Understanding optimal methods for monitoring glycaemia in people with type 2 diabetes in low resource settings.

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PhD Thesis 2022

Understanding optimal methods for monitoring glycaemia in people with type 2 diabetes in low resource settings.

Submitted by Anxious J Niwaha of the College of Health and Medicine to the University of Exeter Medical School as a thesis for the degree of Doctor of Philosophy in Medical studies, August 2022.

I certify that all material in this thesis which is not my own work has been identified and that any material that has previously been submitted and approved for the award of a degree by this or any other University has been acknowledged.

Abstract

Monitoring glycaemic control in type 2 diabetes is essential to allow appropriate titration of medication and prevent diabetes complications. In developed countries glucose control is monitored mainly by glycated haemoglobin (HbA1c) testing or intensive home capillary glucose measurements. HbA1c is not used routinely in low resource settings because of cost, and a number of conditions that are relatively common in this population may result in HbA1c results that poorly reflect blood glucose levels; for example sickle cell and other haemo-globinopathies; anaemia, malaria, renal disease. The alternative measures to HbA1c recommended for monitoring (i.e., fructosamine and glycated albumin, or single glucose measures) in situations where HbA1c may be unreliable have not been well studied in African populations. Current clinical practice in such settings varies, with a single fasting glucose measure used by many clinicians to inform treatment titration, but others routinely use non fasting 'random' measurements.

A key question for use of fasting glucose in monitoring glycaemic burden is whether it is significantly affected by exercise (prolonged walking to the clinic). This is because majority of the patients in Uganda and other low resource settings (e.g., SSA countries) walk long distances to the diabetes clinics and fasting/non-fasting blood glucose will often be measured after an abnormally prolonged fast (of more than the recommended 8 hours) and/or very long walk to clinic.

On the other hand, a key barrier to therapy intensification in the management of type 2 diabetes is fear of hypoglycaemia. As intensive glucose monitoring is not possible in low resource settings, often sulphonylurea and insulin glucose lowering therapy (two of the 3 therapy classes widely available) are only started and maintained at glycaemic

thresholds far higher than recommended elsewhere (therapeutic inertia) because of the fear of hypoglycaemia. It is not clear whether this fear of hypoglycaemia is justified. Little is known about hypoglycaemia in the patients receiving these treatments in sub-Saharan Africa, with the only a small number of retrospective studies that have not used objective measurements.

The aim of the thesis is to determine the optimal method for monitoring glycaemic burden and impact of exercise on fasting glucose and to understand the rates and determinants of hypoglycaemia with sulphonylurea and insulin treatment.

In Chapter 1 we review the current literature for monitoring glycaemic control in the clinical management of diabetes

In Chapter 2 we compare the performance of three tests (HbA1c, fasting and non-fasting/random glucose) commonly used for monitoring glycaemic burden in type 2 diabetes patients. We show that HbA1c is the overall best measure of glycaemic burden, despite high prevalence of other medical conditions that may affect its accuracy (e.g. anaemia, haemoglobinopathies). We also demonstrate that fasting plasma glucose and random plasma glucose strongly correlate with CGM glucose and HbA1c, have reasonable sensitivity and specificity to detect poor glycaemic control and the difference in performance between these tests is modest.

In Chapter 3 we assess the performance of glycated albumin and fructosamine against continuous glucose monitoring in comparison to other measures as an assessment of glucose burden in participants with type 2 diabetes and determine whether a recently developed automated glycated albumin assay can improve performance over and above fructosamine.

In chapter 4 we assess the impact of prolonged walking on fasting glucose in type 2 diabetes patients. We demonstrate that fasting plasma glucose is not significantly affected by walking to the clinic.

In Chapter 5 we assess the rates and determinants of continuous glucose monitoring measured hypoglycaemia in patients receiving insulin or sulfonylurea treatment in the Ugandan population, in comparison to those receiving metformin or diet treatment. We show that in a low-resource sub-Saharan African setting, hypoglycaemia is infrequent among people with type 2 diabetes receiving sulphonylurea treatment, and the modest excess occurs predominantly in those with tight glycaemic control.

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Acknowledgments

I am very grateful to my supervisors Prof. Angus Jones, Dr. Beverley Shields, Dr. Lauren Rodgers and Prof. Moffat J Nyirenda for the support, guidance and motivation they have given me during the course of my research.

I want to also thank my colleagues in the Exeter Clinical Diabetes research team, the NIHR Global Health group and MRC/UVRI & LSHTM Research unit. Particularly, I would like to extend my sincere gratitude to my classmates Jean Claude Katte and Wisdom P Nakanga, whose presence and health competition kept me motivated.

I would like to thank Aisha Karungi and Winnie Ayuru, the research assistants that assisted with data collection at the diabetes clinics. I am thankful to the staff at the two laboratories; the central Biochemistry and clinical diagnostic laboratory services (CDLS) laboratory at the MRC/UVRI & LSHTM Research Unit Entebbe Uganda and the Exeter clinical laboratory that performed the various assays. My sincere thanks also go to all the people living with diabetes who participated in this study, to whom this thesis is dedicated.

This work was supported by the National Institute of Health Research, UK (award reference 17/63/131).

Last but not least, there are many people who have supported me in the background as I dived in yet again another 3 years of academia. I am grateful to them. However, I would like to specifically thank my family, Nixon Akampurira, and Ikwaput Faith. I would also like to thank my colleagues at the MRC/UVRI & LSHTM Research Unit who have supported me in various ways throughout this journey.

Abbreviations

AACC	American Association for Clinical Chemistry
ADA	American Diabetes Association
AUC	Area Under The Curve
Beta (β)	Regression coefficient, change in outcome variable for a 1 unit change in covariate
CGM	Continuous glucose monitoring
DM	Diabetes Mellitus
eGFR	Estimated Glomerular Filtration Rate
EPO	Erythropoietin
G6PD	Glucose-6 Phosphate Dehydrogenase
GA	Glycated Albumin
GLUT	Glucose transporter
HbA1c	Glycated haemoglobin
HPLC	High performance liquid chromatography
HIV	Human immunodeficiency Virus
IRMM	Institute for Reference Materials and Measurements
LMICs	low-middle income countries
NGSP	National Glyco-haemoglobin Standardization Program
NBT	Nitroblue tetrazolium
RBCs	Red blood cells
r	Correlation coefficient
R^2	r squared
SCT	Sickle cell trait
SSA	Sub-Saharan Africa
T2DM	Type 2 diabetes Mellitus

Chapter 1

Introduction

Chapter 1: Introduction

Structure

The introduction is divided into three parts. Part one describes the structure and aims of the thesis. Part two reviews the approaches used for monitoring glycaemic control in the clinical management of diabetes and the specific challenges in the context of low resource settings and sub-Saharan Africa. Part three discusses rates and determinants of hypoglycaemia with sulfonylurea and insulin treatment, and their relationship to glycaemic.

Chapter 1: Table of contents

Introduction part 1: Structure and aims of thesis

Structure and aims of thesis

The overall aim of this thesis is to determine the optimal method for monitoring glycaemic burden in patients with Type 2 diabetes in Uganda and to assess the rate of short term hypoglycaemia associated with sulphonylurea and insulin therapy, and its relation to glycaemic control. The findings provide important implications for improving diabetes care in low resource settings.

Chapter 2

The aim of this chapter is to determine the accuracy of fasting glucose, non-fasting 'random' glucose and HbA1c as measures of glucose burden in individuals living with Type 2 diabetes in Uganda. Financial constraints mean that the monitoring of diabetes, and decisions to intensify treatment in Uganda and much of Sub-Saharan Africa (SSA), are predominantly based on the measurement of a single glucose value rather than HbA1c and home-based glucose monitoring. Current clinical practice varies with fasting glucose used by many clinicians, but others routinely use non fasting 'random' measurements. The comparative accuracy of these measures as assessment of glucose burden in SSA setting is unknown.

Chapters 3

The aim of chapter 4 is to assess the performance of glycated albumin and fructosamine in comparison to other measures as an assessment of glucose burden in those with type 2 diabetes, and determine whether a recently developed automated glycated albumin assay can improve performance over and above fructosamine. The widely used glucose tests for monitoring glucose burden have limitation and HbA1c is said to be unreliable in SSA populations. Recent recommendations from diabetes

organisations including ADA is to use fructosamine and glycated albumin as alternative measures of glycaemia. However, there is little evidence to inform clinical practice about their performance in a setting where conditions thought to alter HbA1c reliability are common.

Chapter 4

The aim of this chapter is to assess whether walking in the fasted state significantly affects the reliability of fasting glucose as a monitoring test in patients with type 2 diabetes. This information is important to inform choice of test where patients have a long walk to clinic. There is very limited previous evidence on the acute effects of moderate exercise such as walking on ambient blood glucose levels in diabetes, with no studies assessing impact of walking in the fasting state. African physicians commonly prefer random glucose due to concerns that fasting glucose will be falsely low due to prolonged walking to clinic.

Chapter 5

The purpose of glucose monitoring is to assess if glucose lowering therapy should be initiated or intensified. A key barrier to therapy intensification in many SSA populations is fear of hypoglycaemia. The aim of chapter 5 is to understand the rates and determinants of hypoglycaemia with sulfonylurea and insulin treatment (two of the 3 therapy classes widely available), and their relationship to glycaemic control. At present these treatments are initiated at glucose levels higher than used elsewhere because of fear of hypoglycaemia. While food insecurity and lack of access to glucose monitoring may raise specific concerns for hypoglycaemia in sub-Saharan African populations, there is little objective evidence to inform hypoglycaemic risk with these

agents in this population. Understanding of hypoglycaemia risk with sulphonylurea and insulin treatment in this setting will be important to help determine treatment thresholds in clinical practice.

Chapter 6

In this chapter I summarise the findings of the previous chapters and discuss limitations, subsequent work and potential areas of future research.

Introduction part 2: Monitoring of glycaemic control in the management of diabetes

2.1 Background

Diabetes Mellitus (DM) is a metabolic disorder that manifests as chronic hyperglycaemia and is characterised by dysregulated carbohydrate, fat and protein metabolism secondary to defects in insulin secretion, insulin action or a combination of the two.[1 2] If left untreated, the persistently high plasma glucose levels lead to the development of microvascular complications that include retinopathy (which may lead to blindness), nephropathy (with subsequent kidney failure) and neuropathy.[3] Diabetes is also associated with the development of macrovascular complications.[3] DM, particularly type 2 diabetes (T2DM), is a significant cause of morbidity and mortality worldwide and reduces the overall life expectancy in people below 60 years by 4 to 10 years.[4] In people living with DM, the risk of all-cause mortality, cardiovascular complications, and microvascular complications is markedly increased independent of other traditional risks factors.[5 6]

The burden of DM in sub-Saharan Africa and other developing countries

Globally, the prevalence of diabetes has been rapidly rising, principally driven by increases in type 2 diabetes. The number of people living with diabetes worldwide increased from 211.2 million (196.0–228.5) in 1990 to 476.0 million (436.6–522.8) in 2017- a 129.7% increase;[7] and currently, approximately 537 million adults (20-79 years) are living with diabetes, and the total number is projected to rise to 643 million by 2030 and 783 million by 2045.[8]

Most people living with diabetes (approximately 80%) live in low-middle income countries (LMICs).[8] SSA, a continent historically known for communicable diseases (infectious diseases), is now at the crossroads of communicable and non-communicable diseases (NCDs). T2DM, previously thought to be rare in SSA, has

increased markedly since 1980.[9] Notably, results from an analysis of pooled population-based studies in Africa showed that the age-standardised diabetes prevalence increased from 3.4% (1.5-6.3) to 8.5% (6.5-10.8) in men and 4.1% (2.0-7.5) to 8.9% (6.9-11.2) in women from 1980 to 2014.[10] The most recent data show that 1 in 22 adults in SSA was living with diabetes in 2021 (of whom the vast majority have Type 2 diabetes).[8] The SSA region is said to harbour the highest proportion of undiagnosed T2DM, with 54% of those with glucose in the diabetes range thought to be undiagnosed. For example, data from a nationwide population-based NCD survey in Uganda showed that approximately 50% of people with glucose in the diabetes range were unaware of their hyperglycaemia.[11] In addition, SSA is projected to have the greatest future increase in the burden of diabetes.[12 13]

Additionally, SSA has the highest proportion of people who die from T2DM below 60 years.[14] In Uganda, about 716,000 people were living with Diabetes Mellitus, with an age-adjusted comparative prevalence of 4.6%.[8] Over 57% of people with diabetes remain undiagnosed, diabetes-associated deaths reached 10,416, and the proportion of diabetes-related deaths in people under 60 years was 5% in 2021.[8] These figures may be an underrepresentation given the scarcity of data on causal mortality and high rates of undiagnosed disease. The rise in the prevalence of T2DM has been driven by a combination of factors, including ageing populations and increasing prevalence of DM risk factors (obesity, physical inactivity and unhealthy diet) in developing countries that are undergoing rapid urbanisation.[4 15]

Compounding the unfavourable data in most developing nations are insufficient infrastructure, fragmented healthcare systems, health illiteracy and poor accessibility of optimal medications leading to suboptimal identification and treatment of people living with DM.[9]

Monitoring Diabetes

The importance of controlling blood glucose in diabetes became obvious after discovering insulin in 1922. With insulin therapy, the survival of type 1 diabetes patients dramatically improved, allowing them to survive longer than ever. However, those who survived longer developed various vascular complications later attributed to poor glycaemic control.[16] Subsequent studies such as UKPDS proved that achieving a glycaemic control close to the non-diabetic range prevented and delayed the development of microvascular complications and reduced macrovascular complications.[17-21]

To control blood glucose and prevent complications of diabetes, there is a need for monitoring glycaemic control among patients in clinical practice. This is essential to guide the intensification of treatment, prevent hyperglycaemia complications, and avoid hypoglycaemia, a significant side effect of some of the medicines used in diabetes management.

Regular monitoring of glucose levels has always been an integral part of the effective management of diabetes.[22 23] Over the past decades, there has been a series of changes in the methods used for monitoring glucose targets. At first, glucose targets were monitored using crude measures such as relief and improvement in diabetes-related symptoms or colour changes observed after boiling a mixture of the patient's urine, water and benedict's solution.[22 24] Later in the 1940s and early 1950s, the first urine test strip was introduced and used to monitor glucose control for a long time.[22 24 25] This urine test allowed instant monitoring of glycaemic control; however, the number of pitfalls associated with urine glucose measurement motivated the pursuit of the development of better alternatives including, blood glucose methods.[22]

Blood glucose monitoring (measured directly or indirectly) became possible in the 1960s. The first direct measurement of glucose test to be introduced involved placing a large drop of blood on strips (these utilised the glucose-peroxidase system) and waiting for 1 minute for it to generate a colour that was then compared to a series of colours on the chart on the bottle.[22] These colour chart comparisons gave a semi-quantitative assessment of blood glucose. Further improvements saw the introduction of the first glucose monitoring device (Ammes Reflectance Meter) that enabled self-monitoring of glucose. Over the years, these have undergone further refinements to more improved devices.

Blood glucose provides information about the day-to-day level of glucose control, but the wide intra-individual variation, makes interpretation of a single measure, difficult; particularly in the non-fasting setting.

The discovery of glycated haemoglobin (HbA1c), an indirect measure of blood glucose control changed the paradigm of glucose monitoring.[26] HbA1c provides a measure of average glycaemic burden over the previous 2-3 months and therefore enables clinicians to reasonably assess therapeutic response and the need for therapy intensification.[27]

The details of both direct and direct glucose measurements are discussed below.

2.2: The utility of Glycated haemoglobin (HbA1c) as a monitoring test for glycaemic burden in diabetes

Background

Glycated haemoglobin (HbA1c) is formed by a spontaneous non-enzymatic reaction involving a glucose molecule forming a keto-amine on the N-terminus of the haemoglobin (Hb) Beta chain.[28] Glucose enters the red blood cells (RBCs) at a rate proportional to the extracellular concentration through the glucose-transporter channels (GLUT-1), constitutively active, rendering an almost equilibrium glucose environment between the intracellular and extracellular compartments. Therefore, the extent of haemoglobin glycation is directly proportional to the concentration of blood glucose and RBCs' duration of glucose exposure, which depends on the RBCs' age (and, therefore, lifespan).[29] Usually, RBCs spend approximately 120 days (4 months) in circulation before they are removed by the macrophages residing in the spleen.[30 31] Therefore, HbA1c represents the average glucose concentration exposed to the Hb over 120 days. However, the RBC lifespan is reduced in conditions characterised by increased oxidative stress, abnormal RBC size, shape and deformability and other metabolic abnormalities.[32] It is worth noting that though HbA1c may be affected by glucose up to 3 months prior, glucose closer to HbA1c measurement has a more significant contribution to HbA1c variation, with about 50% of the variance in HbA1c determined by blood glucose variation over 14 – 30 days, 25% by glucose variation over 30 – 60 days and the remaining 25% by glucose variation 60 – 120 days.[27 33]

The discovery of HbA1c as a marker of glycaemic control dates back to the 1960s when Samuel Rahbar observed unusual haemoglobin that was markedly raised (7.5 – 10.6%) in people living with diabetes relative to those without diabetes (4 – 6%).[34 35]

Subsequent data from animal and human studies showed that HbA1c positively correlated with glycaemia, i.e., HbA1c increased with increasing blood glucose levels and declined with reducing glucose levels. Data from large clinical trials, i.e. DCCT and UKPDS, demonstrated that the risk of developing complications was directly proportional to glycaemic control as measured by HbA1c.[26] In the DCCT, patients who were intensively treated and achieved a mean HbA1c of 7.4% had a significantly lower incidence of microvascular complications than patients who achieved a mean HbA1c of 9.1%.[19] Similarly, data from a long-term follow-up of patients in the DCCT trial demonstrated that lowering HbA1c (i.e., early intensive glycaemic control) in the initial years following diagnosis reduces the risk of developing cardiovascular complications later in life. Similarly, data from UKPDS showed a statistically significant risk reduction in microvascular endpoints among T2D patients who achieved a lower HbA1c compared to those whose HbA1c was higher, i.e.., median HbA1c 7.0 vs 7.9[17], and median HbA1c 7.4 vs 8.0.[36] Because of these reasons, most developed and developing countries have widely adopted HbA1c as their preferred test for monitoring glycaemic control. [37] [38] Other advantages of HbA1c in comparison to other tests available for monitoring glycaemic control include; the no need for fasting, samples can be obtained any time of the day, the sample is stable, is not altered by external factors like stress and exercise, it reflects long-term glycaemic load (over the previous 2-3 months, with ~50% of the value resulting from the previous four weeks glucose).[27] [33]

2.2.1 The measurement of HbA1c

The HbA1c molecule poses an electrical (chemical) charge that differs from the charges present on the other parts of the Hb molecule. Similarly, the HbA1c molecule

differs in size and structure from the other Hb components. HbA1c can therefore be separated from other HbA components in blood and several methods have been developed for its measurement. In these methods, the glycated haemoglobin is separated from the non-glycated haemoglobin based on their differences in charge or structure. These methods are ion-exchange chromatography (differences in charge), electrophoresis (differences in charge), boronate affinity chromatography (structure differences), and immunoassay (structure differences).

Following its discovery, commercial assays used to measure HbA1c were available in the late 1970s; however, it was not until 1985 that the WHO acknowledged the importance of HbA1c measurement.[39 40] After that, other organizations, including the American Diabetes Association (ADA), recommended HbA1c assessment to monitor patients living with DM.

Methods utilizing differences in charge

Following the attachment of glucose on the N-terminal valine of the HbA beta-chain, an extra negative charge on the HbA1c molecule is generated. Cation-exchange chromatography and electrophoresis methods of HbA1c measurement utilise this charge difference to separate and quantify HbA1c. Cation-exchange chromatography is a procedure that involves the separation of proteins based on the charge properties of their molecules; charged haemoglobins and other haemoglobin components are eluted at varying times depending on the net charge of the molecule in relation to a gradient of increasing ionic strength buffers passed through a cation-exchange column.

Capillary electrophoresis utilizes the principle of liquid-flow capillary electrophoresis in free solution where charged molecules are separated by their electrophoretic mobility

in an alkaline buffer at a specific pH.[41] Separation of HbA1c and HbA0 occurs due to a charge difference coming from elimination of one positively charged amino group in the HbA1c molecule after the attachment of the glucose. The second option is where Hb is analysed as anions in alkaline conditions with selectivity to HbA1c induced by a cis-diol interaction of its glucose molecule with a borate anion from background electrolyte. The pros and cons of these methods are summarised in table 1 below.

Methods utilizing differences in structure

Boronate affinity chromatography is based on the covalent binding of cis-diols of glucose in the glycated Hb to a boronate matrix.[42] Non-glycated Hb does not bind to the boronate and is eluted directly from the column.[42] The Initially bound glycated Hb is released when buffers with higher affinity for the boronate binding site are applied, thereby displacing the bound HbA1c. The result is a chromatogram showing two peaks; the non-glycated Hb peak and the HbA1c peak.[43]

The immunoassay methods such as the latex enhanced immunoassay and the immunoturbidimetry use specific anti-HbA1c antibodies that recognise the first three to five amino acids and the glucose attached to the N-terminal of the beta-chain of the Hb molecule.[42] Total Hb is measured separately using a bichromatic assay and the ratios of the two components are calculated.

The enzymatic method principle comprises of enzymatic cleavage and quantification of glycated dipeptides and then measurement of total Hb.[42] The reference method for measuring Hba1c involves enzymatic cleavage; reverse-phase high performance liquid chromatography (HPLC) to separate the N-terminal hexapeptides; and their subsequent quantification by electro-spray ionisation-mass spectrometry or capillary

electrophoresis. The pros and cons of these methods are summarised in table 1 below.

Table 1: Different methods of measuring HbA1c and their advantages and disadvantages

HbA1c assay	**Principle**	**Advantages**	**Disadvantages**
Ion Exchange Chromatography	HbA1c has lower isoelectric point and migrates faster than other Hb components	Can inspect chromograms for Hb variants; measurements with great precision	Variable interference from haemoglobinopathies, HbF and carbamylated Hb but the current ion exchange assays correct for HbF and carbamylated Hb does not interfere
Boronate Affinity	Glucose binds to m-aminophenylboronic acid	Minimal interference from haemoglobinopathies, HbF and carbamylated Hb	Measures not only glycation of N-terminal valine on β chain, but also β chains glycated at other sites and glycated α chains
Immunoassays	Antibody binds to glucose and between 4- 10 N-terminal amino acids on β chain	Not affected by the common Hb variants such as HbAS, HbAC, HbE, HbD or carbamylated Hb. Can achieve high throughput volumes given its fully automated systems. Relatively easy to implement.	May be affected by haemo-globinopathies with altered amino acids on binding sites. Some interference with HbF

Standardization of HbA1c

Before early 1993, these methods measured HbA1c, HbA1 (HbA1a + HbA1b + HbA1c), or total glycated haemoglobin (GHB). As a result, a single sample would produce widely varying results among methods. This variation in results led to the formation of a Subcommittee on Glyco-haemoglobin Standardization by the American Association for Clinical Chemistry (AACC) in 1993. Furthermore, the National Glyco-haemoglobin Standardization Program (NGSP), formed in July 1996, aimed to standardise HbA1c methods so that HbA1c results from different laboratories would be comparable to those reported in the DCCT study.[44]

Standardisation has a reference measurement procedure and a clearly defined and characterised analyte available as reference material. The HbA1c reference materials are pure A1c and pure A0, registered with the Institute for Reference Materials and Measurements (IRMM).[45] Harmonisation, on the other hand, aims to achieve comparable results among different measurement procedures of the same analyte and typically has no reference measurement procedure and no defined reference material or calibrator (as a result of heterogeneity in measurement principles, common with hormones and antibody measurements).[46] Harmonisation is commonly achieved by exchanging samples and adjusting the results with a factor (slope or an intercept) to match the two comparator methods, i.e., aligning results.[46] For standardisation, the aim is to get near identical results by having calibration traceable to a reference measurement procedure and a primary reference calibrator.

Despite efforts to standardise HbA1c, there are over 30 different methods in use for measurement. Manufacturers provide calibration factors for individual machines, and

a global laboratory network maintains and monitors the relationship between the different standards.

2.2.2 Conditions that may affect the reliability of HbA1c

Because HbA1c is now widely used for monitoring of type 2 diabetes and more recently for diagnostic purposes, understanding factors besides glycaemic burden (non-glycaemic variables) that may alter HbA1c measurement is imperative to ensure accurate interpretation of results. Inaccurate quantification of glycaemic burden by HbA1c has immediate clinical implications. For example, as a monitoring test, underestimation of glycaemic burden may potentially result in suboptimal treatment. Contrariwise, overestimation of glycaemic burden leads to overtreatment, wastage of resources (misappropriate allocation of the already scarce drugs) and increased risk of hypoglycaemia.

A number of conditions, can either falsely lower or raise HbA1c results independent of the glycaemic burden (Table 2). These non-glycaemic conditions (including; sickle cell and other haemo-globinopathies; anaemia, malaria) are relatively common among populations of African ancestry (SSA).[47-51]

The mechanism of how these conditions alter HbA1c are partly explained by red blood cell/haemoglobin factors as shown in Figure 2. These conditions, unrelated to the level of glycaemia or glucose burden are denoted non-glycaemic factors (Figure 2).

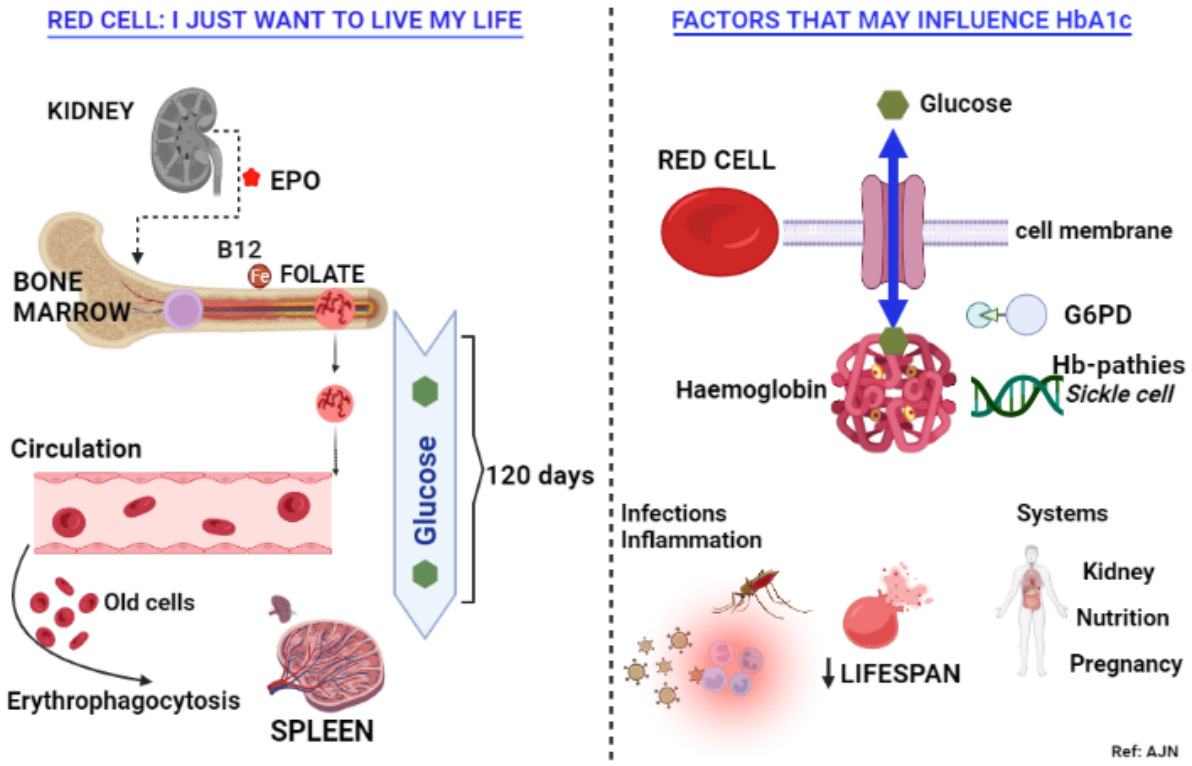

Figure 2: Red blood Cell and factors that may influence HbA1c. EPO denotes erythropoietin, B12; Vitamin B_{12}, and G6PD; Glucose-6 Phosphate Dehydrogenase (G6PD)

Table 2: A summary of the conditions that may alter HbA1c results, the direction of the effect and the possible mechanisms.

Condition	Effect on HbA1c	Probable mechanisms
Age[52 53]	↑	Mechanisms are unclear but are associated with decreasing RBC count with age.
Ethnicity[54]	↓ ↑	Differences in erythrocytes survival, variations in the glycation gap, heterogeneity in the glucose concentration gradient across the erythrocyte membranes and differences in the passage of glucose mediated by GLUT transporters into the erythrocyte
Iron deficiency with and without anaemia	↑	Malondialdehyde, which is increased in patients with iron deficiency anaemia enhances the glycation of haemoglobin.[55]
Chronic renal failure[56]	↓	Shortened red cell lifespan; increased ratio of immature red cells following the administration of erythropoietin and/or iron therapy. Interference from carbamylated haemoglobin with immunoassay methods.
Hb variants[57]	↓ ↑	May cause alterations in red cell lifespan or affect glycation. Additionally, variants may alter the charge of the molecule and interfere with certain assays.
G6PD[58]	↓	G6PD deficiency will affect red blood cell turnover and shortens RBC lifespan
Glycation gap	↓ ↑	The mechanism remains unclear. However, genetic and red blood cell lifespan variations may result in individual differences in glycation rates.
Serum indices and drugs		Lipids, urea, glucose (and labile HbA1c), aspirin, vitamin C and bilirubin may potentially cause assay interferences. Lipids cause a significant negative relative bias in capillary electrophoresis once triglycerides exceed ≈ 15 mmom/L and cholesterol 8.5 mmol/L.
HIV and other chronic inflammatory conditions	↓	Low grade haemolytic state that reduces HbA1c.[59]
Other systemic conditions e.g chronic alcoholism, and pregnancy		interfere with some assay methods.

RBC lifespan

Any condition that decreases the mean red blood cell age will falsely lower HbA1c results regardless of the assay method, e.g., recovery from acute blood loss or haemolytic anaemia.[60] Understanding the factors determining red cell survival and its lifespan is crucial since HbA1c can significantly be altered by factors affecting RBC lifespan.

Normal RBCs survive in the blood circulation for 120 days after their release from the bone marrow (where they are formed). An erythropoietin-dependent increase in RBC production can compensate for a mild to moderate decrease in RBC lifespan. However, severe and acute shortening leads to haemolytic anaemia. All human RBCs have approximately the same lifespan and exhibit non-random removal, unlike other animals like the mouse, where both younger and older cells are removed. Several conditions may alter the RBC lifespan, potentially leading to significant inter-individual variation in mean RBC age without necessarily causing noticeable haematological changes on a complete blood count. Conditions that are associated with impaired erythropoiesis (RBC formation) tend to increase the mean RBC age.[60] These include iron and vitamin B12 deficiency, renal failure (due to a lack of erythropoietin), pregnancy and alcoholism due to bone marrow suppression.[60] Conditions associated with increased erythropoiesis (e.g., haemolytic anaemia) lead to an increased number of young RBCs (reticulocytes) with a subsequent reduction in HbA1c.[60]

Haemoglobin variants

Haemoglobin comprises four globin chains. The main types of haemoglobin in adults are; fetal haemoglobin (HbF $\alpha 2^{\gamma}_{2}$), which is predominant at birth and is mostly undetectable by six months but may persist in individuals who have haemoglobinopathies, haemoglobin A2 ($\alpha\gamma\delta\beta$): a minor Hb after birth (2 – 3%),

Haemoglobin A (α2β2): the most abundant form in most adults (95 – 98%).[61] HbA comprises of 4 sub-types HbA0; HbA1a1; HbA1b and HbA1c. HbA1c represents the majority of HbA1 and results from non-enzymatic glycosylation of the N-terminal of the beta-globin chain in the presence of free sugars. The most common haemoglobinopathies are caused by single amino acid substitutions in the β-chain, including HbS, HbE, HbC and HbD.[57] There are a several ways in which Hb variants interfere with HbA1c measurement: 1) Change the Hb molecule's net charge, resulting in potential interferences with methods such as HPLC and electrophoresis HbA1c results[62], 2) alter the rate of glycation, and 3) reduce RBC life span. The impact of haemo-globinopathies on the different HbA1c assays are given in section 2.2.1 (see Table 1 above).

Sickle cell effect

Sickle cell trait (SCT) is a benign medical condition resulting from a point mutation in only one of the 2-beta globin genes on chromosome 11. Valine is substituted for glutamic acid substitution at position 6 of the β chain.[57] SCT is ubiquitous in SSA, especially within the Eastern region but remains underdiagnosed given its benign nature, even among people living with diabetes. It is not clear how SCT affects HbA1c reliability, but the joint hypothesis is that SCT may reduce the lifespan of RBCs. However, there's limited data to support this hypothesis and it is still unknown whether the lifespan of RBC in SCT patients is reduced relative to haematologically normal patients and whether this reduction impacts HbA1c (in those with effective/intact erythropoiesis).[63]

A systematic review by Gordon et al. (2020) showed that studies during which an NGSP-certified method was used to compare HbA1c in patients with and without SCT showed contrasting results.[63] NGSP-certified methods are said to have no clinically

significant interference by HbS. However, other studies showed that persons of African ancestry, the group to which the bulk of SCT patients belong, had higher HbA1c than non-Hispanic whites, just based on race, and a greater probability of getting G6PD deficiency, which lowers HbA1c.[63]

According to the ADA, HbA1c measurements in SCT patients should be performed using an NGSP-certified device to minimise HbS interference. This would imply that using an NGSP-certified device without HbS interference in an SCT patient would give reliably similar HbA1c results for estimated average glucose over the previous 120 days compared to haematologically normal patients. However, results from a systematic review of 11 studies that only used NGSP-certified machines that do not experience HbS-interference gave conflicting results; some stated that HbA1c was higher, others stated it was lower and some stated high variability higher-lower or same-lower.[63] The included studies had some limitations: no assessment for alpha-thalassaemia, low numbers of SCT (information bias), and analysis based on single measurement e.g., fasting glucose, and exclusion of patients with missing SCT information (which could have potentially caused selection bias)

HbA1c and racial effect

Racial disparity in HbA1c has been observed among people living with diabetes, those with impaired glucose tolerance and those who are normoglycemic. It is now accepted that there are racial differences in the absolute levels of HbA1c irrespective of mean blood glucose (MBG) though the mechanisms behind these differences are unclear. Moreover, HbAS haemoglobinopathy occurs more frequently in black people than in non-Hispanic whites. A systematic review of 12 studies using data from approximately 50,000 patients concluded that in patients without Diabetes, HbA1c was higher in blacks, Asians and Latinos than whites.[54] A meta-analysis of non-diabetic participants

demonstrated statistically significantly higher levels of HbA1c in black (2.8 mmol/mol, 95% CI 0.18 – 0.33), Asian (2.6 95% CI 0.16 – 0.33) and Latino Cohorts 10.9 mmol/mol 95% CI 0.06 – 0.10) compared with Caucasians.[54] In a prospective study using CGM and comparing 104 black and 104 white patients with known T1D over 12 weeks, black patients had, on average, an HbA1c higher by 0.4% than whites for comparable average glucose measure.[50]

The reasons for these observed differences between races are unknown but may include; differences in Hb glycation, non-glycaemic genetic factors, red blood cell survival, and differences in extracellular and intracellular glucose balance. Of particular notice is the increased association of SCT with African ancestry and Glucose-6 Phosphate Dehydrogenase (G6PD) deficiency. The most extensive GWAS meta-analysis of approximately 160,000 persons from 82 cohorts identified 60 common genetic variants associated with HbA1c.[64] In particular, 22 affect the structure, lifespan and function of RBCs (erythrocyte variants) and 19 influence glucose control (glycaemic variants). A longitudinal follow-up of 33,000 people from 5 ancestry groups showed that the higher the number of glycaemic variants, the greater the risk of diabetes (OR = 1.05 per HbA1c-raising allele). Contrastingly, the more erythrocyte variants a person had did not increase the risk of diabetes. Moreover, some erythrocyte variants, especially G6PD, lowered HbA1c independent of glucose concentration and led to a missed diagnosis. The erythrocyte variant G6PD was responsible for the massive difference in HbA1c among Africans between patients with more glycaemic and erythrocytic variants; this finding or difference was minimal/reduced in individuals of other descent.

In those without diabetes, factors other than glucose have a relatively higher contribution to HbA1c variation, however as glucose increases the proportion of

variation explained by glucose becomes stronger. Therefore these studies in people without diabetes may not apply to those with established dysglycaemia and use of Hba1c for monitoring.

Understanding the performance of HbA1c in SSA and other LMIC settings

Very few studies have assessed the performance of HbA1c measurement as a measure of glycaemic burden in diabetes monitoring in SSA. [65] [66] Majority of the previous studies have compared HbA1c against single glucose measures. Rasmussen et al [66], found a strong correlation (r = 0.73 overall and 0.77 after excluding 8 insulin treated patients) between HbA1c and a single random blood glucose measure in 78 type 2 diabetes patients living in Africa. In these studies, the sample sizes were small and the impact of common glycaemic comorbidities thought to alter HbA1c reliability was not assessed for. Another study in India where HbA1c was compared to each of fasting and random glucose among 1,000 people living with diabetes, HbA1c showed good correlation with both fasting glucose and random glucose (0.739 and 0.601 respectively).[67] A similar study by El-Kebbi et al., among an African-American predominant population in the US, HbA1c was correlated strongly with random non-fasting glucose collected 1-4 hour post meal. [68] This was a predominantly insulin treated population.[68]. In contrast, in studies where a fixed post meal time point was used, non-fasting (post-prandial) glucose was a slightly better correlate of HbA1c than fasting glucose.[69] When compared to glucose measures (fasting and 2hr-oral glucose measures) in screening for T2DM in Africa, the diagnostic accuracy of HbA1c was poor (missing almost half of the individuals with T2D based on blood glucose measures) SSA.[70]These results underscore the need for assessing the performance of HbA1c as a measure of glycaemic burden in diabetes monitoring in SSA.

Few studies have assessed the performance of HbA1c against a more robust standard glycaemic measure such as against continuous glucose monitoring in low-resource settings. Previous studies are predominantly from developed countries including the DCCT study which recruited Type 1 diabetes participants in the USA and showed a strong relationship between the mean plasma glucose and HbA1c (Pearson correlation coefficient of 0.82) [71]. In the ADAG study, where over 500 participants with and without diabetes were recruited predominantly from the US and Europe, HbA1c and mean glucose were closely correlated ($r = 0.89$, $P < 0.0001$) and this was true across both insulin treated and non-insulin treated patients as well as across all ages [72]. However, it should be noted that participants with analytical concerns for HbA1c measurement were excluded.

Therefore, studies are needed that assess the reliability of HbA1c as a measure of glycaemic burden in LMICs especially in SSA where patients are likely to manifest with multiple of these comorbidities (i.e., iron deficiency without anaemia, vitamin B12 and folate deficiencies, haemoglobinopathies, among others). Given the limitations of single glucose measures, future studies should employ long-term glucose measurement such as CGM as the standard measure against which to assess HbA1c's performance.

2.3: Glucose monitoring in diabetes

Background

Glucose can be measured either as plasma glucose during a fasted or random non-fasted state at the hospital/clinic (laboratory-based glucose monitoring) or as interstitial glucose using a glucometer or subcutaneously with an automated glucose sensor obtained out of clinic (non-laboratory based techniques). A single plasma glucose measurement at the clinic provides a one-time snapshot of glycaemic control. However, blood glucose is never static, it continuously fluctuates over time. In diabetes, the fluctuations of blood glucose are more prominent than in health and are more amplified by the diminished or absent glycaemic auto-regulation and the person's day-to-day activities including work-related activities, eating, sleeping and other environmental factors[27]. Therefore, a single glucose measurement taken at the clinic may not represent the person's true glycaemic burden. This is especially true in cases where plasma glucose fluctuates rapidly for example in type 1 diabetes. In such cases, more than one glucose reading obtained out of clinic is recommended to give a broader picture of one's glycaemic status and this can be done either by self-monitoring of blood glucose with a glucose meter or continuous glucose monitoring.

Laboratory-based plasma glucose monitoring

Fasting and non-fasting plasma glucose

The International Diabetes Federation (IDF) recommended use of a fasting plasma glucose (FPG) for monitoring glycaemic control in developing countries like Uganda where HbA1c services are not readily available[73]. This is because fasting glucose is cheap, widely available and easy to do. Nevertheless, it has its limitations. FPG is highly affected by pre-analytical factors such as food consumption, sample collection,

storage[27]. Moreover, its large biological variability up to a coefficient of variation between 5.7- 8.3% for intra-individual and 12.5% for inter-individual variation and diurnal variations means that it lacks reproducibility[27]. The patient is required to fast for more than 8 hours.

The other key limitation of using a single glucose measurement for diabetes monitoring is a single measurement may not reflect longer term glucose control, for example patients may be more likely to strive for low glucose levels immediately prior to an appointment.

Substantial evidence is needed on the acute effects of walking long distances to clinics and prolonged fasting on glycaemia diabetes.

In addition to the already known limitations of using glucose measures for monitoring, most of the patients in Uganda and other SSA countries walk long distances to the diabetes clinics normally operated at regional and district hospitals.[74]

As a consequence, fasting/non-fasting blood glucose will often be measured after an abnormally prolonged fast (of more than the recommended 8 hours) and a very long walk to the clinic. Whether this affects the fasting glucose is unclear.

To understand the potential impact of prolonged walking (aerobic exercise) on fasting glucose, it is imperative first to appreciate the physiological or metabolic changes that occur during these distinct states.

Review of the physiology of metabolic changes during a fasted state.

Fasting is generally defined as the act of restraining food or drink intake over some time. There are two main types of fasting, i.e., short-term and prolonged fasting.

However, in this review, we shall focus on short-term fasting (overnight fasting), hereafter referred to as fasting and its acute effects.

During fasting, the post-absorptive period (first stage) lasts 3 to 8 hours, depending on the content and size of the meal. During the early stages of fasting, blood glucose is kept stable by the continuous breakdown of the liver glycogen to release glucose into the circulation.[75] Even during fasting, there is a continuous need for oxidative metabolism to meet the energy needs. It is widely accepted that the rate of carbohydrate utilisation is decreased during fasting, and an increased rate of fat oxidation meets the energy demand. This is important to spare the body's limited carbohydrate reserves for specific tissues that are obligatory users of glucose, e.g., the brain and red blood cells.

Therefore, one of the significant responses to fasting is the mobilisation of free fatty acids (FFAs), an alternative muscle fuel source.[76] [77] This happens through the breakdown of triglycerides primarily stored in the adipose tissue and increased free fatty acid levels in circulation. Similarly, there is increased release of glycerol (a valuable precursor for gluconeogenesis in the liver) from the adipose, thereby contributing to the pool of available glucose.[77] This integrated metabolic response that involves both mobilisations of FFAs and hepatic gluconeogenesis is regulated by changes in the hormonal milieu, including a reduction in the plasma insulin concentration and increased circulating concentrations of counter-regulatory hormones such as glucagon, catecholamine, and growth hormone.[78] This process initially depends on the availability of glycogen stores in the liver and skeletal muscle. The glycogen stored in the liver contributes the greatest to the maintenance of glucose during the first hours of a fast.

Fasting is associated with a set of well-coordinated metabolic/physiologic changes designed to maintain a stable supply of glucose for the brain. These changes include reduced insulin and increased glucagon levels in circulation. These changes activate the breakdown of glycogen into glucose released in circulation, thereby reducing the glucose stores in the form of glycogen. As a result, there is attenuated hepatic glycogen synthesis and glycolysis. In fasting conditions, FFA and ketones are the primary energy sources for most cells; this transition is called metabolic switching.[79] Therefore the fasting period is characterised by an increase in the systemic levels of free fatty acids (FFA) and ketones together with activation of gluconeogenesis (from amino acids, glycerol and ketone bodies).[79] These modifications result from reduced insulin concentrations, increased glucagon levels, and enhanced sympathetic activity.[79]

Understanding the impact of exercising on glycaemia

Walking is a form of physical activity (exercise) that involves body movement orchestrated by skeletal muscle contractions. Thus, it is an active process that requires fuel and increases energy expenditure. Several factors determine what fuel will be used during an exercise, including the intensity and duration of the physical activity. However, any form of activity causes a shift from predominant reliance on free-fatty acids (FFAs) at rest to a mixed dependence on fat, glucose and muscle glycogen and lesser extent, amino acids.[80] As the intensity of the activity increases, there is greater reliance on carbohydrates, provided sufficient quantities in the muscles and blood.

Glycogen is the primary fuel source for the exercising muscles in the first few minutes of physical activity. However, as glycogen stores get depleted, muscles increase their uptake and use of circulating blood glucose, along with FFAs released from the

adipose tissue. With increased uptake by the contracting muscles, blood glucose levels are maintained by glucose production from the liver through glycogenolysis and gluconeogenesis.[80]

There are two distinct pathways by which the muscle takes up glucose.[80] The first is the insulin-stimulated pathway which is the main pathway at rest and is impaired in T2D. The second happens during physical activity, where muscular contractions stimulate blood glucose uptake transport via separate insulin-independent mechanisms that are not affected by insulin resistance or T2D.[81] Glucose transport into the skeletal muscle is accomplished via glucose transporters, in particular GLUT4 modulated by both exercise and insulin.[82]

The acute (immediate) effects of exercising on blood glucose control in people living with type 2 diabetes is unclear

The effects of exercise on blood glucose will vary with duration, intensity and subsequent diet. During moderate-intensity exercise in normal individuals, the rise in peripheral glucose uptake primarily by contracting muscles is balanced by an equal rise in hepatic glucose production. As a result, blood glucose levels remain stable except for prolonged exercise, where glycogen stores are depleted. The endocrine and sympathetic nervous systems coordinate glucose stabilisation during these states.

However, in individuals living with T2D, blood glucose uptake and utilisation by contracting muscles is higher than hepatic glucose production, and therefore glucose tends to decline.[83] The declining blood glucose leads to a simultaneous reduction in plasma insulin levels making the risk of exercise-induced hypoglycaemia minimal unless the patient is on insulin or insulin-secretagogues.

Previous studies, however, have documented hyperglycaemia during brief, intense exercises caused by a marked rise in plasma catecholamine levels that then enhance endogenous glucose production. The resultant hyperglycaemia is said to persist up to 1 – 2 hours since catecholamines persist in circulation and glucose production does not return to normal immediately after physical activity.[84]

It is known that skeletal muscle contraction (during exercise) mainly regulates glucose transport by translocating the glucose transporter into the plasma membrane, resulting in increased glucose uptake and utilisation by the exercising muscles.[81 85] This increase in glucose uptake, is independent of insulin, directly proportional to the duration of exercise, and persists for several hours post-exercise.

The impact of exercise on glucose metabolism in the fasted state remains unclear. Some studies in non-diabetic healthy individuals have documented lower blood glucose levels when exercise is performed in a fasted state compared to a fed state. However, substantial data also reports elevated or stable blood glucose concentrations when exercise is performed in a fasted state. The discordant results may be as a result of varying protocols including different exercise intensities and duration. In healthy individuals, hypoglycaemia is unlikely since blood glucose levels are kept stable during exercise, especially in the initial 60 minutes.[80] However, as exercise continues, blood glucose decreases between 60 and 120 minutes.

The stabilisation of fasting blood glucose levels during exercise has been attributed to lower insulin levels before exercising; this leads to smaller hepatic and muscle glucose uptake, while elevated levels of epinephrine levels increase hepatic glucose production. This is contrary to exercising in the post-prandial phase when insulin levels are elevated; in this case, exercising in the hyperinsulinaemic state, coupled with

increased acute insulin sensitivity and markedly increased muscle glucose uptake, lowers glucose levels significantly.

There is minimal previous evidence on the acute effects of moderate exercises, such as walking in a fasted state, on ambient blood glucose levels in T2D, especially in low-resource settings. The few studies in developed countries reported discordant results and used poor study designs, comparing exercising in a fasted and post-prandial state. The unique characteristics in low-resources settings, such as a higher likelihood of having less than two meals a day (potentially leading to low glycogen stores), and distinct T2D phenotype (young, leaner), mean that the magnitude of change in plasma glucose after a moderate activity like walking is unknown. One study in a Swedish population (mean age 63) showed that blood glucose levels reduced at least 2.0 mmol/l after walking a short distance.[86] The study activities were performed in the afternoon, and the time and composition of the last meal were not recorded. Therefore, there is a need to determine how well fasting glucose reflects overall glycaemia in patients with diabetes who walk long distances to clinics/hospitals and assess whether this measure is altered by prolonged fast or walking a considerable distance.

2.3.2: Non-laboratory based glucose monitoring

Self-monitoring of blood glucose using glucometers

Self-monitoring of blood glucose (SMBG) is crucial in the self-management of DM and adjustment of medication. Although more important for people living with type 1 diabetes, SMBG is also very crucial for insulin-treated type 2 diabetes who are likely to experience rapid glucose fluctuations. SMBG allows patients to evaluate their individual response to therapy and assess whether they are meeting their treatment targets as well as ensuring that they are free from hypoglycaemia.

The recommendation from international guidelines is that patients who are using intensive insulin regimens including multiple daily injections or insulin pumps should assess glucose levels prior to meals and snacks, at bedtime, postprandial, prior to exercise and when they suspect or after treating hypoglycaemia.[87] For type 1 diabetes or insulin treated type 2 diabetes, a frequency of 3 to 4 times per day SMBG is recommended.[88] SMBG in individuals on noninsulin therapies has not consistently shown clinical significance and the recommended frequency for non-insulin treated type 2 diabetes is unclear with some researchers suggesting only modest role of SMBG in this sub-group.[87] Both national and international organisations like the international Diabetes Federation (IDF) recommend the utilisation of SMBG part of self-management of diabetes and titration of medication. A number of detection kits for blood glucose have been developed in the form of portable or implantable glucometers. Despite the clear benefits of SMBG, patient compliance is very low. The low compliance is said to result from the discomfort and pain associated with the finger pricks as well as the complexity of glucometers. For patients in low resource settings, financial constraints mean that majority of the patients will not afford SMBG and is not well funded by healthcare systems in most of SSA, and is beyond the financial means and literacy skills of a large proportion of those who have diabetes.[9 89]

Interstitial glucose monitoring Subcutaneous Continuous glucose monitoring

Following the recent advancements in technology, it is now possible to measure glucose in the skin interstitium.[90] Continuous glucose monitoring (CGM) complements the assessment of glycaemic control by offering the opportunity of measuring glucose in day to day living over a period of days to weeks and is widely used in high income countries and some LMICs.[91]

The utility of the methods of monitoring glycaemic control has not been robustly assessed, and each potential method will have specific considerations for use in SSA. Due to costs, this is unlikely to be widely used in SSA for clinical care but could offer important insights on day to day glucose levels in a research setting

2.4: Glycated serum proteins (Fructosamine and albumin)

Glycated serum proteins (Fructosamine and albumin)

As described above, the two most recommended tests for monitoring diabetes (FPG and HbA1c) have potential issues that may affect their reliability in LMIC and sub-Saharan African settings. Recently, the American Diabetes Association (ADA) recommended that in patients in whom HbA1c is unreliable (for example, populations of African ancestry, iron deficiency anaemia, subjects with increased red cell turnover, e.g., haemolytic anaemia, other systemic diseases such as end-stage renal disease, heavy alcohol consumption, haemoglobin variants among others), other markers of chronic glycaemia may be used.[92] These alternative measures are Fructosamine and glycated albumin.

Understanding Fructosamine and Glycated Albumin

Fructosamine refers to all ketoamines resulting from the glycation of nearly all plasma proteins. Since albumin is the most abundant serum protein, fructosamine is predominantly a measure of glycated albumin. However, other circulating proteins like glycated lipoproteins and glycated globulins may contribute to the total concentration of fructosamine.[93]

Albumin is the most abundant protein in circulation and accounts for approximately 60 to 70% of total serum proteins. Its plasma concentrations range between 35 to 55 g/L.[94] It is a globular protein with a serum half-life of about 20 days. Albumin consists of 585 amino acid residues organised in a single polypeptide chain and stabilised by disulphide bridges.[95] Its principal function is the maintenance of osmotic pressure. The other functions include binding, stabilisation and transportation of metabolic products, regulatory mediators, nutrients, ions and other proteins.[94]

Similar to other proteins like haemoglobin, albumin undergoes glycation. Both fructosamine and GA levels increase in states of hyperglycaemia and can therefore be used to monitor glycaemic control. Relative to haemoglobin, whose life span is approximately 90 – 120 days, the lifespan of non-immunoglobulin serum proteins is much lower (14 – 21 days). The glycation process of serum proteins is non-enzymatic similar to that of haemoglobin.[94] Similarly, glucose reacts spontaneously with amino-terminal residuals of serum proteins, specifically lysine and arginine. Initially, an unstable, reversible Schiff base product (aldimine intermediate) that can be reconverted to glucose and protein is formed. The intermediate product then undergoes further changes called to form a stable Amadori product (ketoamine derivative).[96]

Therefore, the measurement of fructosamine or GA provides information on glycaemic control in the preceding two weeks.[97] The glycation rate of albumin is 9 – 10 fold higher than that of haemoglobin, and thus greater susceptibility to glycation of serum proteins compared to haemoglobin is said to give them an edge in the early detection of rapid glucose changes.[94] Unlike Hb, which is an intracellular protein, fructosamine and GA are extracellular proteins; therefore, these factors are not affected by factors that affect the RBCs or haemoglobin, e.g., haemoglobinopathies, variation in glucose transport into red blood cells or the mean age of these cells. It is easy to measure fructosamine and GA given that the current assays are rapid, technically easy, and inexpensive. However, it is should be noted that changes in protein concentration and half-life affect fructosamine and GA. Conditions other than glycaemia, including the nephrotic syndrome, thyroid dysfunction, hepatic cirrhosis, smoking, hyperuricemia, and hypertriglyceridemia alter GA.[98] It is proposed that fructosamine and GA may enable early identification of suboptimal glycaemic control before any significant HbA1c

changes and, therefore, may play a crucial role in the monitoring of patients with fluctuating or poorly controlled diabetes.[99]

Measurement of fructosamine and glycated albumin

Fructosamine has been in use longer, given that the method for its measurement was developed way back in the 1980s. This method is based on the ability of serum Fructosamine to reduce nitroblue tetrazolium (NBT) to formazane and change the dye's absorbance.[100] The rate of formazane formation is directly proportional to the concentration of fructosamine and can be measured by spectrophometry. The accuracy and sensitivity of the test were improved by minimising interference from uric acid and polylysine by addition of a non-ionic detergent containing uricase.[101]

There are a number of methods used to quantify glycated albumin and each method has its advantages and disadvantages in terms of ease of use, skills, and availability. These methods include calorimetry, spectroscopy, enzymatic assays, immunoassays, high pressure liquid chromatography (HPLC) or mass spectroscopy (MS). HPLC was one of the first methods used to measure glycated albumin but it is very expensive, needs skilled technical know-how and is reported to have a low throughput. Besides being different, these methods measure glycation at different sites (that is, they measure different target molecules) and therefore will provide varying results. For example, HPLC defines GA as the ratio of GA molecules to total albumin molecules. Enzymatic assays and MS measure the concentration as the ratio of GA amino acids to total albumin.[102] In order to optimise the interpretation of results and Takei, I., et al developed an equation to match enzymatic assay GA percentages to the HPLC results.[103]

Over the past decade more accurate and user-friendly enzymatic assays based on albumin-specific enzymatic protease and ketoamine-oxidase have been developed. These enzymatic assays are expanding the global use of GA. Of these, the most widely used and extensively evaluated assay is the one developed in Japan.[104] This assay is widely used in Japan, China, Taiwan and Korea but not yet approved in Europe and the US. Another method approved in the US is also commercially available.[105] In order to achieve standardisation, the committee on Diabetes Mellitus Indices of the Japan Society of Clinical Chemistry developed an isotope dilution liquid chromatography/tandem mass spectrometry method as a reference measurement procedure, and a certified reference material for glycated albumin measurement.[103]

Evidence supporting glycated albumin and fructosamine as markers of glycaemic control

GA reflects glycaemic burden over the past 14 to 21 days. Much controversy remains on whether these alternative markers of glycaemia have any clinical utility in monitoring diabetes. The clinical reliability of these glycated proteins above and beyond the existing traditional tests (HbA1c and fasting glucose) is unknown in SSA, where conditions that may potentially affect HbA1c are common. Only one study has explored the association between fructosamine and GA with glucose measures in SSA, and this study was limited to diagnosis utility.[106] The few studies that have assessed the correlation of fructosamine or GA with other markers of glycaemia such as HbA1c and fasting glucose were limited to developed countries.[107]

A few studies carried out in a small select group of patients and using single-point glucose measures demonstrated that fructosamine and GA are strongly associated with other established markers of glycaemic control, including HbA1c and FPG.[108 109]

Studies incorporating repeat assessments of glucose and CGM are needed to assess the clinical utility of these alternative glycaemic markers for monitoring glycaemic control in type 2 diabetes. In a tiny (limited to 26 T1D children) study where CGM was utilised, glycated serum proteins had comparable correlations with HbA1c against mean glucose from CGM.[110] Other studies that showed strong correlation between fructosamine and GA with HbA1c and mean blood glucose excluded patients with liver disease and kidney failure.[111]

Increasing evidence from developed countries shows that GA is closely related to the risk of the onset of diabetes, diabetes complications including retinopathy, nephropathy, peripheral neuropathy and macrovascular complications such as myocardial infarction, heart failure and stroke.[112] One large study in the US showed that the associations of fructosamine and GA measured at baseline with the risk of incident diabetes, prevalent retinopathy, and risk of CKD were more modest compared with that for HbA1c.[113]

Therefore, given the limitations of most of these studies including small numbers, highly select group of participants, single point glucose measures, exclusion of patients with conditions thought to alter HbA1c (kidney disease), studies are needed to establish the clinical utility of fructosamine and GA as markers for monitoring glycaemic control in SSA.

2.5: Key issues for monitoring of glycaemic burden in SSA.

The optimum method of monitoring glycaemic control in sub-Saharan African populations is unclear

In developed countries, glucose control is monitored mainly by HbA1c testing or intensive home capillary glucose measurements.[114 115] There are a number of reasons why these approaches may not be appropriate for SSA countries like Uganda. First of all, home glucose monitoring is too expensive for the majority of Ugandans especially in rural areas, they will not afford to buy a blood glucose monitor and blood glucose test strips.[116] This means blood glucose monitoring for most patient with diabetes is only undertaken when they attend clinic appointments at public or private hospitals/health centres.[117] Therefore glycaemic control, and whether glucose lowering medication is intensified, is commonly determined by a single glucose measure either performed as fasting or as a 'random' test in a non-fasting state.

Measuring HbA1c is expensive relative to glucose tests, and is therefore not readily available for many people living with diabetes SSA.[9] In Uganda, HbA1c is unavailable in more than 50% of major hospitals including regional and general hospitals.[116 118] In Kenya, Park. H. et al, noted that laboratory Hba1c measurement gives reliable, accurate measurements however the laboratories were are faced with less capacity to afford upfront machine cost, machine maintenance, electricity challenges, technical staff and delays on result turnaround time.[74] Therefore, HbA1c is not used routinely in most centres because of cost. An additional issue for use of HbA1c in SSA is that where it is available its use may not be appropriate because of potential reduced reliability in SSA populations. The non-glycaemic conditions which have been shown to interfere with the HbA1c are common in populations of African ancestry. These

conditions include anaemia, iron deficiency, renal impairment, haemo-globinopathies (such as sickle cell), and glucose 6 phosphate deficiency [58 119 120].

The International Diabetes Federation (IDF) recommended use of a fasting glucose for monitoring glycaemic control in developing countries like Uganda where HbA1c services are not readily available[121]. This is because fasting glucose is cheap, widely available and easy to do. However, it has its limitations[122] including lack of reproducibility, a patient is required to fast for more than 8 hours, large biological variability. [123] The key limitation of using a single glucose measurement for diabetes monitoring is a single measurement may not reflect longer term glucose control, for example patients may be more likely to strive for low glucose levels immediately prior to an appointment[124]. There are a number of specific concerns for use of fasting glucose in sub-Saharan Africa. Due to food insecurity, and sometimes long waits in clinics, fasting time may be prolonged. In addition many patients make long walks to attend a centralised clinic[125]; these factors may lead to glucose values that are falsely low. For these reasons many clinicians use non-fasting (random) glucose monitoring in clinic, without the requirement to fast[124]. While these tests have been compared to HbA1c in the LMICs setting, [66 69] given the limitations of HbA1c itself in these populations, the performance of fasting and random tests as measures of average glucose is unclear.

Of particular concern is the confusion that surrounds the cut-off points for optimal glycaemic control of non-fasting glucose. A study in Kenya showed that only the morning non-fasting had a linear correlation with HbA1c and a non-fasting glucose of 126 mg/dl (7.0 mmol/l) had a sensitivity of about 93% and specificity of about 60% for an HbA1C of ≤7.8% [126]. Another study recommended a non-fasting glucose cut off of 135mg/dl for a sensitivity of 76 % and specificity of 70% for an HbA1C of ≤ 7.0%[127].

Also in another study by Gill et al, it was shown that clinic-measured random blood glucose levels below 10.0mmoll^{-1} predict acceptable overall glycaemic control in non-insulin dependent diabetic patients especially those on diet management only[128]. However, non-fasting (fasting) is of limited value when above 10mmol^{-1}[1128]. So, there are inconsistencies on what is the right cut-off point for poor control; most cases these studies have been small and have not taken into consideration other important factors such as distance walked to the clinic, time of the last meal.

Therefore, studies that compare HbA1c and glucose tests to blood glucose levels obtained at different times of the day with CGM and determine the best predictor of good glycaemic in a typical African population are needed.

Introduction Part 3: Treatment related hypoglycaemia among individuals living with type 2 diabetes in sub-Saharan Africa

3.1 Glycaemic control and treatment targets in type diabetes patients

The morbidity and mortality from complications of type 2 diabetes can be prevented by optimal blood glucose control, which is usually achieved through a combination of lifestyle change and glucose-lowering medication. The lifestyle interventions target dietary and physical activity habits to improve glucose, blood pressure and lipid levels and ultimately promote weight loss or at least avoid weight gain. However, because type 2 diabetes is a progressive disease and many patients are diagnosed late with high levels of glycaemia, especially in the developing world, maintenance of glycaemic targets with lifestyle intervention is often limited and possible for only a few years, among other individuals. Therefore, ultimately patients will be started on single or combination therapy consisting of one or more oral and injectable drugs (Table 2), and finally, administration of exogenous insulin. In addition to the long-standing drugs (metformin and sulphonylureas), other newer drugs are now available in the market, including SGLT2i, GLP-1 RA and DPP4i (Table 2).

Most guidelines recommend a patient-centred approach. Metformin remains the first-line drug for patients with type 2 diabetes in most developed and developing nations unless specifically contraindicated.[129-131] Metformin is an effective and safe drug that reduces hepatic glucose output, enhances peripheral tissue sensitivity, and stimulates GLP-1 secretion (Table 2 and Figure 3). New recommendations from developed countries include the addition of another drug in particular cases such as SGLT2i in individuals with established or increased risk of cardiovascular or renal complications. While several options exist when metformin alone is inadequate in achieving glycaemic goals in developed countries there is only one oral option after metformin

(i.e., SUs) for most people living with T2DM in SSA, then insulin. Other newer oral drugs with lower hypoglycaemia risk are not available.

Table 3: Type 2 diabetes pharmacological treatments and their mechanism of action.[132]

Class	Primary Mechanism of Action	Agent(s)
a-Glucosidase inhibitors	Delay carbohydrate absorption from intestine	Acarbose
Biguanide	Decrease Hepatic Glucose Production Increase glucose uptake in muscle	Metformin
DPP4 inhibitors	Increase glucose-dependent insulin secretion Decrease glucagon secretion	Linagliptin Saxagliptin Sitagliptin
Sulfonylureas	Increase insulin secretion	Glibenclamide Glimepiride Glipizide Glyburide
Thiazolidinediones	Increase glucose uptake in muscle and fat Decrease HGP	Pioglitazone Rosiglitazone
GLP1 receptor agonists	Increase glucose-dependent insulin secretion Decrease glucagon secretion Slow gastric emptying Increase satiety	Dulaglutide Exenatide Exenatide XR Liraglutide
SGLT2 inhibitors	Increase urinary excretion of glucose	Canagliflozin Dapagliflozin Empagliflozin

DPP4, dipeptidyl peptidase; HGP, hepatic glucose production. GLP1, glucagon-like peptide; HGP, hepatic glucose production; SGLT2, sodium glucose cotransporter 2

Achieving and maintaining tight glycaemic control is very important to avoid diabetes-related complications. The DCCT, a prospective randomized controlled trial in patients with type 1 diabetes was the first to demonstrate that intensive (mean HbA1c of ≈ 53 mmol/mol) versus standard (mean HbA1c ≈ 75 mmol/mol) glycaemic control, reduced the development and progression of microvascular (retinopathy, neuropathy, and diabetic kidney disease) complications.[16] Follow up studies established that the benefits of reduced microvascular risk among the intensively treated patients persisted for many years despite glycaemic deterioration.[18] Similar studies among type 2 diabetes patients confirmed that intensive glycaemic control significantly decreased rates of microvascular and macrovascular complications and the effects were long lasting.[19 20]

Therefore, these studies demonstrated that achieving HbA1c targets of <53 mmol/mol reduces microvascular complications when instituted early in the course of disease. However, subsequent studies subsequent studies have raised concerns about very intensive treatment to lower HbA1c targets in high risk populations (e.g., longer type 2 diabetes duration and elderly).[133-135] For example, increased mortality rates were documented among patients with type 2 diabetes who were treated to glucose levels that are much lower than HbA1c targets of <53 mmol/mol (near normal).[133] As a result, the widely recommended glycaemic target is blood glucose levels that correlate with achievement of an HbA1c of <7% (53 mmol/mol) for non-pregnant adults and this may be relaxed among some individuals such as older patients with multiple coexisting chronic illnesses, and cognitive impairment.[130 131 136]

Poor glycaemic control is common in patients with Type 2 diabetes in sub-Saharan Africa

A large proportion of diabetes patients in Africa have poorly controlled glucose levels.[137-139] In Uganda poor glycaemic control (HbA1c ≥ 7.0%/53 mmol/mol) rates above 60% have been reported both in public and in private settings.[140 141] This is similar in other countries of SSA like Guinea and Cameroon where a recent large study demonstrated that 3 out of every 4 patients with type 2 diabetes had poor glycaemic control (HbA1C ≥ 7.0%/53 mmol/mol). [142] The 2015 GBD reported 145,189 DM associated deaths and over 5.5 Million DALYS; this is an increase of approximately 90% between 1990- 2010.[143] In order to reverse the tide caused by T2DM associated catastrophes, DM has to be diagnosed early and glycaemic control optimised.

3.2 Hypoglycaemia-associated risk of type 2 diabetes treatment agents

Hypoglycemia is defined as the decrease of blood glucose level below normal. Clinically significant hypoglycaemia is considered to be plasma glucose of <3.5 mmol/l. In other guidelines, glucose < 3.9 mmol/L (70 mg/dL) is also considered as an important biochemical level of hypoglycaemia.[144] Other definitions of hypoglycaemia are largely based on one's ability to self-treat when exposed to low blood glucose.[145] Hypoglycaemia is classified as mild if self-treatment is possible, irrespective of the nature or intensity of the symptoms experienced; it is classified as severe if the hypoglycaemia episode requires external assistance.[145]

Hypoglycemia is also defined by the international consensus on use of CGM guidelines as follows: (1) Percentage of CGM values that are below 54 mg/dL (3.0 mmol/L) or the number of minutes or hours below this threshold, (2) Number hypoglycaemic events that occur over the given CGM reporting period.[146]

CGM readings below the 3.0 mmol/L (54 mg/dL) threshold for at least 15 min is considered a clinically significant (level 2) hypoglycaemic event. A CGM hypoglycaemia event is considered to end when readings are ≥70 mg/dL (3.9 mmol/L) for 15 min at. Prolonged hypoglycemia is defined as CGM levels <54 mg/dL (3.0 mmol/L) for consecutive 120 min or more. It is recommended by the consensus that for clinical study CGM outcomes studies or reports, hypoglycemia values <54 mg/dL (3.0 mmol/L) are given more weight or importance than those <70–54 mg/dL (3.9–3.0 mmol/L).[146]

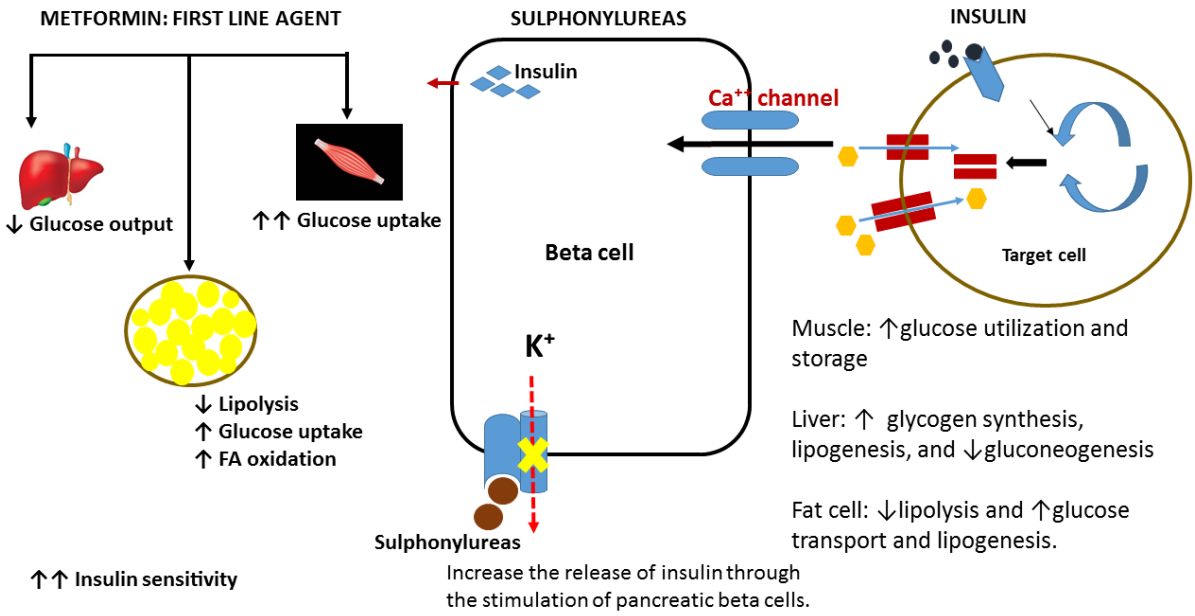

Figure 3: Mechanisms of action of the readily available type 2 diabetes drugs in low-resource settings

Usually, hypoglycemia is rare among non-diabetic individuals; physiologically, as glucose levels fall below normal, endogenous insulin secretion is inhibited, and other counter-regulatory physiological mechanisms are turned on.[144] These include increased secretion of glucagon (a powerful counter-regulatory hormone)by the alpha cells stimulating the release of glucose from the liver through glycogenolysis.[144] In DM management, drugs that increase insulin concentrations in circulation irrespective of

the ambient glucose (e.g., exogenous insulin, SUs) inevitably carry the risk of intermittent hypoglycaemia.[147] Individuals with insulin-treated diabetes or those taking insulin secretagogues (sulfonylureas) are at increased hypoglycaemic risk because the circulating insulin levels from these drugs are not dependent on glucose levels. Hypoglycaemia therefore is a critical consequence of diabetes treatment and when severe leads to falls, dysrhythmias, confusion, and neuroglycopenia, presenting a significant burden for patients and health workers.[148]

Observational and trial data from high-income countries suggest that severe hypoglycaemia is rare in patients taking sulphonylureas. However, in those with well-controlled diabetes, non-severe hypoglycaemia may be expected.[149 150] According to a 2015 systematic review and meta-analysis of population-based studies in high-income countries, the prevalence and incidence of mild/moderate hypoglycaemia in SU-treated patients were 30% and 2 events per person-year, respectively. Severe episodes were rare in the SU group (prevalence of 5% and incidence 0.01 events per person-year).[151]

For patients on insulin, the prevalence and incidence of mild/moderate hypoglycaemia episodes were 50% and 23 events per person-year, respectively. Severe episodes were also relatively common among insulin-treated patients, with a prevalence and incidence of 21% and 1 event per person-year, respectively.[151] The major insulin regimes used were insulin analogues including intermediate-acting insulin and combinations of short-acting or rapid acting insulin analogues.

Sulfonylureas have been in the market for more than six decades now and therefore their safety profile is well-established.[152] They work by stimulating endogenous insulin secretion from the pancreas and as such they are associated with an increased risk

of hypoglycaemia. The risk of hypoglycaemia differs between the different generations of sulphonylureas; newer agents have a lower hypoglycaemic risk compared to the older generational drugs.[153]

Table 3: Pharmacokinetics of sulphonylureas

PK/PD Properties	Glibenclamide	Gliclazide	Glip	Glimepiride	GlipXL	Glic MR
Generation	2nd	2nd	2nd	3rd	3rd	3rd
Year of dev't	1984	1984	1984	1995	1995	1995
Duration	16-24	10 - 24	12 - 24	24	> 24	> 24
V_d (L)	9 -10	13 - 24	10 - 11	19.8 - 37.1	10	19
Bioavailability	99	80	100	100	100	97
Metabolism	Hepatic (active metabolites)			Hepatic (no active metabolites)		
$T_{½}$	10	8 - 12	2 - 5	5	2 - 5	16
Time to peak	2 - 4	2 - 4	1 - 3	2 - 3	6 - 12	6 - 7
Excretion (%)	50 renal	80 renal	80 renal	60 renal	80 renal 10 faeces	< 60-70 renal 10 – 20 faeces

Legend: PK- Pharmacokinetics; PD-Pharmacodynamics; Glip- glipizide; GlipXL- glipizide extended release; Glic MR- Gliclazide modified release; $T_{1/2}$ – Half-life; V_d – volume of distribution

3.3 Hypoglycaemia in SSA

Risk of treatment related hypoglycaemia in sub-Saharan Africa

The high rates of complications and premature mortality due to suboptimal glycaemic control in these countries underscore the need for optimization of glycaemic control using guidelines based on locally generated data and context. It is well established that intensive glucose control to near normal levels prevents and delays onset and progression of complications.[154] [155] A key barrier to intensifying glucose lowering therapy in low resource healthcare settings is fear of hypoglycaemia.[156] [157] As intensive glucose monitoring is not possible with newer glucose-lowering agents which

are very costly, sulphonylureas (SUs) and insulin- two of the cheapest and most widely available glucose lowering therapies are the ones likely to be used to intensify treatment in these settings. However, they are started and maintained at glycaemic thresholds far higher than recommended than recommended in international guidelines because of the fear of hypoglycaemia.[158 159]

Previous studies investigating the burden of hypoglycaemia among type 2 diabetes patients in low-resource settings are limited and the available data is predominantly from developed countries. Real-world data from developed countries suggest that severe hypoglycaemia is rare in patients taking sulphonylureas. Real-world hypoglycaemia studies are lacking in SSA and the available data are from small studies that are limited by their cross sectional and retrospective nature. A systematic and meta-analysis of 46 population studies published in 2015 showed that hypoglycaemia was highly prevalent in type 2 diabetes patients on insulin therapy (50% for mild-moderate episodes and 30% for severe episodes).[151] However, this meta-analysis did not include any study from sub-Saharan Africa (SSA) and these trials achieved glucose levels far lower than seen in clinical care.

It is not clear whether this fear of hypoglycaemia among type 2 diabetes patients in SSA who are treated with insulin and sulphonylureas is justified. Data from developed countries may not apply to resource poor settings where use of older generation SUs (e.g., glibenclamide) with higher hypoglycaemia risk compared to newer generation SUs (e.g., gliclazide and glimepiride) and food insecurity (and therefore missed meals) are common. Therefore, it is important to assess the burden of hypoglycaemia in these settings so as to address therapeutic inertia among clinicians that may potentially

hamper the efforts geared towards treatment intensification to optimise glycaemic control using readily available drugs in low resource settings.

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Chapter 2

HbA1c performs well in monitoring glucose control even in populations with medical conditions that may alter its reliability.

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Published in BMJ Open Diabetes Res Care. 2021 Sep; 9(1):e002350.

Acknowledgements of co-authors and contributions to the paper

Myself, Beverley Shields, Moffat Nyirenda and Angus Jones conceptualised and designed the study. I set up the study in Uganda and obtained ethical approval (with assistance of supervisors), and led the study in Uganda researching the data including undertaking all aspects of recruitment and data collection assisted by our research nurse team. I analysed the data with assistance from Lauren Rodgers, Beverley Shields and Angus Jones. I drafted the paper which was critically reviewed and edited by all authors.

ABSTRACT

Introduction

The utility of HbA1c to estimate glycaemic control in populations of African and other low resource countries has been questioned because of high prevalence of other medical conditions that may affect its reliability. Using continuous glucose monitoring (CGM), we aimed to determine the comparative performance of HbA1c, fasting plasma glucose (FPG) (within 5 hours of a meal) and random non-fasting glucose (RPG) in assessing glycaemic burden.

Research Design and Methods

We assessed the performance of HbA1c, FPG and RPG in comparison to CGM mean glucose in 192 Ugandan participants with type 2 diabetes. Analysis was undertaken in all participants, and in subgroups with and without medical conditions reported to affect Hba1c reliability. We then assessed the performance of FPG and RPG, and optimal thresholds, in comparison to HbA1c in participants without medical conditions thought to alter HbA1c reliability.

Results

32.8% (63/192) of participants had medical conditions that may affect HbA1c reliability: anaemia 9.4% (18/192), sickle cell trait and/or HbC 22.4% (43/192), or renal impairment 6.3% (12/192). Despite high prevalence of medical conditions thought to affect HbA1c reliability, HbA1c had the strongest correlation with CGM measured glucose in day to day living (0.88, 95% CI 0.84, 0.91), followed by FPG (0.82, 95% CI 0.76, 0.86), and RPG (0.76, 95% CI 0.69, 0.81). Among participants without conditions thought to affect HbA1c reliability, FPG and RPG had a similar diagnostic performance in identifying poor glycaemic control defined by a range of HbA1c thresholds. FPG of

≥ 7.1 mmol/L and RPG of ≥ 10.5 mmol/L correctly identified 78.2% and 78.8%, respectively, of patients with an HbA1c of ≥ 7.0%.

Conclusions

HbA1c is the optimal test for monitoring glucose control even in low and middle-income countries where medical conditions that may alter its reliability are prevalent; FPG and RPG are valuable alternatives where HbA1c is not available.

INTRODUCTION

Diabetes is a global problem disproportionately affecting low and middle-income countries (LMICs) with 80% of the global 463 million people with diabetes living in LMICs.[160] Unlike high income countries, diabetes healthcare in LMICs is underfunded,[160] and lacks quality, pragmatic and contextualised guidelines.[161] As such, LMICs are heavily impacted by high rates of poorly controlled glucose levels, [162-164] and subsequently high rates of diabetes-related complications and poor quality of life among people living with diabetes.

Monitoring glycaemic control is essential to allow appropriate titration of medication and improve outcomes among diabetes patients, but regular monitoring can be challenging in LMICs. In high income countries, HbA1c is the recommended measure used for assessing glucose control and titrating medications, often supported by home glucose capillary or interstitial glucose monitoring.[114 115] However, financial constraints mean that the monitoring of diabetes, and decisions to intensify treatment in much of the low-income regions, are predominantly based on testing of a single glucose measure.[9] This is because HbA1c testing is not routinely available in most centres,[9] and HbA1c is often too expensive for the majority of patients.[165] Even where testing is available, there has been substantial concern that HbA1c measurement may be

unreliable in LMIC populations,[166-168] due to high prevalence of haemoglobinopathies such as sickle cell and thalassaemia, and other medical conditions that might affect test reliability including anaemia and malaria.[169] Home glucose monitoring is not well funded by healthcare systems in LMICs, and is beyond the financial means and literacy skills of a large proportion of those who have diabetes.[9 89]

International organisations recommend the use of plasma glucose for monitoring glycaemic control in developing countries where HbA1c services are not readily available.[170] However, assessment of glycaemic control in such settings is normally after long walks by the patients to attend a centralised clinic every 2 -3 months, coupled with prolonged fasting, and long waiting times.[74] As such, many clinicians rely on a random glucose without the requirement to fast to assess glycaemia.[74] While these tests have been compared to HbA1c in the LMICs setting, [66 69] given the limitations of HbA1c itself in these populations, its performance as a measure of average glucose is unclear. Continuous glucose monitoring (CGM) offers the opportunity of measuring glucose in day to day living over a period of days to weeks and is widely used in high income countries and some LMICs.

In the OPTIMAL study, we aimed to compare, in an African population with type 2 diabetes, the accuracy of fasting plasma glucose (FPG), random non-fasting plasma glucose (RPG), and HbA1c in comparison to CGM as an independent measure of glycaemic control, and assess the impact of other medical conditions that may affect HbA1c reliability to monitor glycaemia in people with established diabetes.

METHODS

Study population

Participants were recruited from diabetes clinics in Masaka regional referral hospital (rural, public) and St. Francis hospital Nsambya (urban, private not-for profit) in Uganda and met the following inclusion criteria: a clinical diagnosis of type 2 diabetes, diagnosed at the age of 18 years and above, more than 12 months' diabetes duration, no initial insulin requirement for at least 1 year since the time of diagnosis, no change in glucose lowering therapy 3 months prior, and able to give informed consent. Participants who were pregnant or judged by their clinician to need an immediate change in glucose lowering medication were excluded from recruitment.

Study visits

Participants were scheduled for three visits. The overview of the study design is presented in supplementary Figure S1.

At the baseline visit, participants came to the clinic in a non-fasted state. Following assessment of clinical features and demographics non-fasting (within 5 hours of a meal) random blood sample was collected for measurement of RPG, HbA1c, full blood count, lipid profile, renal function and assessment of haemoglobin variants. Continuous glucose monitoring was carried out using the Freestyle Libre Pro Flash Glucose Monitoring System (Abbott Laboratories, Illinois, USA), a professional continuous glucose monitoring (CGM) device which records interstitial glucose every 15 minutes for up to two weeks. Freestyle Libre Pro is blinded, meaning data could not be viewed by the wearer.

All participants returned in a fasted state (at least 8 hours) in the second week of CGM between days 7 and 10 from the baseline visit, and for their final visit, between days

12 and 14 from the baseline visit, in a non-fasted state (within 5 hours of a meal). At both of these visits, CGM data were downloaded and a venous blood sample was collected for measurement of HbA1c and RPG (visit 1 and 3) and FPG (visit 2). The study was carried out in accordance with 2008 revised principles of the Declaration of Helsinki and all participants provided informed consent before study activities.

Patient and public involvement (PPI)

Patients were not involved in the setting of the research question or outcome measures. In addition, patients were not involved in the design and conduct of the study. However, they were central to dissemination of the results by choosing to have some of the results sent to their respective clinicians, and other information to be shared in PPI meetings.

Laboratory procedures

Blood samples for glucose measurement were collected in a vacutainer with sodium fluoride (NaF), centrifuged and separated into two cryovials (aliquots) immediately and kept in an icebox at 4-8^{O}C before being transported to the central laboratory for immediate testing (within 8 hours of collection). Whole blood samples for full blood count and HbA1c were collected in vacutainers containing EDTA. All analytical measurements were performed at the central Biochemistry and clinical diagnostic laboratory services (CDLS) laboratory at the MRC/UVRI & LSHTM Research Unit Entebbe Uganda. Laboratory analyses were performed on a Roche cobas 6000 analyser, (Hitachi high technologies corporation, Tokyo, Japan). Plasma glucose was measured by the glucokinase method. HbA1c was also measured on Cobas 6000 by the immunoassay technique; calibrated to the International Federation of Clinical

Chemistry (IFCC). Haemoglobinopathies (sickle cell trait and HbC) were assessed by Hb electrophoresis.

CGM Measures

Raw glucose readings were downloaded from the Libreview software and CGM summary variables (including mean CGM glucose) were calculated using R v3.6.1. Sensor data was considered for analysis if the total duration of CGM wear was at least 5 days.

For CGM validation we matched plasma FPG at visit 2 with a nearest CGM glucose value within 15 minutes. We then determined the relationship between the plasma glucose and the CGM glucose value using Bland Altman analysis to assess the degree of bias and levels of agreement between the sensor and plasma glucose.

Statistical analysis

Data were analysed using Stata V16.1 (StataCorp LLC, USA).

Comparison of glucose and HbA1c measures with CGM measured glucose in daily living

We assessed the strength of the relationship between CGM assessed mean glucose over two weeks and each of FPG, RPG and HbA1c using Pearson's correlation coefficients and linear regression. Analysis was based on RPG and HbA1c tests performed on the last visit (visit 3), unless not available, in which case values from visit one were used instead (n = 9). To assess the impact of other medical conditions (anaemia, haemoglobinopathies, and renal impairment) on HbA1c reliability, we subdivided the cohort into those without medical conditions that may alter HbA1c reliability and those with medical conditions that may alter HbA1c reliability. HbA1c performance in comparison to CGM was assessed in all participants regardless of

comorbidities, and by presence or absence of medical conditions thought to affect test performance (see below). Equivalent thresholds for predicting sub-optimal glycaemic control (defined as CGM glucose values ≥ 8mmol/L and ≥ 10mmol/L) were derived from linear regression equations. We compared the performance of RPG and FPG and HbA1c to identify participants with CGM glucose values ≥ 8mmol/L and ≥ 10mmol/L using Receiver Operating Characteristic (ROC) curve analysis, and assessed the sensitivity, specificity and positive/negative predictive values of these tests using the equivalent cut offs derived from linear regression equations.

Comparison of FPG and RPG measurement with HbA1c

As HbA1c is the measure which has been robustly validated against clinical outcomes we performed additional analysis, where we assessed the strength of the relationship between HbA1c and each of the FPG and RPG tests in the absence of medical conditions that might affect HbA1c reliability. Participants were considered to have no other medical conditions that may affect HbA1c reliability if they met the following characteristics: no haemoglobinopathies (sickle cell trait and haemoglobin C), absence of anaemia (Hb in women ≥ 120g/L, men ≥ 130 g/L),[171] and no renal impairment (EGFR ≥ 60 ml/min/1.73m2). In participants without these medical conditions, we determined diagnostic performance of the glucose tests for suboptimal glucose control defined by HbA1c at the following thresholds: HbA1c ≥ 48 mmol/mol (6.5%), ≥ 53 mmol/mol (7.0 %), 58 mmol/mol (7.5%), 64 mmol/mol (8.0%), 69 mmol/mol (8.5%) and 75 mmol/mol (9.0%). Equivalent thresholds of FPG and random glucose for predicting sub-optimal glycaemic control were obtained by linear regression analysis.

RESULTS

Baseline characteristics

A total of 213 adults were enrolled in the study. 9.86% (21/213) participants were excluded for insufficient data of which 61.9% (13/21) were female with a median BMI of 28.5, IQR (27.6, 33.8) (supplementary table 1). 192 of 213 participants had sufficient data for inclusion in the final analysis (See flow chart: Supplementary Figure S2). The median CGM duration was 14 (IQR: 13 – 14) days. Participant characteristics are presented in Table 1. Average glycaemic control was poor with a median (IQR) HbA1c of 67 (52.0, 90.0) mmol/mol [8.3% (6.9, 10)]. The other medical conditions that may affect HbA1c reliability were common, occurring in 32.8% (63/192) of participants, of whom 9.4% (18/192) had anaemia, 22.4% (43/192) had haemoglobinopathies (sickle cell trait (n = 43) and/or HbAC (n = 1)), and 6.3% (11/190) had renal impairment (EGFR <60 ml/min/1.73m2). Characteristics according to absence or presence of medical conditions that may affect HbA1c reliability are shown in supplementary Table 2.

Table 1: Participant characteristics (n = 192)

	Median (IQR) for continuous variables, % (n) for proportions
Clinical	
Female, n (%)	58.3 (112/192)
Age, years	56 (50, 63)
Duration of diabetes, years	6 (3, 10)
BMI, kg/m^2	26.8 (24.0, 30.5)
Current management n (%)	
Metformin only	15.6 (30/192)
SU (+/- metformin)[a]	57.3 (110/192)
Insulin (+/- other diabetes drug)[b]	26.0 (50/192)

Diet [c]	1.0 (2/192)
Glycaemia	
CGM glucose, mmol/L	8.6 (6.8, 12.3)
HbA1c, %	8.3 (6.9, 10.0)
HbA1c, mmol/mol	67 (52.0, 90.0)
FPG, mmol/L	8.2 (6.1, 11.4)
RPG, mmol/L	13.5 (8.8, 17.2)
Other laboratory	
Hb (g/L)	14.2 (13.2, 15.0)
Anaemia	9.4% (18/192)
Haemoglobinopathies, n (%)	22.4% (43/192)
eGFR	111.5 (92.3, 121.0)
Renal impairment, n (%)	6.3% (12/192)

Legend: a: sulphonylureas with or without metformin, **b:** insulin with or without any other oral therapy, **c:** Two participants were on non-pharmacological management (diet) only. Haemoglobinopathies was defined as the presence of sickle cell trait (HbAS) or HbAC. Anaemia was defined as an Hb of < 120g/L in women and < 130 g/L in men. FPG; Fasting plasma glucose, RPG; random non fasting plasma glucose, eGFR; estimated glomerular filtration rate

FPG and CGM glucose are highly correlated

FPG and CGM glucose (closest value, within 15 minutes) were highly correlated (Pearson's r= 0.97 95% CI: 0.96 – 0.98). CGM values showed a modest bias towards lower glucose than FPG, with CGM values mean 1.3 (95% CI: 1.1 -1.5) mmol/L lower – this was consistent across the range of glycaemic control (Figure S3).

HbA1c has the strongest relationship with CGM glucose in an African population, even in participants with comorbidities thought to alter HbA1c reliability

The relationship between HbA1c, FPG and RPG tests and average CGM glucose is shown in Figure 1. There was a strong correlation between all the three tests and mean CGM glucose. HbA1c had the strongest correlation, (0.88; 95% CI 0.84, 0.91), followed by FPG (0.82; 95% CI 0.76, 0.86), and RPG (0.76; 95% CI 0.69, 0.81).

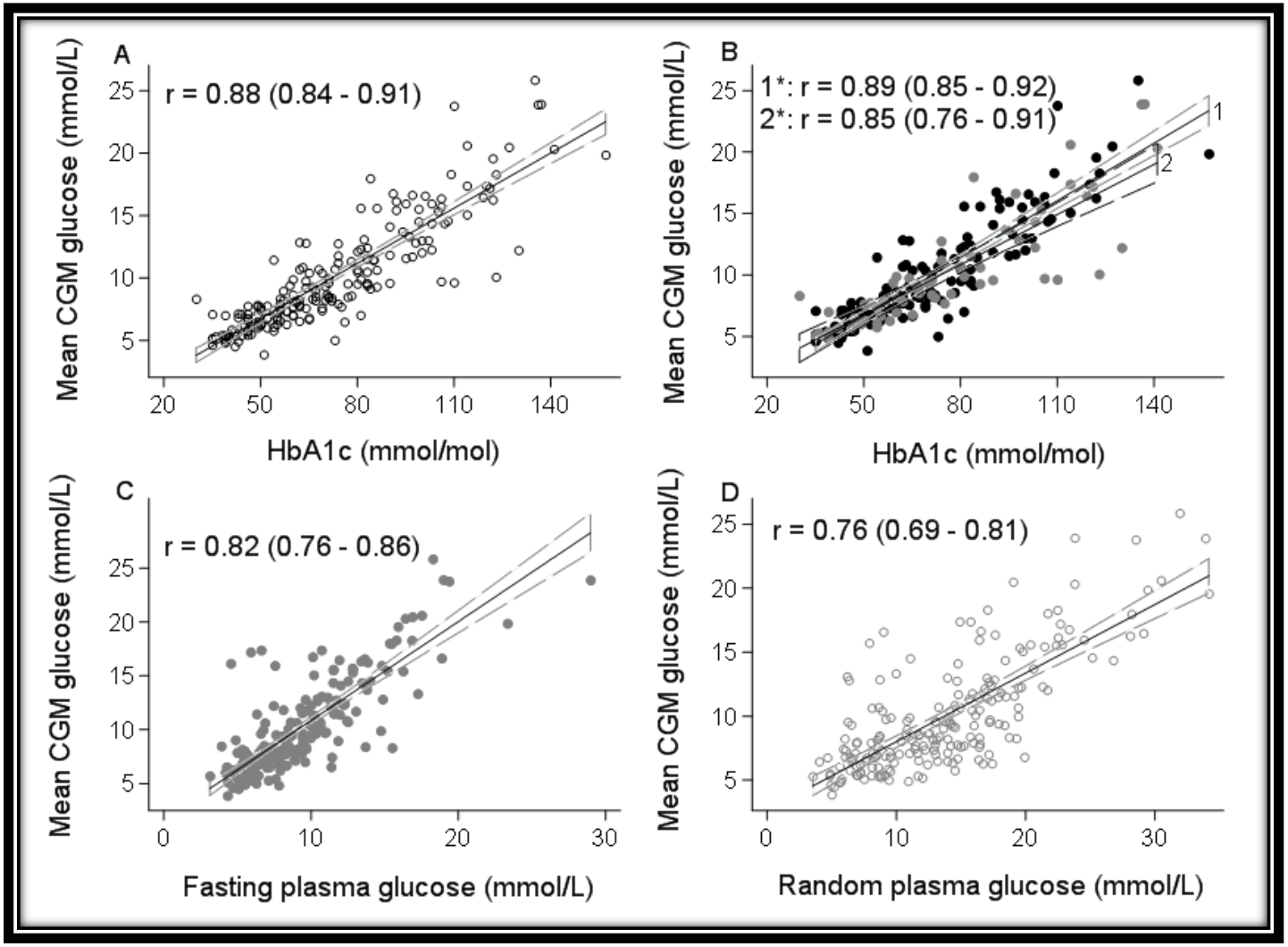
A
r = 0.88 (0.84 - 0.91)
Mean CGM glucose (mmol/L)
HbA1c (mmol/mol)
B
1*: r = 0.89 (0.85 - 0.92)
2*: r = 0.85 (0.76 - 0.91)
1
2
Mean CGM glucose (mmol/L)
HbA1c (mmol/mol)
C
r = 0.82 (0.76 - 0.86)
Mean CGM glucose (mmol/L)
Fasting plasma glucose (mmol/L)
D
r = 0.76 (0.69 - 0.81)
Mean CGM glucose (mmol/L)
Random plasma glucose (mmol/L)

Figure 1: Comparison of (A) HbA1c of the overall sample population and, (B) HbA1c without (1; black circles) and with (2; grey circles) conditions thought to alter HbA1c reliability with mean CGM glucose. Comparison of (C) FPG and (D) RPG with mean CGM glucose. Solid straight line denotes the line of best fit and the dashed lines represent the 95% confidence interval. The Pearson's correlation coefficient (r) and 95% confidence intervals are shown for each graph. Conditions thought to alter HbA1c reliability include haemoglobinopathies including sickle cell trait and HbAC, anaemia, and renal impairment.

The derived linear equations for estimating mean glucose from HbA1c, FPG and RPG among diabetes patients are shown in supplementary table 3. The diagnostic performances of HbA1c, FPG and RPG tests for diagnosing suboptimal glucose control (defined by illustrative mean CGM thresholds of 8 and 10 mmol/L) are shown in Table 2. There was a very modest loss of diagnostic performance using FPG compared to HbA1c, at equivalent thresholds. HbA1c was the most sensitive and specific test followed by FPG.

Table 2: Ability of HbA1c, FPG and RPG to define sub-optimal glucose control using CGM thresholds <8 mmol/L and <10 mmol/L

CGM Cut-off	Test	n	AUROC (95% CI)	Optimal threshold	Sensitivity (95% CI)	Specificity (95% CI)	Correctly classified	PPV (95% CI)	NPV (95% CI)
≥ 8.0	HbA1c	191	0.95 (0.92 – 0.98)	≥ 61.7	90.2 (83.1- 95.0)	83.5 (73.5 – 90.9)	87.4	88.6 (81.3 – 93.8)	85.7 (75.9- 92.6)
	FPG	191	0.90 (0.86- 0.95)	≥ 7.6	84.8 (76.8 – 90.9)	81.0 (70.6 – 89.0)	83.3	86.4 (78.5 – 92.2)	79.0 (68.5 -87.3)
	RPG	192	0.82 (0.77 – 0.88)	≥ 11.6	78.6 (69.8 – 85.8)	64.6 (53.0 – 75.0)	72.8	75.9 (67.0 – 83.3)	68.0 (56.2 -78.3)
≥ 10.0	HbA1c	191	0.94 (0.90 – 0.97)	≥ 72.1	88.9 (79.3 – 95.1)	84.9 (77.2 – 90.8)	86.4	78.0 (67.5 – 86.4)	92.7 (86.0- 96.8)
	FPG	191	0.90 (0.85 – 0.95)	≥ 9.1	83.6 (73.0 – 91.2)	83.1 (75.0 – 89.3)	83.3	75.3 (64.5 – 84.2)	89.1 (81.7- 94.2)
	RPG	192	0.85 (0.79 – 0.91)	≥ 13.8	84.7 (74.3 – 92.1)	72.3 (63.3 – 80.1)	77.0	64.9 (54.4 – 74.5)	88.7 (80.6- 94.2)

Table 2 legend: AUROC: area under ROC curve, PPV: positive predictive value, NPV: negative predictive value. FPG; fasting plasma glucose, RPG; random non fasting plasma glucose. The units used are; HbA1c- mmol/mol and mmol/L for fasting and random non-fasting glucose.

HbA1c maintained the strongest relationship with CGM glucose even in (those with other medical conditions that might affect HbA1c reliability) (Figure 1). In those with and without conditions that might affect HbA1c reliability, the relationship between CGM glucose and HbA1c was similar, with no difference in correlation (0.85; 95%CI 0.76, 0.91) versus (0.89; 95% CI 0.85, 0.92) (Figure 1) and the difference in linear regression slopes was modest (mean CGM glucose =0.14*HbA1c – 0.02 and 0.16*HbA1c – 1.07 with and without conditions that may affect HbA1c reliability respectively) (supplementary Table 3). This was also similar when examining only those with haemoglobinopathy (r=0.90, 95% CI: 0.82 – 0.94, n=42, supplementary figure S4).

FPG and RPG have broadly similar diagnostic performance in identifying patients with poor glycaemia control

Among participants without conditions thought to alter HbA1c reliability (including haemoglobinopathies, anaemia and renal impairment), RPG and FPG had similar correlation with HbA1c (0.74; 95% CI 0.65, 0.80) and (0.78; 95% CI 0.71, 0.84) respectively (Figure 2).

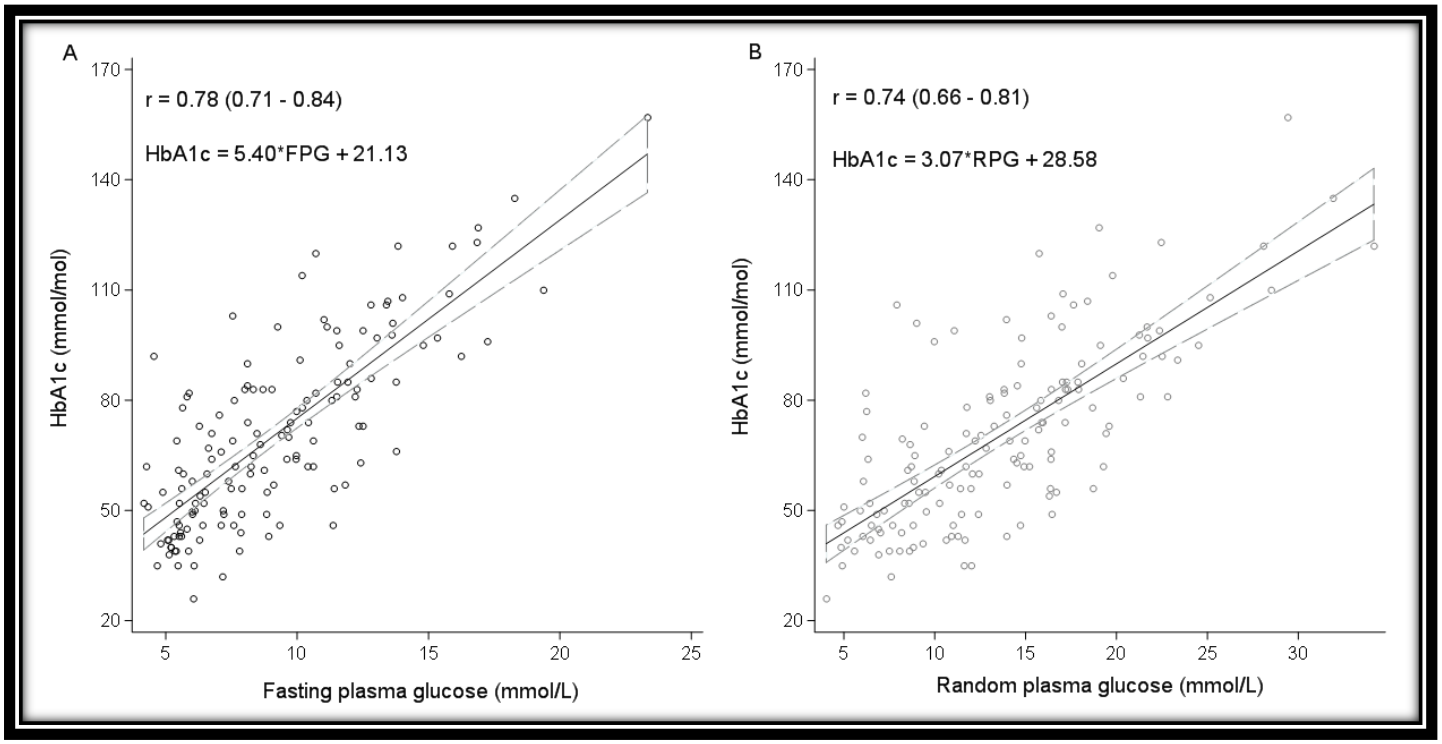

Figure 2: (A) and (B) Comparison of FPG and RPG with HbA1c in type diabetes patients without conditions thought to alter HbA1c reliability. Solid straight line denotes the line of best fit and the dashed lines represent the 95% confidence interval. The Pearson's correlation coefficient (r) and 95% confidence intervals are shown for each graph. Conditions thought to alter HbA1c reliability include haemoglobinopathies including sickle cell trait and HbAC, anaemia, and renal impairment.

The equivalent thresholds and diagnostic performances of FPG and RPG for predicting HbA1c defined sub-optimal glucose control (at different HbA1c thresholds), restricted to those without conditions thought to alter HbA1c reliability, are shown in Table 3. FPG and RPG had very similar performance in identifying those with suboptimal glycaemic control (Table 3). For the widely used HbA1c target of 7.0%, the AUC ROC for these tests was similar (FPG 0.76, RPG 0.77). At their respective optimal thresholds (FPG ≥ 7.1 mmol/L and RPG ≥ 10.5 mmol/L), the tests had a similar sensitivity (FPG - 81.0, 95% CI: 71.9 – 88.2 vs RPG – 81.6, 95% CI: 72.7 – 88.5) and specificity (FPG - 71.4, 95% CI: 55.4 – 84.3 vs RPG - 72.1, 95% CI: 56.3 – 84.7) for identifying sub-optimal glycaemic control. The linear equations for estimating HbA1c from FPG and RPG among diabetes patients were; HbA1c (mmol/mol) = 5.40*FPG + 21.3 and HbA1c = 3.07* RPG + 28.58 respectively for patients without comorbidities thought to alter HbA1c (supplementary table 4).

Table 3: Ability of FPG and RPG to predict sub-optimal glucose control among type 2 diabetes patients without medical conditions thought to alter HbA1c reliability using different HbA1c thresholds

HbA1c Cut-off	Test	n	AUROC (95% CI)	Equivalent threshold (mmol/L)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Correctly classified (%)
48 (6.5%)	FPG	142	0.84 (0.77 – 0.92)	6.6	79.5 (70.8 – 86.5)	73.3 (54.1 – 87.7)	91.8 (84.4 – 96.4)	48.9 (33.7 – 64.2)	78.2
	RPG	145	0.86 (0.80 – 0.92)	9.6	79.1 (70.6 – 86.1)	71.0 (52.0 – 85.8)	91.0 (83.6 – 95.8)	47.8 (32.9 – 63.1)	77.4
53 (7.0%)	FPG	142	0.87 (0.81 – 0.93)	7.1	81.0 (71.9 – 88.2)	71.4 (55.4 – 84.3)	87.1 (78.5 – 93.2)	61.2 (46.2 – 74.8)	78.2
	RPG	145	0.88 (0.83 – 0.94)	10.5	81.6 (72.7 – 88.5)	72.1 (56.3 – 84.7)	87.5 (79.2 – 93.4)	62.0 (47.2 – 75.3)	78.8
58 (7.5%)	FPG	142	0.85 (0.79 – 0.91)	7.7	76.7 (66.6 – 84.9)	76.9 (63.2 – 87.5)	85.2 (75.6 – 92.1)	65.6 (52.3 – 77.3)	76.8
	RPG	145	0.84 (0.77 – 0.90)	11.4	78.5 (68.8 – 86.3)	71.7 (57.7 – 83.2)	83.0 (73.4 – 90.1)	65.5 (51.9 – 77.5)	76.0
64	FPG	142	0.86	8.4	74.0	81.5	82.6	72.6	77.5

(8.0%)			(0.80 – 0.92)		(62.8 – 83.4)	(70.0 – 90.1)	(71.6 – 90.7)	(60.9 – 82.4)	
	RPG	145	0.84 (0.78 – 0.90)	12.4	78.8 (68.2 – 87.1)	80.3 (68.7 – 89.1)	82.9 (72.5 – 90.6)	75.7 (64.0 – 85.2)	79.5
69 (8.5%)	FPG	142	0.85 (0.79 – 0.91)	9.0	73.5 (61.4 – 83.5)	83.8 (73.4 – 91.3)	80.6 (68.6 – 89.6)	77.5 (66.8 – 86.1)	78.9
	RPG	145	0.85 (0.79 – 0.91)	13.3	76.1 (64.5 – 85.4)	78.7 (67.7 – 87.3)	77.1 (65.6 – 86.3)	77.6 (66.6 – 86.4)	77.4
75 (9.0%)	FPG	142	0.85 (0.78 – 0.92)	9.6	74.1 (60.3 – 85.0)	80.7 (70.9 – 88.3)	70.2 (56.6 – 81.6)	83.5 (73.9 – 90.7)	78.2
	RPG	145	0.85 (0.78 – 0.92)	14.4	75.0 (61.6 – 85.6)	77.8 (67.8 – 85.9)	67.7 (54.7 – 79.1)	83.3 (73.6 – 90.6)	76.7

Table 3 legend: AUROC, Sensitivity, specificity, % correctly classified, PPV and NPV are given for the respective optimal thresholds of the test. This was restricted to HbA1c where there were no conditions thought to alter HbA1c reliability like anaemia, sickle cell traits and renal impairment. AUROC: area under ROC curve, PPV: positive predictive value, NPV: negative predictive value. FPG; fasting plasma glucose, RPG; random non fasting plasma glucose. The units used are mmol/L for fasting and random non-fasting glucose.

DISCUSSION

The international guidelines recommend HbA1c for monitoring glycaemic control and blood glucose test where HbA1c is unavailable. Despite this guidance, there remains concerns about the accuracy of HbA1c in populations with high frequency of other medical conditions that may alter its reliability. In this study, we used CGM to compare the accuracy of HbA1c, FPG and RPG tests in assessing glycaemic control among diabetes patients under conditions of everyday life in low-resource settings. The prevalence of other medical conditions that may alter HbA1c reliability was remarkably high. However, we found that HbA1c remained the most accurate test of average glucose control, despite the high prevalence of haemoglobinopathies, anaemia and renal impairment. Similarly, FPG and RPG demonstrated reasonable accuracy as measures of average glycaemic control, providing confidence that glucose tests provide a good measure of glycaemia where HbA1c is not available. Furthermore, the very modest loss of diagnostic test performance using RPG provides some reassurance for use of this test in situations where a RPG is the only or most practical measure available.

In the current study, we have compared FPG, RPG and HbA1c in the same study and more importantly against an independent measure of day-to-day glycaemic burden. CGM was used as an independent marker of glycaemic burden to allow assessment of the relative performance of HbA1c, FPG and RPG in assessing glycaemic burden. This is a major strength of our analysis in contrast to previous studies which have compared between measures such as HbA1c and FPG, with no independent comparison. Further, we assessed performance of HbA1c in presence of other medical conditions that may alter its effect. This gave us the opportunity to assess the overall impact on HbA1c reliability.

However, the present study has some limitations that should be taken into consideration. First, although CGM was the best available option for direct measurement of glucose in day to day living and allowed us to compare the relative performance of HbA1c and glucose tests, it should be noted that glycaemia was measured using a CGM sensor over median 14 (IQR: 13 – 14) days and yet HbA1c estimates glycaemia over a longer duration.[172] Secondly, we used HbA1c immunoassay, one of the most widely used HbA1c assays, particularly in low resource settings. However, our results for the performance might not apply to other HbA1c assay types, which are known to have different susceptibility to the effects of haemoglobinopathies.[173] Furthermore, although we screened for a number of potential comorbidities thought to alter HbA1c, with the available sample size and very modest subgroup numbers, we were unable to do further subgroup analyses to assess the impact of other individual underlying non-glycaemic conditions.[59] In addition, the impact of glucose-6 phosphate dehydrogenase (G6PD) variants, another common condition that may affect HbA1c results reliability was not assessed.[64]

Our results showing a strong relationship between HbA1c and mean glucose from CGM are consistent with studies that have compared these two measures in high income settings. The DCCT study of participants in the USA with Type 1 diabetes showed a strong relationship between the mean plasma glucose and HbA1c with a Pearson correlation (r) of 0.82.[71] Similarly, results from the ADAG study, which included 507 participants with and without diabetes predominantly from the US and Europe, and excluded participants with other medical conditions thought to alter HbA1c reliability, showed HbA1c and mean glucose were closely correlated ($r = 0.89$, $P< 0.0001$).[72] Our similar results ($r = 0.88$) in an African population, and without

exclusion of participants with analytical concerns for HbA1c measurement, is reassuring for the use of HbA1c testing in this region.

Our results are broadly consistent with previous studies that have reported the relationship between glucose tests and HbA1c. El-Kebbi et al. showed, in 1,827 predominantly African American living in the US, that RPG collected 1-4 hour post meal was correlated strongly with HbA1c although in this predominantly insulin treated population the correlation (r 0.63) was lower than observed in our study (0.74).[68] In a study that compared both FPG and RPG to HbA1c among 1,000 patients with diabetes living in India, FPG showed a better correlation with HbA1c than RPG (0.739 vs 0.601).[67] In contrast, in studies where a fixed post meal time point was used, RPG was a slightly better correlate of HbA1c than FPG.[69] Unfortunately, studies comparing performances of glucose tests against HbA1c in Africa are very few, with small sample sizes, and in these studies the impact of common medical conditions that may alter HbA1c reliability was not assessed.[65 66]

Our data suggest that there is a high prevalence of other medical conditions that may alter HbA1c reliability justifying the questioning of HbA1c utility. However, even with these comorbidities, HbA1c, when measured with an immunoassay method, correlated strongly with mean glucose, outperforming glucose measures, and only displayed a modest improvement when patients with comorbidities were excluded. This suggests that HbA1c remains the optimal laboratory method of monitoring glucose burden even where prevalence of conditions that may affect its reliability is high. The strong correlation of HbA1c with glucose despite the prevalence of other medical conditions that may alter HbA1c reliability deserves further exploration. However, there are some reasons why the impact of these conditions on HbA1c reliability may be modest in this setting. First, in line with the National

Glycohaemoglobin Standardisation Program (NGSP) recommendation, modern HbA1c immunoassays are not directly affected by the presence of haemoglobin variants like HbAS.[173] Secondly, while comorbidities that affect red cell life will alter the accuracy of any HbA1c method, the predominant haemoglobinopathy in our study population was HbAS (Sickle cell) trait, and previous research has been conflicting as to whether this meaningfully alters red cell lifespan.[63]

While our results support the use of HbA1c (where available) rather than glucose measures in LMIC populations, the small subgroup numbers in our study limited the power to definitively determine the impact of some of these comorbidities on HbA1c performance. To accurately determine the impact of individual comorbidities, larger multi-national studies involving other regions in Africa and LMICs with enrichment for these comorbidities would be needed. Furthermore, while our data show that HbA1c (measured using an immunoassay method) has the closest relationship with average glucose, even with comorbidities, it is possible that the overall relationship between glucose and HbA1c is different in this population, therefore the thresholds used internationally are not appropriate, and bespoke HbA1c thresholds are needed for different populations. This further underscores the need for much larger studies, ideally incorporating risk of microvascular complications, to determine whether the HbA1c targets used internationally are appropriate for LMIC populations.

In conclusion our results suggest that HbA1c is the optimal test for monitoring glucose control even in low and middle-income countries where medical conditions that may alter its reliability are prevalent; FPG and RPG are valuable alternatives where HbA1c is not available.

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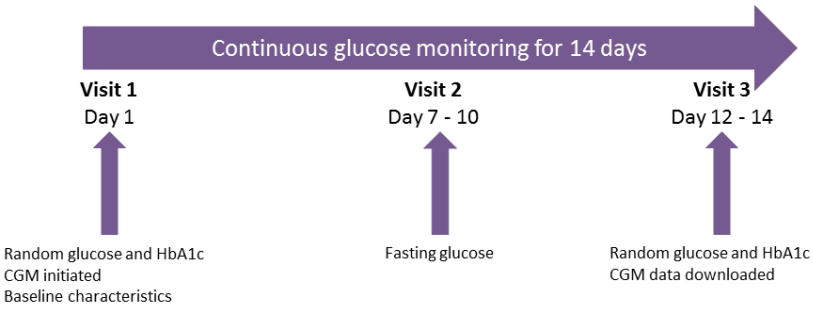

Figure S1: Overview of the study design

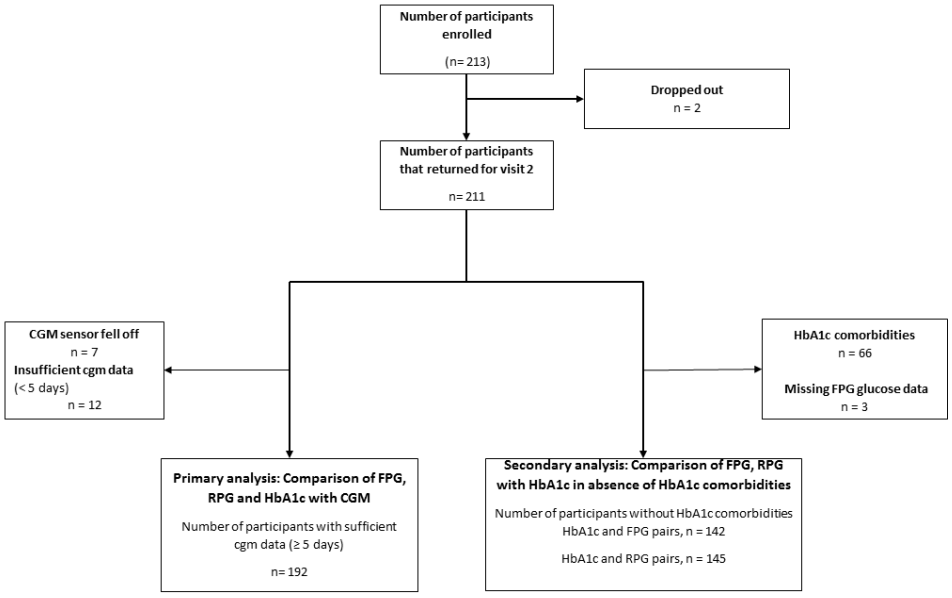

Figure S2: Participant flow chart. Conditions thought to alter HbA1c reliability (HbA1c comorbidities) include haemoglobinopathies (sickle cell trait and HbAC), anaemia, and renal impairment.

Supplementary Table 1: Participant characteristics of those included in the final analysis (n = 192) versus those excluded (n = 21)

	Median (IQR) for continuous variables, n (%) for proportions	
	Included	Not included
Number, n (%)	192 (90.1)	21 (9.9)
Clinical		
Female, n (%)	112 (58.3)	13 (61.9)
Age, years	56 (50, 63)	52 (48, 60)
Duration of diabetes, years	6 (3, 10)	7 (1, 11)
BMI, kg/m2	26.8 (24.0, 30.5)	28.5 (27.6, 33.8)
Current management n (%)		
Metformin only	30 (15.6)	2 (9.5)
SU (+/- metformin)a	110 (57.3)	13 (61.9)
Insulin (+/- other diabetes drug)b	50 (26.0)	6 (28.6)
Diet c	2 (1.0)	0 (0.0)
Glycaemia		
HbA1c, %	8.3 (6.9, 10.0)	7.7 (6.0, 9.1)
HbA1c, mmol/mol	67 (52.0, 90.0)	61.0 (42.5, 76.0)
Fasting plasma glucose, mmol/L	8.2 (6.1, 11.4)	7.0 (5.8, 12.3)
Random plasma glucose, mmol/L	13.5 (8.8, 17.2)	10.8 (7.6, 17.2)
Other laboratory		
Hb (g/L)	14.2 (13.2, 15.0)	14.5 (14.1, 15.5)
eGFR	111.5 (92.3, 121.0)	117.8 (96.7, 124.7)

Supplementary Table 2: Participant characteristics presence (Group 2) or absence (Group 1) of HbA1c comorbidities

	Median (IQR) for continuous variables, % (n) for proportions	
Clinical	Group 1	Group 2
N (%)	67.2 (129/192)	32.8 (63/192)
Female, n (%)	60.5 (78/192)	54.0 (34/192)
Age, years	55 (50, 61)	58 (50, 64)
Duration of diabetes, years	6 (3, 10)	9 (4, 12)
BMI, kg/m2	27. 1 (24.3, 30.3)	25.8 (23.1, 30.6)
Current management n (%)		
Metformin only	18.6 (24/129)	9.5 (6/63)
SU (+/- metformin)a	57.4 (74/129)	57.1 (36/63)
Insulin (+/- other diabetes drug)b	22.5 (29/129)	33.3 (21/63)
Diet c	2 (1.5)	0
Glycaemia		
CGM glucose, mmol/L	8.4 (6.8, 12.3)	9.3 (7.0, 12.3)
HbA1c, %	8.2 (6.7, 9.8)	8.7 (7.1, 10.7)
HbA1c, mmol/mol	66.0 (50.0, 85.0)	70.5 (54.0, 97.0)
Fasting plasma glucose, mmol/L	8.3 (6.1, 11.3)	7.8 (5.9, 11.5)
Random plasma glucose, mmol/L	13.0 (8.8, 16.8)	14.1 (8.7, 18.4)

Group 1 includes all those without HbA1c comorbidities (n = 129) and Group 2 includes all those with HbA1c comorbidities (n = 63). HbA1c comorbidities are the non-glycaemic biological conditions thought to alter HbA1c reliability e.g., haemo-globinopathies including sickle cell, anaemia, and renal impairment.

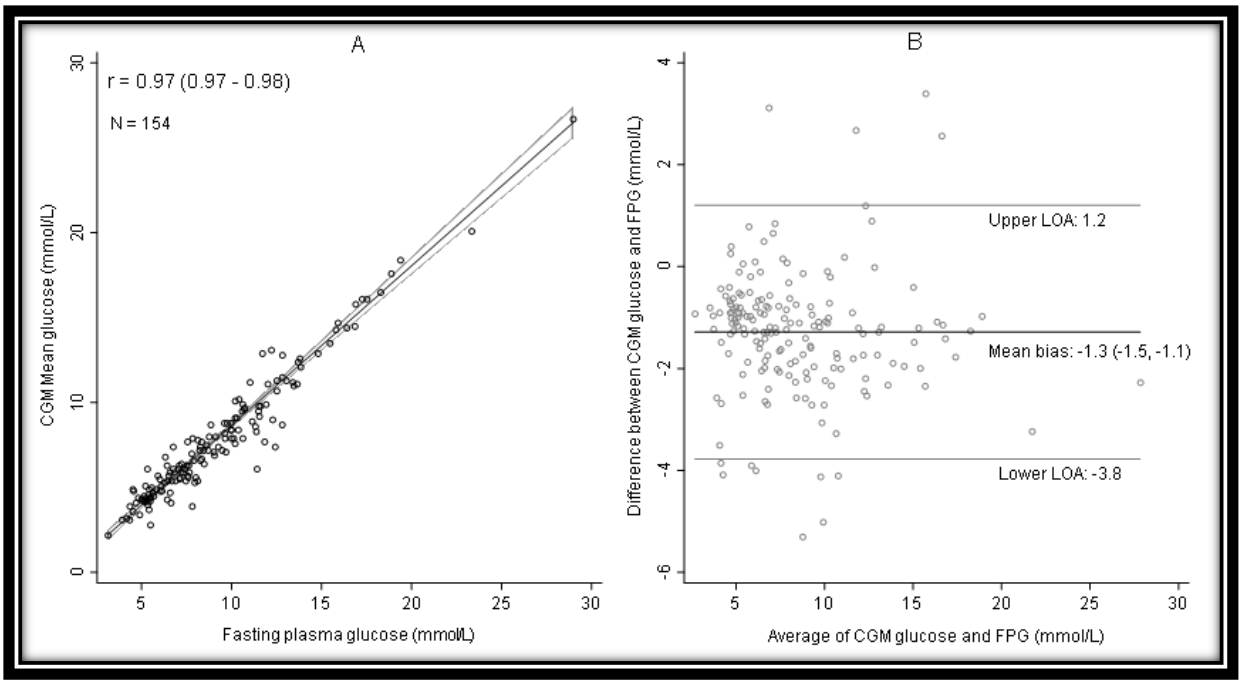

Figure S3: Comparison of CGM glucose and Fasting plasma glucose (A). A Bland Altman Plot of Fasting Plasma Glucose test and CGM sensor glucose. The black solid line denotes the mean bias between the fasting plasma glucose tests and average cgm glucose and the grey solid lines denote upper and lower limits of agreement (LOA). Overall CGM underestimated plasma glucose by 1.3 mmol/L, with LOA from ranging between − 3.8 to 1.2 mmol/L

Supplementary Table 3: Glycaemic measures correlated with mean day-to-day glucose measured by CGM stratified by presence or absence of comorbidities thought to alter HbA1c reliability

	Overall	Group 1	Group 2
Fasting			
N	192	129	63
r (95% CI)	0.82 (0.76 – 0.86)	0.84 (0.78 – 0.89)	0.78 (0.67 – 0.86)
LR equation	Mean CGM = 0.91 (fasting) + 1.77	Mean CGM = 1.02 (fasting) + 0.71	Mean CGM = 0.77(fasting) + 3.13
Random			
N	192	129	63
R (95% CI)	0.76 (0.69 – 0.81)	0.74 (0.65 – 0.81)	0.80 (0.69 – 0.87)
LR equation	Mean CGM = 0.53(random) + 2.66	Mean CGM = 0.53(random) + 2.80	Mean CGM = 0.55(random) + 2.37
HbA1c			
N	192	129	63
R (95% CI)	0.88 (0.84 – 0.91)	0.89 (0.85 – 0.92)	0.85 (0.76 – 0.91)
LR equation	Mean CGM = 0.15(HbA1c) - 0.61	Mean CGM = 0.16(HbA1c) – 1.07	Mean CGM = 0.14(HbA1c) – 0.02

Group 1 includes all those without HbA1c comorbidities (n = 129) and Group 2 includes all those with HbA1c comorbidities (n = 63). Comorbidities are the non-glycaemic biological conditions thought to alter HbA1c reliability e.g., haemo-globinopathies including sickle cell, anaemia, and renal impairment.

Supplementary Table 4: Short-term glycaemic measures correlated with HbA1c stratified by presence or absence of comorbidities thought to alter HbA1c reliability

	Overall	Group 1	Group 2
Fasting			
N	208	142	66
r (95% CI)	0.70 (0.62 – 0.76)	0.78 (0.71 – 0.84)	0.57 (0.38 – 0.71)
LR equation	HbA1c = 4.62(fasting) + 29.55	HbA1c = 5.40(fasting) + 21.13	HbA1c = 3.57(fasting) + 41.92
Random			
N	211	145	66
r	0.74 (0.68 – 0.80)	0.74 (0.66 – 0.81)	0.74 (0.61 – 0.83)
LR equation	HbA1c = 3.09(random) + 29.01	HbA1c = 3.07 (random) + 28.58	HbA1c = 3.12(random) + 30.39

Group 1 includes all those without HbA1c comorbidities (n = 129) and Group 2 includes all those with HbA1c comorbidities (n = 63). Comorbidities are the non-glycaemic biological conditions thought to alter HbA1c reliability e.g., haemo-globinopathies including sickle cell, anaemia, and renal impairment.

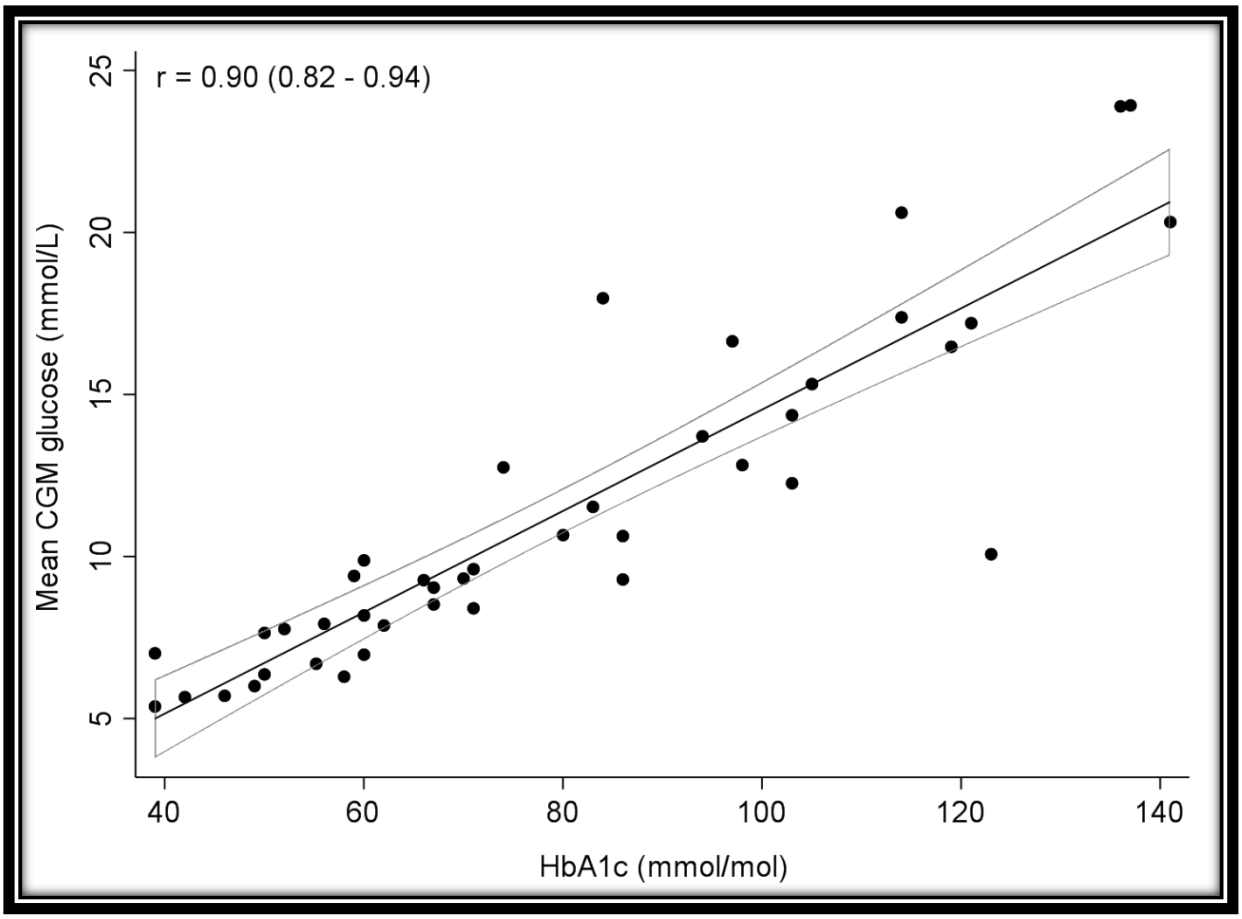

Figure S4: Comparison of HbA1c to mean CGM glucose among those with haemoglobinopathies. Dark thick straight line denotes the line of best fit and the thin lines represent the 95% confidence interval. The Pearson's correlation coefficient (r) and 95% confidence intervals are shown on the left upper corner of the graph.

Chapter 3

Examining the role of fructosamine and glycated albumin in the assessment of glycaemic control in individuals living with type 2 diabetes in Uganda.

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Acknowledgements of co-authors and contributions to the paper

Myself, Beverley Shields, Moffat Nyirenda and Angus Jones conceptualised and designed the study. Myself, Beverley Shields, Moffat Nyirenda and Angus Jones conceptualised and designed the study. I set up the study and researched the data including undertaking all aspects of recruitment and data collection assisted by our research nurse team. I analysed the data with assistance from Lauren Rodgers, Beverley Shields and Angus Jones. I drafted the paper which was critically reviewed and edited by all authors. Minor revisions suggested by supervisors were incorporated.

ABSTRACT

Introduction

HbA1c may be unreliable in low income settings and populations of African ancestry, and the use of alternative markers such as fructosamine and glycated albumin (GA) have been suggested for these populations. We aimed to assess the performance of GA and fructosamine tests in determining glycaemic control among Ugandan participants with type 2 diabetes.

Methods

We compared fructosamine, GA, and HbA1c (measured by immunoassay) to mean glucose from 14 days of continuous glucose monitoring in 192 participants with type 2 diabetes. We assessed whether the relationship between these assays and continuously measured glucose was altered by the presence of conditions reported to affect HbA1c reliability (sickle cell trait, anaemia and renal impairment), including specific subgroup analysis of anaemia, sickle cell trait and renal impairment, with impact of these conditions on the relationship between each measure and CGM glucose assessed using interaction terms in linear regression..

Results

A total of 192/213 completed 14-day continuous glucose monitoring and had sufficient (≥ 5 days) data. 43/192 (22.4%) had haemoglobinopathies (predominantly sickle cell trait), 18/192 (9.4%) had anaemia, and 12/192 (6.3%) had renal impairment. The overall association of HbA1c, GA and fructosamine with CGM assessed glucose was similar (r = 0.88 (95% CI: 0.84 – 0.91) vs 0.84 (0.79 – 0.88) and 0.84 (0.79 – 0.88) for fructosamine and GA). Within those with conditions, the correlation between mean CGM glucose and each of HbA1c, GA and fructosamine was similar (r = 0.85 (95%

CI: 0.76 – 0.91)), 0.76 (0.62 – 0.86), 0.74 (0.60 – 0.84) respectively). There was no evidence that the presence of a sickle cell trait altered the relationship between HbA1c, fructosamine or GA with CGM glucose (p value for interaction >0.3 for all). Those with anaemia had a lower HbA1c for a given mean CGM glucose ((β= 0.07 (95% CI: 0.04 – 0.09) mmol/l increase in glucose per 1mmol/mol increase in HbA1c) than those without anaemia (β= 0.15 (0.14 – 0.17), p for interaction <0.001. Similarly, the anaemia group had a lower fructosamine for a given mean CGM glucose (beta = 0.01, 95% CI: 0.01 – 0.02) mmol/l increase in glucose per 1umol/l increase in fructosamine compared to those without (beta = 0.02, 95% CI: 0.02 – 0.02); p-value for interaction =0.004). There was no evidence of difference in slopes for the association of GA with average CGM glucose between those with anaemia and those without (p-value for interaction = 0.216). HbA1c, glycated albumin and fructosamine were lower for a given mean CGM glucose among those with renal impairment than those without renal impairment (p value for interaction < 0.001 for all).

Conclusion

HbA1c has the best overall performance in monitoring glucose control even in SSA where medical conditions that may alter its reliability are prevalent. Fructosamine and glycated albumin do not improve performance over and above HbA1c. The accuracy of HbA1c, glycated albumin and fructosamine in reflecting glycaemic control is not affected by sickle cell trait. In those with anaemia and renal impairment the relationship between HbA1c and glucose is altered, but fructosamine and GA do not improve assessment of glycaemic burden.

INTRODUCTION

Diabetes mellitus, primarily type 2 diabetes remains a significant public health concern in low and middle-income countries (LMICs) with approximately 80% of the people living with diabetes worldwide, residing in LMICs.[8] Optimal control of plasma glucose levels is crucial to prevent or minimise the risk of developing long-term complications of diabetes. Monitoring glycaemic control among diabetes patients is an integral part of diabetes management to guide the intensification of treatment, prevent hyperglycaemia complications, and avoid hypoglycaemia, a significant side effect of many diabetes treatments.

For most developed countries, the standard test for monitoring diabetes is glycated haemoglobin (HbA1c). HbA1c reflects average glycaemic control better than a single glucose measurement and HbA1c levels are strongly related to long-term diabetes complications. Reducing HbA1c levels to lower optimal levels reduces the risk of developing such complications.[26] As such, HbA1c thresholds for optimal glycaemic control derived from large clinical trials serve as a guide for treatment intensification.[174-176]

HbA1c reflects average glycaemic control over the previous 2 – 3 months (the lifespan of red blood cells) and hence may not be appropriate for evaluating short-term therapeutic response or glucose variations. In addition, HbA1c may be affected by conditions unrelated to glucose control, e.g., haemoglobin variants, end-stage renal disease, iron deficiency with/without anaemia and other systemic conditions that cause anaemia or reduce the red blood cell lifespans.[177] Therefore, HbA1c levels may not accurately reflect the actual glycaemic control status in these conditions. Moreover, studies have suggested that HbA1c may be unreliable in populations of African ancestry.[178] This may partly be attributed to the high prevalence of conditions

mentioned above that may affect HbA1c reliability such as haemoglobin variants, iron deficiency (with/without anaemia).[47 48] Additionally, evidence suggests that HbA1c overestimates mean blood glucose in individuals of African ethnicity.[49 50]

The American Diabetes Association (ADA) recommends using other markers of chronic glycaemia, including fructosamine and glycated albumin (GA), in patients for whom HbA1c may be unreliable.[92] Fructosamine and glycated albumin provide information on glycaemic control in the preceding two weeks, are not influenced by non-glycaemic factors that affect HbA1c, and are more sensitive to glycation than HbA1c.[26 94] Some countries have fully embraced fructosamine and GA in routine practice and have included them in their country-specific guidelines.[179] These tests may potentially be useful particularly in the SSA where conditions that may potentially affect HbA1c reliability are common. However their utility in this population is not known. Studies are needed to support using fructosamine and glycated albumin as alternative tests.

In this study, we aimed to compare the performance of HbA1c, fructosamine and GA as an assessment of glucose burden in participants with type 2 diabetes.

METHODS

Study design

This study was a prospective, multicenter study at a rural-based hospital (Masaka regional referral hospital) and an urban-based hospital (St. Francis hospital Nsambya). People living with type 2 diabetes and attending routine scheduled diabetes clinics were recruited.

Study population

Eligible individuals were aged 18 years and above with a diagnosis of type 2 diabetes, more than 12 months' diabetes duration, no initial insulin requirement for at least 1 year since the time of diagnosis and treated with metformin, sulfonylurea or insulin, and no change in glucose lowering therapy 3 months prior to recruitment. All participants provided written informed consent before entering the study. Participants who were pregnant or judged by their clinician to need an immediate change in glucose lowering medication were excluded from recruitment.

Study visits

Details of the study visits have already been fully described in chapter 2. Briefly, participants came to the clinic in a non-fasted state for baseline visit. Following assessment of clinical features and demographics non-fasting (within 5 hours of a meal) random blood sample was collected for measurement of HbA1c, full blood count, renal function and assessment of haemoglobin variants. Continuous glucose monitoring was carried out using the Freestyle Libre Pro Flash Glucose Monitoring System (Abbott Laboratories, Illinois, USA), a professional continuous glucose monitoring (CGM) device which records interstitial glucose every 15 minutes for up to two weeks. Freestyle Libre Pro is blinded, meaning data could not be viewed by the wearer. Raw glucose readings were downloaded from the Libreview software and CGM summary variables (including mean CGM glucose) were calculated using R v3.6.1. Sensor data was considered for analysis if the total duration of CGM wear was at least 5 days.

All participants returned in a fasted state (at least 8 hours) in the second week of CGM between days 7 and 10 from the baseline visit, and for their final visit, between days

12 and 14 from the baseline visit, in a non-fasted state (within 5 hours of a meal). At both of these visits, CGM data were downloaded and a venous blood sample was collected for measurement of HbA1c, GA and fructosamine (visit 1 and 3) and FPG (visit 2). The study was carried out in accordance with 2008 revised principles of the Declaration of Helsinki and all participants provided informed consent before study activities.

Laboratory procedures

Plasma glucose and HbA1c were measured at the central Biochemistry and clinical diagnostic laboratory services (CDLS) laboratory at the MRC/UVRI & LSHTM Research Unit Entebbe, Uganda. Glucose was measured by the glucokinase method. HbA1c was measured on Cobas 6000 by the immunoassay technique; calibrated to the International Federation of Clinical Chemistry (IFCC).

Two assays (calorimetric and enzymatic assays) were used to measure serum fructosamine at the two laboratories. The calorimetric assay on the Roche COBAS INTEGRA 400 plus (Roche Diagnostics, Indianapolis, IN) was used at the CDLS laboratory (MRC/UVRI & LSHTM Research Unit, Uganda). This assay is based on the ability of fructosamine to reduce nitrotetrazolium-blue to formazan in an alkaline solution. The formation rate of formazane is directly proportional to the fructosamine concentration and is measured photometrically at 552 nm. The enzymatic assay (Randox Laboratories Limited, UK), was used to measure fructosamine on Cobas 8000, c702 module analyser at the Exeter clinical laboratory (Exeter, UK).

The GA value (%) was obtained from GA concentration and the albumin concentration measured in the serum sample. Glycated albumin was measured with the enzymatic assay in serum on the Roche Cobas 8000 series using Lucica GA-L enzymatic assay

(Asahi Kasei Pharma, Tokyo, Japan) kindly provided by Werfen - the European distributor https://www.werfen.com/en. In this method, the sample reacts with a ketoamine oxidase to eliminate endogenous glycated amino acids by conversion to glucosone, amino acids, and hydrogen peroxide. The resultant solution reacts with Albumin specific protease, which converts GA (Glycoalbumin) to Glycated amino acids. Thereafter, Glycated amino acids reacts with ketoamine oxidase to form glucosone, amino acids, and hydrogen peroxide. Peroxidase catalyses the reaction of N, N-bis-3-Methylaniline Disodium Salt, 4-Aminoantipyrine and hydrogen peroxide forming a quantitatively a blue-purple pigment. The resultant colour is proportional to the GA concentration in a sample and is quantified by absorbance.

Statistical analysis

Data were analysed using Stata V16.1 (StataCorp LLC, USA).

We used descriptive statistics to summarise all results in the study. We summarised continuous variables by median and interquartile ranges, while categorical variables were summarised by number and percentage. We subdivided the cohort into those with anaemia, those with haemoglobinopathies, those with renal impairment and those without any of the HbA1c comorbidities (anaemia, haemoglobinopathies, and renal impairment). We used scatterplots and linear regression to determine the strength of the association between CGM assessed mean glucose over 14 days and each of HbA1c and fructosamine.

We used linear regression to determine the slopes and intercept of the lines of best fit separately for individuals with and without each of the HbA1c comorbidities (sickle cell trait, anaemia and renal impairment). In addition, we added an interaction term to the

linear regression models to test the hypothesis that the relationships between the mean CGM glucose and each of HbA1c, GA and fructosamine was altered by the presence of sickle cell trait, anaemia and renal impairment, including specific subgroup analysis of anaemia, sickle cell trait and renal impairment. Analysis was based on fructosamine and HbA1c tests performed on the last visit (visit 3). We carried out a sensitivity analysis where we repeated the above analyses using fasting plasma glucose in place of CGM glucose as the outcome.

Fructosamine Inter- assay comparison and reproducibility

Furthermore, to assess the reproducibility of fructosamine, we measured the strength of the relationship between fructosamine results obtained on visit one and visit 3 (10 – 14 days) using Pearson correlation coefficient.

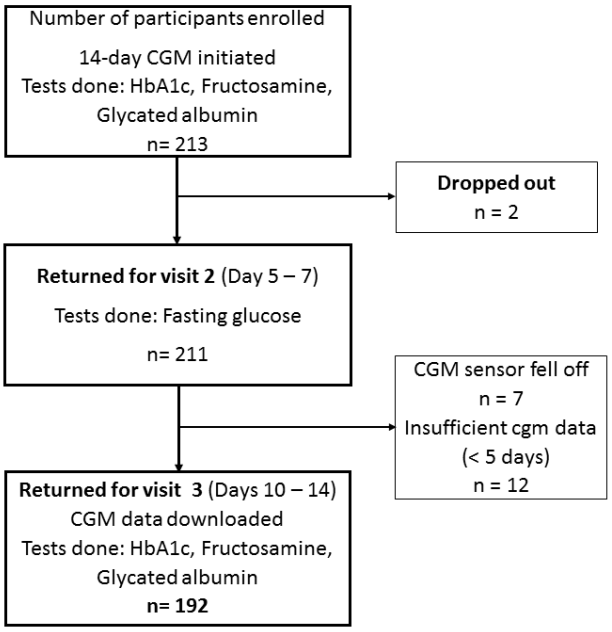

Figure 1: Participant flow chart

RESULTS

Out of 213 recruited, 192 participants had at least 5 days of CGM data and were considered for analysis (Figure 1).43/192 (22.4%) had haemoglobinopathies (All but one (HbAC) had sickle cell trait), and 18/192 (9.4%) had anaemia, 12/192 (6.3%) had renal impairment (Table 1). 129/192 (67.2%) did not have any of the above conditions (no HbA1c comorbidities).

Fructosamine results obtained from the enzymatic assay (UK Laboratory) had a better correlation with CGM glucose than those obtained from the calorimetric assay (MRC Uganda Laboratory); 0.84 (95% CI: 0.79 – 0.88) vs 0.60 (0.50 – 0.69) (Supplementary figure 1). As the enzymatic assay was performed in the same laboratory as HbA1c and GA, this assay was therefore used for primary analyses.

HbA1c has a similar relationship to CGM assessed glucose like fructosamine and GA, even in participants with comorbidities that may affect HbA1c reliability.

The relationship between each of HbA1c, GA and Fructosamine and mean CGM glucose is shown in figure 2. The overall association of HbA1c, GA and fructosamine with CGM assessed glucose was similar (r = 0.88 (95% CI: 0.84 – 0.91) vs 0.84 (0.79 – 0.88) and 0.84 (0.79 – 0.88) (Figure 2). Within those with conditions, the correlation between mean CGM glucose and each of HbA1c, GA and fructosamine was similar (r = 0.85 (95% CI: 0.76 – 0.91), 0.76 (0.62 – 0.86), 0.74 (0.60 – 0.84)) respectively (Figure 2).

Sickle cell trait does not alter the relationship between HbA1c, fructosamine and GA with CGM glucose

Among those with sickle cell trait, HbA1c, GA and fructosamine had similar association with CGM glucose (r = 0.90 (95% CI: 0.82 – 0.94), (0.79 (0.63 – 0.89), 0.79 (0.63 – 0.88) respectively). There was no evidence of a difference in the relationship between each of HbA1c, fructosamine or GA with CGM glucose among those with sickle cell trait and those without (Figure 3). Those with and without sickle cell trait had similar HbA1c for a given mean CGM glucose (beta= 0.15, 95% CI: 0.13 – 0.18 vs 0.14, 95% CI: 0.13 – 0.17 mmol/l increase in glucose per 1 mmol/mol increase in HbA1c, p for interaction = 0.319). Likewise, GA and fructosamine were similar for a given mean CGM glucose among those with and without HbAS (beta = 0.74 (0.54 – 0.94) vs 0.77 (0.69 – 0.85 mmol/l increase in glucose per 1% increase in GA and 0.02 (0.01 – 0.03) vs 0.02 (0.02 – 0.02) mmol/l increase in glucose per 1 umol/l increase in fructosamine for GA and fructosamine respectively, p for interaction > 0.60 for all)) (Figure 3).

Anaemia significantly affected the relationship between HbA1c and fructosamine with CGM.

In individuals without anaemia, the association of HbA1c with CGM glucose (r = 0.89, 95% CI: 0.86 – 0.92) was similar to that of GA (r = 0.84, 95% CI: 0.79 – 0.88) and fructosamine (r= 0.86, 95% CI: 0.81 – 0.89) respectively. Likewise, in those with anaemia, HbA1c, GA and fructosamine had similar associations with mean CGM glucose(r = 0.80 (95% CI: 0.54 – 0.92), 0.77 (0.47 – 0.91) and 0.76 (0.44 – 0.91) respectively) (Figure 3).

Those with anaemia had a lower HbA1c for a given mean CGM glucose than those without anaemia (anaemia group β= 0.07 (95% CI: 0.04 – 0.09) mmol/l increase in glucose per 1mmol/mol increase in HbA1c vs β= 0.15 (0.14 – 0.17) for the non-anaemia group, p for interaction <0.001, Figure 3, supplementary table 1). Similarly,

those with anaemia had a lower fructosamine for a given mean CGM glucose (beta = 0.01, 95% CI: 0.01 – 0.02) umol/l compared to those without (beta = 0.02, 95% CI: 0.02 – 0.02) umol/l; p=0.004, supplementary table 1). In contrast, there was no evidence of difference in slopes for the association of GA with average CGM glucose between those with anaemia and those without (beta= 0.54, 95% CI: 0.29 – 0.78 vs 0.77, 95% CI: 0.69 – 0.85, p = 0.216, Figure 3, supplementary table 1).

Renal impairment significantly affected the relationship between HbA1c and fructosamine with CGM.

In individuals without renal impairment (12/192 (6.3%)), HbA1c had the strongest association with CGM glucose (r = 0.88 (95% CI: 0.85 – 0.91) compared to GA and fructosamine (0.86(0.81 – 0.89) and 0.86(0.81 – 0.89) respectively) (Figure 3). Individuals with renal impairment had a lower HbA1c for a given mean CGM glucose than those without renal impairment (renal impairment group β =0.04 (95% CI: 0.01 – 0.08) mmol/l increase in glucose per 1mmol/mol increase in HbA1c vs β = 0.15 (0.14 – 0.16) for individuals without renal impairment (p for interaction < 0.001) (Figure 3, supplementary table 1). Similarly, those with renal impairment had a lower glycated albumin for a given mean CGM glucose (beta = 0.20, 95% CI: 0.03 – 0.37) mmol/l per 1% increase in glycated albumin compared to those without (beta = 0.79, 95% CI: 0.72 – 0.87); p-value for interaction < 0.001, Figure 3 and supplementary table 1). Fructosamine was also significantly lower in those with renal impairment (beta= 0.01, 95% CI: 0.00 – 0.01) mmol/l increase in glucose per 1 umol/l in fructosamine compared to those without renal impairment (beta = 0.02, 95% CI: 0.02 – 0.02); p-value for interaction < 0.001, Figure 3, supplementary table 1).

Table 1: Baseline characteristics

	Median (IQR) for continuous variables, % (n) for proportions				
Clinical	**No HbA1c comorbidities**	**HbA1c comorbidities**	**HbAS**	**Anaemia**	**Renal impairment**
N (%)	**67.2 (129/192)**	**32.8 (63/192)**	**22.4 (43/192)**	**9.4 (18/192)**	**6.3 (12/192)**
Female, % (n)	60.5 (78/192)	54.0 (34/192)	60.5 (26/43)	61.1 (11/18)	16.7 (2/12)
Age, years	55 (50, 61)	58 (50, 64)	55 (49, 63)	57.5 (50, 65)	62 (55.5, 68.5)
Duration of diabetes, years	6 (3, 10)	9 (4, 12)	8 (3, 12)	8.5 (4, 12)	10.5 (5.5, 17)
BMI, kg/m^2	27. 1 (24.3, 30.3)	25.8 (23.1, 30.6)	25.8 (22.7, 29.7)	25.5 (21.5, 31.7)	26.1 (24.7, 28.5)
Haemoglobin (g/dL)	14.3 (13.4, 15.1)	13.5 (11.9, 15.0)	14.5 (12.9, 15.4)	11.4 (10.8, 11.9)	13.5 (12.2, 14.1)
Total protein (g/dL)	73.0 (70.0, 77.0)	74.0 (68.0, 80.0)	73.0 (66.0, 79.0)	75.0 (68.0, 80.0)	73.0 (70.0, 78.0)
Serum albumin (g/dL)	44.0 (42.0, 46.0)	43.0 (39.0, 47.0)	43.0 (39.5, 46.5)	41.0 (37.0, 44.0)	43.5 (39.5, 46.0)
Iron deficiency, % (n)	31.0 (40/129)	22.2 (14/63)	11.6 (5/43)	55.6 (10/18)	8.3 (1/12)
Current management n (%)					
Metformin only	18.6 (24/129)	9.5 (6/63)	9.3 (4/43)	11.1 (2/18)	0.0
SU (+/- metformin)[a]	57.4 (74/129)	57.1 (36/63)	62.8 (27/43)	33.3 (6/18)	66.7 (8/12)
Insulin (+/- other diabetes drug)[b]	22.5 (29/129)	33.3 (21/63)	27.9 (12/43)	55.6 (10/18)	33.3 (4/12)

Diet [c]	1.5 (2/129)	0	0	0	0.0
Glycaemia					
CGM glucose, mmol/L	8.4 (6.8, 12.3)	9.3 (7.0, 12.3)	9.6 (7.8, 15.3)	8.3 (6.1, 9.8)	7.4 (6.1, 8.3)
HbA1c, %	8.2 (6.7, 9.8)	8.7 (7.1, 10.7)	8.8 (7.5, 11.1)	8.1 (6.4, 9.4)	6.9 (6.0, 9.0)
HbA1c, mmol/mol	66.0 (50.0, 85.0)	70.5 (54.0, 97.0)	71.0 (58.0, 103.0)	64.5 (46.0, 80.0)	52.0 (42.5, 73.5)
Fasting plasma glucose, mmol/L	8.3 (6.1, 11.3)	7.8 (5.9, 11.5)	8.6 (6.5, 12.8)	7.6 (5.3, 10.9)	6.5 (5.3, 7.5)
Random plasma glucose, mmol/L	13.0 (8.8, 16.8)	14.1 (8.7, 18.4)	14.9 (9.5, 19.5)	11.1 (6.9, 17.2)	12.1 (9.1, 14.3)
Glycated albumin (%)	11.9 (9.4, 15.2)	11.9 (10.3, 16.1)	12.2 (10.6, 17.7)	11.9 (9.0, 14.3)	10.6 99.5, 12.8)
Fructosamine (umol/L)	362 (306, 505)	420 (358, 559)	431 (369, 568)	384 (317, 509)	403 (205, 601)

HbA1c comorbidities are the non-glycaemic biological conditions thought to alter HbA1c reliability e.g., including haemo-globinopathies (sickle cell trait), anaemia, and renal impairment

The linear equations for estimating HbA1c, GA and fructosamine from mean CGM glucose are shown in supplementary table 1. The results were broadly similar when we used fasting plasma glucose (in place of CGM glucose as the outcome) (supplementary figure 2).

The relationship between HbA1c with GA and Fructosamine is shown in Supplementary figure 3. Fructosamine had a slightly stronger association with HbA1c than GA both in the absence (r = 0.86, 95% CI: 0.81 – 0.90) vs 0.81 (0.74 – 0.87) and presence (r = 0.75 (0.61 – 0.84) vs 0.70 (0.54 – 0.82) (Supplementary figure 3).

GA and fructosamine were highly correlated both in the presence and absence of HbA1c comorbidities; 0.89 (95% CI: 0.82 – 0.93) and 0.92 (0.89 – 0.95) respectively (Supplementary figure 4).

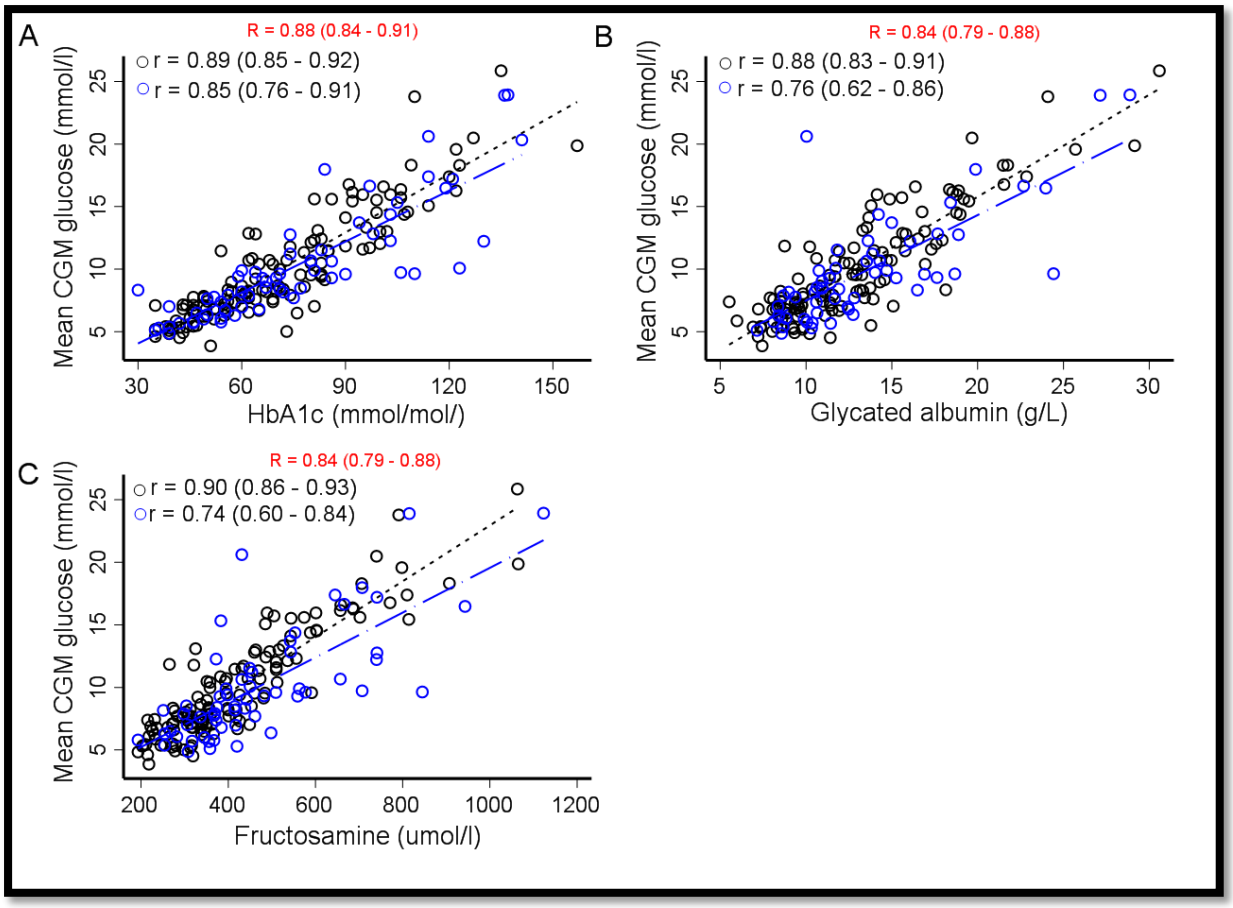

Figure 2: Scatterplots of the relationship between mean glucose obtained through continuous glucose monitoring and HbA1c (A) and fructosamine (B) and Glycated albumin (C) in type 2 diabetes patients with and without HbA1c comorbidities (Sickle cell trait, anaemia and renal impairment). The relationship between each marker of diabetic control and mean glucose was analysed using a linear regression analysis. The black hollow circles (○) and dotted line represent subjects with HbA1c comorbidities the blue open circles (○) and dashed line represent those without HbA1c comorbidities. Straight lines denote the lines of best fit. The overall Pearson's correlation coefficient (R) and 95% confidence intervals are shown for each graph in red colour. The Pearson's correlation coefficient (r) and 95% confidence intervals stratified by presence or absence of HbA1c comorbidities are also shown for each graph in black colour.

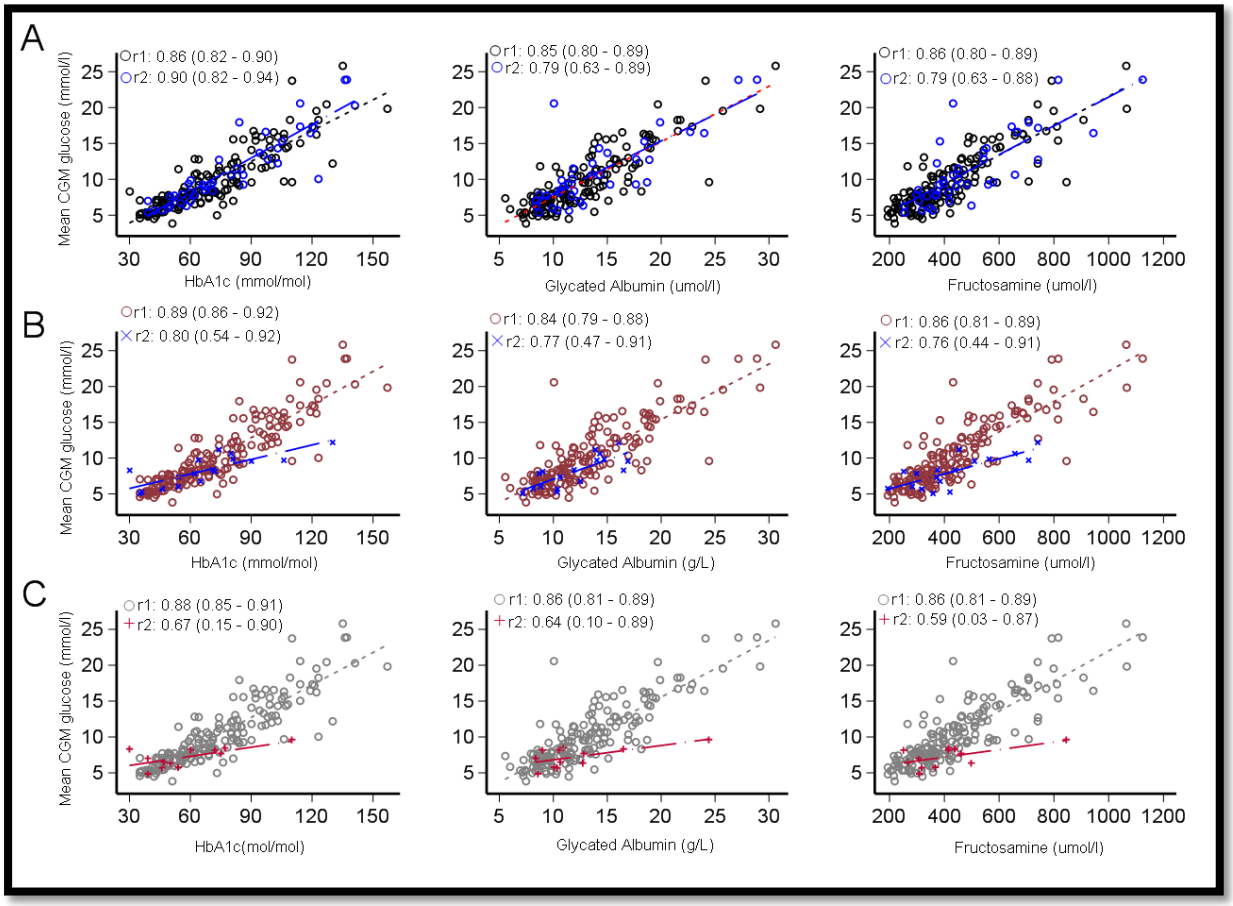

Figure 3: Scatterplots of the relationship between mean glucose obtained through continuous glucose monitoring and each of HbA1c, GA and fructosamine in type 2 diabetes patients with and without A) Sickle cell trait, B) anaemia and C) renal impairment. The relationship between each marker of diabetic control and mean CGM glucose was analysed using a linear regression analysis. The top row (A) shows the relationship between HbA1c, GA and fructosamine with mean CGM glucose in those with (black open circles) and without (blue open circles) HbAS. The middle row (B) shows graphs among those with (maroon open circles) and those without (blue x) anaemia. The bottom row (C) shows the association between mean CGM glucose with the respective tests among subjects with (cranberry plus signs) and without (grey open circles) renal impairment. The p-values are for evidence of interaction by the HbAS or anaemia on the association between HbA1c and fructosamine with mean CGM glucose. The strength of association (R-squared) are shown for each graph. Straight lines denote the lines of best fit. The Pearson's correlation coefficient (r) and 95% confidence intervals stratified by presence or absence of sickle cell trait (A), Anaemia (B) and renal impairment (C).

DISCUSSION

We found in this SSA population that using fructosamine or GA did not improve overall accuracy in assessing glycaemic burden compared to HbA1c. There was no evidence that these alternative markers are more reliable than HbA1c in those with non-glycaemic conditions reported to affect the reliability of HbA1c. The accuracy of HbA1c, GA and fructosamine in reflecting glycaemic control was not affected by sickle cell trait. In contrast, in those with anaemia or renal impairment, HbA1c, fructosamine and GA were all unreliable in reflecting glycaemic burden. These findings have important implications for the choice of measurement for monitoring glucose in people with these conditions. Our findings raise uncertainty regarding the recent recommendation from diabetes organisations such as ADA to use fructosamine and glycated albumin as alternative glycaemic control markers in patients with whom HbA1c is unreliable.

Our results show a broadly similar overall correlation between HbA1c, GA, and fructosamine with mean CGM glucose and are consistent with earlier studies from developed countries. In a recent US prospective multicenter conducted among people living with type 1 or type 2 diabetes (including people of African ancestry), HbA1c, GA and fructosamine had similar correlations with 7-day mean blood glucose obtained by self-monitoring of blood glucose.[111] Similar relationships among HbA1c and the alternate glycaemic markers (fructosamine and GA) with CGM were seen in a study of 56 obese youth with prediabetes and type 2 diabetes.[180] In another tiny study (limited to 26 T1D children) where CGM was utilised, glycated serum proteins had comparable correlations with HbA1c against mean glucose from CGM.17 However, it should be noted that these studies either recruited small numbers or highly select

groups of participants or excluded patients with conditions thought to alter HbA1c (kidney disease or liver disease).[111 180]

Likewise, studies that have utilised single glucose measures (fasting glucose or random non-fasting glucose) have shown similar correlation coefficients between HbA1c, GA and fructosamine and glucose. In a large Swedish type 1 and 2 diabetes population, investigators reported a similar association between each HbA1c and fructosamine with single glucose (fasting or non-fasting) measure, with an r of 0.75.[181] However, in this Swedish study, Glycated albumin was not measured. A single blood glucose measurement was a limitation rather than the more accurate average glucose obtained from CGM used in our research. Additionally, we found similar relations between fructosamine and HbA1c as in previously published studies.[182 183] To our knowledge, no studies have examined the clinical utility of fructosamine and GA as alternative tests for monitoring glycaemic control in SSA. Only one study in SSA has assessed the diagnostic performances of GA and fructosamine against OGTT in screening for diabetes.[106 184] Similar strong correlations have been reported in studies among other types of diabetes (type 1 diabetes, gestational diabetes mellitus) and non-diabetic individuals.[185] Unlike the current study, where the association of HbA1c and fructosamine were assessed differently for individuals with and without HbA1c comorbidities, the previous studies only reported the overall correlations.

In the current study, the presence of sickle cell trait did not affect the correlation of HbA1c, GA and fructosamine with mean CGM glucose. In line with Lacy, M et al., those with and without sickle cell trait had similar HbA1c for given glucose.[166] Some studies reported lower levels of HbA1c and others higher or same HbA1c levels in carriers of SCT compared to non-carriers.[63] In the present study, HbA1c was measured with the HbA1c immunoassay. HbA1c immunoassay is among the most

widely used HbA1c assays, particularly in low-resource settings. Although modern HbA1c assays (e.g., Tinaquant immunoassay reagents) have been shown to provide accurate results for the most common heterozygous variants, our results may not apply to other HbA1c assays methodologies, which are known to have different susceptibility to the effects of different haemoglobinopathies.[173] However, in another study where HbA1c was measured using the immunoassay method, higher levels of HbA1c were observed in carriers of SCT compared to non-carriers.[166]

Findings are inconsistent even when NGSP-certified methods were used to measure HbA1c: A recent systematic review of 11 published studies aimed to assess how SCT affects HbA1c showed conflicting results across studies.[63] Sickle cell trait was the predominant haemoglobinopathy in our study. However, it should be noted that other haemoglobinopathies such as HbC, common in other parts of SSA, were rare in this setting.[48] The impact of other common genetic variants, e.g., the HbC trait and other rare Hb variants thought to interfere with the measurement of HbA1c,[57] remains unknown. For example, recent evidence shows that some rare Hb variants (especially those with substitutions close to the beta N-terminus) may lead to inaccurate HbA1c results even with modern assays.[62] These issues merit a more extensive multinational study enriched with common haemoglobinopathies to properly evaluate their impact on the reliability of HbA1c and the alternative glycaemic markers in assessing glycaemic control.

In contrast, anaemia and renal impairment significantly affected the relationship between HbA1c, GA and fructosamine. In those with anaemia, HbA1c maintained a better correlation than HbA1c and GA, implying that GA and fructosamine may not necessarily improve glycaemic monitoring accuracy among those with anaemia and renal impairment. Previous studies that evaluated the performance of HbA1c to assess

glycaemic control in renal impairment compared with alternative markers of glycaemia have not been conclusive.[186] However, the performance of fructosamine as a glycaemic marker control in patients with renal failure has been consistently poor.[187-189] In another study, the correlations between all the three tests with glucose were broadly poor among patients with renal impairment. However, GA and fructosamine had slightly better correlations with glucose than HbA1c (r: 0.54 vs 0.38).[190] Similar to our study, the correlation between mean CGM glucose and HbA1c in patients with severe nephropathy was very poor but good in those with no nephropathy (r: 0.38 vs 0.66).[190] Results from previous studies have been inconsistent, with some studies reporting better and other similar or poorer correlation of glucose with GA compared to HbA1c in patients with renal impairment.[191] In an extensive cross-sectional analysis involving 1665 diabetes patients Jung et.al, al showed that correlations of HbA1c, GA and fructosamine with fasting glucose were lowest in those with severe kidney disease; HbA1c: r = 0.52, GA: r = 0.39, fructosamine: r = 0.41).[191] The authors concluded that glycated albumin or fructosamine may have no particular advantage over HbA1c for monitoring glycaemic control in CKD.[191]

One of the strengths of this study is that glycaemic control was continuously monitored in day-to-day living over 14 days using CGM. However, the following limitations should be considered when interpreting our results. First, it should be noted that the fructosamine assay is not well standardised across laboratories, and there has been concern regarding its validity and reliability.[192] We had two fructosamine assays, but one was used in a different laboratory to all the other tests. The enzymatic fructosamine results (Exeter laboratory) had the best correlation with mean CGM glucose compared to the calorimetric assay (MRC/UVRI Uganda laboratory). This assay was used for primary analyses as the enzymatic assay was performed in the

same laboratory as HbA1c and GA. The different fructosamine results from the two fructosamine assays may be explained by differences between laboratories or from sample handing and transport rather than by the test itself. Assessing differences between fructosamine assays in the same laboratory should be a focus of future research. Our results for the fructosamine performance might not apply to other assay types.

Secondly, even though our study population had a substantial number, we had a minimal number of patients with renal impairment and anaemia, with wide confidence intervals around our results for these subgroups. Further characterisation of people with these comorbidities was impossible (e.g., mild anaemia vs severe anaemia or mild renal impairment vs severe renal impairment). Nonetheless, our results showed statistically significant differences between the slopes of those with versus without renal impairment and anaemia. In the present study, we could not assess the impact of other individuals underlying non-glycaemic conditions.[59] For example, the impact of glucose-6 phosphate dehydrogenase (G6PD) variants and a common condition among people of African ancestry that may affect HbA1c results reliability was not assessed.[64]

The result that GA and fructosamine- glycated serum proteins are not affected by red blood cell factors does not improve accuracy in people with renal impairment and anaemia is counterintuitive. The poor correlation of fructosamine and GA with glucose in people with renal impairment and anaemia deserves further exploration. However, some reasons these conditions are thought to affect only HbA1c may alter fructosamine and GA includes: First, fructosamine and GA reliability may be influenced by clinical conditions that affect protein metabolism (concentration).[112] Pathologic and physiologic conditions associated with reduced serum protein, such as malnutrition,

nephrotic syndrome and thyroid disease, may alter the reliability of fructosamine and albumin.[193] [194] Similarly, abnormally elevated immunoglobulins (especially IgA) present during infectious episodes may alter fructosamine levels.[195] These conditions, broadly classified as protein-losing states or diminished protein production, include nephrotic syndrome, hepatic cirrhosis and thyroid disease and have been associated with altered GA and fructosamine. Anaemia in this setting is likely to be associated with infections. For example, patients with anaemia had lower serum albumin concentration but modestly higher total serum protein. Whether the half-life of albumin or fructosamine is altered by anaemia or renal impairment is unknown.

Our findings that HbA1c has a similar relationship to CGM and fasting glucose in an African population than fructosamine and GA, even in those with comorbidities reported to affect HbA1c validity, supports the continued use of HbA1c in this population. These findings further suggest that GA and fructosamine are unlikely to have utility for glycaemic monitoring in those with diabetes. However, these findings may not apply to diagnosis where the impact of non-glycaemic factors on HbA1c reliability is likely to be more marked. Consistent with previous research, our results also suggest that sickle cell trait is not an important issue. Though in small numbers, our findings in renal impairment and anaemia raise caution that HbA1c and the alternative markers underestimate glycaemic burden, but this needs further research. There is a need for more extensive multi-national studies involving other regions in Africa and LMICs with enrichment for HbA1c-associated comorbidities, ideally incorporating further subgroup characterisation and analysis to determine whether GA and fructosamine (alternative markers of glycaemia) offer any advantage above and beyond HbA1c in this setting.

In conclusion, HbA1c has the best overall performance in monitoring glucose control, even in SSA, where medical conditions that may alter its reliability are prevalent. Fructosamine and glycated albumin do not improve performance over and above HbA1c. The accuracy of HbA1c, glycated albumin and fructosamine in reflecting glycaemic control is not affected by sickle cell trait. In those with anaemia and renal impairment, the relationship between HbA1c and glucose is altered, but fructosamine and GA do not improve the assessment of glycaemic burden.

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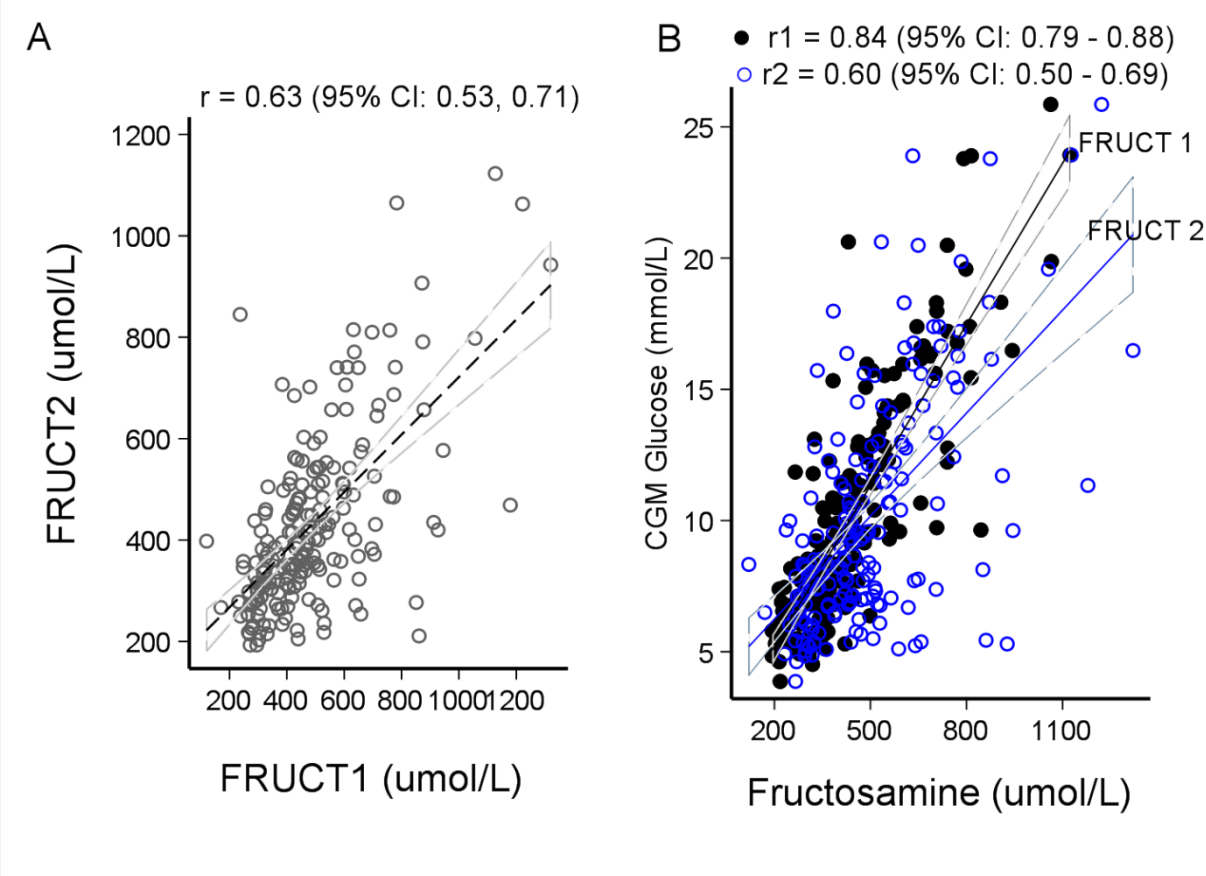
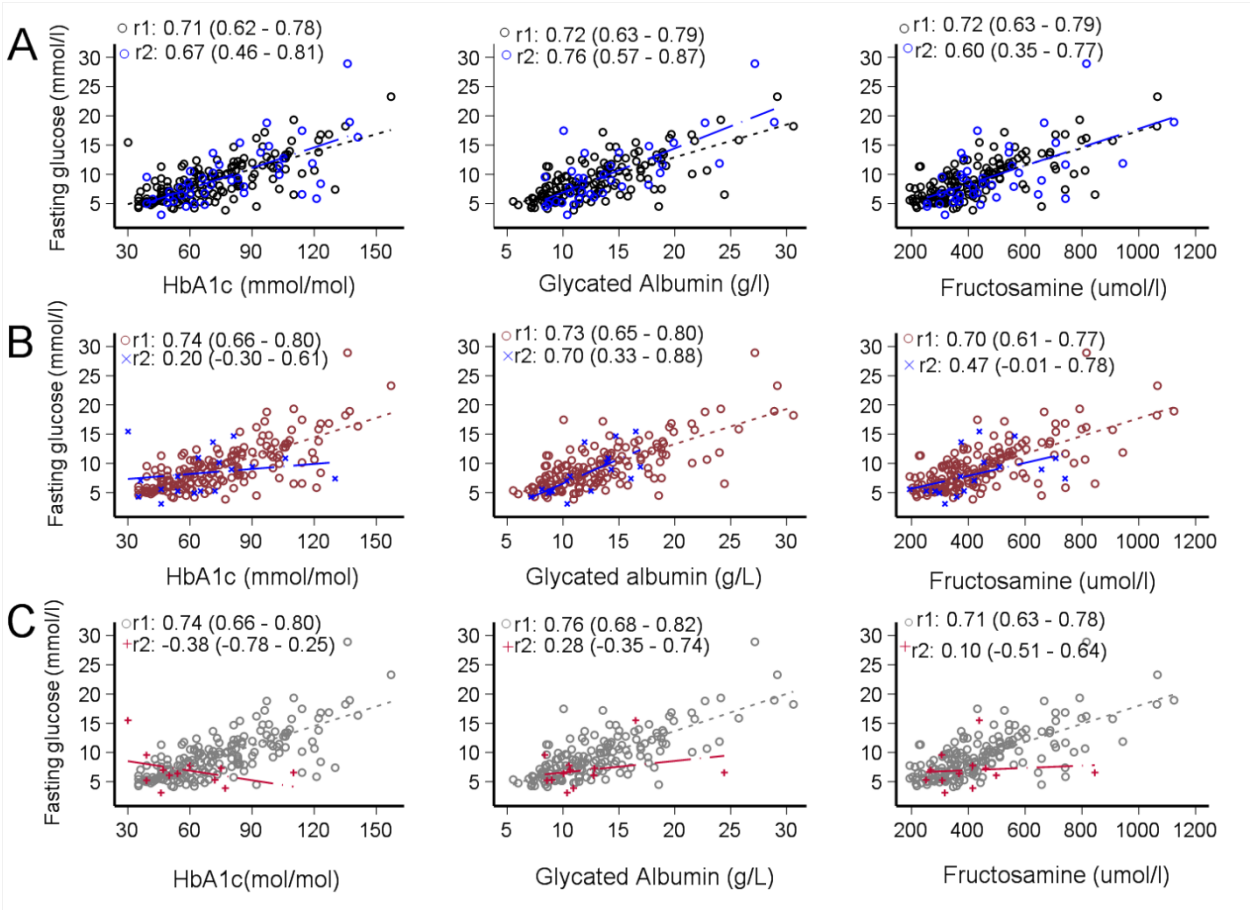


Supplementary Figure 1: Plots illustrating the correlation of fructosamine results (A) between enzymatic assay and calorimetric assay. Graph B compares the correlation of CGM glucose with fructosamine from the two assays in diabetic patients. The Pearson's correlation coefficients (r) and their 95% CIs (parentheses) are shown for each graph. FRUCT 1 denotes fructosamine measured at baseline and FRUCT 2 is fructosamine measured at visit 3 (10 -14 days from baseline).



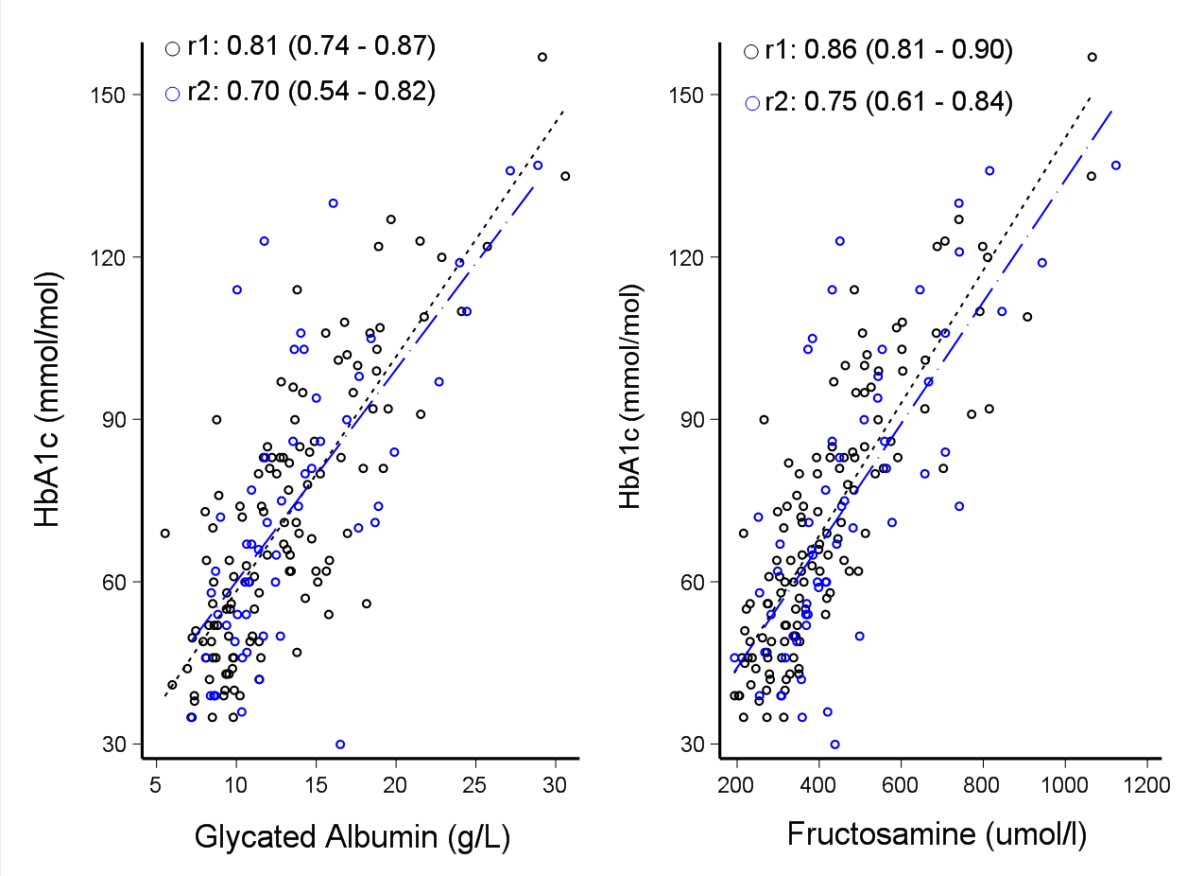
Supplementary Figure 2: Relationship between HbA1c, GA and fructosamine tests with fasting plasma glucose. The relationship between each marker of diabetic control and mean glucose was analysed using a linear regression analysis. The top row (A) shows the relationship between HbA1c, GA and fructosamine with fasting glucose in those with (black open circles) and without (blue open circles) HbAS. The middle row (B) shows graphs among those without (maroon open circles) and those with (blue x) anaemia. The bottom row (C) shows the association between mean CGM glucose with the respective tests among subjects with (cranberry plus signs) and without (grey open circles) renal impairment. The p-values are for evidence of interaction by the HbAS or anaemia on the association between HbA1c and fructosamine with fasting glucose. The Pearson's correlation coefficient (r) and 95% confidence intervals are shown for each graph.

Supplementary Table 1: Glycaemic measures and their linear relationships with mean day-to-day glucose measured by CGM stratified by presence or absence of comorbidities thought to alter HbA1c reliability

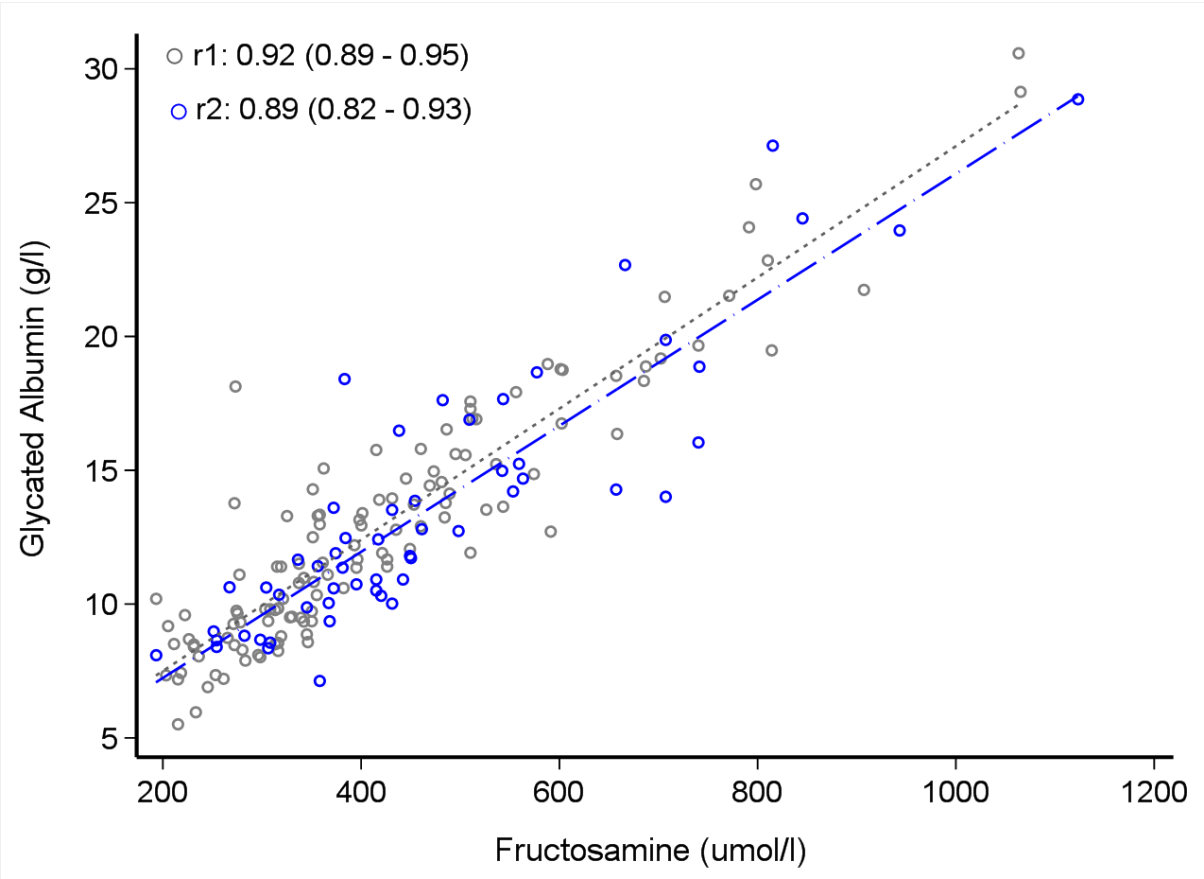
	HbA1c	**GA**	**Fructosamine**
HbA1c comorbidities			
No	CGM = HbA1c*0.16 - 1.07	CGM = GA*0.82 - 0.54	CGM = FRUC*0.02 - 0.63
Yes	CGM = HbA1c*0.14 - 0.01	CGM = GA*0.68 + 0.65	CGM = FRUC*0.02 + 1.65
Haemoglobinopathies			
No	CGM = HbA1c*0.14 - 0.38	CGM = GA*0.77 - 0.24	CGM = FRUC*0.02 + 1.05
Yes	CGM = HbA1c*0.16 - 1.10	CGM = GA*0.74 + 0.56	CGM = FRUC*0.02 + 1.38
Anaemia			
No	CGM = HbA1c*0.15 - 0.99	CGM = GA*0.77 - 0.06	CGM = FRUC*0.02 + 0.98
Yes	CGM = HbA1c*0.07 + 3.74	CGM = GA*0.54 + 1.71	CGM = FRUC*0.01 + 3.64
Renal impairment			
No	CGM = HbA1c*0.15 – 0.82	CGM = GA*0.79 - 0.26	CGM = FRUC*0.02 + 1.01

Yes	CGM = HbA1c*0.04 + 4.76	CGM = GA*0.20 + 4.82	CGM = FRUC*0.01 + 5.09

HbA1c comorbidities are the non-glycaemic biological conditions thought to alter HbA1c reliability e.g., haemo-globinopathies including sickle cell, anaemia, and renal impairment.



Supplementary figure 3: Plots illustrating the correlation between HbA1c with GA and fructosamine in type 2 diabetes patients with and without HbA1c comorbidities (Sickle cell trait, anaemia and renal impairment). The relationship between each marker of diabetic control and mean glucose was analysed using a linear regression analysis. The blue open circles (○) and, dotted line represent subjects with HbA1c comorbidities while the black open circles (○) and dashed line represent those without HbA1c comorbidities. Straight lines denote the lines of best fit. The Pearson's correlation coefficients (r) and 95% confidence intervals are shown for each graph.



Supplementary figure 4: Plots illustrating the correlation between GA and fructosamine in type 2 diabetes patients with and without HbA1c comorbidities (Sickle cell trait, anaemia and renal impairment). The relationship between each marker of diabetic control and mean glucose was analysed using a linear regression analysis. The grey open circles (○) and dotted line represent subjects with HbA1c comorbidities the blue open circles (○) and dashed line represent those without HbA1c comorbidities. Straight lines denote the lines of best fit. The Pearson's correlation coefficient (r) and 95% confidence intervals are shown for each graph.

Chapter 4

The impact of prolonged walking on fasting plasma glucose in type 2 diabetes: A Randomised controlled crossover study

Anxious J. Niwaha, Lauren R. Rodgers, Andrew T. Hattersley, Beverley M. Shields, Angus G. Jones, Moffat J. Nyirenda

Acknowledgements of co-authors and contributions to the paper

Myself, Beverley Shields, Moffat Nyirenda and Angus Jones conceptualised and designed the study. I set up the study in Uganda and obtained ethical approval (with assistance of supervisors), and led the study in Uganda researching the data including undertaking all aspects of recruitment and data collection assisted by our research nurse team. I analysed the data with assistance from Lauren Rodgers, Beverley Shields and Angus Jones. I drafted the paper which was critically reviewed and edited by all authors.

ABSTRACT

Background and aims

In many low-resource countries, fasting glucose is the primary measure of glycaemic control used for treatment titration, as HbA1c is often unavailable or unaffordable. Many patients in these countries walk long distances to the clinic, but the impact of walking on fasting glucose in type 2 diabetes is unknown. We aimed to determine whether this prolonged walking affects the reliability of fasting plasma glucose as a measure of glycaemic control.

Materials and Methods

In a randomised crossover trial, the change in glucose from baseline in the fasting state was compared between walking on a treadmill at a predetermined speed of 4.5 km/hour for 1 hour and not walking (resting) in people with type 2 diabetes. Glucose was measured every 30 minutes for 2 hours post-baseline. The pre-specified co-primary outcomes were glucose difference at 1 and 2 hours.

Results

Forty-five participants were enrolled, with all participants completing both visits. 21/45 (46.7%) were female, and the median age was 51. Glucose was similar during and after walking and at rest; glucose difference (walking minus rest) was -0.15 (95% CI: -0.55, 0.26) and -0.10 (95% CI: -0.50, 0.31) mmol/L at 1 and 2 hours respectively, p>0.4 for both). In a mixed-effects model examining glucose change throughout each test, the exercise intervention did not explain further variability in glucose (LR test chi2=9.0, p=0.25).

Conclusions

Fasting plasma glucose is not meaningfully affected by prolonged walking in participants with type 2 diabetes; therefore, the reliability of fasting glucose for monitoring glycaemic burden is unlikely to be altered in patients who walk to the clinic.

INTRODUCTION

HbA1c and home capillary or subcutaneous glucose monitoring are the primary measures used to monitor glycaemic control and guide treatment titration in diabetes in high-income countries. However, these approaches are often unavailable or unaffordable [9 74] for many of the 432.7 million people with diabetes who live in low or middle-income countries.[8] In this setting international organisations recommend the use of fasting glucose to monitor glycaemic control and titrate glucose-lowering therapy, and this remains the primary means of glucose monitoring for a substantial proportion of those living with diabetes worldwide.[170]

In many low and middle-income countries, diabetes clinics are operated at regional and district hospitals, and access to transport services may be unreliable or unaffordable. Therefore, many patients will walk long distances to attend diabetes clinics. [124] While exercises such as walking result in increased glucose uptake and utilisation by the exercising muscles[196-198], it remains unclear whether the fasting plasma glucose measure is affected by a single bout of exercise such as walking in individuals with type 2 diabetes (T2D). This question is of high clinical relevance in most low-income countries where fasting glucose (obtained at the clinic) is still widely used to monitor glycaemia among people living with T2D. We, therefore, conducted a randomised crossover trial to quantify the immediate impact of a single bout of

continuous aerobic exercise performed in a fasted state on fasting glucose in people living with T2D.

MATERIAL AND METHODS

Study Design and Patients

The study was a multicentre randomised two-period crossover design to test the impact of walking on a treadmill for one hour compared to not-walking on fasting glucose changes in participants with type 2 diabetes. The Uganda Virus Research Institute (UVRI) Institutional Review Board and the Uganda National Council of Science and Technology (UNCST) approved the study.

We enrolled 45 non-insulin-treated type 2 diabetes patients attending diabetes outpatient clinics at one rural and one urban hospital in Uganda. Exclusion criteria included pregnancy, acute illness, and clinical need to immediately increase their glucose-lowering medication and those unable to arrive at the clinics with minimal activity due to location or access to transport. All participants provided written informed consent before participating. The study was registered in the Pan African Clinical Trial Registry (https://pactr.samrc.ac.za/) (PACTR202009486614518). The overview of the study design is presented in Supplementary Figure S1.

Study visits and procedures

Participants attended two study visits: an exercise visit and a resting visit, in random order, as assigned by an independent person using computer-generated random numbers. There was a five-day washout period between visits. Blinding was not possible for this study.

Exercise visit

Participants were asked to come to the clinic in a fasting state before 9 am and were required not to have taken their morning medications. They exercised at a moderate walking pace of 3 mph (1.34 m/s) on a treadmill for 1 hour. Blood samples were taken for laboratory glucose measurement at 0, 30 and 60 minutes, and post-exercise (resting) every 30 minutes for a further 2 hours (3 hours total monitoring).

Rest visit

Participants attended this visit in a fasting state before 9 am. In place of walking on the treadmill, participants rested (while seated) for 3 hours. Samples were taken for laboratory glucose measurement at 0, 30 and 60 minutes, every 30 minutes for a further 2 hours (3 hours total monitoring).

Primary Outcome

The pre-specified co-primary outcomes were change in fasting glucose between the walking and rest visits at 60 minutes and 120 minutes post-commencing the intervention.

Statistics

The study aimed for a sample size of at least 38 with the primary outcome, which would have 80% power to detect a 1mmol/L (0.47 standard deviation) difference in fasting glucose change at 1 and 2 hours from the start of the intervention (exercise or rest) between the walking and rest visit with an alpha of 0.05.

We assessed carryover and period effects using mixed-effects models with 60-minute glucose as the outcome, intervention group, period, and intervention group*period interaction as independent variables, and participant ID as the random effect. We compared the difference in glucose change from baseline between resting and

exercising visits, at 60 and 120 minutes from the start, using paired t-tests. The overall impact of walking (across all the time points 0-180 minutes) was assessed using mixed-effect models adjusted for baseline (time 0) glucose with time point as fixed effects and patient as random effects. We also examined whether the exercise intervention explained further variability in glucose over the total duration using the likelihood ratio test to compare with the rest intervention model.

RESULTS

All 45 recruited participants completed both study visits (Supplementary figure S1). The characteristics of study participants are shown in table 1. 21/45 (46.7%) were female, and the median age was 51. Median (IQR) BMI was 29.7 (26.7, 32.7) and median (IQR) HbA1c of 60.0 (46.0, 82.0) mmol/mol. Median (IQR) fasting glucose was 7.9 (5.5, 10.0) mmol/L and 28/45 participants (62.2%) had fasting glucose ≥ 7 mmol/L.

There was no evidence of period effect (p = 0.29) or carryover effect (p-value = 0.56).

Walking was not associated with a change in fasting glucose at the end of the exercise.

Walking for 1 hour was not associated with changes in fasting glucose at the end of the exercise or after an additional hour of rest. Compared to the resting (control visit) glucose change from baseline (pre-intervention) with exercise was -0.15 (95% CI: -0.55, 0.26) mmol/L (p=0.48) and -0.10 (95% CI: -0.50, 0.31) mmol/L (p=0.64) at 60 and 120 minutes, respectively (Figure 1). Glucose difference was similar across all other post-baseline time points (Figure 1). The absolute values for exercise and rest visits separately are shown in supplementary figure S2.

Table 1: Baseline characteristics (n = 45)

Variables	Median (IQR), n (%)
	Overall
Age	51 (46, 56)
Female	21 (46.7)
Diabetes duration, years	4 (2.0, 7.0)
Treatment	
Metformin (+/-diet only)[a]	07 (15.6)
SU (+/- metformin)[b]	35 (77.8)
Other diabetes drugs [c]	03 (06.6)
BMI, kg/m^2	26.7 (24.0, 29.7)
Body fat, (%)	33.3 (23.7, 44.5)
Visceral adiposity, (%)	9 (6, 11)
Fasting glucose, mmol/L	7.9 (5.5, 10.0)
HbA1c, mmol/mol	66.0 (46.0, 82.0)
C-peptide, pmol/L	1310 (878, 2030)

Legend: Categorical data is presented as frequency (%), continuous data as median (IQR). BMI: Body mass index. a: metformin and diet only (only 1 patient was on diet alone without pharmacological treatment), b: sulphonylureas with or without metformin, c: insulin with or without any other oral therapy, c: Other diabetes drugs include.

Walking was not associated with differences in overall post baseline glycaemia

When assessing all time points using a mixed-effects model, there was no difference in glucose between visits (p=0.67) over the 3 hours post-baseline (supplementary

figure S2). The addition of exercise into the model did not explain further variability in glucose (LR test chi2=9.0, p=0.25).

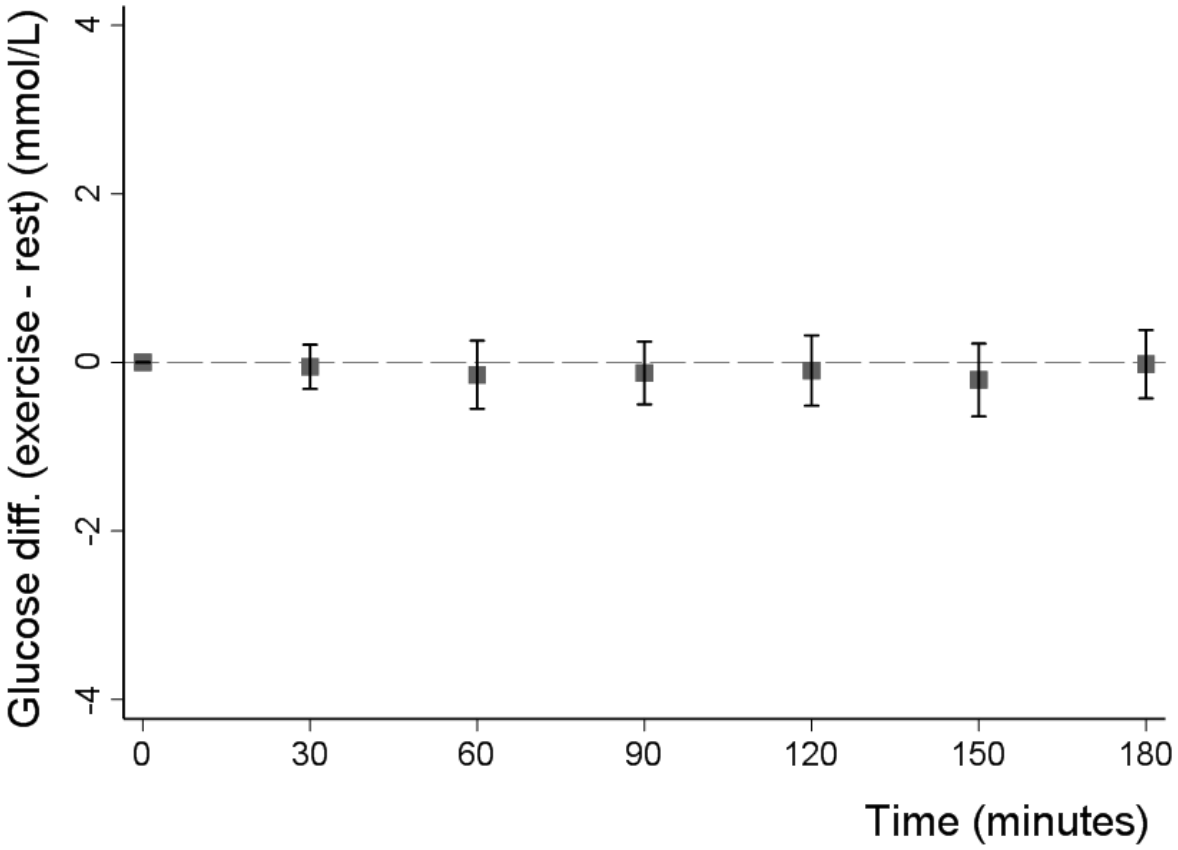


Figure 1: Mean difference (with 95% CIs) in glucose change from baseline between the exercise (walking) and resting visits. Y-Axis shows the difference in glucose change from baseline between the exercise and rest visits (exercise minus rest). The X-axis shows time in minutes from baseline (0 minute) up to 180 minutes from the start of the visits.

DISCUSSION

Our findings demonstrate that in people with type 2 diabetes and significant fasting hyperglycaemia, fasting glucose is not meaningfully altered by 1 hour of walking, with no meaningful change in fasting glucose observed at any point up to 3 hours after commencing exercise.

In this study, we used a randomised crossover trial design to assess the acute effects of continuous walking on fasting plasma glucose. This is one of the key strengths of our study, given that there are few well-designed studies that report the effects of a single bout of exercise on fasting glucose.[199] Additionally, we had sufficient participant numbers and recruited both genders compared to previous studies that recruited a minimal number of participants, majority or all of whom were males (or females in very few studies).[200 201] Furthermore, by allowing a 5-day washout period between the exercise and rest visits, we minimised potential carryover effect from exercise that could have altered the overall effect of rest. This time was sufficient as published data suggest that the effects of exercise last 72 hours (equivalent to 3 days).[202 203] However, the interpretation of our findings should consider the following limitations. First, while we standardised the walking speed, it should be noted that people walk at different speeds in the real world and have different aerobic powers. Therefore, exercise intensity could have varied given the differences in age and gender at the standardised walking speed of 3 miles per hour. Secondly, we excluded insulin-treated patients; our findings cannot necessarily be extrapolated to this subgroup.

To our knowledge very few studies have assessed the acute impact of walking on glucose in the fasting state.[199] In a recent systematic review, only one study was identified that examined the acute effects of single-bout of a walking exercise in adults with type 2 diabetes in a fasted state.[199] Karstoft et al examined the impact of continuous walking and interval walking for 1 hour (60 minutes) in a small study of 10 participants, and found no impact of exercising in a fasted state on fasting plasma glucose.[204] Consistent with our finding, studies assessing the overall effect of exercise on CGM measured glucose among persons living with type 2 diabetes showed no acute effect on fasting glucose.[200 201] In contrast, post-prandial glucose is significantly

affected by a single-bout of exercise and this is well established by majority of studies.[86 204] In comparison to these studies we have studied a far larger population, used a randomised cross over design, measured both pre-and post-exercise glucose and therefore we were able to control for baseline difference in the exercise and non-exercise (control) arms. Similarly, our finding of no meaningful change in fasting glucose post a single bout of continuous exercise is consistent with findings from studies performed in healthy individuals.[205]

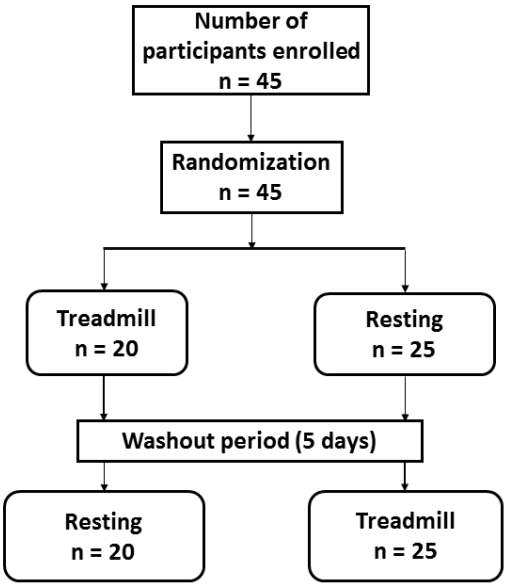
The stable fasting glucose in the walking (exercising) visit suggests that short-term exercise does not affect fasting glucose, although exercising increases glucose intake and utilisation by the contracting muscles via separate mechanisms that are not impaired by insulin resistance, a hallmark of T2D[206]. The mechanisms explaining this finding are unclear. However, the widely accepted hypothesis is that exercise has a more significant impact on post-prandial glucose, which is more correlated with muscle insulin resistance than fasting glucose, which is more related to hepatic insulin resistance.[201] Moreover, it has been established that during fasting, glucose is less utilised as the major source of fuel by peripheral tissues including muscles is minimised.[77] Exercising in the fasted state leads to changes in the hormonal milieu (e.g., reduced insulin levels, increased glucagon, and rise in catecholamine) that may potentially favour fasting glucose stabilisation through glycogenolysis and increased mobilisation of alternative fuel sources like FFAs from triglycerides in the adipose tissue.[80 204 205]

These findings are reassuring for clinicians working in low and middle-income countries where most patients with type 2 diabetes reside. In settings where HbA1c is not readily available/affordable and patients have a long walk to the clinic, fasting

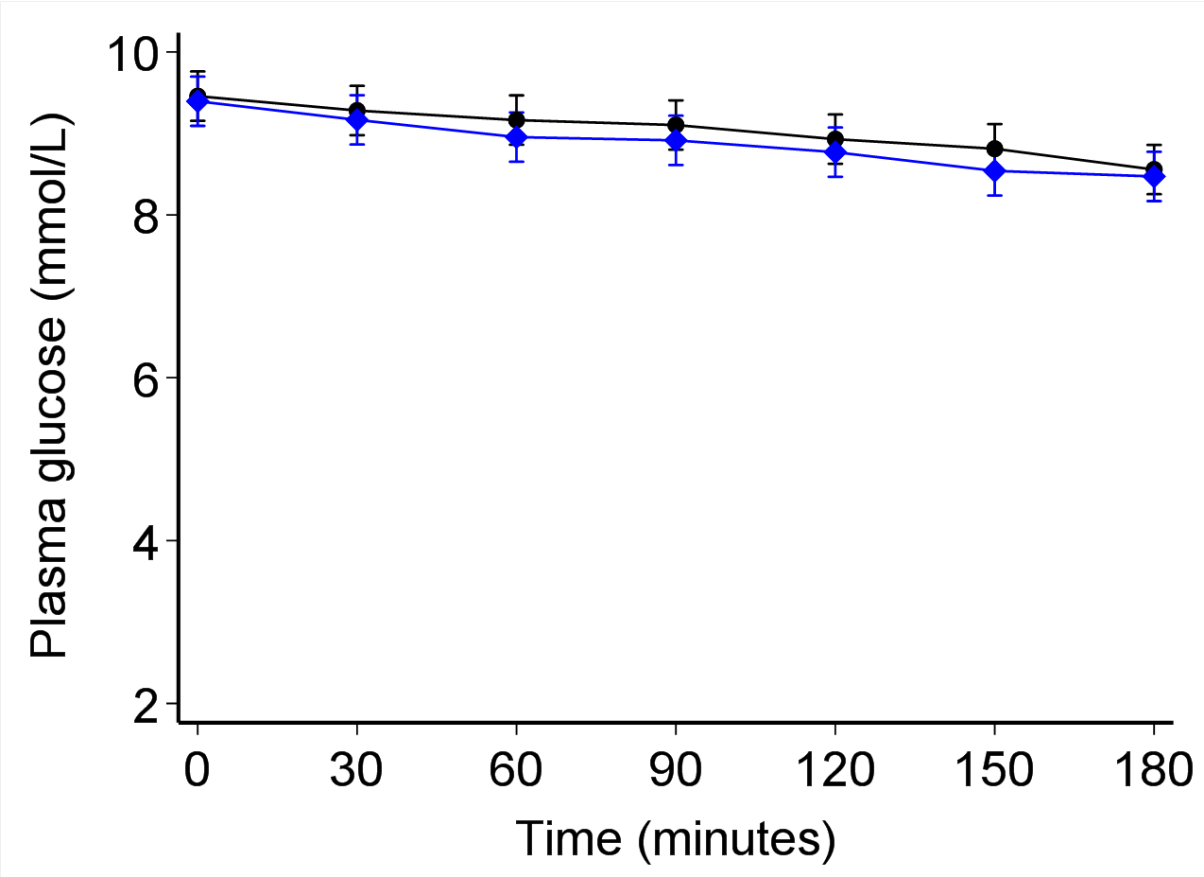
glucose can be utilised to monitor glycaemia and titrate patient medication without worrying about the effects of walking.

In conclusion, fasting plasma glucose is not meaningfully affected by prolonged walking in participants with type 2 diabetes; therefore, the reliability of fasting glucose for monitoring glycaemic burden is unlikely to be altered in patients who walk to the clinic.

Supplementary materials



Supplementary figure S1: Participant flow chart: The cross over study design showing participant flow. Treadmill visit: Participant walks on a treadmill at a speed of 4.5 Km/hr for 60 minutes. Then rests for the next 2 hours. Resting visit: Participant rests for 3 hours.



Supplementary figure S2: Glucose trends between the exercise (Blue diamonds) and resting visit (Black circles) after adjusting for the baseline differences between the two visits. Adjusted means adjusting for baseline differences at the two visits. The error bars denote 95% confidence intervals.

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Chapter 5

Continuous glucose monitoring demonstrates low risk of clinically significant hypoglycaemia associated with sulphonylurea treatment in an African type 2 diabetes population.

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BMJ Open Diabetes Research and Care 2022; 10:e002714.

Acknowledgements of co-authors and contributions to the paper

Myself, Beverley Shields, Andrew Hattersley, Moffat Nyirenda and Angus Jones conceptualised and designed the study. I set up the study in Uganda and obtained ethical approval (with assistance of supervisors), and led the study in Uganda researching the data including undertaking all aspects of recruitment and data collection assisted by our research nurse team. I analysed the data with assistance from with assistance from Alice Carr, Lauren Rodgers, Beverley Shields and Angus Jones. I drafted the paper which was critically reviewed and edited by all authors. I drafted the paper which was critically revised by all authors. All authors approved the final manuscript.

ABSTRACT

Introduction

People living with diabetes in low-resource settings may be at increased hypoglycaemia risk due to food insecurity and limited access to glucose monitoring. We aimed to assess hypoglycaemia risk associated with sulphonylurea and insulin therapy in people living with type 2 diabetes in a low-resource sub-Saharan African setting.

Research design and methods

This study was conducted in the outpatients' Diabetes clinics of two hospitals (one rural and one urban) in Uganda. We used blinded continuous glucose monitoring (CGM), and self-report to compare hypoglycaemia rates and duration in 179 Type 2 diabetes patients treated with sulphonylureas (n=100) and insulin (n=51) in comparison to those treated with metformin only (n=28). CGM-assessed hypoglycaemia was defined according to the international consensus on use of CGM guidelines as the number of hypoglycaemic events that occur over the given CGM reporting period. Clinically significant hypoglycaemic event was defined as readings below the 3.0 mmol/L (54 mg/dL) threshold for at least 15 min in people with type 2 diabetes.

Results

CGM recorded hypoglycaemia was infrequent in sulphonylurea treated participants and did not differ from metformin: median minutes/week of glucose <3mmol/L were 39.2, 17.0 and 127.5 for metformin, sulphonylurea and insulin respectively (metformin vs SU, p=0.6). Hypoglycaemia risk was strongly related to HbA1c and fasting glucose, with most episodes occurring in those with tight glycaemic control. After adjusting for

HbA1c, time <3mmol/L was 2.1 (95% CI: 0.9, 4.7) and 5.5 (2.4, 12.6) times greater with sulphonylurea and insulin respectively than metformin alone.

Conclusions

In a low-resource sub-Saharan African setting, hypoglycaemia is infrequent among people with type 2 diabetes receiving sulphonylurea treatment, and the modest excess occurs predominantly in those with tight glycaemic control.

INTRODUCTION

The prevalence of type 2 diabetes is rapidly increasing especially in low and middle income countries (LMICs) where the majority of people living with type 2 diabetes reside[207]. While complications of type 2 diabetes can be reduced by maintaining glucose control [154 155], glycaemic control for people living with type 2 diabetes in LMICs is often poor [208]. A key barrier to intensifying glucose lowering therapy in low resource healthcare settings is fear of hypoglycaemia. [156 157] Sulphonylureas (SUs) and insulin remain the most available treatments after metformin for people living with diabetes in LMICs[209 210]. Because of limited resources, treatments with lower risk of hypoglycaemia such as the newer classes of SUs (e.g., gliclazide and glimepiride) and analogue insulins, are not readily available in LMICs[210] and robust glucose monitoring is often unaffordable, even for those treated with insulin[9]. Concerns about hypoglycaemia associated with older generation SUs like glibenclamide mean that they may be started at far higher glycaemic thresholds than recommended in international guidance[158 159]

It is not clear whether this fear of hypoglycaemia among type 2 diabetes patients in low resource settings is justified**.** Previous studies investigating the burden of

hypoglycaemia among type 2 diabetes patients in low-resource settings are limited, with available data predominantly from high income countries.[211] Observational and trial data from high income countries suggest that severe hypoglycaemia is rare in patients taking sulphonylureas, but in those with well controlled diabetes non-severe hypoglycaemia may be common[149 150] Studies in high income countries suggest substantially higher rates of hypoglycaemia with insulin than SUs.[151 212] However, these data may not apply in resource poor settings where use of older SUs, with higher hypoglycaemia risk compared to newer generation SUs (e.g., gliclazide and glimepiride) and food insecurity (and therefore missed meals) are common. In addition, due to resource constraints, the majority of those receiving treatment associated with hypoglycaemia will not be able to access capillary glucose monitoring.

We therefore aimed to assess hypoglycaemia risk with SUs and insulin therapy (in comparison to metformin) in people living with type 2 diabetes in a low-resource sub-Saharan African setting.

METHODS

We compared continuous glucose monitoring (CGM) and self-reported hypoglycaemia in people treated with metformin, sulfonylureas or insulin attending diabetes clinics in Uganda. CGM was used to obtain an objective assessment of hypoglycaemia.

Study population

People living with type 2 diabetes attending a routinely scheduled diabetes clinic in a rural-based hospital (Masaka regional referral hospital) and urban-based hospital (St. Francis hospital Nsambya) were invited consecutively. Eligible individuals were aged 18 years and above and treated with metformin, sulfonylurea or insulin. All participants provided written informed consent before entering the study.

Patient and public involvement (PPI)

Patients were involved in prioritization of the research question. Patients were not involved in the design and conduct of the study. However, they were central to dissemination of the results by choosing to have some of the results sent to their respective clinicians, and will continue to be involved in ongoing study dissemination.

Study procedures

We used questionnaires to record baseline patient characteristics including socio-demographic, diabetes medical history, current treatment information, and history of severe hypoglycaemia in the previous 12 months.

We assessed glucose levels over a 14-day period from the baseline visit using the blinded Freestyle Libre Pro Glucose Monitoring System (Abbott Laboratories, Illinois, USA) as previously described.[213]

Hypoglycaemia assessment

CGM-assessed hypoglycaemia was defined according to the international consensus on use of CGM guidelines as the number of hypoglycaemic events that occur over the given CGM reporting period[146]. Clinically significant hypoglycaemic events defined as readings below the 3.0 mmol/L (54 mg/dL) threshold for at least 15 min were considered for this study. The end of a CGM hypoglycaemic event was defined at the point where glucose was at least 3.9 mmol/L (70 mg/dL) for 15 minutes. Hypoglycaemia rate and duration below 3mmol/mol were standardised to events/week and minutes/week per week respectively, to account for variation in duration of CGM measurement. Self-reported hypoglycaemia data was collected using a questionnaire that captured the history of hypoglycaemia requiring assistance of another person,

history and number of times the participant was hospitalised due to hypoglycaemia in the previous 12 months.

Statistical analysis

Statistical analysis was performed using Stata V16.1 (StataCorp LLC, USA).

Medians and interquartile ranges are reported for descriptive data due to skewed nature of most variables. We compared median hypoglycaemia event rate per week and the median minutes below 3mmol/L per week across treatment classes using the nonparametric Wilcoxon rank-sum test. Frequency of self-reported hypoglycaemia and hospital admission due to hypoglycaemia was assessed, and proportions were compared across the three treatment groups using Chi square or Fischer's exact tests.

Hypoglycaemia rate and minutes below 3 mmol/L per week results were positively skewed following a Poisson distribution. We therefore assessed whether the differences in hypoglycaemia rates between the 3 treatment groups were due to confounding by differences in clinical features associated with hypoglycaemia using Poisson regression models. To ensure model assumptions of variance, we fitted Poisson regression with robust standard errors[214]. The differences in minutes below 3 mmol/L were also assessed using Poisson regression; the Poisson regression with robust standard errors (Huber-White-Sandwich linearized estimator of variance) was preferred to log-linear regressions for easy interpretation of results and due to the presence of numerous natural zeros in the outcome of interest (minutes below 3 mmol/L) and overdispersion[215]. We assessed the rates and the minutes below 3 mmol/L, with and without adjustment for glycaemic control (HbA1c or fasting plasma glucose/FPG), age, sex, diabetes duration and BMI. We then visually assessed the

relationship between FPG and HbA1c using scatter plots, and compared rate and duration at different HbA1c and FPG values.

The adjusted means of hypoglycaemia rates and minutes below 3 mmol/L per week were then estimated using the margins command for each treatment class (i.e., metformin only, SUs and insulin) holding HbA1c or FPG (or other adjusted covariates) at the sample population mean. We also estimated adjusted mean rates of hypoglycaemia and minutes per week below glucose levels of 3 mmol/L at clinically relevant HbA1c and FPG thresholds.

RESULTS

Baseline characteristics

179 participants met analysis inclusion criteria (supplementary figure 1). 28 participants were treated with metformin only, 100 were treated with SUs (with or without metformin) and 51 were treated with insulin (with or without metformin and/or SU) (supplementary figure 1). Of the 100 participants treated with SUs, 67 patients (67%) were prescribed glibenclamide, 26 (26%) were prescribed glimepiride and 7 (7%) were prescribed Gliclazide. 42/51 (78.8%) of the patients taking insulin were on mixtard insulin. The median duration of CGM was 14 (IQR: 13, 14) days. Baseline characteristics are shown in Table 1. Participants treated with SU and insulin had substantially higher glycaemia than those treaded with metformin: median HbA1c (mmol/mol) of 66 (IQR: 2, 83), 84 (IQR: 67, 102) and 46 (IQR: 39.5, 63.5) respectively.

Table 1: Characteristics of CGM-assessed and self-reported hypoglycaemia in type 2 diabetes according to treatment

	Median (IQR) for continuous variables, n (%) for proportions		
Variable	**Metformin Group**	**SU Group**	**Insulin Group**
Number	28	100	51
Female, n (%)	18 (64.3)	57 (57.0)	31 (60.8)
Age, years	56.5 (49.5, 61.5)	55.5 (50.0, 62.0)	55.0 (49.0, 64.0)
Diabetes duration, years	5.0 (2.0, 8.0)	6.0 (3.0, 9.0)	10.0 (8.0, 17.0)
BMI, kg/m^2	26.9 (24.2, 29.9)	26.7 (23.7, 30.1)	25.8 (23.1, 30.2)
eGFR	113.4 (96.8, 123.7)	112.8 (93.8, 121.0)	110.8 (92.3, 121.8)
Renal impairment, n (%)	0 (0)	6 (6.0)	4 (7.8)
Glycaemic control			
CGM duration	14 (13, 14)	14 (13, 14)	14 (13, 14)
Average CGM glucose (mmol/L)	6.8 (5.4, 9.9)	8.5 (7.0, 12.0)	10.1 (8.2, 14.5)
HbA1c (%)	6.4 (5.8, 8.0)	8.2 (6.9, 9.6)	9.8 (8.2, 11.3)
HbA1c (mmol/mol)	46 (40, 64)	66 (52, 83)	84 (67, 102)
Fasting glucose	7.2 (5.5, 10.2)	8.2 (6.2, 10.7)	9.3 (7.0, 12.3)
Glucose variability (cv)	0.29 (0.26, 0.33)	0.34 (0.29, 0.39)	0.39 (0.33, 0.47)
SD	2.06 (1.65, 2.93)	3.16 (2.59, 3.85)	4.0 (3.3, 5.2)
Percent time spent in optimal range	78.1 (55.3, 86.4)	60.1 (33.8, 73.9)	40.1 (22.2, 55.4)
Percent time above 10	10.9 (1.3, 35.3)	31.9 (14.3, 66.0)	49.3 (30.8, 74.2)
CGM Hypoglycaemia per week			
Episodes < 3mmol/L	1 (0, 2.3)	0.5 (0, 3.0)	2 (0, 6.0)
Total time/week <3mmol/L, minutes	39.2 (0, 174.8)	17.0 (0, 229.3)	127.5 (0, 637.5)

Percent time < 3mmolL (%)	0.39 (0, 1.74)	0.17 (0, 2.26)	1.27 (0, 6.42)
Self-reported hypoglycaemia, n (%)			
History of hypoglycaemia events, n (%)	7 (25.0)	28 (28.0)	23 (45.1)
Hospitalised for hypoglycaemia in the previous 12 months, yes	1 (3.6)	3 (3.0)	6 (11.8)
Hospitalised for hypoglycaemia in the previous 12 months, % (95% CI)	3.6 (0.1, 18.3)	3.0 (0.6, 8.5)	11.8 (4.4, 23.9)

Legend: Metformin Group includes patients being treated with Metformin only, SU Group includes patients on Sulfonylureas and metformin, and Insulin Group includes patients being treated with insulin with metformin and/or sulfonylureas. Sulfonylureas used included glibenclamide by the majority of patients (70/100), other SUs included glimepiride, Gliclazide, and glipizide. Mixtard was the mainly used insulin (44/51), followed by Glargine (4/51), Actrapid (2/51) and Lente (1/51). Renal impairment was defined as an eGFR (estimated glomerular filtration rate) < 60 ml/min/1.73m^2. Percent time spent in optimal range was defined as the percentage of readings and time spent between 3.9–10.0 mmol/L (70–180 mg/dL).

Hypoglycaemia was infrequent in participants with sulphonylurea treated diabetes, and did not differ from metformin.

Median minutes and rate below 3 mmol/L per week of CGM defined hypoglycaemia were low in those treated with SUs, and similar to rates observed in those treated with metformin (Figure 1 and Table 1).

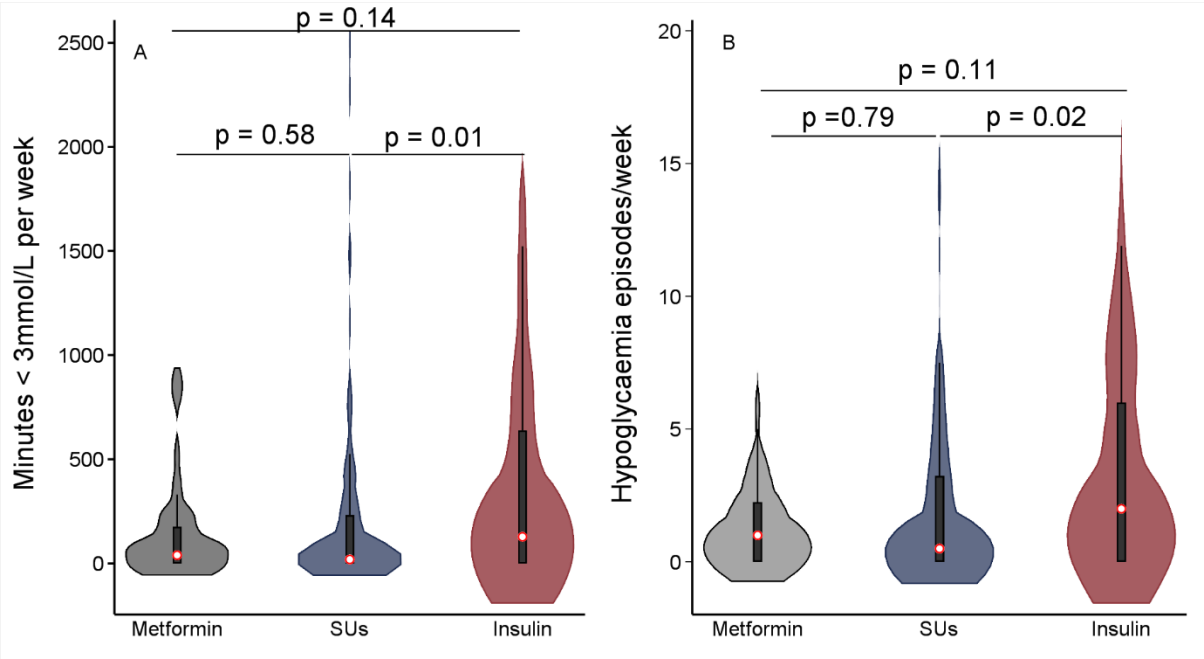

Figure 1: The distributions of hypoglycaemia measured by CGM in individuals treated with metformin only, or sulphonylureas (with or without metformin) and insulin (with or without metformin and/or sulfonylureas)

Median (IQR) minutes below 3 mmol/L per week were 39.2 (0, 174.8), 17.0 (0, 229.3) and 127.5 (0, 637.5) with metformin, SU and insulin respectively. Median hypoglycaemic events/week were 1 (IQR: 0, 2.3), 0.5 (0, 3.0) and 2 (0, 6.0) with metformin, SU and insulin respectively. Self-reported hypoglycaemia results were broadly consistent with CGM findings, with numerically similar proportions of reported hypoglycaemia related hospitalisation with SU (3.0% (95% CI: 0.6, 8.5) and metformin (3.6% (0.1, 18.3)), and higher rates in those treated with insulin (11.8% (4.4, 23.9) (Table 1).

Hypoglycaemia risk was strongly associated with glycaemic control, with most episodes occurring in tightly controlled diabetes.

In those treated with SU and Insulin time spent in hypoglycaemia and hypoglycaemic event rate were strongly associated with glycaemic control, with differences in HbA1c

explaining 33.1% (p = <0.001) and 20.7% (p = 0.005) of variation in time below 3mmol/l for SU and insulin respectively (figure 2).

The majority of hypoglycaemia occurred in those with lower HbA1c or fasting glucose (figure 2 (time <3mmol/L) and supplementary figure 2 (hypoglycaemia rate). Participants with HbA1c below 53 mmol/mol (7%) spent 2.34% (IQR: 0.60, 4.49) and 5.61% (0.34, 13.80) of their total time per week in hypoglycaemia (<3mmol/L), for SU and insulin respectively. In comparison, those who had an HbA1c ≥ 53 mmol/mol on SU spent 0.0% (IQR: 0.00, 0.92) and those on insulin spent 1.27% (0.00, 5.75) of their total time per week in hypoglycaemia (<3mmol/L).

Participants with fasting glucose <7mmol/L spent 2.40% (IQR: 0.60, 4.98) and 6.52% (IQR: 1.24, 13.50) of their total time per week in hypoglycaemia, for SU and insulin respectively, in comparison to only 0.0% (IQR: 0.00, 0.46) and 0.67% (IQR: 0.00, 3.44) for those who had fasting glucose ≥ 7 mmol/L (supplementary table 1).

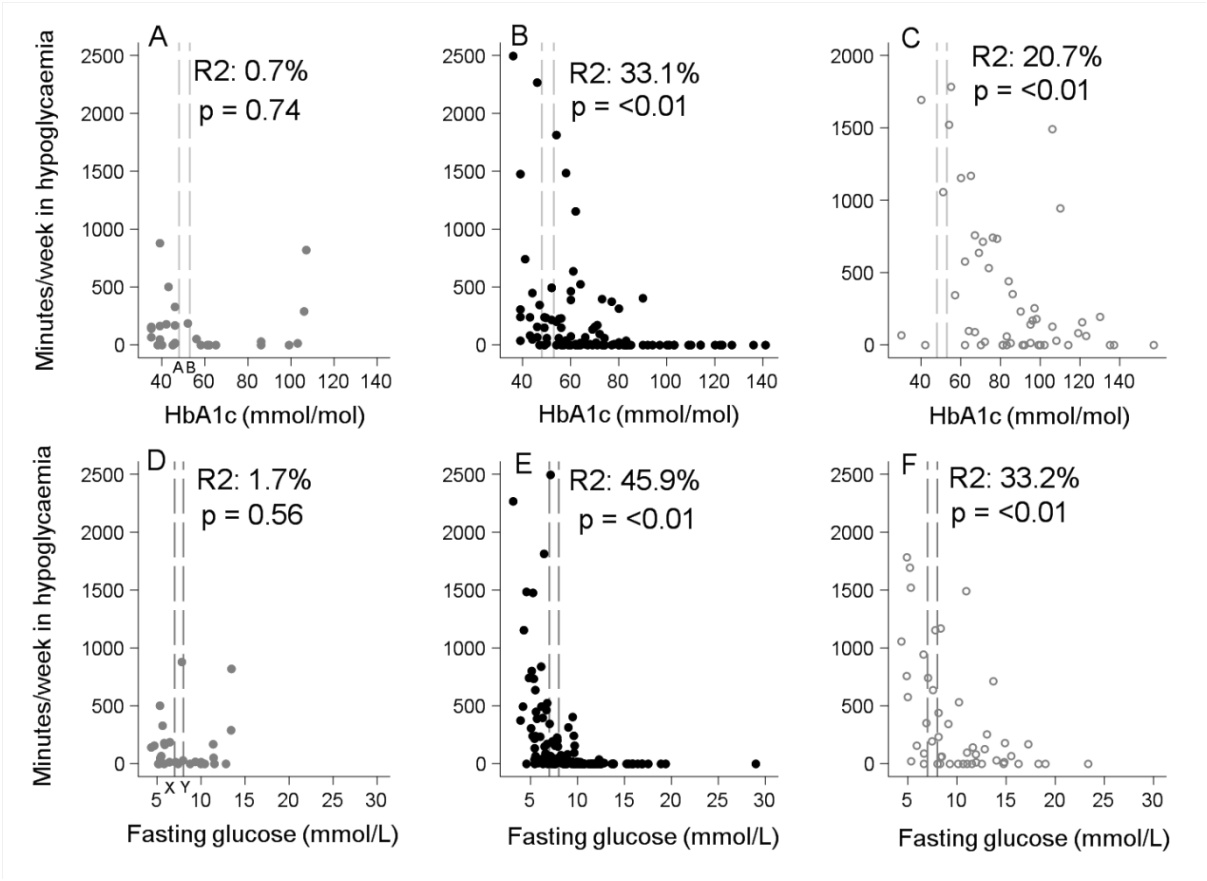

Figure 2: Comparison of Glycaemic control and Hypoglycaemia duration (minutes per week <3mmol/L). Graphs in the top row show the relationship between HbA1c and the number of minutes spent in hypoglycaemia per week for metformin (A), sulphonylureas (B) and insulin (C) treated participants respectively. The bottom row shows the relationship between Fasting glucose and number of minutes spent in hypoglycaemia per week for metformin (D), sulphonylurea (E) and insulin (F) treated participants respectively. The long-dashed lines denote glycaemic thresholds, HbA1c 6.5% (48 mmol/mol) and 7.0% (53 mmol/mol) (top row), fasting glucose 7.0 mmol/L and 8.0 mmol/L (bottom row). R^2 = R-Squared

In analysis adjusted for HbA1c participants receiving SU or insulin treatment experienced 2 and 5 times more hypoglycaemia respectively than those receiving metformin.

Table 2 shows mean and rate ratio for minutes in hypoglycaemia by treatment (relative to metformin), unadjusted and with adjustment for HbA1c (model 2) and HbA1c, age, diabetes duration, BMI and sex (Model 3). In unadjusted analysis, the mean number

of minutes <3 mmol/L per week for SU and metformin treatment did not substantially differ (duration ratio SU vs metformin 1.4 (95% CI: 0.69, 2.91), p = 0.35), but duration in hypoglycaemia substantially higher with insulin than metformin (duration ratio 2.5 (95% CI: 1.3, 5.0), p=0.009). After adjusting for HbA1c, differences between therapies were accentuated, with minutes <3mmol/mol 2.1 (95%CI: 0.9 - 4.7, p value = 0.067) and 5.5 (95% CI: 2.4 - 12.6, p value = <0.001) times greater than metformin with SU and insulin respectively. Findings were not substantially altered by further adjustment for age, BMI, diabetes duration, renal impairment and sex.

Table 2: Number of minutes <3 mmol/L per week in type 2 diabetes patients on different glucose-lowering agents before and after adjusting for HbA1c and clinical features

	Variables	Minutes< 3 mmol/L (95% CI)	Duration ratio (vs metformin)	P-value
Model 1	Metformin (Ref)	146.0 (60.6, 231.3)	1.0	
R2 = 0.05	SU	206.7 (119.2, 294.2)	1.4 (0.7, 2.9)	0.345
	Insulin	365.9 (229.9, 501.9)	2.5 (1.3, 5.0)	0.009
Model 2	Metformin	74.0 (14.6, 133.4)	1.0	
R2 = 0.23	SU	156.9 (97.6, 216.3)	2.1 (0.9, 4.7)	0.067
	Insulin	405.7 (262.1, 549.3)	5.5 (2.4, 12.6)	<0.001
Model 3	Metformin	96.4 (20.2, 172.6)	1.0	
R2 = 0.30	SU	157.5 (97.6, 217.4)	1.6 (0.7, 3.6)	0.230
	Insulin	355.0 (212.7, 497.2)	3.7 (1.5, 9.3)	0.006

Model 1: Unadjusted, Model 2: Adjusted for HbA1c, Model 3: Adjusted for HbA1c, Age, Diabetes duration, BMI, sex and renal impairment. Adjusted minutes < 3 mmol/L are adjusted to the mean value for the covariate for the cohort (mean cohort HbA1c 73.2 mmol/mol). 95% CI are shown in the parentheses. Renal impairment was defined as an eGFR (estimated glomerular filtration rate) < 60 ml/min/1.73m^2.

When adjusting to HbA1c of 53mmol/mol (7%), an internationally recognised target for glycaemic control, estimated minutes in hypoglycaemia (per week) were 137.2 (95% CI: 49.6, 224.7), 290.9 (168.8, 413.0) and 751.9 (433.9, 1070.0) with metformin, SU and insulin respectively (supplementary materials figure 3). Findings were similar for hypoglycaemia rates per week, with rates approximately 2 and 5 times higher with SU and insulin than metformin after adjustment for HbA1c (Table 3). Estimated adjusted mean rates of hypoglycaemia at a range of clinically relevant HbA1c (and FPG) thresholds are shown in supplementary figure 4.

Table 3: Hypoglycaemia rates in type 2 diabetes patients on different glucose-lowering agents before and after adjusting for HbA1c and clinical features

	Variables	Rates (95% CI)	Rate ratio (vs metformin)	P-value (verses metformin)
Model 1	Metformin	1.3 (0.7, 1.9)	1.0	
R^2 = 0.03	(Reference)			
	SUs	2.1 (1.4, 2.8)	1.6 (0.9, 2.7)	0.108
	Insulin	3.2 (2.1, 4.2)	2.4 (1.4, 4.2)	**0.002**
Model 2	Metformin (reference)	0.6 (0.3, 1.0)	1.0	
R^2 = 0.21	SUs	1.5 (1.1, 2.0)	2.4 (1.4, 4.1)	**0.001**
	Insulin	3.8 (2.3, 4.6)	5.4 (3.0, 9.9)	**<0.001**
Model 3	Metformin (reference)	0.7 (0.3, 1.1)	1.0	
R^2 = 0.24	SUs	1.6 (1.1, 2.0)	2.1 (1.2, 3.6)	**0.006**
	Insulin	3.2 (2.0, 4.4)	4.4 (2.2, 8.7)	**<0.001**

Table 2 legend: Model 1: Unadjusted, Model 2: Adjusted for HbA1c, Model 3: Adjusted for HbA1c, Age, Diabetes duration, BMI, sex and renal impairment. Adjusted rates are adjusted to the mean value for the covariate for the cohort (mean cohort HbA1c 73 mmol/mol). Renal impairment was defined as an eGFR (estimated glomerular filtration rate) < 60 ml/min/1.73m^2.

DISCUSSION

This study has demonstrated that both CGM assessed and self-reported clinically significant hypoglycaemia in participants treated with sulfonylureas in Uganda is infrequent among patients who receive SU treatment. While observed hypoglycaemia rates and duration were similar in those treated with metformin and SU, hypoglycaemia risk was strongly associated with glycaemic control, and after adjusting for differences in HbA1c, the risk of hypoglycaemia doubled and quintupled in those treated with SUs and insulin respectively. The modest hypoglycaemia excess associated with SUs in comparison to metformin occurred predominantly in those with tight glycaemic control. Hypoglycaemia was more common in insulin treated diabetes than those treated with SU, further increasing upon adjustment for glycaemic control.

Studies comparing hypoglycaemia risk across different treatments in type 2 diabetes are limited in low and middle income countries, especially Sub-Saharan Africa. The few hypoglycaemia-related studies among people with type 2 diabetes patients in Sub-Saharan Africa that have assessed the incidence and prevalence of hypoglycaemia have predominantly used self-reported hypoglycaemia and documented increased risk with insulin use.[216] The majority of these studies either included only patients on insulin and or grouped SUs together with other oral glucose lowering agents.[159 216 217] Our finding that SU treatment is associated with a modest risk of clinically significant hypoglycaemia among those with type 2 diabetes is consistent with studies in other popualtions[218 219] However, it should be noted that the SUs in these studies are of newer generation, like gliclazide and glimepiride, that are known to have a lower hypoglycaemia risk compared to glibenclamide.[209] The present study, although not designed to compare intra SU-class differences showed a modest hypoglycaemia risk even when majority (two out of three) of our patient population were taking

glibenclamide, an older agent with higher hypoglycaemia risk.[209] Moreover, the modest hypoglycaemia excess in the SUs group mainly occurred in a small proportion of patients with tightly controlled diabetes, below international glycaemic targets.[220-222]

A key strength of this study is the objective assessment of hypoglycaemia through use of blind CGM monitoring. This removed potential biases that could arise from patient reactivity to glucose measurements, differences in glucose testing by treatment, hypoglycaemia unawareness and recall bias that may affect studies assessing self-reported hypoglycaemia or using medical records. An additional strength is comparison across therapies. It is well known that CGM can report occurrence of hypoglycaemia in those who do not have diabetes, or are treated with medications not associated with hypoglycaemia risk[223 224], meaning the absolute risk of meaningful hypoglycaemia by CGM will be over-estimated. By including a metformin 'control' arm in our study, we ensured to avoid this overestimation by assessing the excess risk. A notable limitation of our study was that routine capillary glucose monitoring is not available to the vast majority of people with diabetes in Uganda, due to cost. Therefore, self-reported hypoglycaemia is very unlikely to have been confirmed by glucose testing, and is likely to be inaccurate in a population like ours where healthy literacy including hypoglycaemia education is not good. Such testing may even be limited in a healthcare setting. Additionally, the modest number of participants treated with only metformin will have impacted our ability to detect modest differences in hypoglycaemia risk in comparisons against metformin, as shown by the large confidence intervals of estimates for metformin treated participants. Lastly, the majority of participants with SU and insulin treated diabetes had poor glycaemic control, while this reflects current practice in this region, given the strong relationship between glycaemic control and hypoglycaemia risk it is likely that hypoglycaemia rates would be substantially higher

were glycaemic control improved in this population, as suggested by our adjusted analysis.

Glycaemic control is the cornerstone of lowering microvascular complications among people living with diabetes. While there is no doubt that there is an association between SUs (especially the older agents like glibenclamide) and insulin treatment and hypoglycaemia, the high rates of poor glycaemic control in type 2 diabetes patients and relatively low hypoglycaemic events among patients taking SUs suggest that there is room for optimizing glycaemic control using these cheap, readily available and effective agents, despite the specific challenges of food insecurity and lack of glycaemic monitoring in many LMIC populations . This supports the recommendations to optimize glycaemic control using these readily available and affordable agents including metformin and SUs.[130] [210] The modest excess of hypoglycaemia was predominantly seen in a small proportion of patients taking SUs whose fasting glucose was less than 7 mmol/L or HbA1c < 7% (53mmol/mol) (thresholds often recommended by international guidelines) suggesting caution is needed when treating below these levels.[222]

In conclusion in a low resource sub-Saharan African setting, clinically significant hypoglycaemia is infrequent among people with type 2 diabetes receiving Sulphonylurea treatment, and the modest excess occurs predominantly in those with tight glycaemic control.

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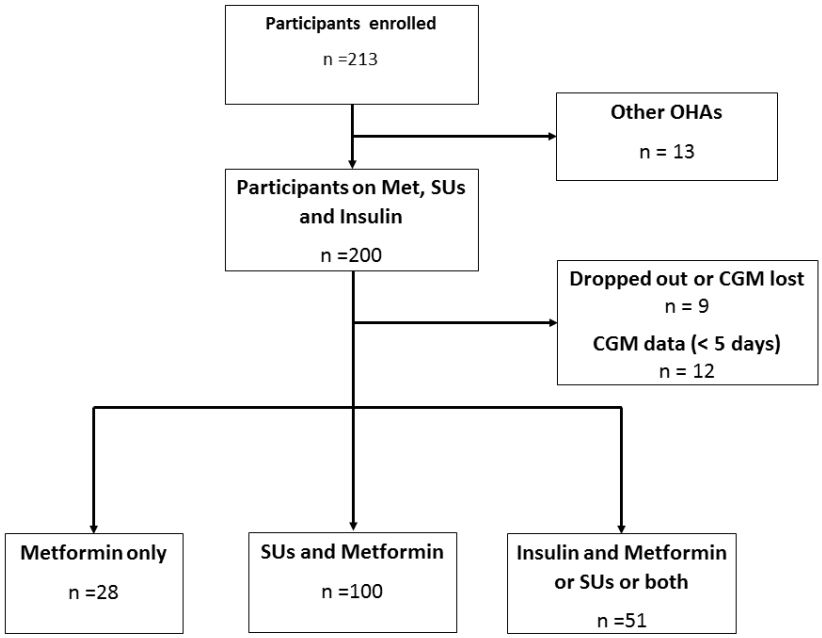
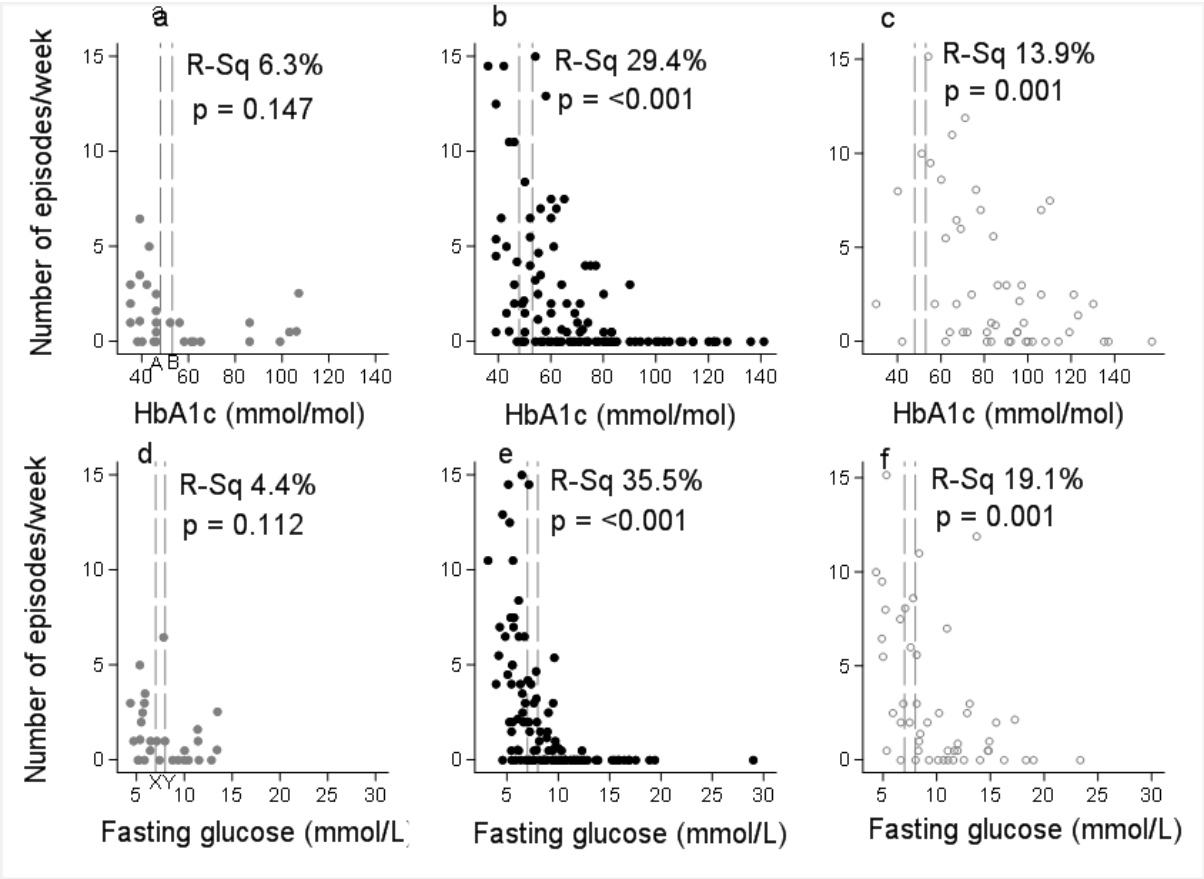


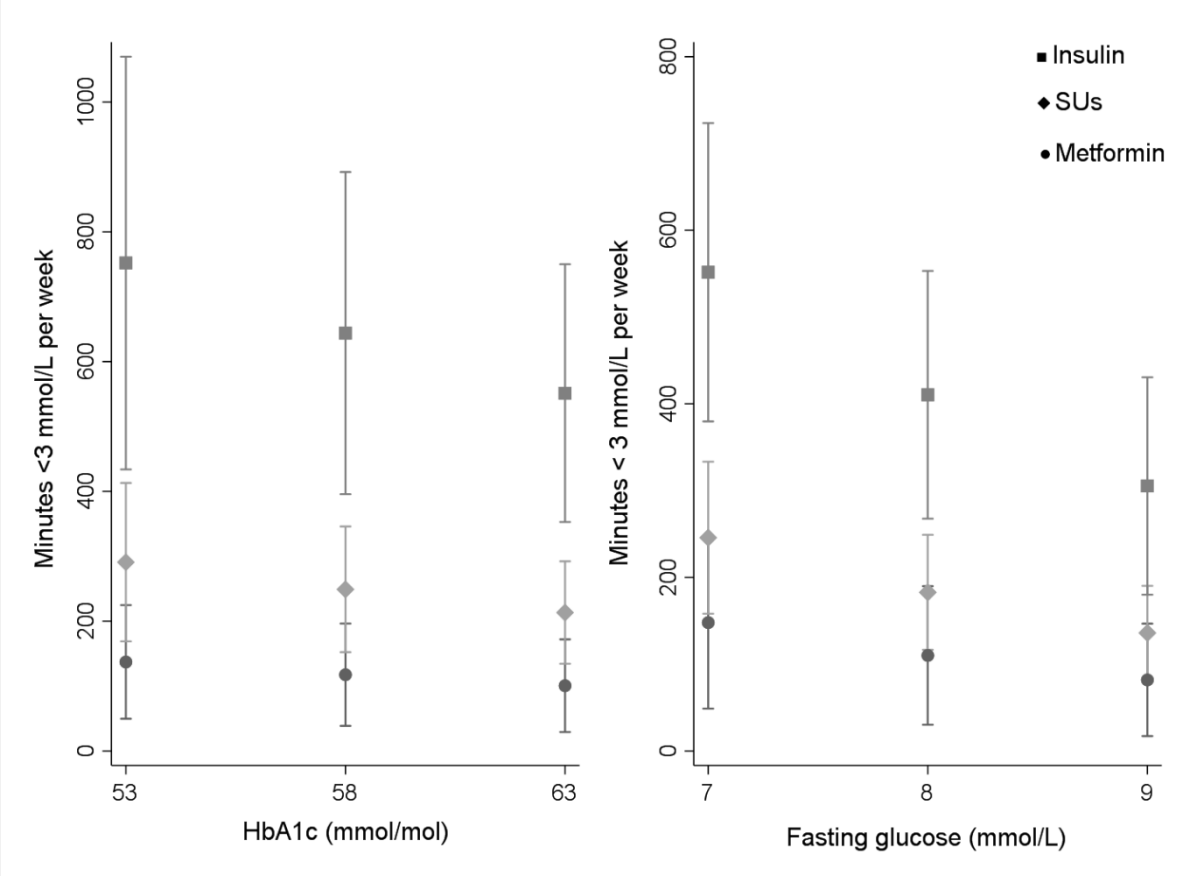
Supplementary figure 1: Participant flow chart

Supplementary Table 1: Percentage of time below 3 mmol/L per week stratified by type of medication and glycaemia control

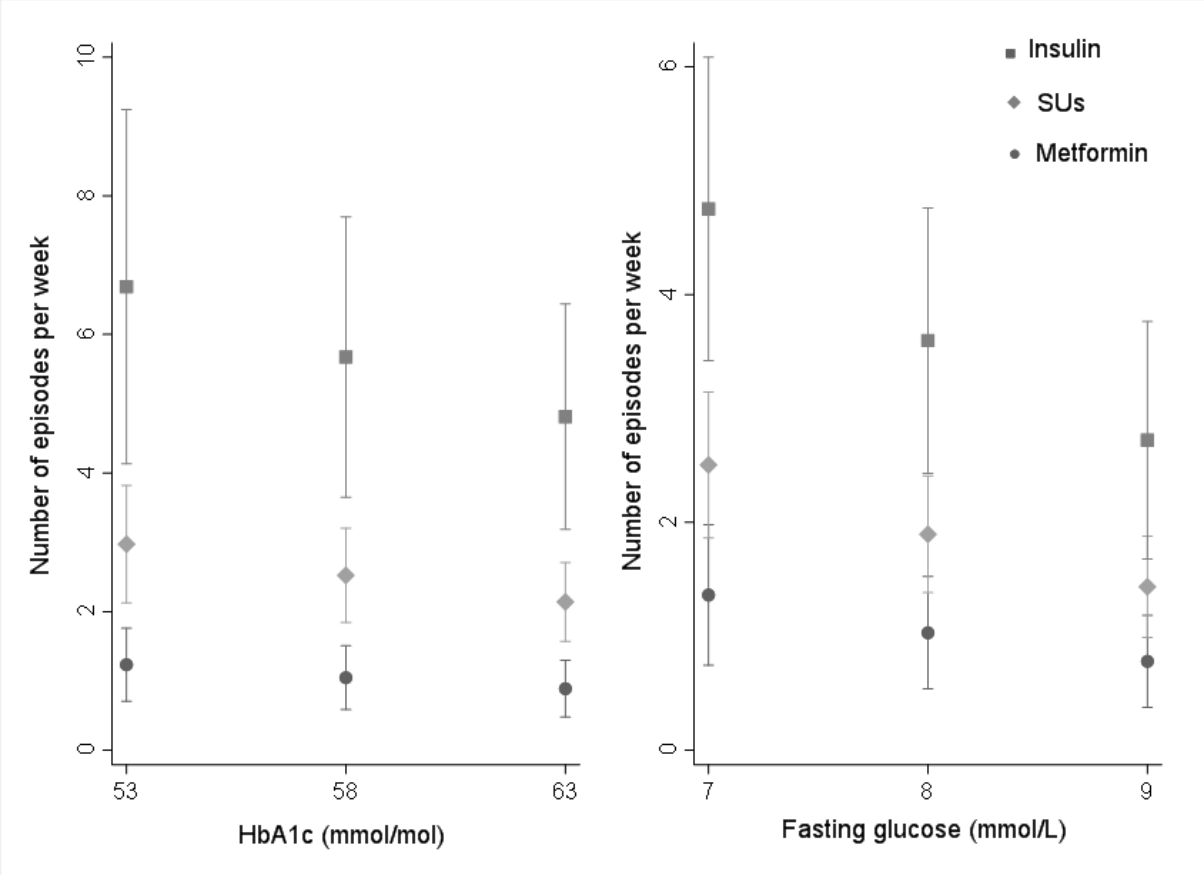
	Metformin		**SUs**		**Insulin**	
HbA1c (mmol/mol)	**HbA1c < 53**	**HbA1c ≥ 53**	**HbA1c < 53**	**HbA1c ≥ 53**	**HbA1c < 53**	**HbA1c ≥ 7.0 %**
% time < 3 mmol/L per week	1.42 (0.15, 1.8)	0.00 (0.00, 0.52)	2.34 (0.60, 4.49)	0.00 (0.00, 0.92)	5.61 (0.34, 13.80)	1.27 (0.00, 5.75)
FPG (mmol/L)	**FPG < 7.0**	**FPG ≥ 7.0**	**FPG < 7.0**	**FPG ≥ 7.0**	**FPG < 7.0**	**FPG ≥ 7.0**
% time < 3 mmol/L per week	1.42 (0.15, 1.8)	0.15 (0.00, 1.68)	2.40 (0.60, 4.98)	0.00 (0.00, 0.46)	6.52 (1.24, 13.50)	0.67 (0.00, 3.44)



Supplementary figure 2: Comparison of HbA1cand Hypoglycaemia rates and duration per week.



Supplementary figure 3: Estimated number of minutes below 3 mmol/L per week at different HbA1c and fasting plasma glucose levels. The error bars denote 95% confidence intervals.



Supplementary figure 4: Estimated number of minutes below 3 mmol/L per week at different HbA1c and fasting plasma glucose levels. The error bars denote 95% confidence intervals.

Chapter 6: Discussion

The work presented in this thesis demonstrates that HbA1c is the optimal method for assessing glycaemic control, even in populations with high prevalence of conditions reported to affect test reliability. Fasting and random glucose are valuable alternatives where HbA1c is not available even in patients who walk to the clinic since it is not meaningfully affected by prolonged walking.

We have shown that the accuracy of HbA1c, GA and fructosamine in reflecting glycaemic control is not affected by sickle cell trait. We have demonstrated that in patients with anaemia and renal impairment, the relationship between HbA1c and glucose is altered, but fructosamine and GA do not appear to improve assessment of glycaemic burden.

We have also used continuous glucose monitoring to demonstrate that clinically significant hypoglycaemia is infrequent among people with type 2 diabetes receiving Sulphonylurea treatment, and the modest excess occurs predominantly in those with tight glycaemic control.

This chapter gives an overview of the main findings of this thesis and discusses the work's conclusions, implications, limitations and potential areas for further research.

Chapter 2: HbA1c performs well in monitoring glucose control even in populations with medical conditions that may alter its reliability.

This chapter compares the performance of HbA1c, fasting plasma glucose and Random non-fasting plasma glucose to mean CGM glucose, and assesses the overall impact of comorbidities reported to alter HbA1c reliability. We also compare fasting plasma glucose and random non-fasting plasma glucose to HbA1c in a subset of people living with T2DM without HbA1c comorbidities (anaemia, sickle cell trait and renal impairment).

We highlight the pros and cons and factors that may alter the performances of these methods. Most importantly, we draw attention to HbA1c, the widely recommended test for assessing glycaemic control. Furthermore, we summarise the main findings and discuss the conclusions, implications, limitations, and questions that remain unanswered to data that may be potential areas for future research.

Conclusion

Assessing glycaemic control in settings with limited resources can be challenging, especially when there is no capacity to measure HbA1c, which is a long-term marker of glycaemia and when there are no resources to rule out comorbidities that can alter its accuracy. We found a high prevalence (32.8%) of medical conditions thought to affect HbA1c reliability. Specifically, the prevalence of anaemia, sickle cell trait and renal impairment was 9.4% (18/192), 22.4% (43/192), and 6.3% (12/192), respectively. Despite high prevalence of medical conditions thought to affect HbA1c reliability, HbA1c had the strongest correlation with mean CGM glucose (Pearson correlation coefficient, r= 0.88, 95% CI: 0.84, 0.91), followed by FPG (0.82, 95% CI: 0.76, 0.86), and RPG (0.76, 95% CI 0.69, 0.81).

HbA1c maintained a similar relationship with CGM glucose in those with and without conditions that might affect HbA1c reliability ((0.85 (95%CI: 0.76, 0.91) versus (0.89 (0.85, 0.92) respectively) and the difference in linear regression slopes was modest (mean CGM glucose =0.14*HbA1c – 0.02 and 0.16*HbA1c – 1.07 with and without conditions that may affect HbA1c reliability respectively). Among participants without conditions thought to alter HbA1c reliability, the correlation between HbA1c and each Fasting plasma glucose and Random non-fasting plasma glucose was similar (0.74 (95% CI: 0.65, 0.80) and 0.78 (0.71, 0.84) respectively). In conclusion, HbA1c appears to have better overall performance than single glucose measures, even in those with conditions reported to affect reliability. Our results support the use of single glucose measurements (fasting glucose or random glucose) where HbA1c is not available as the demonstrated high sensitivity, specificity and predictive values for predicting sub-optimal glucose control.

Implications of study findings

HbA1c performs well and should be the preferred test to single glucose were available and affordable. Moreover, HbA1c remained more accurate than single glucose measures for assessing glycaemic burden even in non-glycaemic conditions. Therefore, in settings where it is impossible to detect sickle cell trait, renal impairment or anaemia (which is the case in most up-country health facilities in Uganda and the rest of SSA), HbA1c can still be reliably utilised.

Single glucose measures (Fasting plasma glucose and random non-fasting plasma glucose) perform reasonably well in identifying poor glycaemic control. Therefore, clinicians in low-resource settings, especially in the periphery settings where HbA1c is unavailable like most rural clinics, can manage people based on single glucose

measures because they are cheap, easy to do and provide immediate results. The very modest loss of diagnostic test performance using random non-fasting plasma glucose provides some reassurance that random non-fasting glucose- the most practical measure in situations where fasting is impossible can be used.

Limitations

This study was among the first to use an independent marker of glycaemic burden to assess the performance of HbA1c or single glucose measures compared to glucose measured in day-to-day living and the impact of HbA1c analytical issues on HbA1c performance in a low resource setting. The study was performed in clinically diagnosed type 2 diabetes patients. However, it would be good to extend this study to include type 1 diabetes patients and other patient sub-populations, for example, children. Glycaemia was measured using a CGM sensor over median 15 (IQR: 13 – 14) days, and while an estimated 50% of HbA1c variation reflects the previous two weeks of glucose, hba1c is influenced by glucose over the previous 2-3 months[1], which our measure would not have captured. Although we consider CGM the best available option for the direct measurement of average glucose, these meters have imperfect accuracy. Therefore while this measure allows us to compare the relative performance of HbA1c and glucose tests robustly against an independent assessment of mean glycaemia, a discrepancy on an individual level between CGM and another measure may reflect an error in either measurement. We also did not assess for thalassaemia, another common regional haemoglobinopathy that may affect HbA1c results. Also, we grouped individual non-glycaemic conditions that are thought to alter HbA1c reliability. These conditions (anaemia, sickle cell trait and renal impairment) may have different effects on HbA1c, and we did not examine them individually. Even though our study was powered to assess the overall impact of these conditions on

HbA1c performance, we had a minimal number of patients with renal impairment and anaemia. However, in chapter 4, where we compared the performance of HbA1c to fructosamine and glycated albumin, we went on to assess the impact of these individual conditions on HbA1c and the other alternative measures. Moreover, we used the HbA1c immune assay, one of the widest available – and therefore results for the performance might not apply to other HbA1c assay types, which may have different susceptibility to the effects of haemoglobinopathy.

Future research

HbA1c remains the optimal test for monitoring glycaemic control displaying the best correlation with mean blood glucose even in LMIC settings with a high prevalence of comorbidities thought to affect the accuracy of HbA1c including anaemia and haemoglobinopathies. However, the small subgroup numbers in our study limited the power to determine the impact of these comorbidities on HbA1c performance. This underscores the need for more extensive studies in which the sample population is enriched with enough patients with these comorbidities to give us enough power to compare HbA1c accuracy in those with and without (controls) such comorbidities. Moreover, the clinical impact of these comorbidities may not be very significant when considered separately, but in LMIC settings where patients are likely to manifest with multiple of these comorbidities (i.e., iron deficiency without anaemia, vitamin B12 and folate deficiencies, haemoglobinopathies, among others), their constellation in one patient has the potential to significantly impact HbA1c.

Furthermore, while our data show that HbA1c (measured using an immunoassay method) has the closest relationship with average glucose, even with comorbidities, the overall relationship between glucose and HbA1c may be different in this

population; therefore, the thresholds used internationally are not appropriate, and bespoke HbA1c thresholds are needed for different populations.

While a lot of work remains to be done in regards to understanding the performance of HbA1c in LMIC settings that are heavily impacted with increasing rates of type 2 diabetes and poor glycaemic control, our overall recommendation is that HbA1c performs well and should be the preferred test to single glucose where available and affordable for monitoring glycaemic control in LMIC settings where haemoglobinopathies and anaemia are prevalent. However, where HbA1c testing is not available or affordable, fasting glucose and random non-fasting glucose perform reasonably well in identifying poor glycaemic control and can be used. Furthermore, studies are needed to assess the clinical utility and cost-effectiveness of point of care measurement of HbA1c as this is likely to be more affordable and more accessible for people with DM than the laboratory based method used in these studies.

Chapter 3: Examining the role of fructosamine and glycated albumin in assessing glycaemic control in individuals living with type 2 diabetes in Uganda.

In this study, we aimed to assess the performance of GA and fructosamine tests in determining glycaemic control among Ugandan participants with type 2 diabetes.

We compared fructosamine, GA, and HbA1c (measured by immunoassay) to mean glucose from 14 days of continuous glucose monitoring in 192 participants with type 2 diabetes. In addition, we assessed whether the relationship between these assays and continuously measured glucose was altered by the presence of conditions reported to affect HbA1c reliability (sickle cell trait, anaemia and renal impairment), including specific subgroup analysis of anaemia, sickle cell trait and renal impairment. 43/192 (22.4%) had haemoglobinopathies (predominantly sickle cell trait), 18/192 (9.4%) had anaemia, and 12/192 (6.3%) had renal impairment.

Conclusion

The key finding of this chapter is that the overall association of HbA1c, GA and fructosamine with CGM assessed glucose was similar [(r = 0.88 (95% CI: 0.84 – 0.91), 0.84 (0.79 – 0.88) and 0.84 (0.79 – 0.88) respectively]. Also, within those with conditions reported to affect HbA1c reliability, the correlation between mean CGM glucose and each of HbA1c, GA and fructosamine was similar (r = 0.85 (95% CI: 0.76 – 0.91)), 0.76 (0.62 – 0.86), 0.74 (0.60 – 0.84) respectively). We also found that sickle cell trait did not alter the relationship between HbA1c, fructosamine or GA with CGM glucose (p-value for interaction >0.3 for all).

However, patient who were anaemic had a lower HbA1c (β= 0.07 (95% CI: 0.04 – 0.09) than those without anaemia (β= 0.15 (0.14 – 0.17) for a given level of glucose

(p for interaction <0.001). Similarly, the anaemia group had a lower fructosamine (beta = 0.01, 95% CI: 0.01 – 0.02) than those without anaemia (beta = 0.02, 95% CI: 0.02 – 0.02) for a given mean CGM glucose (p for interaction =0.004). There was no difference in slopes for the association of GA with average CGM glucose between those with anaemia and those without (p = 0.216). HbA1c, glycated albumin and fructosamine were lower for a given mean CGM glucose among those with renal impairment than those without renal impairment (p-value for interaction < 0.001 for all).

In conclusion, HbA1c has the best overall performance in monitoring glucose control, even in SSA, where medical conditions that may alter its reliability are prevalent. Fructosamine and glycated albumin do not improve performance over and above HbA1c. The accuracy of HbA1c, glycated albumin and fructosamine in reflecting glycaemic control is not affected by sickle cell trait. In those with anaemia and renal impairment, the relationship between HbA1c and glucose is altered, but fructosamine and GA do not improve the assessment of glycaemic burden.

Implications of study findings

Our results suggest that switching to GA or fructosamine would not improve the accuracy of glycaemic monitoring among African type 2 diabetes patients above and beyond HbA1c. Therefore, these findings do not support recent recommendations from diabetes organisations such as ADA to use fructosamine and glycated albumin as alternative glycaemic control markers in patients with whom HbA1c is unreliable. Furthermore, our work suggests that the accuracy of HbA1c (measured by HbA1c immunoassay), GA and fructosamine in reflecting glycaemic control is not affected by sickle cell trait. In contrast, in those with anaemia or renal impairment, HbA1c, fructosamine and GA may all be unreliable in reflecting glycaemic burden.

Limitations

To our knowledge, this is the first study to simultaneously evaluate HbA1c, GA and fructosamine as glycaemic monitoring tools assessed against an independent CGM in an African type 2 diabetes population. The first limitation of this study is that even though our study population had a substantial number, subdividing them according to the various HbA1c comorbidities led to smaller subgroups. As a result, findings may differ depending on the severity of the condition; for example, mild anaemia vs severe anaemia or mild renal impairment vs severe renal impairment). Furthermore, further characterisation of people with these comorbidities was impossible given the limited number of patients with renal impairment and anaemia.

Secondly, sickle cell trait was the predominant haemoglobinopathy in our study. Therefore our findings cannot be generalised to other haemoglobinopathies such as HbC, common in other parts of SSA, were rare in this setting[2]. A further limitation of the study is that we measured HbA1c using the immunoassay method only and therefore our results may not apply to other HbA1c assays methodologies, which are known to have different susceptibility to the effects of different haemoglobinopathies.

Our findings are limited to three non-glycaemic conditions thought to alter HbA1c reliability (anaemia, sickle cell trait and renal impairment). In the present study, we could not assess the impact of other individual underlying non-glycaemic conditions[3]. For example, we have not examined the impact of glucose-6 phosphate dehydrogenase (G6PD) variants, and a common condition among people of African ancestry that may affect HbA1c results reliability was not assessed.

Future research

We need more research to confirm these results and assess their clinical implications in SSA. Although there were marked differences in HbA1c, GA and fructosamine between type 2 diabetes patients with anaemia and renal impairment compared to those who did not have these comorbidities, there were wide confidence intervals due to the small sample size for participants with these comorbidities. Furthermore, not everyone with anaemia or renal impairment had low HbA1c values. It would be good to replicate these findings in a multi-regional observational study containing a more significant number of participants with these HbA1c comorbidities. Such a study would ideally be enriched with patients at different stages of the disease, i.e., mild anaemia, severe anaemia and renal impairment. A similar cohort of type 1 diabetes patients and insulin-treated type DM would allow a comparison of the performance of HbA1c and these alternative markers of glycaemia in assessing glycaemic control. This is of high clinical relevance given that the impact of non-glycaemic factors on HbA1c reliability may be more marked in younger African children likely to suffer from severe malaria and subsequent haemolytic anaemia, iron deficiency.

Likewise, it would be interesting to investigate the role of fructosamine and glycated albumin in optimising the diagnosis of diabetes in SSA. The SSA region harbours the highest proportion of undiagnosed diabetes patients and is projected to have the most significant future increase in the burden of diabetes[4]. Historically, for a long time fasting plasma glucose (FPG) or 2-hour oral glucose tolerance (OGTT) and HbA1c are the primary tests used for diagnosis among asymptomatic people. Given that all 3 of the current tests have limitations, searching for alternative tests to improve screening and diagnosis of DM in our setting is worth it. For example, both FPG and the OGTT require

fasting, reducing their use for “opportunistic screening” of a patient who presents to health centres for other medical reasons.

Additionally, the OGTT requires substantial pre-test preparation and is more demanding and unpleasant for many people. The utility of HbA1c testing (widely used for diabetes diagnosis in developed countries) has also been questioned due to the high prevalence of comorbidities that may affect its reliability (such as haemoglobinopathies, anaemia, and HIV), and potential variations in HbA1c with ethnicity[3]. Therefore, rigorous studies in which the performances of these tests are measured at a single point against alternative tests (Glycated albumin and fructosamine) are needed. Finally, it would be good to explore the relationship of these tests (HbA1c, GA, and fructosamine) with diabetes-related complications (microvascular and macrovascular complications).

Chapter 4: The impact of prolonged walking on fasting plasma glucose in type 2 diabetes: A Randomised controlled crossover study

In this study, we investigated the immediate impact of a single bout of continuous aerobic exercise performed in a fasted state on fasting glucose in people living with T2D.

We aimed to determine the change in fasting glucose between the walking and rest visits at 60 minutes and 120 minutes post-commencing the intervention. We compared the difference in glucose change from baseline between resting and exercising visits at 60 and 120 minutes from the start. We assessed the overall impact of walking (across all the time points 0-180 minutes). Also, we examined whether the exercise intervention explained further variability in glucose over the total duration of the assessment.

Conclusions

Walking for 1 hour was not associated with meaningful changes in fasting glucose at the end of the exercise or after an additional hour of rest. Compared to the resting (control visit) glucose change from baseline (pre-intervention) with exercise was -0.15 (95% CI: -0.55, 0.26) mmol/L (p=0.48) and -0.10 (95% CI: -0.50, 0.31) mmol/L (p=0.64) at 60 and 120 minutes, respectively.

When assessing all time points using a mixed-effects model, there was no difference in glucose between visits (p=0.67) over the 3 hours post-baseline (supplementary figure S2). Furthermore, the addition of exercise into the model did not explain further variability in glucose (LR test chi2=9.0, p=0.25).

Implication of findings

This study demonstrates that fasting glucose remains relatively stable following a short bout of continuous walking (60 minutes). This suggests that short-term exercise does not affect fasting glucose in people with non-insulin treated diabetes.

These findings are reassuring for clinicians working in low and middle-income countries where most patients with type 2 diabetes reside. In settings where HbA1c is not readily available/affordable and patients have a long walk to the clinic, fasting glucose can be utilised to monitor glycaemia and titrate patient medication even where patients have long walks to clinic.

Limitations

The first limitation of our study is that we standardised the walking speed. However, people walk at different speeds in the real world and have different aerobic powers. This means that the exercise intensity could have varied given the differences in age and gender at the standardised walking speed of 3 miles per hour. Secondly, we excluded insulin-treated patients; our findings cannot be extrapolated to this subgroup.

Future research

Our results suggesting that moderate exercise (walking) in a fasted state does not impact fasting glycaemia despite known insulin-independent effects of exercise deserves further investigation. It would be good to examine the underlying mechanisms by measuring changes in other metabolic markers beyond plasma glucose, including free fatty acids, insulin and glucagon hormonal changes. The impact of walking on fasting glucose might vary by treatment, and the numbers did not allow subgroup analysis. Assessing individuals treated with insulin would be essential. Therefore, future studies on the impact of exercise in a fasted state should assess the

effect of walking on fasting glycaemia in the different treatment groups and the impact at different exercise intensities and duration. In addition, I intend to investigate this further in the main study, where I performed and collected accelerometer data from about 100 participants. Using these data, I plan to assess whether self-reported and accelerometer-measured exercise prior to the fasting glucose test alters the relationship between fasting glucose and HbA1c compared with effects on non-fasting (random glucose).

Chapter 5: Continuous glucose monitoring demonstrates a low risk of clinically significant hypoglycaemia associated with sulphonylurea treatment in an African type 2 diabetes population.

A significant problem in SSA is the high prevalence of poor glycaemic control among people living with type 2 diabetes despite the availability of at least three classes of glucose-lowering therapy. A key barrier to intensifying glucose-lowering therapy which may partly explain the poor glycaemic control often reported in SSA cohorts, is fear of hypoglycaemia because treatments with lower risk of hypoglycaemia such as the newer classes of SUs (e.g., gliclazide) and analogue insulins, are not readily available and robust glucose monitoring is often unaffordable, even for those treated with insulin.

This chapter assesses hypoglycaemia risk with SU and insulin therapy (compared to metformin) in people living with type 2 diabetes in a low-resource sub-Saharan African setting.

Conclusion

Clinically significant CGM recorded hypoglycaemia (<3mmol/L) was infrequent in sulphonylurea-treated participants and did not differ from metformin. The median minutes/week of glucose <3mmol/L were 39.2, 17.0 and 127.5 for metformin, sulphonylurea and insulin, respectively (metformin vs SU, p=0.6). In those treated with SU and Insulin, time spent in hypoglycaemia and hypoglycaemic event rate were strongly associated with glycaemic control, with differences in HbA1c explaining 33.1% (p = <0.001) and 20.7% (p = 0.005) of variation in time below 3mmol/l for SU and insulin respectively After adjusting for HbA1c, time <3mmol/L was 2.1 and 5.5 times greater with sulphonylurea and insulin respectively than metformin alone.

Participants with HbA1c below 53 mmol/mol (7%) spent 2.34% (IQR: 0.60, 4.49) and 5.61% (0.34, 13.80) of their total time per week in hypoglycaemia (<3mmol/L), for SU and insulin respectively. In comparison, those who had an HbA1c ≥ 53 mmol/mol on SU spent 0.0% (IQR: 0.00, 0.92), and those on insulin spent 1.27% (0.00, 5.75) of their total time per week in hypoglycaemia (<3mmol/L). Hypoglycaemia risk was strongly related to HbA1c and fasting glucose, with most episodes occurring in those with tight glycaemic control. CGM assessed and self-reported clinically significant hypoglycaemia in participants treated with sulfonylureas was infrequent among patients who received SU treatment. Modest hypoglycaemia excess was associated with SUs compared to metformin in those with tight glycaemic control. In contrast to SUs, hypoglycaemia rates were substantially higher in insulin-treated diabetes independent of glycaemic control. In conclusion, in a low-resource sub-Saharan African setting, clinically significant hypoglycaemia is infrequent among people with type 2 diabetes receiving Sulphonylurea treatment, and the modest excess occurs predominantly in those with tight glycaemic control.

Implications of study findings

Glycaemic control is the cornerstone of preventing microvascular complications among people living with diabetes. While there is no doubt that there is an association between SUs (especially the older agents like glibenclamide) and insulin treatment and hypoglycaemia, the high rates of poor glycaemic control in type 2 diabetes patients and relatively low hypoglycaemic events among patients taking SUs suggest that there is room for optimising glycaemic control using these cheap, readily available and effective agents, despite the specific challenges of food insecurity and lack of glycaemic monitoring in many LMIC populations. This supports the recommendations to optimise glycaemic control using these readily available and affordable agents,

including metformin and SUs. However, the modest excess of hypoglycaemia was predominantly seen in a small proportion of patients taking SUs whose fasting glucose was less than 7 mmol/L or HbA1c < 7% (53mmol/mol) (thresholds often recommended by international guidelines), suggesting caution is needed when treating below these levels.

Limitations

A key strength of this study is the objective assessment of hypoglycaemia through the use of blind CGM monitoring. This removed potential biases that could arise from patient reactivity to glucose measurements, differences in glucose testing by treatment, hypoglycaemia unawareness and recall bias that may affect studies assessing self-reported hypoglycaemia or using medical records. An additional strength is a comparison across therapies. It is well known that CGM can report the occurrence of hypoglycaemia in those who do not have diabetes or are treated with medications not associated with hypoglycaemia risk, meaning the absolute risk of meaningful hypoglycaemia by CGM will be over-estimated. By including a metformin 'control' arm in our study, we ensured to avoid this overestimation by assessing the excess risk. A notable limitation of our study was that routine capillary glucose monitoring is unavailable to the vast majority of people with diabetes in Uganda due to cost. Therefore, self-reported hypoglycaemia is unlikely to have been confirmed by glucose testing and is likely to be inaccurate in a population like ours where healthy literacy, including hypoglycaemia education, is not good. Such testing may even be limited in a healthcare setting. Additionally, the modest number of participants treated with only metformin will have impacted our ability to detect modest differences in hypoglycaemia risk in comparisons against metformin, as shown by the large confidence intervals of estimates for metformin-treated participants. Lastly, the

majority of participants with SU and insulin-treated diabetes had poor glycaemic control. While this reflects current practice in this region, given the strong relationship between glycaemic control and hypoglycaemia risk, it is likely that hypoglycaemia rates would be substantially higher were glycaemic control improved in this population, as suggested by our adjusted analysis. Additionally, I did not explore the causes of poor glycaemic control in my studies. It remains unknown whether the likely causes of the poor glycaemic control are due poor access to medication/lack of adherence but drugs are available or treatment inertia because of provider apathy.

Future research

While our results suggest modest hypoglycaemia excess associated with SUs compared to metformin in those with tight glycaemic control, we had limited numbers to carry out subgroup analysis. Overall, 28 participants were treated with metformin only, 111 were treated with sulphonylureas (with or without metformin), and 51 were treated with insulin. I would love to replicate these findings in a large study with substantial numbers of people within the respective drug groups and other newer oral agents. Unfortunately, a narrow range of patients was recruited, resulting in only modest numbers of high-risk individuals. The median (IQR) age of the patients in this study was 56 (50, 63). Generally, patients had a shorter duration of diabetes (5 years in the metformin and SU groups and 10 years in the insulin group). Those treated with SU and insulin had substantially high glycaemia. Therefore our findings may not be generalisable to other patient subgroups like the elderly diabetic patients (above 65 years), those with longer diabetes duration and in settings of tightly controlled glycaemia. For example, renal impairment was very rare in our sample population, with only 4/51 patients in the insulin-treated group and 6/100 in the sulphonylurea-treated group. The modest hypoglycaemia risk in the present study even when the

majority (two out of three) of our patient population receiving SU were taking glibenclamide (an older agent with higher hypoglycaemia risk[5]), makes it more interesting to design a larger observational study in which we are comparing Intra SU-class differences in hypoglycaemic risk. Hypoglycaemia was only assessed over 14 days, and a longer assessment duration may better capture the overall risk. Another critical issue to study is the impact of hypoglycaemia on this population's quality of life and wellbeing. Without home monitoring, it is impossible to accurately assess the risk of severe or symptomatic hypoglycaemia. Therefore, a study with home glucose monitoring and robust prospective assessment of severe and symptomatic hypoglycaemia would be beneficial.

Overall conclusions from thesis

This thesis demonstrates that HbA1c has better overall performance than single glucose measures, even in those with conditions reported to affect reliability. Fructosamine and glycated albumin do not improve performance over and above HbA1c, and the accuracy of HbA1c, glycated albumin and fructosamine in reflecting glycaemic control is not affected by sickle cell trait. In those with anaemia and renal impairment, the relationship between HbA1c and glucose is altered, but fructosamine and GA do not improve the assessment of glycaemic burden.

We have shown that single glucose measures (Fasting plasma glucose and random non-fasting plasma glucose) perform reasonably well in identifying poor glycaemic control that can be used where HbA1c is unavailable. This thesis also demonstrates that short-term exercise does not affect fasting glucose in people with non-insulin-treated diabetes. Therefore, fasting glucose can be utilised to monitor glycaemia and titrate patient medication even when patients have long walks to the clinic.

Further extensive research is needed to explore the relationship of these tests (HbA1c, GA, and fructosamine) with glycaemic burden and diabetes-related complications to determine the optimal test for monitoring and diagnosing diabetes in SSA.

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BMJ Open Diabetes Research & Care

HbA1c performs well in monitoring glucose control even in populations with high prevalence of medical conditions that may alter its reliability: the OPTIMAL observational multicenter study

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To cite: Niwaha AJ, Rodgers LR, Greiner R, *et al*. HbA1c performs well in monitoring glucose control even in populations with high prevalence of medical conditions that may alter its reliability: the OPTIMAL observational multicenter study. *BMJ Open Diab Res Care* 2021;**9**:e002350. doi:10.1136/bmjdrc-2021-002350

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/bmjdrc-2021-002350).

Received 27 April 2021
Accepted 22 August 2021

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ABSTRACT

Introduction The utility of HbA1c (glycosylated hemoglobin) to estimate glycemic control in populations of African and other low-resource countries has been questioned because of high prevalence of other medical conditions that may affect its reliability. Using continuous glucose monitoring (CGM), we aimed to determine the comparative performance of HbA1c, fasting plasma glucose (FPG) (within 5 hours of a meal) and random non-fasting glucose (RPG) in assessing glycemic burden.

Research design and methods We assessed the performance of HbA1c, FPG and RPG in comparison to CGM mean glucose in 192 Ugandan participants with type 2 diabetes. Analysis was undertaken in all participants, and in subgroups with and without medical conditions reported to affect HbA1c reliability. We then assessed the performance of FPG and RPG, and optimal thresholds, in comparison to HbA1c in participants without medical conditions thought to alter HbA1c reliability.

Results 32.8% (63/192) of participants had medical conditions that may affect HbA1c reliability: anemia 9.4% (18/192), sickle cell trait and/or hemoglobin C (HbC) 22.4% (43/192), or renal impairment 6.3% (12/192). Despite high prevalence of medical conditions thought to affect HbA1c reliability, HbA1c had the strongest correlation with CGM measured glucose in day-to-day living (0.88, 95% CI 0.84 to 0.91), followed by FPG (0.82, 95% CI 0.76 to 0.86) and RPG (0.76, 95% CI 0.69 to 0.81). Among participants without conditions thought to affect HbA1c reliability, FPG and RPG had a similar diagnostic performance in identifying poor glycemic control defined by a range of HbA1c thresholds. FPG of ≥7.1 mmol/L and RPG of ≥10.5 mmol/L correctly identified 78.2% and 78.8%, respectively, of patients with an HbA1c of ≥7.0%.

Conclusions HbA1c is the optimal test for monitoring glucose control even in low-income and middle-income countries where medical conditions that may alter its reliability are prevalent; FPG and RPG are valuable alternatives where HbA1c is not available.

SIGNIFICANCE OF THIS STUDY

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT?

⇒ HbA1c is the gold standard for monitoring glycemic control.
⇒ The value of HbA1c measurement among populations living in low-resource settings has been questioned because of high prevalence of other medical conditions that may affect test reliability, such as hemoglobinopathies or anemia.

WHAT ARE THE NEW FINDINGS?

⇒ HbA1c is the overall best measure of glycemic burden, despite high prevalence of other medical conditions that may affect its accuracy (eg, anemia, hemoglobinopathies).
⇒ Fasting plasma glucose (FPG) and random non-fasting glucose (RPG) were strongly correlated with continuous glucose monitoring (CGM) glucose and HbA1c, and had reasonable sensitivity and specificity to detect poor glycemic control.
⇒ The difference in performance between these tests is modest.

HOW MIGHT THESE RESULTS CHANGE THE FOCUS OF RESEARCH OR CLINICAL PRACTICE?

⇒ HbA1c is the optimal laboratory method for assessing glycemic control, even in populations with high prevalence of conditions reported to affect test reliability.
⇒ FPG and RPG measurements correlate strongly with both CGM and HbA1c, perform reasonably well in identifying poor glycemic control and can therefore be used when HbA1c is unavailable.

INTRODUCTION

Diabetes is a global problem disproportionately affecting low-income and middle-income countries (LMICs), with 80% of the global 463 million people with diabetes

living in LMICs.[1] Unlike high-income countries, diabetes healthcare in LMICs is underfunded[1] and lacks quality, pragmatic and contextualized guidelines.[2] As such, LMICs are heavily impacted by high rates of poorly controlled glucose levels,[3–5] and subsequently, high rates of diabetes-related complications and poor quality of life among people living with diabetes.

Monitoring glycemic control is essential to allow appropriate titration of medication and improve outcomes among patients with diabetes, but regular monitoring can be challenging in LMICs. In high-income countries, HbA1c (glycosylated hemoglobin) is the recommended measure used for assessing glucose control and titrating medications, often supported by home glucose capillary or interstitial glucose monitoring.[6 7] However, financial constraints mean that the monitoring of diabetes and decisions to intensify treatment in much of the low-income regions are predominantly based on testing of a single glucose measure.[8] This is because HbA1c testing is not routinely available in most centers,[8] and HbA1c is often too expensive for the majority of patients.[9] Even where testing is available, there has been substantial concern that HbA1c measurement may be unreliable in LMIC populations,[10–12] due to high prevalence of hemoglobinopathies such as sickle cell and thalassemia, and other medical conditions that might affect test reliability including anemia and malaria.[13] Home glucose monitoring is not well funded by healthcare systems in LMICs and is beyond the financial means and literacy skills of a large proportion of those who have diabetes.[8 14]

International organisations recommend the use of plasma glucose for monitoring glycemic control in developing countries where HbA1c services are not readily available.[15] However, assessment of glycemic control in such settings is normally after long walks by the patients to attend a centralized clinic every 2–3 months, coupled with prolonged fasting and long waiting times.[16] As such, many clinicians rely on a random glucose without the requirement to fast to assess glycemia.[16] While these tests have been compared with HbA1c in the LMIC setting,[17 18] given the limitations of HbA1c itself in these populations, its performance as a measure of average glucose is unclear. Continuous glucose monitoring (CGM) offers the opportunity of measuring glucose in day-to-day living over a period of days to weeks and is widely used in high-income countries and some LMICs.

In the OPTIMAL study, we aimed to compare, in an African population with type 2 diabetes, the accuracy of fasting plasma glucose (FPG), random non-fasting plasma glucose (RPG), and HbA1c in comparison to CGM as an independent measure of glycemic control, and assess the impact of other medical conditions that may affect HbA1c reliability to monitor glycemia in people with established diabetes.

METHODS

Study population

Participants were recruited from diabetes clinics in Masaka Regional Referral Hospital (rural, public) and St. Francis Hospital Nsambya (urban, private not-for-profit) in Uganda and met the following inclusion criteria: a clinical diagnosis of type 2 diabetes, diagnosed at the age of 18 years and above, more than 12 months' diabetes duration, no initial insulin requirement for at least 1 year since the time of diagnosis, no change in glucose-lowering therapy 3 months prior, and able to give informed consent. Participants who were pregnant or judged by their clinician to need an immediate change in glucose-lowering medication were excluded from recruitment.

Study visits

Participants were scheduled for three visits. The overview of the study design is presented in online supplemental figure S1.

At the baseline visit, participants came to the clinic in a non-fasted state. Following assessment of clinical features and demographics, non-fasting (within 5 hours of a meal) random blood sample was collected for measurement of RPG, HbA1c, full blood count, lipid profile, renal function and assessment of hemoglobin variants. CGM was carried out using the Freestyle Libre Pro Flash Glucose Monitoring System (Abbott Laboratories, Illinois, USA), a professional CGM device which records interstitial glucose every 15 min for up to 2 weeks. Freestyle Libre Pro is blinded, meaning data could not be viewed by the wearer.

All participants returned in a fasted state (at least 8 hours) in the second week of CGM between days 7 and 10 from the baseline visit, and for their final visit, between days 12 and 14 from the baseline visit, in a non-fasted state (within 5 hours of a meal). At both of these visits, CGM data were downloaded and a venous blood sample was collected for measurement of HbA1c and RPG (visits 1 and 3) and FPG (visit 2). The study was carried out in accordance with the 2008 revised principles of the Declaration of Helsinki and all participants provided informed consent before study activities.

Patient and public involvement (PPI)

Patients were involved in prioritization of the research question. Patients were not involved in the design and conduct of the study. However, they were central to dissemination of the results by choosing to have some of the results sent to their respective clinicians, and will continue to be involved in ongoing study dissemination.

Laboratory procedures

Blood samples for glucose measurement were collected in a vacutainer with sodium fluoride (NaF), centrifuged and separated into two cryovials (aliquots) immediately and kept in an icebox at 4°C–8°C before being transported to the central laboratory for immediate testing (within 8 hours of collection). Whole blood samples for

full blood count and HbA1c were collected in vacutainers containing EDTA. All analytical measurements were performed at the Central Biochemistry and Clinical Diagnostic Laboratory Services (CDLS) laboratory at the MRC/UVRI & LSHTM Research Unit Entebbe Uganda. Laboratory analyses were performed on a Roche Cobas 6000 analyzer (Hitachi High Technologies, Tokyo, Japan). Plasma glucose was measured by the glucokinase method. HbA1c was also measured on Cobas 6000 by the immunoassay technique, calibrated to the International Federation of Clinical Chemistry. Hemoglobinopathies (sickle cell trait and hemoglobin C (HbC)) were assessed by Hb electrophoresis.

CGM measures

Raw glucose readings were downloaded from the Libreview software and CGM summary variables (including mean CGM glucose) were calculated using R V.3.6.1. Sensor data were considered for analysis if the total duration of CGM wear was at least 5 days.

For CGM validation, we matched plasma FPG at visit 2 with a nearest CGM glucose value within 15 min. We then determined the relationship between the plasma glucose and the CGM glucose value using Bland-Altman analysis to assess the degree of bias and levels of agreement between the sensor and plasma glucose.

Statistical analysis

Data were analyzed using Stata V.16.1 (StataCorp LLC, USA).

Comparison of glucose and HbA1c measures with CGM measured glucose in daily living

We assessed the strength of the relationship between CGM assessed mean glucose over 2 weeks and each of FPG, RPG and HbA1c using Pearson's correlation coefficients and linear regression. Analysis was based on RPG and HbA1c tests performed on the last visit (visit 3), unless not available, in which case values from visit 1 were used instead (n=9). To assess the impact of other medical conditions (anemia, hemoglobinopathies, and renal impairment) on HbA1c reliability, we subdivided the cohort into those without medical conditions that may alter HbA1c reliability and those with medical conditions that may alter HbA1c reliability. HbA1c performance in comparison to CGM was assessed in all participants regardless of comorbidities, and by presence or absence of medical conditions thought to affect test performance (see below). Equivalent thresholds for predicting suboptimal glycemic control (defined as CGM glucose values ≥8 and ≥10 mmol/L) were derived from linear regression equations. We compared the performance of RPG and FPG and HbA1c to identify participants with CGM glucose values ≥8 and ≥10 mmol/L using receiver operating characteristic curve analysis, and assessed the sensitivity, specificity and positive/negative predictive values of these tests using the equivalent cut-offs derived from linear regression equations.

Comparison of FPG and RPG measurement with HbA1c

As HbA1c is the measure which has been robustly validated against clinical outcomes, we performed additional analysis, where we assessed the strength of the relationship between HbA1c and each of the FPG and RPG tests in the absence of medical conditions that might affect HbA1c reliability. Participants were considered to have no other medical conditions that may affect HbA1c reliability if they met the following characteristics: no hemoglobinopathies (sickle cell trait and HbC), absence of anemia (Hb in women ≥120 g/L, men ≥130 g/L),[19] and no renal impairment (estimated glomerular filtration rate (eGFR) ≥60 mL/min/1.73 m^2). In participants without these medical conditions, we determined diagnostic performance of the glucose tests for suboptimal glucose control defined by HbA1c at the following thresholds: HbA1c ≥48 mmol/mol (6.5%), ≥53 mmol/mol (7.0 %), 58 mmol/mol (7.5%), 64 mmol/mol (8.0%), 69 mmol/mol (8.5%) and 75 mmol/mol (9.0%). Equivalent thresholds of FPG and random glucose for predicting suboptimal glycemic control were obtained by linear regression analysis.

RESULTS

Baseline characteristics

A total of 213 adults were enrolled in the study. Of these participants, 9.86% (21/213) were excluded for insufficient data. Characteristics of excluded participants were broadly similar to those included in analysis, as shown in online supplemental table 1. Out of 213 participants, 192 had sufficient data for inclusion in the final analysis (see flow chart: online supplemental figure S2). The median CGM duration was 14 (IQR 13–14) days. Participant characteristics are presented in table 1. Average glycemic control was poor with a median (IQR) HbA1c of 67 (52.0–90.0) mmol/mol (8.3% (6.9–10)). The other medical conditions that may affect HbA1c reliability were common, occurring in 32.8% (63/192) of participants, of whom 9.4% (18/192) had anemia, 22.4% (43/192) had hemoglobinopathies (sickle cell trait (n=43) and/or hemoglobin AC (HbAC) (n=1)), and 6.3% (11/190) had renal impairment (eGFR <60 mL/min/1.73 m^2). Characteristics according to absence or presence of medical conditions that may affect HbA1c reliability are shown in online supplemental table 2.

FPG and CGM glucose are highly correlated

FPG and CGM glucose (closest value, within 15 min) were highly correlated (Pearson's r=0.97, 95% CI 0.96 to 0.98). CGM values showed a modest bias toward lower glucose than FPG, with CGM values mean 1.3 (95% CI 1.1 to 1.5) mmol/L lower—this was consistent across the range of glycemic control (online supplemental figure S3).

Table 1 Participant characteristics (N=192)

	Median (IQR) for continuous variables, % (n) for proportions
Clinical	
Female, n (%)	58.3 (112/192)
Age, years	56 (50–63)
Duration of diabetes, years	6 (3–10)
BMI, kg/m^2	26.8 (24.0–30.5)
Current management, n (%)	
Metformin only	15.6 (30/192)
SU (±metformin)*	57.3 (110/192)
Insulin (±other diabetes drug)†	26.0 (50/192)
Diet‡	1.0 (2/192)
Glycemia	
CGM glucose, mmol/L	8.6 (6.8–12.3)
HbA1c, %	8.3 (6.9–10.0)
HbA1c, mmol/mol	67 (52.0–90.0)
FPG, mmol/L	8.2 (6.1–11.4)
RPG, mmol/L	13.5 (8.8–17.2)
Other laboratory	
Hb (g/L)	14.2 (13.2–15.0)
Anemia§	9.4% (18/192)
Hemoglobinopathies, n (%)¶	22.4% (43/192)
eGFR	111.5 (92.3–121.0)
Renal impairment, n (%)	6.3% (12/192)

*Sulfonylureas with or without metformin.
†Insulin with or without any oral therapy.
‡Two participants were on non-pharmacological management (diet) only.
§ Anemia was defined as a Hb of <120 g/L in women and <130 g/L in men.
¶Hemoglobinopathies was defined as the presence of sickle cell trait (HbAS) or HbAC.
BMI, body mass index; CGM, continuous glucose monitoring; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; Hb, hemoglobin; HbAC, hemoglobin AC; HbA1c, glycosylated hemoglobin; RPG, random non-fasting plasma glucose.

HbA1c has the strongest relationship with CGM glucose in an African population, even in participants with comorbidities thought to alter HbA1c reliability

The relationship between HbA1c, FPG and RPG tests and average CGM glucose is shown in figure 1. There was a strong correlation between all the three tests and mean CGM glucose. HbA1c had the strongest correlation (0.88; 95% CI 0.84 to 0.91), followed by FPG (0.82; 95% CI 0.76 to 0.86) and RPG (0.76; 95% CI 0.69 to 0.81). The derived linear equations for estimating mean glucose from HbA1c, FPG and RPG among patients with diabetes are shown in online supplemental table 3. The diagnostic performances of HbA1c, FPG and RPG tests for diagnosing suboptimal glucose control (defined by illustrative mean CGM thresholds of 8 and 10 mmol/L) are shown in table 2. There was a very modest loss of diagnostic performance using FPG compared with HbA1c, at equivalent thresholds. HbA1c was the most sensitive and specific test followed by FPG.

HbA1c maintained the strongest relationship with CGM glucose even in those with other medical conditions that might affect HbA1c reliability (figure 1). In those with and without conditions that might affect HbA1c reliability, the relationship between CGM glucose and HbA1c was similar, with no difference in correlation (0.85; 95% CI 0.76 to 0.91) versus (0.89; 95% CI 0.85 to 0.92) (figure 1) and the difference in linear regression slopes was modest (mean CGM glucose=0.14*HbA1c–0.02 and 0.16*HbA1c–1.07 with and without conditions that may affect HbA1c reliability, respectively) (online supplemental table 3). This was also similar when examining only those with hemoglobinopathy (r=0.90, 95% CI 0.82 to 0.94, n=42, supplementary figure S4).

FPG and RPG have broadly similar diagnostic performance in identifying patients with poor glycemia control

Among participants without conditions thought to alter HbA1c reliability (including hemoglobinopathies, anemia and renal impairment), RPG and FPG had similar correlation with HbA1c (0.74; 95% CI 0.65 to 0.80) and (0.78; 95% CI 0.71 to 0.84), respectively (figure 2). The equivalent thresholds and diagnostic performances of FPG and RPG for predicting HbA1c defined suboptimal glucose control (at different HbA1c thresholds), restricted to those without conditions thought to alter HbA1c reliability, are shown in table 3. FPG and RPG had very similar performance in identifying those with suboptimal glycemic control (table 3). For the widely used HbA1c target of 7.0%, the AUC ROC for these tests was similar (FPG 0.76, RPG 0.77). At their respective optimal thresholds (FPG ≥7.1 mmol/L and RPG ≥10.5 mmol/L), the tests had a similar sensitivity (FPG –81.0, 95% CI 71.9 to 88.2 vs RPG –81.6, 95% CI 72.7 to 88.5) and specificity (FPG –71.4, 95% CI 55.4 to 84.3 vs RPG –72.1, 95% CI 56.3 to 84.7) for identifying suboptimal glycemic control. The linear equations for estimating HbA1c from FPG and RPG among patients with diabetes were HbA1c (mmol/mol)=5.40*FPG+21.3 and HbA1c=3.07*RPG +28.58, respectively, for patients without comorbidities thought to alter HbA1c (online supplemental table 4).

DISCUSSION

The international guidelines recommend HbA1c for monitoring glycemic control and blood glucose test where HbA1c is unavailable. Despite this guidance, there remains concerns about the accuracy of HbA1c in populations with high frequency of other medical conditions that may alter its reliability. In this study, we used CGM to compare the accuracy of HbA1c, FPG and RPG tests in assessing glycemic control among patients with diabetes under conditions of everyday life in low-resource settings.

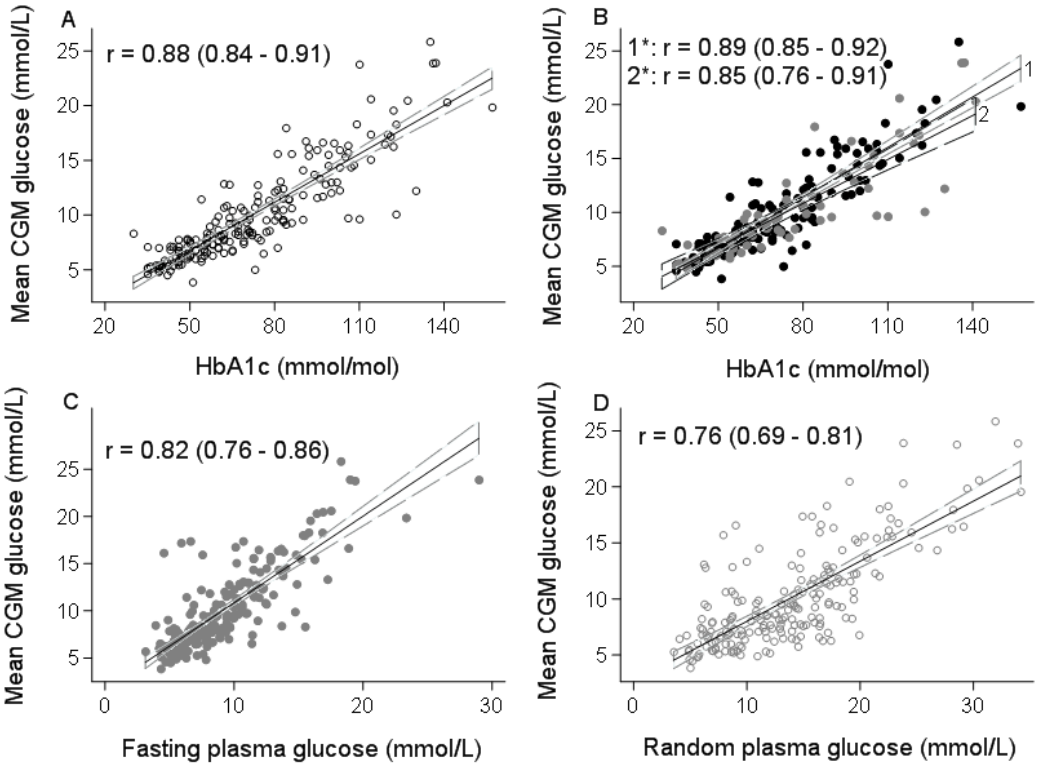

Figure 1 Comparison of (A) HbA1c (glycosylated hemoglobin) of the overall sample population and (B) HbA1c without (1; black circles) and with (2; gray circles) conditions thought to alter HbA1c reliability with mean continuous glucose monitoring (CGM) glucose. Comparison of (C) fasting plasma glucose (FPG) and (D) random non-fasting plasma glucose (RPG) with mean CGM glucose. Solid straight line denotes the line of best fit and the dashed lines represent the 95% CI. The Pearson's correlation coefficient (r) and 95% CIs are shown for each graph. Conditions thought to alter HbA1c reliability include hemoglobinopathies including sickle cell trait and hemoglobin AC (HbAC), anemia, and renal impairment.

The prevalence of other medical conditions that may alter HbA1c reliability was remarkably high. However, we found that HbA1c remained the most accurate test of average glucose control, despite the high prevalence of hemoglobinopathies, anemia and renal impairment. Similarly, FPG and RPG demonstrated reasonable accuracy as measures of average glycemic control, providing confidence that glucose tests provide a good measure of glycemia where HbA1c is not available. Furthermore, the very modest loss of diagnostic test performance using RPG provides some reassurance for use of this test in situations where a RPG is the only or most practical measure available.

Table 2 Ability of HbA1c, FPG and RPG to define suboptimal glucose control using CGM thresholds <8 and <10 mmol/L

CGM cut-off	Test	N	AUROC (95% CI)	Optimal threshold	Sensitivity (95% CI)	Specificity (95% CI)	Correctly classified	PPV (95% CI)	NPV (95% CI)
≥8.0	HbA1c	191	0.95 (0.92 to 0.98)	≥62 mmol/mol	90.2 (83.1 to 95.0)	83.5 (73.5 to 90.9)	87.4	88.6 (81.3 to 93.8)	85.7 (75.9 to 92.6)
	FPG	191	0.90 (0.86 to 0.95)	≥7.6 mmol/l	84.8 (76.8 to 90.9)	81.0 (70.6 to 89.0)	83.3	86.4 (78.5 to 92.2)	79.0 (68.5 to 87.3)
	RPG	192	0.82 (0.77 to 0.88)	≥11.6 mmol/l	78.6 (69.8 to 85.8)	64.6 (53.0 to 75.0)	72.8	75.9 (67.0 to 83.3)	68.0 (56.2 to 78.3)
≥10.0	HbA1c	191	0.94 (0.90 to 0.97)	≥72 mmol/mol	88.9 (79.3 to 95.1)	84.9 (77.2 to 90.8)	86.4	78.0 (67.5 to 86.4)	92.7 (86.0 to 96.8)
	FPG	191	0.90 (0.85 to 0.95)	≥9.1 mmol/l	83.6 (73.0 to 91.2)	83.1 (75.0 to 89.3)	83.3	75.3 (64.5 to 84.2)	89.1 (81.7 to 94.2)
	RPG	192	0.85 (0.79 to 0.91)	≥13.8 mmol/l	84.7 (74.3 to 92.1)	72.3 (63.3 to 80.1)	77.0	64.9 (54.4 to 74.5)	88.7 (80.6 to 94.2)

The units used are as follows: HbA1c—mmol/mol and mmol/L for fasting and random non-fasting glucose.
AUROC, area under receiver operating characteristic curve; CGM, continuous glucose monitoring; FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin; NPV, negative predictive value; PPV, positive predictive value; RPG, random non-fasting plasma glucose.

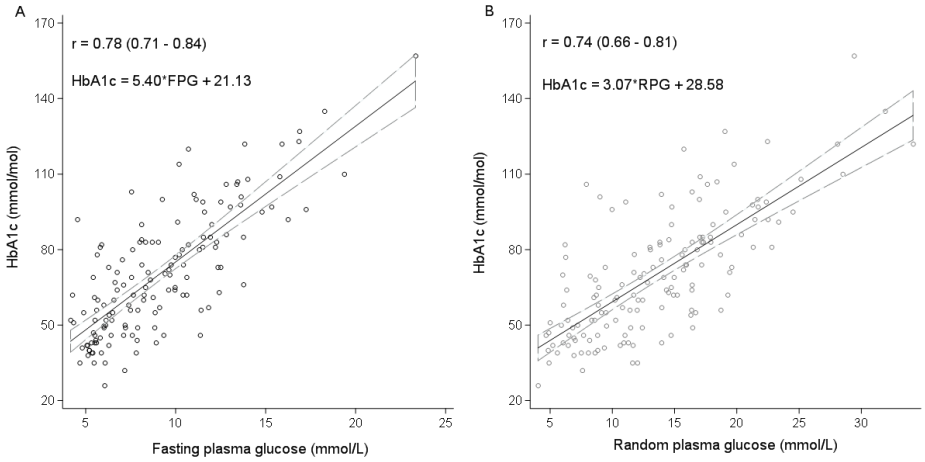

Figure 2 (A, B) Comparison of fasting plasma glucose (FPG) and random non-fasting plasma glucose (RPG) with HbA1c (glycosylated hemoglobin) in participants without conditions thought to alter HbA1c reliability. Solid straight line denotes the line of best fit and the dashed lines represent the 95% CI. The Pearson's correlation coefficient (r) and 95% CIs are shown for each graph. Conditions thought to alter HbA1c reliability include hemoglobinopathies including sickle cell trait and hemoglobin AC (HbAC), anemia, and renal impairment.

In the current study, we have compared FPG, RPG and HbA1c in the same study and more importantly against an independent measure of day-to-day glycemic burden. CGM was used as an independent marker of glycemic burden to allow assessment of the relative performance of HbA1c, FPG and RPG in assessing glycemic burden. This is a major strength of our analysis in contrast to previous studies which have compared between measures such as HbA1c and FPG, with no independent comparison. Further, we assessed performance of HbA1c in the

Table 3 Ability of FPG and RPG to predict suboptimal glucose control among patients with type 2 diabetes without medical conditions thought to alter HbA1c reliability using different HbA1c thresholds

HbA1c cut-off	Test	N	AUROC (95% CI)	Equivalent threshold (mmol/L)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Correctly classified (%)
48 (6.5%)	FPG	142	0.84 (0.77 to 0.92)	6.6	79.5 (70.8 to 86.5)	73.3 (54.1 to 87.7)	91.8 (84.4 to 96.4)	48.9 (33.7 to 64.2)	78.2
	RPG	145	0.86 (0.80 to 0.92)	9.6	79.1 (70.6 to 86.1)	71.0 (52.0 to 85.8)	91.0 (83.6 to 95.8)	47.8 (32.9 to 63.1)	77.4
53 (7.0%)	FPG	142	0.87 (0.81 to 0.93)	7.1	81.0 (71.9 to 88.2)	71.4 (55.4 to 84.3)	87.1 (78.5 to 93.2)	61.2 (46.2 to 74.8)	78.2
	RPG	145	0.88 (0.83 to 0.94)	10.5	81.6 (72.7 to 88.5)	72.1 (56.3 to 84.7)	87.5 (79.2 to 93.4)	62.0 (47.2 to 75.3)	78.8
58 (7.5%)	FPG	142	0.85 (0.79 to 0.91)	7.7	76.7 (66.6 to 84.9)	76.9 (63.2 to 87.5)	85.2 (75.6 to 92.1)	65.6 (52.3 to 77.3)	76.8
	RPG	145	0.84 (0.77 to 0.90)	11.4	78.5 (68.8 to 86.3)	71.7 (57.7 to 83.2)	83.0 (73.4 to 90.1)	65.5 (51.9 to 77.5)	76.0
64 (8.0%)	FPG	142	0.86 (0.80 to 0.92)	8.4	74.0 (62.8 to 83.4)	81.5 (70.0 to 90.1)	82.6 (71.6 to 90.7)	72.6 (60.9 to 82.4)	77.5
	RPG	145	0.84 (0.78 to 0.90)	12.4	78.8 (68.2 to 87.1)	80.3 (68.7 to 89.1)	82.9 (72.5 to 90.6)	75.7 (64.0 to 85.2)	79.5
69 (8.5%)	FPG	142	0.85 (0.79 to 0.91)	9.0	73.5 (61.4 to 83.5)	83.8 (73.4 to 91.3)	80.6 (68.6 to 89.6)	77.5 (66.8 to 86.1)	78.9
	RPG	145	0.85 (0.79 to 0.91)	13.3	76.1 (64.5 to 85.4)	78.7 (67.7 to 87.3)	77.1 (65.6 to 86.3)	77.6 (66.6 to 86.4)	77.4
75 (9.0%)	FPG	142	0.85 (0.78 to 0.92)	9.6	74.1 (60.3 to 85.0)	80.7 (70.9 to 88.3)	70.2 (56.6 to 81.6)	83.5 (73.9 to 90.7)	78.2
	RPG	145	0.85 (0.78 to 0.92)	14.4	75.0 (61.6 to 85.6)	77.8 (67.8 to 85.9)	67.7 (54.7 to 79.1)	83.3 (73.6 to 90.6)	76.7

AUROC, sensitivity, specificity, % correctly classified, PPV and NPV are given for the respective optimal thresholds of the test. This was restricted to HbA1c where there were no conditions thought to alter HbA1c reliability like anemia, sickle cell traits, and renal impairment.
The units used are mmol/L for fasting and random non-fasting glucose.
AUROC, area under receiver operating characteristic curve; FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin; NPV, negative predictive value; PPV, positive predictive value; RPG, random non-fasting plasma glucose.

presence of other medical conditions that may alter its effect. This gave us the opportunity to assess the overall impact on HbA1c reliability.

However, the present study has some limitations that should be taken into consideration. First, although CGM was the best available option for direct measurement of glucose in day-to-day living and allowed us to compare the relative performance of HbA1c and glucose tests, it should be noted that glycemia was measured using a CGM sensor over median 14 (IQR 13–14) days and yet HbA1c estimates glycemia over a longer duration.[20] Second, we used HbA1c immunoassay, one of the most widely used HbA1c assays, particularly in low-resource settings. However, our results for the performance might not apply to other HbA1c assay types, which are known to have different susceptibility to the effects of hemoglobinopathies.[21] Furthermore, although we screened for a number of potential comorbidities thought to alter HbA1c, with the available sample size and very modest subgroup numbers, we were unable to do further subgroup analyses to assess the impact of other individual underlying non-glycemic conditions.[22] In addition, the impact of glucose-6-phosphate dehydrogenase variants, another common condition that may affect HbA1c results reliability, was not assessed.[23]

Our results showing a strong relationship between HbA1c and mean glucose from CGM are consistent with studies that have compared these two measures in high-income settings. The Diabetes Control and Complications Trial (DCCT) of participants in the USA with type 1 diabetes showed a strong relationship between the mean plasma glucose and HbA1c with a Pearson correlation (r) of 0.82.[24] Similarly, results from the ADAG (A1c Derived Average Glucose) study, which included 507 participants with and without diabetes predominantly from the USA and Europe, and excluded participants with other medical conditions thought to alter HbA1c reliability, showed HbA1c and mean glucose were closely correlated (r=0.89, p<0.0001).[25] Our similar results (r=0.88) in an African population, and without exclusion of participants with analytical concerns for HbA1c measurement, is reassuring for the use of HbA1c testing in this region.

Our results are broadly consistent with previous studies that have reported the relationship between glucose tests and HbA1c. El-Kebbi *et al* showed, in 1827 predominantly African–American living in the USA, that RPG collected 1–4 hour post meal was correlated strongly with HbA1c, although in this predominantly insulin-treated population, the correlation (r=0.63) was lower than observed in our study (0.74).[26] In a study that compared both FPG and RPG to HbA1c among 1000 patients with diabetes living in India, FPG showed a better correlation with HbA1c than RPG (0.739 vs 0.601).[27] In contrast, in studies where a fixed post meal time point was used, RPG was a slightly better correlate of HbA1c than FPG.[18] Unfortunately, studies comparing performances of glucose tests against HbA1c in Africa are very few, with small sample sizes, and in these studies, the impact of common medical conditions that may alter HbA1c reliability was not assessed.[17 28]

Our data suggest that there is a high prevalence of other medical conditions that may alter HbA1c reliability justifying the questioning of HbA1c utility. However, even with these comorbidities, HbA1c, when measured with an immunoassay method, correlated strongly with mean glucose, outperforming glucose measures, and only displayed a modest improvement when patients with comorbidities were excluded. This suggests that HbA1c remains the optimal laboratory method of monitoring glucose burden even where prevalence of conditions that may affect its reliability is high. The strong correlation of HbA1c with glucose despite the prevalence of other medical conditions that may alter HbA1c reliability deserves further exploration. However, there are some reasons why the impact of these conditions on HbA1c reliability may be modest in this setting. First, in line with the National Glycohemoglobin Standardization Program (NGSP) recommendation, modern HbA1c immunoassays are not directly affected by the presence of hemoglobin variants like HbAS.[21] Second, while comorbidities that affect red cell life will alter the accuracy of any HbA1c method, the predominant hemoglobinopathy in our study population was HbAS (sickle cell) trait, and previous research has been conflicting as to whether this meaningfully alters red cell lifespan.[29]

While our results support the use of HbA1c (where available) rather than glucose measures in LMIC populations, the small subgroup numbers in our study limited the power to definitively determine the impact of some of these comorbidities on HbA1c performance. To accurately determine the impact of individual comorbidities, larger multinational studies involving other regions in Africa and LMICs with enrichment for these comorbidities would be needed. Furthermore, while our data show that HbA1c (measured using an immunoassay method) has the closest relationship with average glucose, even with comorbidities, it is possible that the overall relationship between glucose and HbA1c is different in this population, therefore the thresholds used internationally are not appropriate, and bespoke HbA1c thresholds are needed for different populations. This further underscores the need for much larger studies, ideally incorporating risk of microvascular complications, to determine whether the HbA1c targets used internationally are appropriate for LMIC populations.

In conclusion, our results suggest that HbA1c is the optimal test for monitoring glucose control even in LMICs where medical conditions that may alter its reliability are prevalent; FPG and RPG are valuable alternatives where HbA1c is not available.

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Acknowledgements The authors would like to thank Edwin Gale for providing editorial suggestions and assistance in drafting and proofreading of the paper. The authors acknowledge Alice Carr for assisting with CGM coding and analysis. The authors are grateful to Aisha Karungi and Winnie Ayuru, the research assistants that carried out the study activities at the diabetes clinics. We are thankful to the staff at the Central Biochemistry and Clinical Diagnostic Laboratory Services (CDLS) laboratory at the MRC/UVRI & LSHTM Research Unit Entebbe Uganda that performed the glucose and HbA1c assays. The authors also thank Esther Nkiinzi (employee of MRC/UVRI & LSHTM Research unit, Entebbe) and Maddy Geen (employee of University of Exeter) for assistance in project management. We are thankful to the hospital ethical committees and staff of the diabetes clinics of Masaka regional referral hospital and St. Francis hospital, Nsambya in Uganda. We dedicate this work to all the diabetic patients that participated in this study and all the others living in LMICs.

Contributors AJN, BMS, MJN and AGJ conceptualized and designed the study. AJN researched the data with assistance from PAB, RM, TJM, MJN and AGJ. AJN, RG and LRR conducted data cleaning. AJN analyzed the data with assistance from LRR, BMS and AGJ. AJN drafted the paper which was critically revised by all authors. AJN, LRR, BMS and AGJ had full access to all of the data in the study and took responsibility for the accuracy and the integrity of the data of analysis. All authors approved the final manuscript.

Funding This study is funded by the National Institute of Health Research, UK (award reference 17/63/131).

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The study was approved by the UVRI REC (UVRI-121/2019) and the Uganda National Council of Science and Technology (HS 2588).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. Data analysed in this study are not available for public use but to researchers upon reasonable request from the corresponding author.

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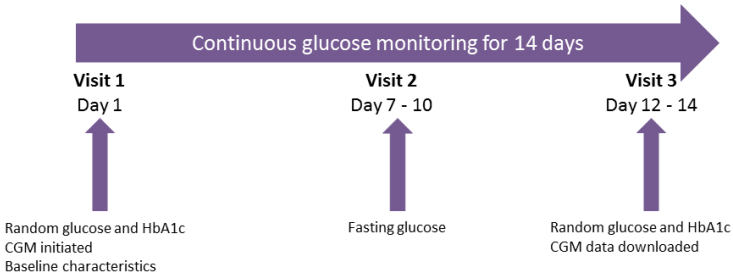

Figure S1: Overview of the study design

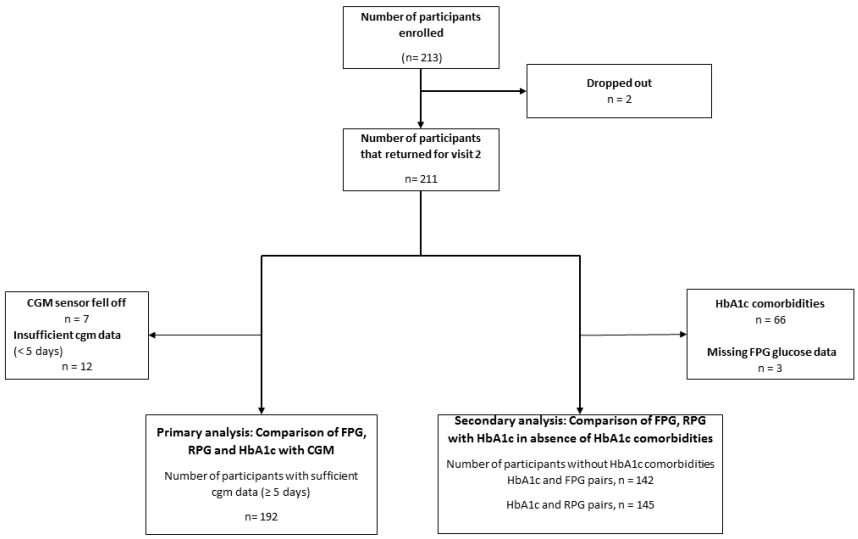

Figure S2: Participant flow chart. Conditions thought to alter HbA1c reliability (HbA1c comorbidities) include haemoglobinopathies (sickle cell trait and HbAC), anaemia, and renal impairment.

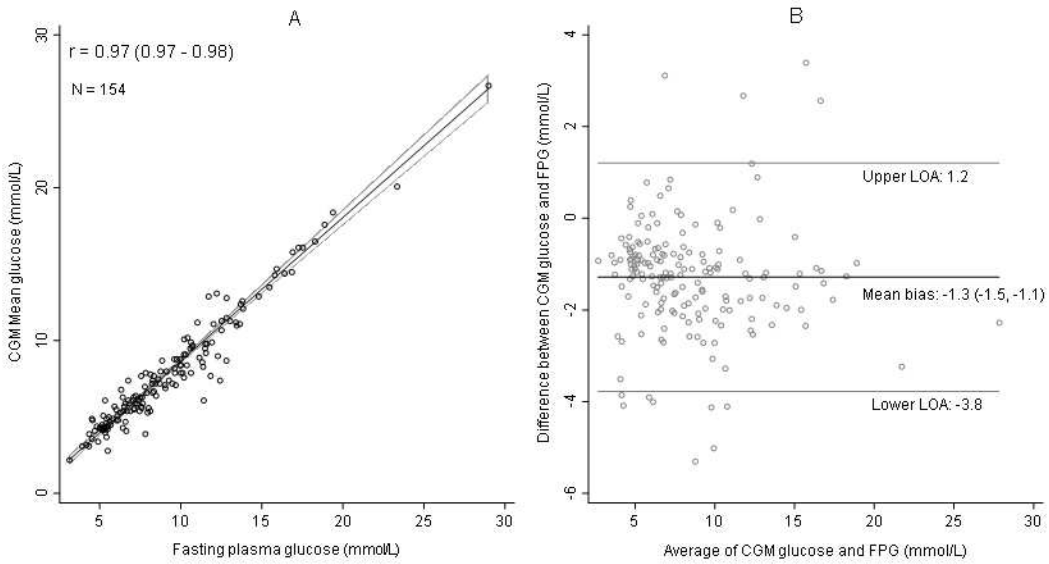

Figure S3: Comparison of CGM glucose and Fasting plasma glucose (A). A Bland Altman Plot of Fasting Plasma Glucose test and CGM sensor glucose. The black solid line denotes the mean bias between the fasting plasma glucose tests and average cgm glucose and the grey solid lines denote upper and lower limits of agreement (LOA). Overall CGM underestimated plasma glucose by 1.3 mmol/L, with LOA from ranging between − 3.8 to 1.2 mmol/L

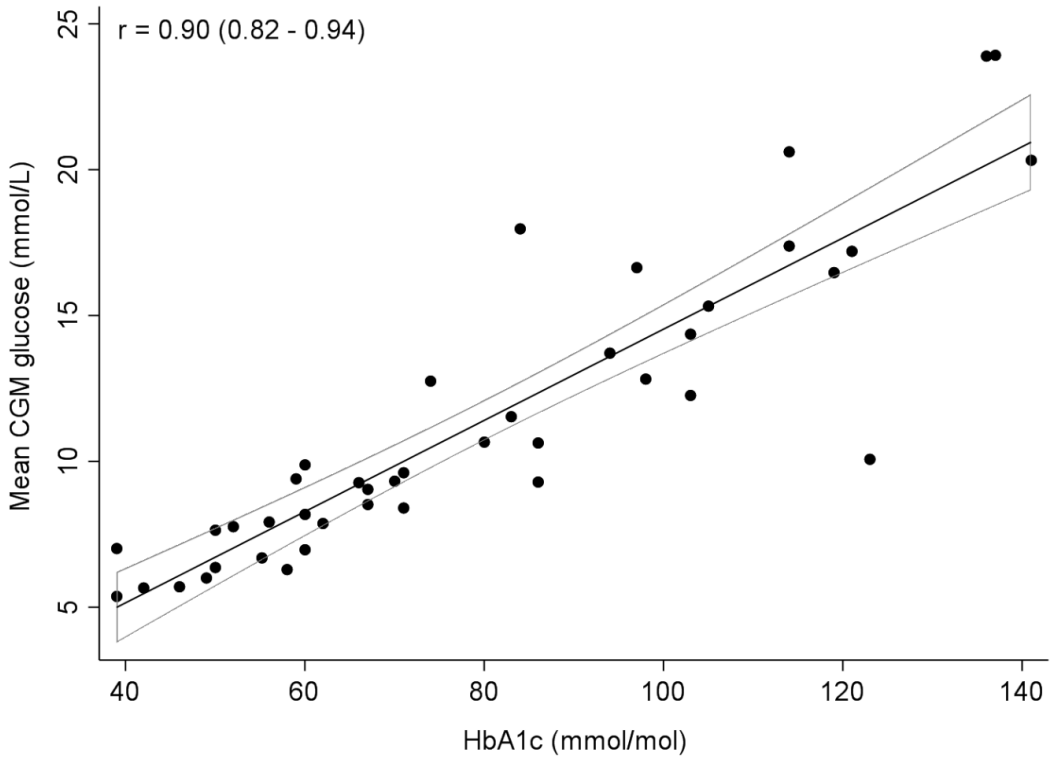

Figure S4: Comparison of HbA1c to mean CGM glucose among those with haemoglobinopathies. Dark thick straight line denotes the line of best fit and the thin lines represent the 95% confidence interval. The Pearson's correlation coefficient (r) and 95% confidence intervals are shown on the left upper corner of the graph.

Supplementary Table 1: Participant characteristics of those included in the final analysis (n = 192) versus those excluded (n = 21)

	Median (IQR) for continuous variables, n (%) for proportions	
	Included	Not included
Number, n (%)	192 (90.1)	21 (9.9)
Clinical		
Female, n (%)	112 (58.3)	13 (61.9)
Age, years	56 (50, 63)	52 (48, 60)
Duration of diabetes, years	6 (3, 10)	7 (1, 11)
BMI, kg/m^2	26.8 (24.0, 30.5)	28.5 (27.6, 33.8)
Current management n (%)		
Metformin only	30 (15.6)	2 (9.5)
SU (+/- metformin)[a]	110 (57.3)	13 (61.9)
Insulin (+/- other diabetes drug)[b]	50 (26.0)	6 (28.6)
Diet [c]	2 (1.0)	0 (0.0)
Glycaemia		
HbA1c, %	8.3 (6.9, 10.0)	7.7 (6.0, 9.1)
HbA1c, mmol/mol	67 (52.0, 90.0)	61.0 (42.5, 76.0)
Fasting plasma glucose, mmol/L	8.2 (6.1, 11.4)	7.0 (5.8, 12.3)
Random plasma glucose, mmol/L	13.5 (8.8, 17.2)	10.8 (7.6, 17.2)
Other laboratory		
Hb (g/L)	14.2 (13.2, 15.0)	14.5 (14.1, 15.5)
eGFR	111.5 (92.3, 121.0)	117.8 (96.7, 124.7)

Supplementary Table 2: Participant characteristics presence (Group 2) or absence (Group 1) of HbA1c comorbidities

	Median (IQR) for continuous variables, % (n) for proportions	
Clinical	**Group 1**	**Group 2**
N (%)	**67.2 (129/192)**	**32.8 (63/192)**
Female, n (%)	60.5 (78/192)	54.0 (34/192)
Age, years	55 (50, 61)	58 (50, 64)
Duration of diabetes, years	6 (3, 10)	9 (4, 12)
BMI, kg/m^2	27. 1 (24.3, 30.3)	25.8 (23.1, 30.6)
Current management n (%)		
Metformin only	18.6 (24/129)	9.5 (6/63)
SU (+/- metformin)[a]	57.4 (74/129)	57.1 (36/63)
Insulin (+/- other diabetes drug)[b]	22.5 (29/129)	33.3 (21/63)
Diet [c]	2 (1.5)	0
Glycaemia		
CGM glucose, mmol/L	8.4 (6.8, 12.3)	9.3 (7.0, 12.3)
HbA1c, %	8.2 (6.7, 9.8)	8.7 (7.1, 10.7)
HbA1c, mmol/mol	66.0 (50.0, 85.0)	70.5 (54.0, 97.0)
Fasting plasma glucose, mmol/L	8.3 (6.1, 11.3)	7.8 (5.9, 11.5)
Random plasma glucose, mmol/L	13.0 (8.8, 16.8)	14.1 (8.7, 18.4)

Group 1 includes all those without HbA1c comorbidities (n = 129) and Group 2 includes all those with HbA1c comorbidities (n = 63). HbA1c comorbidities are the non-glycaemic biological conditions thought to alter HbA1c reliability e.g., haemo-globinopathies including sickle cell, anaemia, and renal impairment.

Supplementary Table 3: Glycaemic measures correlated with mean day-to-day glucose measured by CGM stratified by presence or absence of comorbidities thought to alter HbA1c reliability

	Overall	Group 1	Group 2
Fasting			
N	192	129	63
r (95% CI)	0.82 (0.76 – 0.86)	0.84 (0.78 – 0.89)	0.78 (0.67 – 0.86)
LR equation	Mean CGM = 0.91 (fasting) + 1.77	Mean CGM = 1.02 (fasting) + 0.71	Mean CGM = 0.77(fasting) + 3.13
Random			
N	192	129	63
R (95% CI)	0.76 (0.69 – 0.81)	0.74 (0.65 – 0.81)	0.80 (0.69 – 0.87)
LR equation	Mean CGM = 0.53(random) + 2.66	Mean CGM = 0.53(random) + 2.80	Mean CGM = 0.55(random) + 2.37
HbA1c			
N	192	129	63
R (95% CI)	0.88 (0.84 – 0.91)	0.89 (0.85 – 0.92)	0.85 (0.76 – 0.91)
LR equation	Mean CGM = 0.15(HbA1c) - 0.61	Mean CGM = 0.16(HbA1c) – 1.07	Mean CGM = 0.14(HbA1c) – 0.02

Group 1 includes all those without HbA1c comorbidities (n = 129) and Group 2 includes all those with HbA1c comorbidities (n = 63). Comorbidities are the non-glycaemic biological conditions thought to alter HbA1c reliability e.g., haemo-globinopathies including sickle cell, anaemia, and renal impairment.

Supplementary Table 4: Short-term glycaemic measures correlated with HbA1c stratified by presence or absence of comorbidities thought to alter HbA1c reliability

	Overall	Group 1	Group 2
Fasting			
N	208	142	66
r (95% CI)	0.70 (0.62 – 0.76)	0.78 (0.71 – 0.84)	0.57 (0.38 – 0.71)
LR equation	HbA1c = 4.62(fasting) + 29.55	HbA1c = 5.40(fasting) + 21.13	HbA1c = 3.57(fasting) + 41.92
Random			
N	211	145	66
r	0.74 (0.68 – 0.80)	0.74 (0.66 – 0.81)	0.74 (0.61 – 0.83)
LR equation	HbA1c = 3.09(random) + 29.01	HbA1c = 3.07 (random) + 28.58	HbA1c = 3.12(random) + 30.39

Group 1 includes all those without HbA1c comorbidities (n = 129) and Group 2 includes all those with HbA1c comorbidities (n = 63). Comorbidities are the non-glycaemic biological conditions thought to alter HbA1c reliability e.g., haemo-globinopathies including sickle cell, anaemia, and renal impairment.

BMJ Open Diabetes Research & Care

Continuous glucose monitoring demonstrates low risk of clinically significant hypoglycemia associated with sulphonylurea treatment in an African type 2 diabetes population: results from the OPTIMAL observational multicenter study

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To cite: Niwaha AJ, Rodgers LR, Carr ALJ, *et al*. Continuous glucose monitoring demonstrates low risk of clinically significant hypoglycemia associated with sulphonylurea treatment in an African type 2 diabetes population: results from the OPTIMAL observational multicenter study. *BMJ Open Diab Res Care* 2022;**10**:e002714. doi:10.1136/bmjdrc-2021-002714

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/bmjdrc-2021-002714).

Received 29 November 2021
Accepted 27 March 2022

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ABSTRACT

Introduction People living with diabetes in low-resource settings may be at increased hypoglycemia risk due to food insecurity and limited access to glucose monitoring. We aimed to assess hypoglycemia risk associated with sulphonylurea (SU) and insulin therapy in people living with type 2 diabetes in a low-resource sub-Saharan African setting.

Research design and methods This study was conducted in the outpatients' diabetes clinics of two hospitals (one rural and one urban) in Uganda. We used blinded continuous glucose monitoring (CGM) and self-report to compare hypoglycemia rates and duration in 179 type 2 diabetes patients treated with sulphonylureas (n=100) and insulin (n=51) in comparison with those treated with metformin only (n=28). CGM-assessed hypoglycemia was defined as minutes per week below 3mmol/L (54mg/dL) and number of hypoglycemic events below 3.0 mmol/L (54 mg/dL) for at least 15 minutes.

Results CGM recorded hypoglycemia was infrequent in SU-treated participants and did not differ from metformin: median minutes/week of glucose <3 mmol/L were 39.2, 17.0 and 127.5 for metformin, sulphonylurea and insulin, respectively (metformin vs sulphonylurea, p=0.6). Hypoglycemia risk was strongly related to glycated haemoglobin (HbA1c) and fasting glucose, with most episodes occurring in those with tight glycemic control. After adjusting for HbA1c, time <3 mmol/L was 2.1 (95% CI 0.9 to 4.7) and 5.5 (95% CI 2.4 to 12.6) times greater with sulphonylurea and insulin, respectively, than metformin alone.

Conclusions In a low-resource sub-Saharan African setting, hypoglycemia is infrequent among people with type 2 diabetes receiving sulphonylurea treatment, and the modest excess occurs predominantly in those with tight glycemic control.

Significance of this study

What is already known about this subject?

- Evidence from high-income countries suggest that severe hypoglycemia is rare in patients taking sulphonylureas, but in those with well-controlled diabetes, non-severe hypoglycemia may be common.
- People treated with sulphonylureas in low-income countries may be at increased of hypoglycemia because of food insecurity, lack of access to glucose monitoring, and use of older sulphonylurea agents that have higher hypoglycemia risk; however, the risk of hypoglycemia with these agents in low-income populations is unclear.

What are the new findings?

- Both continuous glucose monitoring assessed and self-reported hypoglycemia were infrequent in participants with sulphonylurea-treated diabetes and did not differ from metformin.
- Hypoglycemia risk was strongly associated with glycemic control, with most episodes occurring in those with tight glycemic control.
- After adjusting for glycemic control (HbA1c), participants receiving sulphonylurea or insulin treatment experienced two and five times more continuous glucose monitoring assessed hypoglycemia, respectively, than those receiving metformin.

How might these results change the focus of research or clinical practice?

- The high rates of poor glycemic control in type 2 diabetes patients and relatively low hypoglycemic events among patients taking sulphonylureas suggest that there is room for optimizing glycemic control using these cheap, readily available and effective agents in low-resource settings.

INTRODUCTION

The prevalence of type 2 diabetes is rapidly increasing especially in low-income and middle-income countries (LMICs) where the majority of people living with type 2 diabetes reside.[1] While complications of type 2 diabetes can be reduced by maintaining glucose control,[2 3] glycemic control for people living with type 2 diabetes in LMICs is often poor.[4] A key barrier to intensifying glucose-lowering therapy in low-resource healthcare settings is fear of hypoglycemia.[5 6] Sulphonylureas (SUs) and insulin remain the most available treatments after metformin for people living with diabetes in LMICs.[7 8] Because of limited resources, treatments with lower risk of hypoglycemia, such as the newer classes of SUs (eg, gliclazide and glimepiride) and analog insulins, are not readily available in LMICs,[8] and robust glucose monitoring is often unaffordable, even for those treated with insulin.[9] Concerns about hypoglycemia mean that SUs may be started at far higher glycemic thresholds than recommended in international guidance.[10 11]

It is not clear whether this fear of hypoglycemia among type 2 diabetes patients in low-resource settings is justified. Previous studies investigating the burden of hypoglycemia among type 2 diabetes patients in low-resource settings are limited, with available data predominantly from high-income countries.[12] Observational and trial data from high-income countries suggest that severe hypoglycemia is rare in patients taking SUs, but in those with well-controlled diabetes, non-severe hypoglycemia may be common.[13 14] Studies in high-income countries suggest substantially higher rates of hypoglycemia with insulin than SUs.[15 16] However, these data may not apply in resource poor settings where use of older SUs, with higher hypoglycemia risk compared with newer generation SUs (eg, gliclazide and glimepiride) and food insecurity (and therefore missed meals) are common. In addition, due to resource constraints, the majority of those receiving treatment associated with hypoglycemia will not be able to access capillary glucose monitoring.

We therefore aimed to assess hypoglycemia risk with SUs and insulin therapy (in comparison with metformin) in people living with type 2 diabetes in a low-resource sub-Saharan African setting.

METHODS

We compared continuous glucose monitoring (CGM) and self-reported hypoglycemia in people treated with metformin, sulfonylureas or insulin attending diabetes clinics in Uganda. CGM was used to obtain an objective assessment of hypoglycemia.

Study population

People living with type 2 diabetes attending a routinely scheduled diabetes clinic in a rural-based hospital (Masaka regional referral hospital) and urban-based hospital (St. Francis Hospital Nsambya) were invited consecutively. Eligible individuals were aged 18 years and above and treated with metformin, SU or insulin. All participants provided written informed consent before entering the study.

Patient and public involvement (PPI)

Patients were involved in prioritization of the research question. Patients were not involved in the design and conduct of the study. However, they were central to dissemination of the results by choosing to have some of the results sent to their respective clinicians and will continue to be involved in ongoing study dissemination.

Study procedures

We used questionnaires to record baseline patient characteristics including sociodemographic, diabetes medical history, current treatment information, and history of severe hypoglycemia in the previous 12 months.

We assessed glucose levels over a 14-day period from the baseline visit using the blinded Freestyle Libre Pro Glucose Monitoring System (Abbott Laboratories, Illinois, USA) as previously described.[17]

Hypoglycemia assessment

CGM-assessed hypoglycemia was defined according to the international consensus on use of CGM guidelines as the number of hypoglycemic events that occur over the given CGM reporting period.[18] Clinically significant hypoglycemic events were defined as readings below the 3.0 mmol/L (54 mg/dL) threshold for at least 15 minutes. The end of a CGM hypoglycemic event was defined at the point where glucose was at least 3.9 mmol/L (70 mg/dL) for 15 min. Hypoglycemia rate and duration below 3 mmol/mol were standardized to events/week and minutes/week per week, respectively, to account for variation in duration of CGM measurement. Self-reported hypoglycemia data were collected using a questionnaire that captured the history of hypoglycemia requiring assistance of another person, history and number of times the participant was hospitalized due to hypoglycemia in the previous 12 months.

Statistical analysis

Statistical analysis was performed using Stata V.16.1 (StataCorp LLC).

Medians and IQrs are reported for descriptive data due to skewed nature of most variables. We compared median hypoglycemia event rate per week and the median minutes below 3 mmol/L per week across treatment classes using the non-parametric Wilcoxon rank-sum test. Frequency of self-reported hypoglycemia and hospital admission due to hypoglycemia was assessed, and proportions were compared across the three treatment groups using χ^2 or Fischer's exact tests.

Hypoglycemia rate and minutes below 3 mmol/L per week results were positively skewed following a Poisson distribution. We therefore assessed whether the differences in hypoglycemia rates between the three treatment groups were due to confounding by differences in clinical features associated with hypoglycemia using Poisson

regression models. To ensure model assumptions of variance, we fitted Poisson regression with robust SEs.[19] The differences in minutes below 3 mmol/L were also assessed using Poisson regression; the Poisson regression with robust SEs (Huber-White-Sandwich linearized estimator of variance) was preferred to log-linear regressions for easy interpretation of results and due to the presence of numerous natural zeros in the outcome of interest (minutes below 3 mmol/L) and overdispersion.[20] We assessed the rates and the minutes below 3 mmol/L, with and without adjustment for glycemic control (glycated haemoglobin (HbA1c) or fasting plasma glucose (FPG)), age, sex, diabetes duration and body mass index (BMI). We then visually assessed the relationship between FPG and HbA1c using scatter plots and compared rate and duration at different HbA1c and FPG values.

The adjusted means of hypoglycemia rates and minutes below 3 mmol/L per week were then estimated using the margins command for each treatment class (ie, metformin only, SUs and insulin) holding HbA1c or FPG (or other adjusted covariates) at the sample population mean. We also estimated adjusted mean rates of hypoglycemia and minutes per week below glucose levels of 3 mmol/L at clinically relevant HbA1c and FPG thresholds.

RESULTS

Baseline characteristics

One hundred and seventy-nine participants met analysis inclusion criteria (online supplemental figure 1). Twenty-eight participants were treated with metformin only, 100 were treated with SUs (with or without metformin) and 51 were treated with insulin (with or without metformin and/or SU) (online supplemental figure 1). Of the 100 participants treated with SUs, 67 patients (67%) were prescribed glibenclamide, 26 (26%) were prescribed glimepiride and 7 (7%) were prescribed gliclazide. Forty-two of 51 (78.8%) of the patients taking insulin were on mixtard insulin. The median duration of CGM was 14 (IQR: 13–14) days. Baseline characteristics are shown in table 1. Participants treated with SU and insulin had substantially higher glycemia than those treaded with metformin: median HbA1c (mmol/mol) of 66 (IQR: 2–83), 84 (IQR: 67–102) and 46 (IQR: 39.5–63.5) respectively.

Metformin group includes patients being treated with metformin only, SU group includes patients on SUs and metformin, and insulin group includes patients being treated with insulin with metformin and/or SUs. Renal impairment was defined as an estimated glomerular filtration rate (eGFR)<60 mL/min/1.73 m^2. Per cent time spent in optimal range was defined as the percentage of readings and time spent between 3.9–10.0 mmol/L (70–180 mg/dL).

Hypoglycemia was infrequent in participants with SU-treated diabetes and did not differ from metformin

Median minutes and rate below 3 mmol/L per week of CGM defined hypoglycemia were low in those treated with SUs and similar to rates observed in those treated with metformin (figure 1 and table 1). Median (IQR) minutes below 3 mmol/L per week were 39.2 (0–174.8), 17.0 (0–229.3) and 127.5 (0–637.5) with metformin, SU, and insulin, respectively. Median hypoglycemic events/week were 1 (IQR: 0–2.3), 0.5 (0–3.0) and 2 (0–6.0) with metformin, SU, and insulin, respectively. Self-reported hypoglycemia results were broadly consistent with CGM findings, with numerically similar proportions of reported hypoglycemia-related hospitalization with SU (3.0% (95% CI 0.6 to 8.5) and metformin (3.6% (95% CI 0.1 to 18.3)) and higher rates in those treated with insulin (11.8% (95% CI 4.4 to 23.9) (table 1).

Hypoglycemia risk was strongly associated with glycemic control, with most episodes occurring in tightly controlled diabetes

In those treated with SU and insulin, time spent in hypoglycemia and hypoglycemic event rate was strongly associated with glycemic control, with differences in HbA1c explaining 33.1% (p=<0.001) and 20.7% (p=0.005) of variation in time below 3 mmol/L for SU and insulin, respectively (figure 2). The majority of hypoglycemia occurred in those with lower HbA1c or fasting glucose (figure 2 (time <3 mmol/L) and online supplemental figure 2) (hypoglycemia rate). Participants with HbA1c below 53 mmol/mol (7%) spent 2.34% (IQR: 0.60–4.49) and 5.61% (0.34–13.80) of their total time per week in hypoglycemia (<3 mmol/L), for SU and insulin, respectively. In comparison, those who had an HbA1c ≥53 mmol/mol on SU spent 0.0% (IQR: 0.00–0.92) and those on insulin spent 1.27% (0.00–5.75) of their total time per week in hypoglycemia (<3 mmol/L). Participants with fasting glucose <7 mmol/L spent 2.40% (IQR: 0.60–4.98) and 6.52% (IQR: 1.24– 13.50) of their total time per week in hypoglycemia, for SU and insulin, respectively, in comparison with only 0.0% (IQR: 0.00–0.46) and 0.67% (IQR: 0.00–3.44) for those who had fasting glucose ≥7 mmol/L (online supplemental table 1).

In analysis adjusted for HbA1c participants receiving SU or insulin treatment experienced two and five times more hypoglycemia, respectively, than those receiving metformin

Table 2 shows mean and rate ratio for minutes in hypoglycemia by treatment (relative to metformin), unadjusted and with adjustment for HbA1c (model 2) and HbA1c, age, diabetes duration, BMI and sex (model 3). In unadjusted analysis, the mean number of minutes <3 mmol/L per week for SU and metformin treatment did not substantially differ (duration ratio SU vs metformin 1.4 (95% CI 0.69 to 2.91), p=0.35), but duration in hypoglycemia substantially higher with insulin than metformin (duration ratio 2.5 (95% CI 1.3 to 5.0), p=0.009). After adjusting for HbA1c, differences between therapies were accentuated, with minutes

Table 1 Characteristics of CGM-assessed and self-reported hypoglycemia in type 2 diabetes according to treatment

	Median (IQR) for continuous variables, n (%) for proportions		
Variable	**Metformin group**	**SU group**	**Insulin group**
Number	28	100	51
Female, n (%)	18 (64.3)	57 (57.0)	31 (60.8)
Age, years	56.5 (49.5–61.5)	55.5 (50.0–62.0)	55.0 (49.0–64.0)
Diabetes duration, years	5.0 (2.0–8.0)	6.0 (3.0–9.0)	10.0 (8.0–17.0)
BMI, kg/m^2	26.9 (24.2–29.9)	26.7 (23.7–30.1)	25.8 (23.1–30.2)
eGFR	113.4 (96.8–123.7)	112.8 (93.8–121.0)	110.8 (92.3–121.8)
Renal impairment, n (%)	0 (0)	6 (6.0)	4 (7.8)
Glycemic control			
CGM duration	14 (13–14)	14 (13–14)	14 (13–14)
Average CGM glucose (mmol/L)	6.8 (5.4–9.9)	8.5 (7.0–12.0)	10.1 (8.2–14.5)
HbA1c (%)	6.4 (5.8–8.0)	8.2 (6.9–9.6)	9.8 (8.2–11.3)
HbA1c (mmol/mol)	46 (40–64)	66 (52–83)	84 (67–102)
Fasting glucose	7.2 (5.5–10.2)	8.2 (6.2–10.7)	9.3 (7.0–12.3)
Glucose variability (cv)	0.29 (0.26–0.33)	0.34 (0.29–0.39)	0.39 (0.33–0.47)
SD	2.06 (1.65–2.93)	3.16 (2.59–3.85)	4.0 (3.3–5.2)
Percent time spent in optimal range	78.1 (55.3–86.4)	60.1 (33.8–73.9)	40.1 (22.2,–55.4)
Percent time above 10	10.9 (1.3–35.3)	31.9 (14.3–66.0)	49.3 (30.8–74.2)
CGM hypoglycemia per week			
Episodes <3 mmol/L	1 (0–2.3)	0.5 (0–3.0)	2 (0–6.0)
Total time/week <3 mmol/L, min	39.2 (0–174.8)	17.0 (0–229.3)	127.5 (0–637.5)
Per cent time <3 mmol/L (%)	0.39 (0, 1.74)	0.17 (0, 2.26)	1.27 (0, 6.42)
Self-reported hypoglycemia, n (%)			
History of hypoglycemia events, n (%)	7 (25.0)	28 (28.0)	23 (45.1)
Hospitalized for hypoglycemia in the previous 12 months, yes	1 (3.6)	3 (3.0)	6 (11.8)
Hospitalized for hypoglycemia in the previous 12 months, % (95% CI)	3.6 (0.1 to 18.3)	3.0 (0.6 to 8.5)	11.8 (4.4 to 23.9)

BMI, body mass index; CGM, continuous glucose monitoring; eGFR, estimated glomerular filtration rate; HbA1c, glycated haemoglobin; SU, sulphonylurea.

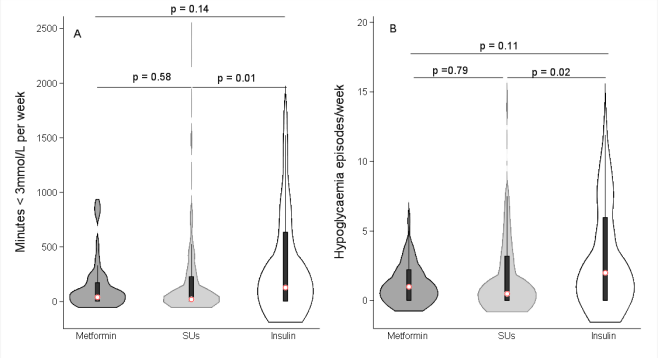

Figure 1 The distributions of hypoglycemia measured by CGM in individuals treated with metformin only, or sulphonylureas (SU) (with or without metformin) and insulin (with or without metformin and/or sulfonylureas). CGM, continuous glucose monitoring.

<3 mmol/mol 2.1 (95% CI 0.9 to 4.7, p value=0.067) and 5.5 (95% CI 2.4 to 12.6, p value=<0.001) times greater than metformin with SU and insulin, respectively. Findings were not substantially altered by further adjustment for age, BMI, diabetes duration, renal impairment and sex.

When adjusting to HbA1c of 53 mmol/mol (7%), an internationally recognized target for glycemic control, estimated minutes in hypoglycemia (per week) were 137.2 (95% CI 49.6 to 224.7), 290.9 (168.8 to 413.0) and 751.9 (433.9 to 1070.0) with metformin, SU and insulin, respectively (online supplemental material 3). Findings were similar for hypoglycemia rates per week, with rates approximately two and five times higher with SU and insulin than metformin after adjustment for HbA1c (table 3). Estimated adjusted mean rates of hypoglycemia at a range of clinically relevant HbA1c (and FPG) thresholds are shown in online supplemental figure 4).

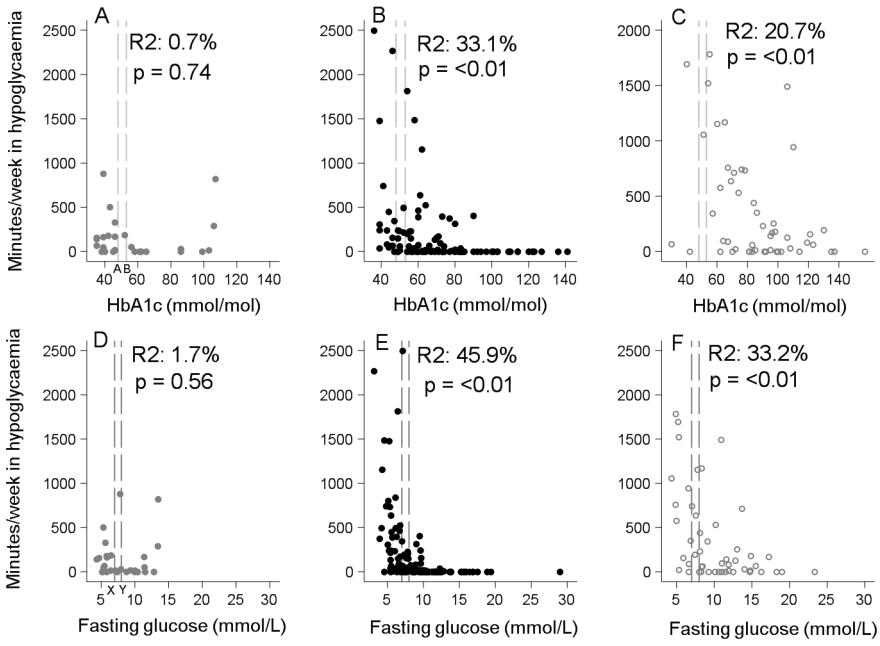

Figure 2 Comparison of glycemic control and hypoglycemia duration (minutes per week <3 mmol/L). Graphs in the top row show the relationship between HbA1c and the number of minutes spent in hypoglycemia per week for metformin (A), sulphonylureas (B), and insulin (C) treated participants, respectively. The bottom row shows the relationship between fasting glucose and number of minutes spent in hypoglycemia per week for metformin (D), sulphonylurea (E) and insulin (F) treated participants, respectively. The long-dashed lines denote glycemic thresholds, HbA1c 6.5% (48 mmol/mol) and 7.0% (53 mmol/mol) (top row), fasting glucose 7.0 mmol/L and 8.0 mmol/L (bottom row). HbA1c, glycated haemoglobin.

DISCUSSION

This study has demonstrated that both CGM assessed and self-reported clinically significant hypoglycemia in participants treated with SUs in Uganda is infrequent among patients who receive SU treatment. While observed hypoglycemia rates and duration were similar in those treated with metformin and SU, hypoglycemia risk was strongly associated with glycemic control, and after adjusting for differences in HbA1c, the risk of hypoglycemia doubled and quintupled in those treated with SUs and insulin, respectively. The modest hypoglycemia excess associated with SUs in comparison with metformin occurred predominantly in those with tight glycemic control. Hypoglycemia was more common in insulin treated diabetes than those treated with SU, further increasing on adjustment for glycemic control.

Table 2 Number of minutes <3 mmol/L per week in type 2 diabetes patients on different glucose-lowering agents before and after adjusting for HbA1c and clinical features

	Variables	Minutes <3 mmol/L (95% CI)	Duration ratio (vs metformin)	P value
Model 1 R^2=0.05	Metformin (Ref)	146.0 (60.6 to 231.3)	1.0	
	SU	206.7 (119.2 to 294.2)	1.4 (0.7 to 2.9)	0.345
	Insulin	365.9 (229.9 to 501.9)	2.5 (1.3 to 5.0)	**0.009**
Model 2 R^2=0.23	Metformin	74.0 (14.6 to 133.4)	1.0	
	SU	156.9 (97.6 to 216.3)	2.1 (0.9 to 4.7)	0.067
	Insulin	405.7 (262.1 to 549.3)	5.5 (2.4 to 12.6)	**<0.001**
Model 3	Metformin	96.4 (20.2 to 172.6)	1.0	
R^2=0.30	SU	157.5 (97.6 to 217.4)	1.6 (0.7 to 3.6)	0.230
	Insulin	355.0 (212.7 to 497.2)	3.7 (1.5 to 9.3)	**0.006**

Model 1: unadjusted; model 2: adjusted for HbA1c; model 3: adjusted for HbA1c, age, diabetes duration, BMI, sex, and renal impairment. Adjusted minutes <3 mmol/L are adjusted to the mean value for the covariate for the cohort (mean cohort HbA1c 73.2 mmol/mol). 95% CIs are shown in the parentheses. Renal impairment was defined as an estimated glomerular filtration rate <60 mL/min/1.73 m^2.
Values shown are mean (95 % CIs) and p-value. Bold values denote statistical significance at the p < 0.005 level.
BMI, body mass index; HbA1c, glycated haemoglobin; SU, sulphonylurea.

Table 3 Hypoglycemia rates in type 2 diabetes patients on different glucose-lowering agents before and after adjusting for HbA1c and clinical features

	Variables	Rates (95% CI)	Rate ratio (vs metformin)	P value (verses metformin)
Model 1 R^2=0.03	Metformin (reference)	1.3 (0.7 to 1.9)	1.0	
	SUs	2.1 (1.4 to 2.8)	1.6 (0.9 to 2.7)	0.108
	Insulin	3.2 (2.1 to 4.2)	2.4 (1.4 to 4.2)	**0.002**
Model 2 R^2=0.21	Metformin (reference)	0.6 (0.3 to 1.0)	1.0	
	SUs	1.5 (1.1 to 2.0)	2.4 (1.4 to 4.1)	**0.001**
	Insulin	3.8 (2.3 to 4.6)	5.4 (3.0 to 9.9)	**<0.001**
Model 3 R^2=0.24	Metformin (reference)	0.7 (0.3 to 1.1)	1.0	
	SUs	1.6 (1.1 to 2.0)	2.1 (1.2 to 3.6)	**0.006**
	Insulin	3.2 (2.0 to 4.4)	4.4 (2.2 to 8.7)	**<0.001**

Model 1: unadjusted; model 2: adjusted for HbA1c; model 3: adjusted for HbA1c, age, diabetes duration, BMI, sex and renal impairment. Adjusted rates are adjusted to the mean value for the covariate for the cohort (mean cohort HbA1c 73 mmol/mol). Renal impairment was defined as an estimated glomerular filtration rate <60 mL/min/1.73 m^2.
Values shown are mean (95% CIs) and p-value. Bold values denote statistical significance at the p < 0.05 level.
BMI, body mass index; HbA1c, glycated haemoglobin; SUs, sulphonylureas.

Studies comparing hypoglycemia risk across different treatments in type 2 diabetes are limited in LMICs, especially sub-Saharan Africa. The few hypoglycemia-related studies among people with type 2 diabetes patients in sub-Saharan Africa that have assessed the incidence and prevalence of hypoglycemia have predominantly used self-reported hypoglycemia and documented increased risk with insulin use.[21] The majority of these studies either included only patients on insulin and or grouped SUs together with other oral glucose-lowering agents.[11 21 22] Our finding that SU treatment is associated with a modest risk of clinically significant hypoglycemia among those with type 2 diabetes is consistent with studies in other popualtions.[23 24] However, it should be noted that the SUs in these studies are of newer generation, like gliclazide and glimepiride, that are known to have a lower hypoglycemia risk compared with glibenclamide.[7] The present study, although not designed to compare intra-SU class differences, showed a modest hypoglycemia risk even when majority (two out of three) of our patient population were taking glibenclamide, an older agent with higher hypoglycemia risk.[7] Moreover, the modest hypoglycemia excess in the SUs group mainly occurred in a small proportion of patients with tightly controlled diabetes, below international glycemic targets.[25–27]

A key strength of this study is the objective assessment of hypoglycemia through use of blind CGM monitoring. This removed potential biases that could arise from patient reactivity to glucose measurements, differences in glucose testing by treatment, hypoglycemia unawareness and recall bias that may affect studies assessing self-reported hypoglycemia or using medical records. An additional strength is comparison across therapies. It is well known that CGM can report occurrence of hypoglycemia in those who do not have diabetes, or are treated with medications not associated with hypoglycemia risk,[28 29] meaning the absolute risk of meaningful hypoglycemia by CGM will be overestimated. By including a metformin 'control' arm in our study, we ensured to avoid this overestimation by assessing the excess risk. A notable limitation of our study was that routine capillary glucose monitoring is not available to the vast majority of people with diabetes in Uganda, due to cost. Therefore, self-reported hypoglycemia is very unlikely to have been confirmed by glucose testing and is likely to be inaccurate in a population like ours where healthy literacy including hypoglycemia education is not good. Such testing may even be limited in a healthcare setting. Additionally, the modest number of participants treated with only metformin will have impacted our ability to detect modest differences in hypoglycemia risk in comparisons against metformin, as shown by the large CIs of estimates for metformin treated participants. Lastly, the majority of participants with SU and insulin treated diabetes had poor glycemic control, while this reflects current practice in this region, given the strong relationship between glycemic control and hypoglycemia risk, it is likely that hypoglycemia rates would be substantially higher were glycemic control improved in this population, as suggested by our adjusted analysis.

Glycemic control is the cornerstone of lowering microvascular complications among people living with diabetes. While there is no doubt that there is an association between SUs (especially the older agents like glibenclamide) and insulin treatment and hypoglycemia, the high rates of poor glycemic control in type 2 diabetes patients and relatively low hypoglycemic events among patients taking SUs suggest that there is room for optimizing glycemic control using these cheap, readily available and effective agents, despite the specific challenges of food insecurity and lack of glycemic monitoring in many LMIC populations. This supports the recommendations

to optimize glycemic control using these readily available and affordable agents including metformin and SUs.[8 30] The modest excess of hypoglycemia was predominantly seen in a small proportion of patients taking SUs whose fasting glucose was less than 7 mmol/L or HbA1c <7% (53mmol/mol) (thresholds often recommended by international guidelines) suggesting caution is needed when treating below these levels.[27]

In conclusion in a low resource sub-Saharan African setting, clinically significant hypoglycemia is infrequent among people with type 2 diabetes receiving SU treatment, and the modest excess occurs predominantly in those with tight glycemic control.

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Acknowledgements The authors would like to thank Aisha Karungi and Winnie Ayuru, the research assistants that assisted with data collection at the diabetes clinics. We are thankful to the staff at the central Biochemistry and clinical diagnostic laboratory services (CDLS) laboratory at the MRC/UVRI & LSHTM Research Unit Entebbe Uganda that performed the glucose and HbA1c assays. The authors would also like to thank the hospital ethical committees, the staff of the respective diabetes clinics, and all the people with diabetes who participated in this study.

Contributors AJN, BMS, MJN, and AGJ conceptualized and designed the study. AJN researched the data with assistance from PAB, RM, MJN, and AGJ. AJN and LRR conducted data cleaning. AJN analyzed the data with assistance from ALJC, LRR, BMS, and AGJ. AJN drafted the paper, which was critically revised by all authors. AJN, BMS and AGJ were fully responsible for the work and conduct of the study, had full access to all of the data and took responsibility for the accuracy and the integrity of the data of analysis. All authors approved the final manuscript.

Funding This work was supported by the National Institute of Health Research, UK (award reference 17/63/131).

Disclaimer The funder was not involved in the design of the study, data collection, data analysis, and preparation of the manuscript.

Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by UVRI REC (UVRI-121/2019) Uganda National Council of Science and Technology (HS 2588). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. Data analyzed in this study are available to researchers on reasonable request from the corresponding author.

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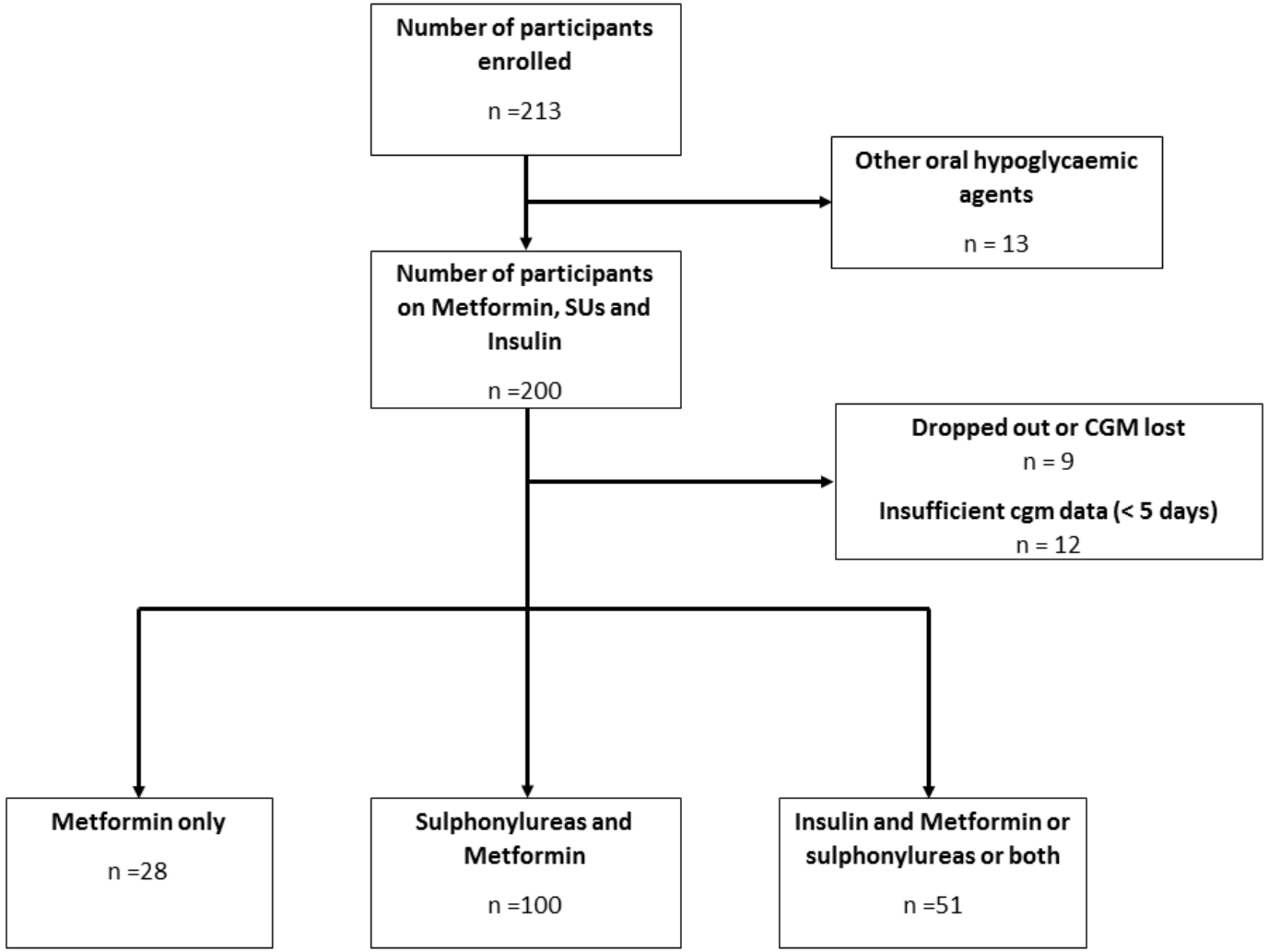
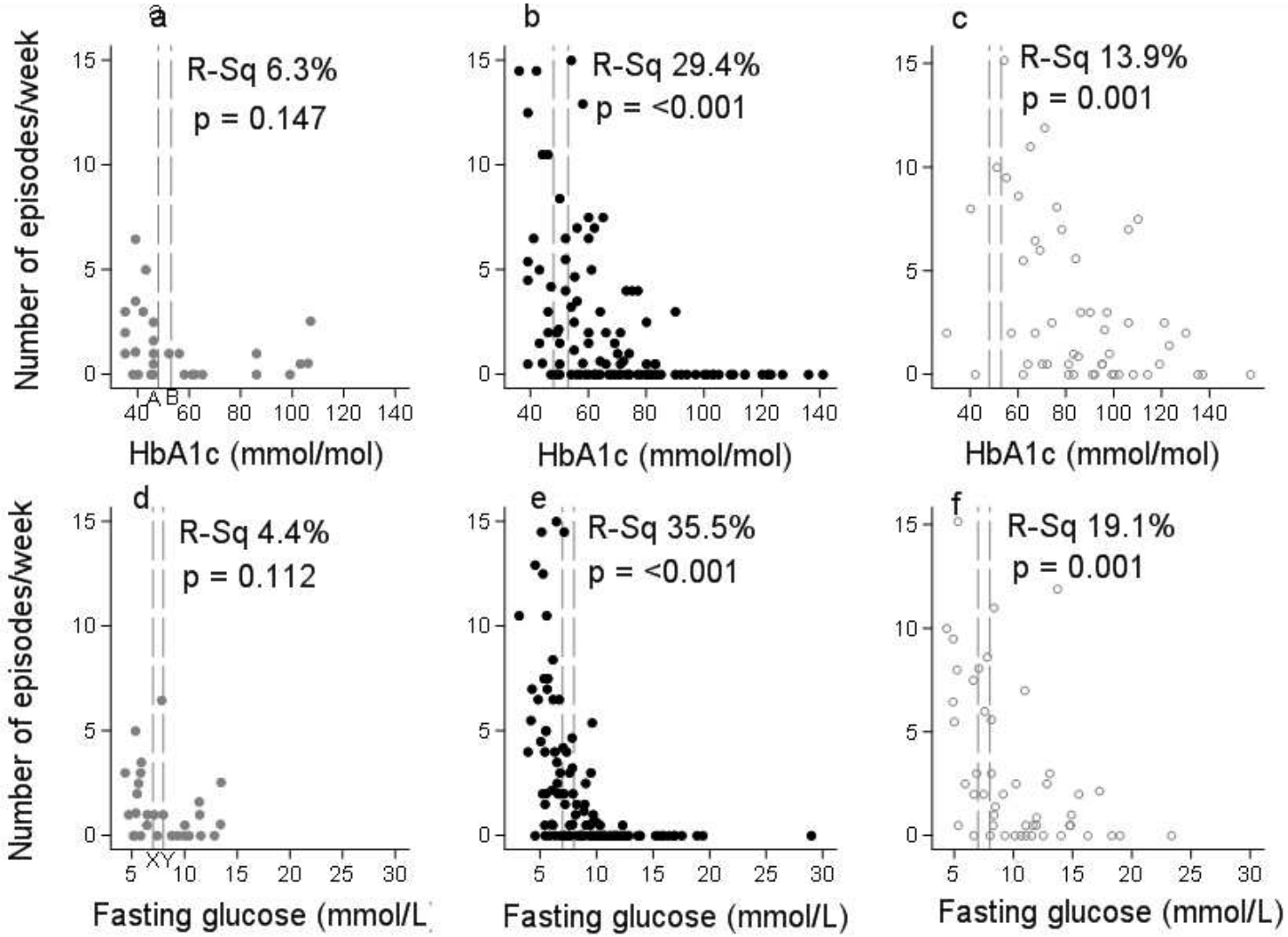


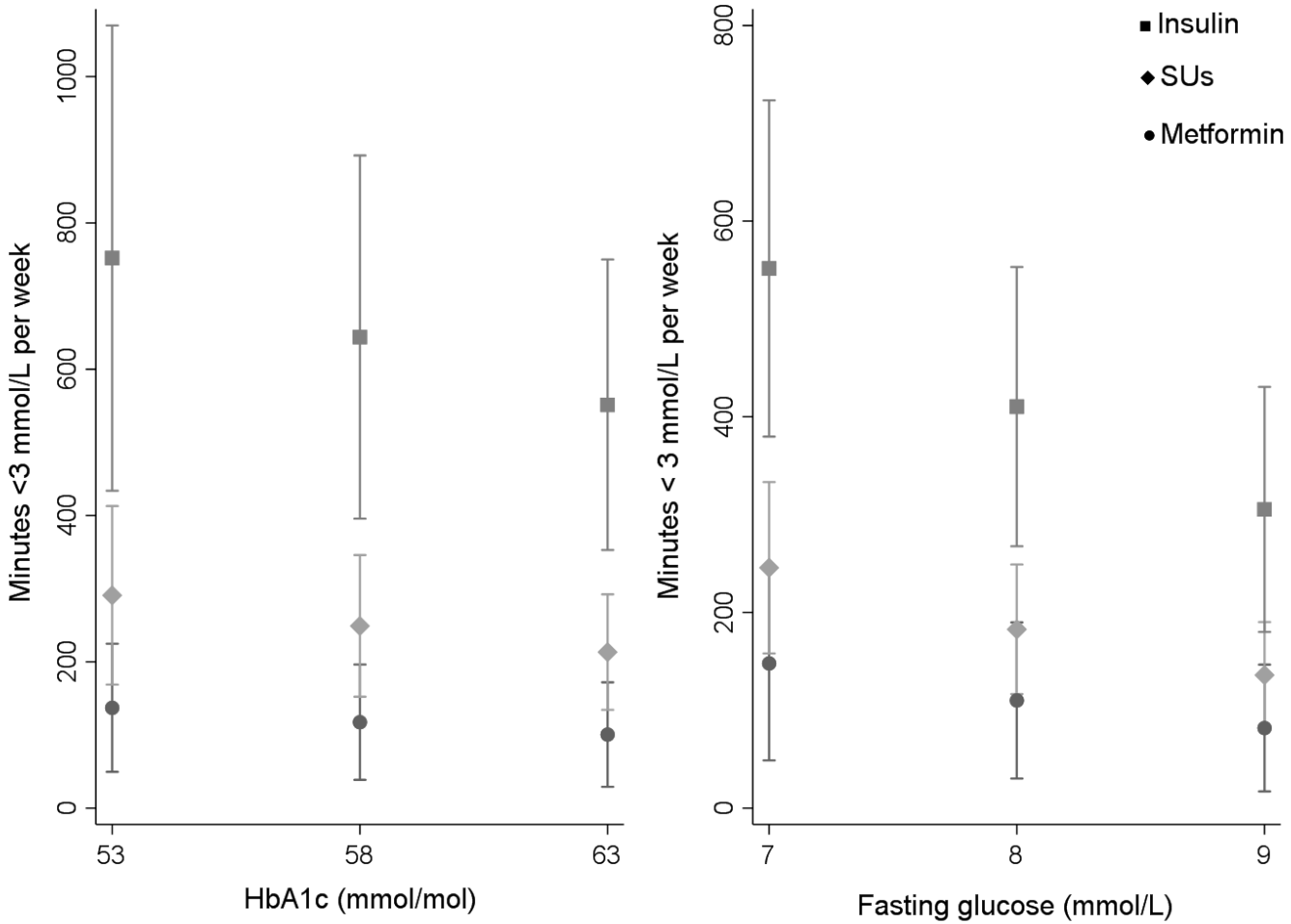
Supplementary figure 1: Participant flow chart

Supplementary Table 1: Percentage of time below 3 mmol/L per week stratified by type of medication and glycaemia control

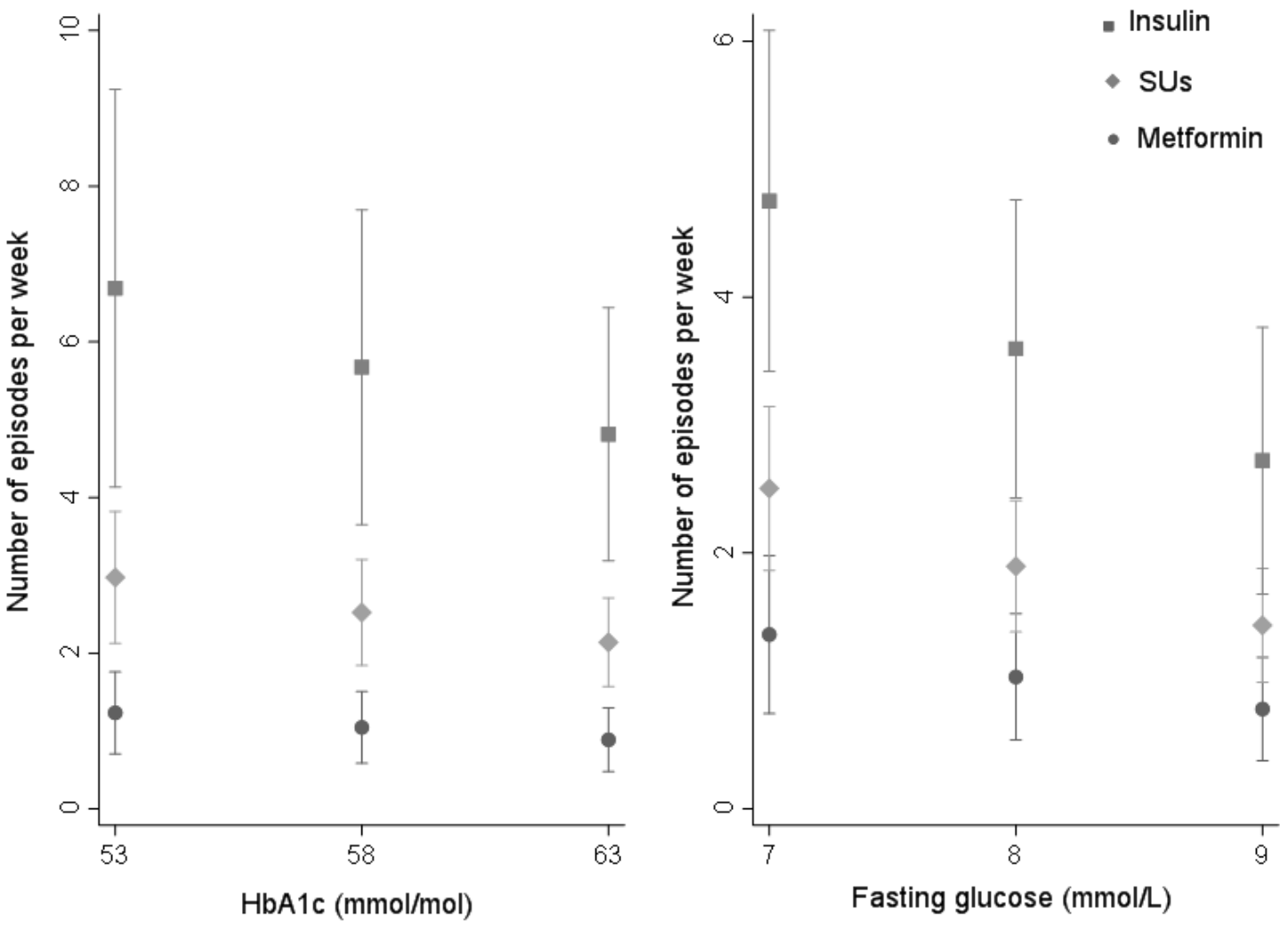
	Metformin		**SUs**		**Insulin**	
HbA1c (mmol/mol)	HbA1c < 53	HbA1c ≥ 53	HbA1c < 53	HbA1c ≥ 53	HbA1c < 53	HbA1c ≥ 7.0 %
Percentage time < 3 mmol/L per week	1.42 (0.15, 1.8)	0.00 (0.00, 0.52)	2.34 (0.60, 4.49)	0.00 (0.00, 0.92)	5.61 (0.34, 13.80)	1.27 (0.00, 5.75)
FPG (mmol/L)	FPG < 7.0	FPG ≥ 7.0	FPG < 7.0	FPG ≥ 7.0	FPG < 7.0	FPG ≥ 7.0
Percentage time < 3 mmol/L per week	1.42 (0.15, 1.8)	0.15 (0.00, 1.68)	2.40 (0.60, 4.98)	0.00 (0.00, 0.46)	6.52 (1.24, 13.50)	0.67 (0.00, 3.44)



Supplementary figure 2: Comparison of HbA1cand Hypoglycaemia rates and duration per week.



Supplementary figure 3: Estimated number of minutes below 3 mmol/L per week at different HbA1c and fasting plasma glucose levels. The error bars denote 95% confidence intervals.



Supplementary figure 4: Estimated number of minutes below 3 mmol/L per week at different HbA1c and fasting plasma glucose levels. The error bars denote 95% confidence intervals.