Accuracy and utility of fasting and stimulated glucose for diagnosis of diabetes in Sub-Saharan Africa

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I certify that all material in this thesis that is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University

Signature…….

Abstract

The oral glucose tolerance test (OGTT) is regarded as the gold standard for diagnosing diabetes and impaired glucose tolerance, and it is widely used in epidemiological studies to estimate the prevalence of diabetes, prediabetes and gestational diabetes mellitus in different populations, including those in sub-Saharan Africa. However, there is a lot of debate on the usefulness of this test, especially in low-resource settings. This thesis aims to determine the accuracy and utility of OGTT in sub-Saharan Africa through four different studies done in Uganda and Malawi.

OGTT results in sub-Saharan Africa are influenced by challenges in sample handling before the analysis. In Chapter 2, we show that in the absence of the recommended sodium fluoride (NaF) sample collecting tubes for glucose measurement, the readily available EDTA tubes can be used provided that the samples are kept in a cooler box with ice and are centrifuged or analysed within six hours.

The accuracy of OGTT based prevalence studies in regions with high food insecurity is unknown. In Chapter 3, we conducted a randomised cross over study in rural Uganda to explore factors that impact fasting and post-load glucose results. We demonstrated that the OGTT is affected by alteration of a single evening meal before the test. The two-hour glucose results are significantly higher after a low-carbohydrate evening meal compared to after a normal carbohydrate evening meal, even if the total daily carbohydrate intake was the recommended amount. The prevalence of abnormal glucose tolerance doubled after a restricted evening meal.

This finding raises questions on the utility of OGTT in populations with high levels of food insecurity.

In Chapter 4, we followed up participants with prediabetes classified by impaired fasting glucose levels in urban and rural Malawi. By a period of 4 years, we found that the progression to diabetes in Malawi is high, with 30% of participants progressing to diabetes in the study period. The incident rate of diabetes was 63 per 1000 person-years. We also found that the waist circumference and the baseline glucose levels were the strongest predictors of progression. A simple chart with probabilities of progression based on these risk factors could be used to identify those at risk of developing diabetes in this population.

In Chapter 5, we analysed the relationship between birth weight and adverse pregnancy outcomes with maternal fasting plasma glucose and stimulated glucose in Uganda. We found that the contribution of maternal glucose to birthweight is much lower in this population than what has been reported in other populations. Fasting plasma glucose was just as good at predicting large for gestational age babies than either one or two-hour glucose results.

An overview of the major findings of each chapter, their implications, and potential future research are discussed in Chapter 6.

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Abbreviations

BMI	-	Body Mass Index
CI	-	Confidence Interval
CV	-	Coefficient of Variation
DM	-	Diabetes Mellitus
FPG	-	Fasting Plasma Glucose
GDM	-	Gestational Diabetes Mellitus
GPV	-	Glucose Pre-analytic Validation Exercise
HAPO	-	Hyperglycaemia and Adverse Pregnancy Outcome
HbA1C	-	Glycated haemoglobin
HIC	**-**	High-Income Countries
HOMA2 IR	-	Homeostatic model assessment insulin resistance
HOMA2%S	-	Homeostatic model assessment insulin sensitivity
IADPSG	-	International Association of the Diabetes and Pregnancy Study Group
IDF	-	International Diabetes Foundation
IFG	**-**	Impaired Fasting Glucose
IGI	-	Insulinogenic Index
IGT	-	Impaired glucose tolerance
LGA	**-**	Large for Gestational Age
LMIC	-	Low and Middle-Income Countries
NCD	-	Non-Communicable Disease
OGTT	-	Oral glucose tolerance test
OR	-	Odds Ratio
SD	-	Standard Deviation

SSA	-	sub-Saharan Africa
TOGA	-	The impact of a small evening meal on Oral Glucose tolerance test in Africa
WHO	-	World Health Organization

Aims and structure of thesis

The overall aim of this thesis is to establish the accuracy and utility of the Oral Glucose Tolerance Test (OGTT) in sub-Saharan Africa and to inform novel practices that can improve its usage in the region.

The introduction reviews the literature describing: diabetes in sub-Saharan Africa, the OGTT, its advantages and limitations in comparison to other methods of determining levels of glycaemia; and outlines previously described approaches that can be used to improve its utility in the region.

The Introduction chapter is divided into six sections. Section one presents an overview of diabetes in sub-Saharan Africa. Section two discusses the diagnosis of diabetes. Sections three and four discuss the diagnosis of prediabetes and Gestational Diabetes Mellitus in sub-Saharan Africa. Section five elaborates on factors that affect the accuracy and utility of OGTT. Section six introduces the areas that will be studied in the subsequent chapters

The subsequent chapters are presented in paper format. Chapter 2 has been published and the rest are prepared for submission.

In Chapter 2, we aim to assess the stability of glucose under different conditions with a view to identifying alternative pre-analytical sample handling procedures that can provide accurate glucose results in the absence of the recommended optimal conditions.

In Chapter 3, we aim to assess the impact of consuming meals of different sizes and carbohydrate content the evening before an OGTT on plasma glucose levels. This is very relevant in the many regions of sub-Saharan Africa, where access to food can often be limited.

In Chapter 4, we aimed to assess the incidence and risk factors of developing Type 2 diabetes patients with impaired fasting glucose in sub-Saharan Africa. We followed up, for approximately four years, 144 participants with Impaired Fasting Glucose (IFG) recruited in Malawi. This result will inform potential strategies to identify those at risk of progression.

In Chapter 5, we aimed to examine the relationship between foetal birth weight with maternal glucose in Uganda and to compare with data from other studies in other ethnic groups and in different continents. The impact of maternal glucose on offspring being large for gestational age allows us to assess whether international criteria for the diagnosis of gestational diabetes are appropriate in sub-Saharan Africa.

Chapter 6 is a discussion of the main findings, conclusions, limitations and future work generated by the work done in chapters, 2,3, 4 and 5.

Chapter 1

Introduction

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Diagnosis of Diabetes in sub-Saharan Africa

1.1 Overview

Oral glucose tolerance test involves measuring fasting and 2-hour glucose after 75 grams glucose load. For over 60 years, this test has been the gold-standard test for diagnosing diabetes mellitus worldwide. But over the years, its utility has been highly debated, especially more recently, with wider acceptance of alternative methods of estimating glycaemic concentrations. This introduction discusses methods of diagnosing glycaemic states, focusing on low resources settings.

1.1.1 Definition of diabetes

Diabetes mellitus, commonly known as diabetes, is a group of metabolic disorders characterised by chronic hyperglycaemia (elevation of glucose concentration in the blood) with disturbances of carbohydrate, fat and protein metabolism (1). It results from inadequate insulin production or reduced tissue sensitivity to insulin (2). Type 2 diabetes mellitus accounts for 90% to 95% of all diabetes and affects 425 million people worldwide (3). The long-term complications of diabetes include developing progressive disease of the retina (retinopathy), kidneys (nephropathy), and damage to the peripheral nerves (neuropathy). People with diabetes are also at increased risk of other diseases, including heart, peripheral arterial and cerebrovascular diseases and infectious diseases such as tuberculosis.

1.1.2 Diabetes in sub-Saharan Africa

While diabetes was previously thought of as a disease of the wealthy, it has become a disease associated with low and middle-income groups with a disproportionate burden of disease lying with those of low social-economic status. Approximately 79.4% of people with diabetes live in low and middle-income countries (4). Sub-

Saharan Africa, a region south of the Sahara desert, where 36% of low-income countries are found, is experiencing the largest increase in diabetes worldwide (5). In 2019, 19.4 million people in the region had diabetes. This is projected to rise to 34.2 million people, an increase of 143%, by 2045, the highest predicted increase worldwide (4, 6). This rise is expected to correspond with a similar surge in the number of people with prediabetes, a transition stage between normal glucose tolerance and diabetes(7-10). The rise in diabetes is commonly attributed to population ageing, increasing urbanisation and economic development leading to more sedentary lifestyles and greater consumption of unhealthy foods associated with obesity (11).

Diabetes has a greater impact on morbidity and mortality related to the disease in sub-Saharan Africa than any other region in the world (12). In 2019, 366200 deaths (6.8 % of all-cause mortality) in the region were attributable to the disease. The disease mainly affects working-age persons between 20 to 70 years and has high complications and devastating economic implications (13). The region also has the highest death rate of people with diabetes under 60 years.

Sub-Saharan Africa has a large loss to care at the stage of detection with a high prevalence of people living with diabetes who are undiagnosed (14). The high burden of undiagnosed diabetes and low awareness of diabetes and control are ongoing concerns in the region (15). Over half (60%) of adults aged 20 – 79 years with diabetes are undiagnosed, the highest proportion of undiagnosed diabetes worldwide (13).

1.1.3. Conclusion

Unless urgent action is taken, the rising diabetes burden will add tremendous pressure to overstretched health systems and pose a significant challenge to development in Africa (16). The burden of diabetes in people living with HIV/AIDS in sub-Saharan Africa remains to be fully determined (17). This extension of the devastating consequences of chronic diseases to the underserved sub-Saharan Africa populations indicates the need for urgent preventative solutions and focus on prediabetes to avert their full development (18).

1.2 Diagnosis of diabetes

Blood glucose analysis has been the cornerstone of both the diagnosis and monitoring of therapy in patients with diabetes for more than a hundred years (19). The diagnosis of diabetes is based on three different measurements: the oral glucose tolerance test (OGTT), fasting plasma glucose (FPG), and HbA1c. Each provides different information about glucose metabolism, reflects different physiological mechanisms, and identifies different people without previous diagnoses as having diabetes (20). Consequently, the various diagnostic criteria have their advantages and limitations discussed in this section.

1.2.1 Oral glucose tolerance test

1.2.1.1 History of Oral glucose tolerance test (OGTT)

The development of the OGTT followed the discovery, in 1913, that carbohydrates influenced blood glucose concentration. This led to the first standard glucose load test conducted in 1917 (19, 21). By the end of the 1960s, it was widely agreed that the use of fasting plasma glucose alone for diagnosis identified individuals too late in the natural history of diabetes. This observation resulted in the OGTT being established as the gold standard for diagnosing diabetes. At least six different recommendations for glucose loads varying from 50 to 100g were proposed, including one by the American Diabetes Association (ADA), who suggested using estimated body surface area to determine the suitable glucose load for the OGTT (22, 23). Finally, in 1985 the World Health Organization (WHO) set out the procedures for diagnosing diabetes. The glucose load was fixed at 75g as a compromise between the 100g load used in the United States and the 50g used in

many parts of Europe (24). The key values of an OGTT were also simplified to two (fasting and two-hour post-load blood).

1.2.1.2 The procedure of conducting an OGTT

The WHO recommends that OGTT should be done after three days of unrestricted diet (25, 26). The procedure of conducting an OGTT involves the participant fasting overnight (8 to 14 hours, water allowed). The patient rests, remain seated for the test duration, and should not eat, drink or smoke during the test. Fasting and two hours samples are necessary for diagnosis. Fasting plasma glucose of over 7mmol/L or 2-hour post-load plasma glucose results of >11.0 mmol/l are the diagnostic cut-offs for diabetes.

1.2.1.3 Use of OGTT in epidemiological and clinical settings

Table 1.1 describes the advantages and disadvantages of the different blood glucose tests: OGTT, FPG and HbA1c. OGTT is used in epidemiological studies to define the prevalence of diabetes and gestational diabetes mellitus in various countries, including those in sub-Saharan Africa (27). OGTT has several advantages as a tool for the diagnosis of diabetes. Firstly, it includes fasting plasma glucose and 2-hour glucose after the oral glucose load. The FPG reflects glucose homeostasis in the post-absorptive state, while the 2-hour plasma glucose primarily reflects the disposal of an exogenous glucose load (28). As a result, it identifies more individuals with dysglycaemia than the FPG or HbA1c (29-31).

However, there is continuing debate about the place of OGTT for clinical purposes, and routine use of the test in clinical practice is discouraged in favour of fasting plasma glucose and HbA1c. In clinical settings, an OGTT is performed when the

patient's fasting glucose is equivocal (6.1 – 6.9 mmol/L), or during pregnancy to test for gestational diabetes (26).

1.2.1.4 Disadvantages of OGTT

The disadvantages of OGTT are that it requires an overnight fast, and it is associated with nausea and vomiting in a subset (~2-5%) of individuals after ingestion of 75 g glucose load. In a setting where the health systems are constrained with limited health workers and a lack of medical supplies, OGTTs are not practical and are seldom done in SSA. OGTT are also sensitive to day-to-day differences due to diet, physical activity and other factors that will be further discussed in the chapters below.

1.2.1.5 Reproducibility of OGTT results

The reproducibility of OGTT in Africa is an area of controversy (32). The OGTT is a highly variable test with a coefficient of variation (CV) of up to 40% on a day-to-day basis within individuals. In one study, the reproducibility of OGTT was 57.9% (33). However, another study determined that the diagnostic reproducibility of OGTT for type 2 diabetes was excellent (84%) but only moderate for prediabetes (51%), suggesting that a single OGTT positive for diabetes is sufficient to guide care and inform epidemiologic study design (34). The reproducibility of OGTT in pregnant women is 78% (35).

1.2.2 Fasting plasma glucose

1.2.2.1 Advantages

The main advantages of FPG are that it requires only a single blood draw making it cheaper and easy to measure than other measurements. The current optimal cut-off of FPG is 7.0 mmol/L and was based on cross-sectional population studies

examining the relationship between the glycaemic threshold and diabetic retinopathy (36). The majority of data on the prevalence of diabetes in sub-Saharan Africa is based on studies in the World Health Organization STEPwise chronic disease risk factor surveillance programme (WHO STEPs). The diagnosis of diabetes in these studies is based on fasting plasma glucose (FPG) using either venous or, mostly, finger-prick capillary glucose.

1.2.2.2 Disadvantages

Low sensitivity of fasting compared to two-hour OGTT in diagnosing diabetes in sub-Saharan Africa

The main limitations with FPG are that it requires a participant to fast before the test, and it is less sensitive than the OGTT (37). Since the 1960s, it has been widely acknowledged that the FPG alone for diagnosis identified individuals too late in the natural history of diabetes.

Fasting glucose also appears to have a very low sensitivity for diagnosing diabetes in sub-Saharan Africa (38-40). Many people with diabetes only meet WHO diabetes criteria on the post-OGTT sample, termed isolated postprandial hyperglycaemia (IPH) (38, 40-43). The International Diabetes Federation (IDF) estimates that 19.4 million adults aged 20-79 years have diabetes in sub-Saharan Africa, representing a prevalence of 3.9% (4). However, two thirds (64%) of the countries lack high-quality in-country data sources. The estimates for these countries are extrapolated from data from countries matched by geographical location, income group, ethnicity, language, and region (4). Only four countries (Comoros, Kenya, Seychelles and Zimbabwe) have data based on Oral Glucose Tolerance Tests (OGTT) (44-46), which is regarded as the gold standard for diagnosis of diabetes worldwide (47).

Some of these countries with OGTT have the highest age-adjusted prevalence of diabetes in adults aged 20-79 in the region: Seychelles (12.3%) and Comoros (12.3%), suggesting that the real prevalence of diabetes might be higher than previously reported (4).

1.2.3 HbA1c

Over the past decade, HbA1c has become universally accepted for diagnosing and monitoring diabetes. Due to the limitations in measuring the FPG and OGTT, the International Expert Committee (IEC) recommended HbA1c for diagnosing diabetes (48). In 2010, the International Diabetes Federation (IDF) and American Diabetes Association (ADA) approved HbA1c as an alternative to the OGTT for the diagnosis of diabetes (49). Since then, the test has been widely adopted in clinical care, especially in High-Income countries (HIC).

1.2.3.1 Advantages

HbA1c has several advantages over the other glucose measurements. Firstly, the test is the simplest test for diabetes because it avoids the need for fasting sample collection and measurement of glucose concentrations at 2-hours as with an OGTT. Secondly, HbA1c estimates the average blood glucose levels of the previous two to three months period; therefore, it correlates strongly with overall glycaemia. HbA1c also has less within-individual variation and better predicts microvascular and macrovascular complications compared to FPG and OGTT (50). HbA1C also leads to few false positives compared with the other glucose-based definitions (51).

1.2.3.2 Disadvantages

Whilst HbA1C is increasingly used as a diagnostic tool in HIC, its use in sub-Saharan Africa faces many challenges. The test is relatively expensive and unavailable in many low-and middle-income countries (52). Another major limitation

of the HbA1c is that it is less sensitive than other measures of glucose measurement. An HbA1c 6.5% for the diagnosis of diabetes has a high specificity (~99%), but the sensitivity is poor (~20-40%) when compared to 75-gram OGTT (53). HbA1c also fails to identify most patients with impaired glucose tolerance (54). Using an HbA1c based definition alone in health surveys does not identify a substantial proportion of previously undiagnosed people who would be considered as having diabetes by OGTT or FPG test (20).

Another key challenge to using HbA1C in sub-Saharan Africa is that it relies on the integrity of red blood cells, which can be affected by several conditions prevalent in the region, including malaria or sickle cell anaemia(55, 56). The impact of these haemoglobinopathies on accuracy of HbA1C results is mostly dependent on the assay method used. HbA1C is measured using the ion exchange chromatography, electrophoresis, boronate affinity chromatography and immunoassay methods (57, 58). Boronate affinity chromatography, a method that measures total glycated haemoglobin is not subject to interferences arising from the presence of most haemoglobin variants and is used as a reference or comparative method in many studies (59).

There are also concerns that black persons have higher HbA1c levels than white persons across the full spectrum of glycaemia, and the differences increase as glucose intolerance worsens (29). However, cut-offs for diabetes with HbA1C have not been established for African populations. These concerns limit the use of HbA1C as a screening tool (31). The World Health Organization (WHO) supports the use of HbA1C >6.5% for diabetes diagnosis but does not support the use of HbA1c for the

diagnosis of prediabetes because of poor performance at lower levels of glycaemia (47).

Table 1.1 summarises the advantages and disadvantages of the traditional diabetes measurements

Screening test	Advantages	Limitations
Fasting plasma glucose	• It can be performed as a single blood draw • The most commonly used test • The majority of the global diabetes prevalence epidemiology studies were based on the FPG criteria	• Requires overnight fast • Less sensitive than the OGTT
Oral glucose tolerance test	• Includes assessment of both fasting plasma glucose and 2-hour glucose after the oral glucose load • Allows assessment of the glucose response after an oral glucose challenge. • Identifies more individuals with dysglycaemia than the FPG or HbA1C	• Requires overnight fast. • Associated nausea and vomiting in a subset (~2-5%) of individuals after ingestion of 75 g glucose load. • Two hours testing duration • Sensitive to day-to-day differences due to diet and or physical activity • It can vary according to the time of day of testing. • Reproducibility is not as good as the FPG or HbA1C.
HbA1C	• Reflects integrated glucose levels over the preceding 3 months • Convenient • Does not require fasting, or patient preparation. • Can be performed as a single blood draw • High reproducibility (precision). • Less day to day variations	• Less sensitive than the FPG and OGTT • Interpretation and accuracy can be affected by the presence of haemoglobin variants (i.e. sickle cell trait), chronic renal failure, iron deficiency anaemia, differences in red blood cell lifespan, and differences with age and race. • Weakly associated with diabetes pathophysiology (e.g. insulin sensitivity, and b-cell function) • May be high or low relative to underlying average glucose levels (accuracy – HbA1c “mismatches” as a reflection of average glucose levels).

1.2.4 High prevalence of diabetes in people with low or normal BMI in sub-Saharan Africa

Though published data is limited, there appear to be distinct phenotypes of diabetes in sub-Saharan Africa, with most patients being young and relatively lean in body size. In contrast to high-income countries, epidemiological studies in low-and-middle-income countries (LMIC), like those in sub-Saharan Africa, have found an increased risk of Type 2 diabetes mellitus (T2DM) at relatively low or normal BMI and in all ages (38, 60). However, no clear determination has been made as to why T2DM is more common in the non-obese population in low-income countries than in high-income countries (38).

1.2.5 Unknown risk of progression in people with discordant fasting and 2-hr glucose results

Furthermore, studies have demonstrated that individuals who have a normal (<6.1 mmol/l) or slightly higher fasting glucose (6.1 – 6.9 mmol/L) but have a 2hour glucose on OGTT >11.2 mmol/L, termed Isolated Post-prandial Hyperglycemia (IPH), have an increased risk of developing cardiovascular diseases and mortality (61-64). IPH in high-income countries is a known risk factor for cardiovascular diseases and mortality (61, 62). But whether IPH carries a similar risk in sub-Saharan Africa, where individuals with IPH often lack the classical characteristics of type 2 diabetes, being thin, young and often from poor/rural settings, is unknown (65).

1.2.6 Conclusion

In population-based health examination surveys, different biomarkers and definitions for diabetes can lead to different estimates of population prevalence of diabetes, with

the highest prevalence when diabetes is defined based on FPG-or-OGTT and the lowest when based on HbA1c alone(20, 39).

The question of which test to use for diabetes prevalence studies (FPG, OGTT or HbA1c) in sub-Saharan Africa is complex because decisions have to consider costs, convenience, and reliability (39). No longitudinal studies have been done in sub-Saharan Africa to determine the best method for the diagnosis of diabetes (66). In a setting where the health systems are constrained with limited health workers and a lack of medical supplies, OGTT is not practical and are seldom done in SSA. HbA1c is also not frequently used because of cost limitations, high prevalence of haemoglobin variants and anaemia (55, 56). Fasting plasma glucose (FPG) is cheaper, easy to measure, and frequently used in clinical practice to diagnose and monitor diabetes in sub-Saharan Africa. However, with the apparently high prevalence of IPH in sub-Saharan Africa, are we missing a lot of people with diabetes, or is most of this IPH relatively benign and therefore we should not be worried?

1.3 Diagnosis of prediabetes

Prediabetes is defined as a transition stage with blood glucose levels higher than normal but not high enough to be diagnosed as diabetes. It is characterised by elevated blood glucose levels defined by the World Health Organization (WHO) or the American Diabetes Association (ADA) and the International Experts Committee (IEC) with different cut-offs (Table 1.2) (67, 68).

Prediabetes is made up of impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or both (69). IFG and IGT are similar in clinical definition but are physiologically different. In IFG, too much glucose is released into the bloodstream from the liver overnight, resulting in raised blood glucose on waking. Whereas, in IGT, the insulin produced by the pancreas does not work properly, is inadequate to meet the demand, or both, resulting in raised blood glucose levels throughout the day and after meals (70). The pathophysiology of IFG includes the following key defects: increased hepatic insulin resistance (71), pancreatic beta-cell dysfunction (72) or chronic low beta-cell mass, deranged glucagon-like peptide-1 secretion, and glucagon hypersecretion (73). Conversely, IGT is characterised by heightened peripheral insulin resistance, normal hepatic insulin sensitivity, progressive beta-cell dysfunction, reduced secretion of insulinotropic hormones, and deranged glucagon secretion (74). Individuals with combined IFG and IGT have peripheral and hepatic insulin sensitivity defects and a progressive loss of beta-cell function.

The importance of IGT and IFG is three-fold: first, they signify a risk of the future development of type 2 diabetes; second, IGT and IFG correspond to an increased risk of cardiovascular diseases (CVD) (7-9); and, third, their detection opens the door to interventions that can lead to the prevention of diabetes. Therefore, individuals

with prediabetes offer a unique target population for identification and intervention (7-10, 75).

Unfortunately, there is relatively little agreement between FPG, OGTT and HbA1c as markers of prediabetes (76, 77). Significant differences in prevalence can depend on whether prediabetes is defined by IFG or IGT, and age and ethnic group of the patients and the criteria used (78).

The global prevalence of prediabetes is currently at 8.3% and is expected to rise to 9.3% by 2035 (79). This high prevalence of prediabetes is worrisome as this implies a huge population at risk of developing diabetes in the future (69). Without lifestyle changes, 15%-30% of people with prediabetes are projected to develop type two diabetes within five years (67). However, there is a lack of data on the prevalence of prediabetes in sub-Saharan Africa (80). Slowing the diabetes epidemic in sub-Saharan Africa requires an understanding of the rate of progression from impaired fasting glucose to diabetes and improved detection of prediabetes (81).

Longitudinal studies in other settings have demonstrated that 40 per cent of people who progressed to diabetes over five years had normal glucose tolerance (NGT) at baseline, suggesting prediabetes status is not sufficient in identifying a large proportion at risk for diabetes (82). Therefore, reliance on established prediabetes criteria may not only miss a large subset of individuals at high risk of developing diabetes but may identify them later than optimal for lifestyle interventions when earlier treatment may be more effective. It has been established that diabetes can be delayed or prevented through lifestyle modification, including dietary change, weight loss and increasing exercise (83). Clinical trials in individuals with impaired glucose

tolerance have shown that diet, exercise or agents such as metformin have a marked effect in deferring the onset of diabetes (10) (84).

Table 1.*2* Definitions of prediabetes recommended by ADA *(1)*, WHO *(47)*, IEC
ADA=American Diabetes Association. WHO=World Health Organization.
IEC=International Experts Committee

	ADA	**WHO**	**IEC**
Fasting glucose concentration (impaired fasting glucose)	5.6-6.9 mmol/L (100-125 mg/dL)	6.1-6.9 mmol/L (110-125 mg/dL)	…
2 hr glucose concentration after 75 g glucose load (impaired glucose tolerance)	7.8-11.0 mmol/L (140-199 mg/dL)	7.8-11.0 mmol/L (140-199 mg/dL)	…
HbA1c (sub-diabetic HbA1c)	5.7-6.4% (39-47 mmol/mol)	…	6.0-6.4% (42-46 mol/mol)

1.4 Diagnosis of gestational diabetes mellitus

An OGTT is also recommended for screening Gestational Diabetes Mellitus (GDM) between the 24th and 28th weeks of pregnancy but is conducted earlier in pregnancy for high-risk women (85).

GDM is diagnosed for the first time during pregnancy and may occur anytime but mostly after 24 weeks (86). Adverse outcomes of GDM include maternal (C-section, hypertension and cardiovascular diseases) and neonatal (macrosomia, neonatal hypoglycaemia, perinatal death) (87, 88).

It is estimated that 20.4 million or 15.8% of live births to women in 2019 had some form of hyperglycaemia in pregnancy, of which 83.6% were due to GDM, while 7.9% were due to diabetes detected before pregnancy and 8.5% due to diabetes (including type 1 and type 2) first detected in pregnancy(4). The vast majority of GDM are seen in low-and-middle-income countries, where access to antenatal care is often limited.

1.4.1 The HAPO study used to define the diagnosis criteria for Gestational Diabetes Mellitus (GDM) did not recruit any participants in sub-Saharan Africa

The diagnostic criteria for GDM were controversial because they lacked correlation to maternal or perinatal outcomes (89). A gold standard for the screening of GDM is lacking. There are wide inconsistencies regarding the method for screening and the diagnostic criteria of GDM. This lack of consensus for screening for and diagnosis GDM led to various diagnostic glucose thresholds being used internationally (Table 1.3) (90).

The Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) was a landmark study conducted to clarify the risks of adverse outcomes associated with various degrees of maternal glucose intolerance less severe than overt diabetes mellitus (91). Based on the HAPO study, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) consensus panel recommended new thresholds for diagnosing (GDM), which aimed to reduce obesity risk by identifying infants who were large for gestational age (LGA). They also recommended universal screening of all pregnant women for Gestational Diabetes Mellitus (GDM) with lower diagnostic cut-offs than previous guidelines (92). This criterion has been adopted by several international boards, including the World Health Organization (WHO), American Diabetes Association (ADA) and the International Federation of Gynecology and Obstetrics (FIGO) (86, 93). However, other international bodies have not incorporated IADPSG criteria, citing that (1) the benefit of treating women with mild GDM is not well established; (2) the increased prevalence of GDM will lead to additional healthcare costs; (3) caesarean section rates and neonatal intensive care units admission rates would increase; and (4) patients identified as having GDM will develop additional psychological burdens which will decrease their quality of life (94).

1.4.2 The impact of maternal glucose on foetal birth weight and adverse pregnancy outcomes in SSA is not known.

However, the HAPO study did not recruit from Africa, a continent with 1.2 billion people. Therefore, it is unknown if a similar relationship between maternal glucose and adverse pregnancy outcomes exists in this population and if the guidelines apply to the context of sub-Saharan Africa (SSA).

The pooled prevalence of gestational diabetes in sub-Saharan Africa is 9.6% (95% CI 7-12%), and the risk factors for GDM are like what has been found in other

settings (95, 96). However, the relationship between maternal glucose and foetal birth weight and adverse pregnancy outcomes in sub-Saharan Africa is not well understood. Many factors contribute to birth weight, including genetics, maternal constraints and glucose. Maternal fasting glycaemia is known to explain approximately 6% of the variance in birth weight in western white populations (97, 98). However, it is unclear what proportion is explained by maternal glycaemia in sub-Saharan Africa.

Table 1.3 Various criteria for gestational diabetes mellitus (GDM) diagnosis using oral glucose tolerance test (OGTT)
Abbreviations: ADA, American Diabetes Association; NICE National Institute for Health and Care Excellence; IADPSG, International Association of Diabetes and Pregnancy Study Group; WHO, World Health organisation

Criteria	Pregnancies	Timing of OGTT	Steps	Glucose Load (g)	Glucose Threshold (mmol/L)			
					Fasting	1 h	2 h	3 h
O'Sullivan, 1964 (99)	All	24-28 weeks	2	100	5.0	9.2	8.1	6.9
WHO, 1999	All	24-28 weeks	1	75	7.0	-	7.8	-
American Diabetes Association (ADA), 2004 (100)	High and medium risk	14-18 weeks for high risk, 28-32 weeks for medium risk	2	100	5.3	10.0	8.6	7.8
National Institute for Health and Care Excellence (NICE), 2015 (101)	High risk	As early as possible	1	75	5.6	-	7.8	-
IADPSG, 2010. WHO,2013 ADA,2016 (92)	All	24-28 weeks	1	75	5.1	10.0	8.5	-

1.5 Factors that affect the accuracy of the OGTT

There is a long and ongoing debate around the accuracy and utility of OGTT in clinical and epidemiological practice (102). The main challenges concerning OGTT can be divided into three pre-analytical, analytical and post-analytical.

1.5.1 Pre-analytical factors

1.5.1.1 Preparatory diet and food intake before OGTT

Several studies have analysed the impact of carbohydrate restriction before an OGTT, a summary of which is provided in Table 1.4. It is known that reduced carbohydrate intake before an OGTT adversely affects the glucose tolerance of normal people (103) (104). Claude Benard first described the phenomenon, which was later termed “starvation diabetes” or “hunger diabetes” (105). Hofmeister first used the term hunger diabetes in 1889 to refer to the glycosuria that occurred in starving dogs when they were given a carbohydrate load. He suggested that during periods of food restriction, glucose use is decreased. In these circumstances, falsely elevated glucose values may occur when a carbohydrate load is given. Himsworth also studied the effect of dietary factors on glucose tolerance and insulin sensitivity in healthy men. He found that glucose tolerance was affected by the amount of carbohydrate in the diet and might not be related to the total caloric intake, protein or fat, or the carbohydrate/fat ratio in the diet (106).

Initially, ingestion of 300g of carbohydrate for at least three days before the OGTT was recommended to avoid misdiagnosis. Wilkerson et al. showed that only having 20g of carbohydrate per day in the three days before the test resulted in 2-hour values in the diabetes range in almost all subjects, with normal glucose tolerance

when given at least 150g of carbohydrates per day for three days (107).

Kaneko et al. also demonstrated that marked carbohydrate restriction in the final evening meal before the fast alone could result in impaired glucose tolerance (IGT) which was not seen if the same individuals were given a high carbohydrate meal at this time. They also noted that this impairment was accompanied by decreased insulinogenic index and increased fasting plasma free-fatty acid concentration (104, 108). However, in this study, the meals were kept iso-caloric, so it is unclear whether these results could be attributed to the foods low carbohydrate or high fat composition due to the Randle effect (109). Free fatty acids levels are known to be greatest during the period of starvation (110). Decreased carbohydrate intake is more important than increased fat intake in glucose intolerance by a low-carbohydrate/high-fat diet (111).

Table 1.4 Review of studies that have analysed the impact of low carbohydrate meals on the OGTT

	Author/ Citation	Title	Aim	N, Summary of methods	Major Findings
1	2021, Rosenberg (112)	Relationship between carbohydrate intake and OGTT results among pregnant women	To evaluate the relationship between self-reported carbohydrate intake and OGTT results in pregnancy	24-hour dietary recall and OGTT in 95 pregnant women at 26 weeks gestation.	Lower carbohydrate intake predicts higher glucose levels
2	2020, Chen (113)	Low-carbohydrate diet & maternal glucose metabolism in Chinese pregnant women	A study to evaluate the relationship between Low card diet and maternal glucose concentration with the hypothesis that LCD may be associated with impaired glucose tolerance	Food diaries divided 1018 gravid mothers into ten carbohydrate groups from low to high as % of food consumption. Models used to compare groups	Low-carb dietary patterns were associated with higher postprandial 1-h glucose
3	2018, Clayton (114)	24-hour severe energy restriction impairs postprandial glycaemic control in young, lean males	To investigate the acute effects of 24hr severe energy restriction in lean males on indices of glycaemic control	14 lean men in randomised crossover trial consisting of a 24hr period of either energy balanced or energy restriction followed by OGTT	Severe energy restriction acutely impairs postprandial glycaemia control in lean men, despite reducing HOMA2-IR.
3	2012, Numao (115)	Short term low carb high-fat diet intake increases post prandial glucose and GLP during OGTT	Determine whether 3-day low carb/high fat alters post prandial glucose & incretins	Cross over study. Nine healthy men, isocaloric diet	Low carb/high fat increased post prandial glucose and GLP levels and decreased first-phase insulin release
4	2003, Buhlin (116)	No influence of high & low carb diet on OGTT in pregnancy	To determine the influence of carb content of diet preceding OGTT in pregnancy.	Cross over study. 34 women, food diaries, Low-carb week: 40%carb 164g carb 1815kcal, high-carb week: 50%carb 254g 2097kcal	Prevalence of GDM not significantly diff: 2 after LCH and three after HCH,
5	2000, Crowe (117)	OGTT and preparatory diet	To evaluate the necessity of a 3-day preparatory diet containing > 150g of carbohydrate	20 healthy obstetric patients. 3-day dietary juice, candy and baked goods =150g carbs or	No difference in OGTT number with abnormal results with or without diet restriction. No

				normal diet for three days. Cross over study	difference in mean glucose values
6	1999, Kaneko (118)	Impairment of glucose tolerance following lowered carbohydrate intake	Extended the period of carb restriction to three days	1-day and 3-day test diet before OGTT. High carb diet: 80%carbohydrate, 15%protein, 5%fat Low carbohydrate diet: 10%carb, 25%protein, 65%fat	High: FPG: 4.7±0.3 120min: 5.0±0.8 Low: FPG: 4.3±0.3 120min: 7.1±1.5 3/12 & 2/8 classified as IGT after 1 & 3 days, IGT accompanied with increase in fasting FFA
7	1998, Kaneko (117, 119)	Low carbohydrate intake before OGTT	Re-assessed the importance of carbohydrates before OGTT	8 men and 4 women. Last meal either: Normal: 80% carbs, 15% proteins, 5% fat, Low carb: 10% carbs, 25% proteins, 65% fat, carbs 3g/kg	3 men and one woman had IGT after low card evening meal
8	1998, Entrekin (120)	Does a high carbohydrate preparatory diet affect the 3 hr OGTT in pregnancy	To determine the effect of high carb on the performance of OGTT	354 women. 108- 150g/d carbs	Mean intergroup fasting, 1, 2, and 3 hr glucose values were similar p=0.35-0.99
9	1960, Wilkerson (107)	The effect of prior carbohydrate intake on OGTT	To determine whether glucose of healthy individuals is impaired by restricting carbohydrate	9 male and 9 female participants. 20g carbohydrates, 1600 kcal in low carb meals or 150g carbohydrate, 1640 kcal in normal carb meals. Five days duration	OGTT should be done after three days of unrestricted diet >150g/day

Food security remains a serious challenge in many households in sub-Saharan Africa, with one out of every four people being undernourished (121, 122). In these populations, decreased consumption of carbohydrates could be due to low intake of calories rather than changes in food groups. Most families consume two meals a day (lunch and dinner) with no breakfast and no snacks in between, but during periods of food shortage, many families may have only one meal a day (123). Seasonal calorie availability in sub-Saharan Africa can vary from a low of 1520 Kcal/capita per day to 2250Kcal /capita/day (124). The effect of changes in meal size the night before an OGTT has not been looked at.

1.5.1.2 Time of fasting

Another factor that could affect the accuracy of the OGTT is the fasting period before the test. OGTT is performed after an overnight fast. However, fasting is not standardised and varies between eight and 16 hours. This variation in fasting before testing could alter the OGTT results. Studies conducted during Ramadan found decreased glucose after a complete fasting period of 13 hours in healthy young men compared to before the Ramadan period (125).

1.5.1.3 Glucose load

There are also challenges concerning the glucose load given during the OGTT. The WHO defined the glucose load given during the OGTT as 1.75 g/kg body weight with a maximum of 75 g (24) (126). This amount was a compromise between the 100g load used in the United States and the 50g used in many parts of Europe (127). Practically, this means that all patients over 43 kg are tested using the maximal dose of 75 g glucose. However, studies have shown an association between a person's height and 2-hour glucose values during an OGTT (128, 129). As the size of the

glucose load is fixed, the amount consumed per kg body weight or pre litre extracellular fluid will vary between individuals; in undernourished populations can be up to 2 g/kg some populations, whilst in grossly obese persons, the dose may be as low as 0.5 g/kg. Participants who are undernourished or elderly are likely to have a decrease in muscle mass which is the primary tissue for muscle glucose absorption after a meal. This variation would influence the person's response to the glucose load.

Finally, the WHO recommends 75 g of anhydrous glucose. Numerous epidemiological studies have used 75 g glucose monohydrate, containing only 68 g of true glucose. Whilst this might only cause a small difference, cumulatively, this might have an effect (130).

1.5.1.4 Sample type and collection tube

Next to pre-testing variability, blood glucose analysis depends on pre-analytical conditions (fasting or postprandial), specimen type (whole blood, capillary or venous blood, serum or plasma), presence of glycolytic inhibitor in the collection tube as well as time from sampling until cell separation (131).

However, part of the problem of performing proper OGTT in sub-Saharan Africa is cost. Venous plasma glucose is the standard method for measuring and reporting. Glucose drinks must be provided together with syringes, needles, blood tubes and trained personnel, as well as glucose analysers. Obtaining accurate results requires careful sample handling, including correct blood-collecting tubes, sample processing and timely analysis. The current guidelines recommend using tubes containing sodium fluoride (NaF) for sample collection due to its ability to inhibit ex-vivo glycolysis (132, 133). These pre-analytical requirements can be challenging in

resource-poor settings, such as sub-Saharan Africa (SSA) (134, 135). For example, the supply of NaF tubes may be erratic or might be thought uneconomical to use such tubes because they are only suitable for glucose analysis (136).

1.5.1.5 Storage and transport

In addition, there are delays in sample analysis, commonly due to challenges in transporting samples to laboratories that are typically many miles away from blood collection sites. These delays are coupled with inconsistent power supply and outages that are endemic in the region (137, 138). Glucose stability also becomes important because of the high ambient temperature in many sub-Saharan African countries. Previous studies of glucose stability at 'room temperature' have used temperatures often far below those experienced in many African situations. Therefore, these studies may not apply to the SSA setting.

1.5.2 Physiological factors that affect OGTT results

1.5.2.1 Exercise

Physical activity influences the way our body processes nutrients. Therefore, exercise before an OGTT is another factor that can alter the results. An exercise session carried out 14 hours before having a high carbohydrate meal significantly reduces postprandial levels of glucose (139). The intensity of exercise, with a combination of diet, is thought to play a crucial role in the variability of OGTT results. Low and moderate-intensity exercise sessions positively improve insulin sensitivity and fasting plasma glucose (140). A combination of moderate exercise and diet restriction usually reduces fasting glucose levels (141).

The amount of physical activity done before an OGTT is not usually recorded, and this provides another concern on the accuracy of OGTT results. In sub-Saharan

Africa, large proportions of people rely on subsistence farming which involves variation in levels of physical activities throughout the year (142). Epidemiological prevalence of diabetes, therefore, might also vary according to the season and the related levels of physical activities at the time of the survey, which is not usually recorded (143).

1.5.2.2 Hydration

OGTT results are also affected by the amount of water consumed before the test(144). Epidemiological research has demonstrated that low daily water intake is associated with an increased diagnosis of hyperglycaemia. Many studies have found that higher prevalence of diabetes and GDM during the summer months with higher 1-hour and 2-hour glucose values during an OGTT and no impact on fasting glucose levels(145-148). While these results may be attributed to other factors discussed before, such as nutritional quality and exercise, hydration status may be considered to explain the differences observed. A possible explanation could be that increased temperature is not usually accompanied by adequate fluid intake, which could lead to hypo-hydration, hypovolaemia, and therefore increased glucose concentration (94). Hydration could play an even more important role in sub-Saharan Africa, where access to clean drinking water varies with seasons, accompanied by intense outdoor physical activity like farming that requires a lot of time in the sun resulting in increased de-hydration.

1.5.2.3 Stress

In populations not used to the procedures of an OGTT or venepuncture, it has been found that the apparent prevalence of diabetes halved when subjects were retested within a week of the initial test, which cannot be merely explained by regression to

the mean (149). A similar description has been reported for white coat hypertension when a patient's blood pressure is raised due to the Stress of being in the clinic.

1.5.3 Analytical factors

1.5.3.1 Central laboratory or point of care device

Glucose is analysed and measured by either a central laboratory or point of care. There are two reference methods for blood glucose measurement: isotope dilution mass spectrometry (ID-MS (150)) and enzymatic (Hexokinase-Glucose-6-Phosphate Dehydrogenase). These tests have been recommended by the Joint Committee for Traceability in Laboratory Medicine, which ensures global standardisation of clinical assays (151). In most clinical laboratories, glucose is measured using three common enzymatic methods: hexokinase, glucose 1-dehydrogenase and glucose oxidase in reactions that are coupled to a chromophore or generate an electric current.

Point of care devices includes blood glucose meters where glucose is measured using capillary blood concentrations. These use enzymes that oxidise the glucose, and then the electron transfer to the electrode is measured (152).

1.5.4 Post-analytical factors

The last phase in the processing pathway is the post-analytical stage which includes processing results into a report format and identifying and communicating the results. As discussed previously in this chapter, the diagnostic criteria for prediabetes and GDM are not uniform, and the classification of the condition varies according to the references used.

1.6 Summary

This introduction explored the use of OGTT to diagnose diabetes, prediabetes and GDM in sub-Saharan Africa. Factors that affect its accuracy and reliability were also discussed. Areas that will be studied in this thesis to understand and improve the use of glucose measurement in the region include:

1.6.1 Problems faced in pre-analytical handling of samples

Challenges in sample handling before analyses might influence OGTT results in sub-Saharan Africa (153). For example, shortages of specific test tubes are a common occurrence. Thus, when Sodium Fluoride (NaF) tubes are not available, Ethylenediaminetetraacetic acid (EDTA) or plain serum tubes are often used to collect blood for measuring glucose. Delay in processing samples is another problem. The high heat environment in many sub-Saharan African countries may compound the issues of glucose stability.

1.6.2 Impact of low carbohydrate intake on OGTT results

Food security remains a serious challenge in many households in sub-Saharan Africa, with one out of every four people being undernourished (121, 122). Seasonal calorie availability in sub-Saharan Africa can vary from a low of 1520 Kcal/capita per day to 2250Kcal/capita/day (124). High 2-hour OGTT glucose would be expected if these persons were carbohydrate depleted in the previous 1-3 days or even the night before the OGTT. These individuals would be classified to have diabetes, although their glucose would be normal in day to day living, and will therefore not be at risk of diabetes complications. Therefore, we hypothesised that isolated postprandial hyperglycaemia after OGTT, and therefore the epidemiological prevalence of "diabetes" in these populations, may not reflect sustained hyperglycemia but rather a

specific response to the high glucose load as a result of the previous carbohydrate restriction.

1.6.3 Risk factors and progression of prediabetes

OGTT is mainly used in epidemiology rather than clinical practice. In clinical practice, fasting plasma glucose is mainly used for diagnosis. Therefore, we need to understand the impact using fasting rather than OGTT will have on both the prevalence and phenotype of diabetes in sub-Saharan Africa. The incidence of diabetes in people with prediabetes in sub-Saharan Africa is not well documented. It is unknown whether identifying impaired fasting glucose (IFG) correctly identifies this population's future risk of diabetes. It is plausible that these individuals are in a steady state of mildly impaired fasting glucose and glucose tolerance, and intervening in everyone with prediabetes might not be cost-effective (154, 155). Long-term follow-up of participants with prediabetes to determine progression to diabetes can establish the significance of prediabetes in sub-Saharan Africa.

1.6.4 Use of OGTT to diagnose GDM

The one situation where OGTT is proposed in clinical practice throughout the world is in pregnancy to define patients with gestational diabetes (91). It is unknown if the criteria proposed from the Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO) study for gestational diabetes are appropriate in sub-Saharan Africa as no participants from the continent of Africa were included in this international study. In addition, when resources are limited, is there a clear benefit from performing a full OGTT and not just a fasting glucose test?

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Chapter 2

Alternative pre-analytic sample handling techniques for glucose measurement in the absence of fluoride tubes in low resource settings.

Plos One

Acknowledgements of co-authors and contributions to the paper

I conceived and designed the study with input from Andrew Hattersley, Moffat Nyirenda, Rob Andrews. I carried out the study. Beverly Shields and I analysed the data. I wrote the first draft of the manuscript. All authors provided support for the interpretation of results, critically revised the manuscript, and approved the final draft of the manuscript.

2.0 Abstract

Introduction

Sodium fluoride (NaF) tubes are the recommended tubes for glucose measurements, but these are expensive, have a limited use, and are not always available in low resource settings. Alternative sample handling techniques are thus needed. We compared glucose stability in samples collected in various tubes exposed to different pre-analytical conditions in Uganda.

Methods

Random (non-fasted) blood samples were drawn from nine healthy participants into NaF, Ethylenediaminetetraacetic acid (EDTA), and plain serum tubes. The samples were kept un-centrifuged or centrifuged with plasma or serum pipetted into aliquots, placed in a cool box with ice or at room temperature, and stored in a permanent freezer after 0, 2, 6, 12 and 24 hours post blood draw before glucose analysis.

Results

A rapid decline in glucose concentrations was observed when compared to baseline in serum (declined to 64%) and EDTA-plasma (declined to 77%) after 6 hours when samples were un-centrifuged at room temperature whilst NaF-plasma was stable after 24 hours in the same condition. Un-centrifuged EDTA-plasma kept on ice was stable for up to 6 hours, but serum was not stable (degraded to 92%) in the same conditions. Early centrifugation prevented glucose decline even at room temperature regardless of the primary tube used with serum, EDTA-plasma and NaF-plasma after 24 hours.

Conclusion

We recommend using EDTA tubes placed in a cool box with ice and analysed within 6 hours as an alternative to NaF tubes in low resource settings. Alternatively, immediate separation of blood with manual hand centrifuges will allow any tube to be used even in remote settings with no electricity.

2.1 Introduction

Glucose measurement is crucial for appropriate diagnosis and management of diabetes. However, obtaining accurate results requires careful sample handling, including correct use of blood collecting tubes, sample processing and timely analysis. The current guidelines recommend use of tubes containing sodium fluoride (NaF) for glucose measurement due to its ability to inhibit ex-vivo glycolysis (1, 2). These pre-analytical requirements can be challenging in resource-poor settings, such as in many parts of Sub Saharan Africa (SSA) (3, 4). For example, the supply of NaF tubes may be erratic or might be thought uneconomical to use such tubes because they are only suitable for glucose and lactate analysis (5). In addition, there are delays in sample analysis, commonly due to challenges in transporting samples to laboratories that are typically away from sites of blood collection. This is coupled with inconsistent power supply and outages that are endemic in the region (6, 7). The question of glucose stability also becomes important because of the high ambient temperature in many SSA countries. Previous studies of glucose stability at 'room temperature' have used temperatures often far below those that may be experienced in many African situations, and therefore they may not apply to the SSA setting.

Alternative pre-analytic sample handling techniques to optimise glucose measurement have been suggested. These include immediate centrifugation and placing samples in ice water for analysis within one-hour post-collection (2, 8). But none of these studies have been in the context of SSA. We, therefore, set out to assess the effect of different pre-analytical conditions on measured glucose

concentration, with a view to identifying alternative pre-analytic sample handling procedures that can provide accurate glucose results in the absence of NaF.

2.2 Methods

The study was approved by the Uganda Virus Research Institute Research Ethics Committee (ref: GC/127/19/03/700), Uganda National Council for Science and Technology (ref: HS 2566), and London School of Hygiene and Tropical Medicine Ethics Committee (ref: 17514). Written informed consent was obtained from all participants.

Participants with no history of diabetes were invited to the MRC/UVRI and LSHTM Unit laboratory in Uganda for the blood tests.

Sixty mls of non-fasted venous blood was taken in the morning (glucose range 4.1–8.0 mmol/L), using an 18 g needle and 20 ml syringes, and allocated into 20 NaF, 20 Ethylenediaminetetraacetic (EDTA) and 20 plain tubes (figure 2.1). Four NaF, 4 EDTA and 4 plain tubes were centrifuged at 3000G for 10 minutes within 30 minutes from being taken and allocated into tubes and stored in a -20^{o}C freezer. These were used as our reference specimens.

Eight NaF, 8 EDTA and 8 plain tubes were centrifuged at 3000G for 10 minutes within 30 minutes from being taken and allocated in aliquot tubes such that there were 8 aliquots from the NaF bottles, 8 from the EDTA and 8 from the plain tubes. 4 of each of these were kept at room temperature and 4 in cool box with ice. At time points 2, 6, 12 and 24 hours one aliquot from each tube from the room temperature and cool box were placed in the -20^{o}C freezer. 4 NaF, 4 EDTA and 4 plain tubes

were stored at room temperature with 1 of each of these bottles centrifuged, aliquoted and placed in -20^{o}C freezer at 2, 6, 12 and 24 hrs from when the sample was taken. Lastly 4 NaF, 4 EDTA and 4 plain tubes were stored in the cool box with the same procedure followed with the specimens stored at room temperature.

The samples were analysed as a batch at the MRC/UVRI and LSHTM Clinical Diagnostic Laboratory in Entebbe, Uganda (coefficient of variation for glucose measurement 1%) using the glucose oxidase method on the Cobas 6000 analyser (Roche/Hitachi, Tokyo, Japan). We compare the results of samples kept in four different pre-analytic conditions: (1) un-centrifuged kept at room temperature, (2) un-centrifuged kept in a cool box with ice, (3) centrifuged kept at room temperature, (4) centrifuged kept in a cool box with ice.

Statistical analysis

The baseline glucose concentrations of all the samples were compared using analysis of variance. Results are presented as mean percentage change from baseline (sample centrifuged, separated and frozen at time point zero). The difference between the baseline and glucose concentration at different time points was assessed by Wilcoxon signed-rank test. We considered samples to be the same if there was a mean change of less than 10 per cent from baseline and a P-value of greater than 0.05.

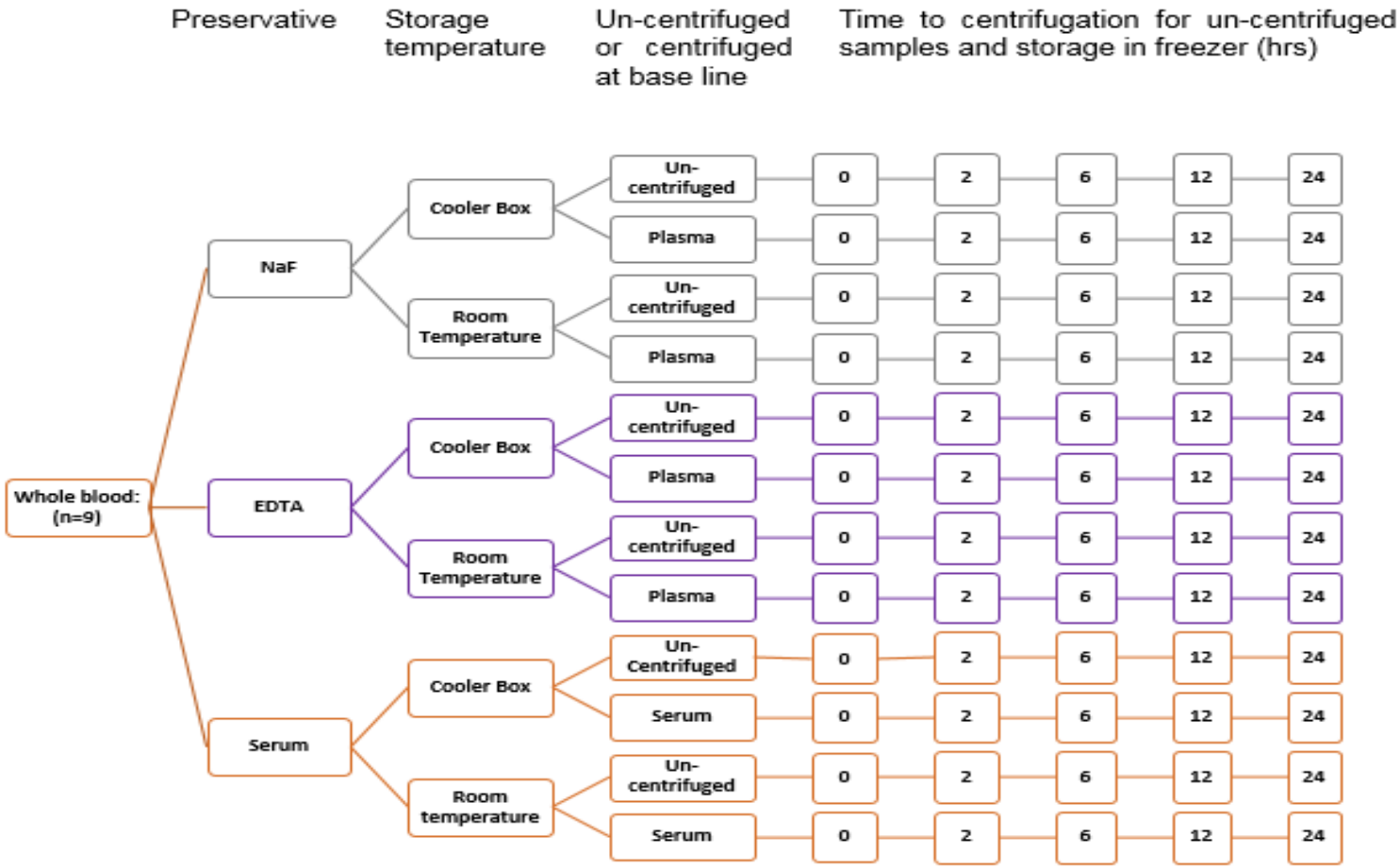

Figure 2.1 Flow diagram detailing the sample collection protocol for the study over 24 hours. At each time-point the samples were centrifuged for 10 minutes. The supernatant was frozen at -20^{o}C.

2.3 Results

Nine people (seven males, two females, mean age 30.8 years, range: 24-50 years) volunteered to take part in the experiment and attended in September 2019 for their blood test. The mean baseline glucose levels from NaF, EDTA and serum tubes were similar 5.4mmol/L, 5.4mmol/L and 5.2mmol/L, respectively (P=0.77). The mean ambient room temperatures after 6 and 24 hours was 28^{o}C and 25^{o}C, which were higher than their corresponding temperatures in the cool box, 6^{o}C and 15^{o}C.

Rapid decline in glucose if left un-centrifuged at room temperature unless in NaF

Un-centrifuged plasma and serum from EDTA and Serum tubes decreased to 77% (P=0.004) and 64% (P=0.004) respectively from baseline after 6 hours and by 34% (P=0.004) and 6% (P=0.004) after 24 hours (figure 2.2A and table 2.1). In contrast, samples kept un-centrifuged in NaF tube had only declined to 94% (P=0.18) at 6 hours and after that remained stable up to 24hours.

Un-centrifuged EDTA plasma but not serum was stable for 6hours if kept on ice at 6^{o}C

Un-centrifuged NaF and EDTA plasmas that were kept in a cool box were stable after 6 hours 100% (P=1) and 97% (P=0.18), respectively (figure 2.2B). Serum from plain tubes stored in a cool box was stable after 2 hours, 99% (P=1) but decreased to 92% (P=0.004) from baseline 6 hours after blood draw.

Early centrifugation prevented glucose decline even at room temperature

All NaF, EDTA and serum samples that were centrifuged immediately after collection, and plasma or serum separated from cells and placed in a cool box with ice were stable at 24 hours (figure 2.2D). Plasma and serum samples centrifuged immediately after collection were also stable at room temperature after 24 hours regardless of the primary tube used, NaF 103% (P=0.17), EDTA 100%(p=0.51) and plain tube 103% (P=0.5) (figure 2.2C)

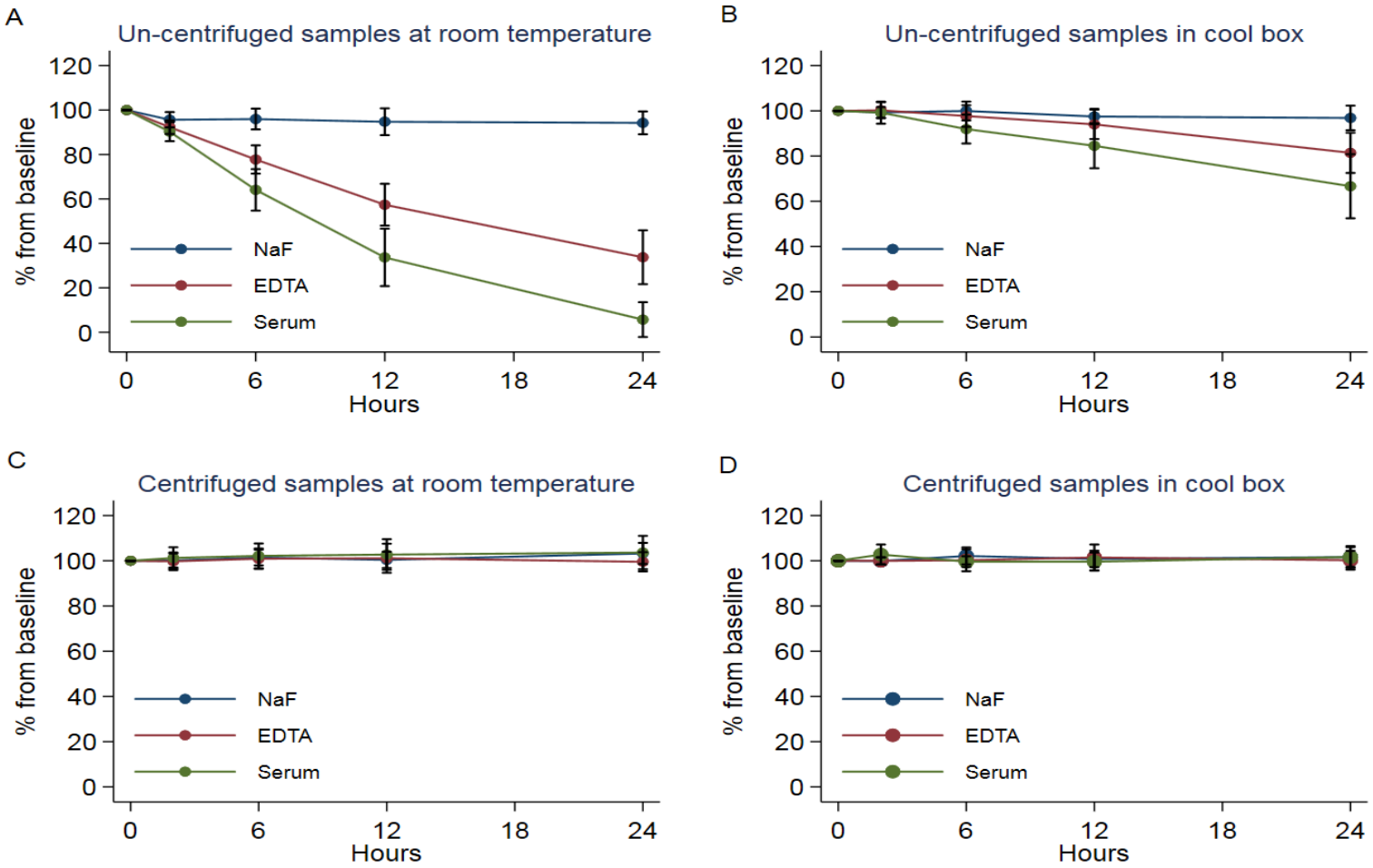

Figure 2.2 The stability over time for A: Un-centrifuged samples at room temperature, B: Un-centrifuged samples in cool box, C: Centrifuged samples at room temperature and D: Centrifuged samples in cool box. Mean percentage change from baseline shown ± 95% confidence interval (CI).

Table 2.1 Mean glucose reduction 6 and 12 hours after collecting in different sampling tubes and stored in different pre-analytical conditions. * Wilcoxon signed-rank test was applied

Condition	Sample collection Tube	Mean % reduction at 6hrs (SD)	P-Value *	Mean % reduction at 24hrs (SD)	P-Value*
Ice uncentrifuged	NaF	100 (4.2)	1	97 (5.5)	0.17
Ice uncentrifuged	EDTA	98 (4.8)	0.17	81(8.9)	0.004
Ice uncentrifuged	Serum	92 (6.4)	0.004	67 (14.1)	0.004
Ice Centrifuged	NaF	102 (3.7)	0.18	102 (4.9)	1
Ice Centrifuged	EDTA	100 (4.8)	1	100 (4.1)	0.28
Ice Centrifuged	Serum	100 (2.4)	0.73	102 (4.3)	1
Room temperature uncentrifuged	NaF	96 (4.7)	0.18	94 (5.1)	0.18
Room temperature uncentrifuged	EDTA	77 (6.3)	0.004	34 (12.1)	0.004
Room temperature uncentrifuged	Serum	64 (9.3)	0.004	6 (7.8)	0.004
Room temperature centrifuged	NaF	101 (3.5)	0.73	103 (4.7)	0.17
Room temperature centrifuged	EDTA	100 (4.5)	1	99 (9.6)	0.51
Room temperature centrifuged	Serum	102 (5.5)	1	104 (7.4)	0.51

2.4 Discussion

In resource-limited settings, where NaF tubes are unavailable, glucose can be accurately measured with appropriate pre-analytical handling. We have shown that if serum or plasma from EDTA and plain tubes is separated within 30 minutes, it can be stored at room temperature and used for up to 24 hours to accurately measure glucose. In addition, if stored on ice, an EDTA specimen can be separated up to 6 hours after being taken and still provide an accurate measure of glucose. This offers potential viable alternative techniques for blood collection and handling that can be utilised in clinical and research settings in SSA.

Glucose falls rapidly in un-centrifuged samples kept at room temperature, even by two hours, in the absence of a glycolytic inhibitor, fluoride (9). This emphasises the need for appropriate sample handling techniques, for example, the use of a cooling agent or rapid centrifugation in scenarios where delays in analysis may occur. However, even when NaF tubes were used, a small decline was observed in the first two hours, with stability achieved thereafter up to 24 hours. This is because the fluoride acts by inhibiting enolase enzyme, which occurs further down the glycolytic pathway. It does not inhibit the enzymes upstream of enolase which continue to metabolise glucose 6 phosphates (10).

The pragmatic approach of not replacing the ice blocks in the cool box resulted in the stability of EDTA-plasma glucose for up to six hours. The decreased temperature results in a corresponding decrease in cellular metabolism leading to preservation of glucose (11). However, we found that un-centrifuged serum was not stable even on ice. This has also been found in other studies (12). We recommend that EDTA tubes

be used in sample collection for glucose analysis if samples are placed on ice immediately after blood draw and analysed or centrifuged within 6 hours.

Glucose was best preserved when the samples were centrifuged immediately, even in serum tubes. The use of serum could enable many biochemical measures to be analysed in a single sample (13). Innovations such as 3D printed hand-held centrifuges could offer solutions to the obvious challenges with advocating for immediate separation of blood in remote areas where conventional centrifuges and electricity are not readily available (14). The glucose concentration in the EDTA-plasma was slightly lower than in the comparable tubes, which could be attributed to the presence of platelets that also undergo glucose metabolism (7).

Illustrated weakness of the study is the limited range of glucose results. It is unclear how the EDTA tubes will perform in very high or low glucose values. Another drawback is that we had a limited sample size and therefore were unable to observe differences, for example, in the baseline plasma and serum glucose reported in other studies (15). Lastly, other blood parameters known to alter glucose concentration, for example, white blood cells and platelets, were not measured. However, this study is amongst the first to assess the impact of several conditions on glucose results on the same samples in a low resource setting in a region with high ambient temperatures.

2.5 Conclusion

NaF tubes remain the preferred collection tubes for glucose measurement in research and clinical setting. In the absence of these, we recommend the use of EDTA tubes, provided they are immediately placed on ice and analysed or centrifuged within 6 hours. If facilities for immediate centrifugation are available, then any collection tube can be used for glucose measurement, and samples can be placed at room temperature, and measurement of glucose made up to 24 hours from sampling.

2.6 Acknowledgement

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Chapter 3

The effect of having a low caloric/low carbohydrate evening meal before an oral glucose tolerance test, implication on screening for diabetes in populations with high levels of food insecurity: a randomised cross over study.

Acknowledgement of co-authors and contributions to the paper

I conceived and designed the study with input from Andrew T. Hattersley, Rob C. Andrews, Moffat J. Nyirenda. I conducted the study and prepared and analysed the data. I drafted the manuscript. All authors provided support for interpretation of results, critically revised the manuscript, and approved the final draft of the manuscript.

3.0 Abstract

Background

Oral glucose tolerance test (OGTT) is widely used globally to determine the prevalence of diabetes and glucose intolerance. However, its performance in settings of food restriction, as is common in sub-Saharan Africa, is unclear. We aimed to determine the impact of the size of the evening meal the day before an OGTT on test results.

Methods

Participants with no history of diabetes were recruited from rural Uganda for a randomised crossover study. The day before the OGTT, participants were given a normal midday meal (1065 kcal, 139g carbohydrate) and then, randomly, either a normal calorie/normal carbohydrate (1065 kcal, 139g carbohydrate) or a small (58 kcal, 14g carbohydrate) evening meal. OGTT was performed the next morning with 30 minutes of blood sampling for two hours. The protocol was repeated after one week with the alternative evening meal. The primary outcome was the difference in the fasting, and two-hour plasma glucose levels in the OGTT performed after the two different sized meals. The trial is registered with Pan-African Clinical Trials, PACTR202007803347704.

Results

Forty (mean (SD) (age 43.8 (15.9) yrs, BMI 23.3 (2.9) kg/m^2, 21 male) participants were randomly assigned to start with either the small evening meal (n=23) or normal evening meal (n=17). All the participants completed all visits. Fasting plasma glucose

levels were higher after normal evening meal versus small evening meal (5.2(0.6) mmol/L vs 4.9 (0.5) mmol/L, p<0.001). Conversely, the 2-hour glucose concentrations were higher after a small evening meal versus a normal evening meal (7.2(1.5) mmol/L vs 6.3(1.7) mmol/L, p=0.003). Overall, glucose tolerance was worse after a small evening meal (mean Area under plasma glucose-time curve 925 mmol min/L(small) vs 841mmol min/l(normal) p<0.001). Twice as many individuals showed impaired glucose tolerance (≥ 7.8 mmol/L) after a small evening meal, compared to a normal evening meal (10 vs 5 p= 0.09). The insulinogenic index, a marker of insulin secretion, was lower post small evening meal (4.55(0.75) (small) vs 4.82(0.76) (normal), p=0.014).

Discussion

This study demonstrates that short-term food restriction the evening before can induce “physiological” glucose intolerance unrelated to sustained hyperglycaemia. This may lead to overestimating the burden of diabetes when OGTT-based estimates are used in populations where food insecurity is common.

3.1 Introduction

Food insecurity in Sub-Saharan Africa and impact on oral glucose tolerance test

The oral glucose tolerance test (OGTT) is regarded as the gold-standard test for diagnosing diabetes and impaired glucose tolerance. It is widely used in epidemiological studies to estimate the prevalence of diabetes, including in sub-Saharan Africa (SSA) (1). The World Health Organization recommend that an OGTT be done after three days of unrestricted diet but does not make specific recommendations for the meal the evening before the test (2, 3). However, many countries worldwide continue to experience seasonal variability in food availability and a high prevalence of malnutrition (4). This is particularly prevalent in SSA, where food security remains a serious challenge, with one out of every four people being undernourished (5-7). During periods of food shortage, many families may have only one meal a day (8). It is unclear how these variations in food supplies affect the glucose results seen with an OGTT and, therefore, the documented prevalence of diabetes or impaired glucose tolerance in populations with food insecurity.

OGTT and burden of diabetes and IGT in SSA

The burden of diabetes is increasing rapidly in low and middle-income countries (LMIC), like those in SSA. The limited data available also suggest that diabetes in LMIC may have unique clinical and diagnostic characteristics. For example, Type 2 diabetes mellitus (T2DM) diabetes is common in young individuals and those with relatively low BMI (9, 10). In addition, fasting glucose appears to have low sensitivity for diagnosing diabetes in SSA, with many people with diabetes only meeting WHO

diabetes criteria on the post-OGTT glucose levels, termed isolated post-prandial hyperglycaemia (IPH) (9, 11-14). The reasons for these discrepancies are not known. IPH in high-income countries is a known risk factor for cardiovascular diseases and mortality (15, 16). But, it is unknown whether it carries a similar risk in SSA, where individuals with IPH often lack the classical characteristics of T2DM (being thin, young and often from poor/rural settings) (17).

Impact of low carbohydrate intake on OGTT results

Several factors can influence OGTT glucose results and, therefore, the accuracy of estimates of diabetes prevalence. These include sample handling techniques and the participant's preparation before the OGTT. The amount of carbohydrate intake before the test is thought to be particularly important; reduced carbohydrate intake before an OGTT worsens post glucose load glycaemia in people with no history of diabetes (18-20).

While previous studies have examined dietary intake prior to OGTT, there have mainly focused on a low carbohydrate diet for up to 72 hours before the OGTT. Moreover, this has involved a low carbohydrate diet in the absence of reduced caloric intake by replacing carbohydrate food with fat and protein. Thus, the impact of food restriction (i.e. reduced calories) rather than the iso-caloric reduction in carbohydrates is not known. In SSA, especially in rural areas, when carbohydrates are not available, they are not replaced with more fats or proteins. The reduction in food intake is commonly in the form of reduced plate size or the number of meals eaten per day. We, therefore, aimed to assess the impact of consuming meals of different sizes immediately before an OGTT on plasma glucose levels in a region of high food insecurity.

3.2 Methods

3.2.1 Study design

We conducted a single-centre, open-label, randomised, repeated-measure, crossover study in Uganda. The Uganda National Council for Science and Technology (HS 2578), the Uganda Virus Research Institute Research Ethics Committee (GC/127/19/03/706), and the London School of Hygiene and Tropical Medicine Ethics Committee (17641) approved the study protocol. The study was registered with the Pan African Clinical Trial Registry, PACTR202007803347704.

3.2.2 Setting

This study took place at the Medical Research Council/Uganda Virus Research Institute and London School of Hygiene & Tropical Medicine (MRC/UVRI & LSHTM) Uganda Research Unit in Kyamulibwa, a rural area in South-Western Uganda, approximately 140 km from the capital city of Uganda, Kampala.

3.2.3 Participants

The MRC/UVRI & LSHTM Uganda Research Unit has a cohort comprising of approximately 22,000 people with men and women in equal proportions who work primarily in agriculture who have agreed to be contacting about research project. We held meetings in the communities where these people lived to inform them about the study.

Those willing to participate formed two straight lines grouped according to sex. Every second person was recruited from each line starting from the nth participant (the nth number was randomly generated from 1 to 5). All participants provided written

informed consent. Eligible participants were 18 to 70 years and free of diabetes. Exclusion criteria were pregnancy, a diagnosis of diabetes and those likely to change medication in the eight-day study period.

3.2.4 Randomisation and masking

Eligible participants were randomly assigned by use of a computer-generated sequence to one of two sequences of either the low calorie/low carbohydrate evening meal (small evening meal), followed by crossover to normal calorie/normal carbohydrate evening meal (normal evening meal) after a seven day wash-out period, or the opposite sequence (i.e. normal evening meal followed by the small evening meal) (figure 3.1). The randomisation was done by an individual who was not involved in the study in blocks. Both the participants and the investigators knew the assigned sequence of the evening meals.

3.2.5 Procedures

Participants arrived at the research unit at around 7 am after an overnight fast of 10 hours. Once consent was obtained, demographic and health information was obtained through the use of an interviewer-administered questionnaire, followed by measurement of anthropometric measurements. Household food security was assessed using the household food insecurity access scale for measurement of food access (21), and participants were classified as either: food secure or mild, moderate and severely food insecure. The participants then underwent an OGTT by ingesting a 75g anhydrous glucose in a 300ml solution with venous samples taken before load and 2 hours post-load. This initial OGTT was done to exclude participants with a

diagnosis of diabetes and was repeated at both visits so that both arms were of the study were similar.

The participants then proceeded to have a normal calorie/normal carbohydrate (1065 Kcal/ 139 g carbohydrates) afternoon meal at noon. The participants were kept at the unit and randomised into either a normal evening meal containing 1065 Kcal/ 139 g or a small evening meal (53 Kcal/14g carbohydrates) between 6:30 and 7:00 in the evening (table 3.1). Block randomisation was done so that the participants who arrived on the same day had the same meals sequence. All meals were consumed within 30 minutes, and the participants ate all of the food given to them. Total daily caloric and carbohydrate content was 2419 Kcal/ 349g on the normal evening meal and 1412Kcal/ 224g on the day of the small evening meal.

The participants slept at the Research Unit and were monitored to ensure no other foods were taken. In the morning, the participants underwent another OGTT with samples drawn at baseline, 30, 60, 90 and 120 minutes for glucose, insulin and c-peptide measurement. The participants then went home and returned for visit two after a seven-day wash-out period when they had the alternative evening meal. Similar study procedures were undertaken during both visits.

Samples for glucose measurement were collected in Sodium Fluoride (NaF) tubes and Ethylenediaminetetraacetic acid (EDTA) tubes were used for insulin and C-peptide measurement. All samples were centrifuged within 15 minutes after blood draw and transported, under cold-chain storage, to the central laboratory for glucose measurement, within 24 hours, or stored at -80^{o}C for batch processing of insulin and C-peptide analyses.

Plasma glucose was measured by the glucose oxidase method using the Cobas 6000 analyser (Roche/Hitachi, Tokyo, Japan). The insulin and C-peptide were measured using enzyme-linked immunosorbent assays on the Cobas 8000 analyser. HbA1c was measured using the immunoassay method.

3.2.6 Outcomes

The primary outcomes of interest were the difference in the fasting and two-hour glucose concentrations in the OGTT performed after the two meals. Secondary outcomes were differences in insulin secretion and insulin resistance after the two meals as measured by homeostatic model assessment of insulin sensitivity, the Matsuda insulin sensitivity index and the Insulinogenic Index.

Table 3.1: Diet menu of the food consumed during the two study visits.
Nutrition content of the foods are obtained from the Food Composition Table for Central and Eastern Uganda developed by harvest plus

Food Description	Food Quantity (g)	Energy (kcal)	Protein (g)	Lipid Total (g)	Carbohydrate (g)
Morning					
Glucose	75	290	0	0	71.3
Afternoon meals					
Rice	100	130	2.4	0.2	28.6
Banana, Matooke	250	335	3.5	1	87.8
Groundnuts paste	100	588	25.1	50.4	19.6
Cabbage	50	11.5	0.7	0.1	2.8
Total	**500**	**1064.5**	**31.7**	**51.7**	**138.8**
Normal calorie/carbohydrate (normal) evening meal					
Rice	100	130	2.4	0.2	28.6
Banana, Matooke	250	335	3.5	1	87.8
Groundnuts paste	100	588	25.1	50.4	19.6
Cabbage	50	11.5	0.7	0.1	2.8
Total	**500**	**1064.5**	**31.7**	**51.7**	**138.8**
Low calorie/carbohydrate (small) evening meal					
Cabbage	250	57.5	3.3	0.3	13.8
Day totals for high carbohydrate evening meal diet		2419	63.4	103.4	348.9
Day totals for low carbohydrate evening meal diet		1412	35	52	223.9

3.2.7 Statistical analyses

Based on a two-sided type 1 error level of 0.05, we calculated that 40 participants would provide 80% power to detect a mean difference of 1 mmol/L between the two-hour OGTT results after the different evening meals. Data were analysed in Stata V.16. The design of the trial and wash-out was to minimise any possible carry-over effect.

Baseline characteristics are reported as means (SDs) for normally distributed continuous variables, as medians (IQRs) for skewed continuous variables, and as counts (percentages) for categorical variables. The changes in the plasma glucose levels following glucose load were displayed as the area under the plasma glucose-time curve (AUC) for the period between the fasting and two-hour post glucose load using the trapezoidal method.

The formula for Area under the glucose curve using the trapezoidal rule :

0.5 X fasting + 30 min glucose + 1.5X60 min glucose + 1.5X90 min glucose + 120 min glucose

Student's paired t-tests were used at each time point and for the overall AUC to evaluate the effects of calorie and carbohydrate restriction on glucose. The results of the OGTT after the different meals were classified as normal or impaired using the WHO classification criteria and were compared using Mc Nemar's chi-square test.

To examine the mechanism of the changes in glucose seen, we utilised the insulin measures during the OGTT. To measure insulin sensitivity, we used the homeostatic model assessment of insulin sensitivity (HOMA2 %S), using freely available online

software (http://www.dtu.ox.ac.uk/homa-calculator), and the Matsuda insulin sensitivity index (22) (23).

The formula for the Matsuda Index (22)

$$\left(\frac{1000}{\sqrt{(fasting\ glucose\ X\ fasting\ insulin\ X\ mean\ glucose\ X\ mean\ insulin)}}\right)$$

To measure insulin secretion in the two tests, we used the Insulinogenic Index (IGI) (24), a ratio of insulin concentration at 30 minutes minus fasting insulin to the difference of glucose at the same time, was measured after each meal and compared using the Student's paired t-test.

The formula for the insulinogenic index (24):

$$\frac{30\ min\ insulin - fasting\ insulin}{30\ min\ glucose - fasting\ glucose}$$

3.2.8 Role of funding source

The study's funder had no role in research design, data collection, data analysis, data interpretation, or report writing. The corresponding authors had full access to all the data in the study and had the final responsibility to submit for publication.

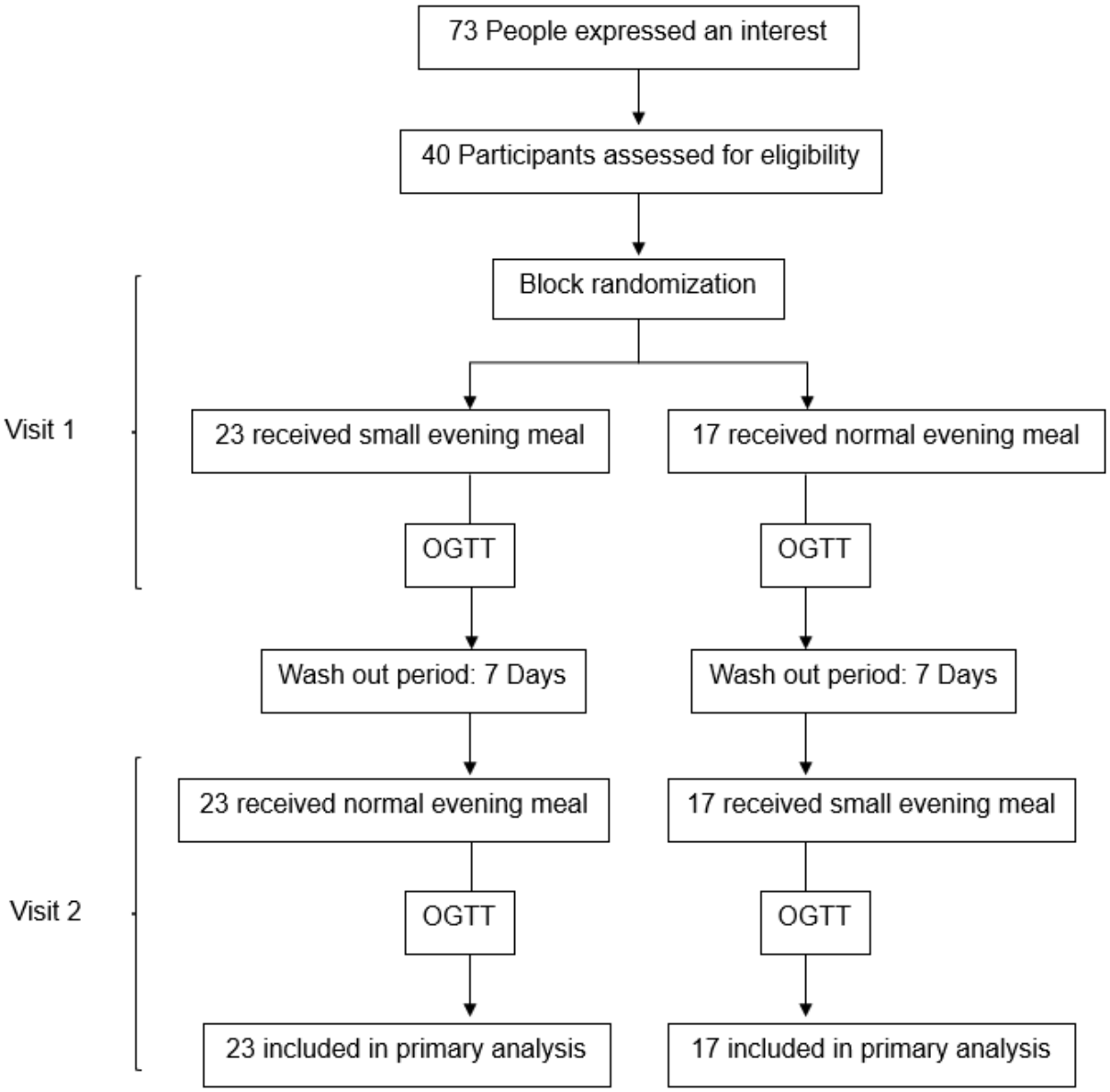


Figure 3.1 Study flow chart All 40 participants completed both study visits and were included in the primary analysis: Normal evening meal consisted of 1065 Kcal/ 139 g carbohydrates, and the small meal consisted of 53 Kcal/14g carbohydrates

3.3 Results

Baseline characteristics

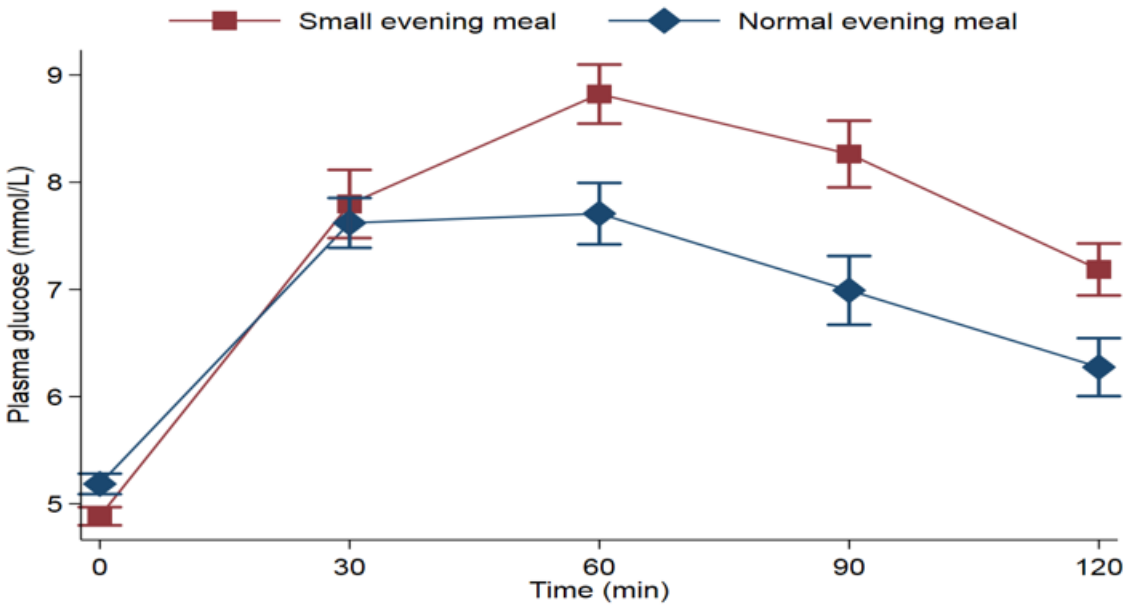
Forty participants were screened for eligibility and enrolled in the study, with 23 randomised to a small evening meal and 17 to a normal evening meal in the first visit before the cross over. All study participants completed both visits and were included in the study analysis (figure 3.1). The baseline characteristics of participants are provided in (table 3.2). There was a high level of moderate and severe food insecurity amongst the participants, with 62 per cent of participants being moderately or severely food insecure.

Fasting and 2 hr glucose results change after a small evening meal

We did not expect any carry-over effects, and no sequence or period effects were observed on testing. The analysis of the primary endpoints showed that the small meal the evening before the OGTT resulted in a lower fasting glucose than the normal evening meal (4.9 (0.5) v 5.2(0.6) mmol/L vs, $p< 0.001$), and a higher 2-hour glucose (7.2(1.5) mmol/L vs 6.3 (1.7) mmol/L, $p=0.003$). Overall plasma glucose concentrations were higher in participants after the small evening meal compared to when they had the normal evening meals (glucose AUC 926(166) mmol min/L vs 841(165) mmol min/L, $p<0.001$) (table 3.2) with a greater and more prolonged elevation of plasma glucose concentration (figure 3.2).

Table 3.2 Participant characteristics at baseline Data are mean (SD), n (%), or median (IQR)

	Analysis population (n=40)
Age (years)	43.8 (15.9)
Male	21 (52%)
Household food insecurity assessment	
Food secure, n (%)	14(35%)
Mildly food insecure, n (%)	1(3%)
Moderately food insecure, n (%)	18(45%)
Severely food insecure, n (%)	7(17%)
Bodyweight, kg	60.7(10)
Waist circumference, cm	78.3 (75.2-87.1)
BMI (kg/m^2)	23.3 (2.9)
HbA1C, %	4.6 (0.6)
HbA1C, mmol/mol	27.6 (6.6)



	Small meal	Normal meal	Mean Difference (95% CI)	P Value*
Fasting glucose (mmol/L)	4.9(0.5)	5.2 (0.6)	-0.3 (-0.5 to -0.1)	<0.001
30 min glucose (mmol/L)	7.7(2.1)	7.6 (1.5)	0.2 (-0.4 to 0.8)	0.68
60 min glucose (mmol/L)	8.8 (1.7)	7.7 (1.8)	1.1 (0.6 to 1.6)	<0.001
90 min glucose (mmol/L)	8.3 (2.0)	7.0 (2.0)	1.3 (0.7 to 1.8)	<0.001
120 min glucose (mmol/L)	7.2 (1.5)	6.3 (1.7)	0.9 (0.3 to 1.5)	0.003
AUC glucose (mm min)	926 (166)	841 (165)	85 (41 to 128)	<0.001
Impaired fasting glucose N(%)	1(2.5)	3(7.5)		0.16†
Impaired glucose tolerance N(%)	10(25)	5(12.5)		0.09†

Figure 3.2 Plasma glucose time curves during the OGTT after the small evening meal (red) or normal evening meal (blue) The graph show plasma glucose after a small evening meal containing 53 Kcal/14g and a normal evening meal consisting of 1065Kcal/139 g carbohydrates for the 40 participants. The intervals in the figure are standard errors (SE). The table below shows the outcomes after the different meals. Data are mean (SD), mean difference (95% CI).*Paired t-test was applied unless otherwise stated. † Mc Nemar's chi-square test was applied.

Diagnosis of impaired glucose tolerance doubled after a small evening meal

The number of people with impaired fasting glucose (≥ 6.1mmol/L) was lower after the small evening meal than a normal evening meal (1 vs 3, p=0.16). In contrast, ten of the 40 (25%) participants were classified as impaired glucose tolerance (≥ 7.8 mmol/L) after a small evening meal versus five after the normal evening meal (p=0.09).

High post-OGTT hyperglycaemia after a small evening meal is associated with a reduction in early insulin secretion.

To assess the mechanism associated with the changes in glycaemia in the OGTT, we assessed insulin sensitivity and insulin secretion using insulin measures measured in the OGTT. The insulin sensitivity was increased in the presence of the small meal compared to the normal meal shown either by HOMA2%S analysis (4.90 (0.38) vs 4.70 (0.30), p=<0.001) and the Matsuda insulin sensitivity index (3.04 (0.67) vs 2.84 (0.62) p=0.001) (table 3.3, figure 3.3). Consistent with a defect in early insulin secretion, the insulinogenic index was lower post small evening meal (4.55 (0.75) vs 4.82(0.76), p=0.014).

Table 3.3 Insulin sensitivity and insulin secretion measured from glucose, insulin and C-peptide measurement during the OGTT after a small evening meal or normal evening meal Insulin sensitivity was measured from the HOMA2 model using fasting glucose and C-peptide (23) and by the Matsuda Index (22) using glucose and insulin measurements in the OGTT. IGI, Insulinogenic Index. Data is presented in the log-transformed form and is displayed as mean (SD) or mean difference (95% CI). *Paired t-test was applied.

	Small meal	Normal meal	Mean difference (95% CI)	P Value*
Log HOMA2%S	4.84 (0.31)	4.64 (0.27)	0.20 (0.10 to 0.21)	<0.001
Log Matsuda Index	3.04 (0.67)	2.84 (0.62)	0.21 (0.08 to 0.33)	0.002
Log IGI	4.55 (0.75)	4.82 (0.76)	- 0.27 (-0.03 to -0.51)	0.014

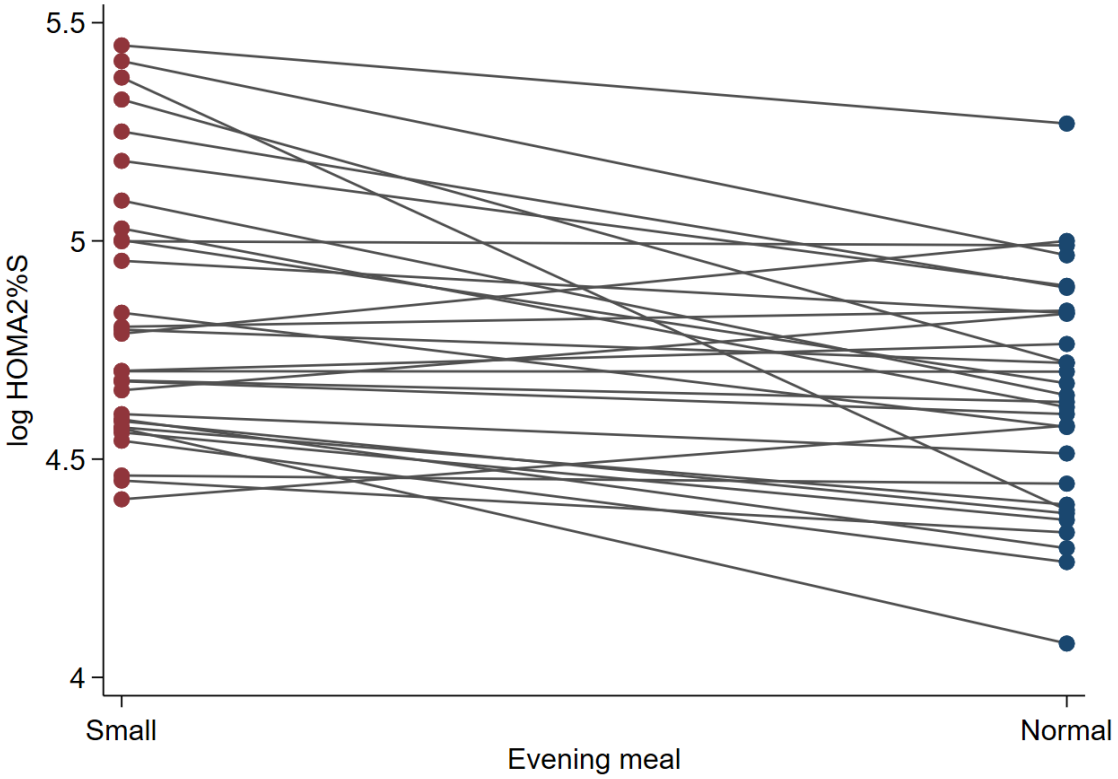


Figure 3.3 Paired plot of log HOMA2%S (n=30) After a small evening meal in red and a normal evening meal in blue

3.4 Discussion

We have shown that oral glucose tolerance test results are altered by the size of the meal eaten the evening before it is performed. A small evening meal with reduced calories and carbohydrates slightly reduces fasting glucose but markedly raises the 2-hour glucose. This has epidemiological and clinical implications.

In 2006 the WHO resolved to maintain the OGTT as a diagnostic tool mainly because fasting fails to diagnose approximately 30% of cases of previously undiagnosed diabetes and is the only means of assessing IGT. According to the IDF 2019, the majority (72.2%) of people with IGT live in LMIC like those in SSA (25). Similarly, diabetes is increasingly in non-obese individuals (BMI <30 kg/m2); this is particularly common in LMIC, including SSA (10). Several factors have been proposed to explain this phenomenon. For example, it is thought that undernutrition early in life, including during the prenatal period, can increase the risk of diabetes, independent of adulthood lifestyle risk factors, generally referred to as the thrifty phenotype hypothesis(26). Similarly, environmental exposures in SSA may impair pancreatic beta-cell function and lead to specific forms of diabetes that are not associated with obesity, such as ketosis-prone diabetes (27, 28). However, our data, which show a doubling in the number of people with IGT merely by giving a lower carbohydrate/ lower calorie evening meal, suggest a potential confounding effect of food insecurity and restriction on OGTT performance. This raises the questions, in these contexts, of the practical utility of OGTT results when the contents of the evening meal are not standardised.

Our findings are consistent with previous studies showing that large changes in dietary content can substantially impact the result of OGTTs (4, 29). Wilkerson et al.

showed that only having 20g of carbohydrate per day in the three days before the test resulted in 2-hour values in the diabetes range in almost all subjects, with normal glucose tolerance when given at least 150g of carbohydrates per day for three days (20). Another study demonstrated that marked restriction in the final evening meal before the fast alone could result in impaired glucose tolerance (IGT) which was not seen if the same individuals were given a high carbohydrate meal at this time (18, 19). However, in this study, the meals were kept iso-caloric, so it is unclear whether these results could be attributed to the meals low carbohydrate or the high fat composition (30) (31). To our knowledge, our study is the first study to assess the impact of different meal sizes consumed immediately before the OGTT on glucose levels and insulin secretion in people in a region of high food insecurity. The high prevalence of food insecurity in our study population is likely to be representative of many settings in SSA and other LMICs where these findings will be relevant.

The potential mechanisms of how low carbohydrate diets impact glucose metabolism are complex and not yet fully understood. Some propose that the mechanism is partly due to loss of first-phase insulin release resulting in decreased peripheral and hepatic glucose uptake and incomplete suppression of hepatic glucose production (32-34). Our findings support this hypothesis as we observed a lower change in the insulin concentration in the early stages of the OGTT after the small evening meal compared to the normal evening meal. Loss of first-phase insulin is well characterised in both type 1 and type 2 diabetes and occurs early in the evolution of the disease (35, 36). However, several other factors known to impact glucose tolerance will need further investigation. Studies have found a paradoxical rise in free fatty acid levels in the participants after a carbohydrate-restricted evening meal.

Fatty acid oxidation is associated with decreased glycolysis, glucose uptake, and glucose oxidation, all of which can ultimately raise glucose (37, 38).

The variations in the fasting glucose results could also be explained by alterations in glucose availability after the different evening meals. Short periods of calorie and carbohydrate restriction may deplete hepatic glycogen stores and reduce endogenous glucose production (39). Consequently, circulating glucose and insulin are also reduced (40).

3.5 Strengths and limitations

Strengths of our study include the randomised cross over design, allowing the overall impact of the two interventions to be compared within participants, and substantially increasing statistical power for a given sample size. Additional strengths include that experiments were undertaken in very controlled conditions, with overnight admission and observation of all participants, minimising the potential influence of dietary change beyond that specified in the study protocol.

Limitations of the study include giving all the participants the same quantity of food regardless of weight or sex. Furthermore, sample collection did not allow investigation of all potential mechanisms that have been proposed to cause impaired glucose tolerance after a low carbohydrate meal, for example, free fatty acids and incretins.

3.6 Conclusion

This randomised cross over study demonstrates that OGTT results are altered by the size of the last meal before the test and that abnormal glucose tolerance results may not reflect sustained hyperglycaemia but a response to the glucose load as a result of the previous short-term food restriction. Our findings have broader implications on the use of OGTTs for research and clinical purposes in regions without food insecurity, where both low carbohydrate meal plans and intermittent fasting have gained in popularity. One way around this would be to provide a standardised evening meal before the OGTT in areas of food insecurity.

3.7 Acknowledgements

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Chapter 4

Incidence and predictors of progression to diabetes in Malawi: 4-year prospective cohort study of people with impaired fasting glucose

Acknowledgement of co-authors and contributions to the paper

I conceived and designed the study with input from Amelia C. Crampin and Moffat Nyirenda. I implemented the study. I performed the analysis and drafted the manuscript. All authors provided support for interpretation of results, critically revised the manuscript, and approved the final draft of the manuscript.

4.0 Abstract

Introduction

Sub-Saharan Africa is projected to have the highest increase in the number of people with diabetes worldwide. However, the drivers of diabetes in this region have not been clearly elucidated. The aim of this study was to evaluate the incidence of diabetes and the predictors of progression in a population-based cohort with impaired fasting glucose (IFG) in Malawi.

Methods

We used data from an extensive rural and urban non-communicable disease survey. One hundred seventy-five, of 389 individuals with impaired fasting glucose (IFG) at baseline, age 48 ±15 years and body mass index 27.5 ±5.9 kg/m2 were followed up for a median of 4.2 years (714 person-years). Incidence rates were calculated, and predictors of progression to diabetes were analysed using multivariable logistic regression models, with overall performance determined using receiver operator characteristics (ROC) curves.

Results

The median follow-up was 4.2 (IQR 3.4 – 4.7) years. Forty-five out of 175 (26%) progressed to diabetes. Incidence rates of diabetes were 62.9 per 1000 person-years 95% CI, 47.0-84.3. The predictors of progression were higher; age (odds ratio [OR] 1.48, 95% CI 1.01-2.19, P=0.046), BMI (OR 1.98, 95% CI 1.34-2.94, P=0.001), waist circumference (OR 2.50, 95% CI 1.60-3.91,P<0.001), waist-hip ratio (OR 1.40, 95%CI 0.98-2.01, P=0.03), systolic blood pressure (OR 1.56, 95% CI 1.10-2.21,

P=0.01), fasting plasma glucose (OR 1.53, 95%CI 1.08-2.16, P=0.01), cholesterol (OR 1.44, 95% CI 1.00-2.08, P=0.05) and low-density lipoprotein cholesterol (OR 1.80, 95% 1.23-2.64, P=0.002). A simple model combining fasting plasma glucose and waist circumference was predictive of progression to diabetes (ROC area under the curve=0.79)

Conclusion

The incidence of diabetes in people with IFG in Malawi is higher than those seen in Europe (35.0 per 1,000 person-years) but similar to those seen in India (61.0 per 1,000 person-years). Predictors of progression are like those seen in other populations. A simple chart with probabilities of progression to diabetes based on waist circumference and fasting plasma glucose could be used to identify those at risk of progression in clinical settings in sub-Saharan Africa.

4.1 Introduction

Sub-Saharan African (SSA) will face the largest increase in diabetes worldwide unless drastic interventions are implemented. Presently 19 million people in the region have diabetes, and this is projected, by the International Diabetes Federation (IDF), to rise to 34.2 million people, an increase of 143%, by 2045 (1). This rise is expected to correspond with a similar surge in the number of people with prediabetes, a transition stage between normal glucose tolerance and diabetes. Individuals with prediabetes have a higher risk of developing diabetes and macrovascular disease than those with normal glucose tolerance and therefore offer a unique target population for identification and intervention (2-5).

Few studies have determined the incidence rate and the modifiable risk factors that predispose to the development of diabetes amongst the population with prediabetes in SSA (6). The factors that determine the progression of prediabetes have been widely studied in high-income countries and include positive family history, obesity and age (7, 8). But these factors cannot be generalised to SSA, where patients with diabetes are younger and thinner than in other regions (9, 10). Moreover, the rate of progression in prediabetes individuals has been shown to vary between different study populations (11, 12). In SSA the rate of progression may further be influenced by the distinct diabetes phenotypes in addition to early environmental exposures to malnutrition and infections.

The diagnosis of prediabetes can be based on measurement of fasting glucose, oral glucose tolerance test (OGTT) or HbA1c. However, in a setting where the health systems are constrained with limited health workers and a lack of medical supplies, OGTT is not practical and are seldom done in SSA. HbA1c is also not frequently

used because of cost limitations, high prevalence of haemoglobin variants and anaemia (13, 14). Fasting plasma glucose (FPG) is cheaper, easy to measure, and frequently used in clinical practice for diagnosing and monitoring diabetes in SSA. Prediabetes diagnosed solely based on fasting glucose is specifically called impaired fasting glucose (IFG) and is defined by the World Health Organization and the American Diabetes Association using different cut-offs.

In this study, we attempt to determine the incidence rate of diabetes in people with IFG and to assess the predictors of progression in the follow-up cohort of an epidemiological survey, conducted in a representative urban and rural population in Malawi.

4.2 Methods

4.2.1 Study population

The population for this study was a subset of participants with IFG who took part in the Malawi Epidemiology and Intervention Research Unit Non-Communicable Disease Survey. This cross-sectional population study was conducted on a representative sample of 13,878 adults (>18 years) participants from a rural district, Karonga, and 15,013 from the urban city, Lilongwe, between 2013 and 2016. The methodology and results of the baseline cross-sectional study have been published elsewhere (15, 16). From the total of 28,891 participants, all the participants with IFG according to the WHO classification an FPG greater or equal to 6.1 mmol/L and less than 7.0 mmol/L with no history of diabetes diagnosis and not taking diabetes medication (n=389) were followed-up. All activities were approved by the local ethics

committee (protocol no. 17/12/1953, National Health Sciences Research Committee, Lilongwe. Malawi) and complied with the Declaration of Helsinki.

We used the Cochran formula to calculate the power of the study. Assuming a prevalence of 4% conversion to diabetes per year, 389 subjects would give us a 1.1% absolute precision for a 95% confidence level. As only 175 subjects could be recruited, this gave us a 2.1% absolute precision for a 95% confidence level.

4.2.2 Measures

4.2.2.1 Demographic, education and health-related behaviours

A trained field team visited the participant at home. Demographic, socioeconomic status, medical and family history, tobacco and alcohol use were captured using a structured interviewer-administered questionnaire. Family history was considered "positive" if immediate family (parents or siblings) were reported to have been diagnosed with diabetes. We generated proxy wealth scores using locally determined monetary values of household assets, categorised into thirds across the total baseline population (17). We combined self-reported physical exercise duration (minutes) and intensity (pre-coded activities, grouped into high exertion, low exertion, or sedentary) in the previous week (both at work and during leisure time) to generate average metabolic equivalent of task (MET) data per day, and categorised these into whether or not the WHO recommendations of at least 600 Total Physical Activity MET minutes per week were met.

4.2.2.2 Anthropometric measurements

Height, weight, waist circumference and hip circumference were measured twice, in light clothing and without shoes, using calibrated Seca scales, stadiometers, and

flexible tape measures. The mean of the two measurements was used in the analyses. BMI was calculated by dividing weight (kg) by height squared (m2). Three seated blood pressure measurements, with 5 min rest in between, were collected on the right arm using portable sphygmomanometers (OMRON-Healthcare-CoHEN-7211; Kyoto, Japan). The mean of the last two blood pressure readings was used in the analysis. The same protocol was used to take these measures at follow-up.

4.2.3 Biochemical analyses

Fasting blood samples were drawn in the morning (from 0500 h) after a minimum 8 h fast by a trained nurse who also provided HIV screening using a rapid diagnostic test according to standard operating procedures. The samples were transported, on ice, to the onsite project laboratory for processing (mean time between collection and processing 2.6 hours). Samples for glucose measurement were collected in sodium fluoride (NaF) tubes. We used the hexokinase glucose-6-phosphate dehydrogenase method (Beckman Coulter AU480 Chemistry Analyser, Johannesburg, South Africa), with sufficient sensitivity (range 0.555-44.4 mol/L) to determine glucose concentration in the study samples. Serum total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were measured using enzymatic assays on the Beckman Coulter Chemistry Analyser. The same protocol was used to take these measures at follow-up.

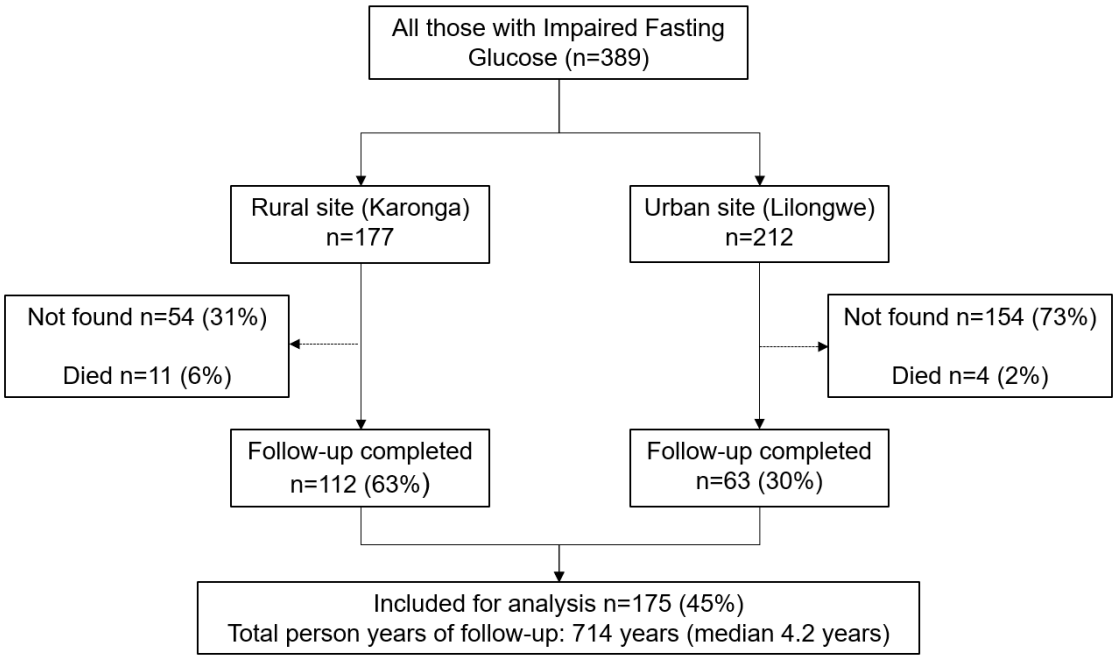

Figure 4.4 Flow diagram of study participants by site

4.2.4 Outcome

The participants were classified as having diabetes at follow-up if: the participant had since received a diagnosis of diabetes by health personnel, they were currently on diabetes medication, or the FPG at follow up was greater than or equal to 7.0 mmol/L. IFG was diagnosed if FPG was greater or equal to 6.0 mmol/L and less than 7.0 mmol/L. Normal Glucose Tolerance (NGT) individuals were those with FPG less than 6mmol/L.

4.2.5 Statistical Analysis

Statistical analyses were performed using Stata version 16 (StataCorp LP, College Station, TX). The baseline characteristics are expressed as mean ± SD or median (interquartile range [IQR]) for continuous variables or proportions for categorical variables. Person-years for diabetes were calculated from baseline until the last examination. Incidence of diabetes with 95% confidence interval (CI) was calculated per 1000 person-years, using the number of participants who developed diabetes during follow-up as the numerator and the total person-time as the denominator. Only 29% of those who had progressed to diabetes knew of their diagnosis before the follow-up visit. 71% were identified at follow-up. Thus exact time to progression from baseline, for the majority of participants is unknown. Therefore, we used logistic regression rather than survival analysis to interpret our data.

Variables known or suspected to attribute to the risk of progression were included in univariate analysis and then in multivariable logistic models adjusting for three important covariates: age, BMI and waist circumference. All models were adjusted for the duration of follow-up. The covariates included were age, BMI, waist

circumference, waist-hip ratio, systolic blood pressure, baseline FPG, cholesterol, triglycerides, HDL-C and LDL-C, sex, site, wealth score, education, smoking history and HIV status. Longitudinal changes of risk factors in those that progressed to diabetes, remit to normal glucose regulation, or remained IFG, were calculated and one-way ANOVA was used to compare the three progression groups.

Models of risks associated with the development of diabetes within four years using simple measures routinely taken in the clinical care performed were developed with performance determined by pseudo R2 and area under the curve (AUC). We provide a risk classification tool with probabilities of developing diabetes, using the most practical model to guide clinical interventions.

Sensitivity analysis was performed to assess any differences between those who were followed up and those who could not be found at follow up.

4.3 Results

The follow-up study was conducted in 2018-2019 with a median of 4.2 years (714.9 person-years to follow-up). A total of 190 participants, of the 389 who had IFG in the population, were successfully traced during the follow-up period representing 49% identification. Fifteen of those traced were confirmed to have died, and we report the results of 175 participants (figure 4.1). Baseline characteristics of those followed up and lost to follow up are shown in supplementary table 4.1. The participants successfully followed-up were more likely to be rural residents and older than those not followed-up. There were no differences in the sex or baseline glucose results between the two populations.

Forty-five (26%) participants with IFG at baseline progressed to diabetes (incidence rate of 62.9 per 1000 person-years 95% CI 47.0 - 84.3). Of those who developed diabetes, the majority, 32 (71%), did not know their status and were detected during the follow-up. 106 (61%) participants regressed to normal glucose tolerance, and 24 (14%) remained as IFG at follow up

Table 4.1 shows the clinical, biochemical and demographic characteristics of the participants at baseline based on their glycaemic status at follow-up. The individuals who progressed from IFG were older, and had higher BMI, waist circumference, systolic and diastolic BP, FPG, cholesterol, triglycerides and LDL-C compared to those who did not. There were no significant differences in changes of clinical characteristics between those who remitted to normal glucose tolerance remained IFG or progressed to diabetes in the study period, apart from the change in age, which reflects the differences in the follow-up period (supplementary table 4.2).

Table 4.2 shows the multivariable logistic regression analysis representing the odds ratio (OR) and 95% CI for progression to diabetes. Standardised logistic regression adjusted for follow-up for each variable showed the strongest predictors of progression were BMI (OR 1.98, 95% CI 1.34-2.94, P=0.001), waist circumference, (OR 2.50, 95% CI 1.60-3.91, P<0.001) and LDL-C (OR 1.80, 95% 1.23-2.64, P=0.002). Higher waist-hip ratio (OR 1.40, 95%CI 0.98-2.01, P=0.03), systolic blood pressure (OR 1.56, 95% CI 1.10-2.21, P=0.01) and FPG (OR 1.53, 95%CI 1.08-2.16, P=0.01), were also associated with progression.

In models adjusted for both age and follow-up, BMI and waist circumference remained strong predictors; waist-hip ratio, FPG and LDL-C remained associated with progression, but systolic blood pressure was not. However, in models adjusted

for waist circumference and follow-up period, only FPG was associated with progression. Among the measures of adiposity, waist circumference was the biggest predictor of progression to diabetes in the population. Analysis using participants only from the rural area which had a greater follow-up rate produced similar results (Supplementary Table 4.2).

Table 4.1 Baseline characteristics of participants with IFG (n=175) based on their glycaemic status at follow-up. Values are presented as mean ± SD for continuous variables and n(%) for proportions. NGT, Normal Glucose Tolerance; IFG, Impaired Fasting Glucose

Variable	**n**	**Regressed to NGT 106(60.6)**	**Remained as IFG 24(13.7)**	**Progressed to DM 45(25.7)**
Location: Rural, n (%)	112 (64)	75(67)	13(11.6)	24(21.4)
Urban, n (%)	63 (36)	31(49.2)	11(17.5)	21(33.3)
Sex: Male, n (%)	54 (30.8)	35(64.8)	6(11.1)	13(24.1)
Female, n (%)	121(69.1)	71(58.7)	18(14.9)	32(26.4)
Age, (years)	175	44.1±14.5	57.4±12.5	52.4±13.1
BMI, (kg/m2)	173	25.7±5	29±6.3	30.9±6
Waist Circumference, (cm)	173	87±10.7	94±10.5	99±11.2
Waist Hip Ratio	173	0.9±0.1	0.9±0.1	0.9±0.1
Systolic BP, (mmHg)	175	131±22	144±25	146±28
Fasting plasma glucose, (mmol/L)	175	6.3±0.2	6.4±0.2	6.5±0.3
Serum Cholesterol, (mmol/L)	175	4.5±1.2	4.9±0.9	5.1±1.1
Serum Tryglycerides, (mmol/L)	175	1.3±0.8	1.9±1.2	1.9±1
HDL Cholesterol, (mmol/L)	173	1.1±0.4	1.1±0.2	1.1±0.2
LDL Cholesterol, (mmol/L)	175	2.9±0.9	3.4±0.8	3.6±0.8
HIV Status: Positive, n (%)	15	13(86.7)	1(6.7)	1(6.7)
Positive family history of diabetes, n (%)	26 (14.8)	13(50)	5(19.2)	8(30.8)
Current consumption of Alcohol, n (%)	27(15.4)	20(74.1)	2(7.4)	5(18.5)
Wealth score: Poorest, n (%)	40 (22.8)	29(72.5)	6(15)	5(12.5)
Middle, n (%)	75 (42.9)	46(61.3)	8(10.7)	21(28)
Wealthiest, n (%)	60 (34.3)	31(51.7)	10(16.7)	19(31.7)
Physical activity: High (%)	154 (88)	95(89.6)	20(83.3)	39(86.7)
Moderate (%)	15 (0.09)	8(7.6)	4(16.7)	3(6.7)
Low (%)	6 (0.03)	3(2.8)	0	3(6.7)

Table 4.2 Multivariate logistic regression of risk factors for progression of Impaired Fasting Glucose (IFG) to diabetes.

Variables	Model 1: Standardised logistic regression adjusted for follow-up		Model 2: Adjusted for follow-up and age		Model 3: Adjusted for follow-up and BMI		Model 4: Adjusted for follow-up and waist circumference	
	OR (95% CI)	P Value	OR (95% CI)	P Value	OR (95% CI)	P Value	OR (95% CI)	P Value
Age	1.48 (1.01 - 2.19)	0.046	-		1.41 (0.93 - 2.13)	0.11	1.27 (0.82 - 1.95)	0.28
BMI	1.98 (1.34 - 2.94)	0.001	1.95 (1.31 - 2.91)	0.001	-		1.01 (0.53 - 1.93)	0.98
Waist circumference	2.50 (1.60 - 3.91)	<0.001	2.39 (1.52 - 3.76)	<0.001	2.48 (1.19 - 5.16)	0.02	-	
Waist hip ratio	1.40 (0.98 - 2.01)	0.03	1.30 (0.90 - 1.88)	0.16	1.47 (1.01 - 2.14)	0.046	1.05 (0.70 - 1.56)	0.82
Systolic BP	1.56 (1.10 - 2.21)	0.01	1.43 (0.97 - 2.11)	0.07	1.40 (0.98 - 2.01)	0.06	1.30 (0.90 - 1.87)	0.16
Fasting plasma glucose	1.53 (1.08 - 2.16)	0.01	1.45 (1.02 - 2.06)	0.04	1.54 (1.07 - 2.22)	0.02	1.55 (1.07 - 2.26)	0.02
Cholesterol	1.44 (1.00 - 2.08)	0.05	1.31 (0.88 - 1.94)	0.19	1.23 (0.83 - 1.82)	0.30	1.18 (0.79 - 1.76)	0.42
Triglycerides	1.36 (0.95 - 1.94)	0.09	1.28 (0.89 - 1.83)	0.18	1.22 (0.84 - 1.77)	0.31	1.07 (0.72 - 1.57)	0.75
HDL- Cholesterol	1.14 (0.80 - 1.61)	0.47	1.10 (0.77 - 1.57)	0.58	1.21 (0.83 - 1.77)	0.32	1.31 (0.88 - 1.94)	0.18
LDL- Cholesterol	1.80 (1.23 - 2.64)	0.002	1.69 (1.14 - 2.52)	0.01	1.52 (1.01 - 2.27)	0.04	1.45 (0.96 - 2.17)	0.08
Site	1.36 (0.64 - 2.85)	0.41	1.53 (0.71 - 3.27)	0.27	0.93 (0.41 - 2.07)	0.85	1.16 (0.53 - 2.54)	0.72
Sex	1.09 (0.50 - 2.35)	0.84	1.12 (0.51 - 2.46)	0.77	0.72 (0.31 - 1.66)	0.44	0.97 (0.42 - 2.21)	0.93
Wealth	1.40 (0.86 - 2.28)	0.18	1.44 (0.88 - 2.36)	0.15	1.09 (0.64 - 1.85)	0.76	0.99 (0.57 - 1.71)	0.97
Physical activity	0.88 (0.43 – 1.81)	0.72	0.96 (0.46 – 2.01)	0.92	0.83 (0.36 –1.83)	0.64	0.91 (0.40 – 2.07)	0.83

Models of risks associated with progression to diabetes using simple measures routinely taken in clinical care (age, BMI, waist circumference and FPG) are presented in table 4.3. The AUC values of models of follow-up period with age, BMI and waist circumference were 0.69, 0.75 and 0.77, respectively. In models adjusted for follow-up, a model with two covariates, waist circumference and FPG, had the higher pseudo R2 and AUC (0.159 and 0.77); adding age to this model only marginally improved these measures. A graph of probabilities of developing diabetes over four years based on the model of follow-up period, waist circumference and FPG is represented in figure 4.2.

Table 4.3 Models of risks associated with progression to diabetes

Model No:	Variables	N	Pseudo R2	AUC	95% CI
1	Follow up period, Age	175	0.0831	0.695	0.61 – 0.78
2	Follow up period, Fasting glucose	175	0.0910	0.702	0.62 – 0.79
3	Follow up period, BMI	173	0.1284	0.754	0.67 – 0.84
4	Follow up period, Waist	173	0.1590	0.767	0.69 – 0.85
5	Follow up period, Waist, Age	173	0.1648	0.77	0.69 – 0.85
6	Follow up period, Waist, BMI	173	0.1650	0.767	0.69 – 0.84
7	Follow up period, Waist, BMI, Age	173	0.1650	0.775	0.69 – 0.85
8	Follow up period, Waist, Fasting glucose	173	0.1861	0.786	0.71 – 0.86
9	Follow up period, Waist, Fasting glucose, Age	173	0.1881	0.792	0.72 – 0.87

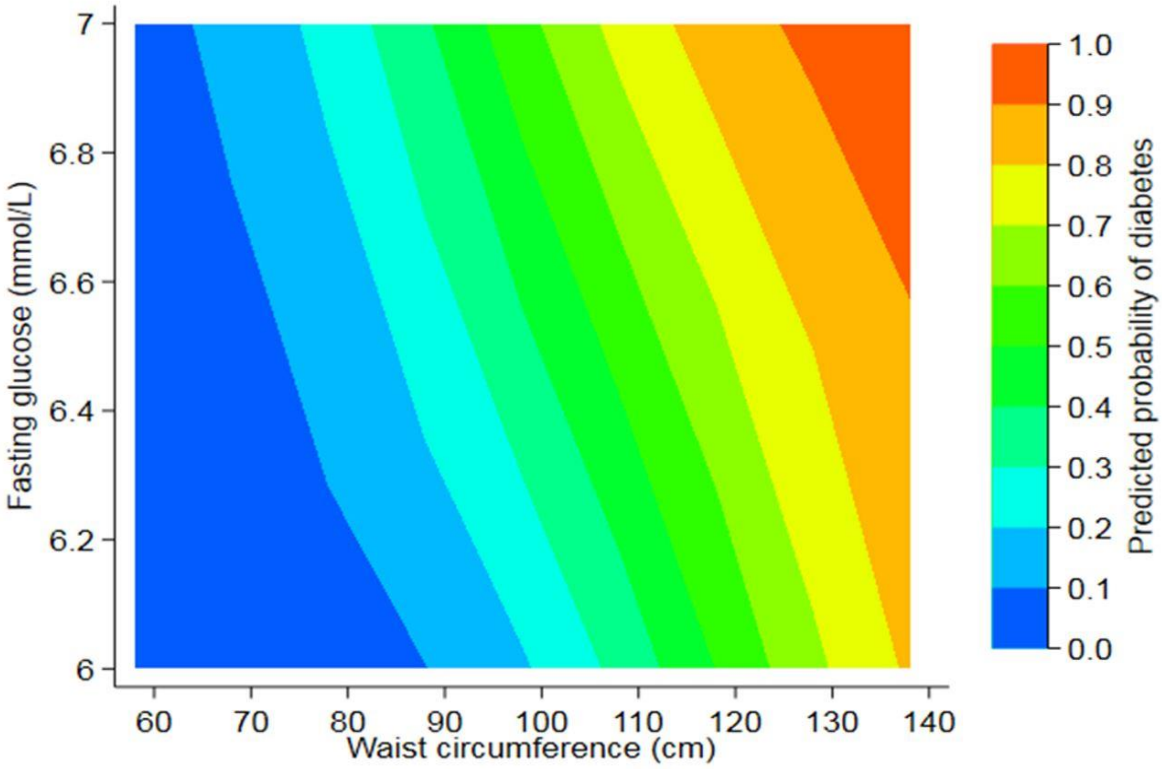

Figure 4.2 Probabilities of progression to diabetes within four years based on a simple regression model of waist circumference and fasting glucose over four years. Predicted probability of 1.0 represents 100% risk of developing diabetes.

4.4 Discussion

This study is among the first to determine the incidence of diabetes in participants with IFG, as defined by WHO classification, in a rural and urban population in Malawi. We found a high incidence of diabetes, with 26% of participants progressing in 4.2 years. Waist circumference and baseline FPG were the two most significant predictors, and a model of these risk factors can potentially be used as a tool in screening for those at risk of progression to diabetes.

Comparison with previous research

The incidence rate of diabetes in our cohort is 62.9 per 1000 person-years, which is higher than previously reported in studies of white Europeans (35.0-40.0 per 1,000 person-years) and Iran (34.5 per 1,000 person-years) (18) but similar to that reported in Chennai India (61.0 per 1,000 person-years) (19). The incidence is also similar to that reported in ethnic groups with high levels of obesity and diabetes, such as the Pima Indians (87.3 per 1,000 person-years), the Micronesian population of Nauru (62.8 per 1,000 person-years) and the Native Americans (66.1 per 1,000 person-years) (20).

Our data shows that the risk of progression to diabetes significantly increased as FPG and waist circumference increased. Several studies have demonstrated that the progression from prediabetes to diabetes is accompanied by worsening weight gain, insulin resistance, and beta-cell dysfunction (21). Waist circumference is an inexpensive marker of insulin resistance and visceral adipose tissue (22). Individuals with high waist circumference would be ideal candidates for intervention programs aimed at screening and preventing

diabetes, which can potentially decrease the high rate of undiagnosed diabetes found in our study and others in the region. Lifestyle interventions, including either physical activity alone or combined with dietary advice aiming at weight loss, have been shown to significantly reduce the progression of prediabetes to diabetes in other populations (23-25). However, these prevention programs have shown variable levels of success in some ethnicities, and it is not known if they can have an impact on SSA populations. Determining whether waist circumference reduction through a trial of dietary and physical activity to reduce the incidence of diabetes in rural and urban Malawi would be an appropriate next step.

Blood pressure and triglycerides are highly correlated with obesity. Several studies also found that higher triglycerides and blood pressure at baseline in those with prediabetes were associated with progression to diabetes. However, we found that the relationship apparent in univariate analysis was lost when adjusted for BMI or waist circumference. In contrast to most studies, we observed that the association between age and incident diabetes was weak and no longer significant after adjustment for BMI or waist circumference.

We did not observe a significant difference in the incidence of diabetes between men and women. This is contrary to several studies that have reported a higher incidence in women than men usually ascribed to higher adiposity and less physical activity (19). Our baseline survey identified self-reported physical activity levels well above the WHO recommendation for men and women (15). Contrary to other reported studies, we did not find that family history was a significant risk factor for progression to diabetes in people with IFG. This might be because some family members were unaware that they had diabetes, did

not survive long enough to develop diabetes, or were not exposed to the same precipitating factors as their index relatives.

Implications

A large proportion of individuals with prediabetes at baseline regressed to normal glucose regulation at follow-up. This has also been observed in most cohort studies in different regions and questions the usefulness of surveys as a tool for diabetes prevention (26-28). It is possible that many of the populations may pass in and out of prediabetes without a diagnosis, and many people found in surveys could well have transient diabetes. This places greater importance on identifying those individuals at greatest risk of developing diabetes.

The Diabetes Prevention Program trial demonstrated that intensive lifestyle intervention, to a lesser extent, metformin use reduced the risk of diabetes progression in high-risk adults (5, 29). Our data suggest that a simple contour map with probabilities of progression to diabetes based on waist circumference and FPG could be used in a clinical setting to identify those at risk of progression (figure 4.2). Glucose tests are now available worldwide, so this map is a practical and pragmatic tool that health officials could use to communicate the likelihood of patients with IFG to progress to diabetes using simple clinical features without expensive tests or age, which is not always accurate. Further tests are needed to validate this map in this population and other SSA populations.

Strengths and limitations

Strengths of the study include that the study recruited participants from both urban and rural populations. The participants were visited at home, early in the

morning, so there is no effect of physical activity on glucose results. Furthermore, all the tests were done to a standard protocol with the same equipment.

The study's limitations are the lack of year-by-year follow-up data and the relatively low response rate, especially among men and urban participants. Loss to follow-up remains a significant challenge in conducting long term research and intervention studies in many countries in SSA. Our study had a better follow-up in the rural area (75%), and a repeat analysis using participants only from this region produced similar results. We recruited fewer participants than anticipated which means that the 95% confidence is broad, 47.0 - 84.3 per 1000 person-years. However, this is the first information on the progression from intermediate hyperglycaemia in sub-Saharan Africa and so it will inform future planning in the region.

Another limitation of the study was that oral glucose tolerance tests were not conducted, meaning isolated impaired glucose tolerance could not be identified. However, several other studies have also found that fasting glucose alone identified most of the individuals with prediabetes, suggesting that glucose tolerance testing would not be cost-effective (30). In a study of OGTTs in 800 participants from Malawi, we found only ten per cent of participants with prediabetes had isolated impaired glucose tolerance (Authors unpublished observation).

4.5 Conclusion

We found that the incidence rate of diabetes in people with IFG in Malawi is higher than that seen in Europe or the USA but similar to that seen in India. However, the predictors of progression to diabetes are similar, with waist circumference and glycaemia being the strongest predictors of progression. Based on modelling, we suggest that a simple chart with probabilities of progression to diabetes based on waist circumference and FPG could be developed for use in a clinical setting in sub-Saharan Africa to identify those at risk of progression.

4.6 Acknowledgement

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4.9 Supplementary information

Supplementary Table 4.1 Comparison between participants who were found and not found at follow up

	Not Followed up (N=199)	**Followed up (N=175)**	**P Value**
Location: Rural (%)	38(25)	112(75)	<0.001
Urban (%)	136(68)	63(32)	
Sex: Male (%)	72(57)	54(53)	0.66
Female (%)	102(46)	121(54)	
Age (years)	38.3±14.4	48±14.7	<0.001
BMI (kg/m^2)	27.3±6.6	27.5±5.9	0.89
Waist (cm)	88.4±15.4	91.1±11.9	0.20
Fasting plasma glucose (mmol/L)	6.3±0.2	6.4±0.2	0.13

Supplementary Table 4.2 Longitudinal changes of risk factors in those that remit to Normal Glucose Tolerance (NGT) remained Impaired Fasting Glucose (IFG) or progressed to diabetes

	n	Regressed to NGT 106(60.6)	Remained as IFG 24(13.7)	Progressed to DM 45(25.7)	P-Value
Follow-up (years)	175	4±2.6	4.3±0.9	5±1.9	<0.001
BMI	168	0.3±2	0.2±1.9	-0.6±3.2	0.115
Waist Circumference (cm)	166	-0.1±6.5	1.4±5.1	-0.6±6.8	0.452
Waist-hip ratio	166	0±0.1	0±0.1	0±0.1	0.484
Systolic BP (mmHg)	170	0.5±17.5	2.3±22.9	-2.9±25.5	0.537
Fasting Plasma Glucose (mmol/L)	173	-1.3±0.6	0±0.3	4.1±4.7	<0.001
Cholesterol (mmol/L)	174	-0.1±0.8	0.4±0.7	0.1±1.2	0.068
Tryglycerides (mmol/L)	173	0.1±0.8	0.2±0.9	0.3±1.1	0.665
HDL-C (mmol/L)	172	0.1±0.3	0.1±0.2	0.1±0.3	0.684
LDL-C (mmol/L)	174	-0.4±0.6	-0.2±0.7	-0.5±1	0.141

Supplementary Table 4.3 Multivariate logistic regression of risk factors to the progression of IFG using participants from the rural area

Variables	Model 1 Standardised logistic regression adjusted for follow-up		Model 2 Adjusted for follow-up and age		Model 3 Adjusted for follow-up and BMI		Model 4 Adjusted for follow-up and waist circumference	
	OR (95% CI)	**P Value**	**OR (95% CI)**	**P Value**	**OR (95% CI)**	**P Value**	**OR (95% CI)**	**P Value**
Age	1.68 (0.99 - 2.85)	0.054	-		1.60 (0.91 – 2.80)	0.10	1.40 (0.77 – 2.52)	0.27
BMI	1.90 (1.08 – 3.34)	0.03	1.80 (1.01 – 3.17)	0.04	-	0.03	0.53 (0.18 - 1.59)	0.26
Waist circumference	2.54 (1.42 – 4.53)	0.002	2.36 (1.30 – 4.27)	0.005	4.29 (1.43 – 12.9)	0.009	-	
Waist hip ratio	2.21 (1.23 – 3.96)	0.007	1.98 (1.07 – 3.66)	0.03	2.14 (1.17 – 3.92)	0.014	1.57 (0.81 – 3.7)	0.18
Systolic BP	1.40 (0.89 – 2.20)	0.14	1.18 (0.71 – 1.95)	0.53	1.24 (0.78 – 1.97)	0.36	1.03 (0.63 – 1.70)	0.89
Fasting plasma glucose	1.18 (0.74 – 1.89)	0.49	1.04 (0.64 – 1.70)	0.86	1.21 (0.75 – 1.97)	0.43	1.21 (0.73 – 1.99)	0.47
Cholesterol	1.52 (0.93 - 2.49)	0.09	1.32 (0.77 – 2.25)	0.31	1.32 (0.77 – 2.24)	0.31	1.27 (0.74 – 2.20)	0.39
Triglycerides	1.25 (0.76 – 2.05)	0.38	1.09 (0.64 – 1.85)	0.76	1.06 (0.61 – 1.85)	0.82	0.88 (0.49 – 1.59)	0.68
HDL-Cholesterol	1.14 (0.72 – 1.80)	0.58	1.16 (0.72 – 1.86)	0.54	1.18 (0.72 – 1.93)	0.51	1.31 (0.76 – 2.19)	0.35
LDL- Cholesterol	2.03 (1.19 – 3.48)	0.01	1.84 (1.04 – 3.26)	0.03	1.82 (1.03 – 3.20)	0.04	1.74 (0.97 – 3.11)	0.06
Sex	0.99 (0.37 – 2.64)	0.98	1.12 (0.37 – 2.76)	0.99	0.71 (0.25 – 2.03)	0.52	0.83 (0.29 – 2.35)	0.72
Wealth	2.86 (0.64 – 12.7)	0.17	2.84 (0.62 – 13.0)	0.18	1.36 (0.69 – 2.69)	0.38	1.95 (0.41 – 9.26)	0.40

Chapter 5

The contribution of maternal glucose to birth weight is less in Uganda (sub-Saharan Africa) compared to White and Black ethnic groups in the HAPO study

Acknowledgements of co-authors and contributions to the paper

I designed the analysis with input from Andrew Hattersley, Moffat Nyirenda, Rob Andrews and Bev Shields. I collected, analysed the data and drafted the manuscript. All authors provided support for the interpretation of results and critically revised the manuscript.

5.0 Abstract

Introduction

Maternal glycaemia has a significant influence on fetal birth weight and maternal and fetal morbidity. Glycaemia measured in a third-trimester oral glucose tolerance test shows a linear relationship with birth weight and other birth complications. The Hyperglycaemia Adverse Pregnancy Outcomes (HAPO) study established a similar relationship between glucose and birth weight in multiple ethnic groups, and they proposed cut-off criteria where the risk of large for gestational age (LGA) was 1.75 times greater than the population risk. However, the HAPO study did not include any cohorts from sub-Saharan Africa (SSA), where 17% of the world population lives. No studies have examined the relationship between maternal glycaemia and fetal birth weight in SSA. This study aims to address this.

Methods

We compared the relationship between oral glucose tolerance test (OGTT) glucose measures and obstetric outcomes in participants from Uganda (n=2544), black participants in HAPO (n=1224) and white participants in HAPO (n=7679). We used multivariable linear regression to assess the correlation between birth weight corrected for gestational age and sex with maternal glucose concentration. Logistic regression was used to determine the association of LGA (defined as birthweight > 90th percentile) and maternal fasting glucose.

Results

The contribution of maternal fasting glucose to birth weight is lower in Uganda than in other settings (β-coefficient (95%CI) 104(58.6 to 149)g/mmol/L in Uganda, 203(137 to 269) HAPO-black, and 239(214 to 265) HAPO-white. Likewise, the risk of

LGA with higher fasting glucose was smaller in Uganda compared to the other studies (adjusted odds ratio (95%CI) 1.07 (0.94 to 1.23) in Uganda, 1.29 (1.22 to 1.38) HAPO-black, and 1.34(1.24 to 1.45) HAPO-white. Analysis using 1-hr and 2-hr post glucose load concentrations produced similar results.

Conclusion

We found that associations between maternal glucose and fetal birth weight are substantially lower in our SSA cohort than in countries recruited into the HAPO study. This suggests that cut-offs for pregnancy generated from other study populations need to be confirmed in SSA before they are adopted.

5.1 Introduction

The HAPO study, used to define diagnostic criteria for Gestational Diabetes Mellitus (GDM), did not recruit any participants in sub-Saharan Africa

The Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) was a landmark study conducted to clarify the risks of adverse outcomes associated with various degrees of maternal glucose intolerance less severe than overt diabetes mellitus (1). This large, multinational study recruited patients from nine countries (four ethnicities) and found a strong, continuous association of maternal glucose levels measured by a 75g OGTT at 24-32 weeks gestation adverse pregnancy outcomes such as LGA (birth weight above 90th percentile), Caesarean section, neonatal hypoglycaemia and shoulder dystocia. No differences in the relationship between maternal glucose and pregnancy complications in the different ethnicities were reported.

The similar relationship between maternal glucose and pregnancy complications in the different ethnicities allowed universal cut-offs to be proposed. Based on the HAPO study, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) consensus panel recommended new international thresholds for diagnosing Gestational Diabetes Mellitus (GDM), which aimed to reduce adverse pregnancy outcomes by identifying mothers who were at or above a 1.75 fold increased probability of having infants who were large for gestational age (LGA). They recommended universal screening of all pregnant women for Gestational Diabetes Mellitus (GDM) with lower diagnostic cut-offs than previous guidelines (fasting: 5.1 mmol/L, 1-hour: 10.0 mmol/L, and 2-hour: 8.5) (2). The World Health Organization (WHO) adopted the IADPSG guidelines in 2013.

However, the HAPO study did not recruit patients from any country in sub-Saharan Africa, which has a combined 1.2 billion people (17% of the world population). Therefore, it is unclear if a similar relationship between maternal glucose and adverse pregnancy outcomes exists in this population and if the guidelines are applicable in the sub-Saharan Africa (SSA) context.

The impact of maternal glucose on fetal birth weight and adverse pregnancy outcomes in SSA is not known.

The relationship between maternal glucose and fetal birth weight and adverse pregnancy outcomes in SSA is not known. This will be important to know when deciding whether to use the IADASG cut-offs. The overall prevalence of gestational diabetes in pregnancy in SSA is 9.6% (95% CI 7-12%) (3). The risk factors for GDM are similar to those found in other settings, but the prevalence is lower (4, 5). Maternal fasting glycaemia is known to explain approximately 6% of the variance in birth weight in western white populations (6, 7). However, it is unclear what proportion is explained by maternal glycaemia in SSA.

We, therefore, aimed to examine the relationship, in Uganda, between maternal glycaemia and fetal birth weight and the associated adverse pregnancy outcomes of LGA with maternal glucose. This data was compared with other ethnic groups from the HAPO study.

5.2 Methods

5.2.1 Study design

We assessed the relationship between maternal glucose and fetal birth weight in a cohort study from Uganda and compared this relationship with cohort studies done in other regions, namely the black and white populations in the HAPO study.

5.2.2 Data collection

Data collection methods for each of the studies have been described elsewhere for Uganda (8) and HAPO (1, 9). All studies collected data on sociodemographic variables, anthropometric measurements and blood samples. Babies were weighed following delivery using clinically validated scales at birth.

5.2.2.1 Uganda Gestational Diabetes Mellitus (GDM) Study

This study aimed to provide data on the burden and risk factors of GDM in Uganda, a low-income country in East Africa, and to inform on screening strategy for GDM in the region. Women attending antenatal care at five major hospitals in urban and peri-urban areas of central Uganda between 13th June 2018 and 31st October 2019 were screened for inclusion and exclusion criteria and invited to participate in the study.

All participants were aged >18 yrs at recruitment and between 24 and 28 weeks of gestation. Women were not recruited if they were known to have diabetes prior to the current pregnancy, if they were unable to give informed consent, if they had significant medical conditions (e.g. heart failure, renal disease, severe anaemia, autoimmune disease or preeclampsia), or if they had a multiple pregnancy.

History was taken using prior specified questionnaires. Detailed anthropometric measurements using validated scales, together with fasting plasma glucose and 1-hr and 2-hr glucose concentration after 75 g glucose load, were measured at

approximately 27 weeks. No medical intervention was provided except when plasma glucose concentrations were outside the predefined values (fasting > 5.8 mmol/L, and/or two hours > 11.1 mmol/l). These patients were informed of their status and excluded from the analysis

Samples were immediately centrifuged at study sites, and plasma was stored on ice and returned immediately to the central laboratory. All samples were analysed for glucose centrally at the MRC/UVRI and LSHTM Clinical and Diagnostics Laboratory in Entebbe, Uganda, within 4 hours of collection. The plasma glucose was measured by the glucose oxidase method using the Cobas 6000 analyser (Roche/Hitachi, Tokyo, Japan).

5.2.2.2 HAPO study

The HAPO study protocol has previously been reported (10). Pregnant women were recruited at 15 centres in nine countries (Australia (2 centres), Barbados (1), Canada (1), China (1), Israel (2), Thailand (1), Singapore (1), United Kingdom (2) and the United States of America (4). They were from four predominant ethnicities White, Blacks, Asian, Hispanics, which were self-defined. All subjects underwent a 75 g oral glucose tolerance test (OGTT) at 24 to 32 weeks of gestation between July 2000 and April 2006.

All pregnant women at a given centre were eligible to participate unless they had one or more of the following exclusion criteria (10): age younger than 18 years, a plan to undergo delivery at another hospital, an uncertain date of last menstrual period and no ultrasonographic estimation between 6 and 24 weeks of gestational age, inability to complete OGTT within 32 weeks of gestation, multiple pregnancy, conception

using gonadotropin ovulation induction or in-vitro fertilisation, glucose testing before recruitment or a diagnosis of diabetes before the current pregnancy, participation in another study that could interfere with the HAPO study, infection with the human immunodeficiency virus or hepatitis B or C virus, previous participation in the HAPO study, or inability to converse in the language used on centre forms without the aid of an interpreter.

Detailed anthropometric measurements, using validated scales, together with fasting plasma glucose (FPG), 1-hr, and 2 hr glucose concentration after 75 g glucose load were measured. Data were masked except when plasma glucose concentrations were outside the predefined values (fasting > 5.8 mmol/L, and/or two hours > 11.1 mmol/l or any value <2.5 mmol/L); such patients were unmasked and removed from the main study. Otherwise, participants received obstetrical and neonatal care routinely provided in their centre. Glucose analysis was done at the central laboratory using the oxidase/peroxidase method (9).

5.2.3 Subjects included in the analysis

We used similar enrolment criteria to the HAPO study for the Uganda GDM study. We, therefore, analysed all women with singleton pregnancies without pre-existing diabetes. Individuals were only included if they had birthweight and maternal OGTT results. They were excluded if they had marked hyperglycaemia (fasting glucose >5.8 mmol/L and 2 hr glucose > 11.1 mmol/l).

5.2.4 Outcomes

We assessed the association of maternal glucose concentrations with the corrected fetal birth weight. Secondly, we assessed associations of maternal glucose

concentration with the adverse outcome of LGA (defined as birth weight greater than the 90th percentile for gestational age in the individual populations).

5.2.5 Statistical Analyses

The major comparison was between the black Ugandan participants, white participants in HAPO, and black participants in HAPO. Descriptive statistics were reported for continuous (mean, SD) and categorical (number, percentage) variables, respectively. The “corrected birth weight” variable was prepared by saving residuals from a linear regression analysis of birth weight (g) against sex and gestational age. Multiple linear regressions were used to analyse associations between corrected birth weight and maternal fasting and post-load glucose with maternal glucose as continuous variables. Multiple logistic regression was used to determine the association between LGA and maternal glucose. Odds ratios (ORs) and regression coefficients were calculated for a 1-SD increase in fasting, 1-hr and 2-hr glucose measurement. The final models were adjusted for maternal age and body mass index at the time of the OGTT. A sensitivity analysis was performed where we excluded women with an FPG >5.1 mmol/L (the threshold recommended by the IADSPG for diagnosis of gestational diabetes mellitus). We also compared the characteristics of those excluded because of incomplete data and those included in the analysis.

We repeated all the analyses using 1-hr and 2-hr post-load glucose concentrations for both the Uganda and HAPO cohort. Pearson product-moment correlations were used to assess associations among glucose measures within the Uganda and HAPO studies.

All analyses were performed using Stata 16 (StataCorp LP, College Station, TX) or R version 1.3. Ethics approval was obtained from the Uganda Virus Research Institute (approval GC/127/19/04/625) and the Uganda National Council for Science and Technology (approval HS2340).

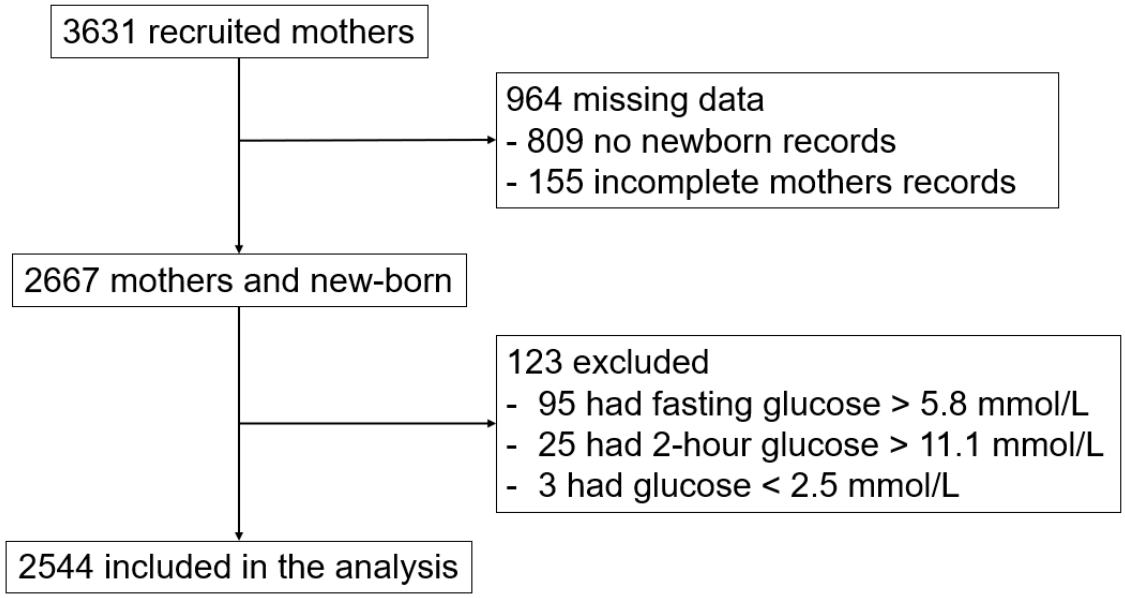

Figure 5.1 Study flow chart for the Uganda study data

5.3 Results

Eligible subjects in Uganda cohort

Figure 5.1 shows the effect of study exclusion criteria and missing data in the Uganda GDM study cohort. Of the 3631 participants recruited, 964 could not be included in the analysis due to missing data. One hundred and twenty-three were not included because they did not meet the inclusion criteria (fasting glucose >5.8 mmol/L and 2 hr glucose > 11.1 mmol/l). The participants with missing data compared to the included participants were slightly younger (25.7 v 27.0 yrs) and slightly slimmer (27.2 v 27.8), but they had similar glycaemia at all points of the OGTT and similar blood pressure (supplementary table 5.1).

Participants

The numbers in the three cohorts after exclusions were 2544 Ugandan cohort, 1224 HAPO black cohort and 7679 HAPO white cohort). Characteristics of the mothers and newborns and pregnancy outcomes are summarised in Table 5.1. All the participants in the Uganda cohort were black. White women in HAPO were the oldest, and black women in HAPO were the youngest (29.9±5.6 years, HAPO-white; 26.7±5.5 years, Uganda; 25.5±5.7 years HAPO-black). The mean fasting glucose was lower in the Uganda study than in the other studies (4.2±0.4 Uganda, 4.5±0.4 HAPO-black, 4.5±0.4 HAPO-white). Black women in HAPO had the smallest babies, and white women in HAPO had the largest babies (3227±449 g, HAPO-black; 3285±525 g, Uganda; 3348±475 g HAPO-white).

Table 5.1 Characteristics of the study participants and their offspring

Mean ±SD for continuous variables n (%) for proportions

	Uganda N=2544	**HAPO – Black N=1224**	**HAPO – White N= 7679**
Maternal Characteristics			
Age (yrs)	26.7±5.5	25.5±5.7	29.9±5.6
Body mass index (kg/m²)	27.6± 5.0	27.8±6.0	28.1±4.5
Systolic blood pressure (mmHg)	103±10	103±9.9	107±10
Plasma glucose (mmol/l)			
Fasting	4.2±0.4	4.5±0.4	4.5±0.4
1-hr	6.3±1.4	6.8±1.4	7.2±1.7
2-hr	5.8±1.1	6.0±1.2	5.9±1.2
Length of gestation at time of OGTT (wk)	25.9±1.2	25.5±5.7	29.9±5.5
Number of mothers parity >1 %	1775 (63.5)	355(29.3)	2146 (28.2)
Any prenatal smoking - %	17 (0.7)	5 (0.4)	941 (12.3)
First degree relative with diabetes - %	650 (24.7)	209 (17.2)	1523 (30.9)
New-born Characteristics			
Gestational age at delivery (wk)	38.7±1.8	39.8±1.2	39.9±1.3
Birth weight (g)	3285±525	3227±449	3348±475
Male - %	1274(50.4)	634 (51.8)	3918 (51.0)

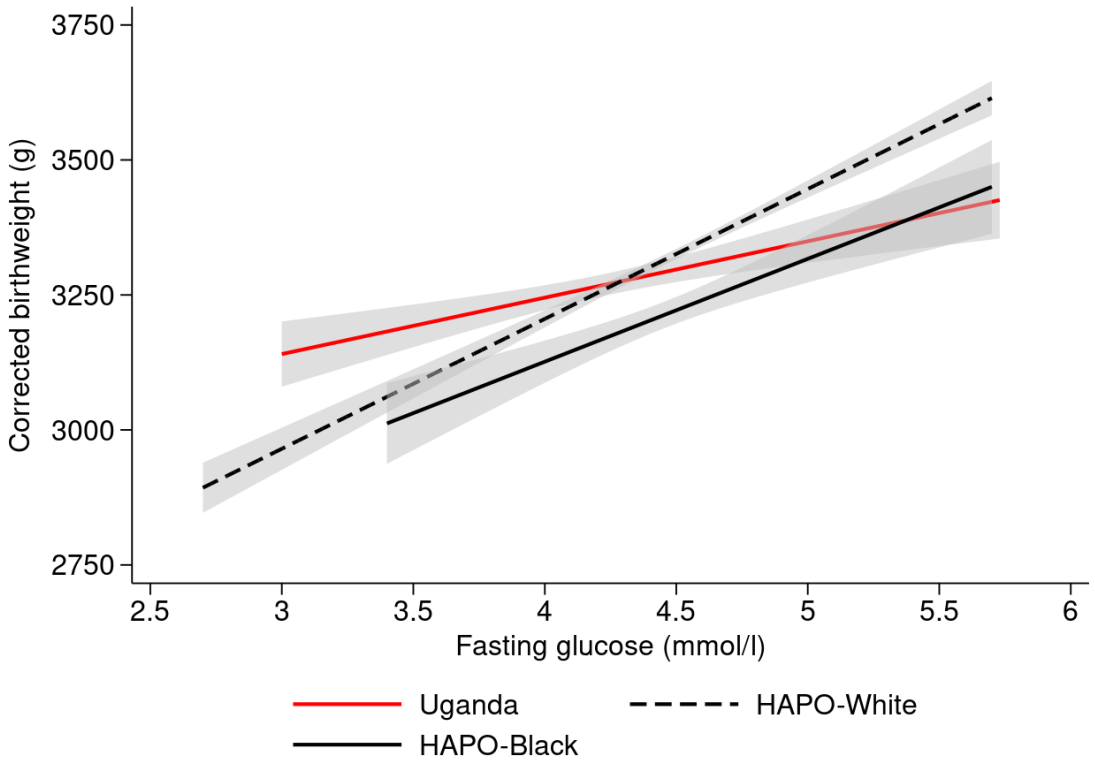

Figure 5.2 Relationship between corrected birth weight with maternal glucose for Uganda (n= 2544) red solid line, HAPO-Black (n=1224) black solid line and HAPO-White (n=7679) black dotted line. The shading in grey reflects the 95% confidence limits of the mean. Corrected birthweight was internally calculated using birthweight corrected for gestational age and sex

The contribution of maternal fasting glucose to fetal birth weight is substantially smaller in Uganda compared to black and white cohorts from the HAPO study.

Figure 5.2 shows the association between maternal fasting glucose and the corrected fetal birth weight. There is a linear relationship between maternal fasting glucose and corrected fetal birth weight in Uganda, as was observed in the other studies. However, the relationship was weaker in Uganda compared to the other populations. In univariate linear regression analysis, the β-coefficients for the relationship between corrected fetal birth weight and maternal fasting glucose were significantly lower in Uganda than in the two HAPO cohorts in unadjusted analysis (β-coefficient (95%CI) 104 (58.6 to 149) in Uganda, 203 (137 to 270) HAPO-black, and 239 (214 to 265) HAPO-white,(Table 5.2). Similarly, the variance in the birth weight explained by glucose (measured as R^2) was marked less in the Uganda population (0.008 Uganda vs 0.036 HAPO black and 0.043 HAPO white. The results were similar when adjusted for maternal age, BMI and parity (Table 5.2 supplementary).

In contrast, the relationship between corrected birth weight and maternal age, BMI, parity, family history of diabetes were similar between the studies (Table 5.2).

Weaker relationship between maternal glycaemia and adverse pregnancy outcome of large for gestational age (birthweight > 90th percentile in Uganda) compared to other cohorts

Table 5.3 shows the association of maternal glucose as a continuous variable with the adverse pregnancy effect of LGA, including adjusted odds ratios and 95% confidence intervals for each 1-SD change in glucose concentration with

adjustments for confounders as in HAPO (1). An increase in glucose by 1 SD was associated with an increased odds of LGA in all the studies. However, the effect was smaller in Uganda compared to the other studies (adjusted odds ratio (95%CI) 1.07 (0.94 to 1.23) in Uganda, 1.29 (1.22 to 1.38) HAPO-black and 1.34(1.24 to 1.45) HAPO-white.

Weaker relationship between maternal post-load glucose concentration and corrected fetal birth weight and LGA in Uganda compared to the other studies

We conducted the same analysis using one-hour and two-hour post glucose load comparing Uganda with the HAPO-black and white groups. The relationship was similar to what was observed with the fasting glucose results (figure 5.3), with the Uganda population showing a reduced association of 1 and 2 hours stimulated glucose with birth weight compared to the white HAPO cohort. The relationship was, however, similar between Uganda and the black HAPO cohort. Similar findings were observed when adjusted for maternal BMI and age at the time of OGTT (supplementary Table 5.2)

Table 5.2 The correlation of corrected birth weight with maternal glycaemia and other maternal characteristics in the three populations. Birthweight corrected for sex and gestational age. β, beta coefficient; CI, confidence interval, HAPO, Hyperglycaemia and Adverse Pregnancy Outcome Study

	Uganda (n=2544)			HAPO-Black (n=1224)			HAPO-White (n=7679)		
	β(95% CI)	**P Value**	**R^2**	**β(95% CI)**	**P Value**	**R^2**	**β(95% CI)**	**P Value**	**R^2**
Fasting glucose (mmol/L)	104 (58.6 – 149)	<0.001	0.008	203 (137 – 270)	<0.001	0.036	239 (214 – 265)	<0.001	0.043
1-hr glucose (mmol/L)	29.4 (15.3 – 43.5)	<0.001	0.007	45.1 (28.4 – 61.7)	<0.001	0.020	45.8 (40.0 – 51.1)	<0.001	0.030
2-hr glucose (mmol/L)	33.2 (15.0 – 51.4)	<0.001	0.005	56.2 (35.6 – 76.8)	<0.001	0.020	57.7 (50.0 – 65.3)	<0.001	0.030
Maternal Age (yrs)	6.0 (2.3 – 9.7)	0.001	0.004	10.7 (6.4 – 15.0)	<0.001	0.010	8.34 (6.6 – 10.1)	<0.001	0.012
Maternal BMI	10.7 (7.8 – 13.6)	<0.001	0.020	13.7 (9.7 – 17.7)	<0.001	0.050	20.2 (18.3 – 22.1)	<0.001	0.055
Parity	88.3 (46.5 – 130.1)	<0.001	0.006	85.8 (31.9 – 140)	<0.002	0.007	117 (95.3 – 138)	<0.001	0.015
Systolic BP (mmHg)	0.53 (-1.45 – 2.50)	0.601	0.000	3.32 (0.87 – 5.76)	0.008	0.006	4.49 (3.53 – 5.44)	<0.001	0.011

Table 5.3 Adjusted Odds Ratio for the association between maternal glycaemia as a continuous variable and Large for Gestational Age (Corrected Birthweight >90th Percentile). Odds ratios were the increase in LGA for an increase in the glucose level of 1 SD in the individual studies. AOR, Adjusted Odds Ratio; CI, confidence interval; HAPO, Hyperglycaemia and Adverse Pregnancy Outcome Study

	Uganda (n=2544)		HAPO-Black (n=1224)		HAPO-White (n=7679)	
	AOR (95% CI)	**R-squared**	**AOR (95% CI)**	**R-squared**	**AOR (95% CI)**	**R-squared**
Fasting glucose	1.13 (1.00 – 1.29)	0.0023	1.38 (1.15 – 1.66)	0.0152	1.57 (1.46 – 1.69)	0.0297
1-hr glucose	1.21 (1.07 – 1.37)	0.0052	1.23 (1.03 – 1.48)	0.0064	1.45 (1.34 – 1.56)	0.0198
2-hr glucose	1.12 (0.99 – 1.27)	0.0018	1.19 (1.00 – 1.42)	0.0045	1.37 (1.28 – 1.47)	0.0150

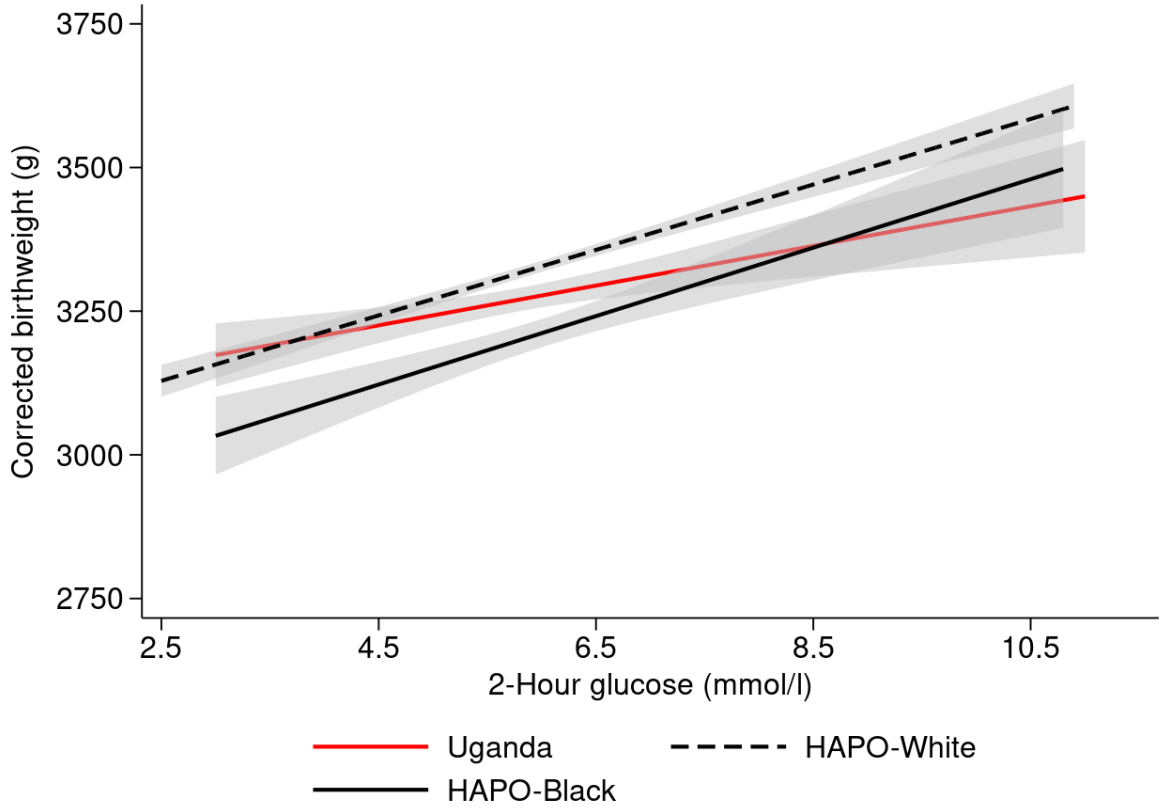

Figure 5.3 Relationship between corrected birthweight with maternal 2-hour glucose for Uganda (n= 2544), HAPO-Black (n=1224) and HAPO-White (n=7679)

5.4 Discussion

We have found a smaller association of maternal glucose to corrected fetal birth weight and large for gestational age in Uganda in comparison to other studies

By comparing three birth cohorts, we have shown that the maternal fasting glucose contribution to fetal birthweight is substantially lower in women in Uganda than in women in the HAPO studies despite having similar BMI. We also found that an increase of glucose by 1 SD in Ugandan women was associated with a smaller odds ratio of having a large for gestational age baby than in the other populations. Taken together, this suggests that fasting glucose cut-offs for treatment in pregnancy need to be population-based.

Possible explanations for the low contributions of maternal glucose to birthweight in Uganda

There are notable differences in the epidemiology of type 2 diabetes and GDM in terms of ethnicity; black women have a higher prevalence of type 2 diabetes but not of GDM when compared to white women (11-13). Many factors are thought to influence birth weight, including the infant sex, maternal race, parity and the mother's weight. The maternal glucose concentration is also known to play a crucial role in the growth of the foetus. Glucose, the main substrate for fetal growth, is transported across the placenta in proportion to its concentration in the bloodstream and stimulates the foetus's endogenous production of insulin and insulin-like growth factors (14). In healthy women, reduced glucose transfer from mother to foetus is linked to slower fetal growth, smaller birth size, reduced risk of LGA birth, and an increased risk of fetal growth restriction. Increased risk of LGA at higher glucose

concentrations is mainly due to the mother's increased insulin resistance leading to a higher amount of blood glucose passing through the placenta into the fetal circulation. This extra glucose in the foetus is stored as body fat, causing macrosomia (14).

The reason maternal glycaemia might have a smaller impact on birth weight in SSA is unclear. In our study, we found that women in Uganda had lower post-challenge glucose concentrations than white women but comparable levels of fasting glucose, suggesting increased insulin secretion or upregulated beta-cell function. Most available data also seems to suggest that black people are more insulin resistant and have upregulated beta-cell function than whites (15).

The financial implications of universal screening and using lower cut-offs to diagnose GDM in SSA need to be debated

Concerns have been raised on the cost implications of the IADPSG guidelines that recommend lower fasting plasma glucose cut off of 5.1 mmol/L for diagnosis of GDM. It has been demonstrated that the IADPSG criteria increase GDM diagnosis by almost two-fold in some populations, and this has drastic economic implications that impact the utility of this test, especially in low resource settings (16). The guidelines also recommend that all pregnant women should be tested for GDM (universal screening) (2). However, universal screening for hyperglycaemia during pregnancy is not practical in many low resource settings, including those in SSA (17). Many health care systems in Africa employ a selective screening approach for GDM whereby only women with certain risk factors are tested (18). This approach is viewed as the most cost-effective in resource-poor settings (19). However, the disadvantage of selective screening is the lack of data on the actual prevalence of the condition and also the risk of affected women being missed; selective screening

is thought to miss around 50% of women with GDM (20). The gold-standard test for diagnosing GDM is the OGTT performed at 24 – 28 weeks gestation (21). In resource-poor settings where OGTTs are not feasible, a fasting plasma glucose screen may be an alternative option for detecting women with GDM. There has been some debate around whether fasting plasma glucose reading alone is sufficient for diagnosing GDM (22, 23) and predicting adverse neonatal outcomes(24). Fasting plasma glucose reading alone appears to have a high sensitivity in detecting GDM in SSA (25, 26). In a study involving 1906 pregnant women in South Africa, fasting plasma glucose reading had a high sensitivity (83.3% (95% CI 77.0, 88.5) in diagnosing GDM (27). Our study found similar results using fasting and post glucose load concentrations.

Strengths and weaknesses

A strength of this study is that it compares a large, well-characterised population in Africa with the HAPO study that was used to determine the diagnostic criteria for GDM. Limitations are that the study only represents findings from one country in SSA, and it is unclear whether the results can be generalised to the entire continent. A further limitation is that there were limited data available to enable exploration of factors that might further explain the differences observed, for example, maternal and chord c-peptide to compare levels of insulin secretion and resistance between the populations. Fasting plasma glucose was measured at earlier gestational age in Uganda than in the HAPO study. Our study did not look at other adverse effects attributed to GDM, for example, caesarean section. However, other predictors for a caesarean section have been shown to play a crucial role in deciding to conduct a caesarean section in many SSA and other regions. Cultural preference seems to play an important role in the caesarean section rate (28).

5.5 Conclusion

The contribution of gestational diabetes to maternal and neonatal ill-health in SSA is not well documented (29). We found that associations between maternal glucose and fetal birth weight and LGA are lower in SSA than in the countries involved in the HAPO study. This study underscored the need that cut-offs that have been generated from other study populations need to be confirmed in SSA before they are adopted.

5.6 Acknowledgements

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5.8 Supplementary information

Supplementary Table 5.1 Sensitivity analysis comparing participants excluded in the study because of incomplete data and those included in the analysis.
Mean ±SD for continuous variables n (%) for proportions

Variable	With Missing data N=974	With Complete data N=2657	P-Value
Mothers age	25.7±5.5	27.0±5.5	<0.001
BMI	27.2±4.9	27.8±6.8	0.011
Systolic BP	104±10.6	104±10.1	0.023
Fasting glucose	4.3±0.6	4.3±0.7	0.161
1-hour	6.3±1.6	6.4±1.6	0.572
2-hour	5.8±1.5	5.9±1.4	0.409
Length of gestation at time of OGTT (wk)	25.8±1.2	25.9±1.2	0.002

Supplementary Table 5.2 Adjusted Odds Ratio for the association between maternal glycaemia as a continuous variable and Large for Gestational Age (Corrected Birthweight >90th Percentile). As in the HAPO analysis (1), all models are adjusted for maternal BMI and age at the time of the OGTT. Odds ratios were the increase in LGA for an increase in the glucose level of 1 SD in the individual studies. AOR, Adjusted Odds Ratio; CI, confidence interval; HAPO, Hyperglycaemia and Adverse Pregnancy Outcome Study;

	Uganda (n=2544)		HAPO-Black (n=1224)		HAPO-White (n=7679)	
	AOR (95% CI)	**R-squared**	**AOR (95% CI)**	**R-squared**	**AOR (95% CI)**	**R-squared**
Fasting glucose	1.07 (0.94 – 1.23)	0.009	1.29 (1.22 – 1.38)	0.045	1.34 (1.24 – 1.45)	0.053
1-hr glucose	1.15 (1.01 – 1.32)	0.012	1.11 (0.97 – 1.35)	0.018	1.29 (1.20 – 1.40)	0.051
2-hr glucose	1.07 (0.94 – 1.22)	0.009	1.07 (0.88 – 1.30)	0.018	1.25 (1.15 – 1.34)	0.050

Chapter 6

Discussion

Chapter 6: Discussion

This chapter gives an overview of the main findings of the thesis and discusses the works' conclusions, implications, limitations and potential areas for further research. I will discuss each chapter in turn.

6.1 Overview of thesis

The work presented in this thesis evaluated the accuracy and utility of fasting and postprandial glucose results during an oral glucose tolerance test (OGTT) in sub-Saharan Africa.

We first assessed glucose stability in different pre-analytic conditions, and identified alternative sample handling procedures that could provide accurate glucose results in the absence of the recommended collection tubes.

We then conducted a randomised cross-over study to explore the impact of consuming meals of different sizes immediately before an OGTT and showed that OGTT results are altered by the size of the meal eaten the evening before it is performed.

We also conducted a prospective cohort study of patients with impaired fasting glucose recruited in Malawi after a mean of four years and found that the incidence of diabetes is high and is predicted by adiposity and fasting glucose at baseline.

Lastly, we examined the relationship between foetal birth weight and the associated adverse pregnancy outcomes of large for gestational age (LGA) with maternal glucose and found that maternal glucose has less impact on foetal growth in Uganda than in other settings.

6.2.0 Chapter 2: Alternative pre-analytic sample handling techniques for glucose measurement in the absence of fluoride tubes in low resource settings.

One of the obstacles in obtaining accurate glucose results is how the samples are handled before analysis. The importance of the pre-analytical phase of the testing process accounts for 46-68% of all laboratory errors (1). Obtaining accurate results requires careful sample handling, including correct blood-collecting tubes, sample processing and timely analysis. The current guidelines recommend using tubes containing sodium fluoride (NaF) for sample collection (2, 3). These pre-analytical requirements can be challenging in resource-poor settings (4, 5). For example, the supply of NaF tubes may be erratic or might be thought uneconomical to use because they are only suitable for glucose analysis (6).

This chapter describes the comparison of glucose results collected in different pre-analytic environments. We assessed the effect of different pre-analytical conditions on measured glucose concentration to identify alternative pre-analytic sample handling procedures that can provide accurate glucose results in the absence of NaF.

6.2.1 Conclusion

The key finding of this chapter is that where resources are limited, appropriate pre-analytic handling could allow accurate glucose measurement, avoiding costly NaF tubes. Early centrifugation prevented glucose decline even at room temperature, with glucose concentration in all the tubes (serum, EDTA and NaF) remaining at 100%

from baseline 24 hours after collection. Uncentrifuged EDTA plasma but not serum is stable for six hours if kept on ice.

6.2.2 Implication of findings

The main implication of this study is that NaF tubes remain the preferred collection tubes for glucose measurement in research and clinical setting. In the absence of NaF, appropriate pre-analytic sample handling can ensure accurate glucose measurement. In a low resource setting, where NaF tubes are unavailable, we recommend using EDTA tubes, provided they are immediately placed on ice and analysed or centrifuged within 6 hours. These are simple procedures, that use readily available equipment, and therefore offer a feasible alternative within these settings. If facilities for immediate centrifugation are available, then any collection tube can be used for glucose measurement, samples can be placed at room temperature, and measurement of glucose is made up to 24 hours from sampling.

These additional protocols for specimen collection for glucose measurement using readily available tubes may offer viable alternatives to point-of-care glucose measurements, which use capillary blood for glucose measurement and are not accurate.

6.2.3 Limitations

A limitation of the study is that we did not examine how the EDTA bottles will perform with very high or low glucose concentrations. Random non-fasted blood from participants with no history of diabetes was used in the analysis, with the glucose

range being 4.1-8.0 mmol/L. Higher glucose concentrations will be expected in those with poorly controlled diabetes. However, it is likely that the conclusions will still hold.

Similarly, the study does not consider the performance of the tubes in very extreme temperatures. The mean ambient room temperature six hours from baseline was 28^{o}C which is considerably higher than the studies in Europe where room temperature was 20^{o}C. Higher temperatures with varying humidity levels may be experienced in surveys and blood collecting exercises in other parts of sub-Saharan Africa, and it is unclear if the same conclusions will be observed in these settings.

Lastly, we had a limited sample size and therefore could not observe small differences; for example, in the baseline plasma and serum glucose, there was a 2% difference reported in other studies (7).

6.2.4 Future research

It would be helpful to study glucose stability in a larger population, including those with diabetes, to further assess the utility of EDTA tubes in extreme glucose ranges. Future work might also involve repeating the study in alternative conditions, including high ambient temperatures and outside environments.

Another area of research is to establish whether the errors and additional per test cost introduced by using point of care capillary glucose in sub-Saharan Africa outweigh the need to store and process samples in a laboratory setting.

Furthermore, research is needed to explore novel solutions that could enable immediate centrifugation and separation of whole blood in low resource settings. Potential mechanisms include the use of manual or solar powered centrifuges, which

could be used in remote locations with unreliable sources of electricity. The practicalities of using a cooler box with ice blocks also need to also be explored.

Lastly, studies on the stability of other related assays, for example, insulin and c-peptide, could increase their utility in sub-Saharan Africa.

6.3.0 Chapter 3: The effect of having a low caloric/low carbohydrate evening meal before an oral glucose tolerance test, implication on screening for diabetes in populations with high levels of food insecurity: a randomised cross over study.

Oral glucose tolerance test (OGTT) is the “gold -standard” test used globally to determine the prevalence of diabetes and glucose intolerance. However, many factors affect its accuracy, including the food intake before the test. There are no guidelines on adequate food consumption in the evening before an OGTT. As such, the performance of the OGTT in settings of food restriction, common in sub-Saharan Africa, is unclear.

In this study, we assessed the impact of consuming meals of different sizes and carbohydrate content the evening before an OGTT on plasma glucose levels.

6.3.1 Conclusions

The key finding of this chapter is that OGTT results are altered by the size of the last meal before the test. Fasting plasma glucose levels were lower after a small evening meal versus a normal evening meal (4.9(0.5) mmol/L vs 5.2(0.6) mmol/L, $p<0.001$). Conversely, the 2-hour glucose concentrations were higher after a small evening meal versus a normal evening meal (7.2(1.5) mmol/L vs 6.3(1.7) mmol/L, $p=0.003$). Overall, glucose tolerance was worse after a small evening meal (mean Area under plasma glucose-time curve 925 mmol min/L(small) vs 841mmol min/l(normal) $p<0.001$). Twice as many individuals showed impaired glucose tolerance (≥ 7.8 mmol/L) after a small evening meal, compared to a normal evening meal (10 vs 5 p=

0.09). Mechanism of the reduced fasting glucose was increased insulin sensitivity, and the mechanism of the raised postprandial glucose was a result of reduced insulin secretion

6.3.2 Implication of findings

This study has an important implication that the true burden of diabetes in sub-Saharan Africa is unknown. Dietary intake before an OGTT is rarely recorded or analysed, even in robust surveillance studies in the region. Short-term food restriction can induce glucose intolerance unrelated to sustained hyperglycaemia. As such, OGTT based estimates may not reflect the actual burden of diabetes in populations where food insecurity is common. This is highly relevant for epidemiological studies aiming to determine the prevalence of diabetes in areas with high levels of food insecurity.

These findings also have a broader implication on the utility of OGTT in food-secure settings where low carbohydrate meal plans and intermittent fasting have become common.

There is a need for clear guidelines concerning dietary intake before an OGTT. Participants should receive clear dietary instructions that must be adhered to in clinical and epidemiological studies before an OGTT. We recommend that the last evening meal before the OGTT should include at least 50 grams of carbohydrates. This is in addition to the current recommendation by the WHO of at least three days of moderate to high carbohydrate intake containing more than 150 grams of carbohydrates daily (8, 9).

6.3.3 Limitations

One limitation is that every participant was given the same quantity of food regardless of weight or sex. Participants who are undernourished or elderly are likely to have a decrease in muscle mass which is the primary tissue for muscle glucose absorption after a meal (10, 11). This variation would influence the person's response to the glucose load.

Another potential limitation of the study is we do not know whether an adequate evening meal can correct long term food depletion prior to the day before the test. The dietary intake in the days before the OGTT was not recorded, and we only monitored the food consumed the day prior to the OGTT.

Other potential mechanisms behind the finding were not explored. For example, the role of free fatty acids and incretins, whose role in role in glucose homeostasis might explain our results, were not analysed.

6.3.4 Future research

The next phase of research in this area is to assess, in epidemiological studies, whether makers of low food availability and/or malnutrition are associated with isolated postprandial dysglycaemia in sub-Saharan African populations.

In clinical studies, OGTT is mainly used in pregnancy to define patients with Gestational Diabetes Mellitus. Therefore, an important area for future work would be a replication of the study in pregnant women to determine if the size of the last meal before the test OGTT altered the results in this population as well.

Further studies to determine the mechanism behind the change in glucose control after meals would also be helpful. There are some suggestions that there are sex

differences in glucose tolerance. Future studies are needed to determine if there are variations in glucose tolerance after a restricted evening meal between men and women, old and young, underweight, and overweight. This also includes replicating the study in larger populations and settings, such as urban populations with varying eating habits than their rural counterparts.

Research to determine the long-term impacts of small evening meals on glucose control are also needed. There is a need to understand how other pre-analytic factors that affect OGTT alter the accuracy of the test in low-resource settings. Further studies could therefore be aimed at understanding methodological improvements to account for the factors that could affect the OGTT. This investigation could involve practical interventions, including assessing if giving a prescribed meal the evening before is useful.

Lastly, research should also focus on alternative methods of detecting glycaemic disorders in low resource settings. Several metabolites, including amino acids, lipids, and carbohydrates, have potential as biomarkers for type 2 diabetes (12).

6.4.0 Chapter 4: Incidence and predictors of progression to diabetes in Malawi: a 4-year prospective cohort study of people with impaired fasting glucose.

Individuals with prediabetes have a higher risk of developing diabetes than those with normal glucose tolerance and therefore offer a unique target population for identification and intervention (13-16). Few studies have determined the incidence rate and the modifiable risk factors that predispose to the development of diabetes amongst the population with prediabetes in sub-Saharan Africa.

In this prospective cohort study, we aimed to evaluate the incidence of diabetes and the predictors of progression in a population-based cohort with impaired fasting glucose in Malawi.

6.4.1 Conclusion

The key conclusion of this study is that the incidence rates of diabetes in people with impaired fasting glucose in Malawi are higher than those seen in Europe or the USA but similar to those seen in India. Increased adiposity and fasting glucose at baseline predicted those who went on to develop diabetes. The median follow-up was 4.2 (IQR 3.4 – 4.7) years. Forty-five out of 175 (26%) progressed to diabetes. The incidence rate of diabetes in our cohort was 62.9 per 1000 person-years, which is higher than previously reported in studies of white Caucasians (35.0-40.0 per 1,000 person-years) and Iran (34.5 per 1,000 person-years) (17) but similar to that reported in Chennai India (61.0 per 1,000 person-years) (18). The predictors of progression were higher; age, BMI, waist circumference, waist-hip ratio, systolic blood pressure, fasting plasma glucose, cholesterol and low-density lipoprotein cholesterol. A simple

model combining fasting plasma glucose and waist circumference was predictive of progression to diabetes (ROC area under the curve=0.79)

6.4.2 Implication of findings

The main implication of this work is a practical suggestion to identify those most likely to develop T2D for lifestyle intervention or increased testing for diabetes. An important finding of the study was that waist circumference is an independent risk factor for diabetes. This observation confirms reports from other populations that measures of abdominal adiposity, rather than total body adiposity (BMI), maybe a better indicator for the relationship between obesity and diabetes (19).

A simple chart with probabilities of progression to diabetes based on waist circumference and fasting plasma glucose could be used to identify those at risk of progression in sub-Saharan Africa. This presents a novel, practical, low-cost tool to identify those at risk of progression in the region.

6.4.3 Limitations

The study's limitations are the lack of year-by-year follow-up data and the relatively low response rate, especially among men and urban participants. A much higher loss to follow-up was observed in the urban population, where rates of diabetes are known to be higher than the rural area (20). The true incidence rate in the region could therefore be higher than reported. Loss to follow-up remains a significant challenge in conducting long term research and intervention studies in many countries in sub-Saharan Africa.

Another limitation was that we only had data on the age at which they developed diabetes on 29% of those who progressed to diabetes before follow-up. 71% were identified at follow-up and had not been tested in the previous five years. Thus exact time to progression from baseline is unknown. Therefore, we could not use survival analysis to interpret our data, and we had to use logistic regression instead.

Further study design limitations were that appropriately matched controls with normal glucose tolerance at baseline were not recruited.

Lastly, OGTTs were not conducted both at baseline and follow-up, meaning isolated impaired glucose tolerance could not be identified. Significant differences in prevalence can depend on whether prediabetes is defined by IFG or IGT (21).

6.4.4 Future research

There is a need for more extensive population-based studies to determine the incidence of diabetes and determinants of progression in sub-Saharan Africa. Future research should follow up patients with prediabetes in other populations in sub-Saharan Africa to validate the findings to inform a comprehensive understanding of the disease in the region. This could be combined with studies to determine the mechanisms behind drivers of progression in the region.

Our study suggests that prediabetes in sub-Saharan Africa is associated with a rapid progression to diabetes similar to what has been observed in other low-income populations. The reasons for this are unknown and points to the need for a more detailed investigation on the pathophysiology and natural course of diabetes in people from sub-Saharan Africa.

Further studies are also needed to validate the tool that uses waist circumference and fasting plasma glucose to identify those at risk of developing diabetes and to determine the settings where it would be most helpful. This could include research to determine whether total body or abdominal adiposity is the more significant risk factor for diabetes in sub-Saharan Africa.

A high proportion of prediabetes patients regressed to normal glucose regulation at follow-up. However, the predictors to regression in people with prediabetes are unknown. The use of type 2 genetic risk scores to identify the progression of prediabetes has not been done in sub-Saharan Africa, so this could also be explored.

Another question of interest is whether interventions that have been shown to slow progression in high-income countries will be as effective in sub-Saharan Africa (or LMIC), given the differences in progression rates. The Diabetes Prevention Program trial demonstrated that intensive lifestyle intervention, to a lesser extent, metformin use reduced the risk of diabetes progression in high-risk adults (5, 29).

Finally, research needs to be done to understand how to improve retention of patients in studies in sub-Saharan Africa or follow up people at risk of diabetes or those with the disease. This could involve developing national registers or regular record collection of people with prediabetes or diabetes and qualitative work to understand the barriers and facilitators to retaining people in studies.

6.5.0 Chapter 5: Associations between maternal glucose and foetal birth weight in sub-Saharan Africa and comparison with other regions: analysis of three cohort studies.

Studies in different countries have found that maternal glucose influences the baby's size. As a result, lower fasting and 2-hour glucose than are standardly used to diagnose diabetes are used for diagnosing diabetes in pregnancy. The relationship between maternal glycaemia and foetal birth weight in sub-Saharan Africa is unknown and has not been compared to other regions. In this chapter, we compared the association between maternal glucose and foetal birth weight in a sub-Saharan Africa birth cohort to the finding in white and black participants in the HAPO study.

6.5.1 Conclusion

The key conclusion of this chapter is that maternal glucose has less impact on foetal growth in Uganda than in other settings. The contribution of maternal glucose to birth weight is lower in Uganda than in other settings (β-coefficient (95%CI) 103.0 (58.6 to 149.1) in Uganda, 202.8(136.8 to 269.3) HAPO-black, and 239.4(214.0 to 264.7) HAPO-white. Likewise, the risk of LGA with increased fasting glucose was weaker in Uganda compared to the other studies (adjusted odds ratio (95%CI) 1.07 (0.94 to 1.23) in Uganda, 1.29 (1.22 to 1.38) HAPO-black, and 1.34(1.24 to 1.45) HAPO-white.

6.5.2 Implications of findings

Our findings imply that reference cut-offs for GDM derived from the HAPO study might not be appropriate for Uganda as many women diagnosed with GDM will not

develop the adverse pregnancy outcome of LGA associated with the condition. An important consideration is whether it is more beneficial to treat each risk factor associated with diabetes to predefined targets or consider risk factors collectively and aim to reduce overall risk.

Our study implies that GDM is less of a health priority in sub-Saharan Africa than in other settings. International policies for diagnosing gestational diabetes, that use data from other regions, should not be adopted without evidence that the results apply to the sub-Saharan Africa context. Concerns have been raised on the cost implications of the IADPSG guidelines that recommend lower fasting plasma glucose cut off of 5.1 mmol/L for diagnosis of GDM. It has been demonstrated that the IADPSG criteria increase GDM diagnosis by almost two-fold in some populations, and this has drastic economic implications that impact the utility of this test, especially in low resource settings (22). The guidelines also recommend that all pregnant women should be tested for GDM (universal screening) (23). However, universal screening for hyperglycaemia during pregnancy is not practical in many low resource settings, including those in sub-Saharan Africa (24). Our findings also suggest that universal screening for GDM is not needed in sub-Saharan Africa, and only those with known risk factors for diabetes would benefit from screening.

6.5.3 Limitations

The study represents findings from one country in sub-Saharan Africa and, therefore, is difficult to generalise. These findings need to be replicated in other countries in sub-Saharan Africa.

Limited data is available to explore the mechanism behind the differences observed, for example, cord c-peptide. We did not have data for cord-blood C-peptide concentrations, caesarean sections or neonatal hypoglycaemia rates. High cord-blood C-peptide concentrations were one of the criteria used by the IADPSG to develop their diagnostic criteria; this additional information might have affected the results.

6.5.4 Future research

Research is needed to define risk factors for adverse pregnancy outcomes related to hyperglycaemia in pregnancy in sub-Saharan Africa. These could be used to develop screening tools that predict undiagnosed GDM and women who will benefit from an OGTT and subsequent therapy.

There is a need to establish robust cohorts of pregnant women and their offspring in other populations in sub-Saharan Africa to validate the findings to inform a comprehensive understanding of the GDM in the region. This could also include comparisons between mothers who emigrate from Africa and those who remain to understand the effect of genes and environment.

6.6 Final remarks

The OGTT has been the cornerstone for diagnosing type 2 diabetes, prediabetes and gestational diabetes for over a hundred years (25). Despite this long history, there is still a lot to learn about its utility and accuracy in the sub-Saharan Africa.

This thesis has demonstrated that it is possible to produce reliable glucose measurements, even in resource-poor areas, if appropriate pre-analytical sample handling procedures are followed. Our studies have also questioned the accuracy of OGTT results, where the last meal before the test were not recorded. We have also identified the high incidence of diabetes in individuals with impaired fasting glucose, and have identified simple clinical features that predict progression to diabetes in sub-Saharan Africa. Lastly, we have demonstrated that international policies for diagnosing diabetes that uses data from other regions should not be adopted without evidence that the results apply to the sub-Saharan context.

Further research is needed to identify alternative markers of diabetes in sub-Saharan Africa and cost-effective strategies for screening and managing people with diabetes in the region.

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