of 5.7% and 5.3%, respectively (29, 30). In the Tanzanian study, the prevalence of islet autoantibody positivity increased to 7.3% with additional IA-2A measurement (30). Higher prevalence levels of GADA positivity in adults with type 2 diabetes have also been reported in Madagascar (12%) (31) and West African populations in Nigeria (10.5-14%) (32-34) and Ghana (8.9-14.3%) (35, 36).

Participants who were islet autoantibody positivity were more likely to reside in rural areas, to be initiated on insulin therapy at diagnosis, and to have a lower WC, WC:

height ratio, and fasting C-peptide concentration. These findings are consistent with what has been observed in participants with islet autoantibody positivity in other populations (17, 18, 28, 37, 38). In addition to having lower markers of obesity, a finding of lower fasting and postprandial C-peptide concentrations in islet autoantibody-positive participants as shown in our study is an indicator of reduced pancreatic beta-cell function which is linked to progressive autoimmune-mediated damage of the pancreatic beta-cells (39). A high prevalence of pancreatic beta-cell dysfunction based on a lower fasting C-peptide concentration in participants with islet autoantibody positivity has been widely documented in several similar studies (38, 40-42).

Living in a rural area and being initiated on insulin therapy at the time of diagnosis of diabetes were noted to be independently associated with islet autoantibody positivity in our study population. The association with living in a rural residence is of particular interest. An association between the high frequency of pancreatic autoimmunity and rural residence has been previously reported in other studies performed in Ghana (36) and Ethiopia (43), which reported islet autoantibody positivity rates of 14.3% and 28% in patients living in rural areas, respectively. The underlying mechanisms to explain the increased likelihood of pancreatic autoimmunity in rural areas are unclear but may be related to the effects of chronic malnutrition (44, 45).

Initiation of insulin therapy at the time of diagnosis of diabetes as an independent predictor of islet autoantibody positivity in this study population signifies pancreatic beta-cell dysfunction which is a predictor of early initiation of insulin therapy in islet autoantibody-positive participants in several studies (17, 18, 40, 41, 46).

In European and Asian population-based studies, patients with adult-onset diabetes and positive for islet autoantibodies are significantly younger at diagnosis and have

lower BMI, blood pressure, and a more favourable lipid profile, when compared to those with type 2 diabetes (17-19, 38). In contrast, data from our study and most studies in African patients have not demonstrated these differences between patients with and without islet autoantibody positivity (31-33, 35). This may partly be due to the low number of patients positive for islet autoantibodies.

Given our study finding of a low prevalence of islet autoantibody positivity, pancreatic autoimmunity is an infrequent cause of adult-onset diabetes in our Ugandan population. The documented low prevalence of islet autoantibody positivity also implies that routine measurement of islet autoantibodies in adult patients at the time of diagnosis of diabetes in Uganda would have minimal clinical benefit and would likely result in many false-positive results, especially if the lower manufacturer's cut-offs are used to define autoantibody positivity. The study finding of an association between living in a rural area and islet autoantibody positivity is of unique interest and warrants further investigation.

9.2.2.2 Characterisation of the lean type 2 diabetes phenotype

After excluding participants with islet autoimmunity (those that tested positive for at least one of the three measured islet autoantibodies) and those with missing data on islet autoantibodies, 32% and 68% of participants with confirmed new-onset type 2 diabetes were lean and non-lean, respectively.

The relatively high proportion of individuals with type 2 diabetes who are lean in body size in our study is also in accord with findings from the only nationwide population-based study conducted in Uganda that also reported a high proportion of participants with BMI <25 kg/m^2 of about 81% (47), reflecting that, generally, the majority of adult Ugandans are lean in body size.

The documented frequency of lean type 2 diabetes phenotype in our study population is higher than what has been reported in large-scale studies performed in white European and Asian populations, with prevalence ranging between 5 and 23.5% (48-52).

Compared with the non-lean phenotype, the lean type 2 diabetes phenotype in our study population was associated with a male preponderance, lower levels of body adiposity (total body and visceral fat, WHR, and WHtR), and metabolic syndrome (serum triglycerides, uric acid, leptin, and low HDLC concentrations). Pathophysiologically, pancreatic beta-cell dysfunction (reflected by lower markers of pancreatic beta-cell function, i.e. oral IGI and 120-minute C-peptide concentration) as opposed to insulin resistance was the more predominant underlying pathophysiologic defect.

This well-described distinct lean type 2 diabetes phenotype in our study population and the related metabolic characteristics like the predominance of pancreatic beta-cell dysfunction, lower levels of adiposity, and metabolic syndrome further demonstrate the heterogeneity of type 2 diabetes across populations. This distinct diabetes phenotype may be a manifestation of certain genetic influences or previous environmental exposures like chronic early-life malnutrition, poverty, and infections, and therefore, may be common in other similar low-income settings.

Despite the propensity to have central obesity (high WC and WHR), increased markers of metabolic syndrome (an atherogenic lipid profile, low adiponectin concentrations, and higher systolic and diastolic blood pressures), and visceral adiposity, studies performed in South (51, 53-56) and East Asians (57-59) with the lean type 2 diabetes phenotype have also demonstrated that pancreatic beta-cell secretory dysfunction is the predominant underlying pathophysiological defect (51, 53-56), similar to what we

observed in our study population. These differences in the manifestation of diabetes, especially with the metabolic characteristics, may be influenced by environmental factors, such as dietary patterns.

The relatively high proportion of individuals with type 2 diabetes that are lean in body size in our study population in addition to the predominance of pancreatic beta secretory dysfunction, as opposed to insulin resistance, has significant practice and policy implications. From a pathophysiological perspective, it may not be clinically prudent to manage such patients following a treatment algorithm that recommends lifestyle management and metformin as first-line approaches, similar to what is recommended by the American and European diabetes treatment guidelines. This is because the evidence supporting this management approach is largely derived from studies performed in white populations of European ancestry with type 2 diabetes (60-63), whose diabetes phenotype differs from our Ugandan adult population (a greater proportion of white patients of European ancestry are overweight or obese with a pro-atherogenic lipid profile and insulin resistance).

Because of these population differences in diabetes phenotypes (notably the predominance of pancreatic beta-cell dysfunction) in addition to the high prevalence of lean body size as reflected by the low median BMI in the general adult Ugandan population, it would be prudent to revise the local guidelines for the management of type 2 diabetes in Uganda and advocate more for the use of oral diabetes therapies like the newer-generation sulfonylureas and dipeptidyl peptidase IV (DPP IV) inhibitors as first-line oral therapies for the management of type 2 diabetes in our adult Ugandan population as opposed to the commonly used metformin. From a pathophysiologic perspective, sulfonylureas and DPP IV inhibitors improve and/or preserve pancreatic beta-cell mass and function, hence most effective in patients with pancreatic beta-cell

secretory dysfunction while metformin is most effective in cases where insulin resistance predominates (64, 65). It is worth mentioning that we lack compelling evidence about which of the above oral therapeutic options that improve pancreatic beta-cell mass and/or function should be used as first-line therapy (either as monotherapy or in combination with another agent) in the management of type 2 diabetes, especially in lean individuals.

Because of the predominance of pancreatic beta-cell dysfunction in lean individuals with type 2 diabetes in our study population, assessment of pancreatic beta-cell function by measurement of either fasting, random, or stimulated C-peptide concentrations at the time of diagnosis of diabetes in these individuals would be clinically important to guide individualised diabetes treatment (especially timely initiation of insulin therapy in patients with confirmed absolute insulin deficiency).

9.2.2.3 Burden and predictors of peripheral arterial disease

Of the 255 participants that underwent baseline ABI measurement, we found a relatively high prevalence of PAD (defined as a resting ABI ≤ 0.9 in either or both lower limbs) of 17.3%. The majority of participants with confirmed PAD had severe disease or critical occlusion (≈93%). Despite the severity of the condition, most participants were asymptomatic without intermittent claudication and gangrene on clinical examination.

This documented prevalence of PAD in our study population is almost similar to what has been reported in some African adult populations with long-standing diabetes (66-68) and significantly higher than what has been reported in white and South Asian populations, where prevalence range between 6.6% and 10.4% (69-74). The reasons for the differences in the burden of PAD across populations are unclear but may be

related to genetic diversity, methodological differences in diagnosing PAD, and environmental influences.

In our study population, we also observed an association between the female sex, UACR, FBG, and PAD. The female preponderance in PAD risk has also been reported by another study performed on adult patients with type 2 diabetes in Southwestern Uganda (75) and large studies in white populations of European ancestry (76, 77). The postulated reasons for the increased risk of PAD in women include older age, higher BMI, blood pressure, lipid, and inflammation indices (78, 79).

Microalbuminuria as an independent predictor of PAD has also been reported in Asian and white populations with type 2 diabetes (80-82). In addition to being a predictor of kidney damage, microalbuminuria is also a well-documented predictor of subclinical vascular damage and increased cardiovascular morbidity and mortality in the general population and specific patient populations like patients with diabetes and hypertension (83-85).

Contrary to what is reported in most studies performed in white and Asian populations with type 2 diabetes, we did not observe any association with several conventional cardiovascular risk factors like increasing age, smoking, hypertension, dyslipidaemia, markers of adiposity (BMI, WC, WHR, and WHtR), and hyperuricaemia. The plausible explanation for this clinical difference is not well-documented but may be related to genetic variations and the possible presence of emerging novel cardiovascular risk factors in our study population which need to be investigated.

Intriguingly, despite the high prevalence of HIV co-infection comorbidity in patients with PAD (20.5%), it was not observed to be an independent predictor of PAD in this study population. The absence of an observed association could probably be due to the small sample size of the study.

The absence of clinical features like intermittent claudication and gangrene in patients with severe PAD in our study population also warrants further comprehensive investigation.

Given the study findings, screening for PAD in adult patients with newly diagnosed patients, especially in female patients and those with abnormal UACR levels, should be recommended in clinical practice in Uganda.

9.2.2.4 Burden and predictors of diabetic nephropathy

In our study population, we documented a high prevalence of diabetic nephropathy of ≈34%. The majority of the participants with diabetic nephropathy had an abnormal spot UACR (96%) and only 1.4% had an e-GRF of <60 ml/min/1.73 m^2.

A wide variation in the prevalence of diabetic nephropathy has been documented in African populations, with systematic reviews and meta-analyses reporting prevalence ranging from 7.3% to 83.7% (86-88). This may be explained by differences in diagnostic methods (urine protein and e-GFR measurement) and diagnostic thresholds used, populations studied (long-standing vs newly diagnosed or type 1 vs type 2 diabetes or combined), and study design (population-based vs health facility-based or private vs public health facilities).

In this study population, hypertension comorbidity was noted to have a positive association with DKD while an inverse association was observed with obesity (BMI ≥30 kg/m^2). Hypertension is a well-recognised risk factor of DKD, regardless of phenotype, in African (88-92), white (93, 94), and Asian (95, 96) populations. The mechanisms to explain the association between hypertension and diabetic nephropathy are not well-understood but may be related to augmented sodium retention, pro-inflammatory cascade, renin-angiotensin-aldosterone, and sympathetic

nervous system, endothelial cell dysfunction, and oxidative stress, which ultimately result in glomerular damage (97, 98).

The study finding of a protective effect of obesity towards the onset of diabetic nephropathy in our study population is contrary to what has been described in most studies (99), highlighting the heterogeneity in the pathogenesis of diabetic nephropathy across populations. Obesity is often associated with increased insulin resistance, a highly atherogenic lipid profile, hypoadiponectinemia, and an exaggerated pro-inflammatory state, which all exacerbate renal injury in patients with type 2 diabetes (100).

The reasons to explain the increased odds of developing DKD in individuals with normal or low BMI as shown in our study population have not been fully investigated but could partly be explained by the developmental origins of health and disease (DOHaD) theory or developmental programming. Early-life environmental insults (in-utero or early childhood) like chronic infections, pre-eclampsia, and maternal malnutrition induce epigenetic changes that affect organ development and function later in life. These often result in a low nephron number or mass increasing the susceptibility to hypertension and chronic kidney disease (101).

Universal screening for diabetic nephropathy, especially in adult patients with co-existing hypertension and BMI <30 kg/m^2, should be recommended in clinical practice in Uganda.

9.3 Study strengths

This Ph.D. study had several strengths. To our knowledge, it is the first study to undertake robust clinical, metabolic, and immunologic characterisation of a large cohort of adult patients with newly diagnosed diabetes in SSA. Recruiting patients with newly diagnosed diabetes minimised the effects of increasing the duration of diabetes

on important phenotypic characteristics being investigated like BMI, pancreatic beta-cell function, and frequency and pattern of islet autoantibody positivity.

The study was also undertaken across several tertiary hospitals that serve both urban, peri-urban, and rural populations. All of these hospitals run weekly active outpatient specialised diabetes clinics where all patients diagnosed with diabetes are referred for long-term management. We had a high acceptance rate of participants invited to join the study.

We also used rigorously study protocols to perform a wide array of clinical, metabolic, and immunological tests (including 75g OGTT to obtain fasting and stimulated insulin, C-peptide, and glucose indices to evaluate the pancreatic beta-cell function, insulin resistance, and sensitivity) to comprehensively define the different diabetes phenotypes in our study population.

Unlike all studies performed in SSA, screening for islet autoantibody positivity in our study was based on testing three common autoantibodies and the diagnostic cut-offs to defined positivity were derived from an appropriate healthy adult Ugandan population without diabetes. This approach is widely recommended because it ensures a high test specificity and, therefore, the inclusion of fewer false-positive results.

As part of the islet autoantibody assessment, we used one of the highest performing islet autoantibody assays in the international Islet Autoantibody Standardisation Program and also undertook extensive validation performed on paired samples in an external laboratory (Royal Devon and Exeter NHS Foundation Trust, Exeter UK), to ensure robust results.

9.4 Study limitations

The study participants were recruited only from tertiary hospitals located mainly in Central Uganda. This may be associated with selection bias which ultimately affects the generalisability of study findings to the general adult Ugandan population with diabetes, particularly underestimating the proportion of lean individuals with type 2 diabetes. However, it is important to note that, according to the health system framework in Uganda, diabetes care is mainly centralised in the upper-tier healthcare facilities, and most patients self-refer to these hospitals for chronic diabetes management.

As part of the evaluation of the total body and visceral adiposity, we used BIA, which is a less sensitive approach than computed tomography, magnetic resonance imaging, and dual-energy X-ray absorptiometry. The BIA has not been widely validated in adult African populations with type 2 diabetes.

During the recruitment of participants, we did not immediately enroll critically ill participants at the time of presentation to the hospital (only recruiting them later on re-attending the diabetes clinics when clinically stable but still within three months). This could have led to an underestimation of the study's main outcomes. We, however, tried to follow up with most of these patients following discharge from the hospital and recruited them later at their subsequent clinical review.

Because the study was a cross-sectional study in design, it offers no evidence of a temporal relationship between lean nonautoimmune type 2 diabetes and the well-described pancreatic beta-cell dysfunction.

9.5 Implications for future research

The reasons for the documented pancreatic beta-cell secretory dysfunction associated with lean type 2 diabetes phenotype remain uninvestigated. There is a need for studies to rigorously investigate specific environmental (local toxins, tropical overt or

subclinical bacterial and viral infections), genetic (functional genomics on polymorphisms of genes related to pancreatic development and secretory function), and hormonal (incretin hormone axis) factors that may explain why pancreatic dysfunction predominates in Ugandan adult patients with lean type 2 diabetes.

There is also a need for a robust study to undertake an in-depth investigation of the pancreatic organ size (evaluating the effect of structural defects) and visceral, ectopic, and total body adiposity (evaluating visceral and ectopic fat deposition) using highly sensitive recommended radiological modalities like magnetic resonance imaging or dual-energy Xray absorptiometry studies with an aim of further understanding the underlying pathophysiology of type 2 diabetes in our population.

Because of the documented differences in the diabetes phenotype in our study population and white populations of European ancestry, it implies that treatment guidelines for type 2 diabetes informed by evidence collated from phenotyping studies of white populations of European ancestry cannot be directly extrapolated to our Ugandan adult population with type 2 diabetes. A future RCT to investigate the optimal first-line glucose-lowering therapy(ies) in our adult population with type 2 diabetes is urgently needed. Through collaboration in form of a local and international research consortium, this large-scale clinical research to identify the optimal first-line diabetes therapy in SSA, either as monotherapy or as a combination, can be extended to involve other adult African populations with type 2 diabetes.

Due to the absence of an association between the conventional cardiovascular risk factors and PAD in our study, novel cardiovascular risk factors to explain the relatively high rate of PAD in our adult Ugandan population with diabetes need to be thoroughly investigated. It is interesting that, despite the documented high prevalence of severe PAD in our study population, most patients were asymptomatic (no intermittent

claudication), and none had gangrene on clinical examination. The reasons to explain this clinical observation ought to be investigated in detail, in addition to assessing whether PAD is associated with concurrent macro-vasculopathy in other vascular beds (notably carotid and coronary vasculature).

Mechanisms to explain why obesity reduces the likelihood of having diabetic nephropathy in our study population also warrant an in-depth investigation.

10.0 Conclusions

The narrative review showed that few phenotyping studies with in-depth data on clinical, metabolic, and immunologic characteristics, especially in adult patients with new-onset diabetes exist in SSA. The cross-sectional study provided compelling evidence that, i) Generally, the Ugandan adult diabetes phenotype is distinct from that of white populations of European descent, ii) Pancreatic autoimmunity is an infrequent cause of adult-onset diabetes in the Ugandan population based on the low prevalence of islet autoantibody positivity, iii) The lean type 2 diabetes phenotype is relatively common, occurring in about one in every three adult patients recently diagnosed with diabetes, and pathophysiologically, is associated with predominant features of pancreatic beta-cell dysfunction and fewer markers of total body and visceral adiposity, metabolic syndrome, and insulin resistance, iv) PAD is relatively common in adult Ugandan population with new-onset diabetes, and is independently associated with female sex, UACR, and FBG, v) Diabetic nephropathy is relatively prevalent in adult Ugandan population with new-onset diabetes, and pre-existing hypertension and obesity are independent predictors.

Future rigorous clinical studies to explain some of these study findings in our Ugandan adult population are urgently needed.

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1.0 Appendices

11.1 Local ethical approval from the Uganda Virus Research Institute

UNHRO
Uganda National Health Research Organisation

Uganda Virus Research Institute

Plot 51-59, Nakiwogo Road, Entebbe
P.O. Box 49, Entebbe-Uganda
Tel: +256 414 320 385 / 6
Fax: +256 414 320 483
Email: directoruvri@uvri.go.ug

REPUBLIC OF UGANDA

Our Ref: GC/127/18/05/650
Your Ref:

May 29, 2018

Dear Dr. Davis Kibirige,

RE: UVRI REC review of protocol titled **"An in-depth understanding of the metabolic and immunologic profile of newly diagnosed adult diabetic patients in Uganda: the Uganda Diabetic Phenotype (UDIP) study. Version 2.0 dated 25 May 2018"**

Thank you for submitting the response to the queries addressed to you by the UVRI REC. This is to inform you that your response dated May 25, 2018 was reviewed and met the requirements of the UVRI REC.

UVRI REC annual approval has been given for you to conduct your research up to May 29, 2019. Annual progress report and request for extension should be submitted to UVRI REC prior to the expiry date, to allow timely review.

The reviewed and approved documents included;

1. UVRI REC Application form
2. Study Protocol version 2.0 25 May 2018
3. Informed consent documents
4. Investigators' CVs

You can now continue with your study after registration with the Uganda National Council for Science and Technology (UNCST).

Note: UVRI REC requires you to submit a copy of the UNCST approval letter for the above study before commencement.

You[redacted]

Dr Tom Lutalo
Chair, UVRI REC
C.C Secretary, UVRI REC

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VALID UNTIL
29 MAY 2019
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11.2 Regulatory approval from the Uganda National Council of Science and Technology

Uganda National Council for Science and Technology

(Established by Act of Parliament of the Republic of Uganda)

Our Ref: HS 2431 **10th December 2018**

Dr. Davis Kibirige
MRC/UVRI and LSHTM Uganda Research Unit
Entebbe

Dear Dr. Kibirige,

Re: Research Approval: An In – Depth Understanding of the Metabolic and Immunologic Profile of Newly Diagnosed Adult Diabetic Patients in Uganda: The Uganda Diabetic Phenotype (UDIP) Study

I am pleased to inform you that on **29/10/2018**, the Uganda National Council for Science and Technology (UNCST) approved the above referenced research project. The Approval of the research project is for the period of **29/10/2018** to **29/10/2019**.

Your research registration number with the UNCST is **HS 2431.** Please, cite this number in all your future correspondences with UNCST in respect of the above research project.

As Principal Investigator of the research project, you are responsible for fulfilling the following requirements of approval:

1. All co-investigators must be kept informed of the status of the research.
2. Changes, amendments, and addenda to the research protocol or the consent form (where applicable) must be submitted to the designated Research Ethics Committee (REC) or Lead Agency for re-review and approval **prior** to the activation of the changes. UNCST must be notified of the approved changes within five working days.
3. For clinical trials, all serious adverse events must be reported promptly to the designated local IRC for review with copies to the National Drug Authority.
4. Unanticipated problems involving risks to research subjects/participants or other must be reported promptly to the UNCST. New information that becomes available which could change the risk/benefit ratio must be submitted promptly for UNCST review.
5. Only approved study procedures are to be implemented. The UNCST may conduct impromptu audits of all study records.
6. An annual progress report and approval letter of continuation from the REC must be submitted electronically to UNCST. Failure to do so may result in termination of the research project.

LOCATION/CORRESPONDENCE

Plot 6 Kimera Road, Ntinda
P. O. Box 6884
KAMPALA, UGANDA

COMMUNICATION

TEL: (256) 414 705500
FAX: (256) 414-234579
EMAIL: info@uncst.go.ug
WEBSITE: http://www.uncst.go.ug

Uganda National Council for Science and Technology
(Established by Act of Parliament of the Republic of Uganda)

Below is a list of documents approved with this application:

	Document Title	Language	Version	Version Date
1.	Research proposal	English	3.0	August 2018
2.	Clinical review form (CRF) 1	English	3.0	August 2018
3.	UDIP Informed consent form	Luganda	1.0	April 2018
4.	UDIP Informed consent form	English	2.0	May 2018

Yours sincerely,

[redacted]

Isaac Makhuwa
For: Executive Secretary
UGANDA NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

Copied to: Chair, Uganda Virus Research Institute, Research Ethics Committee

LOCATION/CORRESPONDENCE

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11.3 Participant informed consent form– English version

MRC/UVRI and LSHTM Uganda Research Unit

APPENDIX 2. INFORMED CONSENT FORM

Title of study: An in-depth understanding of the metabolic and immunologic profile of newly diagnosed adult diabetic patients in Uganda: The Uganda Diabetic Phenotype (UDIP) study. Version 2.0_25 May 2018

i) INFORMATION SHEET

Investigator

My name is Dr. Davis Kibirige from Medical Research Council (MRC)/Uganda Virus Research Institute (UVRI) and London School of Hygiene and Tropical Medicine (LSHTM) Uganda Research Unit. The study is being sponsored by MRC/UVRI and LSHTM

Uganda Research Unit.

We are inviting you to take part in a research study. You need to know about the study so that you can decide if you would like to join the study or not. In case you accept to join the study, you will be requested to sign a consent form. A copy of the consent form will be given to you and the original will be retained by the study team, however you may choose to take unsigned copy. Joining the study will not interfere with the normal medical treatment that you regularly receive from your hospital.

Purpose of the study

This study is being conducted by a team of doctors from MRC/UVRI and LSHTM Uganda Research Unit and Exeter University, United Kingdom. Its aim is to clearly understand how patients with diabetes present in Uganda. You have been selected because you have recently been diagnosed with diabetes.

Expectations when you join the study

If you decide to join the study, we will ask you questions and collect information about yourself. We will ask you your age, occupation, marital status, social habits and about your medical conditions. A study nurse or doctor will perform a medical examination to know your weight, height, waist and hip circumference. They will also measure blood pressure from your arm using a machine while you are seated. Three readings will be recorded every after 5 minutes.

About 15 mls of blood will be drawn by an experienced study staff once and sent to the laboratory for testing. The tests that will be done include tests to check how your pancreas (an organ in your body that produces insulin, a drug that helps to reduce sugar levels in blood) is working, kidney and liver tests, tests to measure the levels of fat (cholesterol), uric acid, vitamin D and albumin in blood. Your fasting blood sugar level and glycated haemoglobin (a new test to assess how your blood sugar level has been for the past 3 months) will also be performed. You will be asked to collect 10 mls of urine after you have passed out some of it (midstream urine sample) to also assess the level of function of your pancreas and kidney.

You will later be given a solution of glucose dissolved in about 300 mls of clean water to swallow and blood samples will be drawn by pricking your finger after 30 minutes and 2 hours after swallowing that solution. This is an extra test to assess the level of function of your pancreas.

Sample storage

Some of your blood and urine will be stored for future tests. You will be given another consent form to sign if you agree to have your samples stored for future use.

Total number of participants in this study

A total of 700 newly diagnosed adults with diabetes mellitus plus 100 healthy non-diabetic adults who are the controls, all together making a total of 800 will participate in this study. The healthy non-diabetic adults will be enrolled into the study to help in determining the normal levels of pancreatic function and circulating insulin in the body.

Duration of the study:

Approximately one year.

Benefits

There is no direct benefit for participating in this study. However, in case an abnormal test is found like reduced function of your pancreas or high fat or cholesterol levels on screening, we will refer you to your primary doctor for further management.

Risks and discomforts

You may feel discomfort from the needle pricks as blood is drawn from your forearm or finger. You may later have a bruise or swelling (and rarely, an infection) where the needle goes in your arm. In case you have been started on oral treatment for diabetes, you will be requested to stop your drugs briefly for 2 or 3 days and return later to the clinic for the tests to be performed. This will cause a mild, but harmless increase in your blood sugar level. The study staff can advise you on dealing with any of these challenges.

Confidentiality

We will do everything we can to protect your privacy. You will be assigned a unique number that we will use instead of your name. Your records will be kept in a locked location at the research centre only be accessed by only those working on the study. Your name will not be used in any reports, presentations or publications resulting from this study. The people or groups that may see your records include UVRI Research and Ethics Committee, the Uganda National Council for Science and Technology (UNCST), study monitors, the sponsor and study auditors. Reviewers will keep the information on your files private. By signing this written informed consent form, you or your representative authorise these people or groups to see your records.

Refusal or withdraw from the research

Your taking part in this study is totally voluntary. You will not be deprived of anything if you decide not to participate in the study. You are free to withdraw from this study at any time. Your choice will not affect the care you will receive from the diabetes clinic you are attending. Please contact the study staff if you decide to stop taking part in this study.

Costs to study participants

There is no cost to you for taking part in the study. You do not have to pay anything for the study procedures or any of the tests performed. We will give you 20,000 Ugandan shillings as compensation for your time spent at the clinic and to cover your transport expenses according to the current prevailing fares.

Study results

A report will be written and submitted to the relevant authorities including Ministry Of Health. Results will also be communicated to you and your local leaders. You will get feedback on findings and progress of the study. In case any new information that affects the study or data that

has clinical relevance to you is discovered during the study, we will share these findings with you

Payment if your get injured during the study

It is possible that you could get a problem or injury that would not have happened if you did not take part in this study. If the study staff determine that you had been injured as a direct result of being in the study, your will be given immediate treatment for those injuries at no cost to you and then referred for further care if needed

However the study staff may determine that the illness or injury would have happened even if you did not take part in this study. In that case appropriate care and/or referral will likewise be provided for any illness or injury that occurs during the study.

There are no plans to give you money if this happens to you whether or not the problem or injury was related to taking part in the study.

Questions

If you have any questions about the study or if you want to leave this study, please feel free to ask any member of the study team or Dr. Davis Kibirige the principal investigator at telephone number 0782393955.

The study has been reviewed and approved by the Uganda Virus Research Institute Research Ethics Committee which is accredited and based in Entebbe, Uganda. In case you have any questions regarding your rights and welfare as a study participant in this study, you may contact the Mr. Tom Lutalo, Chairman UVRI Research Ethics Committee on telephone number: 0414321962 at the UVRI, Entebbe

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By signing or putting a thumb print on this form, it means that you have willingly offered your consent to participate in this study.

/

ii) CONSENT FORM

Statement	Please initial or thumbprint* each box

I confirm that I have read the information sheet above for this named study. I have had the opportunity to consider the information, ask questions and have these answered satisfactorily. **OR** I have had the information explained to by study personnel in a language that I understand. I have had the opportunity to consider the information, ask questions and have these answered satisfactorily.	
I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.	
I understand that relevant sections of my medical notes and data collected during the study may be looked at by authorised individuals from MRC/UVRI and LSHTM and any other relevant authorities and I give permission for these individuals to have access to my records.	
I understand that data about/from me/the participant may be shared via a public data repository or by sharing directly with other researchers, and that I will not be identifiable from this information	
I agree to take part in the above named study	

Printed name of participant	Signature /Thumbprint of participant	Date

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Printed name of impartial witness*

Signature of impartial witness*

Date

I attest that I have explained the study information accurately in the information sheet to the participant and was understood to the best of my knowledge by, the participant and that he/she has freely given their consent to participate* in the presence of the above named impartial witness.

Printed name of person obtaining consent	Signature of person obtaining consent	Date

(*Only required if the participant is unable to read and write. the participant's name and date of consent are written the appropriate boxes, initialed and dated by the person obtaining consent

11.4 Participant sample storage consent form – English version

MRC/UVRI and LSHTM Uganda Research Unit

MRC Medical Research Council | Uganda Virus Research Institute | LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

APPENDIX 4. CONSENT FORM FOR STORAGE AND FUTURE USE OF BLOOD AND URINE SAMPLES

Introduction

We are asking you for your consent to store part of your blood and urine samples that you have provided as part of this study.

These blood and urine samples will be used for future research and any other research related purposes.

1. **Will you receive any payments?**

These stored samples will not be sold or used for other purposes apart from research and any other research related use like training/ quality. You will not receive any money or other type of payment from any discovery such as developed products like medicine by use of these stored sample.

2. **How will the stored samples be used?**

After all the tests for this study are done there may be some samples of blood and urine left over or that may have not been used. We are asking now for your permission to store them for future use. Regulatory approvals are required for all researches performed on stored blood samples that are linked.

3. **Where are the samples stored?**

All samples will be stored in a locked location at the MRC/UVRI and LSHTM Uganda Research Unit laboratories in Entebbe. The repositories are designed in such a way that only authorised personnel will have access to the stored samples.

All samples will be anonymised with codes and will not have identifying information.

4. **How long will the samples be stored?**

There is no time limit on how long your samples will be stored.

5. **What if I don't want my samples to be stored?**

You can object to having your blood and urine samples stored for future research. This will not affect your relationship with our research team and other health workers. All the left over samples will be destroyed at the end of the study just in case you do not give for storage.

6. **What happens when I withdraw my sample from storage?**

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You can withdraw your consent for your samples to be used for future research. In this case, your samples will be destroyed only after they are no longer needed for the main study. You would need to tell the study team that you are withdrawing your consent for your samples to be stored for future research. This can be done at any time, for any reason. This will not affect your participation in the main study.

If you withdraw your consent for your samples to be kept and used after the study is over, it is possible that the study team may have already discarded the medical records that link your name to study number. In this case, your samples will no longer be linked to you, and it will not be possible to find your samples for destruction. Your samples will be destroyed after they are no longer needed for the study.

Please initial or put a thumb print to indicate that you have understood and greed to the statements in the table below:

NO	STATEMENT	PLEASE INITIAL/THUMB PRINT
1	I confirm that I have read this consent form for sample storage for future use for the above named study. I have had the opportunity to consider the information, asked questions and have had these answered satisfactorily. **OR** I have had the information explained to me by a study personnel in a language that I understand. I have had the opportunity to consider the information, asked questions and have had these answered satisfactorily.	
2	I understand that I can still participate in the main study even if I don't want my samples stored for future use and can withdraw consent at any time.	
3	I understand that the blood and urine sample collected from me will be used to support other research in the future, or used for training/ quality control and may be shared anonymously with other researchers, for their ethically-approved projects in Uganda, the UK and other countries	

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4	I understand that the results of these future studies are unlikely to have any implications for me personally and that I will not benefit financially if this research leads to the development of a new treatment or medical test.	

Consent to storage of samples

My signature (or thumbprint or identifying mark) below confirms that I agree to the storage of some of my samples (blood and urine) for future use as described in this consent form.		
Participant's Name	**Participant's Signature/Thumbprint/ID mark**	**Date**

NOTE:
For those participants who place a thumb print, the witness who signs in the table below, writes the participants' names and date of consent.

Impartial witness in the event the participant is unable to read and write (those who place a thumb print)

My signature here confirms that I saw the participant being informed that some of his/her samples (blood and urine) will be stored for future use as described in this consent. He /She freely consented verbally.		
Witness Name	**Signature**	**Date**

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Investigator/designee

I have adequately explained this information to the potential participant and to the best of my ability made sure that the participant understood what they have consented for. I also confirm that the participant has not been coerced into giving consent.		
Investigator/Designee Name	**Signature**	**Date**

11.5 Case report form

PARTICIPANT IDENTIFICATION

Participant's initials……. Study Identification No. ……/…….

Study site…….

Address……Participant's phone number …….

Next of kin's initials ……. Relationship …….

Phone no of next of kin ……..

Email if applicable…….

Consent to be contacted in the future....1. Yes 2. No.

Preferred mode of contact……1. Phone 2. Email.

Has the participant fasted (no intake except water) for >8 hours? 1. Yes 2. No

Record (in hours) the length of time since the participant last ate:……

IF NOT FASTED PLEASE REARRANGE VISIT

A. SOCIO-DEMOGRAPHIC CHARACTERISTICS

1. Participant status……1. Case 2. Healthy control.

2. Age …….

3. Date of birth ……. /…../……..

4. Sex 1. Male 2. Female

5. Residence (district): 1. Urban 2. Rural

6. Employment: 1. Employed-full time 2. Employed-part time 3. Employed-casual 4. Unemployed 5. Student. 6. Housewife.

7. Level of Education 1. None 2. Primary level 3. Secondary level 4. Tertiary level.

8. Marital status: 1. Single 2. Married 3. Widow 4. Widower 5. Separated

B. FAMILY SOCIAL HISTORY

1. Any familial history of diabetes: mother?..... 1. Yes 2. No 3. Don't know.

2. Any familial history of diabetes father?... ..... 1. Yes 2. No 3. Don't know.

3. Any familial history of diabetes: any siblings?..... 1. Yes 2. No 3. Don't know.

4. Any familial history of hypertension: mother?..... 1. Yes 2. No 3. Don't know.

5. Any familial history of hypertension: father?..... 1. Yes 2. No 3. Don't know.

6. Any familial history of hypertension: siblings?..... 1. Yes 2. No 3. Don't know.

7. Does any of your children have diabetes?.....1. Yes 2. No 3. Don't know 4. NA

8. Do you smoke?..... 1. Yes 2. No 3. Quit

9. Do you drink alcohol?..... 1. Yes 2. No 3. Quit

C. CLINIC ATTENDANCE…….. 1. I attend clinic 2. I do not attend clinic. 3. Not applicable.

If you don't attend clinic, please explain……..

D. HEALTH FACILITY WHERE YOU ATTEND DIABETES CARE CLINICS……..……1. Government/public hospital. 2. Private hospital 3. Mission or Private-not-for profit hospital 4. Private clinic 5. Traditional healer.

E. CLINIC ACCESSIBILITY

1. Getting to the clinic is easy. 2. Getting to the clinic is okay. 3. Getting to the clinic is difficult. If getting to the clinic is difficult, please explain why…….

F. METHOD USED TO GET TO THE CLINIC

1. Walk 2. Bus/Taxi 3. Personal car 4. Others…….

G. HOW LONG DOES IT TAKE YOU TO GET TO CLINIC……. minutes

<u>H. CO-MORBIDITIES:</u> Have been diagnosed/on treatment for any of these diseases? 1. Hypertension 2. Thyroid diseases 3. Chronic kidney disease 4. HIV 5. Asthma/COPD 6. Dyslipidaemia (high blood cholesterol) 7. Cardiac disease (myocardial infarction, hypertensive heart disease, cardiomyopathy) 8. TB 9. Depression/any psychiatric illness 10. Heart failure 11. Stroke 12. Peripheral arterial disease.

<u>I: MODE OF DIAGNOSIS OF DIABETES:</u>

i. Which test did the healthcare practitioner perform to diagnose your diabetes mellitus?

1. Based on tests 2. Based on symptoms. 3. Using both symptoms and tests. 4. Do not know.

ii. Which tests were used to diagnose your diabetes mellitus? Circle all that apply.

1. Random blood glucose (RBG) test 2. Fasting blood glucose (FBG) test 3. Glycated haemoglobin (HbA1c) test 4. 75g oral glucose tolerance test (OGTT) 5. Urine glucose test 6. Do not know.

iii. Were you admitted to the hospital at diagnosis? 1. Yes 2. No

iv. If admitted to hospital, how many nights did you spend in hospital?.....

v. Were you admitted with diabetic ketoacidosis (DKA)? 1. Yes 2. No 3. I don't know.

<u>J: DURATION SINCE DIAGNOSIS</u>

How long has the participant had diabetes?.....1. < 1 month 2. 1 - 2 months 3. >2-3 months.

K: PLEASE STATE WHICH DIABETES MEDICINES THE PARTICIPANT TAKES

Treatment	**Type (for 'other' please state the name of treatment, e.g. Glibenclamide)**	**DOSE (enter the size of the dose as a number)**	**Frequency (enter the number of times the dose is taken per day)**	**Total daily dose**	**UNITS (e.g. mg, g, IU)**
☐ DIET only					
☐ Metformin	☐ Standard ☐ Modified Release				
☐ Other non-insulin treatment					
	Insulin Group	Insulin Type (write the name of insulin type here)	Number of Injections Per day (please circle)	Total Daily Dose (in units)	
☐ INSULIN	☐ Rapid Acting (soluble)		1 2 3 4 5 6		
	☐ Intermediate/ Long Acting		1 2 3		
	☐ Premixed		1 2 3		
			Total Combined Daily Dose		

L: If you are treated with insulin, how many days after diagnosis did you start insulin?

1. Not insulin treated 2. Within 1 week 3. After 1 week

M. If using insulin how do you store it?.....1. Fridge 2. Drawer 3. Cupboard

4. Cool Box/Cooler 5. Other. If other, please explain…….

N. DO YOU OWN A GLUCOMETER?.....1. Yes 2. No.

O. IF YES:

- How often do you check your blood glucose each week? ……

-Which meter do you use (manufacturer and model)?.....

- Where do you get the strips for your glucose meter? 1. Buy myself 2. Free of charge

–If so who provides them? ……..

-If you buy strips yourself, how much do you spend each month?.....

P. MEDICAL HISTORY: Which other medicine does the participant take?

1. Are you taking medicines for hypertension? 1. Yes 2. No.

2. Are you taking medicines for high blood cholesterol? 1. Yes 2. No.

3. Are you taking cardiac aspirin or clopidogrel? 1. Yes 2. No.

Q: OTHER DRUG HISTORY: Which specify drug are you currently taking?.....1. ACEI 2. ARB 3. ARB/ACEI-D combinations 4. CCB-ARB/ACEI combinations 5. CCB-ARB/ACEI-D combination 6. Statins. 7. OHA (Metformin and SUs) 8. Oral cardiac aspirin or clopidogrel 9. Beta blocker-ACEI/ARB combination 10. Anti-TB drugs 11. ART 12. Levothyroxine 13. Carbimazole.

PART II: PHYSICAL EXAMINATION FINDINGS

1. Is acanthosis nigricans (hyperpigmentation at the base of the neck and armpits) present 1. Yes 2. No

2. Weight ……...kg

3. Height ……cm

4. Waist circumference (WC)……cm

5. Hip circumference …….cm

6. 1st systolic blood pressure (BP) measurement……..mmHg

7. 1st diastolic blood pressure (BP) measurement……..mmHg

8. 1st pulse rate measurement……....b/min.

9. 2nd systolic blood pressure (BP) measurement……..mmHg.

10. 2nd diastolic blood pressure (BP) measurement……..mmHg

11. 2nd pulse rate measurement……....b/min.

12. Total body fat……%

13. Visceral fat level ……

14. Skeletal muscle……..%

15. Resting metabolism…….kcal.

16. BMI……kg/m^2

17. Left ankle brachial index (ABI) measurement ……

18. Right ankle brachial index (ABI) measurement……

TIMING OF BLOOD SAMPLE COLLECTION

1. Time when urine sample is collected……..
2. Time when 0-minute blood samples are drawn……..
3. Time when glucose solution is consumed…….
4. Time when 30-minute blood samples will be drawn…….
5. Time when 120-minute blood samples will be drawn……..

COMPLETED BY:

Name: ……

Signature:……...

Date:……..