Faculty of Infectious and Tropical Diseases
Department of Clinical Research

Sarah Louise Bailey

Thesis submitted in accordance with the requirements for the degree of Doctor of Philosophy, University of London

September 2017

Understanding the effect of hyperglycaemia on tuberculosis control in a southern African setting: the impact of HIV and diabetes control

The DARTZ (Diabetes and Risk of Tuberculosis in Zambia) studies

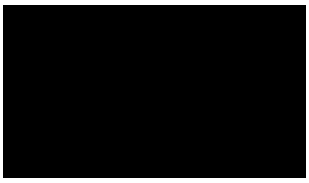
The work contained in this thesis was supported by a Clinical PhD Training Fellowship grant from the Wellcome Trust (100141/Z/12/Z)

Supervisors: Dr Helen Ayles, Professor Peter Godfrey-Faussett, Professor John Yudkin

Declaration

I, Sarah Louise Bailey, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signature Date: 04 September 2017



Abstract

An association between diabetes mellitus and tuberculosis has been established: systematic reviews suggest that individuals with diabetes are three times more likely to develop active tuberculosis disease and have nearly a two-fold increased risk of death from tuberculosis than individuals without diabetes. The context of these associations in southern Africa may differ from the rest of the world due to the high prevalence of HIV and the high prevalence of poorly controlled diabetes mellitus in this region. Most prior studies have measured glycaemia at the time of tuberculosis diagnosis as a proxy for diabetes. Physiological stress from acute TB infection can also cause hyperglycaemia, so we use the term hyperglycaemia to encompass both diagnoses until the two can be differentiated. We conducted a case-control study in Lusaka, Zambia to determine if HIV modifies any association between hyperglycaemia and active tuberculosis. We recruited 3,843 tuberculosis cases and 6,977 controls and found no evidence of an overall association between hyperglycaemia and active tuberculosis, though there was a significant effect modification by HIV: among individuals with HIV there was a positive association. A subset of cases from the case-control study were recruited to a cohort study to determine whether a dose-response relationship exists between hyperglycaemia and tuberculosis treatment outcome. We found no evidence of a relationship. A study of diagnostic accuracy used a further subset of cases from the case-control study to determine the diagnostic accuracy for diabetes mellitus of measures of hyperglycaemia used at the time of tuberculosis diagnosis compared to a reference standard. We found a low proportion of hyperglycaemia measured at the time of tuberculosis diagnosis was due to diabetes mellitus, particularly among individuals co-infected with HIV. Glycated haemoglobin showed no greater test accuracy for diabetes diagnosis at the time of tuberculosis diagnosis than fasting or random blood glucose tests.

Preface

This thesis is written as a research paper style thesis, in accordance with the guidelines and regulations specified by the London School of Hygiene and Tropical Medicine. Linking material is provided to maintain coherence of the thesis and ensure it is presented as one body of work. There are six papers, each as a separate chapter: three in the introduction section and three in the results section. The remainder of the introduction, aims and methods, and discussion sections comprise linking material. Repetition has been minimised wherever possible, though inevitably there is some repetition in papers and linking material, most notably in the methods sections. Nonetheless the thesis does not exceed the word limit of 100,000. The papers are in various stages of publication: three have been published, the others are being prepared for submission. Sarah Lou Bailey is the first author of all papers. Research paper cover sheets giving the publication details are provided at the end of the thesis, directly before the appendix. Regardless of publication status the papers are presented as unformatted manuscripts, to maintain a coherent style throughout the thesis. All papers and all linking material were written by Sarah Lou Bailey.

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Investigators

Research Degree Student:

Dr Sarah Lou Bailey BSc MBChB MRCP PGCHE MSc

Clinical Research Fellow

Clinical Research Department

Faculty of Infectious and Tropical Medicine

London School of Hygiene and Tropical Medicine

The advisory group comprised the following:

Supervisor:

Dr Helen Ayles BSc MBBS MRCP DTM&H MSc PhD

Director, Zambart

Professor of Infectious Diseases and International Health

Clinical Research Department

Faculty of Infectious and Tropical Medicine

London School of Hygiene and Tropical Medicine

Associate Supervisor:

Professor Peter Godfrey-Faussett BA MBBS DTM&H FRCP

Professor of International Health (Currently seconded to UNAIDS)

Clinical Research Department

Faculty of Infectious and Tropical Medicine

London School of Hygiene and Tropical Medicine

Associate Supervisor:

Professor John Yudkin MD FRCP

Emeritus Professor of Medicine

University College London

Statistician:

Sian Floyd BSc MSc

Associate Professor in Epidemiology and Medical Statistics

Department of Infectious Disease Epidemiology

Faculty of Epidemiology and Population Health

London School of Hygiene and Tropical Medicine

PhD Upgrading Examiners:

Professor Liam Smeeth, Professor of Clinical Epidemiology, London School of Hygiene & Tropical Medicine

Dr Philip Gothard, Honorary Senior Lecturer in Medicine, London School of Hygiene & Tropical Medicine and Consultant Physician, Hospital for Tropical Diseases, University College London Hospitals NHS Foundation Trust

Collaborating Institutions

London School of Hygiene and Tropical Medicine, UK

Zambart, Lusaka, Zambia

Chawama, George and Kanyama Health Centres, Lusaka, Zambia

Funding

This work was funded through a Wellcome Trust Clinical PhD Training Fellowship awarded to Dr Sarah Lou Bailey through the London School of Hygiene and Tropical Medicine (100141/Z/12/Z).

Acknowledgements

I would like to thank all those, particularly the members of my advisory group, who have given me academic support throughout the duration of my doctorate studies, from the initial development of my research ideas to completion of this thesis. I am enormously grateful to Helen Ayles for supporting me throughout this journey, and for passing on some of her many pearls of academic wisdom along the way. I could not have completed this work without Sian Floyd's statistical guidance and help. I am extremely grateful for all the time and effort she dedicated to me and this work. Peter Godfrey-Faussett has been inspirational and an utter delight to work with. I am very grateful for his intellectual guidance. This work would have been far inferior without guidance from John Yudkin on all aspects of diabetes and glycaemia. I am enormously grateful for the many hours of his free time that he dedicated to this work, for his inspiration, for his support throughout this work and my earlier career since a medical student, and always for his incredibly speedy response to every email I have ever written to him.

The broad area of investigation for this research arose during my prior work in Zambia when the ZAMSTAR cluster-randomised trial was underway. The idea was initiated by me, Peter Godfrey-Faussett and Helen Ayles. The details of the aims and methods were later developed by me, with guidance from all members of my advisory group.

The participant questionnaire and the relevant study standard operating procedures were based on those from the ZAMSTAR study, in order to maintain comparability with the control group for study 1 of this research. These were originally written by Zambart staff members and have been adapted by me where necessary to meet the requirements of this PhD research.

This thesis has been written and compiled by me. Members of my advisory group have read and commented on near-final versions.

I would like to pay particular thanks to my six research assistants, Violet Bwalya, Justine Chamanga, Mukulumwa Danros, Kalenga Florida Mwila, Sara Phiri, and Sharon Yebo, who were all dedicated to their work throughout the data collection phase of this research and were all a delight to work with.

I would like to thank all the Zambart headquarters staff and the Chawama, George and Kanyama clinic staff who have given me help and information whilst organising and undertaking this research. I am particularly grateful for the help provided by Barry Kosloff and his whole laboratory team at Zambart, for all their hard work processing all the study specimens, and for guiding me through many organisational and administrative hurdles in Lusaka.

I would like to acknowledge Tamara Hurst, Coordinator of the Wellcome Trust Bloomsbury Centre for Global Health Research, who was a solid source of endless administrative and organisational knowledge and support, from the very beginning of the application process to the programme right through to final submission of this thesis, and no doubt beyond. I would also like to thank David Mabey and Alison Grant for their guidance as programme leads, and all my fellow fellows on the Wellcome Trust Clinical PhD Training programme who provided stimulating discussion and inspiration throughout the programme.

I am enourmously grateful to Melanie Newport and David Moore who both enabled me to gain research experience early in my career and have both been sources of inspiration and encouragement early on in my career and subsequently whenever we meet.

Finally, I would like to thank my family, particularly my mother Rosie Bailey and my husband Atilla Kadir, for their support throughout the time I have been working on this research, and particularly over the last year of the write-up, when I have been almost entirely reliant on their help with childcare to have time to complete this thesis.

List of Abbreviations

AFB	Acid-fast bacilli
aOR	Adjusted odds ratio
aRR	Adjusted rate ratio
ART	Antiretroviral therapy
ARTI	Annual risk of infection
BMI	Body mass index
CD4	Cluster of differentiation 4
CI	Confidence interval
DARTZ	The Diabetes and Risk of Tuberculosis in Zambia study
DFT	Test for departure from linear trend
DM	Diabetes mellitus
FBG	Fasting blood glucose
FCG	Fasting capillary blood glucose
HbA_{1c}	Glycated haemoglobin
HIV	Human immunodeficiency virus
ICC	Intraclass correlation
INDEPTH	International Network for the Demographic Evaluation of Populations and Their Health
IQR	Inter-quartile range
LSHTM	London School of Hygiene and Tropical Medicine
MGIT	Mycobacteria Growth Indicator Tube
MTB	*Mycobacterium tuberculosis*
NALC	N-acetyl-L-cysteine
NaOH	Sodium hydroxide
NPV	Negative predictive value
NR	Not reported

NTM	Non-tuberculous mycobacteria
OGTT	Oral glucose tolerance test
OR	Odds ratio
PAF	Population attributable fraction
PDA	Personal digital assistant
PI	Principal Investigator
PPV	Positive predictive value
RBG	Random blood glucose
RNA	Ribonucleic acid
RR	Rate ratio
SD	Standard deviation
SOP	Standard operating procedure
SQL	Structured query language
STROBE	Strengthening the reporting of observational studies in epidemiology
SSA	Sub-Saharan Africa
TB	Tuberculosis
TFT	Test for linear trend
TTC	Time to sputum culture conversion
TTP	Time to initial sputum culture positivity
UTH	University Teaching Hospital
WC	Western Cape
WHO	World Health Organization
Xpert	Xpert MTB/RIF assay
ZAMSTAR	The Zambia South Africa TB and AIDS Reduction study
2hCG	2-hour capillary blood glucose after a standard 75g oral glucose tolerance test

Introduction

Chapter 1: Background

1 Historical context

For centuries it has been recognised that diabetes mellitus (DM) is associated with tuberculosis (TB). Reports of the connection date back to 1000 AD, when Avicenna, a Persian physician and philosopher, observed that phthisis frequently occurred among patients with diabetes.[1] In an ancient text, an Indian Siddhar, Yugimahamuni, discussed varieties of urinary disorders, their general symptoms and signs and the types of sufferings they can cause. He described progression from obesity to impotence, thirst, glycosuria and ultimately to unconsciousness or tuberculosis.[2] In more recent history clinicians have again noted the connection, such as Root, an American physician in the 1930s, who stated: "During the latter half of the nineteenth century, the diabetic patient appeared doomed to die of pulmonary tuberculosis if he succeeded in escaping coma".[3]

In the latter part of the twentieth century, focus on the association diminished. Tuberculosis incidence declined in high income countries alongside improvements in sanitation, nutrition and overcrowding.[4-6] Survival of people with diabetes improved following the discovery and introduction of insulin in 1922.[7, 8] Consequently, the overlap of the two diseases was less discernible. Reports of joint TB-diabetes clinics occurring in the 1950s diminished in later years, possibly following the introduction of TB chemotherapy including streptomycin in 1944, p-aminosalicylic acid in 1949, isoniazid in 1952, pyrazinamide in 1954 and cycloserine in 1955. It is possible that following the introduction of treatment for both TB and diabetes the overlap of the two conditions and consequent need for a joint approach to care diminished.

Over the last two decades, however, interest in the association has intensified. In many low and middle income countries the incidence of tuberculosis is high, particularly where the prevalence of HIV is high.[9] The prevalence of diabetes has been rising globally, particularly in low and middle income countries, due to ageing populations, increasing obesity and sedentary lifestyles.[10-14] Therefore, once again, we have settings with both a high incidence of

tuberculosis and a high prevalence of diabetes. The co-existence of these two diseases in the same localities has stimulated interest in and exploration of the overlaps and associations between them.[15]

2 Prior evidence of association between diabetes and tuberculosis

Following growing interest in the subject, the first systematic review was published in 2007. This paper summarised the evidence from analytical studies reporting the association of diabetes as the exposure to TB incidence or disease as the outcome that had been published between 1995 and 2007.[16] The authors identified nine studies – one prospective cohort study, four case-control studies and four studies based on routine data sources. The studies were located in North America, Asia, and one in Europe. All studies showed a statistically significant association between diabetes and TB, with the odds or risk of TB being between 1.5 and 7.8 times greater for individuals with diabetes compared to those without diabetes, though few studies had controlled for potential major confounders.[16] Heterogeneity of the studies prevented synthesis of findings.

In 2008, a second systematic review was published, this time summarising studies published between 1965 and 2007 that were peer-reviewed and gave a quantitative effect estimate of the relationship between DM as the exposure and active TB as the outcome, and that controlled for possible confounding by age or age groups.[17] The search identified 13 observational studies – three cohort studies, eight case-control studies and two other studies using data from surveys. The studies were located in North America, Asia, and one in Europe. Random effects meta-analysis of the results from the cohort studies gave a risk of TB among individuals with DM of 3.11 (95% CI 2.27-4.26) times greater than the risk of TB among individuals without DM. Heterogeneity of case-control studies prevented synthesis but in all studies the odds of TB among individuals with diabetes was greater than for those without diabetes, ranging from 1.16 to 7.83 times greater.[17]

Subsequent reviews and summaries of the overlaps between TB and diabetes continued to discuss evidence of the association,[18-20] and in 2011 the World Health Organization and the International Union Against Tuberculosis and Lung Disease produced a joint report on the association: Collaborative Framework for Care and Control of Tuberculosis and Diabetes.[15] As part of this report they updated the recent systematic review on the association between diabetes and active tuberculosis that had been published in 2008[17] and found three additional papers, two located in Asia and one in North America.[15] There were now four cohort studies showing the pooled random effects risk of TB to be 2.52 (95% CI 1.53-4.03) times higher in individuals with diabetes compared to those without, and ten case-control studies with odds of TB still ranging from 1.16-7.83 times higher among individuals with diabetes than among those without, but now with a random effects summary OR of 2.2 (95% CI not presented).[15]

Additional systematic reviews and meta-analyses for the collaborative framework found the risk of death for individuals with TB and diabetes to be 1.89 (95% CI 1.52-2.36) times higher than for those with TB but no diabetes, and the risk of TB relapse for individuals with TB and diabetes to be 3.89 (95% CI 2.43-6.23) times higher than for those with TB but no diabetes.[21] The former systematic review identified 23 papers and the latter identified five papers, again located in North America, Asia and Europe, but additionally, two of the papers contributing to both reviews were located in Africa:[21] one in Tunisia[22] and the other in the Republic of the Congo.[23]

Chapter 2 of this thesis presents an updated systematic review of the association between diabetes and active tuberculosis, but focuses specifically on studies located in Africa.

3 Biological plausibility of association

The underlying biological mechanisms that cause hyperglycaemia to increase the risk of TB incidence and worsen TB treatment outcome are currently poorly understood.[17, 18, 21, 24-28] Most studies suggest that diabetes impairs innate immunity, including monocyte,[29, 30] macrophage[31,

[32] and neutrophil function,[33, 34] causing reduced chemotaxis and a decreased capacity to phagocytose *Mycobacterium tuberculosis*.[27, 35]

Hyperglycaemia also has a negative effect on the adaptive immune response, which could be important for protection against *Mycobacterium tuberculosis*.[27] Individuals with diabetes have been seen to have a hyper-reactive antigen-specific T-cell response to TB compared to the response seen in individuals without diabetes, which could contribute to increased lung pathology in individuals with diabetes.[36-39]

However, few studies on this topic have been conducted in humans and so our understanding of these mechanisms remains poor.[26, 27]

4 The effect of HIV

Data on the association between HIV and hyperglycaemia/diabetes mellitus are conflicting. Rather than a direct association being a major concern, the main focus is on a possible causal association between antiretroviral therapy and diabetes in HIV-infected individuals, particularly protease inhibitors which interact with adipose tissue and decrease insulin secretion.[40, 41] A recent 2017 systematic review and meta-analysis of published data from nine studies found no association between the use of protease inhibitors and the development of DM (hazard ratio 1.23, 95% confidence interval (CI) 0.66-2.30) but they did find an association between the use of protease inhibitors and metabolic syndrome (relative risk 2.11, 95% CI 1.28-3.48).[40] Metabolic syndrome is a precursor to DM and so it is possible that studies with a longer duration of follow-up may find an association between the use of protease inhibitors and DM. Another meta-analysis of data from ten observational studies exploring the association between antiretroviral therapy in general (not limited to protease inhibitors) and diabetes did find an association (pooled odds ratio 3.85, 95% CI 2.93-5.07).[42] Further long term data is needed to better understand associations between HIV and hyperglycaemia/diabetes. However, this is not the case for the association between HIV and TB; HIV is well documented to be a major risk factor for TB.[9]

The dual effect of hyperglycaemia and HIV on the risk of developing TB disease or on its clinical evolution is unclear and is therefore a focus of this PhD research. It could be that the relative contribution of hyperglycaemia to TB risk is relatively small in HIV-positive individuals compared with its contribution to TB risk in HIV-negative individuals, as the greatly increased risk of TB among HIV-positive individuals could diminish any additional increased risk from hyperglycaemia. On the other hand, the effect of hyperglycaemia might be exacerbated in the presence of HIV infection if the contributions of each to increased TB risk are synergistic. A systematic review of the association between diabetes and tuberculosis incidence among participants with and without HIV is described in Chapter 2.

5 The impact of diabetes control

The differences in cell mediated immunity and cellular cytokine responses to *M. tuberculosis* in the presence of TB and DM compared to TB without DM are even more marked when there is poor glycaemic control.[28, 30, 32, 36, 37] Effective cellular immunity is important for controlling growth of *M. tuberculosis*[36, 43, 44] so we should expect to see a dose response relationship between hyperglycaemia and TB treatment outcome.

A systematic review of the effect of diabetes control on tuberculosis treatment outcome revealed 4 relevant prior observational studies. No intervention studies to optimise glycaemic control in individuals with hyperglycaemia during TB treatment were identified.[45]

5.1 Systematic Review Methods

EMBASE, Global Health and PubMed databases were searched from 1910 until Jan 2013 using the following search strategy:

1. Diabetes mellitus
2. Hyperglycaemia
3. 1 OR 2
4. Tuberculosis
5. Diabetes control

6. Glycaemic control
7. 5 OR 6
8. 3 AND 4 AND 7

No restrictions on language or location were made. The titles and abstracts were reviewed for relevance to the study question and the full text of articles identified as potentially relevant were examined. The reference lists of final papers included in the review were searched to identify additional relevant papers.

5.2 Systematic Review Results

The database searches identified 75 papers after deduplication. 51 of these were excluded due to irrelevance after examination of the titles and abstracts. The full texts of 24 papers were reviewed, 4 of which were found to contain relevant data.

The findings from the identified studies are conflicting, all but one are retrospective, none are located in Africa and all either exclude participants who are infected with HIV or have a very low prevalence of HIV in their study population.

A retrospective cohort study in South Korea recruited 492 culture-confirmed pulmonary tuberculosis patients.[46] Individuals infected with HIV were excluded. The study examined the effect of diabetes control on tuberculosis clinical and radiological features and found that those with uncontrolled diabetes (HbA_{1C} ≥7.0) had more cavitary lesions (p=0.008) and higher rates of positive sputum smears (p<0.001) compared to those without diabetes. The authors also examined the rate of sputum culture conversion at 2-months. Two-month culture data were available for 214 participants. Of 28 participants with uncontrolled diabetes (HbA_{1c}≥7%) 6 had a positive culture at 2 months. Compared to non-diabetics, uncontrolled diabetes was found to be a significant risk factor for a positive sputum culture at 2 months (OR 4.3, 95% CI 1.3-14.3, p=0.017, adjusting for age, cavities and positive sputum smears before treatment initiation). However, this retrospective analysis was not the primary focus of the paper and had only a small number of participants with uncontrolled diabetes.

A retrospective record review in Kerala State, India, reviewed TB treatment outcome at the end of TB treatment for 3,116 patients.[47] Diabetes status was recorded for 90%, of which, 137 TB cases had uncontrolled diabetes (defined as a fasting blood glucose >100mg/dl or a random blood glucose >140mg/dl or a post-prandial blood sugar >140mg/dl). The prevalence of HIV among study participants was 1.5%. The association of poor glycaemic control and unfavourable TB treatment outcome was of borderline significance (RR 2.00, 95% CI 0.97-4.13).

A retrospective review of notification data and medical records for 1,473 TB cases in Taiwan found 276 individuals with uncontrolled diabetes (HbA_{1c} >9%).[48] The prevalence of HIV was 0.3%. Uncontrolled diabetes was found to be associated with improved TB treatment outcome at the end of TB treatment compared to TB cases with well controlled and no diabetes ($p<0.001$). The effect size for this association was not reported in the study paper.

A prospective cohort study in South Korea determined sputum culture positivity at two months of treatment for 661 individuals with pulmonary TB.[49] Individuals with HIV infection were excluded. 108 participants had uncontrolled diabetes ($HbA_{1C}\geq7.0\%$). Uncontrolled diabetes compared to no diabetes was found to be an independent risk factor for a positive sputum culture after two months of treatment (OR 2.11, 95% CI 1.03-4.31, p=0.042) and also for treatment failure or death (OR 4.11, 95% CI 1.23-13.78) p=0.027).

There were 2 other papers identified that reported associations between diabetes control and tuberculosis incidence, with stronger associations found among those with poorer diabetes control. A population-based case-control study in Denmark recruited 2,950 tuberculosis cases and 14,274 controls without tuberculosis.[50] The prevalence of HIV was 0.1% among both cases and controls. No association was found between TB and diabetes (defined by hospital contact involving diabetes or use of antidiabetic medication; adjusted OR 1.18, 95% CI 0.96–1.45). In a subset of participants with laboratory data for glycated haemoglobin, diabetic individuals with an HbA1c of <7, 7-7.9 and >8% had odds ratios for active tuberculosis of 0.91 (95% CI 0.51-

1.63), 1.05 (95% CI 0.41-2.66), and 1.19 (95% CI 0.61-2.30), respectively, compared to individuals without diabetes. This is suggestive of a linear trend, though no statistical significance is seen. In a cohort study in Hong Kong of 42,116 participants, higher risks of active, culture-confirmed, and pulmonary tuberculosis were observed among those with diabetes with a baseline HbA_{1c} ≥7% compared to those with diabetes with a baseline HbA_{1c} <7%.[51] Hazard ratios for these were 3.11 (95% CI 1.63-5.92) for active TB, 3.08 (95% CI 1.44-6.57) for culture-confirmed TB and 3.63 (95% CI 1.79-7.33) for pulmonary TB. The prevalence of HIV was not reported.

6 Causal association or reverse causality?

When exploring these associations it is important to be certain that individuals diagnosed with diabetes truly have diabetes and do not instead have stress hyperglycaemia secondary to infection with tuberculosis. Diabetes can cause immune impairment that could increase tuberculosis risk (causal association). Physiological stress from tuberculosis infection could cause hyperglycaemia (reverse causality). Hyperglycaemia among newly-diagnosed tuberculosis cases could therefore be due to DM or stress-induced hyperglycaemia. This is an important distinction because individuals with tuberculosis are commonly tested for diabetes at the time of their tuberculosis diagnosis, which is the same time they are most likely to have stress-induced hyperglycaemia secondary to tuberculosis, before commencing treatment for tuberculosis. One would expect hyperglycaemia due to diabetes to be persistent, remaining after treatment and resolution of tuberculosis. Conversely, one would expect hyperglycaemia that is stress-induced to be transient, and return to normoglycaemia once the acute infection or disease has been treated and has resolved. Most prior studies have failed to distinguish between these two causes of hyperglycaemia[52] and therefore risk falsely elevating any association between DM and TB. The focus of this thesis is therefore on hyperglycaemia rather than diabetes, so as to explicitly not presume a diagnosis of diabetes before confirming this.

It may be that hyperglycaemia regardless of cause contributes to worsening tuberculosis outcomes, but the management of hyperglycaemia both immediately and in the long term may differ depending on the underlying cause. Differentiation of the cause of hyperglycaemia among newly-diagnosed tuberculosis cases is therefore important not only for increasing biomedical understanding of these associations but also for the management of individuals and for population public health planning, particularly in the context of rising diabetes prevalence. A systematic review to identify prior studies that differentiate between persistent and transient hyperglycaemia has revealed 6 prior studies with relevant data.

6.1 Systematic Review Methods

EMBASE, Global Health and PubMed databases were searched from 1910 until Jan 2013 using the following search strategy:

1. Hyperglycaemia
2. Tuberculosis
3. 1 AND 2

No restrictions on language or location were made. The titles and abstracts were reviewed for relevance to the study question and the full text of articles identified as potentially relevant were examined. The reference lists of final papers included in the review were searched to identify additional relevant papers.

6.2 Systematic Review Results

The searches identified 96 papers after deduplication. Of these, 65 were excluded due to irrelevance after examination of the titles and abstracts. The full texts of 31 papers were reviewed, along with the reference lists of relevant papers, leading to a total of 6 papers identified to contain relevant data.

A high proportion of transient hyperglycaemia in TB patients was found in all identified studies.

A study in Turkey performed glucose tolerance tests in 30 individuals with active pulmonary TB at the time of TB diagnosis.[53] Impaired glucose tolerance was found in four individuals, but this returned to normal in three of these individuals when tested two months later, after receiving two months of antitubercular therapy.

In 1990 Oluboyo performed sequential OGTTs on 54 Nigerian patients with active pulmonary TB. Only one of eight patients with initial impaired glucose tolerance remained with persisting glucose intolerance after 3 months of TB treatment.[54]

An observational study among 106 patients with pulmonary TB attending a clinic in Pakistan found glucose intolerance (impaired glucose tolerance or diabetes mellitus) in 49% of patients at the time of TB diagnosis based on findings from standardised oral glucose tolerance tests.[55] After antitubercular therapy and sputum conversion the oral glucose tolerance test was repeated in 23 participants with initial glucose intolerance. Of these, 57% had reverted to normal glucose tolerance.

A case-control study in Indonesia recruiting newly-diagnosed pulmonary tuberculosis patients and matched neighbourhood controls found that 13.2% (60 of 454) of tuberculosis patients were hyperglycaemic at the time of tuberculosis diagnosis.[56] Following one month of anti-tuberculosis treatment, the hyperglycaemia reverted in 3.7% of these hyperglycaemic cases.

A prospective cohort study among 317 hospitalised new TB patients in Iran measured glycated haemoglobin in 158 participants at baseline and three months later.[57] There were 54% of participants who had elevated glycated haemoglobin (defined as ≥6.5%) at baseline, but only 24% has persistent hyperglycaemia 3 months later.

In a more recent case-control study in Tanzania, 539 tuberculosis cases underwent sequential DM screening tests.[58] At the time of active TB diagnosis, 4.5%, 6.8% and 9.3% had hyperglycaemia measured by FBG, oral glucose tolerance test (OGTT) and HbA1c respectively. After 5 months of receiving TB treatment and exclusion of patients with previously known DM,

75%, 64% and 71% of these initially hyperglycaemic participants were no longer hyperglycaemic on follow-up FBG, OGTT and HbA1c tests respectively.

7 Choice of study location: Lusaka

The number of adults with diabetes in SSA is predicted to rise from 14.2 million in 2015 to 34.2 million in 2040.[10-12, 59, 60] To appropriately respond to this now and prepare for a future higher prevalence of diabetes, a deeper understanding of associations between tuberculosis and hyperglycaemia in the context of SSA is needed.

Lusaka, the capital of Zambia, which has an estimated TB incidence of 506 per 100,000 pop/year,[61] an adult hyperglycaemia prevalence of 3.2% (Chapter 3), and an HIV prevalence of 15.2%,[62] is well suited for studies of associations between TB, hyperglycaemia and HIV.

A recently conducted population prevalence survey in Lusaka gives the opportunity to obtain background data on hyperglycaemia and tuberculosis prevalence in this location as well as to gain a deeper understanding of diabetes prevalence and risk factors. The Zambia South Africa TB and HIV Reduction (ZAMSTAR) study was a 2 x 2 factorial cluster randomised trial to evaluate the impact of two complex interventions on the prevalence of TB.[63-65] The study took place in 24 communities: 16 communities from 5 provinces in Zambia and 8 communities from the Western Cape province of South Africa. The estimated total population in the study areas was 962,655, with an average population per community of 40,110. A cross-sectional prevalence survey was conducted between January and December 2010 in all study communities to measure the trial's primary endpoint of prevalent TB. Along with TB and HIV data, the prevalence survey collected data on participants' random blood glucose concentration, anthropometry, demographics, socioeconomic position and other risk factors for TB. The results of these analyses relating to hyperglycaemia and its association with TB are detailed in chapters 3 and 4 of this thesis.

One other prior study has investigated the prevalence of diabetes mellitus in Zambia. A population-based cross-sectional study in 2008 used a modified World Health Organization

STEPwise approach to NCD surveillance in Lusaka District and found a prevalence of impaired glucose (fasting blood glucose concentration 5.51-8.49 mmol/L) of 1.3% and a prevalence of diabetes (fasting blood glucose concentration ≥8.5 mmol/L) of 2.7% among 1,800 randomly selected adults aged ≥25 years.[66] The same study found a prevalence of obesity (body mass index ≥30 kg/m^2) of 14.2%,[67] and a prevalence of hypertension (average of three successive blood pressure readings >140/90 mmHg) of 34.8%[68] among 1,928 participants aged ≥25 years. No other published population-based studies have explored the prevalence of diabetes or its major risk factors in Lusaka or Zambia. Prevalence estimates remain largely based on extrapolated data from surrounding similar countries.[69] A greater focus on diabetes, hyperglycaemia and its associations in this setting is needed.

8 Summary

The concept that hyperglycaemia affects TB control is not new. Prior studies suggest that individuals with diabetes mellitus have a two-to-three times increased risk of developing active TB disease than individuals without diabetes. However, few studies contributing to this body of evidence have been based in Africa. The association probably results from pathophysiological changes that are common to all humans so there is no reason to suspect that the association would be different in African populations compared to the rest of the world, however, the context of the association in sub-Saharan Africa differs, primarily in two ways.

Firstly, the prevalence of HIV in Africa is more than five times higher than in any other world region.[62] It is inconclusive from current literature whether or not HIV modifies the association between diabetes and TB.

Secondly, the prevalence of undiagnosed diabetes is high (≥40%)[12] and those who are diagnosed have a high prevalence of poorly controlled diabetes (73% in a recent study in Cameroon).[10, 12] Whilst it seems clear from existing literature that individuals with TB and diabetes have an increased risk of death and TB relapse from TB compared to individuals with TB but no diabetes,[21] it is currently not clear whether or not individuals with higher levels of

hyperglycaemia and poor diabetes control have even worse TB treatment outcomes than individuals with less severe hyperglycaemia and good glycaemic control.

This applies to individuals with persistent hyperglycaemia due to diabetes mellitus and also to those with transient stress hyperglycaemia due to active TB disease. It is often difficult to distinguish between these two causes of hyperglycaemia at the time of active TB disease and so we have chosen to focus on hyperglycaemia in general rather than on diabetes mellitus alone.

This thesis therefore explores associations between hyperglycaemia, TB and HIV in Lusaka, Zambia and differentiates between diabetes mellitus and transient stress hyperglycaemia which resolves after treatment of acute TB disease.

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Chapter 2: Systematic review paper: A systematic review of the association between diabetes mellitus and active tuberculosis in Africa and the effect of HIV

Sarah Lou Bailey[a,b], Helen Ayles[a,b]

[a]*LSHTM TB Centre and Department of Clinical Research, London School of Hygiene and Tropical Medicine, Keppel Street, London, UK;*

[b]*Zambart, Ridgeway Campus, Lusaka, Zambia;*

Corresponding author: Dr Sarah Lou Bailey, email address: slbailey@doctors.org.uk

Word count: 2,600

Abstract

Background: An association between diabetes mellitus and active tuberculosis has been established, though few studies contributing to prior systematic reviews on the topic originate from Africa. Two recent longitudinal studies have suggested that the association may be different among African populations. A possible explanation for this is that other risk factors for tuberculosis could act as effect-modifiers on the association. The most notable difference in the context of the diabetes-tuberculosis association in Africa compared to the rest of the world is the higher prevalence of HIV. Therefore, we aimed to determine current evidence for firstly, the association between diabetes and active tuberculosis in Africa and secondly, how HIV modifies, or not, any association between diabetes and active tuberculosis.

Methods: We conducted a systematic review by searching the EMBASE, Global Health and Medline databases. Studies were eligible for inclusion if they explored the association between diabetes mellitus prevalence and active tuberculosis incidence or prevalence, used a comparison group, were conducted in an African population and adjusted the analysis for at least age. Study characteristics were compared and risk of bias was assessed. The range of effect estimates was determined for the primary association and for effect modification by HIV.

Results: Three eligible studies were identified: two investigated the primary association and two investigated HIV as a potential effect modifier. All studies were case-control studies, including a combined total of 1,958 tuberculosis cases and 2,111 non-tuberculosis controls. Diabetes diagnostic methods and analysis strategies varied between studies. Individual study adjusted odds ratios of active tuberculosis for the effect of diabetes mellitus (unstratified) ranged from 0.88 (95% CI 0.17-4.58) to 10.7 (95% CI 4.5-26.0). Individual study p-values for HIV interaction ranged from 0.01 to 0.83. Quantitative synthesis of individual study data was not performed due to heterogeneity between studies.

Conclusions: Few data currently exist on the association between diabetes and active tuberculosis in Africa, or on the effect of HIV on this association. Existing data is disparate. More regional research is needed to guide policy and practice on the care and control of tuberculosis and diabetes in Africa.

KEYWORDS: Diabetes mellitus, tuberculosis, HIV, Africa, systematic review

Background

An association between diabetes mellitus (DM) and active tuberculosis (TB) has been established: systematic reviews and meta-analyses of studies exploring the relationship suggest that the incidence of active TB is two to three times higher in those with DM compared to those without DM.[1-3] However, the data contributing to this body of evidence originate almost entirely from countries outside of Africa.[1-3] There is longitudinal evidence that suggests this association may differ in African populations. A Danish study evaluated the effect of ethnicity and DM on the risk of incident TB over a follow-up period of 15 years through linking nationwide DM and TB registers at case level.[4] They found a TB rate ratio of 1.9 in individuals with DM compared to individuals without DM, regardless of country of birth, with the exception of African-born individuals who had a rate ratio of 0.5. An ecological longitudinal study covering the years 2000 and 2012 studied the global relationship between the prevalence of DM and the incidence of TB to evaluate their coexistence worldwide.[5] Only countries with a high DM prevalence (>7.6%) showed a significant positive association between DM prevalence and TB incidence based on linear regression time trend analysis ($r=0.17$, $p=0.013$). A non-significant inverse relationship was found for the African region ($r=-0.27$).

Whilst there is no reason to suspect the underlying pathophysiology of the association should differ between ethnographic populations, the context of the association in Africa could be different from the rest of the world and consequently lead to a different overall association. Other risk factors for tuberculosis could act as effect-modifiers on the association. The most notable difference relating to tuberculosis risk factors between Africa and elsewhere is the high prevalence of HIV in Africa, which is more than five times higher than in any other world region.[6]

The dual effect of diabetes and HIV on the risk of developing TB disease or on its clinical evolution is unclear. It could be that the effect of hyperglycaemia on TB risk is relatively small

in HIV-positive individuals compared with its effect in HIV-negative individuals, as the greatly increased risk of TB among HIV-positive individuals could diminish any additional increased risk from hyperglycaemia. On the other hand, the effect of hyperglycaemia might be exacerbated in the presence of HIV infection if the contributions of each to increased TB risk are synergistic.

The prevalence and incidence of tuberculosis remain high in many parts of Africa.[7] The number of adults with diabetes in Africa is predicted to rise from 14.2 million in 2015 (uncertainty interval 9.5-29.4 million) to 34.2 million in 2040 (uncertainty interval 23.7-67.7 million).[8-11] To appropriately respond to this now and prepare for a future higher prevalence of diabetes, a deeper understanding of associations between diabetes and tuberculosis in the context of Africa is needed. Therefore, we aimed to undertake systematic reviews to determine current evidence for firstly, the association between the prevalence of diabetes and the incidence or prevalence of active tuberculosis in Africa and secondly, how HIV modifies, or not, any association between the prevalence of diabetes and the incidence or prevalence of tuberculosis.

Methods

The EMBASE, Global Health and PubMed databases were searched. Studies that investigated the relationship between the prevalence of diabetes and the incidence or prevalence of active tuberculosis, included a comparison group, were conducted in an African population and adjusted for age were eligible for inclusion. As age has the potential to be a major confounder of the association, studies that did not adjust for at least age in the analysis, or present data that enabled this analysis, were not eligible.

Reference lists of identified eligible papers were additionally hand-searched to identify further potentially relevant studies.

The Embase Classic+Embase database was searched from 1947 until June 2016, the Global Health database was searched from 1910 until June 2016 and the Ovid MEDLINE® database

was searched from 1946 until June 2016, including Epub Ahead of Print, In-Process, Other Non-Indexed Citations and Ovid MEDLINE® Daily. The search terms and strategies used were deliberately broad to increase the likelihood of all relevant studies being identified. Searches were restricted to human studies. No restriction on language was made. The following MESH and text search terms were used:

1. Diabetes mellitus.mp. [mp=title, abstract, heading word, original title, keyword]
2. Hyperglycaemia.mp. [mp=title, abstract, heading word, original title, keyword]
3. 1 OR 2
4. Tuberculosis.mp. [mp=title, abstract, heading word, original title, keyword]
5. Africa.mp. [mp=title, abstract, heading word, original title, keyword]
6. 3 AND 4 AND 5

Deduplication of papers identified by the searches was performed using the Ovid database platform. The titles and abstracts were screened for eligibility and the full texts of articles identified as potentially relevant were examined. All studies meeting the eligibility criteria above were included for exploration of the first aim, to determine the association between the prevalence of diabetes and the incidence or prevalence of active tuberculosis in Africa. Studies which met the eligibility criteria and presented the primary age-adjusted association stratified by HIV were also included in assessment of the second aim, to determine how HIV modifies, or not, any association between the prevalence of diabetes and the incidence or prevalence of tuberculosis.

It is possible that studies relevant to the second aim could take place in non-African populations, and so to ensure all relevant papers were identified a second search was performed using the same databases and the same search strategy, except no restriction on location was made and the following MESH and text search terms were used: Diabetes mellitus OR hyperglycaemia AND tuberculosis AND HIV. Studies were eligible only if they investigated the primary association between DM prevalence and TB incidence/prevalence and stratified

the analysis by HIV. Studies that investigated the prevalence of diabetes among TB patients stratified by HIV but did not include a non-TB population were not eligible for inclusion because it is not possible to assess for effect modification without data from a control population for the primary association.

Individual study data were extracted from reports using data collection tables, to identify individual study characteristics, risk of bias and results. When necessary study authors were contacted for clarification of study data. Study characteristics sought were period of data collection, study design, study setting, study size and HIV prevalence among study participants. The risk of bias for individual studies was assessed by ascertaining study definitions for the exposure variable, outcome variable and comparison group and determining the variables adjusted for in the analysis. Qualitative description was used to synthesise the risk of bias results. The principal summary measures sought were the odds ratios or risk ratios for the association between DM and TB, overall and stratified by HIV. The range of effect estimates was determined for the primary association and stratified by HIV. The range of p-values for interaction was also determined.

Results

The database searches for studies investigating the primary association in Africa identified 314 potential papers after deduplication (Figure 1). A further 3 potentially relevant papers were identified from reference lists. Therefore, 317 titles and abstracts were screened, of which 254 were excluded due to failure to meet the eligibility criteria. We examined 63 full texts, of which 3 studies were found to meet the eligibility criteria for inclusion. All 3 papers investigated the association between DM prevalence and TB incidence in an African population,[12-14] though only 2 reported the overall adjusted effect estimate.[13, 14] Of the 3 identified papers, 2 studies went on to also investigate HIV as an effect modifier of the association.[12, 14] No papers investigating the association between DM prevalence and TB prevalence were identified. The additional database searches for any further papers

investigating the primary association plus HIV as an effect modifier identified 800 potentially relevant papers after deduplication, but no further eligible papers were found after screening and full text assessment for eligibility.

The database searches identified 8 review papers that had relevance to associations between TB, diabetes and HIV,[15-22] though none reported current evidence for the association between DM and TB in Africa and none explored how HIV modifies or not this association. Rather, they focused on related factors including current understanding of the underlying mechanisms of diabetes-related and HIV-related increased susceptibility to TB,[15-17] the importance of and challenges faced with TB and diabetes co-management and control,[16, 18-20] current research gaps and prioritised areas for research relating to TB and diabetes,[21] and evidence of association between TB and diabetes from elsewhere in the world.[16, 17, 22] None of the review papers identified additional primary research papers that had not already been identified through the database searches.

Individual study characteristics are shown in Table 1. All 3 studies used a case control design, investigating a combined total of 1,958 tuberculosis cases and 2,111 non-tuberculosis controls. The prevalence of HIV ranged from 23% to 43% among cases and from 10% to 14% among controls. Definitions of DM and TB varied between each study. All studies tested for DM in TB cases around the time of TB treatment initiation or in newly-diagnosed TB patients. Boillat-Blanco *et al* additionally undertook repeat testing for diabetes 5 months after TB treatment initiation.[14] The analysis strategy varied between each study. All adjusted for age and sex. Faurholt-Jepsen *et al* presented the results for two separate analysis strategies, one including adjustment for the acute phase reactant alpha-1-acid glycoprotein and one without.

Table 2 presents the individual study results based on the diabetes tests performed in newly-diagnosed TB cases around the time of enrolment. Adjusted odds ratios of TB for the effect of DM range from 0.88 (95% CI 0.17-4.58) to 10.7 (95% CI 4.5-26.0). Figure 2 shows this graphically, along with odds ratios of TB for the effect of DM measured at follow-up. Boillat-

Blanco *et al* found the prevalence of DM in cases reverted to the background prevalence of DM in controls after treatment for tuberculosis. Adjusted odds ratios of TB for the effect of DM correspondingly reverted to the null.

The individual study effects of HIV on the association between DM and TB were different depending on the definition of diabetes and the factors adjusted for in the analysis (Table 2). Boillat-Blanco *et al* found no evidence for effect modification by HIV other than when diabetes was determined by HbA1c and measured at the time of enrolment, in which case individuals who were uninfected with HIV had a stronger association between DM and TB than individuals infected with HIV (p=0.048). Faurholt-Jepsen *et al* also found evidence for a stronger association among HIV uninfected individuals but only when adjusting for alpha-1-acid glycoprotein (p=0.01).

Quantitative synthesis of individual study data was not performed due to heterogeneity between studies.

Risk of bias

The major risk of bias in all papers included in this systematic review was a lack of study power. Only one of the papers (Faurholt-Jepsen *et al*) had investigation of interaction by HIV as a primary study aim but none were powered for investigating effect modification. There is no mention in any of the papers that sample size calculations were performed for effect modification. Rather, the sample sizes and primary study aims suggest that the sample size calculations were performed for investigation of the primary association between TB and diabetes. Other potential sources of bias were present for each study. Faurholt-Jepsen's study is difficult to interpret fully because they have omitted to report the p-value for effect modification by HIV for the main model adjusting for age, sex and socio-demographic factors, and instead have reported the p-value for a second model which additionally adjusts for an acute phase reactant. This is essentially a measure of acute physiologic stress. There is no clear

explanation for why they have adjusted for this or focused on this model in the results. It the acute phase reactant is a marker of stress hyperglycaemia it is possible that they have adjusted for a factor on the causal pathway of the association between TB and diabetes rather than a confounding factor. Selection bias is a potential source of bias for Haraldsdottir's study as although they recruited community controls they only included individuals who were at home at the time of the afternoon visit, and therefore they were more likely to recruit individuals with lower socioeconomic status and less likely to recruit individuals who were at work rather than at home. As socioeconomic status is known to be associated with both TB and diabetes this could have introduced bias. Boillat-Blanco's study used local clinical guidelines and smear results to diagnose TB rather than culture or molecular methods. Whilst this reflects the situation in practice, it may have led to misclassification of the primary outcome of TB.

Discussion

This systematic review identified only three eligible studies conducted in African populations. Only two of these studies presented data on the adjusted association between DM and TB. One found no evidence of association and the other found evidence of a positive association only when diabetes was measured in cases around the time of TB treatment initiation. There was no evidence of association when DM was measured in TB cases after receiving TB treatment, suggesting that the initial association seen was due to an increase in stress-induced hyperglycaemia among newly-diagnosed TB cases rather than due to true diabetes.

Only two studies stratified their analysis by HIV. The results depended on the analysis strategy and method of DM diagnosis used. There was little evidence of association among either HIV infected or uninfected individuals when DM was measured in cases after TB treatment.

The results seen in this systematic review are in keeping with the idea that the association between DM and active TB may be different among African populations, but existing data is currently too sparse to be conclusive. It remains possible that HIV could modify the association, but again, there is currently insufficient data to be certain.

A limitation at the individual study level was the different criteria used for diabetes diagnosis in each study, both for the methods and the glycaemic cut-offs used to determine diabetes. This made comparison between studies problematic. Conformity with World Health Organization diagnostic criteria and presenting separate rather than combined analyses for each method of diagnosis used would mitigate this limitation.

The results of this systematic review, and any future repeat review, have relevance to health care policy makers, academics and practitioners in Africa. The global collaborative framework for the care and control of tuberculosis and diabetes produced in 2011 by the World Health Organization and the International Union Against Tuberculosis and Lung Disease had few contributory studies from Africa.[23] In 2016, this systematic review suggests there remain few studies based in African populations that can guide local and regional policy and practice on the care and control of TB and diabetes. It remains possible that the association between diabetes and active TB could be different in African populations compared to elsewhere in the world, and it remains possible that HIV could modify the association. Given the continued high incidence of tuberculosis in much of Africa and the predicted rising prevalence of diabetes throughout Africa,[7, 8] further evidence on the nature and magnitude of their association in this setting would be valuable.

Conclusions

Few data currently exist on the association between diabetes and active tuberculosis in Africa, or on the effect of HIV on this association. Exploration of diabetes diagnosed both at the time of TB diagnosis and after TB treatment is valuable to distinguish between diabetes and stress-induced hyperglycaemia secondary to infection with TB. More regional research is needed to guide policy and practice on the care and control of tuberculosis and diabetes in Africa.

FUNDING

SLB is supported by a Wellcome Trust Clinical PhD Fellowship (100141/Z/12/Z). The contents of this manuscript are solely the responsibility of the authors and do not necessarily represent the official views of the Wellcome Trust.

COMPETING INTERESTS

We declare that we have no competing interest.

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Figure 1: Flow diagram of study selection for papers investigating the association between diabetes mellitus prevalence and tuberculosis incidence or prevalence in an African population

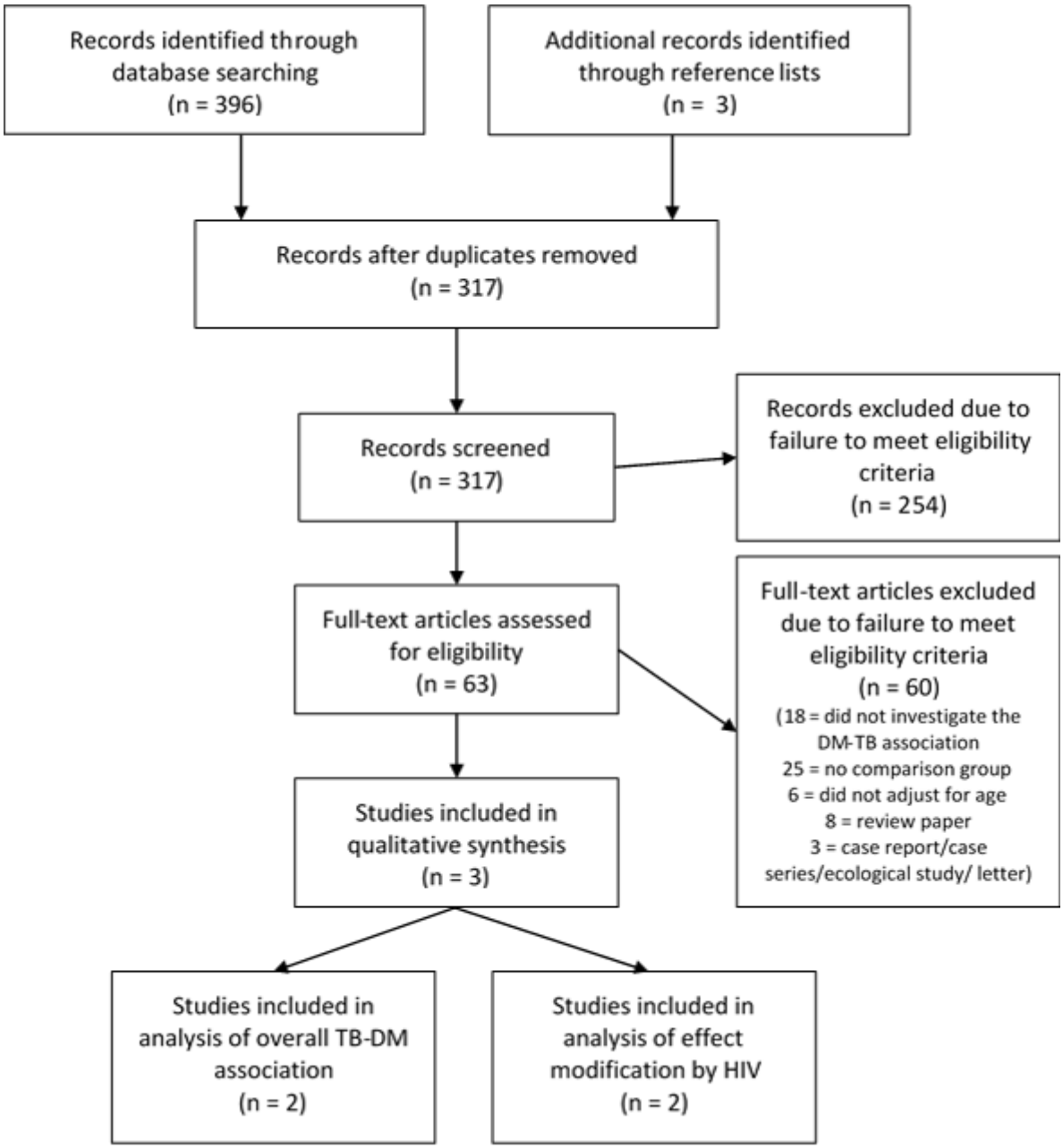
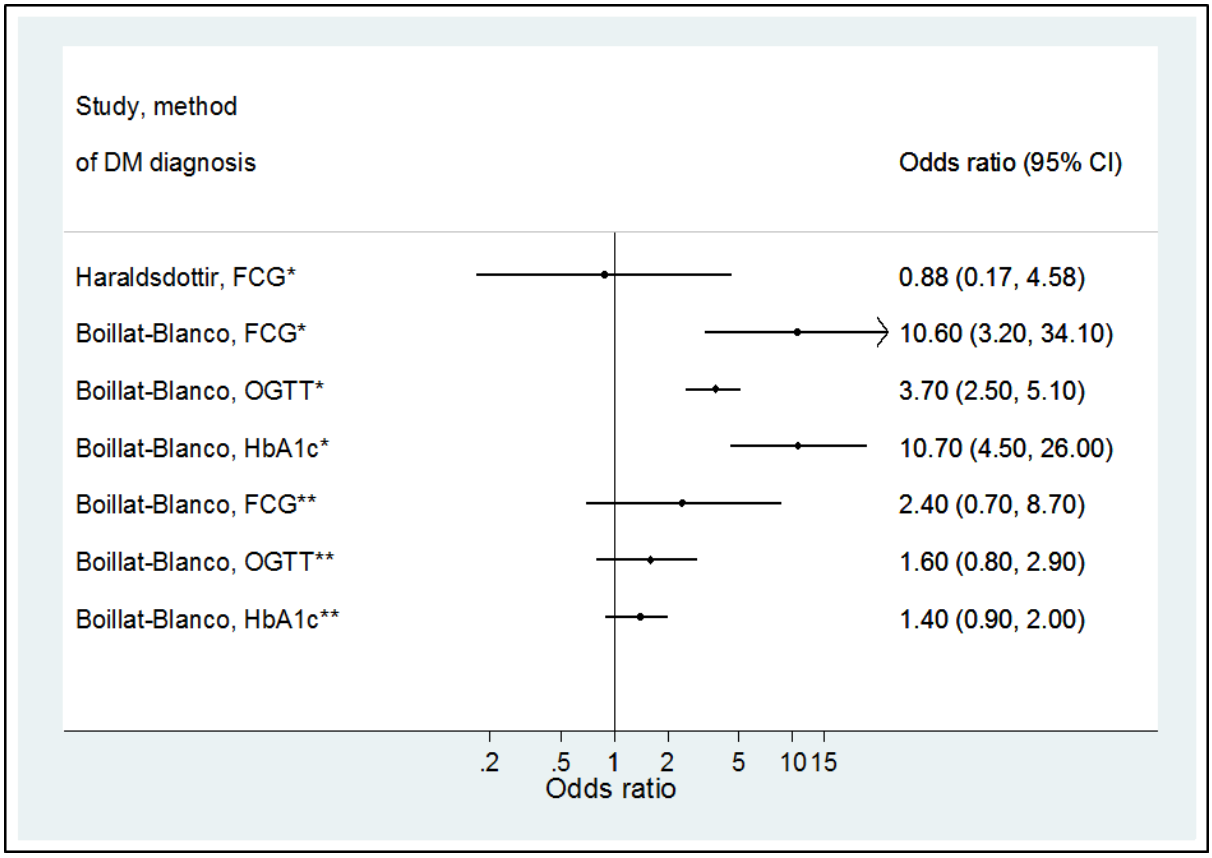


Figure 2: Forest plot for the adjusted odds ratios of active tuberculosis comparing those with diabetes mellitus to those without



DM = diabetes mellitus; FCG = fasting capillary blood glucose; OGTT = oral glucose tolerance test; HbA1c = glycated haemoglobin; *DM in cases measured around the time of TB treatment initiation; **DM in cases measured a median of 5 months after TB treatment initiation

Table 1: Individual study characteristics

Study	Date of data collection	Region, Country	Study design	Study size	Exposure variable	Outcome variable	Primary comparison	HIV prevalence	Variables adjusted for in analysis
Faurholt-Jepsen D *et al*, 2011[12]	April 2006 – January 2009	Mwanza, Tanzania	Case-control	803 cases and 350 controls	DM, determined by FCG>6mmol/L or 2hCG>11mmol/L , measured in cases a few days after initiation of TB treatment	Active pulmonary TB, determined by culture or sputum smear	Newly diagnosed adult TB cases presenting in one of four health facilities and non-TB age- and sex-matched neighbourhood controls	43.2% among cases, 10.0% among controls; determined using two rapid tests and, if equivocal, ELISA	Age, sex, religion, marital status, occupation in model 1; the above plus the acute phase reactant alpha-1-acid glycoprotein in model 2
Haraldsdottir T.L. *et al*, 2015[13]	July 2010 - July 2011	Bissau, Guinea-Bissau	Case-control	110 cases and 572 controls	DM, determined by FCG≥7.0mmol/L. measured in cases when they were newly diagnosed with TB	Active pulmonary TB, determined by sputum smear or chest radiograph plus signs and symptoms suggestive of TB after ineffective antibiotic treatment	Newly diagnosed adult TB cases registered by notification system and non-TB unmatched adult community controls randomly selected from a demographic surveillance database	22.6% among cases, determined using 2 rapid tests; HIV status not determined for control participants	Age, sex, body mass index
Boillat-Blanco N *et al*, 2016[14]	July 2012 – June 2014	Kinondoni District, Tanzania	Case-control	539 cases and 496 controls	DM, determined by FCG≥7.0mmol/L, or 2hCG≥11.1mmol/L or HbA1c≥6.5%, measured in cases at enrolment and confirmed by repeat testing 2-5 days later; then repeated after a median of 5 months of antituberculosis treatment	Active TB diagnosed by the National TB and Leprosy Control Programme	Consecutive adults with new active tuberculosis presenting in participating hospitals and sex- and age-matched non-TB controls selected from adults accompanying patients to the outpatient departments	32% among cases, 14% among controls; determined using two rapid tests	Age, sex, body mass index, socioeconomic status, HIV status (nonstratified models only)

TB = tuberculosis; DM = diabetes mellitus; FCG = fasting capillary blood glucose; 2hCG = 2 hour capillary blood glucose after a standard 75 g oral glucose tolerance test.

Table 2: Individual study estimates of the unadjusted and adjusted odds ratios of active tuberculosis comparing individuals with diabetes mellitus to those without, overall and stratified by HIV, with diabetes mellitus measured around the time of TB diagnosis or initiation of TB treatment

		Overall				HIV uninfected				HIV infected				p-value for HIV interaction (adjusted analysis)
Study	**Method of DM diagnosis**	**Number (%) of cases with DM**	**Number (%) of controls with DM**	**Unadjusted OR of active TB (95% CI)**	**Adjusted OR of active TB (95% CI)**	**Number (%) of cases with DM**	**Number (%) of controls with DM**	**Unadjusted OR of active TB (95% CI)**	**Adjusted OR of active TB (95% CI)**	**Number (%) of cases with DM**	**Number (%) of controls with DM**	**Unadjusted OR of active TB (95% CI)**	**Adjusted OR of active TB (95% CI)**	
Faurholt-Jepsen D *et al*, 2011[12]	FCG and OGTT combined	134 (16.7)	33 (9.4)	2.2 (1.5-3.4)	NR	NR	NR	2.15 (1.35-3.42)	Model 1: 2.14 (1.32-3.46) Model 2: 4.23 (1.54-11.57)	NR	NR	1.94 (0.65-5.75)	Model 1: 2.05 (0.68-6.19) Model 2: 0.14 (0.01-1.81)	Model 1: NR Model 2: 0.01
Haraldsdottir T.L. *et al*, 2015[13]	FCG	3 (2.8)	11 (2.1)	NR	0.88 (0.17-4.58)	NR	NR	NR	NR	NR	NR	NR	NR	NR
Boillat-Blanco N *et al*, 2016*[14]	FCG (enrolment)	24 (4.5)	6 (1.2)	4.2 (1.7-10.3)	10.6 (3.2-34.1)	15 (4.2)	4 (1.0)	4.9 (1.6-15.0)	8.8 (2.1-36.6)	8 (4.8)	2 (3.0)	1.9 (0.4-9.1)	17.1 (1.6-179.4)	0.83
	OGTT (enrolment)	36 (6.8)	15 (3.1)	2.9 (1.6-5.4)	3.7 (2.5-5.1)	23 (6.5)	10 (2.4)	3.5 (1.6-7.4)	3.8 (1.4-10.5)	12 (7.2)	5 (7.6)	1.4 (0.5-4.0)	3.8 (1.0-15.3)	0.73
	HbA1c (enrolment)	49 (9.3)	11 (2.2)	6.5 (3.3-12.9)	10.7 (4.5-26.0)	33 (9.3)	5 (1.2)	11.8 (4.5-31.0)	19.3 (6.1-61.0)	16 (9.6)	6 (9.1)	1.9 (0.7-5.3)	4.7 (1.1-20.8)	0.048

TB = tuberculosis; OR = odds ratio; CI = confidence interval; OGTT = oral glucose tolerance test; NR = not reported; Model 1: adjusted for age, sex, religion, marital status and occupation; Model 2: adjusted for age, sex, religion, marital status, occupation and serum alpha-1-acid glycoprotein; *Results presented are for initial DM prevalence measured at the time of recruitment

Chapter 3: Secondary data analysis paper 1: Diabetes mellitus in Zambia and the Western Cape province of South Africa: prevalence, risk factors, diagnosis and management

Contents available at ScienceDirect

Diabetes Research and Clinical Practice

journal homepage: www.elsevier.com/locate/diabres

International Diabetes Federation

Diabetes mellitus in Zambia and the Western Cape province of South Africa: Prevalence, risk factors, diagnosis and management

Sarah Lou Bailey [a,c,*], Helen Ayles [a,b], Nulda Beyers [c], Peter Godfrey-Faussett [a], Monde Muyoyeta [b], Elizabeth du Toit [c], John S. Yudkin [d], Sian Floyd [e]

[a] LSHTM TB Centre and Department of Clinical Research, London School of Hygiene and Tropical Medicine, Keppel Street, London, UK
[b] Zambart, Ridgeway Campus, Lusaka, Zambia
[c] Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Stellenbosch University, South Africa
[d] University College London, Gower Street, London, UK
[e] LSHTM TB Centre and Department of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine, Keppel Street, London, UK

ARTICLE INFO

Article history:
Received 30 March 2015
Received in revised form
27 April 2016
Accepted 2 May 2016
Available online 7 May 2016

Keywords:
Southern Africa
Cross-sectional study
Epidemiology

ABSTRACT

Aims: To determine the prevalence of and risk factors for diabetes mellitus and examine its diagnosis and management in the study communities.

Methods: This is a population-based cross-sectional study among adults in 24 communities from Zambia and the Western Cape (WC) province of South Africa. Diabetes is defined as a random blood glucose concentration (RBG) $\geqslant$ 11.1 mmol/L, or RBG < 11.1 mmol/L but with a self-reported prior diabetes diagnosis. For individuals with a prior diagnosis of diabetes, RBG < 7.8 mmol/L was considered to be an acceptable level of glycaemia.

Results: Among 45,767 Zambian and 12,496 WC participants the age-standardised prevalence of diabetes was 3.5% and 7.2% respectively. The highest risk groups identified were those of older age and those with obesity. Of those identified to have diabetes, 34.5% in Zambia and 12.7% in WC were previously unaware of their diagnosis. Among Zambian participants with diabetes, this proportion was lower among individuals with better education or with higher household socio-economic position. Of all those with previously diagnosed diabetes, 66.0% in Zambia and 59.4% in WC were not on any diabetes treatment, and 34.4% in Zambia and 32.7% in WC had a RBG concentration beyond the recommended level, $\geqslant$7.8 mmol/L.

Conclusions: The diabetes risk factor profile for our study communities is similar to that seen in high-income populations. A high proportion of individuals with diabetes are not on diabetes treatment and of those on treatment a high proportion have high glycaemic concentrations. Such data may assist in healthcare planning to ensure timely diagnosis and management of diabetes.

* *Corresponding author at:* LSHTM TB Centre and Department of Clinical Research, London School of Hygiene and Tropical Medicine, Keppel Street, London, UK.
E-mail address: slbailey@doctors.org.uk (S.L. Bailey).

http://dx.doi.org/10.1016/j.diabres.2016.05.001
0168-8227/

1. Introduction

The number of adults with diabetes mellitus in sub-Saharan Africa (SSA) is predicted to rise from 19.8 million in 2013 to 41.5 million in 2035 [1–5]. Extensive data exist to guide diabetes public health policies and health systems planning in high income countries [6–8]. In contrast, systematic review suggests there are few data from SSA even for diabetes prevalence and risk factors [1,9–12].

The management of diabetes can be challenging for health systems, as it requires lifelong follow-up and a multidisciplinary approach [3,13,14]. Therefore, the aims of this study are

- to estimate the prevalence of, and identify risk factors for, diabetes mellitus in the study communities in Zambia and in the Western Cape province of South Africa; and
- to estimate the prevalence of undiagnosed diabetes, to determine the proportion of those with a prior diagnosis of diabetes who are on treatment for diabetes, and to determine the levels of glycaemia in those with a prior diagnosis of diabetes.

2. Methods

This population-based cross-sectional study was undertaken between January and December 2010 in 24 communities: 16 from 5 provinces in Zambia and 8 from the Western Cape province of South Africa. The study was nested into a prevalence survey that was conducted to measure the primary endpoint, prevalent tuberculosis, of a large 2 × 2 factorial cluster randomised trial (the ZAMSTAR study) [15–17]. The estimated total population in the study areas was 962,655, with an average population per community of 40,110. Within each community, a two-stage cluster sampling design was used to recruit participants. Exclusion criteria were age <18 years, inability to give informed consent due to disability/incapacitation, refusal to submit a respiratory sample – for purposes of the parent study – and any persons living in institutional settings.

Each participant was required to give written informed consent. Individuals and household heads were interviewed in their homes using structured questionnaires. Finger prick capillary blood was taken for HIV testing and random blood glucose (RBG) measurement, with pre- and post-test counselling for HIV tests. Determine ™HIV-1/2 was used for HIV testing, plus UniGold™HIV-1/2 to confirm all positives. RBG concentration was measured using an Optium Xceed point-of-care glucometer. All research staff were trained on the use of this particular glucometer and were required to undergo proficiency testing. Standardised control solution was used for performance checks on test strips and meters. Height and weight were measured using standard operating procedures. All individuals identified to have abnormal blood glucose or to be HIV positive were referred to existing local health facilities for appropriate management.

Data were electronically entered directly onto personal digital assistants by field staff at the time of data collection, using pre-programmed questionnaires and result sheets. All information was downloaded daily into a SQL (structured query language) database and later exported into Stata.

Ethics approval was granted from the London School of Hygiene and Tropical Medicine Ethics Committee, the University of Stellenbosch Ethics Committee and the University of Zambia Ethics Committee.

2.1. *Definitions*

- Diabetes mellitus was defined as a random blood glucose concentration (RBG) $\geqslant$ 11.1 mmol/L, or RBG < 11.1 mmol/L but with a self-reported prior diabetes diagnosis.
- Body mass index (BMI) was defined as weight in kilograms divided by height squared in metres (weight (kg)/height^2(m)).
- HIV status was defined by a combination of blood sampling and self-report for those with missing biological data.
- Exposures (risk factors) for diabetes were defined as proximal or distal factors. Distal factors include age, sex, household socio-economic position, education, smoking history, HIV status and community. The proximal factor, BMI, may be determined partly by the distal factors and so estimation of its direct effect on diabetes requires controlling for confounding by the more distal factors.
- For assessing the management of those with diabetes, RBG < 7.8 mmol/L was considered to be the recommended level of glycaemia, as specified by the International Diabetes Federation guideline for target postprandial glucose concentration [18].

2.2. *Statistical analyses*

Direct age standardisation for the prevalence of diabetes was calculated by applying the study age-specific diabetes rates (separately for Zambia and the Western Cape) to the 2013 International Network for the Demographic Evaluation of Populations and Their Health (INDEPTH) sub-Saharan African standard population distribution. Univariable and multivariable logistic regression analyses were used to identify risk factors for diabetes, accounting for the cluster sampling design. Principal components analysis was used to create a measure of household socio-economic position separately for each country, using the following variables: main type of dwelling; main type of flooring; main type of household toilet; main source of household drinking water, and presence of household assets including radio, television, refrigerator, bicycle, motorcycle, car, domestic worker and mobile phone.

The variables considered *a priori* as potential risk factors were those known to be risk factors in other populations and settings [19,20]: age, sex, household socio-economic position, education, smoking, ethnicity and adiposity (measured

by BMI). HIV status was also considered given its high prevalence in both localities, and the known effects of some antiretroviral medications on insulin resistance [21]. Rural/urban location was not considered as a binary variable as a finer categorisation of location was required to account for between-community variation in analysis of household and individual-level risk factors.

Household socio-economic position and individual education are expected to be inter-related. For the analyses presented here, education was considered to be the more distal variable and we explored whether the association between education and diabetes prevalence was partly mediated by household wealth.

Body mass index was considered to be a factor that is proximal to household socio-economic position and education on the causal pathway to diabetes, and also to age, sex and HIV status. To estimate the association of these more distal factors with the outcome (to identify high risk groups) it was important first to exclude the measure of obesity from multivariable analyses, and secondly to include it so as to identify how much of any observed associations were mediated by BMI. Therefore, separate final models were created, with and without inclusion of BMI. All data analyses were performed using Stata12.

3. Results

In Zambia, 57,809 (70.8% of eligible) participants from 31,300 (88.6% of eligible) households were enrolled. In Western Cape, 32,792 (77.7% of eligible) participants from 17,095 (85.3% of eligible) households were enrolled (Fig. 1). Complete RBG results were obtained for 45,767 (79.2% of enrolled) participants in Zambia and 12,496 (38.1% of enrolled) participants in Western Cape. Among all participants with an RBG measurement (those forming the final dataset for analyses) 524 (1.1%) in Zambia and 9 (0.1%) in Western Cape had missing data for age, 3883 (8.5%) and 788 (6.3%) respectively had missing data for HIV status, and 3536 (7.7%) and 365 (2.9%) respectively had missing data for BMI.

The distribution of the baseline characteristics for all participants who contributed to the analyses is shown in Tables 1 and 2. The study participants ranged between ages 18–102 (mean 33.3 years) years in Zambia and 18–103 years (mean 37.0 years) in Western Cape. Two-thirds of the participants were female in both countries. The distribution of the baseline characteristics for participants with missing glycaemia data are given in a supplementary table. The only large difference in distribution of individuals with and without glycaemia data occurred for the community variable.

3.1. *Prevalence*

Fig. 2 shows the numbers of people with diabetes according to self-report and positive screen. In Zambia 65.5% (870/1329) of people with diabetes were previously diagnosed whereas in Western Cape the figure was 87.3% (1029/1179). The prevalence of diabetes mellitus among study participants in Zambia was 2.9% and in Western Cape 9.4%. Diabetes prevalence stratified by baseline characteristics is shown in Tables 1 and 2. The prevalence was highest in older participants and in those with a higher body mass index.

The INDEPTH sub-Saharan African standard population age-standardised prevalence of diabetes mellitus was 3.5% for the Zambian communities and 7.2% for the Western Cape communities.

3.2. *Risk factors*

After multivariable analyses (Tables 1 and 2), the distal risk factors for diabetes mellitus identified in these study populations were age, sex, household socio-economic position, HIV status, and community for Zambia, and age, sex, smoking, HIV status and community for the Western Cape communities, with age being the strongest predictor in both settings. In Zambia the association between education and diabetes was strongly confounded by age; after adjusting for age the odds of diabetes increased with education level, and the strong confounding was due to the better educated being younger, on average. The odds of diabetes also increased with higher household socio-economic position in Zambia, and this explained some of the association between education and diabetes (ORs for education grade 3–6, 7–10, 11–12, and College/University were 1.22, 1.39, 1.62, and 1.87, $p < 0.001$, with adjustment for age, sex, community, smoking and HIV but not household socio-economic position). Ethnicity was not identified as a risk factor and was not included in the final multivariable model as it varied little within communities in the Western Cape so that community and ethnicity effects could not be distinguished, and varied little across all the Zambian communities.

There was strong evidence that the proximal risk factor, BMI, was associated with diabetes in both Zambia and the Western Cape communities. Comparison of the models with and without controlling for BMI shows that after adjustment for BMI the higher odds of diabetes among women compared with men is no longer seen for Zambian participants (Table 1) and is slightly reduced in Western Cape participants (Table 2). The association seen between smoking history and diabetes in Western Cape participants is also reduced. The associations with age in both countries, household socio-economic position in Zambian communities, and HIV status in Western Cape communities remain after accounting for the measure of adiposity, but are all reduced in magnitude.

When HIV status is further sub-divided by current use or not of antiretroviral therapy (ART), the odds of diabetes in Western Cape communities is lowest in the group who are HIV positive but not on ART: ORs for HIV negative, HIV positive not on ART and HIV positive on ART were 1, 0.69, and 1.03, $p = 0.006$, with adjustment for all proximal and distal factors. No association remains for the Zambian communities.

Using self-report rather than the RBG-based definition used for the rest of this study removes potential biases resulting from the use of RBG as the measurement tool and simultaneously increases the number of participants without missing data in the fully adjusted models to 43,060 in Zambia and 11,508 in the Western Cape. Of note, fitting the same multivariable models but with the outcome of diabetes defined

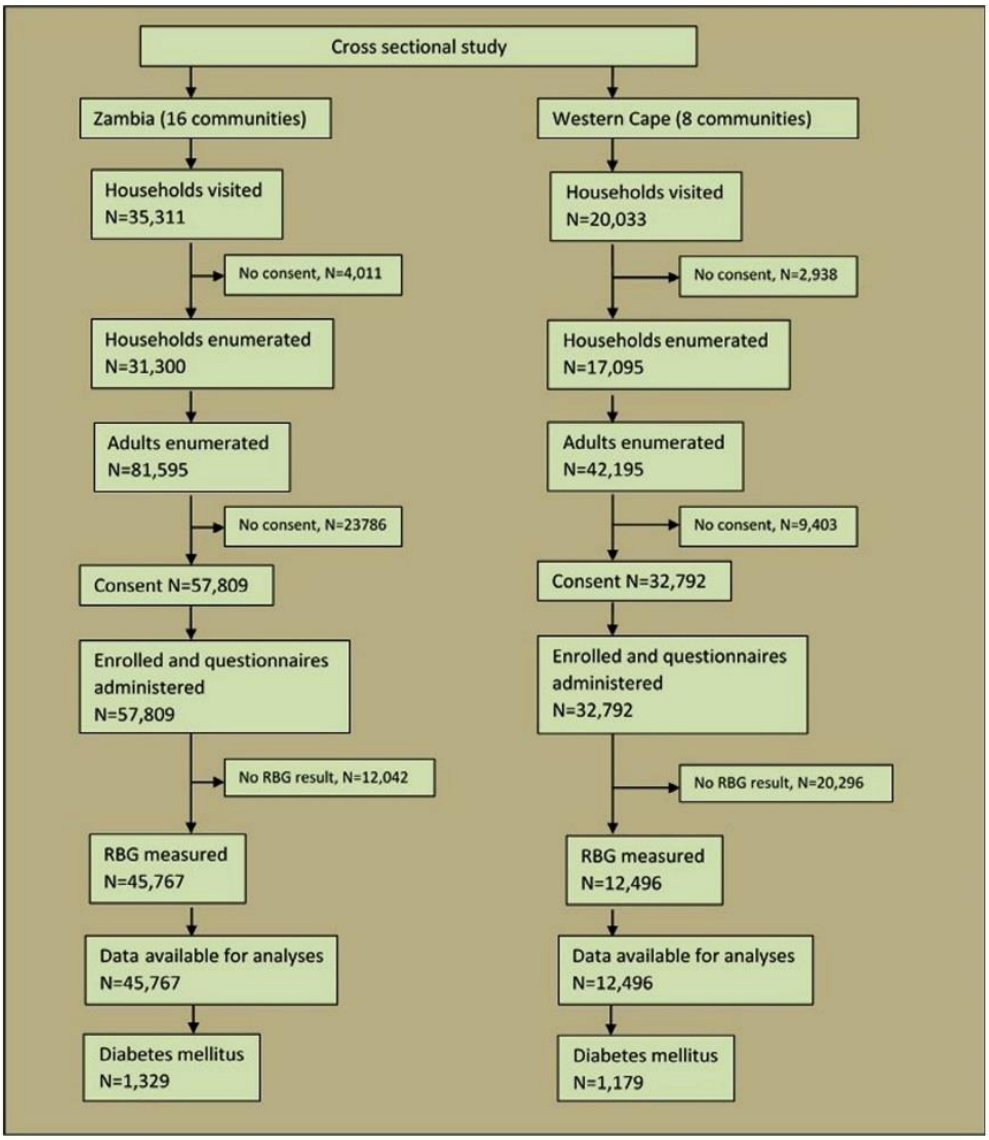


Fig. 1 – Number and flow of study participants and cases in Zambia and the Western Cape of South Africa.

entirely by self-report showed that odds ratios and evidence for associations were similar to the findings summarised in Tables 1 and 2. Likewise, redefining diabetes to include only those with RBG $\geqslant$ 11.1 mmol/L plus those on treatment for diabetes regardless of their RBG concentration confirms the main risk factor profile identified. This definition removes the group who have self-reported diabetes but are not on treatment for diabetes and have a RBG < 11.1 mmol/L. Age and BMI are again identified as the main risk factors for diabetes, and sex is no longer identified as a risk factor in Western Cape.

3.3. *Diagnosis and management*

Fig. 2 shows for Zambia and Western Cape the numbers of participants with diabetes by self-report and RBG diagnosis, along with numbers on treatment for diabetes.

The prevalence of undiagnosed diabetes among the total study population, including among those without diabetes, was 1.0% in the Zambian sites and 1.2% in the Western Cape sites. Stratification by participant characteristics for the prevalence of undiagnosed diabetes among all those with diabetes is shown in Table 3. Strong evidence for unadjusted association with undiagnosed diabetes is seen for household socio-economic position, education and community in the Zambia sites, and age and community in the Western Cape sites.

Of all those who had a prior diabetes diagnosis, 34.0% in Zambia and 40.6% in Western Cape were on diabetes treatment consisting of either dietary management, hypoglycaemic tablets or insulin therapy (Table 4). Among those with a prior diagnosis who were on diabetes treatment, the mean random blood glucose concentration for Zambian participants was 12.8 mmol/L (standard deviation, SD, 6.6 mmol/L). The corresponding mean for Western Cape participants was 11.0 mmol/L (SD 5.8 mmol/L). Among those with a prior diagnosis who were not on any diabetes treatment, the mean RBG concentration for Zambian participants was

Table 1 – Prevalence of diabetes mellitus in the Zambian sites, with corresponding unadjusted and adjusted odds ratios estimated by logistic regression, showing adjustment for distal risk factors (without measure of adiposity) and more proximal factor (body mass index).

Characteristic		Total number (%)	Number (%) with DM	Unadjusted OR (95% CI)	P-value[#]	Adjusted OR excluding BMI (95% CI)[*]	P-value[#]	Adjusted OR including BMI (95% CI)[**]	P-value[#]
Overall		45,767 (100)	1329 (2.9)						
Age (years)	18–24	15,983 (35.3)	202 (1.3)	1	<0.001 (TFT $p < 0.001$)	1	<0.001 (TFT $p < 0.001$)	1	<0.001 (TFT $p < 0.001$)
	25–29	7590 (16.8)	107 (1.4)	1.09 (0.86–1.39)		1.12 (0.88–1.43)		1.04 (0.81–1.35)	
	30–34	5583 (12.3)	122 (2.2)	1.70 (1.35–2.13)		1.73 (1.36–2.21)		1.53 (1.19–1.98)	
	35–39	3971 (8.8)	106 (2.7)	2.23 (1.76–2.84)		2.38 (1.84–3.07)		2.08 (1.59–2.73)	
	40–49	5195 (11.5)	232 (4.5)	3.80 (3.13–4.61)		3.89 (3.14–4.81)		3.03 (2.40–3.81)	
	50–59	3540 (7.8)	277 (7.8)	7.19 (5.95–8.69)		7.50 (6.08–9.26)		5.86 (4.67–7.36)	
	60+	3381 (7.5)	256 (7.6)	7.23 (5.96–8.77)		7.93 (6.27–10.03)		6.79 (5.29–8.72)	
Sex	Male	15,153 (33.1)	403 (2.7)	1	0.022	1	0.010	1	0.893
	Female	30,614 (66.9)	926 (3.0)	1.15 (1.02–1.30)		1.22 (1.05–1.43)		0.99 (0.84–1.17)	
Household socio-economic position	Very low	10,571 (23.1)	229 (2.2)	1	<0.001 (TFT $p < 0.001$)	1	<0.001 (TFT $p < 0.001$)	1	<0.001 (TFT $p < 0.001$)
	Low	12,058 (26.4)	331 (2.8)	1.22 (1.01–1.46)		1.31 (1.07–1.60)		1.23 (1.00–1.53)	
	Medium	11,570 (25.3)	366 (3.2)	1.46 (1.20–1.77)		1.60 (1.29–1.98)		1.45 (1.16–1.83)	
	High	11,568 (25.3)	403 (3.5)	1.83 (1.46–2.30)		2.02 (1.57–2.62)		1.79 (1.37–2.34)	
Highest level of education	None/grade 1-2	3507 (7.7)	142 (4.1)	1	<0.001 (TFT $p < 0.001$)	1	0.074 (TFT $p = 0.004$)	1	0.089 (TFT $p = 0.008$)
	Grade 3-6	5753 (12.6)	191 (3.3)	0.81 (0.65–1.02)		1.21 (0.92–1.58)		1.26 (0.94–1.68)	
	Grade 7–10	22,486 (49.1)	642 (2.9)	0.61 (0.50–0.73)		1.30 (1.01–1.66)		1.35 (1.04–1.77)	
	Grade 11–12	9947 (21.7)	201 (2.0)	0.40 (0.32–0.51)		1.42 (1.06–1.92)		1.49 (1.09–2.05)	
	College/University	4074 (8.9)	153 (3.8)	0.72 (0.57–0.93)		1.56 (1.14–2.14)		1.56 (1.12–2.18)	
Smoking history	Never	39,743 (86.8)	1170 (2.9)	1	0.583 (TFT $p = 0.334$)	1	0.169 (TFT $p = 0.071$)	1	0.397 (TFT $p = 0.194$)
	Ex-smoker	2319 (5.1)	70 (3.0)	0.99 (0.77–1.28)		0.96 (0.72–1.27)		0.97 (0.73–1.29)	
	Current smoker	3705 (8.1)	89 (2.4)	0.89 (0.71–1.11)		0.78 (0.59–1.02)		0.83 (0.63–1.09)	
HIV status[**]	Negative	36,643 (82.7)	947 (2.7)	1	0.169	1	0.041	1	0.646
	Positive	7241 (17.3)	202 (2.8)	0.90 (0.77–1.05)		0.84 (0.71–0.99)		0.96 (0.81–1.14)	
Body Mass Index[***] (weight(kg)/height^2(m))	Recommended weight (18.5–24.9)	26,865 (63.6)	537 (2.0)	1	<0.001 (TFT $p < 0.001$)[##]			1	<0.001 (TFT $p < 0.001$)[##]
	Underweight (<18.5)	4203 (10.0)	98 (2.3)	1.12 (0.90–1.40)				1.09 (0.86–1.38)	
	Overweight (25–29.9)	7429 (17.6)	327 (4.4)	2.26 (1.96–2.61)				1.52 (1.29–1.79)	
	Obese (≥30)	3734 (8.8)	268 (7.2)	3.82 (3.27–4.46)				2.29 (1.89–2.77)	
Community	ZAM1	3675 (8.0)	282 (7.7)	1	<0.001	1	<0.001	1	<0.001
	ZAM2	2944 (6.4)	106 (3.6)	0.51 (0.34–0.77)		0.45 (0.29–0.7)		0.45 (0.29–0.70)	
	ZAM3 (rural)	2516 (5.5)	59 (2.3)	0.37 (0.24–0.56)		0.32 (0.20–0.51)		0.31 (0.19–0.50)	
	ZAM4 (rural)	1302 (2.8)	26 (2.0)	0.28 (0.16–0.51)		0.26 (0.13–0.51)		0.24 (0.12–0.48)	
	ZAM5	2844 (6.2)	79 (2.8)	0.41 (0.27–0.62)		0.41 (0.26–0.66)		0.38 (0.24–0.61)	
	ZAM6	2961 (6.5)	63 (2.1)	0.34 (0.22–0.53)		0.31 (0.19–0.50)		0.29 (0.18–0.48)	
	ZAM7	3540 (7.7)	94 (2.7)	0.43 (0.28–0.64)		0.46 (0.29–0.72)		0.45 (0.28–0.70)	
	ZAM8	3646 (8.0)	78 (2.1)	0.33 (0.22–0.52)		0.38 (0.24–0.60)		0.36 (0.22–0.59)	
	ZAM9	2145 (4.7)	56 (2.6)	0.39 (0.25–0.61)		0.26 (0.16–0.43)		0.27 (0.16–0.46)	
	ZAM10	2392 (5.2)	59 (2.5)	0.37 (0.24–0.57)		0.48 (0.29–0.78)		0.48 (0.27–0.85)	
	ZAM11	3092 (6.8)	94 (3.0)	0.48 (0.31–0.72)		0.31 (0.20–0.50)		0.31 (0.19–0.49)	
	ZAM12	3401 (7.4)	82 (2.4)	0.39 (0.25–0.58)		0.26 (0.16–0.40)		0.25 (0.16–0.40)	
	ZAM13	3032 (6.6)	73 (2.4)	0.35 (0.23–0.54)		0.28 (0.18–0.44)		0.29 (0.18–0.45)	
	ZAM14	2760 (6.0)	58 (2.1)	0.32 (0.20–0.49)		0.37 (0.23–0.61)		0.35 (0.21–0.57)	
	ZAM15 (rural)	2865 (6.3)	64 (2.2)	0.36 (0.22–0.59)		0.33 (0.19–0.56)		0.34 (0.20–0.59)	
	ZAM16 (rural)	2652 (5.8)	56 (2.1)	0.33 (0.21–0.53)		0.35 (0.21–0.58)		0.38 (0.22–0.63)	

DM = diabetes mellitus; CI = confidence interval; OR = odds ratio; TFT = likelihood ratio test for trend with exposure as a linear variable; All analyses accounted for the two-stage clustered sampling design through use of a logistic regression model with random effects for enumeration area and inclusion of community as a fixed-effect.

[*] 41,475 participants included in analysis, adjusted for age, sex, household socio-economic position, education, smoking, HIV status and community.

[**] 38,250 participants included in analysis, adjusted for all variables shown; Missing data: 524 for age, 3883 for HIV status, 3536 for BMI.

[#] Likelihood ratio tests.

[##] Tests for linear trend and departure from linearity calculated with underweight as the baseline group.

[*] Defined as a random blood glucose concentration of ⩾11.1 mmol/L or <11.1 mmol/L with self-reported previous DM diagnosis.

[**] Based on serology plus self-report for those with no available serology.

[***] Grouped according to the International BMI Classification.

Table 2 – Prevalence of diabetes mellitus in the Western Cape sites, with corresponding unadjusted and adjusted odds ratios estimated by logistic regression, showing adjustment for distal risk factors (without measure of adiposity) and more proximal factor (body mass index).

Characteristic		Total number (%)	Number (%) with DM	Unadjusted OR (95% CI)	P-value[#]	Adjusted OR excluding BMI (95% CI)[+]	P-value[#]	Adjusted OR including BMI (95% CI)[++]	P-value[#]
Overall		12,496 (100)	1179 (9.4)						
Age (years)	18–24	3207 (25.7)	115 (3.6)	1	<0.001 (TFT $p < 0.001$)	1	<0.001 (TFT $p < 0.001$)	1	<0.001 (TFT $p < 0.001$)
	25–29	1810 (14.5)	78 (4.3)	1.21 (0.90–1.63)		1.30 (0.96–1.76)		1.16 (0.86–1.58)	
	30–34	1456 (11.7)	75 (5.2)	1.48 (1.10–2.00)		1.59 (1.17–2.17)		1.41 (1.03–1.93)	
	35–39	1290 (10.3)	103 (8.0)	2.34 (1.78–3.09)		2.40 (1.80–3.19)		2.03 (1.51–2.72)	
	40–49	2090 (16.7)	240 (11.5)	3.48 (2.76–4.39)		3.40 (2.65–4.37)		2.88 (2.22–3.72)	
	50–59	1454 (11.6)	263 (18.1)	5.94 (4.71–7.50)		5.37 (4.12–6.99)		4.36 (3.32–5.72)	
	60+	1180 (9.5)	305 (25.9)	9.95 (7.88–12.56)		9.30 (7.07–12.25)		7.63 (5.75–10.12)	
Sex	Male	4155 (33.3)	251 (6.0)	1	<0.001	1	<0.001	1	<0.001
	Female	8341 (66.7)	928 (11.1)	1.97 (1.70–2.28)		1.95 (1.65–2.30)		1.62 (1.35–1.93)	
Household socio-economic position	Very low	2823 (22.6)	188 (6.7)	1	<0.001 (TFT $p < 0.001$)	1	0.149 (TFT $p = 0.067$)	1	0.287 (TFT $p = 0.153$)
	Low	3350 (26.8)	298 (8.9)	1.35 (1.08–1.67)		1.26 (1.00–1.58)		1.21 (0.96–1.53)	
	Medium	3468 (27.8)	362 (10.4)	1.57 (1.26–1.96)		1.26 (0.99–1.60)		1.25 (0.98–1.59)	
	High	2855 (22.9)	331 (11.6)	1.61 (1.27–2.04)		1.33 (1.03–1.71)		1.25 (0.96–1.61)	
Highest level of education	None/grade 1–2	940 (7.5)	151 (16.1)	1	<0.001 (TFT $p < 0.001$)	1	0.341 (TFT $p = 0.483$)	1	0.371 (TFT $p = 0.231$)
	Grade 3–6	1876 (15.0)	275 (14.7)	0.85 (0.68–1.06)		1.05 (0.82–1.33)		1.00 (0.78–1.28)	
	Grade 7–10	4973 (39.8)	503 (10.1)	0.55 (0.45–0.68)		1.05 (0.84–1.33)		0.98 (0.77–1.23)	
	Grade 11–12	4296 (34.4)	226 (5.3)	0.27 (0.22–0.34)		0.87 (0.66–1.15)		0.81 (0.61–1.08)	
	College/University	411 (3.3)	24 (5.8)	0.28 (0.18–0.45)		1.10 (0.67–1.80)		1.02 (0.62–1.69)	
Smoking history	Never	9248 (74.0)	937 (10.1)	1	<0.001 (TFT $p < 0.001$)	1	0.004 (TFT $p = 0.002$)	1	0.041 (TFT $p = 0.090$)
	Ex-smoker	2406 (19.3)	204 (8.5)	0.69 (0.59–0.82)		0.87 (0.72–1.06)		1.01 (0.83–1.23)	
	Current smoker	842 (6.7)	38 (4.5)	0.44 (0.31–0.62)		0.57 (0.40–0.82)		0.63 (0.43–0.92)	
HIV status[**]	Negative	9610 (82.1)	983 (10.2)	1	<0.001	1	<0.001	1	0.008
	Positive	2098 (17.9)	124 (5.9)	0.57 (0.47–0.69)		0.69 (0.57–0.85)		0.76 (0.62–0.93)	
Body Mass Index[***] (weight(kg)/height^2(m))	Recommended weight (18.5–24.9)	4732 (39.0)	251 (5.3)	1	<0.001 (TFT $p < 0.001$)[##]			1	<0.001 (TFT $p < 0.001$)[##]
	Underweight (<18.5)	678 (5.6)	37 (5.5)	1.00 (0.70–1.43)				0.87 (0.60–1.27)	
	Overweight (25–29.9)	2859 (23.6)	298 (10.4)	2.09 (1.75–2.49)				1.47 (1.21–1.78)	
	Obese (⩾30)	3862 (31.8)	549 (14.2)	3.00 (2.56–3.51)				1.78 (1.47–2.14)	
Community	WC1	1258 (10.1)	169 (13.4)	1	<0.001	1	<0.001	1	<0.001
	WC2	2440 (19.5)	266 (10.9)	0.78 (0.56–1.07)		0.96 (0.68–1.35)		0.96 (0.69–1.36)	
	WC3 (rural)	212 (1.7)	12 (5.7)	0.36 (0.18–0.74)		0.70 (0.33–1.51)		0.72 (0.34–1.56)	
	WC4	1809 (14.5)	186 (10.3)	0.76 (0.54–1.05)		1.27 (0.89–1.80)		1.25 (0.88–1.78)	
	WC5	1275 (10.2)	59 (4.6)	0.32 (0.21–0.48)		0.47 (0.30–0.72)		0.48 (0.31–0.74)	
	WC6 (rural)	2684 (21.5)	209 (7.8)	0.56 (0.41–0.77)		0.57 (0.41–0.80)		0.56 (0.40–0.79)	
	WC7	1702 (13.6)	129 (7.6)	0.57 (0.40–0.80)		0.75 (0.52–1.09)		0.73 (0.51–1.07)	
	WC8	1116 (8.9)	149 (13.4)	1.02 (0.74–1.42)		1.28 (0.90–1.81)		1.30 (0.91–1.85)	

DM = diabetes mellitus; CI = confidence interval; OR = odds ratio; TFT = likelihood ratio test for trend with exposure as a linear variable; DFL = likelihood ratio test for departure from linear trend; All analyses accounted for the two-stage clustered sampling design through use of a logistic regression model with random effects for enumeration area and inclusion of community as a fixed effect.

[+] 11,699 participants included in analyses, adjusted for age, sex, household socio-economic position, education level, smoking history and HIV status.

[++] 11,365 participants included in analysis, adjusted for all variables shown; Missing data: 9 for age, 788 for HIV status, 365 for BMI.

[#] Likelihood ratio tests.

[##] Tests for linear trend and departure from linearity calculated with underweight as the baseline group.

[*] Defined as a random blood glucose concentration of ⩾11.1 mmol/L or <11.1 mmol/L but with self-reported previous DM diagnosis.

[**] Based on serology plus self-report for those with no available serology.

[***] Grouped according to the International BMI Classification.

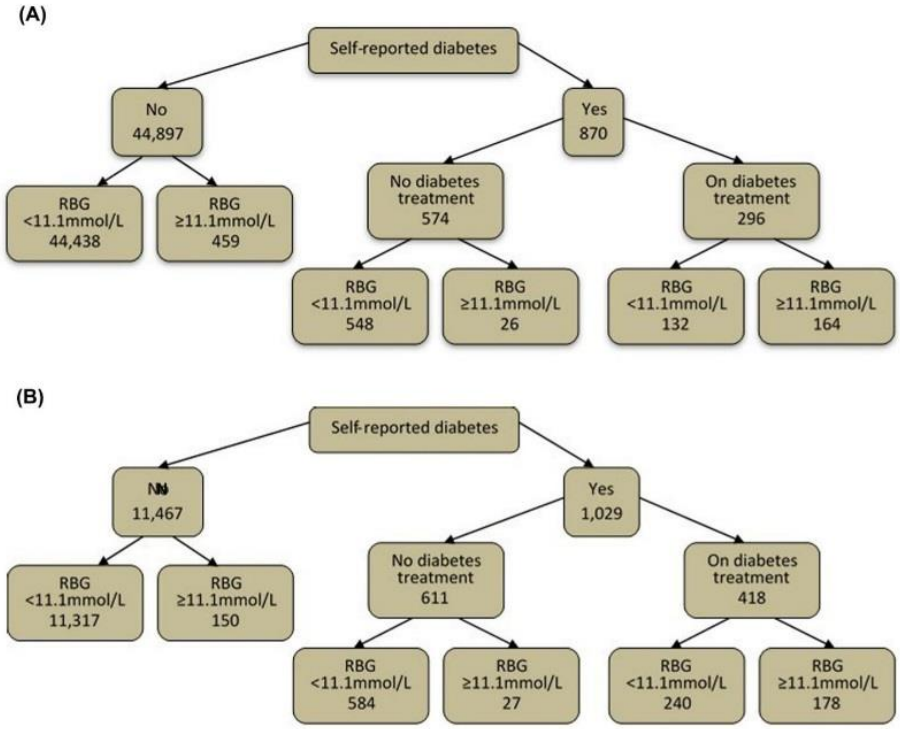


Fig. 2 – Numbers of (a) Zambian and (b) Western Cape participants with diabetes by self-report and RBG diagnosis, and numbers who report they are currently on treatment for diabetes. Legend: treatment = dietary, oral hypoglycaemic agents or insulin.

6.4 mmol/L (SD 3.0 mmol/L) and for Western Cape participants was 6.3 mmol/L (SD 2.7 mmol/L). Table 5 shows these data as numbers of participants by category of RBG concentration.

4. Discussion

The prevalence of diabetes mellitus in these study communities rivals that seen in high-income settings [22,23]. After adjustment for confounding factors, there is strong evidence that the prevalence of diabetes in participants from both countries increases with age and BMI, and differs by community. Among Zambian participants there is strong evidence that the prevalence of diabetes increases with increasing household socio-economic position, and also with level of education attained when the interrelated effect of household socio-economic position is not adjusted for.

Among Western Cape participants there is strong evidence that the prevalence of diabetes is higher in females than males and among those who are HIV negative compared to those who are HIV positive. Participation was higher in women than men in all study communities, most likely because women were more readily found in their homes, the site of enrolment, than men. The same was true for participants who were excluded from the analyses due to missing glycaemia data (supplementary table) and so is unlikely to have caused any systematic bias.

BMI, as a measure of adiposity, entirely explains the male–female differential in diabetes prevalence in the Zambian sites and most of the association with smoking in the Western Cape sites, but only partially explains associations with age, education and household socio-economic position. These distal factors are therefore associated with diabetes independently of adiposity, in addition to the mediating pathway via adiposity. The remaining association with age is explained by differences in self-reporting of diabetes between men and women: the association is removed entirely when the diabetes definition used removes the group who report that they have diabetes but have RBG < 11.1 mmol/L and are not on treatment for diabetes.

Of those with diabetes, individuals who are more likely to have undiagnosed diabetes are those who are older, from a lower household socio-economic position and with a lower level of education. Of those with self-reported previously diagnosed diabetes, many remain on no treatment for their diabetes, not even dietary management. A high proportion have high random blood glucose concentrations, though more so among those who are on treatment for their diabetes rather than among those who are not on treatment. This could indicate that those with the most poorly controlled diabetes are more likely to be given treatment, even though the treatment they are given is sub-optimal, or it could reflect measurement error for self-reported prior diabetes diagnosis.

The risk factor profile seen in this study is similar to that seen for diabetes elsewhere except for the pattern of association seen with HIV status in the Western Cape [11,12,19,24–26]. A reduced risk of diabetes for those with HIV but not on antiretroviral therapy is unexpected. It would be more understandable for those with HIV and on anti-retroviral medication to have an enhanced risk of diabetes, due to

Table 3 – Prevalence of undiagnosed diabetes among all those identified to have diabetes stratified by participant characteristics.

Characteristic		Zambian sites		Western Cape sites	
		Number (%) with undiagnosed DM	P-value*	Number (%) with undiagnosed DM	P-value*
Overall		459 (34.5)	–	150 (12.7)	–
Age (years)	18–24	59 (29.2)	0.596	5 (4.4)	0.007
	25–29	35 (32.7)		4 (5.1)	
	30–34	45 (36.9)		6 (8.0)	
	35–39	36 (34.0)		12 (11.7)	
	40–49	80 (34.5)		39 (16.3)	
	50–59	95 (34.3)		38 (14.5)	
	60+	98 (38.3)		46 (15.1)	
Sex	Male	128 (31.8)	0.160	41 (16.3)	0.053
	Female	331 (35.8)		109 (11.8)	
Household socio-economic position	Very low	100 (43.7)	<0.001	24 (12.8)	0.946
	Low	130 (39.3)		35 (11.7)	
	Medium	100 (27.3)		48 (13.3)	
	High	129 (32.0)		43 (13.0	
Highest level of education	None/grade 1–2	69 (48.6)	<0.001	28 (18.5)	0.147
	Grade 3–6	79 (41.4)		32 (11.6)	
	Grade 7–10	211 (32.9)		63 (12.5)	
	Grade 11–12	59 (29.4)		26 (11.5)	
	College/University	41 (26.8)		1 (4.2)	
Smoking history	Never	391 (33.4)	0.042	125 (13.3)	0.249
	Ex-smoker	24 (34.3)		19 (9.3)	
	Current smoker	44 (49.4)		6 (15.8)	
Body Mass Index***	Recommended weight (18.5–24.9)	166 (30.9)	0.298	19 (7.6)	0.047
	Underweight (<18.5)	38 (38.8)		5 (13.5)	
	Overweight (25–29.9)	115 (35.2)		44 (14.8)	
	Obese (⩾30)	95 (35.5)		77 (14.0)	
HIV status**	Negative	329 (34.7)	0.100	131 (13.3)	0.098
	Positive	58 (28.7)		10 (8.1)	
Self-reported current tuberculosis	No	455 (34.5)	0.898	147 (12.6)	0.146
	Yes	4 (36.4)		3 (27.7)	
Community	ZAM1	33 (11.7)	<0.001	–	–
	ZAM2	27 (25.5)		–	
	ZAM3 (rural)	21 (35.6)		–	
	ZAM4 (rural)	15 (57.7)		–	
	ZAM5	25 (31.7)		–	
	ZAM6	27 (42.9)		–	
	ZAM7	46 (48.9)		–	
	ZAM8	43 (55.1)		–	
	ZAM9	25 (44.6)		–	
	ZAM10	15 (25.4)		–	
	ZAM11	35 (37.2)		–	
	ZAM12	32 (39.0)		–	
	ZAM13	28 (38.4)		–	
	ZAM14	33 (56.9)		–	
	ZAM15 (rural)	38 (59.4)		–	
	ZAM16 (rural)	16 (28.6)		–	
	WC1	–	–	19 (11.2)	0.007
	WC2	–		33 (12.4)	
	WC3 (rural)	–		1 (8.3)	
	WC4	–		13 (7.0)	
	WC5	–		15 (25.4)	
	WC6 (rural)	–		36 (17.2)	
	WC7	–		18 (14.0)	
	WC8	–		15 (12.7)	

DM = diabetes mellitus.

* Pearson's Chi-squared tests; Total number of participants identified to have diabetes and therefore included in these analyses = 1329 in Zambia and 1179 in Western Cape.

** Based on serology plus self-report for those with no available serology.

*** Grouped according to the International BMI Classification.

Table 4 – Proportion of those with known diabetes on diabetes treatment.

Diabetes treatment		Zambian sites	Western Cape sites
		Number (%) of those with known diabetes	
Any treatment		296 (34.0)	418 (40.6)
	Dietary	38 (4.4)	5 (0.5)
	Hypoglycaemic tablets	210 (24.1)	338 (32.9)
	Insulin	48 (5.5)	75 (7.3)
None		574 (66.0)	611 (59.4)

anti-retroviral effects on insulin resistance. The association is partially explained by adiposity: HIV can cause a reduction of adiposity, particularly when not on ART, and consequently a reduction of diabetes risk. Residual confounding by central obesity is a possible explanation but beyond this the association is most likely explained by chance, other residual confounding, or bias.

Diabetes is well known to be associated with urban/rural location [11,12,24,25]. Although not formally explored, an assessment of this can be made in these data through the community variable. For both study populations no clear urban/rural pattern is seen. This could be explained by the nature of the rural communities that were chosen to take part in the ZAMSTAR cluster-randomised trial. These communities are mostly central areas of rural districts rather than remote villages, and so may not be so different from the urban areas.

The prevalence of diabetes was found to be higher in the Western Cape communities than in the Zambian communities, even for comparable age, sex, relative household socioeconomic position and education, and BMI. This could be due to differences in dietary habits, levels of physical activity or genetic factors between the two localities, but as the study was not designed to explore this we are unable to draw definitive conclusions on the cause of the difference in prevalence between the two settings.

Misclassification of outcome data could have resulted in bias. For diabetes, an under-estimate of association is likely as RBG tests have good specificity but sub-optimal sensitivity for diabetes diagnosis [27,28]. It is reassuring for the determination of risk factors, that almost identical associations were seen when diabetes diagnosis was defined in other ways. However, for both the prevalence of diabetes and the prevalence of undiagnosed diabetes the figures determined in this study likely represent the minimum true proportions. Indeed, the proportion of individuals with diabetes who are undiagnosed has been reported to be much higher in other parts of Africa, up to 75% in Northern Africa [13]. The proportion observed among our Western Cape participants is even comparable to that reported from the United States [29]. It is possible that the comparatively low proportion of the total study population who have undiagnosed diabetes is partly due to a low sensitivity of RBG $\geqslant$ 11.1 mmol/L for diabetes diagnosis.

Alternative approaches to the study methods could have been to measure fasting blood glucose or glycated haemoglobin concentrations, or to perform oral glucose tolerance tests. Any of these methods would have resulted in greater sensitivity for diabetes diagnosis, but in a large-scale field study with data collection occurring in the community each of these approaches would have been logistically challenging and likely less acceptable to potential participants. Consequently the likelihood of measurement error and a low uptake of potential participants would have been high. Therefore it was felt that the use of RBG measurement for this study would optimise participant uptake and minimise measurement error.

Even with this approach, the number of participants with missing data for RBG concentration was substantial. However, given that the primary focus of data collection was tuberculosis prevalence, for the purposes of the parent study, these losses are more likely to be due to their lack of prioritisation during the data collection process rather than due to a lack of acceptability to participants. Further, in Western Cape participants were required to attend a mobile clinic for capillary

Table 5 – Random blood glucose concentration among those with known diabetes.

Random blood glucose concentration (mmol/L)	Number (%) of those with known diabetes		
	All participants	Participants on diabetes treatment	Participants not on diabetes treatment
Zambian sites			
<6.0	385 (44.3)	48 (16.2)	337 (58.7)
6.0–7.8	186 (21.4)	38 (12.8)	148 (25.8)
$\geqslant$7.8	299 (34.4)	210 (71.0)	89 (15.5)
Western Cape sites			
<6.0	456 (44.3)	89 (21.3)	367 (60.1)
6.0–7.8	237 (23.0)	73 (17.5)	164 (26.8)
$\geqslant$7.8	336 (32.7)	256 (61.2)	80 (13.1)

Categorisation of glucose concentration based on International Diabetes Federation guidelines for target pre-prandial glucose concentration (<6.0 mmol/L) and target postprandial glucose concentration (<7.8 mmol/L) [18].

blood tests whereas in Zambia the tests were performed in participants' homes, which most likely is the explanation for the higher proportion of missing RBG results seen in Western Cape than Zambia. For these reasons the potential for the missing data to cause bias to the study results, through being associated with glycaemic concentration, is low.

The accuracy of RBG results obtained in our study is a consideration. Although point-of-care capillary blood glucose measurement is more rapid, cost-effective and less invasive than laboratory measurement of plasma glucose concentration, the latter is considered to be the most accurate method. Although performance checks were made on test strips and meters, we have limited quantitation of glucometer characteristics obtained during data collection for this study. Accuracy data from other settings are reassuring: a recently reported study comparing six commonly used point-of-care blood glucose monitoring systems found the Optium Xceed system to have the highest level of accuracy, the lowest occurrence of error messages and to be least influenced by blood haematocrit levels [30]. The Optium Xceed system met current accuracy criteria set by the International Organization for Standardization, having >95% of all readings within ±12.5% from the reference at glucose levels >4 mmol/L and ±0.5 mmol/L at glucose levels <4 mmol/L.[30] Consideration of test accuracy is most relevant to prevalence estimates obtained in this study as inaccurate results could have led to estimates that are either too high or too low. However, there is no reason that glucometer performance would vary systematically by age or gender or other participant characteristic, and so inaccurate performance would only weaken associations between participant characteristics and glucose result. It is reassuring, therefore, that the main risk factors identified in this study are known to be established risk factors for diabetes elsewhere [19,20].

The use of random blood glucose concentration to determine satisfactory control of glycaemia is a major limitation. When interpreting the proportions in this study of those with a prior diabetes diagnosis who were found to have an inadequate level of glycaemia, it is again important to appreciate that this is based on a one-off measurement of random blood glucose concentration, not on a measure of longer-term glycaemic control such as glycated haemoglobin. In this large-scale population-level study setting the measure used and results obtained are certainly suggestive that glycaemic control is sub-optimal, but interpretation beyond this should be made only with caution.

As participants were sampled at random and in sufficiently large numbers, it is possible to generalise these results to the communities from where the participants came. Generalisation beyond this should be made with caution, as the communities were not selected at random from the wider population.

5. Conclusions

The prevalence of diabetes mellitus in the study communities rivals that seen in high-income settings. The risk factor profile is similar to that seen in Caucasian populations, with age $\geqslant$50 years and BMI $\geqslant$ 30 representing the highest risk groups for diabetes. The study findings suggest that many of those with diabetes remain undiagnosed in the community, particularly among those from a lower socio-economic position and with a lower level of education in the Zambian sites. Even if diagnosed, many of those with diabetes remain sub-optimally managed. Further studies to guide effective methods of managing diabetes at the individual and public health levels in low-income sub-Saharan settings are needed. Timely diagnosis and management of this long-term non-communicable disease must be prioritised, with a particular emphasis on redressing the lack of health equity for the poorer and less educated.

Author contributions

All authors contributed to initial study concept and study design. HA, NB and PGF are the principal investigators of the ZAMSTAR study, into which this study is nested. They oversaw participant recruitment and data collection. SLB and SF performed the data analysis. SLB wrote initial drafts and all authors contributed to final editing of the paper.

Conflicts of interest

We declare that we have no conflicts of interest.

Funding

SLB is supported by a Wellcome Trust Clinical PhD Fellowship (100141/Z/12/Z). The ZAMSTAR study was supported by a subcontract from Johns Hopkins University with funds provided by Grant No. 19790.01 from the Bill and Melinda Gates Foundation. The contents of this manuscript are solely the responsibility of the authors and do not necessarily represent the official views of the Bill and Melinda Gates Foundation or the Wellcome Trust.

Acknowledgements

We thank all the members of all study teams who contributed to data collection. We thank the Zambia Ministry of Health, City of Cape Town Health Directorate, the Western Cape Provincial Department of Health, and Zambian and South African National TB Programmes and all the district health managers and health facility staff for allowing us to work in the facilities. We thank the Community Advisory Boards and the members of the community who participated in the trial. We acknowledge the support and scientific guidance of the CREATE consortium leadership, R Chaisson (principal investigator for CREATE) and L Moulton (coprincipal investigator for CREATE biostatistics core) and of the ZAMSTAR Study Advisory Group; M Borgdorff, D Enarson, B Chirwa, P Mwaba, Y Mulla, L Mvusi, M Poolman, I Toms and K Jennings.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.diabres.2016.05.001.

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Chapter 4: Secondary data analysis paper 2: The association of hyperglycaemia with prevalent tuberculosis: a population-based cross-sectional study

Bailey SL[1,2], Ayles H[1,2], Beyers N[3], Godfrey-Faussett P[1], Muyoyeta M[2], Du Toit E[3], Yudkin JS[4], Floyd S[5]

[1]LSHTM TB Centre and Department of Clinical Research, London School of Hygiene and Tropical Medicine, London, UK;
[2] ZAMBART Project, Lusaka, Zambia;
[3]Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Stellenbosch University, South Africa;
[4]University College London, London, UK;
[5]LSHTM TB Centre and Department of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine, London, UK.

Corresponding author: Dr Sarah Lou Bailey, slbailey@doctors.org.uk, +442081234489

Word count: 3,914

Abstract

Background: Systematic reviews suggest that the incidence of diagnosed tuberculosis is two- to- three times higher in those with diabetes mellitus than in those without. Few studies have previously reported the association between diabetes or hyperglycaemia and the *prevalence* of active tuberculosis and none in a population-based study with microbiologically-defined tuberculosis. Most have instead concentrated on cases of diagnosed tuberculosis that present to health facilities. We had the opportunity to measure glycaemia alongside prevalent tuberculosis. A focus on prevalent tuberculosis enables estimation of the contribution of hyperglycaemia to the population prevalence of tuberculosis.

Methods: A population-based cross-sectional study was conducted among adults in 24 communities from Zambia and the Western Cape (WC) province of South Africa. Prevalent tuberculosis was defined by the presence of a respiratory sample that was culture positive for *M. tuberculosis*. Glycaemia was measured by random blood glucose (RBG) concentration. Association with prevalent tuberculosis was explored across the whole spectrum of glycaemia.

Results: Among 27,800 Zambian and 11,367 Western Cape participants, 4,431 (15.9%) and 1,835 (16.1%) respectively had a RBG concentration ≥7.0mmol/L, and 405 (1.5%) and 322 (2.8%) respectively had a RBG concentration ≥11.1mmol/L. In Zambia, the prevalence of tuberculosis was 0·5% (142/27,395) among individuals with RBG concentration <11.1mmol/L and also ≥11.1mmol/L (2/405); corresponding figures for WC were 2·5% (272/11,045) and 4·0% (13/322). There was evidence for a positive linear association between hyperglycaemia and pulmonary prevalent tuberculosis. Taking a RBG cut-off 11.1mmol/L, a combined analysis of data from Zambian and WC communities found evidence of association between hyperglycaemia and TB (adjusted odds ratio=2·15, 95%CI [1·17-3·94]). The population attributable fraction of prevalent

tuberculosis to hyperglycaemia for Zambia and WC combined was 0.99% (95%CI 0·12%-1.85%) for hyperglycaemia with a RBG cut-off of 11.1mmol/L.

Conclusions: This study demonstrates an association between hyperglycaemia and prevalent tuberculosis in a large population-based survey in Zambia and Western Cape. However, assuming causation, this association contributes little to the prevalence of TB in these populations.

KEYWORDS: Zambia; South Africa; logistic regression

BACKGROUND

The number of adults with diabetes mellitus globally is predicted to rise from 382 million in 2013 to 592 million in 2035.[1-6] Of those in the world who currently have diabetes, four out of five live in a low or middle income country.[6] The burden of the anticipated rise in diabetes prevalence will fall largely to these low and middle income countries, the same countries that have some of the highest burdens of tuberculosis (TB) worldwide.[4,7]

Associations between diabetes and tuberculosis are increasingly recognised: systematic reviews and meta-analyses suggest that the incidence of active diagnosed tuberculosis is two- to- three times higher in those with diabetes compared to those without diabetes;[8-10] that diabetes increases the risk of death from diagnosed tuberculosis[11], and that diabetes may increase the risk of tuberculosis relapse.[9, 11] Studies from Africa have shown even stronger associations between diabetes and active diagnosed TB; a four-fold increase in diabetes prevalence was seen in TB patients in Dar es Salaam compared to the general population;[12] an odds ratio for TB of 8·33 was seen in the Congo comparing those with diabetes to those without diabetes.[13] As the prevalence of diabetes rises in locations with a high burden of tuberculosis, a deeper understanding of these associations is increasingly important.

We had the opportunity to measure glycaemia alongside tuberculosis in a population-based cross-sectional survey, which took place as part of the ZAMSTAR (Zambia South Africa TB and HIV Reduction Study) trial.[14-16] This trial was a 2 x 2 factorial community randomised trial to evaluate the impact of two complex interventions on the prevalence of TB in high HIV prevalence settings in Zambia and South Africa. The primary outcome for the study was the prevalence of tuberculosis after three years of intervention, measured through a cross-sectional survey of a random sample of adults from each community. We took this opportunity to also explore the association between hyperglycaemia and prevalent TB.

Both Zambia and South Africa have national TB control programmes, with a structured approach to TB diagnosis, management and control. Sputum smear microscopy is used for the diagnosis of pulmonary TB in Zambia. Few health care centres have access to culture or molecular tests. In South Africa Xpert MTB/RIF was introduced in 2011 for the diagnosis of pulmonary TB, though interruption to the supply of cartridges has been a challenge. Supply of TB medication rarely suffers interruption and is provided free of charge to patients in both countries. The situation for diabetes management is less favourable. In Zambia, point-of-care glucometers are most commonly used for diagnosis, though frequently glucometer strips are unavailable. Metformin and glibenclamide are widely available but access to alternative oral hypoglycaemics can be challenging. Insulin is available but can be difficult to access in remote areas. Storage of insulin is frequently problematic for patients due to lack of access to refrigeration. Unlike for TB, diabetes medication is not provided to patients for free.

Few studies have previously reported the association between diabetes or hyperglycaemia and *prevalent* tuberculosis in the general population, and to our knowledge none in a population-based study with microbiologically-defined tuberculosis. Most have concentrated on cases of tuberculosis that present to, and are diagnosed, at health facilities. Two historical studies did measure the prevalence of tuberculosis in the general population; one in Philadelphia, USA in 1946,[17] the other in Kristianstad, Sweden in 1954.[18] Both identified pulmonary TB by chest radiograph, and identified diabetes through referrals from clinics and through medical records respectively. Rather than being based in a general population, other studies that have focused on tuberculosis prevalence have investigated the prevalence among patients with diabetes in a clinic setting or identified through medical records, using either no comparison group or clinic patients without diabetes as a comparison group.[10, 19, 20] Exploration of the association of hyperglycaemia with prevalent tuberculosis in a

population-based study allows for estimation of the contribution of hyperglycaemia to the population prevalence of tuberculosis.

The aims of this study are therefore to determine the association between hyperglycaemia and prevalent tuberculosis and to estimate the population attributable fractions of prevalent tuberculosis to hyperglycaemia, assuming causation, within our study communities in Zambia and the Western Cape region of South Africa.

METHODS

This population-based cross-sectional study was nested within a 2 x 2 factorial cluster-randomised trial (the ZAMSTAR study[14-16]) and undertaken between January and December 2010 in 24 study communities: 16 from 5 provinces in Zambia and 8 from the Western Cape province of South Africa. Within each community, a two-stage cluster sampling design was used to recruit participants. Exclusion criteria were age <18 years, inability to give informed consent due to disability/incapacitation, refusal to submit a respiratory sample and any persons living in institutional settings.

Each participant was required to give written informed consent. Individuals and household heads were interviewed in their homes using structured questionnaires. Each participant was requested to produce a spot respiratory sample for tuberculosis culture. Finger prick capillary blood was taken for HIV testing and random blood glucose (RBG) measurement, with pre- and post-test counselling for HIV tests. RBG concentration was measured using an Optium Xceed point-of-care glucometer. All individuals identified to have abnormal blood glucose or to be HIV positive were referred to existing local health facilities for appropriate management.

Data were electronically entered directly onto personal digital assistants by field staff at the time of data collection, using pre-programmed questionnaires and result sheets. All information was downloaded daily into a SQL (structured query language) database and later exported into Stata.

All procedures for sputum sample collection and culture were identical in all study sites. Research staff in all sites were trained to instruct participants on adequate expectoration to achieve a lower rather than upper airways sample. Samples were collected daily from field sites and delivered to the laboratory in each study community. A standard liquid culture technique was used to isolate *Mycobacterium tuberculosis* (MGIT, Becton Dickinson). Growth detected by culture was identified using an immunochromatographic assay (Capilia TB), and all Capilia TB assay positive cultures were confirmed by 16S ribosomal RNA sequencing. Detailed methods are described by Ayles *et al.* in the final report of the ZAMSTAR trial.[16]

The Optium Xceed glucometer uses a whole blood capillary sample but is calibrated to report the plasma equivalent result. The results presented here are therefore the plasma equivalent glucose concentrations. This device was chosen because of its documented accuracy in multiple independent studies combined with its availability in Zambia and South Africa. It was found to be one of the most superior glucometers in all published accuracy studies, with between 84% and 100% of the results from the finger prick capillary specimen being within the recommended limits compared to reference plasma estimation on laboratory analysers.[21-27] When inaccuracy was seen, this was mostly for low rather than high glucose concentrations.[24, 25, 27] All research staff were trained on the use of this particular glucometer and were required to undergo proficiency testing. Standardised control solution was used for performance checks on test strips and meters.

Ethics approval was granted from the London School of Hygiene and Tropical Medicine Ethics Committee, the University of Stellenbosch Human Research Ethics Committee and the University of Zambia Biomedical Research Ethics Committee.

Definitions

- Hyperglycaemia, the exposure of interest for this study, is initially examined with RBG concentration as an ordered categorical variable. We then use sequential

RBG cut-offs – 7.0mmol/L, 7.8mmol/L, 9.0mmol/L and 11.1mmol/L – to explore increasing levels of hyperglycaemia. We based our cut-off levels on the current World Health Organisation guidelines for diabetes diagnosis and monitoring,[28] though this was only to allow for exploration of increasing levels of glycaemia and not intended to be indicative of diabetes diagnoses.

- Prevalent pulmonary tuberculosis, the outcome of interest for this study, is defined by the presence of a respiratory sample that is culture positive for *Mycobacterium tuberculosis*.

- HIV status is defined by a combination of blood sampling plus self-report for those with missing biological data.

Analysis strategy

Principal components analysis was used to create a measure of household socio-economic position separately for each country. Unadjusted and adjusted odds ratios of the association between hyperglycaemia and prevalent tuberculosis were estimated using logistic regression analysis, accounting for within-cluster correlation resulting from the sampling design. Analyses were performed separately for Zambian and South African data due to heterogeneity between the two distinct locations. This enabled us to control for confounding differently for each location, which was necessary because of the big differences between the two settings. Fixed-effects meta-analysis of adjusted odds ratios from each country was then performed, with weights according to the inverse variance method to give overall odds ratios of the association between hyperglycaemia and prevalent tuberculosis. Population attributable fractions (PAFs) of prevalent tuberculosis to hyperglycaemia and HIV were calculated separately for each country and for combined estimates. These were calculated using the formula $PAF = \sum p_k'(\theta_k - 1)/\theta_k$ where p' is the proportion of cases exposed in the study population at exposure level k and θ is the adjusted odds ratio. Given that θ and p' were estimated

from the same data, the 95% confidence intervals could be calculated using the following error factor for (1-PAF):

$$Error\ factor = \exp\left\{1.96 \times \sqrt{\frac{n_1 p'^2 V + 2p'(\theta - 1) + p'(1 - p')(\theta - 1)^2}{n_1[\theta(1 - p') + p']^2}}\right\}$$

where n_1 is the total number of cases observed and V the variance of the adjusted log odds ratio (the standard error of the log odds ratio squared). Evidence for effect modification by gender and HIV was explored. All data analyses were performed using Stata 13.

RESULTS

The cross-sectional survey enrolled 57,809 (70·8% of eligible) participants from 31,300 (88·6% of eligible) households in Zambia and 32,792 (77·7% of eligible) participants from 17,095 (85·3% of eligible) households in Western Cape (Figure 1). Evaluable sputum samples and complete RBG results were obtained for 27,800 (48·1% of enrolled) participants in Zambia and 11,367 (34·7% of enrolled) participants in Western Cape. Data from these participants were analysed. Comparison of individuals with evaluable sputum samples with those with non-evaluable samples in Zambia showed them to be much the same (data presented as supplementary material to the ZAMSTAR trial publication).[16]

Among Zambian and Western Cape participants, 15.9% and 16.1% respectively had a RBG concentration ≥7.0mmol/L, and 1.5% and 2.8% respectively had a RBG concentration ≥11.1mmol/L. The prevalence of tuberculosis was approximately 500 per 100,000 (0·5%) among Zambian participants and approximately 2,500 per 100,000 (2·5%) among Western Cape participants. Tuberculosis prevalence stratified by glycaemia and baseline characteristics is shown in Tables 1 and 2.

Among individuals included in the analysis, HIV status was determined by blood sampling for 23,067 (90·2%) participants and by self-reported status for 2,501 (9·8%)

participants in Zambia. Corresponding values for Western Cape participants are 10,106 (94·8%) and 551 (5·2%). Among participants with RBG results, 2,232 participants in Zambia and 710 in Western Cape had missing data for both blood sampling and self-reported HIV status.

Among Zambian participants, as RBG concentration increased, the unadjusted and adjusted odds of prevalent TB initially increased compared to the baseline RBG concentration <5.6 mmol/L, peaking at RBG concentration 9.0-11.0 mmol/L (adjusted OR 4.31, 95% CI [2.07-8.97]). Although the odds of prevalent TB did not continue to increase with increasing RBG concentration beyond this, the number of individuals with a RBG concentration ≥11.1 mmol/L was low and the confidence interval was wide so there was still strong evidence of a linear association between RBG concentration and TB prevalence (p=0.006) after adjusting for age, sex, household socio-economic position, education, body mass index, HIV status and geographical location (Table 1).

On multivariable analysis there was weak evidence of a linear association between glucose concentration and TB prevalence among Western Cape participants (p=0.06). In this location the adjusted odds of prevalent TB compared to the baseline was greatest for individuals with a RBG concentration ≥11.1 mmol/L (OR 2.49, 95% CI [1.29-4.79], Table 2).

Unadjusted and adjusted odds ratios for prevalent TB using sequential RBG cut-offs to give increasing levels of hyperglycaemia are shown in Table 3. The findings from Zambia and Western Cape, combined with fixed-effects meta-analyses, showed increasing adjusted odds of prevalent tuberculosis for increasing cut-off levels of hyperglycaemia, though the increase was small; and, across successive cut-off levels, confidence intervals overlapped (Table 4). There was no evidence that the adjusted odds ratios differed between Zambia and Western Cape (Table 4).

The evidence for association between hyperglycaemia and prevalent tuberculosis strengthened from univariable to multivariable analyses in both Zambia and the

Western Cape communities. In the Zambian communities the predominant negative confounding factors were body mass index and HIV status. The Western Cape communities had the same negative confounding factors, while the predominant positive confounding factor was age.

On combined analysis, there was evidence of a contribution of hyperglycaemia to the population prevalence of tuberculosis throughout the spectrum of hyperglycaemia. (Table 4). However, the PAFs of prevalent TB to hyperglycaemia were small, particularly for the higher RBG cut-offs. For RBG concentration ≥11.1 mmol/L the PAF of prevalent TB was 0.99%, 95% CI [0.12-1.85]. When analysed as separate locations, the confidence intervals for the PAFs were wide and showed less evidence for a contribution of hyperglycaemia to the population prevalence of tuberculosis in both Zambia and Western Cape (Table 3).

When stratified by age, for the highest RBG cut-off, the PAF of prevalent TB to hyperglycaemia increased with increasing age in Western Cape, reflecting the higher prevalence of hyperglycaemia in older age groups (Table 5). In Zambia, for this highest RBG cut-off ≥11.1mmol/L, there remained little evidence for a contribution of hyperglycaemia to the population prevalence of tuberculosis despite the rising prevalence of hyperglycaemia with increasing age, though confidence intervals were wide (Table 5).

For purposes of comparison to HIV, the PAF of prevalent TB to HIV was 12.72%, 95% CI [7.70-17.47] in the Zambian communities, and 11.72%, 95% CI [8.25-15.06] in the Western Cape communities.

For purposes of comparison to self-reported known diabetes, of individuals with a RBG <11.1mmol/L, 1.7% in Zambian and 6.8% in Western Cape communities reported having a previous diagnosis of diabetes, and 0.3% and 2.0% respectively reported being on treatment for diabetes. Of individuals with a RBG ≥11.1mmol/L, 27.9% in Zambian and 57.5% in Western Cape communities reported having a previous

diagnosis of diabetes, and 24.0% and 49.1% respectively reported being on treatment for diabetes. Incorporating participants with self-reported previously diagnosed diabetes into the highest category of hyperglycaemia made little difference to the odds ratio point estimates in Zambia and reduced the association seen with RBG concentration ≥11.1 mmol/L in Western Cape towards the null. Defining diabetes by self-report, the odds of prevalent TB for individuals with diabetes compared to those without was 0.83, 95% CI [0.20-3.46] in Zambia and 0.76, 95% CI [0.44-1.32] in Western Cape. For individuals in Western Cape with self-reported diabetes and RBG ≥11.1mmol/L the odds of prevalent TB was 1.55, 95% CI [0.60-4.01] compared to individuals who did not report diabetes. For those with self-reported diabetes and RBG <11.1mmol/L this odds ratio was 0.61, 95% CI [0.32-1.18].

Subgroup analyses were not possible for the Zambian data using the highest RBG cut-off ≥11.1mmol/L due to limited data, and no difference was seen for gender and HIV categories using the RBG cut-off ≥9.0mmol/L. In the Western Cape using the RBG cut-off ≥11.1 mmol/L, the point estimate of the adjusted odds of hyperglycaemia on prevalent tuberculosis was higher among women than men (for men OR=1·92, 95% CI [0·55-6·68]; for women OR=2·58, 95% CI [1·24-5·35]; but there was no evidence the odds ratio differed for men and women, test for interaction p=0·68). It was higher among those with HIV than those without HIV (among those with HIV OR=5·34, 95% CI [1·56-18·23]; among those without HIV OR=1·90, 95% CI [0·89-4·04]; but the evidence for interaction was weak (p=0·17)).

DISCUSSION

This is the first ever population based study of prevalent tuberculosis diagnosed microbiologically and glycaemia. We used participants who were randomly selected from the community rather than exploring the association among participants who had already been diagnosed with tuberculosis or hyperglycaemia. Among the Zambian participants of our study there was good evidence of a positive linear association

between hyperglycaemia and prevalent tuberculosis, and weak evidence for the same association in Western Cape. When data from the two locations were combined, there was evidence of association between hyperglycaemia and prevalent pulmonary tuberculosis across the spectrum of hyperglycaemia. On combined analysis the odds of prevalent tuberculosis was greatest for individuals with the highest level of glycaemia, though the magnitude of association was small. Those with a RBG concentration ≥11.1 mmol/L, had 2.15 times the odds of prevalent tuberculosis than those with a RBG concentration <11.1 mmol/L.

This association seen in the study communities between hyperglycaemia and prevalent tuberculosis is consistent with the association seen elsewhere in the world between diabetes and prevalent TB[17, 18] and also between diabetes and active diagnosed TB.[8-10]

Assuming causation, hyperglycaemia contributes little to the prevalence of tuberculosis throughout the spectrum of hyperglycaemia in the Zambian and Western Cape populations. This suggests that hyperglycaemia has only a small impact on the prevalence of TB in the study areas of Zambia and Western Cape despite the positive association seen between hyperglycaemia and prevalent TB in these locations. When stratified by age, however, we can see that the contribution of hyperglycaemia to the prevalence of tuberculosis in Western Cape is greater for older age groups, reflecting the higher prevalence of hyperglycaemia among older individuals. The same trend is seen in the Zambian study population, but the lower prevalence of hyperglycaemia in this setting means that confidence intervals are wide and point estimates remain low even for the oldest age groups.

The combined odds ratios were weighted towards the Western Cape estimates due to the larger number of individuals with hyperglycaemia and tuberculosis in this setting, particularly for RBG concentration ≥11.1 mmol/L. Given the uncertainty of the Zambian odds ratio for this higher level of glycaemia the combined analyses yield the more reliable conclusions. However, regardless of which analysis is used, the conclusion

remains the same, that the contribution of hyperglycaemia to the population prevalence of tuberculosis is low.

In this study we measured hyperglycaemia using a single RBG test. To optimise the accuracy of the test research staff were carefully trained and tested, and the point-of-care measure was carefully calibrated using standardised control solution, although not validated against laboratory glucose analyses. Use of this point-of-care test enabled glucose measurement of many participants in a large-scale field study located within the community. Use of a test that is more complicated to administer, such as a fasting blood glucose, oral glucose tolerance test or glycated haemoglobin, would have been logistically challenging and potentially less acceptable to participants, resulting in a much lower uptake of eligible participants and possibly introducing selection bias. Therefore, although not a test to diagnose diabetes, it was felt that the use of a RBG test was most likely to minimise overall bias and loss of study power in this setting of a large-scale population survey, spanning communities and countries. Therefore, rather than diagnosing diabetes, we have measured glycaemia and explored the effect of hyperglycaemia on tuberculosis prevalence. RBG tests normally have good specificity but sub-optimal sensitivity for diabetes.[29, 30] A study in China found measurement of RBG concentration with a cut off of 11·1mmol/L to have a sensitivity for diabetes of only 54·8% compared to an oral glucose tolerance test.[31] This would suggest that association seen with hyperglycaemia based on RBG concentration would be an under-estimate of any association with diabetes. However, in the context of active TB disease the specificity of this test for diabetes could also be reduced, as those with stress-induced hyperglycaemia secondary to their TB disease could also have a high RBG concentration. This would result in an over-estimate of the association with TB based on RBG concentration, compared to association with diabetes. A final consideration is a single RBG measurement fails to give data on chronic hyperglycaemia, so we are unable to explore the effect of chronic hyperglycaemia on tuberculosis prevalence from these data. We did explore the effect of participants

having previously diagnosed diabetes who may be on treatment and therefore may be normoglycaemic at the time of RBG testing but have longer term hyperglycaemia. The number of these participants were few in both Zambia and Western Cape and when incorporated into the categories of hyperglycaemia did not increase the odds ratio point estimates of the association with prevalent TB in either study location. Regardless of whether the hyperglycaemia measured was due to diabetes, was a consequence of TB disease or was transient from any other cause, the conclusion remains that in our study communities hyperglycaemia contributes little to the population prevalence of tuberculosis.

Regression dilution bias is another factor that could have led to an underestimate of the prevalence of hyperglycaemia and consequently an underestimate of PAFs, because a single measurement of a continuous factor tends to underestimate the strength of association of repeated measurements with a given outcome. Further, blood glucose level is continually changing in response to multiple factors including time of day and food consumed and so this particular measure is likely to be particularly susceptible to regression dilution bias.

The population attributable risks presented here should be interpreted with caution because the measure fails to account for onward transmission, which is important when focusing on pulmonary tuberculosis.

The substantial losses of evaluable respiratory samples in this study resulted largely from a failure of the positive mycobacterial control to grow in two of the laboratories in Zambia, causing whole batches to be non-evaluable. This has resulted in reduced study power, but is unlikely to have introduced bias to the study results, as the process could not have been associated with the presence or absence of hyperglycaemia in the individuals affected by these missing data. The missing glycaemic data is similarly unlikely to produce bias and was probably the consequence of the lack of prioritisation during data collection, a consequence of nesting this study within a larger cluster-

randomised trial. Therefore, this too is unlikely to have been associated with the presence or absence of disease and so is also unlikely to have introduced substantial bias.

The participants in this study were randomly selected from their communities and so are representative of the general population within each community. The communities included were from urban and peri-urban settings and so rural populations are under-represented in this study. The prevalence of both hyperglycaemia and tuberculosis would therefore likely be lower in a general population sample.

In subgroup analysis, the association between hyperglycaemia and prevalent TB among those with HIV was stronger than among those without HIV, which could suggest that hyperglycaemia and HIV work synergistically to increase one's risk of TB, or could instead reflect an increase in stress-induced hyperglycaemia among those with HIV compared to those without HIV. These findings should be seen as hypothesis generating as the evidence for effect modification was weak and our data are underpowered for formal assessment of effect modification.

CONCLUSION

In our study communities in Zambia and Western Cape, there is evidence for a positive linear association between hyperglycaemia and prevalent pulmonary tuberculosis. On combined analysis, individuals with RBG concentration ≥11.1 mmol/L had 2.15 times the odds of prevalent tuberculosis than individuals with a RBG concentration <11.1 mmol/L. Despite this, assuming causation, hyperglycaemia contributes little to the tuberculosis prevalence in our study communities. Investigation of the associations between hyperglycaemia, diabetes and active diagnosed tuberculosis in these study communities would be a valuable addition to the findings from this study, and would allow for sub-group analysis of association with smear-negative, smear-positive and drug-resistant tuberculosis.

LIST OF ABBREVIATIONS USED

- CI: confidence interval
- FBG: fasting blood glucose
- HbA_{1c}: glycated haemoglobin
- HIV: human immunodeficiency virus
- OR: odds ratio
- PAF: population attributable fraction
- RBG: random blood glucose
- TB: tuberculosis
- WC: Western Cape

DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethics approval was granted from the London School of Hygiene and Tropical Medicine Ethics Committee, the University of Stellenbosch Human Research Ethics Committee and the University of Zambia Biomedical Research Ethics Committee. Each study participant was required to give written informed consent.

CONSENT FOR PUBLICATION

Not applicable

AVAILABILITY OF DATA AND MATERIALS

Data are from the ZAMSTAR study whose authors may be contacted via Sian.Floyd@lshtm.ac.uk.

COMPETING INTERESTS

The authors declare that they have no competing interest.

FUNDING

SLB is supported by a Wellcome Trust Clinical PhD Fellowship (100141/Z/12/Z). The ZAMSTAR study was supported by a subcontract from Johns Hopkins University with funds provided by grant number 19790.01 from the Bill and Melinda Gates Foundation. The contents of this manuscript are solely the responsibility of the authors and do not necessarily represent the official views of the Bill and Melinda Gates Foundation or the Wellcome Trust.

AUTHORS' CONTRIBUTIONS

All authors contributed to initial study concept and study design. HA, NB and PGF are the principal investigators of the ZAMSTAR study, into which this study is nested. They oversaw participant recruitment and data collection. MM and EDT contributed to the management and supervision of data collection. SLB and SF performed the data analysis. SLB wrote initial drafts and all authors contributed to final editing of the paper.

ACKNOWLEDGEMENTS

We thank all the members of all study teams who contributed to data collection. We thank the Zambia Ministry of Health, City of Cape Town Health Directorate, the Western Cape Provincial Department of Health, and Zambian and South African National TB Programmes and all the district health managers and health facility staff for allowing us to work in the facilities. We thank the Community Advisory Boards and the members of the community who participated in the trial. We acknowledge the support and scientific guidance of the CREATE consortium leadership, R Chaisson (principal investigator for CREATE) and L Moulton (coprincipal investigator for CREATE biostatistics core) and of the ZAMSTAR Study Advisory Group; M Borgdorff, D Enarson, B Chirwa, P Mwaba, Y Mulla, L Mvusi, M Poolman, I Toms and K Jennings.

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Figure 1: Number and flow of participants and cases in this cross sectional study in Zambia and the Western Cape of South Africa

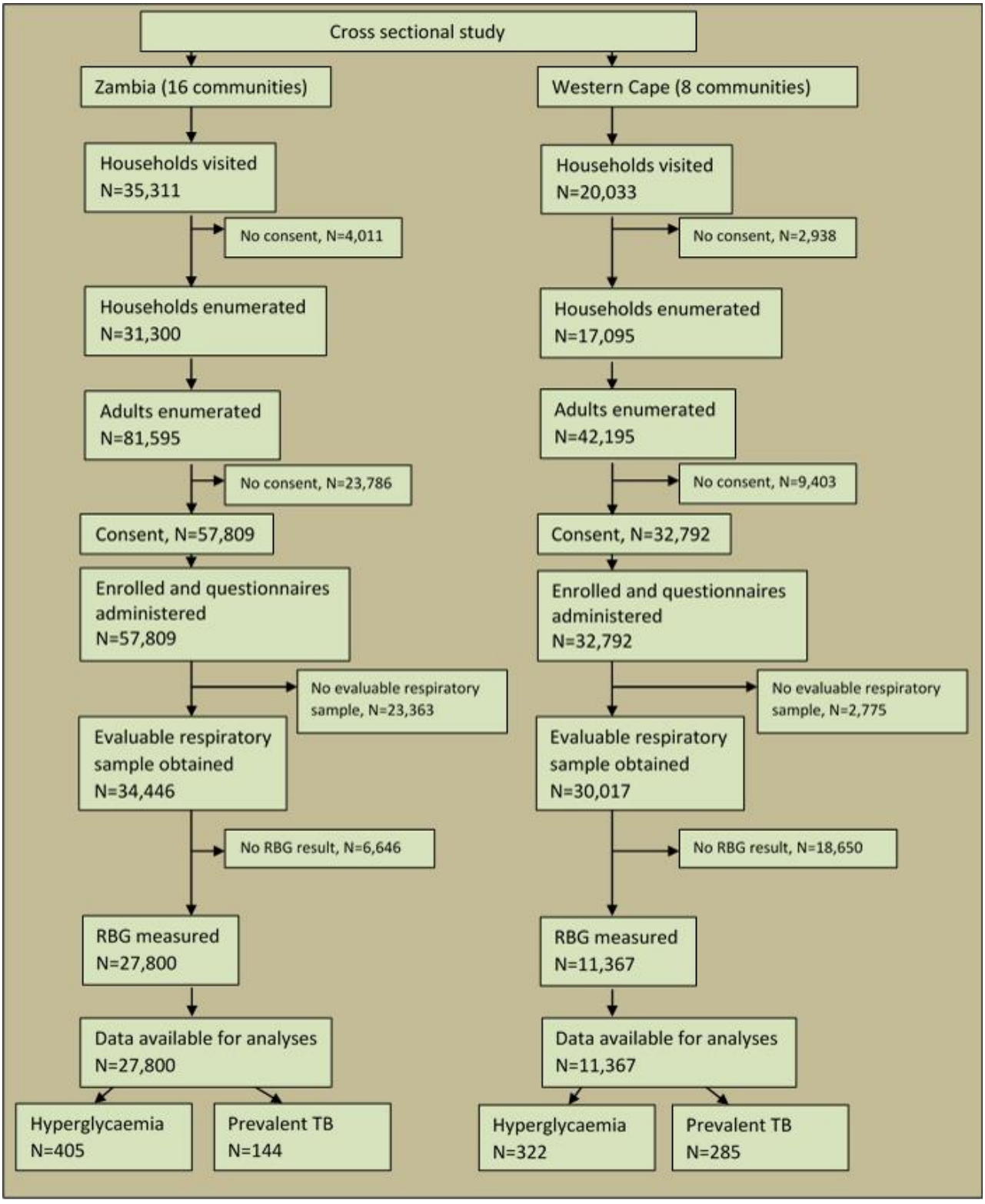

Table 1: Logistic regression estimates of the unadjusted and adjusted odds ratios of prevalent tuberculosis in the Zambian study sites

Characteristic		Total number (%)	Number (%) with prevalent TB	Unadjusted OR (95% CI)	P-value[#]	Adjusted[##] OR (95% CI)	P-value[#]
Overall		27,800 (100)	144 (0·5)	-	-	-	-
Random blood glucose concentration (mmol/L)	<5.6	14,741 (53.0)	67 (0.5)	1	0.029 (test for linear trend p=0.017; test for departure from trend p=0.164)	1	0.011 (test for linear trend p=0.006; test for departure from trend p=0.141)
	5.6-6.9	8,628 (31.0)	42 (0.5)	1.06 (0.72-1.56)		1.13 (0.75-1.71)	
	7.0-8.9	3,447 (12.4)	24 (0.7)	1.51 (0.94-2.41)		1.6 (0.96-2.66)	
	9.0-11.0	579 (2.1)	9 (1.6)	3.44 (1.7-6.96)		4.31 (2.07-8.97)	
	>11.0	405 (1.5)	2 (0.5)	1.06 (0.26-4.35)		0.97 (0.13-7.14)	
Age (years)	18-24	9,733 (35·4)	37 (0·4)	1	0·004	1	0·390
	25-29	4,665 (17·0)	32 (0·7)	1·79 (1·11-2·88)		1.27 (0.75-2.16)	
	30-34	3,438 (12·5)	28 (0·8)	2·15 (1·31-3·52)		1.42 (0.83-2.46)	
	35-39	2,381 (8·7)	16 (0·7)	1·79 (0·99-3·24)		1.09 (0.57-2.09)	
	40-49	3,173 (11·5)	20 (0·6)	1·67 (0·97-2·89)		1.04 (0.56-1.92)	
	50-59	2,113 (7·7)	7 (0·3)	0·87 (0·39-1·96)		0.74 (0.32-1.72)	
	60+	1,998 (7·3)	4 (0·2)	0·55 (0·20-1·55)		0.49 (0.16-1.45)	
Sex	Male	9,265 (33·3)	64 (0·7)	1	0·003	1	0·008
	Female	18,535 (66·7)	80 (0·4)	0·60 (0·43-0·84)		0.59 (0.41-0.87)	
HIV status	Negative	21,103 (82·5)	72 (0·3)	1	<0·001	1	<0·001
	Positive	4,465 (17·5)	66 (1·5)	4·22 (3·00-5·92)		3.57 (2.44-5.23)	
Body Mass Index (weight(kg)/ height^2(m))	Healthy weight (18.5-24.9)	16,497 (64.2)	95 (0.6)	1	<0.001	1	<0.001
	Underweight (<18.5)	2,604 (10.1)	27 (1.0)	1.87 (1.22-2.89)		1.70 (1.09-2.65)	
	Overweight (25-29.9)	4,402 (17.1)	13 (0.3)	0.49 (0.27-0.88)		0.56 (0.3-1.05)	
	Obese (≥30)	2,214 (8.6)	1 (0.1)	0.07 (0.01-0.53)		0.10 (0.01-0.76)	
Region	Rural, low ARTI	7,712 (27·7)	24 (0·3)	1	0·012	1	0·002
	Urban (non-Lusaka), low ARTI	6,033 (21·7)	34 (0·6)	1·83 (1·02-3·29)		2.13 (1.12-4.04)	
	Urban (non-Lusaka), high ARTI	5,158 (18·6)	22 (0·4)	1·39 (0·74-2·63)		1.59 (0.81-3.14)	
	Lusaka, high ARTI	8,897 (32·0)	64 (0·7)	2·34 (1·37-3·98)		2.88 (1.62-5.11)	

TB=tuberculosis; OR=odds ratio; CI=confidence interval; ARTI=annual risk of infection; All analyses accounted for the two-stage clustered sampling design through the use of a logistic regression model with random effects for enumeration area and inclusion of region as a fixed effect, with each region including 4 communities; [#]Likelihood ratio tests; [##]23,414 participants included in analysis (299 missing data for age, 2,083 for BMI, 2,232 for HIV status), adjusted for variables shown plus household socioeconomic position and education.

Table 2: Logistic regression estimates of the unadjusted and adjusted odds ratios of prevalent tuberculosis in the Western Cape study sites

Characteristic		Total number (%)	Number (%) with prevalent TB	Unadjusted OR (95% CI)	P-value[#]	Adjusted[##] OR (95% CI)	P-value[#]
Overall		11,367 (100)	285 (2·5)	-	-	-	-
Random blood glucose concentration (mmol/L)	<5.6	6,068 (53.4)	146 (2.4)	1	0.149 (test for linear trend p=0.328; test for departure from trend p=0.121)	1	0.059 (test for linear trend p=0.098; test for departure from trend p=0.096)
	5.6-6.9	3,464 (30.5)	89 (2.6)	1.06 (0.81-1.39)		1.14 (0.85-1.53)	
	7.0-8.9	1,269 (11.2)	35 (2.8)	1.14 (0.78-1.66)		1.15 (0.76-1.76)	
	9.0-11.0	244 (2.2)	2 (0.8)	0.34 (0.08-1.40)		0.42 (0.10-1.76)	
	>11.0	322 (2.8)	13 (4.0)	1.71 (0.95-3.06)		2.49 (1.29-4.79)	
Age (years)	18-24	2,953 (26·0)	59 (2·0)	1	0·059	1	0·014
	25-29	1,650 (14·5)	40 (2·4)	1·19 (0·79-1·79)		1.25 (0.80-1.95)	
	30-34	1,325 (11·7)	24 (1·8)	0·89 (0·55-1·45)		0.81 (0.47-1.39)	
	35-39	1,175 (10·3)	39 (3·3)	1·67 (1·11-2·52)		1.70 (1.07-2.69)	
	40-49	1,902 (16·7)	52 (2·7)	1·36 (0·93-1·99)		1.34 (0.87-2.08)	
	50-59	1,302 (11·5)	42 (3·2)	1·63 (1·09-2·44)		2.11 (1.31-3.39)	
	60+	1,052 (9·3)	29 (2·8)	1·39 (0·88-2·20)		1.72 (0.96-3.06)	
Sex	Male	3,743 (32·9)	116 (3·1)	1	0·006	1	0·841
	Female	7,624 (67·1)	169 (2·2)	0·71 (0·56-0·90)		0.97 (0.73-1.29)	
HIV status	Negative	8,768 (82·3)	164 (1·9)	1	<0·001	1	<0·001
	Positive	1,889 (17·7)	97 (5·1)	2·83 (2·18-3·66)		2.96 (2.23-3.93)	
Body Mass Index (weight(kg)/ height^2(m))	Healthy weight (18.5-24.9)	4,302 (39.0)	132 (3.1)	1	<0.001	1	<0.001
	Underweight (<18.5)	605 (5.5)	53 (8.8)	2.98 (2.13-4.17)		3.04 (2.14-4.34)	
	Overweight (25-29.9)	2,609 (23.7)	56 (2.2)	0.69 (0.50-0.95)		0.58 (0.41-0.83)	
	Obese (≥30)	3,510 (31.8)	34 (1.0)	0.31 (0.21-0.45)		0.25 (0.16-0.39)	
Community	SA1	1,131 (10·0)	29 (2·6)	1	0·308	1	0·351
	SA2	2,193 (19·3)	49 (2·2)	0·84 (0·49-1·42)		0.85 (0.47-1.55)	
	SA3 (rural)	190 (1·7)	3 (1·6)	0·61 (0·18-2·15)		0.51 (0.11-2.39)	
	SA4	1,618 (14·2)	57 (3·5)	1·33 (0·79-2·25)		1.49 (0.82-2.73)	
	SA5	1,164 (10·2)	20 (1·7)	0·64 (0·34-1·22)		0.71 (0.33-1.51)	
	SA6 (rural)	2,489 (21·9)	65 (2·6)	1·01 (0·61-1·67)		1.01 (0.57-1.78)	
	SA7	1,557 (13·7)	40 (2·6)	1·02 (0·59-1·75)		1.05 (0.55-1.99)	
	SA8	1,025 (9·0)	22 (2·2)	0·83 (0·45-1·52)		0.86 (0.43-1.72)	

TB=tuberculosis; OR=odds ratio; CI=confidence interval; All analyses accounted for the two-stage clustered sampling design through use of a logistic regression model with random effects for enumeration area and inclusion of community as a fixed effect; [#]Likelihood ratio tests; [##]10,336 participants included in analysis (8 missing data for age; 341 for BMI; 710 for HIV status), adjusted for variables shown plus household socioeconomic position and education.

Table 3: Logistic regression estimates of prevalent tuberculosis and population attributable fractions of prevalent tuberculosis to hyperglycaemia for sequential random blood glucose concentration cut-offs

Random blood glucose concentration (mmol/L)	Total number (%)	Number (%) with prevalent TB	Unadjusted OR (95% CI)	P-value[#]	Adjusted OR (95% CI)*	P-value[#]	PAF (95% CI) of prevalent TB to hyperglycaemia (%)
ZAMBIA							
<7.0	23,369 (84.1)	109 (0.5)	1	0.012	1	0.007	7.16 (2.51-11.59)
≥7.0	4,431 (15.9)	35 (0.8)	1.68 (1.14-2.46)		1.82 (1.20-2.75)		
<7.8	25,444 (91.5)	123 (0.5)	1	0.017	1	0.018	4.12 (0.90-7.24)
≥7.8	2,356 (8.5)	21 (0.9)	1.84 (1.15-2.93)		1.94 (1.16-3.25)		
<9.0	26,816 (96.5)	133 (0.5)	1	0.022	1	0.007	2.28 (0.12-4.38)
≥9.0	984 (3.5)	11 (1.1)	2.25 (1.21-4.19)		2.86 (1.46-5.60)		
<11.1	27,395 (98.5)	142 (0.5)	1	0.921	1	0.818	0.00 (0.00-3.21)
≥11.1	405 (1.5)	2 (0.5)	0.93 (0.23-3.79)		0.80 (0.11-5.85)		
WESTERN CAPE							
<7.0	9,532 (83.9)	235 (2.5)	1	0.525	1	0.386	2.34 (0.00-7.08)
≥7.0	1,835 (16.1)	50 (2.7)	1.11 (0.81-1.51)		1.17 (0.82-1.66)		
<7.8	10,320 (90.8)	255 (2.5)	1	0.417	1	0.383	1.66 (0.00-4.96)
≥7.8	1,047 (9.2)	30 (2.9)	1.18 (0.80-1.73)		1.22 (0.79-1.89)		
<9.0	10,801 (95.0)	270 (2.5)	1	0.787	1	0.308	1.35 (0.00-3.55)
≥9.0	566 (5.0)	15 (2.7)	1.08 (0.63-1.83)		1.37 (0.77-2.44)		
<11.1	11,045 (97.2)	272 (2.5)	1	0.099	1	0.015	1.64 (0.28-2.99)
≥11.1	322 (2.8)	13 (4.0)	1.67 (0.94-2.96)		2.38 (1.26-4.50)		

*TB=tuberculosis; OR=odds ratio; CI=confidence interval; PAF=population attributable fraction; All analyses accounted for the two-stage clustered sampling design through the use of a logistic regression model with random effects for enumeration area and inclusion of region or community as a fixed effect; Negative PAFs were given a value of zero; [#]Likelihood ratio tests; *Adjusted for age, sex, HIV status, body mass index, household socioeconomic position and education.*

Table 4: Combined adjusted odds ratios of prevalent tuberculosis for Zambia and Western Cape and associated population attributable fractions of prevalent tuberculosis to hyperglycaemia for sequential random blood glucose concentration cut-offs

Random blood glucose concentration cut-off (mmol/L)	Combined adjusted OR	P-value[#]	I^2 p-value	PAF (95% CI) of prevalent TB to hyperglycaemia (%)
7.0	1.40 (1.07-1.84)	0.013	0.112	4.57 (1.27-7.77)
7.8	1.48 (1.06-2.07)	0.020	0.176	2.82 (0.64-4.95)
9.0	1.87 (1.21-2.90)	0.005	0.104	1.84 (0.56-3.11)
11.1	2.15 (1.17-3.94)	0.013	0.306	0.99 (0.12-1.85)

*OR=odds ratio; CI=confidence interval; PAF=population attributable fraction; ORs combined through fixed-effects meta-analysis; [#]Likelihood ratio tests; All analyses accounted for the two-stage clustered sampling design through the use of a logistic regression model with random effects for enumeration area and inclusion of region or community as a fixed effect; Negative PAFs were given a value of zero; *Adjusted for age, sex, HIV status, body mass index, household socioeconomic position and education.*

Table 5: Population attributable fraction of prevalent tuberculosis to hyperglycaemia for Zambian and Western Cape communities, stratified by age, using random blood glucose concentration cut-off 11.1mmol/L

Age (years)	Zambia		Western Cape	
	Hyperglycaemia prevalence (%)	PAF (95% CI)	Hyperglycaemia prevalence (%)	PAF (95% CI)
18-24	0.42	0.00 (0.00-0.96)	0.27	0.16 (0.00-0.52)
25-29	0.51	0.00 (0.00-1.16)	0.42	0.24 (0.00-0.70)
30-34	1.22	0.00 (0.00-2.72)	0.75	0.43 (0.00-1.05)
35-39	1.18	0.00 (0.00-2.63)	2.04	1.18 (0.07-2.28)
40-49	2.27	0.00 (0.00-4.95)	4.26	2.47 (0.67-4.24)
50-59	4.69	0.00 (0.00-9.88)	7.76	4.50 (1.70-7.22)
60+	4.60	0.00 (0.00-9.70)	8.65	5.01 (1.97-7.97)
Total	1.45	0.00 (0.00-3.21)	2.83	1.64 (0.28-2.99)

PAF=population attributable fraction; CI=confidence interval; Hyperglycaemia defined as a random blood glucose concentration ≥11.1mmol/L; Negative PAFs were given a value of zero.

Aims and methods

Chapter 5: Statement of problem, research questions and rationale

1 Statement of problem

Systematic reviews and meta-analyses of studies exploring the relationship between tuberculosis (TB) and diabetes mellitus (DM) suggest that the incidence of active TB is two to three times higher in those with DM compared to those without DM;[1-3] that DM increases the risk of death from active TB[4] and that DM may increase the risk of TB relapse.[2, 4] The context of these associations in sub-Saharan Africa (SSA) differs from the rest of the world in two ways. Firstly, the prevalence of HIV in Africa is more than five times higher than in any other world region.[5] Secondly, the prevalence of undiagnosed diabetes is high (≥40%)[6] and those who are diagnosed have a high prevalence of poorly controlled diabetes (73% in a recent study in Cameroon).[6, 7]

When exploring these associations between DM and TB it is important to be certain that those diagnosed with diabetes truly have diabetes and do not instead have stress-induced hyperglycaemia secondary to infection with tuberculosis. Prior studies have frequently failed to distinguish between these two causes of hyperglycaemia.

The number of adults with diabetes in SSA is predicted to rise from 14.2 million in 2015 to 34.2 million in 2040.[6-10] To appropriately respond to this now and prepare for a future higher prevalence of diabetes, a deeper understanding of associations between tuberculosis, diabetes mellitus, stress hyperglycaemia and HIV in the context of SSA is needed.

2 Study Questions

2.1 Overall Study Question

In the sub-Saharan Africa context of high HIV prevalence and poor diabetes control, how does hyperglycaemia affect tuberculosis control?

2.2 Specific Study Questions

1. Does HIV modify the association between hyperglycaemia and active tuberculosis?
2. Is there a dose-response relationship between hyperglycaemia and tuberculosis treatment outcome?
3. What proportion of hyperglycaemia among newly-diagnosed tuberculosis cases is due to diabetes mellitus rather than to stress-induced hyperglycaemia?
4. How accurate for the diagnosis of diabetes mellitus are measures of random blood glucose, fasting blood glucose and glycated haemoglobin concentrations measured at the time of tuberculosis diagnosis, compared to the reference standard of fasting blood glucose concentration measured after stabilisation of tuberculosis disease.

3 Research Aims

1. To determine if HIV modifies the association between hyperglycaemia and active tuberculosis.
2. To determine whether a dose-response relationship exists between hyperglycaemia and tuberculosis treatment outcome.
3. To determine the proportion of hyperglycaemia among newly-diagnosed tuberculosis cases that is due to diabetes mellitus rather than to stress-induced hyperglycaemia.
4. To evaluate the accuracy of random blood glucose, fasting blood glucose and glycated haemoglobin concentrations measured at the time of tuberculosis diagnosis for the diagnosis of diabetes mellitus as determined by the reference standard of fasting blood glucose concentration measured after stabilisation of tuberculosis disease.

4 Rationale

The rationale and justification for undertaking these studies lie in the need to improve current understanding of associations between diabetes, stress hyperglycaemia, TB and HIV in sub-Saharan settings. Little is known about these associations in Africa and so these studies will provide new knowledge with specific relevance to this region. The studies will complement

previous and current studies into TB-diabetes associations elsewhere in the world, and are anticipated to justify and propel further research by quantifying the magnitude and strength of associations and the impact on TB treatment outcome. If we find that there are strong and deleterious associations between diabetes, TB and HIV, this study will highlight the need for an intensified focus on diabetes among TB populations (with or without HIV). Furthermore, if diabetes control is found to be negatively associated with tuberculosis treatment outcome, this would suggest that better management of diabetes could improve TB treatment outcomes. This would be beneficial not only to tuberculosis control but also for raising the importance of diabetes control. Should, however, the results show only a minimal association between diabetes and TB/HIV and/or TB treatment outcome in this sub-Saharan setting this would also be useful information to have obtained for public health planning and allocation of future health and research resources.

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Chapter 6: Research Design and Methods

1 Overview

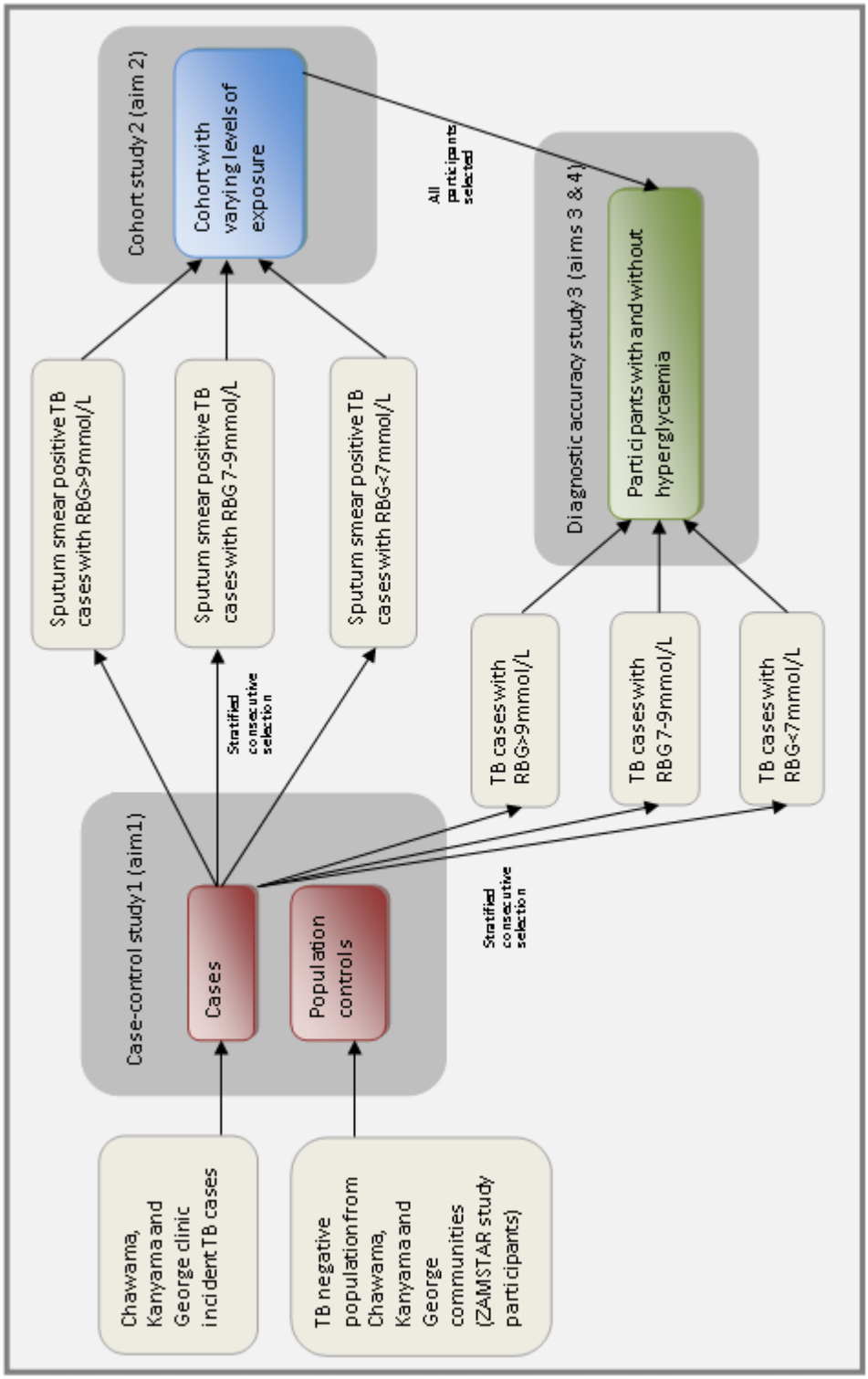
To address the four study aims, three separate but connected studies were undertaken.

- Study 1 addresses aim 1. This was an unmatched case-control study. The study outcome was diagnosed active TB disease and the exposure of interest was hyperglycaemia. Cases with TB were recruited from TB clinics in Lusaka; data for the controls were taken from a recently conducted population-based cross-sectional study (the ZAMSTAR prevalence survey; Appendix I). The analysis explores HIV as a potential effect modifier.
- Study 2 addresses aim 2. This was a cohort study among sputum smear-positive pulmonary TB patients. The exposure was hyperglycaemia, measured by glycated haemoglobin. The outcome was TB treatment outcome, measured by two different recognised surrogate biomarkers: time to sputum culture positivity and time to sputum culture conversion. A subset of cases from study 1 were recruited to study 2 using stratified selection based on random blood glucose concentration, aiming to optimise study power.
- Study 3 addresses aims 3 and 4: This was a study of diagnostic accuracy. All participants of study 2 were also recruited to study 3, along with a further subset of study 1 participants. Stratified selection based on random blood glucose concentration was again used to increase the power of the study. A reference standard test for diabetes diagnosis was performed to distinguish between diabetes and stress-induced hyperglycaemia. This reference standard test was fasting blood glucose concentration measured after stabilisation of TB disease (3 months after TB treatment initiation). Three different tests for diabetes diagnosis taken at the time of TB diagnosis (the index tests) were each compared to the reference standard test, to determine which most

accurately diagnoses diabetes at the time of TB diagnosis. The three index tests used were random blood glucose, fasting blood glucose and glycated haemoglobin.

A pictorial overview of the studies and how they connect to each other is shown in Figure.

Figure 1: Schematic diagram to show participant flow through the connected studies



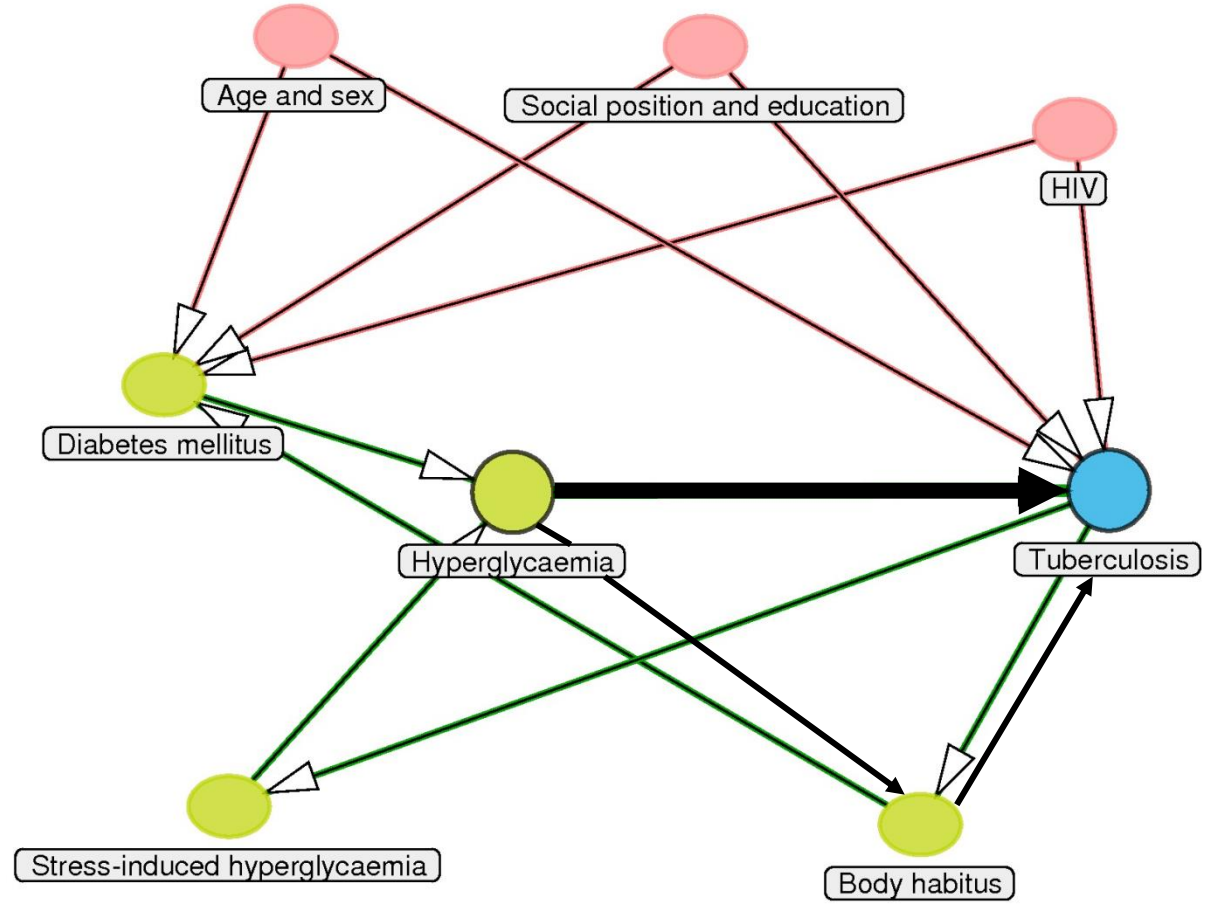
ZAMSTAR = The Zambia South Africa TB and AIDS Reduction study

2 Conceptual Framework

To aid understanding of potential confounding factors, a conceptual framework to link study exposures to outcomes was drawn. This is shown in Figure 2.

For study 1, the exposure was hyperglycaemia and the outcome was diagnosed active tuberculosis disease. Potential measureable confounding factors in this setting were age, sex, household socio-economic position, education and HIV status. HIV status was also considered to be a potential effect modifier. For study 2, the exposure was degree of hyperglycaemia and the outcome was tuberculosis treatment outcome, measured by two different recognised surrogate biomarkers: time to sputum culture positivity and time to sputum culture conversion. The potential confounders are the same as for study 1. Study 3 explores the immediate ancestors to hyperglycaemia, diabetes mellitus and stress-induced hyperglycaemia. This latter ancestor provides a route for reverse causality for the association between hyperglycaemia and tuberculosis. Body habitus has a unique relationship with TB and diabetes. Low body habitus has a causal association with TB incidence and is also an effect of TB disease. Low body habitus is a risk factor for poor TB outcome, but is also a consequence of severe TB disease which is independently a risk factor for poor TB outcome. High body habitus (particularly central/truncal obesity) has a causal association with insulin resistance and diabetes, and thus secondarily with hyperglycaemia, but low body habitus can be an effect of hyperglycaemia from uncontrolled diabetes. Conceptualising body habitus as a potential confounding factor is therefore not satisfactory but was measured in order to explore these associations.

Figure 2: Conceptual framework for studies



Key

exposure outcome ancestor of exposure ancestor of exposure *and* outcome

causal path reverse causality path potentially biasing path

3 Design and methods: study 1

3.1 Study design

Study 1 was an unmatched case-control study. This design provided a cost-effective and time-efficient method for addressing aim 1, to determine if HIV modifies the association between hyperglycaemia and diagnosed active tuberculosis disease.

3.2 Study population

The study took place in Lusaka, Zambia from September 2013 until September 2015, among adults (≥18 years of age) living in the compounds of Chawama, George and Kanyama. These are three areas of the city that have a high incidence of tuberculosis and a high prevalence of HIV. The health centre TB clinics in these three areas collectively receive around 4,000 newly-diagnosed TB patients per year, around 65% of whom are HIV positive.

3.3 Sample size calculations

The sample size calculations for study 1 (table 1) assumed the following: a hyperglycaemia prevalence of 3.2% in the non-TB control population (calculated from the ZAMSTAR prevalence survey data, defined as a random blood glucose (RBG) concentration of ≥11.1, Appendix I); a hyperglycaemia prevalence of between 2 and 3 times this among the active TB population (as suggested by systematic review of prior published studies); an HIV prevalence among incident TB cases of 65% (estimates from TB clinic routine data in Chawama, George and Kanyama, unpublished data), and an odds ratio between HIV strata of between 2 and 3 (taken to be the minimum difference deemed to be clinically important, given that no data existed at the time to guide this estimate).

Table 1: Sample size calculations for study 1, for the range of hyperglycaemia prevalence anticipated to be in the active TB population

1 – α	Power	Hyperglycaemia prevalence in controls	Hyperglycaemia prevalence in cases	Sample size required to test primary association*	Odds ratio between HIV strata	Sample size required for effect modification*
0.95	0.9	3.2%	6.4%	998	2	3992
0.95	0.9	3.2%	8%	521	2.5	2084
0.95	0.9	3.2%	9.6%	336	3	1344
0.95	0.8	3.2%	6.4%	761	2	3044
0.95	0.8	3.2%	8%	400	2.5	1600
0.95	0.8	3.2%	9.6%	259	3	1036

α = probability of a Type I error; *minimum sample size required for each group (cases and controls); rounded up to be conservative

The above estimates were based on sample size equations for the primary association using Stata version 12 and the rule of multiplication by 4 for testing for interaction. Estimates determined by simulation of expected data were comparable, using Stata version 12 to create the simulation followed by repeated sampling from the simulated data, testing for interaction and finally counting the proportion of p-values for interaction that fell below the level of significance.

These sample size estimates, using disease prevalence and anticipated odds ratios, are relevant for standard case-control analysis with exposure and outcome data in binary categories. Given that hyperglycaemia was defined here by a single RBG concentration, it was possible to undertake additional analyses with glycaemia in ordered categories or as a continuous variable. This was valuable for interpretation and clinical applicability. Sample size calculations performed for these scenarios resulted in smaller sample size estimates. As studies of effect modification are frequently underpowered the most conservative estimates (largest sample sizes) were chosen.

Therefore, recruitment of 4,000 participants to each group was anticipated to give >90% power to detect effect modification at the 5% level of significance. The final number of participants was 3,843 cases and 6,977 controls. The prevalence of hyperglycaemia, defined as a random blood glucose concentration ≥11.1mmol/L, was 1.4% among cases and 1.5% among controls, substantially lower than predicted. However, the difference in odds ratios between HIV strata was larger than anticipated (among individuals with HIV, the adjusted odds of TB was 5.47 times higher in those with RBG ≥11.1mmol/L compared to those with RBG <11.1mmol/L; among individuals without HIV this odds ratio was 1.17) and so the study still resulted in sufficient power to detect effect modification.

3.4 Subjects: selection and definitions

Cases for study 1 were adults with diagnosed TB attending the TB clinic at either Chawama, George or Kanyama health centre who had not yet started TB treatment or were within 2 days of starting TB treatment. All eligible potential participants were identified in the TB clinic and invited to participate during a routine clinic attendance. Recruitment ceased when the sample size had been reached. A TB case was defined as any person presenting to a TB clinic with a clinical diagnosis of TB (pulmonary or extra-pulmonary) with or without microbiological confirmation of TB and prescribed a full course of antitubercular chemotherapy, based on the World Health Organization (WHO) TB diagnostic criteria (Appendix III). This is the definition used for TB diagnosis in the study clinics as well as elsewhere in Zambia and much of sub-Saharan Africa and so will facilitate translation of the study results to the clinical setting. It also facilitated recruitment of sputum smear negative and extra-pulmonary tuberculosis cases that did not have microbiological confirmation, but at the same time allowed for sub-group analysis of bacteriologically confirmed (smear or Xpert MTB/RIF positive) cases.

- Inclusion criteria for cases: Newly-diagnosed case of tuberculosis due to commence on a full course of antitubercular chemotherapy.

- Exclusion criteria for cases: <18 years of age, commenced TB treatment >2 days ago.

4,000 new TB patients per year are expected to attend the clinics in Chawama, George and Kanyama, but allowing for 5% who meet the exclusion criteria, 10% who are unwilling to participate, and time for investigator leave, it was anticipated to take up to 21 months to reach the required sample of 4,000. It actually took 24 months to reach the required sample size.

Unmatched controls were adults without TB sampled randomly from all adults living in the clinic catchment area (the compounds of Chawama, George and Kanyama). A cross-sectional survey had recently been conducted comprising approximately 35,000 participants from 16 communities throughout Zambia, including from the Lusaka compounds of Chawama, George and Kanyama, to measure the primary endpoint of prevalent TB in a large cluster randomised trial (total population 1.2 million; the ZAMSTAR study). Along with laboratory diagnosed TB and HIV data, participants have data on RBG concentration. These data have been used to describe the prevalence of hyperglycaemia in Zambia and to quantify the relationship between hyperglycaemia and prevalent TB, as discussed in Chapters 3 and 4. These data were used to provide population controls for study 1. There was therefore no need to repeat data collection among members of the local community. Participants of the ZAMSTAR prevalence survey were randomly sampled within each community using a 2-stage cluster sampling technique. Each community was divided into enumeration areas each containing roughly 1,000 adults. Enumeration areas were randomly selected until 4,000 participants were enumerated. All households of all enumeration areas were eligible and all adults within all households were eligible. Any adult who spent the preceding 24 hours with a household (defined as a group of people who usually eat together and sleep in the same dwelling) were defined as living in the household and were eligible for inclusion.

- Inclusion criteria for controls: ZAMSTAR prevalence survey participants who live in the compounds of Chawama, George or Kanyama.

- Exclusion criteria for controls: <18 years of age; diagnosed prevalent TB at the time of prevalence survey (culture positive sputum for mycobacterium tuberculosis);

diagnosed incident TB at the time of prevalence survey (self-report); previous TB within the year preceding study data collection (self-report).

3.5 Activities

Day 1 of enrolment

1. Newly diagnosed tuberculosis patients attending Chawama, George and Kanyama clinics identified through consultation with clinic staff and clinic records.
2. Individual written informed consent obtained from those meeting inclusion criteria and willing to participate.
3. Structured questionnaire administered.
4. Height, weight and waist circumference measured.
5. Random blood glucose concentration measured.
6. HIV status recorded. If no documented current HIV status available, an HIV test with pre- and post-test counselling as per national guidelines was performed.
7. For HIV +ve participants, CD4 count recorded. If no documented current CD4 count available, CD4 count measured.

3.6 Data collection methods: definitions and measurement

The exposure, hyperglycaemia, was measured by a single capillary RBG concentration using an Optium Xceed point-of-care glucometer for controls and an Accu-Chek Aviva point-of-care glucometer for cases. Manufacture of the Optium Xceed glucometer had been discontinued between the start of data collection for controls and cases. The Accu-Chek Aviva was identified as being a similar and well-performing point-of-care glucometer and was therefore chosen for use instead.[1-4] Measuring an RBG concentration is simple, quick, inexpensive, minimises participant inconvenience (the test requires one finger-prick drop of blood) and enables use of available ZAMSTAR data for the controls. It is not as sensitive for diabetes diagnosis as other measures of glycaemia, such as fasting blood glucose (FBG) or glycated haemoglobin (HbA_{1c}) concentrations, but analysing the data with glycaemia as an ordered categorical or continuous variable will enable assessment of any shift in mean and cumulative proportion of glucose

concentration between cases and controls, rather than having a cut-off definition for hyperglycaemia. Avoiding analysis solely by using a cut-off definition removes potential misclassification error that could exist with the use of this less sensitive measurement.

All research staff were trained on the use of the glucometers and were required to undergo proficiency testing. Standardised control solution was used for performance checks on test strips and meters. The time of last oral intake (excluding water) was recorded. RBG concentration was measured during daylight hours and throughout the year for both cases and controls, so spanning all seasons. For the Accu-Chek Aviva glucometer, intra- and inter-operator variability was measured to assess the validity of glucose measurement. All research assistants contributed to the assessment, using known normoglycaemic volunteers and a standard hyperglycaemic specimen. For intra-operator variability each research assistant repeated the test 5 consecutive times on a combined total of 13 subjects/specimens. For inter-operator variability research assistants each performed the test on a single subject/specimen in the same place at the same time for 7 subjects/specimens.

The potential effect modifier, HIV status, was taken from documented results in TB clinic records. As part of the Zambian National Tuberculosis Control Programme, this was routinely measured in all new-TB cases at the time of TB diagnosis. If no test has been performed since their TB diagnosis (unless already known to be HIV positive) this was measured using point-of-care rapid blood-based kits. Determine™ HIV-1/2 was used as the first line test and Uni-Gold™ HIV as a confirmatory test for individuals with a positive Determine test. Pre- and post-test counselling was offered. Participants who were positive on both tests were referred to the local HIV clinic for management in existing HIV services. Participants who tested HIV negative were counselled and advised to repeat the test as per national guidelines.

In order to adjust for potential confounding factors, a structured questionnaire was administered to all cases by a member of the research team to obtain information on age, sex, household socio-economic position and education level. This information was entered directly

onto a pre-programmed personal digital assistant (PDA) by the researcher. Principal components analysis was used to create a measure of household socio-economic position using the following information: main type of dwelling; main type of flooring; main type of household toilet; main source of household drinking water, and presence of household assets including radio, television, refrigerator, bicycle, motorcycle, car, domestic worker and mobile phone.

Data on baseline characteristics and potential confounding factors for control participants was taken from the ZAMSTAR questionnaire data. All questions, measurement tools (HIV testing kits but not glucometers) and test standard operating procedures were identical for cases and controls.

To allow for sub-group analyses, the type of TB diagnosis (smear positive, smear negative pulmonary or non-pulmonary) was recorded.

Research staff were trained by the principal investigator (PI) on all aspects of data collection and were required to follow standard operating procedures for glucose, HIV and CD4 testing and for measurement of anthropometrics. Research staff were monitored weekly initially and then monthly by the PI for quality control through repetition of non-invasive data collection on 2 randomly selected participants, and observation of invasive sample collection in another 2 randomly selected participants. A pilot study was performed on the first 10 participants recruited to test all data collection tools and procedures.

3.7 Data management and statistical analysis

All point-of-care measurements, test results and questionnaire data were entered directly onto a PDA by research staff. Pre-programmed software was used to ensure that only valid data could be entered and that all data points had an appropriate entry.

With glycaemia as a binary exposure variable, logistic regression was used to quantify the magnitude and strength of association between hyperglycaemia and active tuberculosis.

Interaction terms were used to assess for effect modification by HIV. In addition to defining participants as hyperglycaemic or normoglycaemic, a shift in proportions of glycaemia over the range measured was assessed. Using the binary variable of active tuberculosis as the outcome and an ordered categorical variable of random blood glucose concentration as the exposure, logistic regression was used to further explore the association between hyperglycaemia and active tuberculosis. Interaction terms were again used to assess for effect modification by HIV, classified as a binary variable. We intended to repeat this using hyperglycaemia as a continuous variable if the analysis showed no departure from linear trend. Crude and adjusted odds ratios were calculated. Results are presented in table format in Chapter 7.

In addition to stratification by HIV status, pre-specified sub-group analyses were: stratification by CD4 count, stratification by use of antiretroviral medication (as a proxy for viral load), stratification by type of tuberculosis (smear positive, smear negative pulmonary or non-pulmonary, and also as a binary pulmonary/non-pulmonary variable) and restriction of the outcome definition to microbiologically-confirmed tuberculosis only. The CD4 count sub-groups have been grouped into 4 groups: HIV –ve, CD4>350, CD4 200-350, CD4<200. The use of antiretroviral medication is a binary variable. Aside from stratification by HIV status, sub-group analyses do not have sufficient power for hypothesis testing, but rather are analysed for the purposes of hypothesis generation.

Potential bias was assessed using chi-squared tests of association of glycaemia with time of last oral intake before measurement and time of day of measurement of RBG concentration. Intra- and inter-operator variability were assessed for absolute agreement using a two-way mixed-effects model to calculate intraclass correlations (ICCs).

4 Design and methods: study 2

4.1 Study design

A prospective cohort study allowing for longitudinal follow-up addressed aim 2, to determine whether a dose-response relationship exists between hyperglycaemia and tuberculosis treatment outcome. Hyperglycaemia was determined by glycated haemoglobin level. Tuberculosis treatment outcome was assessed using two surrogate biomarkers: time to initial sputum culture positivity (TTP) and time to sputum culture conversion (TTC).

4.2 Study population

The study took place from September 2013 to December 2015 among smear-positive pulmonary tuberculosis cases who were about to be (or were newly) commenced on antitubercular therapy attending Chawama, George or Kanyama TB clinic in Lusaka, Zambia.

4.3 Sample size calculations

Table 3 gives the predicted sample sizes required for assessing the effect of glycaemia on the surrogate biomarker for tuberculosis treatment outcome of time to initial sputum culture positivity for a range of differences in mean time to culture positivity between the ordered categorical glycaemia groups, assuming a mean time to culture positivity of 7 days (standard deviation 5 days).[52] The differences chosen were the minimum differences deemed to be clinically important.

Table 2: Sample size estimates for study 2: estimates required for analysis of the effect of glycaemia (as an ordered categorical variable) on time to initial sputum culture positivity

1 – α	Power	Mean time to culture positivity (days)	Standard deviation from mean time to culture positivity (days)	Difference in means between groups (days)	Sample size required for group 1*	Sample size required for group 2*	Sample size required for group 3*
0.95	0.9	7	5	3	59	59	59
0.95	0.9	7	5	4	33	33	33
0.95	0.9	7	5	5	22	22	22
0.95	0.8	7	5	3	44	44	44
0.95	0.8	7	5	4	25	25	25
0.95	0.8	7	5	5	16	16	16

α=probability of a Type I error; *rounded up to be conservative; group 1=HbA_{1c}<6.5%, group 2=HbA_{1c} 6.5-9%, group 3=HbA_{1c} ≥9%

Therefore, assuming that 10% were lost to follow-up and 5% were smear positive but culture negative, it was predicted that 70 participants from each group (210 in total) would need to be recruited to give 90% power to detect the minimum clinically important difference at the 5% level of significance. Sample size estimates calculated for survival analysis using a cox proportional hazards model to assess the effect of glycaemia on time to initial sputum culture positivity achieved comparable numbers.

Table 4 gives the sample sizes required for assessing the effect of glycaemia on the surrogate biomarker for tuberculosis treatment outcome of time to culture conversion for a range of differences in mean time to culture conversion between the ordered categorical glycaemia groups, assuming a mean time to culture conversion of 32 days (standard deviation 12 days).[52] The differences chosen were the minimum differences deemed to be clinically significant.

Table 3: Sample size estimates for study 2: estimates required for analysis of the effect of glycaemia (as an ordered categorical variable) on time to sputum culture conversion

1 – α	Power	Mean time to culture conversion (days)	Standard deviation from mean time to culture conversion (days)	Difference in means between groups (days)	Sample size required for group 1*	Sample size required for group 2*	Sample size required for group 3*
0.95	0.9	32	12	6	85	85	85
0.95	0.9	32	12	7	62	62	62
0.95	0.9	32	12	8	48	48	48
0.95	0.8	32	12	6	63	63	63
0.95	0.8	32	12	7	47	47	47
0.95	0.8	32	12	8	36	36	36

α=probability of a Type I error; *rounded up to be conservative; group 1=HbA_{1c}<6.5%, group 2=HbA_{1c} 6.5-9%, group 3=HbA_{1c} ≥9%

Therefore, assuming that 10% were lost to follow-up and 5% were smear positive but culture negative, it was predicted that 100 participants from each group (300 in total) would need to be recruited to give 90% power to detect the minimum clinically important difference at the 5% level of significance. Sample size estimates calculated for survival analysis using a cox proportional hazards model to assess the effect of glycaemia on time to sputum culture conversion achieved comparable numbers.

The final number of participants was 173, though 12 had missing data for random blood glucose concentration and 13 had missing data for baseline glycated haemoglobin. As the prevalence of hyperglycaemia was much lower than anticipated in the population from where the participants were coming, there were only 10 participants with HbA_{1c} ≥6.5, of whom 4 had HbA_{1c} ≥9.0.

The overall mean time to culture positivity of the initial sputum sample was 7.1 days (standard deviation, sd, 3.9 days). Individuals with HbA_{1c} <6.5 had a mean time to culture positivity of 7.3

days days (sd 4.0 days). For individuals with HbA_{1c} 6.5-9.0, this value was 6.5 days (sd 3.9 days), and for individuals with HbA_{1c} ≥9.0 this was 6.0 days (sd 0.9 days). The overall mean time to culture conversion was 30.9 days (sd 18.5 days). Individuals with HbA_{1c} <6.5 had a mean time to culture conversion of 30.8 days (sd 18.3 days). For individuals with HbA_{1c} 6.5-9.0, this value was 25.2 days (sd 24.0 days), and for individuals with HbA_{1c} ≥9.0 this was 47.3 days (sd 15.5 days). As the number of participants with hyperglycaemia was so low, this study lacked power to detect any true difference between groups, even when the groups were re-categorised to have larger numbers in each group.

4.4 Subjects: selection and definitions

Participants were recruited from among the cases of study 1. All eligible potential participants were identified in the TB clinic and invited to participate during a routine clinic attendance. Recruitment ceased when study 1 ceased, regardless of whether or not the number of participants had reached the required size.

- Inclusion criteria were: study 1 participant, smear-positive pulmonary tuberculosis case within 2 days of starting anti-tuberculosis therapy.
- Exclusion criteria were: <18 years of age, smear-negative pulmonary tuberculosis, non-pulmonary tuberculosis

A stratified sampling technique was used to recruit participants from across the glycaemic range. The level of glycated haemoglobin was our glycaemic exposure of interest, as this best reflects the average blood glucose concentration over the time period of interest. However, there are no suitable point-of-care devices to measure HbA_{1c}, and so we were unable to determine participants' HbA_{1c} level at the time of recruitment. For this reason we used RBG concentration as a proxy to HbA_{1c} level to aid recruitment and attempt to recruit participants from across the glycaemic range. We aimed to achieve roughly equal numbers of participants with HbA_{1c} <6.5, 6.5-9.0 and ≥9.0. We therefore based recruitment on RBG concentrations <7.0mmol/L, 7.0-9.0mmol/L and >9.0mmol/L. The first 91 participants with RBG<7mmol/L who

were eligible and willing to be enrolled were recruited. All 42 participants with RBG 7-9mmol/L and all 28 participants with RBG >9mmol/L were recruited.

4.5 Activities

Day 1 of enrolment

1. Written informed consent obtained from consecutive eligible study 1 participants within the three strata of RBG concentration who were willing to participate.
2. Venous blood sample taken for measurement of HbA_{1c} concentration.
3. Sputum specimen collected.

Weekly for 8 weeks post-enrolment

4. Sputum specimen collected.

Week 12 post-enrolment

5. Second venous blood sample taken for measurement of HbA_{1c} concentration.

6 months post-enrolment (or earlier if appropriate)

6. TB register reviewed to determine final TB treatment outcome.

4.6 Data collection methods: definitions and measurement

The exposure, glycaemic control, was measured by HbA_{1c} level, a continuous variable. This was measured at baseline and 12 weeks later.

The outcome, tuberculosis treatment outcome, was assessed using two surrogate biomarkers: time to initial sputum culture positivity (TTP) and time to sputum culture conversion (TTC). TTC is a longstanding recognised and validated surrogate marker for tuberculosis relapse[6]; TTP, which reflects bacterial load and therefore degree of sputum smear positivity, has more recently been validated to predict 2-month sputum culture conversion and relapse.[7, 8]

Results from the first sputum sample were used to determine TTP; results from the subsequent eight sputa determined TTC. For tuberculosis treatment outcome measured by TTP, the HbA_{1c} concentration measured at the time of enrolment (at the time of tuberculosis

treatment commencement) was used to assess exposure status. This best reflects glycaemic concentration leading up to collection of the initial sputum sample. For tuberculosis treatment outcome measured by TTC, the HbA_{1c} concentration measured at 12 weeks into tuberculosis treatment was used to assess exposure status. This reflects glycaemic concentration over the time that TTC was measured.

Data on baseline characteristics and potential confounders were taken from the questionnaire administered in study 1. Potential confounding factors included age, sex, household socio-economic position, and education level attained.

Pin-prick capillary blood was taken for fasting blood glucose measurement and analysed using a point-of-care glucometer (Accu-Chek Aviva). Research staff performed all tests within the TB clinics.

Research staff took a venous blood sample for HbA_{1c} tests. These samples were transported by research staff daily to the ZAMBART laboratory for processing. The samples were processed by Zambart laboratory staff using an Abbott Architect i system.

Sputum samples were collected at the TB clinics at the time of enrolment and then weekly for 8 weeks. Research staff were trained to instruct participants on adequate expectoration to achieve a lower rather than upper airways sample. The samples were transported daily in a cooler box with cold packs to the laboratory and then processed daily using automated mycobacterial culture on liquid media. Each sample was decontaminated using the NaOH/NALC method (final concentration of NaOH = 1.5%), concentrated and then inoculated on to two BD MGIT (Mycobacteria Growth Indicator Tube) culture tubes. MGIT tubes were incubated in the BACTEC MGIT 960 instrument until the instrument detected growth. If the instrument had not declared them to be positive after 42 days the cultures were manually inspected for growth. Any showing growth such as cloudy or crumby particles were treated as a positive sample. Cultures with no visible growth were classified as negative. For MGIT

cultures with growth, a Ziehl-Neelsen stain for acid-fast bacilli (AFB) was performed. Samples with AFB negative stains were classified as contaminated. Samples with AFB positive stains were further identified using the Capilia TB-Neo assay, a rapid immunochromatographic test that confirms the presence of MTB-Complex in the culture supernatant. Capilia positive samples were classified as *Myocbacterium tuberculosis* (MTB). Capilia negative samples were classified as non-tuberculous mycobacteria (NTM). For these participants, the Hain GenoType Mycobacterium CM line-probe assay was used to confirm the presence/absence of MTB-Complex and the presence/absence of an NTM, and to identify the NTM species, if possible. If the CM assay could not identify the NTM species, the Hain GenoType Mycobacterium AS line-probe assay was used.

All contact with study participants took place during routine TB clinic attendances.

Standard operating procedures were followed by field and laboratory staff for collection and processing of all specimens (Appendices IV). Research staff were trained by the PI on all aspects of data collection and were monitored by the PI for quality control. A pilot study was conducted on the first 10 participants recruited to test all data collection tools and procedures.

4.7 Data management and statistical analysis

As per study 1, all point-of-care test results were entered directly onto pre-programmed PDAs by research staff. Laboratory staff entered laboratory results directly into a database using a laboratory data management system.

Cox-proportional hazards models were used to analyse both outcome measures, with HbA_{1c} data in predefined categorical groups (<6.5, 6.5-9.0, >9.0mmol/L; based on clinically relevant categories) and as a linear variable. Results are presented in table and line graph format in Chapter 8.

Stratification by HIV status is the only pre-specified sub-group analysis, and as per study 1 is used for purposes of hypothesis generation rather than hypothesis testing due to insufficient study power for sub-group analyses.

5 Design and methods: study 3

5.1 Study design

A study of diagnostic accuracy addressed aims 3 and 4, to distinguish between DM and stress-induced hyperglycaemia and evaluate the accuracy for DM of measures of glycaemia at the time of tuberculosis diagnosis.

5.2 Study population

This study took place from September 2013 until December 2015 among newly-diagnosed adult cases of tuberculosis (pulmonary and non-pulmonary) who were about to be (or had newly been) commenced on antitubercular therapy at Chawama, George and Kanyama TB clinics in Lusaka, Zambia.

5.3 Sample size calculations

The sample size calculations for study 3 were based on simple estimates of precision for a calculated proportion, using the formula $n = \frac{\left(1.96^2 p(1-p)\right)}{d^2}$ where n=sample size, p=proportion and d=precision. The proportions of interest were measures of index test accuracy (sensitivity, specificity, positive and negative predictive values) as determined by the reference standard. Table 4 shows sample size estimates for a range of anticipated test sensitivities assuming an acceptable level of precision of 0.05.

Table 4: Sample size estimates for study 3

Precision	Index test sensitivity+	Sample size required to calculate proportion*	Total sample size required*#
0.05	0.80	246	492
0.05	0.85	196	392
0.05	0.9	139	278
0.05	0.95	73	146
0.05	0.99	16	32

+Estimates apply equally to other measures of test accuracy (specificity, positive predictive value or negative predictive value); *rounded up to be conservative; #double that required to calculate proportion as stratified sampling aims to recruit roughly half of participants with diabetes mellitus and half without.

Therefore, assuming that 10% were lost to follow-up, it was predicted that 547 participants would need to be recruited to measure the sensitivity (or other measure of test accuracy) of each index test to within a precision of +/-5%. The final number of participants was 490; 205 had a RBG <7.0mmol/L, 179 had a RBG 7.0-8.9mmol/L and 106 had a RBG ≥9.0mmol/L. However, only 8 participants were confirmed to have diabetes mellitus and so this study had poor precision for determining test sensitivity and positive predictive values but good precision for determining test specificity and negative predictive values.

5.4 Subjects: selection and definitions

Participants were recruited from studies 1 and 2.

- Inclusion criteria were: study 1 or 2 participant, tuberculosis case within 2 days of starting antitubercular therapy.
- Exclusion criteria are: <18 years of age

The diagnosis of a case of tuberculosis was again based on WHO guidelines (Appendix II). Pulmonary and non-pulmonary cases of TB were eligible, with or without microbiological confirmation.

As studies 2 and 3 share some of the same activities, participants of study 2 were simultaneously recruited to study 3 when recruited to study 2: the 173 participants recruited to study 2 were included in study 3, with a further 317 recruited directly from study 1.

To simplify recruitment and aim for roughly similar numbers of participants with and without true diabetes, the same stratified sampling technique was implemented for study 3 as for study 2. Therefore, the first 205 willing participants from study 1 with a RBG concentration of <7mmol/L were recruited to study 3; all 179 participants from study 1 with a RBG concentration of 7-9mmol/L and all 106 with a RBG concentration >9mmol/L were recruited.

5.5 Activities

Day 1 of enrolment

1. Potential participants identified
2. Individual written informed consent obtained from potential participants who were willing to be recruited
3. Venous blood sample taken for measurement of HbA_{1c} concentration
4. Participants informed of procedures for fasting blood glucose measurement

Day 2 following enrolment

5. Fasting blood glucose concentration measured

Week 12 post-enrolment

6. FBG measurement repeated

5.6 Data collection methods: definitions and measurement

The index tests were RBG, FBG and HbA_{1c} concentration measured at the time of TB diagnosis. The reference standard test for definitive diabetes diagnosis was FBG concentration measured after stabilisation of TB disease.

Study 2 subjects followed all procedures necessary for both studies 2 and 3 without duplicating procedures (measurement of HbA_{1c} concentration) that were required for both. Participants who were recruited directly from study 1 completed all procedures.

Standard operating procedures based on WHO guidelines were followed for measurement of fasting blood glucose. Capillary blood was sampled and glucose concentration measured using a point-of-care glucometer (Accu-Check Aviva).

The same procedures as for study 2 were followed for measurement of HbA_{1c} concentration.

The reference standard test was measured at 3 months post-treatment commencement, by which time it was anticipated that the participants' acute TB disease should have stabilised.

The diabetes diagnostic criteria for the reference standard test, fasting blood glucose measured after stabilisation of tuberculosis disease, followed the WHO guidelines (Appendix III) i.e. a FBG concentration ≥7.0mmol/L was classified as diabetes.

5.7 Data management and statistical analysis

Analysis for aim 3 required descriptive frequency and percentage statistics only, to give the proportions of hyperglycaemic newly-diagnosed tuberculosis cases (measured separately by RBG, FBG and HbA_{1c}) who truly have diabetes mellitus (measured by FBG after stabilisation of TB disease) rather than stress-induced hyperglycaemia secondary to infection (or other tuberculosis/non-diabetes cause of initial hyperglycaemia).

Analysis for aim 4 required calculation of measures of test accuracy as determined by the reference standard test. Four measures were calculated for each index test: sensitivity, specificity, positive predictive value and negative predictive value.

Results are presented in tabular format in Chapter 9.

6 Project Management

6.1 Personnel – functions and roles

The PI developed all study forms (patient information sheets, informed consent forms, the questionnaire, all SOPs, participant referral letter, diabetes fact sheet); developed the PDA programme for real-time data entry; coordinated submission for ethics approval; recruited and

managed research nurses; oversaw all study activities and data collection; instigated quality control measures; analysed all data, and reported all study findings. Six research assistants recruited participants, administered questionnaire, conducted all point-of-care tests and organised collection of blood and sputum specimens for transport to the laboratory. Ministry of Health employed TB nurses based in the government TB clinics performed phlebotomy to collect the blood samples. Zambart drivers transported specimens from the clinics to the laboratory. Zambart laboratory staff processed specimens and inputted laboratory results to the database. The primary supervisor oversaw study development and execution and provided local liaison to facilitate running of the studies. The associate supervisors provided advice on aspects of the study design and logistics when required. The statistician provided advice on study design, sample size calculations and final data analyses.

6.2 Follow-up procedures

Follow-up of participants was necessary for studies 2 and 3. Follow-up either took place during routine TB clinic visits or through consultation with participant TB clinic records, except for collection of the initial FBG sample. For this attendance, therefore, participants were reimbursed for the cost of their travel to the clinic. For participants who did not attend the clinic for their routine visits and therefore missed their follow up appointments, attempts were made to contact them and request they attend for follow up. Repeated attempts were made via mobile telephone until the participant re-attended or advised that they were unable to re-attend. Participants who did not return to the clinic after exhausting all options for contacting them and encouraging them to return were classified as lost-to-follow-up.

7 Ethical Considerations

The principles of the Helsinki Declaration were taken into account and were followed. The quality of the technical aspects of these studies was assured through rigorous planning and through adopting realistic and achievable methods and goals. Standard operating procedures were used to ensure safe and correct collection of all biological specimens from participants.

Ethics approval was secured prior to study commencement from the University of Zambia Biomedical Research Ethics Committee and the London School of Hygiene and Tropical Medicine Ethics Committee.

Participants may have experienced mild bruising and discomfort associated with capillary and venous blood sampling. Routine safety and comfort measures were incorporated into the SOPs. There were no other hazards resulting from these studies.

All participants who were identified to have an abnormal fasting blood glucose concentration as per WHO guidelines (≥7.0mmol/L; Appendix III; whether at time of TB diagnosis or after TB disease stabilisation) were referred to Chawama/George/Kanyama health centre for further assessment and management. Referral consisted of a participant letter with details of their glucose test results and a diabetes fact sheet for the participant to keep. Close liaison between research and clinic staff facilitated referral of each participant. For participants of study 1 only – who had only a random blood glucose concentration measured – individuals with a RBG≥7.0mmol/L were offered a subsequent FBG and referral following the same procedures if the subsequent FBG was ≥7.0mmol/L.

HIV and CD4 count tests are a routine part of TB management in Lusaka, Zambia. We only performed these tests on participants who had not completed routine TB management procedures. Participant results for these were therefore reported to the TB clinic staff for continuation of management through existing TB and HIV care services, with verbal consent from participants.

The study investigators give assurance that the participants' interests were and will always be prioritised over those of science or society. Formal consent was required of every participant. Each participant received an information sheet detailing the aims and methods of the study and asking if they would like to be included in the study. If they responded favourably they were formally consented and were required to sign two copies of a consent form, one of which

they retained, in order to participate. There was no coercion of those not inclined to participate. Strict confidentiality was maintained throughout the study.

The funding body (the Wellcome Trust) and all investigators have no conflicts of interest to declare.

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Primary research papers

Chapter 7: Study 1 paper: The effect of HIV on the association between hyperglycaemia and active tuberculosis in Zambia, a case-control study

Bailey SL[1,2], Floyd S[1], Yudkin JS[3], Godfrey-Faussett P[1], Ayles H[1,2]

[1]*LSHTM TB Centre and Department of Clinical Research, London School of Hygiene and Tropical Medicine, London, UK*

[2]*Zambart Project, Lusaka, Zambia*

[3]*Division of Medicine, University College London, London, UK*

Corresponding author: Dr Sarah Lou Bailey, slbailey@doctors.org.uk, +442081234489

Word count: 3,454

Abstract

Background: HIV and hyperglycaemia are independently associated with an increased risk of active tuberculosis. However, the dual effect of HIV and hyperglycaemia on the risk of developing tuberculosis disease is unclear. Systematic review identified only two prior studies investigating this. In these studies the effect of HIV on the association between hyperglycaemia and tuberculosis varied depending on the method used to measure hyperglycaemia and on the analysis strategy. Understanding these associations better will help us to respond appropriately to coexistent diabetes mellitus and tuberculosis in countries with a high prevalence of HIV. Therefore, this study aims to determine how HIV modifies or not the association between hyperglycaemia and active tuberculosis in Lusaka, Zambia.

Methods: This is a case-control study among newly-diagnosed adult tuberculosis cases and population controls in three areas of Lusaka. The individual character of interest is hyperglycaemia, determined by random blood glucose (RBG) concentration, measured at the time of recruitment. The outcome of interest is active tuberculosis disease, with cases determined by clinical diagnosis at a National Tuberculosis Programme government clinic. HIV status is determined by serological result. Multivariable logistic regression is used to explore the primary association and effect modification by HIV.

Results: The prevalence of RBG concentration ≥11.1mmol/L among 3,843 tuberculosis cases was 1.4% and among 6,977 controls was 1.5%. Overall, the adjusted odds of active tuberculosis was 1.60 (95%CI 0.91-2.82) times higher among those with RBG concentration ≥11.1mmol/L compared to those with RBG concentration <11.1mmol/L. The corresponding adjusted odds ratio among those with HIV was 5.47 (95%CI 1.29-23.21) and among those without HIV was 1.17 (95%CI 0.61-2.27). The p-value for effect modification by HIV was 0.042. On subgroup analysis, the adjusted odds of smear/Xpert-positive tuberculosis was 2.97 (95%CI 1.49-5.90) times higher among individuals with RBG concentration ≥11.1mmol/L compared to those with RBG concentration <11.1mmol/L. There was no evidence of association when the outcome

was restricted to smear/Xpert-negative or extrapulmonary tuberculosis (ORs 1.27 (95%CI 0.61-2.63) and 1.88 (95%CI 0.58-6.13) respectively).

Conclusions: Overall, no evidence of association between hyperglycaemia and active tuberculosis was found in this study population, though among those with HIV and/or smear/Xpert-positive tuberculosis there was statistical evidence of an association. Differentiation of hyperglycaemia caused by diabetes mellitus and stress-induced hyperglycaemia secondary to tuberculosis infection is important for a better understanding of these findings.

379 words

Keywords: Diabetes mellitus, Africa, Epidemiology

Background

HIV and hyperglycaemia are independently associated with an increased risk of active tuberculosis (TB).[1, 2] However, the dual effect of HIV and hyperglycaemia on the risk of developing TB disease is unclear. It could be that the contribution of hyperglycaemia to TB risk is relatively small in HIV-positive individuals compared with its contribution to TB risk in HIV-negative individuals, as the greatly increased risk of TB among HIV-positive individuals could diminish any additional increased risk from hyperglycaemia. On the other hand, the effect of hyperglycaemia might be exacerbated in the presence of HIV infection if the contributions of each to increased TB risk are synergistic.

Systematic review identifies only two prior studies investigating the effect of HIV on the association between hyperglycaemia or diabetes mellitus (DM) and tuberculosis.[3, 4] Both studies were located in Tanzania and investigated the age- and sex-adjusted association between hyperglycaemia/DM and tuberculosis, stratified by HIV. The effect of HIV on the association varied depending on the analysis strategy used and on the method used to measure hyperglycaemia. Faurholt-Jepsen *et al* found no evidence of effect modification by HIV except for when adjusting the analysis for the acute phase reactant alpha-1-acid glycoprotein, in which case they found a stronger association between DM and active TB among HIV uninfected individuals than among those infected with HIV (p-value for interaction = 0.01).[3] Boillat-Blanco et al found no evidence of effect modification by HIV except for when DM was determined by glycated haemoglobin, in which case again HIV uninfected individuals had the stronger association (p-value for interaction = 0.048).[4] However, the stratified associations reverted to the null when measurement of diabetes was repeated five months after TB treatment initiation.

Global guidelines exist for the care and control of co-existing diabetes mellitus and tuberculosis, but these were developed using evidence from studies largely based in non-African populations and so had little contribution from high HIV prevalence settings.[2, 5, 6] If HIV

does modify the association, this could lead to a different overall association in areas of high HIV prevalence. This is important to understand so that location-specific guidelines can be developed and implemented, to enable the most appropriate use of available resources to optimise the care and control of DM and TB.

Therefore, this study aims to determine how HIV modifies or not the association between hyperglycaemia and active TB in Lusaka, Zambia.

Methods

Study design and setting

This unmatched case-control study took place among adults in three urban communities in Lusaka, Zambia. Each community has a high incidence of TB and a high prevalence of HIV. The exposure of interest was hyperglycaemia and the outcome was diagnosed active TB disease.

Cases

Consecutive newly-diagnosed TB cases who had not yet started TB treatment or were within 2 days of starting TB treatment were recruited from National TB Programme government clinics between September 2013 and September 2015. A TB case was defined as any person presenting to a TB clinic with a clinical diagnosis of TB (pulmonary or extra-pulmonary) with or without microbiological confirmation of TB and prescribed a full course of antitubercular chemotherapy, based on the World Health Organization (WHO) TB diagnostic criteria.[7] This is the definition used for TB diagnosis in the study clinics as well as elsewhere in Zambia and much of sub-Saharan Africa and so use of this definition aimed to facilitate translation of the study results to the clinical setting. At the time of data collection the study clinics were using either sputum smear, Xpert MTB/RIF or both as microbiological tools to diagnose TB, depending on the availability of reagents and equipment. Exclusion criteria for cases were <18 years of age, commenced TB treatment >2 days prior to recruitment or inability to give

consent due to disability/incapacitation. All eligible cases were identified in the TB clinic and invited to participate during a routine clinic attendance.

Controls

Unmatched controls were recruited between January and December 2010 as part of a cross-sectional survey that measured prevalent TB for a large cluster randomised trial (the ZAMSTAR study).[8, 9] The control participants were adults without prevalent TB and who were not on TB treatment on the date they were recruited to the cross-sectional survey. They were sampled randomly from all adults living in the catchment areas of the clinics, using a 2-stage cluster sampling technique within each community. Exclusion criteria for controls were <18 years of age, refusal to submit a respiratory sample, diagnosed TB at the time of the cross-sectional survey (culture positive sputum for *mycobacterium tuberculosis*), currently on treatment for TB at the time of the cross-sectional survey (self-report), inability to give consent due to disability/incapacitation or any persons living in institutional settings. All eligible controls were recruited in their homes.

Data collection

Glycaemia was determined by random capillary blood glucose sampling measured at the time of recruitment. An Optium Xceed point-of-care glucometer was used for controls and an Accu-Chek Aviva point-of-care glucometer was used for cases. Manufacture of the Optium Xceed glucometer had been discontinued between the start of data collection for controls and cases. The Accu-Chek Aviva was identified as being a similar and well-performing point-of-care glucometer and was therefore chosen for use instead. The time of last oral intake (excluding water) was recorded. RBG concentration was measured during daylight hours and throughout the year for both cases and controls, so spanning all seasons. All research staff were trained on the use of the glucometers and were required to undergo proficiency testing. Standardised control solution was used for performance checks on test strips and meters. For the Accu-Chek Aviva glucometer, intra- and inter-operator variability was measured to assess the validity of

glucose measurement. All research assistants contributed to the assessment, using known normoglycaemic volunteers and a standard hyperglycaemic specimen. For intra-operator variability each research assistant repeated the test 5 consecutive times on a combined total of 13 subjects/specimens. For inter-operator variability research assistants each performed the test on a single subject/specimen in the same place at the same time for 7 subjects/specimens. For a subset of cases, fasting blood glucose (FBG) concentration was measured within 3 days of commencement of TB treatment and again 3 months later, to indicate if the hyperglycaemia was transient or persistent. A stratified sampling technique was used to select this subset to give participants with RBG concentrations across the glycaemic range.

The potential effect modifier, HIV, was measured using point-of-care rapid blood-based kits. Determine™ HIV-1/2 was used as the first line test and Uni-Gold™ HIV as a confirmatory test for individuals with a positive Determine test. Pre- and post-test counselling was offered. Participants who tested positive were referred to the local HIV clinic for management in existing HIV services, unless they were already integrated within a service. Self-reported use of antiretroviral therapy (ART) was recorded for individuals who were already known to be infected with HIV.

In order to adjust for potential confounding factors, a structured questionnaire was used to obtain information on age, sex, smoking history, household socio-economic position and education level. Height, weight and waist circumference were measured using standardised methods.

Data were electronically entered directly onto personal digital assistants by research assistants at the time of data collection, using pre-programmed questionnaires and result sheets with error and range checks. All data were downloaded into a SQL (structured query language) database and exported into Stata.

All questions, measurement tools (HIV testing kits but not glucometers) and test standard operating procedures were identical for cases and controls.

Ethics

Each participant was required to give written informed consent. Ethics approval was granted from the London School of Hygiene and Tropical Medicine Ethics Committee and the University of Zambia Biomedical Research Ethics Committee.

Statistical analysis

The sample size for this study was calculated to give sufficient power for determination of effect modification by HIV. Principal components analysis was used to create a measure of household socio-economic position. Hyperglycaemia was initially examined with RBG concentration as an ordered categorical variable. We then used the RBG cut-off 11.1mmol/L to explore hyperglycaemia as a binary variable.

Unadjusted and adjusted odds ratios of the association between hyperglycaemia and active TB were estimated using logistic regression analysis. Interaction terms were used to assess for effect modification by HIV, adjusting for age, sex, socio-economic position, education, body mass index, smoking history and community.

In addition to effect modification by HIV, restriction of the outcome definition separately to sputum smear-positive or Xpert MTB/RIF positive tuberculosis, smear-negative and Xpert MTB/RIF negative pulmonary TB, and extrapulmonary TB were identified *a priori* for sub-group analyses, as was exploration of the impact of ART use on HIV as an effect modifier.

Potential bias was assessed using chi-squared tests of association of glycaemia with time of last oral intake before measurement and time of day of measurement of RBG concentration, each comparing cases to controls. Intra- and inter-operator variability were assessed for absolute agreement using a one-way random-effects model and a two-way random-effects model respectively, to calculate intraclass correlations (ICCs).[10]

Results

There were 3,909 eligible TB cases identified, of which 3,843 (98.3%) consented to participate. There were 11,271 adults randomly selected from the three study communities and enrolled in the ZAMSTAR cross-sectional survey, of which 6,977 (61.9%) were eligible to be control participants for this case-control study (Figure 1).

The characteristics of cases and controls are shown in Table 1. Cases were on average older than controls (p<0.001) and more frequently male (p<0.001). Among cases and controls, 12.9% and 16.2% respectively had a RBG concentration ≥7.0mmol/L, and 1.4% and 1.5% respectively had a RBG concentration ≥11.1mmol/L (Table 1). 65% of cases were living with HIV, compared to 18% of controls.

On unadjusted analysis, there was no evidence of association between hyperglycaemia and tuberculosis with glycaemia as a binary variable. When analysed with RBG concentration as an ordered categorical variable, there was evidence of an association between hyperglycaemia and tuberculosis, but there was strong evidence that the association did not follow a linear trend. We therefore did not examine the association with RBG concentration as a continuous variable. Comparing participants with RBG ≥11.1mmol/L to those with RBG <11.1mmol/L, the unadjusted odds ratio of TB was 0.91 (95% CI 0.65-1.28). The unadjusted odds of TB was 8.74 (95% CI 7.93-9.64) times higher in participants infected with HIV than in uninfected individuals. On unadjusted analysis there was no evidence to suggest that the odds of TB for the effect of hyperglycaemia differed between individuals infected and uninfected with HIV (Table 1).

On adjusting for the effect of age, gender, education, socioeconomic position, body mass index, smoking history and community, there remained no evidence of association between hyperglycaemia and tuberculosis with RBG concentration as a binary variable. As a categorical variable there remained strong evidence of a non-linear association (test for departure from linear trend p<0.001); individuals with RBG concentration 7.0-8.9 mmol/L had a lower odds of TB than individuals in all other categories. This analysis was repeated separately for men and

women: for women the same non-linear direction of association was seen, for men no association was seen. The adjusted odds of TB was 1.60 times higher in those with RBG ≥11.1mmol/L compared to those with RBG <11.1mmol/L (95% CI 0.91-2.82). However, there was evidence that the adjusted association between hyperglycaemia and tuberculosis was different between individuals uninfected and infected with HIV. Among individuals with HIV, the adjusted odds of TB was 5.47 times higher in those with RBG ≥11.1mmol/L compared to those with RBG <11.1mmol/L (95% CI 1.29-23.21). Among individuals without HIV, the adjusted odds of TB was 1.17 times higher for the same comparison (95% CI 0.61-2.27). The p-value for interaction was 0.042. The main confounding factors were age, sex and body mass index. Interaction by age and gender was also seen, after adjusting for HIV, BMI, education, socioeconomic position, smoking history and community. For men aged 25-50 years the adjusted odds of TB was between 4.4 and 6.5 times higher than in men aged 18-25 years. For women aged 25-50 years the adjusted odds of TB was between 1.7 and 3.2 times higher than in women aged 18-25 years (p-value for interaction <0.001).

Of all TB cases, 1,557 (40.9%) had sputum smear or Xpert positive TB, 1,929 (50.6%) had smear or Xpert negative pulmonary TB and 324 (8.5%) had extrapulmonary TB. When the analysis was restricted to these separate categories of TB, there was evidence of association between hyperglycaemia and sputum smear/Xpert-positive tuberculosis (adjusted odds ratio = 2.97, 95% CI 1.49-5.90), and this association was stronger in individuals infected with HIV than in uninfected individuals (p-value for interaction = 0.028, Table 2). There remained no evidence of association between hyperglycaemia and tuberculosis for smear/Xpert-negative TB and extrapulmonary TB, both for the overall association and stratified by HIV (Table 2), though the direction of association was the same for all three categories of TB.

Among individuals infected with HIV, 36.7% of cases and 21.2% of controls were taking ART. When HIV as a variable was re-categorised to include use of ART, there was evidence of association between hyperglycaemia and active tuberculosis among individuals who were HIV

positive and not currently taking ART, but not among individuals who were HIV-positive and taking ART (Table 3).

FBG concentration was measured in 232 participants with TB at baseline and 3 months later. Nine participants had hyperglycaemia at baseline (FBG ≥7.0 mmol/L) and 4 (44.4%) of these had persistent hyperglycaemia 3 months later. Three (75%) of the participants with persistent hyperglycaemia were uninfected with HIV. Four (80%) of the participants with transient hyperglycaemia were infected with HIV.

Participants reported the time of last oral intake prior to measurement of RBG concentration to be a mean of 4.9 hours (standard deviation 4.6 hours). This included all food and drink except water. There was no evidence of a difference in time (< or ≥6 hours) of last oral intake between participants with RBG <11.1 mmol/L and those with RBG ≥ 11.1 mmol/L (p=0.815), nor between individuals with and without TB (p=0.793). All cases had their RBG concentration measured in the morning, between the hours of 7am and 12pm. There was no evidence of a difference in the hour of measurement between participants with RBG <11.1 mmol/L and those with RBG ≥ 11.1 mmol/L (p=0.832).

Assessment of the validity of the Accu-Chek Aviva glucometer measurements gave an intraclass correlation of 0.996 (95% CI 0.991-0.999) for intra-operator variability and 0.983 (95% CI 0.954-0.995) for inter-operator variability.

Discussion

In this case-control study in Lusaka, Zambia, we found no evidence of association between hyperglycaemia and active tuberculosis, except for when TB was restricted to individuals with smear/Xpert-positive pulmonary TB only. There was evidence of effect modification by HIV for the association between hyperglycaemia and active TB. When adjusted for confounding factors, the association was stronger among individuals infected with HIV than among uninfected individuals.

When analysed as an ordered categorical variable with pre-defined categories, there was evidence of a non-linear association between hyperglycaemia and tuberculosis in our study population, as individuals with RBG concentration 7.0-8.9 mmol/L had a lower odds of TB than individuals with lower or higher RBG concentration (p<0.001). This was an unexpected finding and may be due to chance as there is no biological reason to explain this pattern. Aside from this, our primary association findings are in keeping with other studies that investigated the association between hyperglycaemia and tuberculosis in Africa. Haraldsdottir *et al* found no association between DM and active tuberculosis in a case-control study in Guinea-Bissau measuring diabetes in newly-diagnosed TB cases (adjusted odds ratio (OR) of TB for the effect of DM = 0.88, 95% CI [0.17-4.58]).[11] Boillat-Blanco *et al* found a positive association between hyperglycaemia and tuberculosis in Tanzania when hyperglycaemia was measured around the time of TB treatment initiation, but the association disappeared when measurement of diabetes was repeated five months after TB treatment initiation, suggesting that the initial positive association was due to an increase in stress-induced hyperglycaemia among TB cases secondary to acute TB infection rather than due to DM.[4]

Our findings of a stronger association among HIV infected than uninfected individuals could suggest that HIV and hyperglycaemia work synergistically to increase an individual's risk of TB. Another possible explanation is an increase in stress-induced hyperglycaemia among newly-diagnosed TB cases, as seen in Boillat-Blanco's study. It is plausible that the most unwell newly diagnosed TB cases, and therefore the most likely to have stress-induced hyperglycaemia, could be found among individuals with HIV and with smear/Xpert-positive pulmonary TB. This would explain the positive associations seen in our study, and is in keeping with our findings from the subset of cases who had measurement of baseline and follow-up FBG concentration.

This study used a single random blood glucose concentration to measure hyperglycaemia. This method is simple, quick, and minimises participant inconvenience as it requires only one finger-prick drop of blood. It is therefore ideal for use in a large community-based study,

facilitating measurement of glycaemia in control participants in their homes. It is not as sensitive for diabetes diagnosis as other measures of glycaemia, such as fasting blood glucose, 2-hour blood glucose following an oral glucose tolerance test or glycated haemoglobin concentration, and so has likely led to an underestimate of the prevalence of hyperglycaemia. Each of the more sensitive methods would have been challenging to perform on a large scale in the community and could have led to selection bias if they were considered to be unacceptable to healthy control participants. An alternative could have been the use of a clinic-based control population as a proxy to community controls, but this too could have led to selection bias. Our choice to use community controls, measure RBG concentration and analyse the data with glycaemia as an ordered categorical variable has enabled assessment of shifts in proportions of glucose concentration between cases and controls. Avoiding analysis solely by using a binary cut-off definition of hyperglycaemia has limited potential misclassification error that could exist with the use of this less sensitive measurement.

Our definition of hyperglycaemia did not incorporate individuals with previously diagnosed (known) diabetes mellitus who were not hyperglycaemic at the time of measuring their RBG. This was an explicit decision resulting from our prior experience of discussing diabetes with groups and individuals in the community as well as health care workers. It became clear that there is high level of misclassification of known diabetes due to a low level of understanding of diabetes among all groups, and therefore it is difficult to reliably ascertain prior diagnoses of diabetes in our study setting. We therefore chose to focus on a biological measure of hyperglycaemia alone. This could have led to an underestimate of the association between long-term hyperglycaemia and active TB.

Our assessment of intra- and inter-operator variability suggests that measurement of point-of-care random blood glucose among cases in this study was consistent and valid. Additionally to this we explored the possibility of undertaking laboratory validation of glucose measurements by comparing glucometer results to results from laboratory processed venous blood samples

taken simultaneously. This proved not to be possible in our setting, as point-of-care glucose measurement is the principal method for measuring glycaemia, both in the community and centrally. The laboratory alternatives were therefore not equipped to offer a reliable benchmark.

The temporal space between recruitment of controls and cases is another potential source of bias, though the three communities in this study have relatively stable populations and to our knowledge there were no major changes in the prevalence of hyperglycaemia or diabetes, the incidence of TB or the prevalence of HIV during the study period. The use of ART among individuals infected with HIV could feasibly have changed between recruitment of controls and cases: use of ART among the control population would most likely have increased over time. Exploration of the impact of ART on the associations studied showed an association only among individuals with HIV who were not taking ART, and not among individuals taking ART. An increase in use of ART among cases could therefore reduce the overall association seen among individuals with HIV, but an increase in use of ART among controls is unlikely to change the overall association. Further, this pattern fits with our hypothesis that the associations seen in this study could be due to stress-induced hyperglycaemia rather than diabetes. The recruitment gap is therefore unlikely to have introduced bias. However, an unforeseen consequence of the gap was the discontinuation of the initial glucometer model used for control participants. It was therefore necessary to use a different model for case participants. We attempted to choose a similar model to minimise any potential variability between the models, and so any difference in measurement of glycaemia is likely to be small rather than large. All other study procedures were identical for cases and controls.

Control participants are representative of the general population within each community as they were randomly selected from the community. Case participants are representative of TB cases in each community as they were consecutively selected and defined using the same criteria used in the clinical setting. The findings from this study are therefore generalizable to

the study communities and are likely also generalizable to communities elsewhere in Zambia and much of sub-Saharan Africa with similar high incidence of TB and high prevalence of HIV.

Conclusions

Overall, no evidence of association between hyperglycaemia and active tuberculosis was found in this study population, though among those with HIV and those with smear/Xpert-positive pulmonary TB there was evidence of an association. Differentiation of hyperglycaemia caused by diabetes mellitus and stress-induced hyperglycaemia secondary to tuberculosis infection is important to understand these data further.

ACKNOWLEDGEMENTS

We thank all the members of all study teams who contributed to data collection. We thank the Zambian Ministry of Health, the Zambian National TB Programme, the Lusaka District Medical Officer and all health facility staff for allowing us to work in the TB clinics.

AUTHOR CONTRIBUTIONS

All authors contributed to initial study concept and study design. SLB and HA oversaw participant recruitment and data collection. SLB performed the data analysis. SLB wrote initial drafts and all authors contributed to final editing of the paper.

CONFLICTS OF INTEREST

We declare that we have no conflicts of interest.

FUNDING

SLB was supported by a Wellcome Trust Clinical PhD Fellowship (100141/Z/12/Z), and this study was funded by the fellowship. The contents of this manuscript are solely the responsibility of the authors and do not necessarily represent the official views of the Wellcome Trust.

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Figure 1: Number and flow of cases and controls

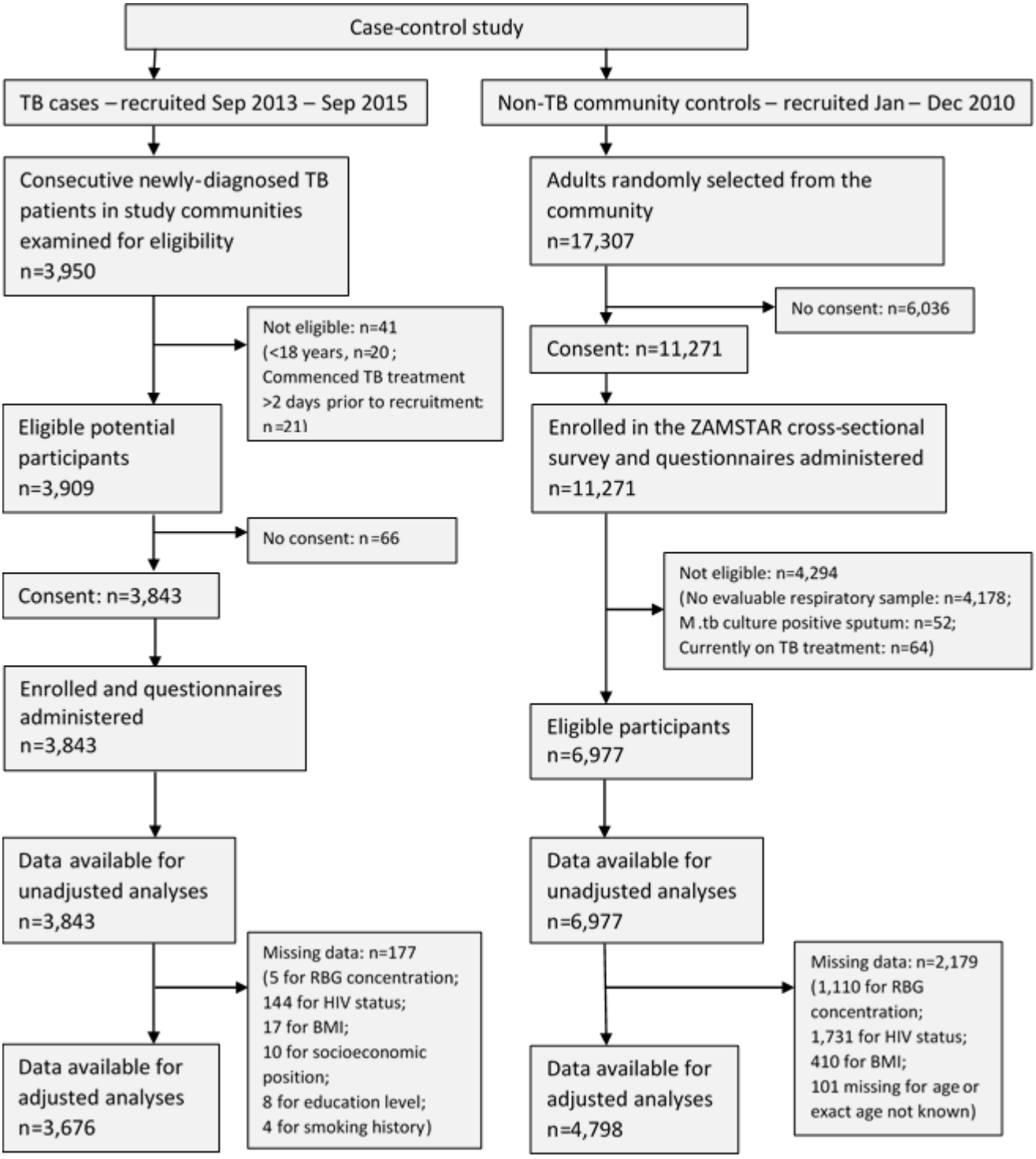

Table 1: Logistic regression estimates of the unadjusted and adjusted odds ratios of tuberculosis, stratified by HIV status

Characteristic		Cases n (%)	Controls n (%)	Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
Overall		3,843 (100)	6,977 (100)	-	-	-	-
Glucose concentration (mmol/L)*	<5.6	2,255 (58.8)	3,069 (52.3)	1	<0.001 TFT: <0.001 DFT: <0.001	1	<0.001 TFT: 0.051 DFT: <0.001
	5.6-6.9	1,089 (28.4)	1,845 (31.5)	0.80 (0.73-0.88)		0.86 (0.74-1.00)	
	7.0-8.9	352 (9.2)	755 (12.9)	0.63 (0.55-0.73)		0.62 (0.50-0.77)	
	9.0-11.0	89 (2.3)	109 (1.9)	1.11 (0.84-1.48)		1.36 (0.84-2.21)	
	>11.0	53 (1.4)	89 (1.5)	0.81 (0.57-1.14)		1.46 (0.83-2.58)	
HIV status*	Uninfected	1,287 (34.8)	4,320 (82.4)	1	<0.001	1	<0.001
	Infected	2,412 (65.2)	926 (17.7)	8.74 (7.93-9.64)		9.35 (8.07-10.85)	
HIV uninfected	<5.6	803 (62.4)	2,258 (53.6)	1	-	1	-
	5.6-6.9	330 (25.7)	1,297 (30.8)	0.72 (0.62-0.83)	<0.001	0.86 (0.71-1.04)	0.125
	7.0-8.9	106 (8.2)	520 (12.3)	0.57 (0.46-0.72)	<0.001	0.63 (0.47-0.85)	0.002
	9.0-11.0	24 (1.9)	71 (1.7)	0.95 (0.59-1.52)	0.832	1.33 (0.71-2.49)	0.372
	>11.0	23 (1.8)	71 (1.7)	0.91 (0.57-1.47)	0.701	1.08 (0.56-2.09)	0.826
HIV infected	<5.6	1,365 (56.6)	447 (49.2)	1	-	1	-
	5.6-6.9	727 (30.2)	316 (34.8)	0.75 (0.64-0.89)	0.001	0.86 (0.68-1.09)	0.209
	7.0-8.9	232 (9.6)	125 (13.8)	0.61 (0.48-0.77)	<0.001	0.61 (0.43-0.85)	0.004
	9.0-11.0	60 (2.5)	16 (1.8)	1.23 (0.70-2.15)	0.474	1.41 (0.66-3.02)	0.371
	>11.0	27 (1.1)	5 (0.6)	1.77 (0.68-4.62)	0.245	4.94 (1.16-21.02)	0.031
Glucose <5.6 mmol/L	HIV uninfected	803 (37.0)	2,258 (83.5)	1	<0.001	1	<0.001
	HIV infected	1,365 (63.0)	447 (16.5)	8.59 (7.51-9.82)		9.26 (7.67-11.19)	
Glucose 5.6-6.9 mmol/L	HIV uninfected	330 (31.2)	1,297 (80.4)	1	<0.001	1	<0.001
	HIV infected	727 (68.8)	316 (19.6)	9.04 (7.56-10.81)		9.26 (7.18-11.95)	
Glucose 7.0-8.9 mmol/L	HIV uninfected	106 (31.4)	520 (80.6)	1	<0.001	1	<0.001
	HIV infected	232 (68.6)	125 (19.4)	9.10 (6.73-12.31)		8.86 (5.85-13.44)	
Glucose 9.0-11.0 mmol/L	HIV uninfected	24 (28.6)	71 (81.6)	1	<0.001	1	<0.001
	HIV infected	60 (71.4)	16 (18.4)	11.09 (5.40-22.79)		9.84 (3.73-25.93)	
Glucose >11.0 mmol/L	HIV uninfected	23 (46.0)	71 (93.4)	1	<0.001	1	<0.001
	HIV infected	27 (54.0)	5 (6.6)	16.67 (5.75-48.30)		42.48 (8.74-206.50)	
Age (years)	18-24	555 (14.4)	2,771 (40.3)	1	<0.001	1	<0.001
	25-29	751 (19.5)	1,222 (17.8)	3.07 (2.70-3.49)		2.68 (2.20-3.26)	
	30-34	861 (22.4)	813 (11.8)	5.29 (4.63-6.04)		3.46 (2.80-4.28)	
	35-39	755 (19.7)	573 (8.3)	6.58 (5.71-7.58)		4.52 (3.59-5.68)	
	40-49	612 (15.9)	727 (10.6)	4.20 (3.65-4.84)		2.96 (2.34-3.74)	
	50+	309 (8.0)	770 (11.2)	2.00 (1.71-2.35)		2.13 (1.66-2.74)	
Sex	Male	2,605 (67.8)	1,955 (28.0)	1	<0.001	1	<0.001
	Female	1,238 (32.2)	5,022 (72.0)	0.19 (0.17-0.20)		0.19 (0.16-0.22)	

Highest level of education	None	387 (10.1)	446 (6.4)	1	<0.001	1	<0.001
	Grade 1-7	1,388 (36.2)	2,474 (35.5)	0.65 (0.56-0.75)		0.42 (0.32-0.56)	
	Grade 8-12	1,969 (51.3)	3,645 (52.2)	0.62 (0.54-0.72)		0.31 (0.24-0.41)	
	College/ University	91 (2.4)	412 (5.9)	0.25 (0.2-0.33)		0.10 (0.07-0.16)	
Smoking history	Never smoked	2,863 (74.6)	6,188 (88.7)	1	<0.001	1	0.326
	Current or ex-smoker	976 (25.4)	789 (11.3)	2.67 (2.41-2.97)		1.10 (0.91-1.32)	
Body Mass Index (weight(kg)/ height^2(m))	Healthy weight (18.5-24.9)	1,985 (51.9)	4,106 (62.5)	1	<0.001	1	<0.001
	Underweight (<18.5)	1,659 (43.4)	541 (8.2)	6.34 (5.68-7.09)		6.49 (5.49-7.68)	
	Overweight (25-29.9)	129 (3.4)	1,265 (19.3)	0.21 (0.17-0.25)		0.24 (0.19-0.31)	
	Obese (≥30)	53 (1.4)	655 (10.0)	0.17 (0.13-0.22)		0.23 (0.16-0.34)	

*OR=odds ratio; CI=confidence interval; TFT = test for linear trend; DFT = test for departure from linear trend; *Estimates from model with no interaction. All other estimates taken from model with HIV interaction; Adjusted analyses adjusted for all variables shown, plus household socioeconomic position and community; 167 cases & 2,179 controls excluded from adjusted analyses due to missing data; p-value for interaction, unadjusted analysis = 0.707; p-value for interaction, adjusted analysis = 0.390;*

Table 2: Logistic regression estimates of the unadjusted and adjusted odds ratios of smear/Xpert-positive, smear/Xpert-negative and extrapulmonary tuberculosis, stratified by HIV status

Characteristic		Cases n (%)	Controls n (%)	Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
Sputum smear/Xpert-positive tuberculosis							
RBG concentration (mmol/L)*	<11.1	1,533 (98.5)	5,778 (98.5)	1	0.911	1	0.003
	≥11.1	23 (1.5)	89 (1.5)	0.97 (0.61-1.55)		**2.97 (1.49-5.90)**	
HIV uninfected	RBG<11.1	588 (98.3)	4,146 (98.3)	1	0.984	1	0.153
	RBG≥11.1	10 (1.7)	71 (1.7)	0.99 (0.51-1.94)		**1.87 (0.79-4.39)**	
HIV infected	RBG<11.1	879 (98.8)	904 (99.5)	1	0.132	1	0.001
	RBG≥11.1	11 (1.2)	5 (0.6)	2.26 (0.78-6.54)		**12.43 (2.63-58.69)**	
Sputum smear/Xpert-negative tuberculosis							
RBG concentration (mmol/L)*	<11.1	1,903 (98.7)	5,778 (98.5)	1	0.593	1	0.518
	≥11.1	26 (1.4)	89 (1.5)	0.89 (0.57-1.38)		1.27 (0.61-2.63)	
HIV uninfected	RBG<11.1	592 (98.2)	4,146 (98.3)	1	0.803	1	0.949
	RBG≥11.1	11 (1.8)	71 (1.7)	1.09 (0.57-2.06)		0.97 (0.42-2.27)	
HIV infected	RBG<11.1	1,260 (98.9)	904 (99.5)	1	0.182	1	0.153
	RBG≥11.1	14 (1.1)	5 (0.6)	2.01 (0.72-5.60)		3.55 (0.62-20.19)	
Extrapulmonary tuberculosis							
RBG concentration (mmol/L)*	<11.1	320 (98.8)	5,778 (98.5)	1	0.675	1	0.324
	≥11.1	4 (1.2)	89 (1.5)	0.81 (0.30-2.22)		1.88 (0.58-6.13)	
HIV uninfected	RBG<11.1	73 (97.3)	4,146 (98.3)	1	0.518	1	0.573
	RBG≥11.1	2 (2.7)	71 (1.7)	1.60 (0.39-6.65)		1.54 (0.34-6.92)	
HIV infected	RBG<11.1	226 (99.1)	904 (99.5)	1	0.576	1	0.321
	RBG≥11.1	2 (0.9)	5 (0.6)	1.60 (0.31-8.30)		2.92 (0.35-24.26)	

*OR=odds ratio; CI=confidence interval; *Estimates from model with no interaction; p-values for interaction: smear/Xpert-positive TB = 0.028, smear/Xpert-negative TB = 0.172, extrapulmonary TB = 0.628*

Table 3: Logistic regression estimates of the unadjusted and adjusted odds ratios of tuberculosis, stratified by HIV status and use of antiretroviral therapy

Characteristic		Cases n (%)	Controls n (%)	Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
RBG concentration (mmol/L)*	<11.1	3,785 (98.6)	5,778 (98.5)	1	0.584	1	0.104
	≥11.1	53 (1.4)	89 (1.5)	0.91 (0.65-1.28)		1.60 (0.91-2.82)	
HIV uninfected	RBG<11.1	1,263 (98.2)	4,146 (98.3)	1	0.800	1	0.574
	RBG≥11.1	23 (1.8)	71 (1.7)	1.06 (0.66-1.71)		1.21 (0.62-2.34)	
HIV infected and currently taking ART	RBG<11.1	878 (99.3)	193 (98.5)	1	0.248	1	0.885
	RBG≥11.1	6 (0.7)	3 (1.5)	0.44 (0.11-1.77)		0.85 (0.09-7.79)	
HIV infected and not currently taking ART	RBG<11.1	1,506 (98.6)	711 (99.7)	1	0.031	1	0.013
	RBG≥11.1	21 (1.4)	2 (0.3)	4.96 (1.16-21.2)		16.79 (1.8-156.75)	

*OR=odds ratio; CI=confidence interval; ART=antiretroviral therapy; *Estimates from model with no interaction.*

Chapter 8: Study 2 paper: The effect of hyperglycaemia on tuberculosis treatment outcome in Lusaka, Zambia; a cohort study

Bailey SL[1,2], Floyd S[1], Munkondya-Gondwe S[2], Mwanza W[2], Maluzi K[2], Cheeba-Lengwe M[2], Chiwele-Kangololo K[2], Kaluba-Milimo D[2], Kosloff B[1,2], Yudkin JS[3], Godfrey-Faussett P[1], Ayles H[1,2]

[1]*LSHTM TB Centre and Department of Clinical Research, London School of Hygiene and Tropical Medicine, London, UK*

[2]*Zambart Project, Lusaka, Zambia*

[3]*Division of Medicine, University College London, London, UK*

Corresponding author: Dr Sarah Lou Bailey, slbailey@doctors.org.uk, +442081234489

Word count: 4,179

Abstract

Background: Hyperglycaemia in individuals with tuberculosis is a risk factor for poor tuberculosis treatment outcome. Diabetes mellitus and hyperglycaemia cause impaired immunity which is more severe for individuals with poor glycaemic control than for those with good glycaemic control. Therefore, we aimed to determine if a dose-response relationship exists between hyperglycaemia and tuberculosis treatment outcome.

Methods: We conducted a prospective cohort study among smear-positive pulmonary tuberculosis cases who were starting antitubercular therapy at three clinics in Lusaka, Zambia. We used a stratified sampling technique to recruit individuals from across the glycaemic range, by screening all potential participants through measurement of their random blood glucose (RBG) concentration. The exposure of interest was glycated haemoglobin (HbA_{1c}). Tuberculosis treatment outcome was assessed using two surrogate biomarkers: time to initial sputum culture positivity (TTP) and time to sputum culture conversion (TTC). Unadjusted and adjusted rate ratios of the association between glycaemia and tuberculosis treatment outcome were estimated using cox-proportional hazards models.

Results: We screened 1,557 individuals with pulmonary tuberculosis, of whom 173 were recruited. Of these, 153 were confirmed on culture to have *Mycobacterium tuberculosis* and were included in the analysis, and 131 (86%) participated in follow up. We identified only 21 individuals with a baseline HbA_{1c} 6.0-6.4% and 9 with HbA_{1c} ≥6.5%. On adjusted cox regression analysis with glycaemia as an ordered categorical variable we found no evidence of association between glycaemia and TTP (RR=1.75, 95%CI 0.73-4.18 comparing participants with HbA_{1c} ≥6.5% to those with HbA_{1c} <6.0%), or between glycaemia and TTC (RR 0.73, 95%CI 0.20-2.60 for the same comparison). We found weak evidence of a linear association between glycaemia and TTP (RR=0.97, 95% CI 0.63-1.49). Using a binary variable to compare individuals with HbA_{1c} ≥6.0% to those with a level <6.0%, there was no evidence of association between glycaemia

and TTP but weak evidence of association between glycaemia and TTC (RR 1.44, 95%CI 0.89-2.33 and RR 0.54, 95%CI 0.27-1.09 respectively).

Conclusions: We found no evidence that a relationship exists between glycaemia as an ordered categorical variable and tuberculosis treatment outcome, though the direction of trend was consistent with a faster time to initial sputum positivity and a slower time to sputum culture conversion for higher levels of glycaemia. We found a low prevalence of hyperglycaemia and so our study was underpowered to detect any true association. This does however suggests that hyperglycaemia is not a major challenge for TB control in this location.

401 words

Keywords: Glycaemic control; Diabetes mellitus; Transient hyperglycaemia; Africa.

Background

Systematic review of the literature suggests that individuals with tuberculosis (TB) and coexistent diabetes mellitus have 1.89 (95% CI 1.36-2.12) times increased risk of death from tuberculosis and 3.89 (95% CI 2.43-6.23) times increased risk of tuberculosis relapse compared to individuals with tuberculosis alone.[1] Transient hyperglycaemia at the time of tuberculosis diagnosis, which was found to normalise after five months of antituberculosis treatment, has also been identified as a risk factor for poor TB treatment outcome:[2] in a study of 530 Tanzanian individuals with tuberculosis, those with a fasting capillary glucose level of ≥6.1 mmol/L at the time of TB diagnosis had 3.32 (95% CI 1.20-9.14) times the adjusted odds of TB treatment failure or death compared to individuals with a fasting capillary glucose of <6.1 mmol/L at the time of TB diagnosis.[2]

Diabetes mellitus causes impaired cell mediated immunity, including T-cell function, which is more severe for individuals with poor glycaemic control than for those with good glycaemic control.[3-7] The differences in cellular cytokine responses to *M. tuberculosis* in TB patients with diabetes compared to TB patients without diabetes are even more marked for patients with poor glycaemic control.[4] Effective cellular immunity is important for controlling growth of *M. tuberculosis*[4, 8, 9] so we might expect to see a dose-response relationship between hyperglycaemia and TB treatment outcome. If this is the case, we could hypothesise that controlling glycaemia during TB treatment could help to improve TB treatment outcome.

Diagnosing diabetes mellitus at the time of active TB infection can be troublesome, as it is difficult to distinguish diabetes from transient hyperglycaemia due to acute infection.[2] In practice, at the time of TB diagnosis, we can distinguish hyperglycaemia from normoglycaemia, either through measurement of blood glucose concentration – reflecting glycaemia at the time the sample was taken – or through measurement of glycated haemoglobin – reflecting the average level of glycaemia over the preceding few weeks. Any hyperglycaemia identified through measurement of either of these could be due to underlying diabetes mellitus or could

be transient from factors such as stress hyperglycaemia from TB infection. We are not able to distinguish diabetes mellitus from transient hyperglycaemia until after resolution of active TB infection; after the window period for potential intervention. For this reason we have chosen to focus on hyperglycaemia at the time of active TB infection irrespective of underlying diagnosis, rather than having a restricted focus solely on diabetes mellitus. Moreover, as transient hyperglycaemia is itself associated with poor TB treatment outcome, the hypothesis that worsening hyperglycaemia could be associated with worsening TB treatment outcome is equally relevant to individuals with transient hyperglycaemia.

We have identified four prior studies that have explored the association between glycaemic control or diabetes control and TB treatment outcome.[10-13] Their findings are conflicting, all but one are retrospective, none are located in Africa and all either exclude participants who are infected with HIV or have a very low prevalence of HIV in their study population. Therefore, we aimed to contribute to the literature on this topic by conducting a prospective study to determine if a dose-response relationship exists between hyperglycaemia and tuberculosis treatment outcome in our study setting of Lusaka, Zambia.

Tuberculosis treatment outcome can be defined in different ways. At the end of treatment, the outcome can be classified as successful, which includes individuals with evidence of microbiological cure and those who have completed treatment, or unsuccessful, which includes individuals with evidence of microbiological treatment failure, those who died, and those who did not complete treatment.[14] Unsuccessful treatment outcome defined as tuberculosis relapse requires measurement at a time point beyond the end of TB treatment. TB treatment outcome can also be measured by numerous surrogate biomarkers at an earlier time point, the most common of which is sputum smear or culture conversion after two months of treatment among individuals with pulmonary tuberculosis.[15-23] This can be measured as a binary variable or as a quantitative time to conversion. The time to sputum culture conversion (TTC) is a longstanding recognised and validated surrogate marker for

tuberculosis relapse as it is a quantifiable measure of response to tuberculosis treatment.[24] Time to initial sputum culture positivity (TTP) at the start of TB treatment reflects bacterial load and therefore degree of sputum smear positivity, so has more recently been validated to predict 2-month sputum culture conversion and relapse.[15, 16]

The use of surrogate markers of TB treatment outcome are particularly useful for settings with low death and treatment failure rates from tuberculosis, such as in our Zambian study setting.[25]

Methods

We conducted a prospective cohort study among smear-positive pulmonary tuberculosis cases who were about to be (or had newly been) commenced on antitubercular therapy at three TB clinics in Lusaka, Zambia. Recruitment took place from September 2013 until September 2015. Participants were followed up for 3 months; data collection finished in December 2015.

Participants

All eligible potential participants were identified in the TB clinics and invited to participate during a routine clinic attendance. Eligible participants were newly-diagnosed cases of smear- or Xpert MTB/RIF-positive tuberculosis due to commence on a full course of antitubercular chemotherapy. Exclusion criteria were <18 years of age, commenced TB treatment >2 days ago, smear/Xpert-negative pulmonary tuberculosis and non-pulmonary tuberculosis. A stratified sampling technique was used to recruit participants from across the glycaemic range. The level of glycated haemoglobin was our glycaemic exposure of interest, as this best reflects the average blood glucose concentration over the time period of interest. However, the only reliable method of measuring glycated haemoglobin in our study setting was a laboratory method, not through use of a point-of-care device, and so we were unable to determine glycated haemoglobin level in potential participants at the time of recruitment. For this reason we used RBG concentration as a proxy to glycated haemoglobin to aid recruitment, as we were

able to determine this at the time of recruitment through use of a point-of-care device. We screened all eligible and willing participants with a random blood glucose (RBG) concentration using an Accu-Chek Aviva glucometer and based stratified recruitment on RBG concentrations <7.0mmol/L and ≥7.0mmol/L. The first 90 participants with RBG <7.0mmol/L and all participants with RBG ≥7.0mmol/L were recruited. Participants were followed up in the TB clinic during routine clinic visits. Sample size calculations were based on estimates for survival analysis using a cox proportional hazards model to assess the effect of glycaemia on both time to initial sputum culture positivity and time to culture conversion. We had aimed to recruit a minimum of 85 participants in each group to give sufficient power to detect the minimum difference deemed to be clinically significant between groups.

The exposure

The exposure, hyperglycaemia, was determined by glycated haemoglobin (HbA_{1c}). Venous blood samples were drawn for HbA_{1c} tests at the time of enrolment and 3 months after commencement of TB treatment. These samples were transported daily from the clinics to the laboratory for storage at 2-8^{o}C before being processed in weekly batches using an Abbott Architect i2000SR analyser. For TB treatment outcome measured by time to initial sputum culture positivity, the HbA_{1c} level measured at the time of enrolment (at the time of tuberculosis treatment commencement) was used to assess exposure status. This best reflects glycaemic concentration leading up to collection of the initial sputum sample. For TB treatment outcome measured by time to sputum culture conversion, the HbA_{1c} level measured at 3 months into tuberculosis treatment was used to assess exposure status. This reflects glycaemic concentration over the time that culture conversion was measured.

All participants who had glycaemia in the diabetes range (HbA_{1c} ≥6.5%) on either test were referred to local health care facilities for follow-up and management.

The outcome

The outcome of interest, tuberculosis treatment outcome, was assessed using two surrogate biomarkers: time to initial sputum culture positivity (TTP) and time to sputum culture conversion (TTC).

Sputum samples were collected at the TB clinics at the time of enrolment and then weekly for 8 weeks. Research staff were trained to instruct participants on adequate expectoration to achieve a lower rather than upper airways sample. The samples were transported daily in a cooler box with cold packs to the laboratory and then processed daily using automated mycobacterial culture on liquid media. Each sample was decontaminated using the NaOH/NALC method (final concentration of NaOH = 1.5%), concentrated and then inoculated on to two BD MGIT (Mycobacteria Growth Indicator Tube) culture tubes. MGIT tubes were incubated in the BACTEC MGIT 960 instrument until the instrument detected growth. If the instrument had not declared them to be positive after 42 days the cultures were manually inspected for growth. Any showing growth such as cloudy or crumby particles were treated as a positive sample. Cultures with no visible growth were classified as negative. For MGIT cultures with growth, a Ziehl-Neelsen stain for acid-fast bacilli (AFB) was performed. Samples with AFB negative stains were classified as contaminated. Samples with AFB positive stains were further identified using the Capilia TB-Neo assay, a rapid immunochromatographic test that confirms the presence of MTB-Complex in the culture supernatant. Capilia positive samples were classified as *Myocbacterium tuberculosis* (MTB). Capilia negative samples were classified as non-tuberculous mycobacteria (NTM). For these participants, the Hain GenoType Mycobacterium CM line-probe assay was used to confirm the presence/absence of MTB-Complex and the presence/absence of an NTM, and to identify the NTM species, if possible. If the CM assay could not identify the NTM species, the Hain GenoType Mycobacterium AS line-probe assay was used.

Results from the baseline sputum sample were used to determine TTP; results from the subsequent seven sputa were used to determine TTC.

Mitigating for potential bias

Data on baseline characteristics and potential confounders were collected through use of a questionnaire, including information on age, sex, household socio-economic position and HIV status.

All data collection methods were identical for all exposure groups. Standard operating procedures were followed by field and laboratory staff for collection and processing of all specimens. Research staff were trained by the PI on all aspects of data collection and were monitored for quality control. All questionnaire data and point-of-care test results were entered at the time of data collection by research staff, directly onto pre-programmed personal digital assistants with error and range checks. Data were downloaded into a SQL (structured query language) database and exported into Stata. Laboratory staff entered laboratory results directly into a database using a laboratory data management system.

Ethics

Each participant was required to give written informed consent. Ethics approval was granted from the London School of Hygiene and Tropical Medicine Ethics Committee and the University of Zambia Biomedical Research Ethics Committee.

Statistical analysis

The exposure variable, glycated haemoglobin, was grouped into predefined categorical groups using clinically relevant cut points (<6.0%, 6.0-6.4% and ≥6.5%). Participants were recruited based on the smear/Xpert result of the sputum sample taken and processed for routine diagnostic purposes in the TB clinics. For analysis, however, each participant was re-classified as having TB, NTM or no mycobacterial disease based on the results of sputum samples collected for this study and processed in our research laboratory. Participants were classified

as having TB if any study sputum sample either at baseline or follow-up was culture positive for *M. tuberculosis* as defined by being AFB positive and Capilia TB positive. Participants were classified as having NTM if any study sputum sample was culture positive for mycobacteria other than tuberculosis as defined by being AFB positive but Capilia TB negative, with subsequent confirmation by the molecular test. Participants were classified as having no mycobacterial disease if all study samples were either MGIT negative, or MGIT positive and AFB negative, indicating sample contamination. Participants with NTM or no mycobacterial disease were excluded from all analyses. For the participants included in the study, each sputum sample was classified as being positive for TB if either or both MGIT cultures were classified as positive for TB, by being AFB and Capilia TB positive. If only one culture was TB positive the TTP for this culture was used in the analysis. If both cultures were positive for TB the mean TTP was calculated and used in the analysis. Sputum samples were classified as negative if both MGIT cultures were classified as negative, or if they were MGIT positive and AFB/Capilia negative. The week of culture conversion was classified as the first week with a negative sputum sample with all subsequent samples remaining negative.

Principal components analysis was used to create a measure of household socio-economic position. Median days to initial sputum culture positivity and median weeks to sputum culture conversion were calculated along with inter-quartile ranges (IQRs). Unadjusted and adjusted rate ratios of the association between glycaemic control and tuberculosis treatment outcome were estimated using cox-proportional hazards models. Potential confounding factors were controlled for through adjusting the survival analysis estimates for age, sex, socio-economic position, community and HIV. Sub-group and interaction analyses were not performed due to insufficient power for these analyses.

Results

There were 1,557 sequential smear-positive TB cases who were screened for eligibility to this cohort study: 173 participants were found to be eligible. All consented for recruitment. After

recruitment, 13 participants had sputum cultures that were persistently negative for mycobacteria and 7 participants were confirmed to have non-tuberculous mycobacterial disease rather than TB. These participants were therefore excluded from the analysis, leaving 153 participants included in the analysis. Of these, 81 (53%) participants had RBG<7mmol/L, 61 (40%) had RBG ≥7mmol/L and 11 (7%) had missing data for RBG concentration. There were 140 (92%) participants who completed the baseline HbA_{1c} test and 88 (56%) who completed the follow-up HbA_{1c} test at 12 weeks. There were 131 (86%) participants who participated in follow up of sputum samples, 97 (63%) completed sputum submission to 7 weeks and 43 (28%) completed sputum submission to 8 weeks. The flow of study participants through recruitment and follow-up is shown in Figure 1.

The median follow-up time for sputum collection was 3 weeks (IQR 1-6 weeks). The combined total follow-up time for all participants submitting sputum samples was 746 weeks. There was no evidence to suggest that participants who completed all follow-up data collection differed in terms of baseline characteristics from individuals with incomplete follow-up data (Table 1).

The participants had a median age of 33 years (IQR 28-38 years). More than two-thirds were male (68.3%) and nearly two-thirds were infected with HIV (62.2%). Of all participants with a baseline HbA_{1c} test, 110 (78.6%) had HbA_{1c} <6.0%, 21 (15.0%) had HbA_{1c} 6.0-6.4% and 9 (6.4%) had HbA_{1c} ≥6.5%. For the follow up HbA_{1c} test, these values were 64 (72.7%), 18 (20.5%) and 6 (6.8%). Two participants reported a prior diagnosis of diabetes mellitus. Neither were on diabetes treatment and both had a baseline HbA_{1c} ≥6.5%.

The median time to positivity of the baseline sputum sample was 6.8 days (IQR 4.9-9.4 days) for participants with a baseline HbA_{1c} <6.0%, 6.0 days (IQR 4.1-8.1 days) for participants with baseline HbA_{1c} 6.0-6.4% and 5.9 days (IQR 4.9-7.1 days) for participants with baseline HbA_{1c} ≥6.5%. Of all participants, 94 (61.4%) achieved documented sputum conversion within the follow-up time. Of the 43 participants who completed sputum submission to 8 weeks, 33 (76.7%) achieved sputum conversion. The median time to sputum culture conversion for

participants with follow-up HbA_{1c} <6.0%, 6.0-6.4% and ≥6.5% was 5 weeks (IQR 4-7 weeks), 6weeks (IQR 3-7 weeks) and 6 weeks (IQR 5-6 weeks) respectively.

On unadjusted cox regression analysis of the baseline data, the rate ratio point estimates for the time to sputum culture positivity of the initial sputum sample collected at the time of TB treatment initiation increase with increasing levels of glycated haemoglobin. However, the confidence intervals are wide and no evidence is seen for an association (p=0.285, Table 2). On unadjusted analysis of the follow-up data there was no evidence of association between glycated haemoglobin level and time to sputum culture conversion (p=0.427, Table 3). Figures 2 and 3 show unadjusted Kaplan-Meier estimates of the time to sputum culture positivity and the time to sputum culture conversion respectively, by glycated haemoglobin level.

Tables 2 and 3 show the cox regression adjusted analyses of the baseline and follow-up data respectively, adjusted for age, sex, socioeconomic position, HIV status and community. The negative confounding factors were age and socioeconomic position. HIV status was a positive confounding factor. There remained no evidence of association between glycated haemoglobin level as a categorical variable and time to sputum culture positivity for the baseline sputum sample (p=0.309), nor between glycated haemoglobin level as a categorical variable and time to sputum culture conversion (p=0.181). The direction of trend was consistent with an association: there was a faster time to initial sputum positivity and a slower time to sputum culture conversion for higher levels of glycaemia, though the RR and p-value for a linear association between glycaemia and time to initial sputum culture positivity showed only weak evidence of a linear association (adjusted RR=0.97 95% CI [0.63-1.49], p=0.125).

Using a binary exposure variable, comparing individuals with glycated haemoglobin ≥6.0% to those with a level <6.0%, there was no evidence of association between glycaemia and TTP of the baseline sputum sample but weak evidence of association between glycaemia and TTC (RR 1.44 95%CI [0.89-2.33], p=0.149 and RR 0.54 95%CI [0.27-1.09], p=0.076 respectively; Tables 2 and 3).

During data collection for this study, 2 participants died, both of whom had a baseline HbA_{1c} <6.0%.

Subgroup analyses were not explored as the number of participants with hyperglycaemia, the risk factor of interest, was low.

Discussion

In this cohort study in Lusaka, Zambia, we found no evidence of association between hyperglycaemia as an ordered categorical variable and two surrogate markers of tuberculosis treatment outcome. Although the direction of trend was consistent with a faster time to initial sputum positivity and a slower time to sputum culture conversion for higher levels of glycaemia, there was weak evidence to support a dose-response relationship between hyperglycaemia and tuberculosis treatment outcome.

However, despite screening over 1,500 smear-positive TB cases we found only 30 individuals with a baseline HbA_{1c} ≥6.0 and only 24 individuals with a follow-up HbA_{1c} ≥6.0. As these were our exposures of interest the study was underpowered to detect an association with TB treatment outcome. Nonetheless these findings suggest that hyperglycaemia is not a major challenge for TB control in our study location.

As our findings do not support the hypothesis that increasing levels of hyperglycaemia are associated with worse TB treatment outcome, they consequently do not support the hypothesis that controlling glycaemia during treatment of tuberculosis could improve TB treatment outcome. As the number of participants with hyperglycaemia was low, however, our findings also do not invalidate these hypotheses; confidence intervals in our study were wide and so we are 95% confident that the rate ratio for association between glycaemia and TTP, comparing participants with HbA_{1c} ≥6.5% to those with HbA_{1c} <6.0%, lies between 0.73 and 4.18. We are 95% confident that the rate ratio for association between glycaemia and TTC

lies between 0.20 and 2.60, also comparing participants with HbA_{1c} ≥6.5% to those with HbA_{1c} <6.0%.

The four prior studies that have explored the association between glycaemic or diabetes control and TB treatment outcome show conflicting results. A retrospective cohort study in South Korea examined the rate of sputum culture conversion at two months for 214 culture-confirmed pulmonary tuberculosis patients.[12] Individuals infected with HIV were excluded. Of 28 participants with uncontrolled diabetes (HbA_{1c}≥7%) 6 had a positive culture at 2 months. Compared to non-diabetics, uncontrolled diabetes was found to be a significant risk factor for a positive sputum culture at 2 months (OR 4.3, 95% CI 1.3-14.3, p=0.017). A retrospective record review in Kerala State, India, reviewed TB treatment outcome at the end of TB treatment for 3,116 patients.[10] Diabetes status was recorded for 90%, of which, 137 TB cases had uncontrolled diabetes. The prevalence of HIV among study participants was 1.5%. Poor glycaemic control was found to be weakly associated with unfavourable TB treatment outcome (RR 2.00, 95% CI 0.97-4.13). A retrospective review of notification data and medical records for 1,473 TB cases in Taiwan found 276 individuals with uncontrolled diabetes.[13] The prevalence of HIV was 0.3%. Uncontrolled diabetes was found to be associated with improved TB treatment outcome at the end of TB treatment compared to TB cases with well controlled diabetes (5.8% of participants with HbA_{1c} >9% died compared to 13.8% for HbA_{1c} 7-9% and 18.3% for HbA_{1c} <7%; p<0.001). A prospective cohort study in South Korea determined sputum culture positivity at two months of treatment for 661 individuals with pulmonary TB.[11] Individuals with HIV infection were excluded. 108 participants had uncontrolled diabetes. Uncontrolled diabetes compared to no diabetes was found to be an independent risk factor for a positive sputum culture after two months of treatment (OR 2.11, 95% CI 1.03-4.31, p=0.042) and also for treatment failure or death (OR 4.11, 95% CI 1.23-13.78) p=0.027). No intervention studies to optimise glycaemic control in individuals with hyperglycaemia during TB treatment have yet taken place.[26] Our study is the first to be located in Africa and in a setting with a high prevalence of HIV.

The main limitation of our study is the low prevalence of hyperglycaemia found among TB patients in these study communities, particularly severe hyperglycaemia. It is possible that our study methods of using a single random blood glucose concentration to screen for hyperglycaemia contributed to this low prevalence of hyperglycaemia because of the low sensitivity of this test. There were too few individuals with hyperglycaemia to detect any potential true association between increasing levels of glycaemia and TB treatment outcome. In particular, it would have been favourable to explore the association among individuals with higher levels of glycaemia than we were able to in our study. Nonetheless, it is clear that hyperglycaemia is not a major driver of poor TB treatment outcome in our study population.

Another potential source of bias was the lack of obtaining complete follow up data for all participants. Although we collected all follow-up data during routine TB clinic attendances, aiming to minimise inconvenience to participants and therefore maximise follow-up, we found that many participants would send relatives to collect their TB medication on their behalf rather than attend the clinic themselves. This was the main cause of missing follow-up data. Whilst this has further led to loss of study power, it is unlikely to have introduced additional bias as there was no evidence to suggest that loss of follow-up data was associated with hyperglycaemia, the exposure of interest. Participant death was a very small contributor to loss of follow-up data as very few participants died during the study and so there is also no reason to suggest that loss of follow-up data might be associated with TB treatment outcome, the outcome of interest.

Rather than measure TB treatment outcome at the end of or beyond TB treatment, we used surrogate markers at an earlier time point. The use of surrogate markers is particularly useful for settings such as ours that have a low prevalence of unsuccessful TB treatment. Without the use of surrogate markers the study would otherwise have been logistically challenging due to the high sample size requirement for a rare outcome. However, contrary to our expectation, many participants did not achieve documented sputum conversion within the follow-up time.

The proportion of participants who did not convert was lower when the analysis was restricted to only those who completed sputum submission to 8 weeks, so the low proportion of participants with documented sputum conversion is likely due, at least in part, to missing data.

A strength of our study was the use of automated liquid culture to process sputum specimens. We were able to determine quantitative time to sputum culture positivity accurately without the potential introduction of human error.

As described above, we have focused on hyperglycaemia rather than diabetes mellitus, so that we also encompass transient stress-induced hyperglycaemia. This reflects the situation in practice, as it is not possible to distinguish between these diagnoses at the time of active TB infection. A consequence of this, however, it was possible that we would not capture individuals with previously diagnosed (known) diabetes mellitus who were not hyperglycaemic at the time of measuring glycaemia. In fact this did not result as a potential source of bias because there were only two individuals in this study who reported a prior diagnosis of diabetes and both were hyperglycaemic at baseline.

The findings from this study are generalizable to the study communities as participants were consecutively selected within glycaemia strata. They are likely also generalizable to communities elsewhere in Zambia and much of sub-Saharan Africa with similar high incidence of TB and low prevalence of hyperglycaemia. However, the findings are less likely to be generalizable to locations with a medium or high prevalence of hyperglycaemia and so repeating this study in other locations would be valuable.

Conclusions

In this cohort study of culture-confirmed pulmonary tuberculosis cases we found no evidence that a dose-response relationship exists between glycaemia as an ordered categorical variable and tuberculosis treatment outcome, though the direction of trend was consistent with a faster time to initial sputum positivity and a slower time to sputum culture conversion for

higher levels of glycaemia. We found weak evidence that a linear dose-response relationship exists between glycaemia and time to initial sputum culture positivity, and weak evidence of association between glycaemia as a binary variable and time to sputum culture conversion. We found a low prevalence of hyperglycaemia in our study population in Lusaka, Zambia and so the study was underpowered to detect any true association. This does however suggest that hyperglycaemia is not a major challenge for TB control in this location. Repeating this study in locations with higher hyperglycaemia prevalence is necessary to determine the impact of hyperglycaemia to TB control in settings with a medium or high prevalence of hyperglycaemia.

ACKNOWLEDGEMENTS

We thank all the members of all study teams who contributed to data collection. We thank the Zambian Ministry of Health, the Zambian National TB Programme, the Lusaka District Medical Officer and all health facility staff for allowing us to work in the TB clinics.

AUTHOR CONTRIBUTIONS

All authors contributed to initial study concept and study design. SLB and HA oversaw participant recruitment and data collection. SLB performed the data analysis. SLB wrote initial drafts and all authors contributed to final editing of the paper.

CONFLICTS OF INTEREST

We declare that we have no conflicts of interest.

FUNDING

SLB was supported by a Wellcome Trust Clinical PhD Fellowship (100141/Z/12/Z), and this study was funded by the fellowship. The contents of this manuscript are solely the responsibility of the authors and do not necessarily represent the official views of the Wellcome Trust.

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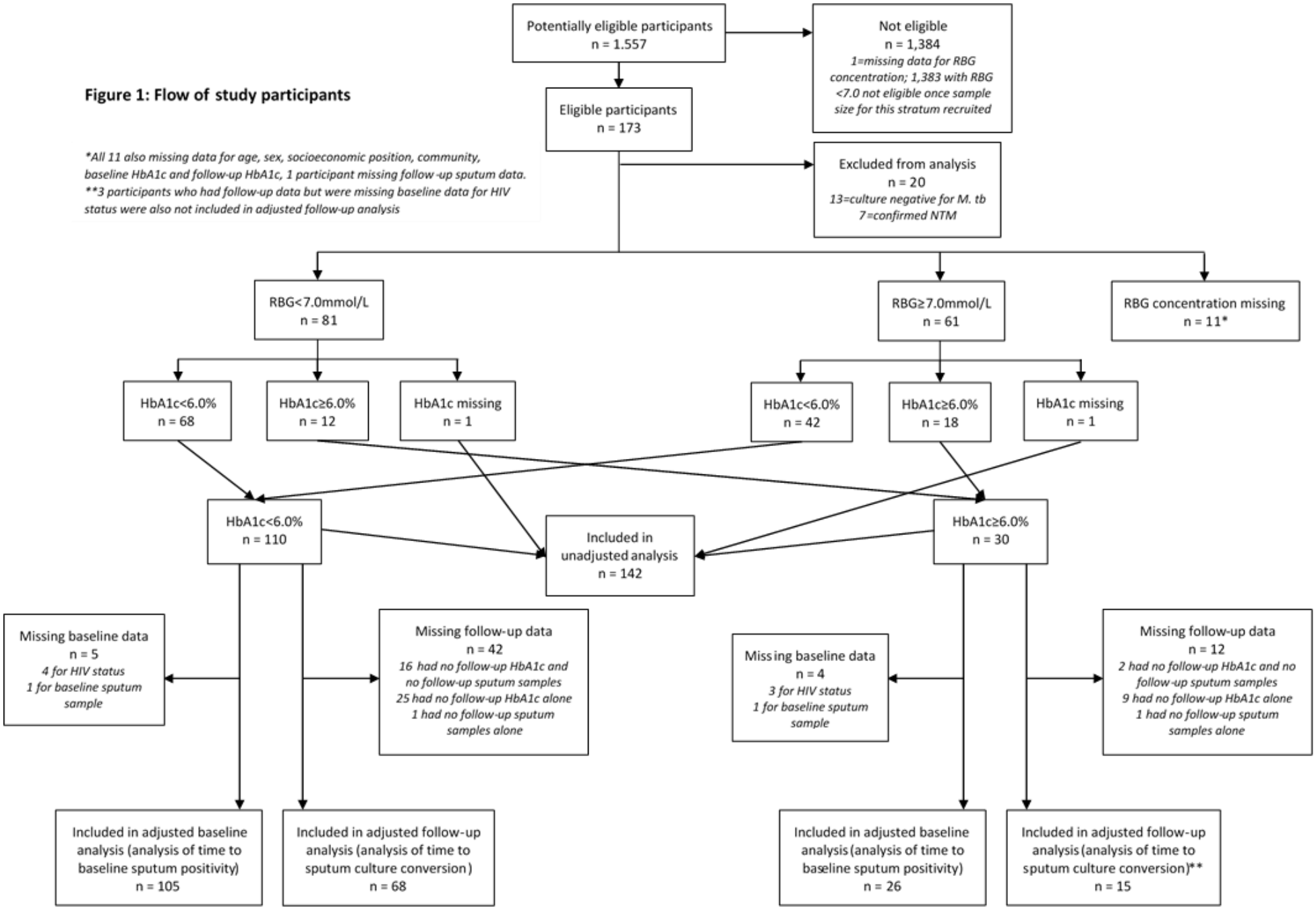
Figure 1: Flow of study participants



*All 11 also missing data for age, sex, socioeconomic position, community, baseline HbA1c and follow-up HbA1c, 1 participant missing follow -up sputum data.
**3 participants who had follow-up data but were missing baseline data for HIV status were also not included in adjusted follow-up analysis

Figure 2: Kaplan-Meier estimates of the time to initial sputum culture positivity by level of glycated haemoglobin

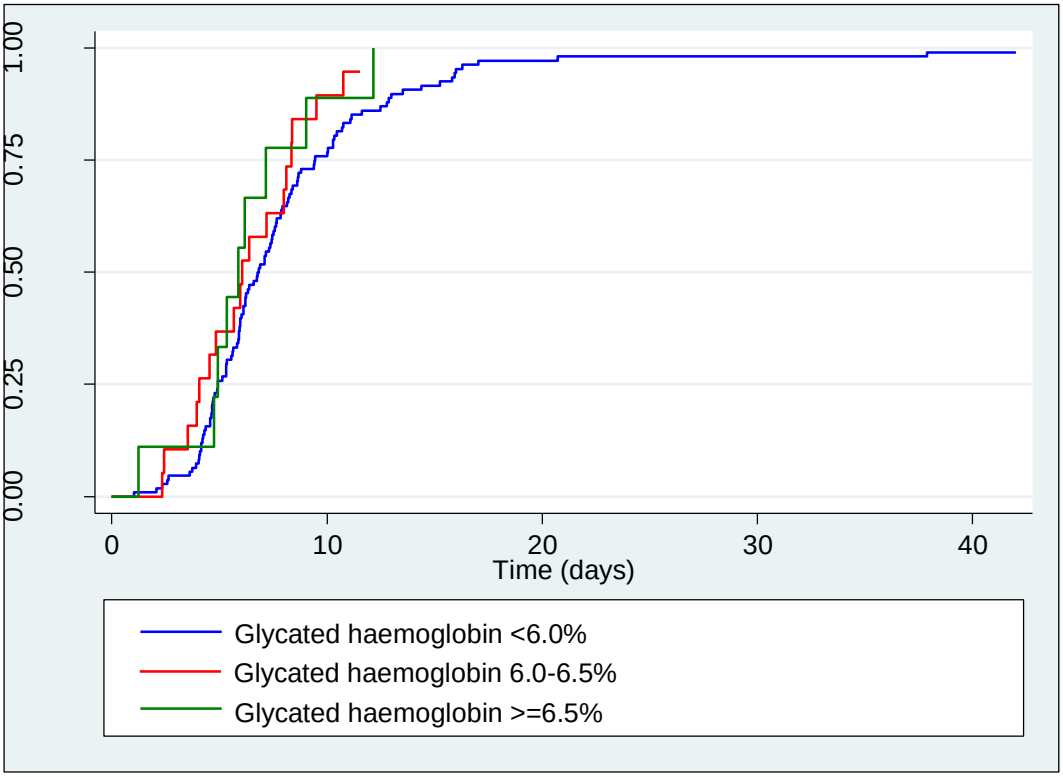


Figure 3: Kaplan-Meier estimates of the time to sputum culture conversion by level of glycated haemoglobin

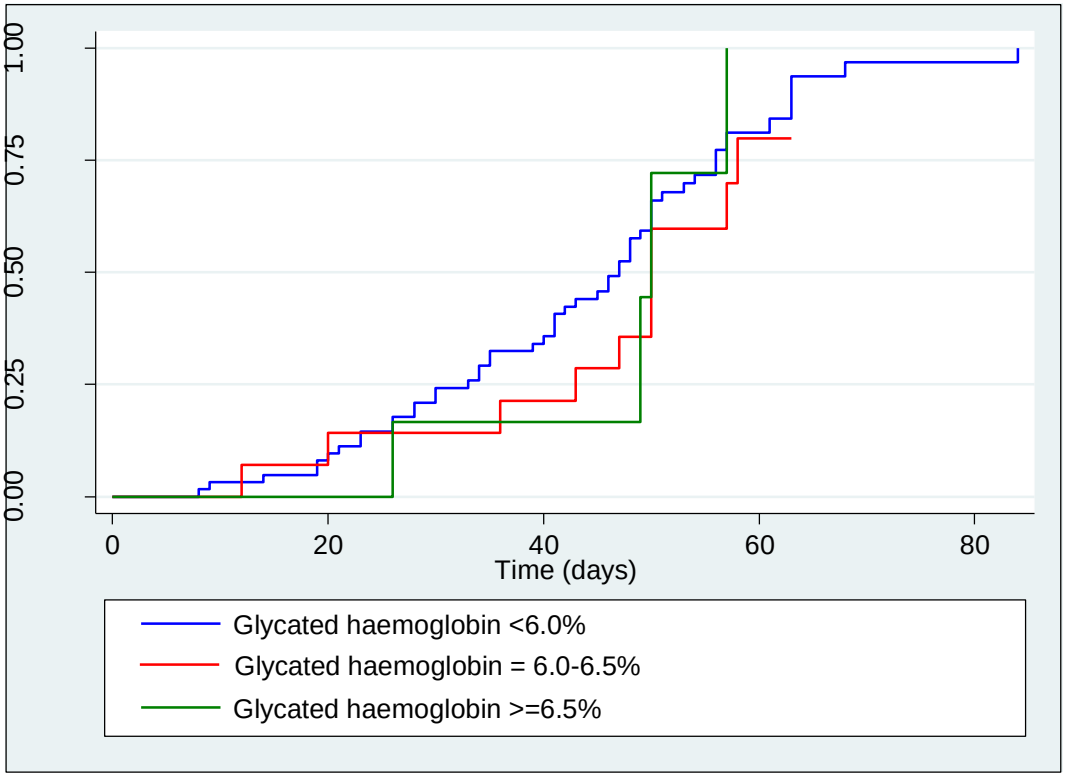


Table 1: Baseline characteristics of participants, comparing individuals with complete and incomplete follow-up data

Characteristic		Participants with complete follow-up data* n (%)	Participants with incomplete follow-up data** n (%)	p-value
Overall		36 (23.5)	117 (76.5)	-
Baseline glycated haemoglobin concentration (%)	<6.0	26 (72.2)	84 (80.8)	0.106
	6.0-6.4	9 (25.0)	12 (11.5)	
	≥6.5	1 (2.8)	8 (7.7)	
HIV status	Uninfected	12 (33.3)	39 (39.4)	0.521
	Infected	24 (66.7)	60 (60.6)	
Age (years)	18-24	4 (11.1)	18 (17.0)	0.211
	25-34	13 (36.1)	53 (50.0)	
	35-44	14 (38.9)	25 (23.6)	
	≥45	5 (13.9)	10 (9.4)	
Sex	Male	24 (66.7)	73 (68.9)	0.806
	Female	12 (33.3)	33 (31.1)	
Household socioeconomic position	Very low	6 (16.7)	15 (14.2)	0.557
	Low	16 (44.4)	59 (55.7)	
	Medium	13 (36.1)	27 (25.5)	
	High	1 (2.8)	5 (4.7)	

*P-values derived from chi-squared tests; *Defined as having data for baseline HbA_{1c}, follow-up HbA_{1c} and sputum culture at 8 weeks after initiation of TB treatment.; **Defined as missing data for any or all of baseline HbA_{1c}, follow-up HbA_{1c} and sputum culture at 8 weeks after initiation of TB treatment.*

Table 2: The median time to sputum culture positivity of the baseline sputum sample taken at the time of TB treatment initiation, with corresponding unadjusted and adjusted rate ratios estimated by cox regression

Characteristic		Number of participants (%)	Median days to sputum culture positivity (IQR)	Unadjusted RR (95% CI)	p-value[+]	Adjusted RR** (95% CI)	p-value[+]
Overall		151 (100)	6.4 (4.8-8.8)	-	-	-	-
Glycated haemoglobin concentration (%)*	<6.0	109 (79.0)	6.8 (4.9-9.4)	1	0.285 (TFT RR=1.27 (0.95-1.71), p=0.126)	1	0.309 (TFT aRR=0.97 (0.63-1.49), p=0.125)
	6.0-6.4	20 (14.5)	6.0 (4.1-8.1)	1.39 (0.84-2.30)		1.34 (0.76-2.35)	
	≥6.5	9 (6.5)	5.9 (4.9-7.1)	1.51 (0.76-3.01)		1.75 (0.73-4.18)	
Glycated haemoglobin concentration (%)*	<6.0	109 (79.0)	6.8 (4.9-9.4)	1	0.117	1	0.149
	≥6.0	29 (21.0)	6.0 (4.5-8.1)	1.43 (0.93-2.19)		1.44 (0.89-2.33)	
HIV status	Un-infected	50 (37.6)	6.0 (4.6-7.7)	1	0.021	1	0.227
	Infected	83 (62.4)	7.5 (5.3-9.5)	0.65 (0.45-0.93)		0.76 (0.49-1.18)	
Age (years)	18-24	22 (15.7)	6.9 (6.0-8.7)	1	0.072	1	0.027
	25-34	65 (46.4)	6.3 (4.6-8.2)	1.14 (0.69-1.87)		1.28 (0.74-2.23)	
	35-44	39 (27.9)	6.2 (4.7-9.5)	1.09 (0.64-1.86)		1.13 (0.63-2.03)	
	45+	14 (10.0)	9.3 (5.2-11.9)	0.53 (0.26-1.10)		0.48 (0.22-1.07)	
Sex	Male	97 (69.3)	6.2 (4.8-8.4)	1	0.122	1	0.905
	Female	43 (30.7)	7.5 (5.3-10.3)	0.75 (0.52-1.08)		0.97 (0.62-1.53)	
Household socio-economic position	Very low	20 (14.3)	7.9 (6.5-10.9)	1	0.496	1	0.676
	Low	74 (52.9)	6.6 (4.7-8.6)	1.30 (0.79-2.14)		1.06 (0.63-1.78)	
	Medium	40 (28.6)	5.9 (4.8-7.5)	1.40 (0.81-2.42)		1.31 (0.71-2.42)	
	High	6 (4.3)	9.0 (8.7-10.3)	0.87 (0.32-2.32)		0.76 (0.25-2.34)	

*IQR=inter-quartile range; RR=rate ratio; aRR=adjusted rate ratio; CI=confidence interval; *Measured at time of tuberculosis diagnosis; TFT=likelihood ratio test for trend with exposure as a linear variable; [+]Likelihood ratio tests; **131 participants included in analysis, adjusted for all variables shown plus community, separately for each categorisation of glycated haemoglobin concentration; Missing data: 11 for age, sex, socioeconomic position and community, 18 for HIV status, 13 for HbA_{1c} concentration and 2 for baseline sputum sample.*

Table 3: The median time to sputum culture conversion, with corresponding unadjusted and adjusted rate ratios estimated by cox regression

Characteristic		Number of participants (%)	Median weeks to sputum culture conversion (IQR)	Unadjusted RR (95% CI)	p-value[+]	Adjusted RR** (95% CI)	p-value[+]
Overall		153 (100)	6 (3-7)	-	-	-	-
Glycated haemoglobin concentration (%)*	<6.0	64 (72.7)	5 (4-7)	1	0.427	1	0.181
	6.0-6.4	18 (20.5)	6 (3-7)	0.65 (0.33-1.29)		0.50 (0.23-1.08)	
	≥6.5	6 (6.8)	6 (5-6)	0.83 (0.30-2.30)		0.73 (0.20-2.60)	
Glycated haemoglobin concentration (%)*	<6.0	64 (72.7)	5 (4-7)	1	0.228	1	0.076
	≥6.0	24 (27.3)	6 (4-6)	0.70 (0.39-1.26)		0.54 (0.27-1.09)	
HIV status	Un-infected	51 (37.8)	6 (4-6)	1	0.687	1	0.811
	Infected	84 (62.2)	5 (3-7)	0.91 (0.59-1.42)		1.08 (0.58-2.01)	
Age (years)	18-24	22 (15.5)	6 (4-7)	1	0.845	1	0.754
	25-34	66 (46.5)	5.5 (3-7)	0.98 (0.55-1.75)		1.66 (0.60-4.59)	
	35-44	39 (27.5)	5 (3-6)	0.79 (0.42-1.50)		1.70 (0.60-4.82)	
	≥45	15 (10.6)	6 (3-7)	0.89 (0.40-1.98)		1.45 (0.44-4.79)	
Sex	Male	97 (68.3)	6 (4-7)	1	0.655	1	0.712
	Female	45 (31.7)	4 (3-6)	1.11 (0.71-1.72)		0.88 (0.46-1.70)	
Household socio-economic position	Very low	21 (14.8)	6 (3-6.5)	1	0.109	1	0.134
	Low	75 (52.8)	5 (3-6)	1.62 (0.85-3.10)		1.79 (0.84-3.82)	
	Medium	40 (28.2)	6.5 (4-7)	1.20 (0.60-2.40)		1.04 (0.42-2.58)	
	High	6 (4.2)	5 (2-6)	3.53 (1.23-10.16)		3.97 (0.65-24.16)	

*IQR=inter-quartile range; RR=rate ratio; CI=confidence interval; *Measured at 2 months post -tuberculosis diagnosis; [+]Likelihood ratio tests; **83 participants included in analysis, adjusted for all variables shown plus community, separately for each categorisation of glycated haemoglobin concentration; Missing data: 11 for age, sex, socioeconomic position and community, 18 for HIV status, 65 for HbA_{1c} concentration and 32 for sputum samples.*

Chapter 9: Study 3 paper: The accuracy of measures of glycaemia for the diagnosis of diabetes mellitus in newly-diagnosed tuberculosis patients in Zambia, a study of diagnostic accuracy

Bailey SL[1,2], Floyd S[1], Maluzi K[2], Cheeba-Lengwe M[2], Kosloff B[1,2], Yudkin JS[3], Godfrey-Faussett P[1], Ayles H[1,2]

[1]*LSHTM TB Centre and Department of Clinical Research, London School of Hygiene and Tropical Medicine, London, UK*

[2]*Zambart Project, Lusaka, Zambia*

[3]*Division of Medicine, University College London, London, UK*

Corresponding author: Dr Sarah Lou Bailey, slbailey@doctors.org.uk, +442081234489

Word count: 3,656

Abstract

Background: A positive association between diabetes mellitus and tuberculosis has been established. When testing for diabetes in individuals with tuberculosis, most prior studies have measured glycaemia at the time of tuberculosis diagnosis. Acute TB disease could also cause hyperglycaemia, because of physiological stress from the infection, and this could lead to false positive test results for diabetes. Therefore, we aimed to distinguish between transient hyperglycaemia, which most likely represents stress hyperglycaemia, and persistent hyperglycaemia, which is indicative of diabetes mellitus, and evaluate the accuracy of measures of glycaemia at the time of tuberculosis diagnosis for the diagnosis of diabetes mellitus.

Methods: In this diagnostic accuracy study we compared three index tests to a reference standard test. Index tests were random blood glucose (RBG) concentration, fasting blood glucose (FBG) concentration and glycated haemoglobin (HbA_{1c}), measured at the time of tuberculosis diagnosis. The reference standard test for diabetes mellitus was a FBG concentration measured 3 months after commencement of tuberculosis treatment, when acute TB disease had stabilised. Participants were newly-diagnosed tuberculosis patients in three health care centres in Lusaka, Zambia. Recruitment was stratified by RBG concentration. Test sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for each index test. Subgroup analyses stratified test accuracy by HIV status.

Results: Of 490 participants recruited, 71 (14.5%) had hyperglycaemia on at least one index test. The reference standard test was performed in 242 (49.4%) participants, of whom 8 (3.3%) had diabetes. Of participants who had hyperglycaemia on the index RBG, FBG and HbA_{1c} tests, 6 (33%), 4 (44%) and 7 (39%) participants respectively had diabetes. All index tests had similar test accuracy, with overlapping confidence intervals. The specificity and NPV were ≥95% with narrow confidence intervals for all index tests. The sensitivity ranged from 57%-88% and the

PPV ranged from 33%-44%, all with wide confidence intervals. Among participants infected and uninfected with HIV, the PPV ranged from 17%-22% and 50%-100% respectively.

Conclusions: In individuals with tuberculosis, a low proportion of hyperglycaemia measured at the time of tuberculosis diagnosis was due to diabetes mellitus, particularly among individuals co-infected with HIV. There was no evidence that glycated haemoglobin had greater test accuracy for diabetes diagnosis at the time of tuberculosis diagnosis than FBG or RBG. When testing for diabetes in individuals with tuberculosis, repeat testing after resolution of acute tuberculosis disease should be considered before a diagnosis of diabetes is made.

399 words

Keywords: Stress-induced hyperglycaemia, transient hyperglycaemia, southern Africa

Background

A positive association between diabetes mellitus (DM) and tuberculosis (TB) has been established. Individuals with DM are around three times more likely to develop active TB disease than individuals without DM. This is thought to be due to the negative effect of diabetes on cell mediated immunity, including altered T-cell function, reduced chemotaxis and reduced oxidative killing potential,[1-6] all of which could impair the control of *M. tuberculosis*.[2, 7, 8]

When testing for diabetes in individuals with tuberculosis, most prior studies have measured glycaemia at the time of tuberculosis diagnosis. However, acute TB disease prior to adequate treatment could also cause hyperglycaemia, because of physiological stress from the infection, and this could lead to false positive test results for diabetes. Stress hyperglycaemia is a well-documented clinical feature of sepsis and critical illness such as acute myocardial infarction, resulting from the interference of stress hormones and cytokines with normal carbohydrate metabolism.[9-11] It is possible that this is also a feature of severe TB disease, and differentiation of this from diabetes mellitus could help to ensure appropriate management of individuals and appropriate organisation of health services.

If stress hyperglycaemia is a common condition among individuals who have untreated TB disease, it would be useful to be able to distinguish this from diabetes mellitus at the time of TB diagnosis, in order to ensure appropriate management for individuals. The World Health Organisation has approved multiple different methods of measuring glycaemia in the diagnosis of diabetes.[12] Most measure an individual's blood glucose concentration at the time of testing, but measurement of glycated haemoglobin quantifies the average blood glucose concentration over the preceding 2-3 months. Consequently, it is possible that this measure is better than others at predicting persistent hyperglycaemia and therefore diabetes, without also giving false positives from transient stress hyperglycaemia.

Therefore, firstly, we aimed to determine the proportion of hyperglycaemia among newly-diagnosed tuberculosis cases that is persistent after resolution of acute TB disease and is thus indicative of diabetes mellitus rather than stress-induced hyperglycaemia. Secondly, we aimed to evaluate the accuracy of random blood glucose (RBG) concentration, fasting blood glucose (FBG) concentration and glycated haemoglobin (HbA_{1c}) measured at the time of tuberculosis diagnosis for predicting diabetes mellitus, as determined by the reference standard of fasting blood glucose concentration measured after stabilisation of tuberculosis disease.

Methods

Study design and participants

We conducted a prospective study of diagnostic accuracy from September 2013 until December 2015 in three tuberculosis clinics in Lusaka, Zambia. Study participants were newly-diagnosed tuberculosis cases who were ≥18 years of age and had commenced TB treatment ≤2 days ago or had not yet started TB treatment. The study was linked to a case-control study which investigated the association between DM and TB in Lusaka, comparing TB cases to non-TB controls. All TB cases of the case-control study were assessed for eligibility for this study of diagnostic accuracy.

A TB case was defined as any person presenting to one of the TB clinics with a clinical diagnosis of pulmonary or extra-pulmonary TB with or without microbiological confirmation of TB and prescribed a full course of antitubercular chemotherapy, based on the World Health Organization TB diagnostic criteria.[13] This is the definition used for TB diagnosis in the study clinics as well as elsewhere in Zambia and much of sub-Saharan Africa and so use of this definition aimed to facilitate translation of the study results to the clinical setting. All potentially eligible participants were identified in the TB clinics and invited to participate during a routine clinic attendance.

A stratified sampling technique was used to recruit adequate numbers of participants with and without diabetes mellitus. As it was not possible to determine the status of diabetes at the time of recruitment we instead based recruitment on the participants' initial random blood glucose (RBG) concentration. We used three strata to facilitate recruitment of participants from across the glycaemic range: RBG <7mmol/L, RBG 7-9mmol/L and RBG >9mmol/L. Within each stratum, consecutive TB cases who were eligible and willing were recruited. Recruitment to a stratum ceased if the target sample size was met before the end of study recruitment.

The eligibility criteria for this study were therefore: age ≥18 years; newly-diagnosed case of TB due to commence on a full course of antitubercular chemotherapy; commenced TB treatment ≤2 days ago if already commenced TB treatment, and RBG concentration within the required stratum.

Test methods

We evaluated three index tests: RBG, FBG and HbA_{1c} concentration measured at the time of TB diagnosis. We compared each of these tests to a reference standard test for diabetes mellitus, which we defined as a repeat FBG concentration measured 3 months after commencement of TB treatment, by which time we expect the TB to have stabilised and only persistent hyperglycaemia to remain.

RBG concentration was measured at the time of recruitment. FBG concentration for the initial index test was measured the day following recruitment or as soon as possible after this, after at least a 6 hour overnight fast of all food and drink except water. Capillary blood was sampled using an auto-lancet and an Accu-Chek Aviva point-of-care glucometer was used to measure the glucose concentration. These point-of-care tests were performed by the research assistants who were also recruiting and interviewing the participants, and so they were aware of demographic and clinical information of each participant at the time of undertaking the index tests.

Venous blood was sampled at the time of recruitment for measurement of HbA_{1c}. The blood samples were transported daily to the laboratory to be stored for up to a week at 5°C. Samples were analysed weekly using an Abbott Architect i system. Laboratory staff who processed the samples were unaware of any participant demographic or clinical information.

The repeat reference standard FBG followed the same procedures as the initial index FBG, but was measured 3 months post-treatment commencement. Research assistants undertaking this point-of-care test again had access to clinical information and index test results for each participant. If participants were taking hypoglycaemic medication they were asked to omit their morning dose until after measurement of FBG concentration.

All test positivity cut-offs for the index and reference standard tests were pre-specified and followed the WHO guidelines for diabetes diagnostic criteria, as follows: RBG concentration ≥11.1mmol/L; FBG concentration (index or reference standard) ≥7.0mmol/L; HbA_{1c} ≥6.5%.[12, 14] All participants who had glycaemia in the diabetes range on any test were referred to local health care facilities for follow-up and management.

A structured questionnaire was used to record participant characteristics and clinical information, including age, sex, household socioeconomic position, education level and details of any prior diabetes diagnosis. Height and weight were measured. This information was entered directly onto a pre-programmed personal digital assistant (PDA) by the researcher, using pre-programmed questionnaires and result sheets with error and range checks. To enable sub-group analyses, HIV status was determined using point-of-care rapid blood-based kits. Determine™ HIV-1/2 was used as the first line test and Uni-Gold™ HIV as a confirmatory test for individuals with a positive Determine test (following the Zambia national algorithm for HIV diagnosis).

Research staff were trained and monitored on all aspects of data collection and were required to follow standard operating procedures for measurement of glucose, HbA_{1c}, anthropometrics and for HIV tests. All research staff were required to undergo proficiency testing for measuring

capillary blood glucose. Standardised control solution was used for glucometer and test strip performance checks. Intra- and inter-operator variability was measured to assess the validity of glucose measurement using the Accu-Chek Aviva glucometer. All research assistants contributed to the assessment, using known normoglycaemic volunteers and a standard hyperglycaemic specimen. For intra-operator variability each research assistant repeated the test 5 consecutive times on a combined total of 13 subjects/specimens. For inter-operator variability research assistants each performed the test on a single subject/specimen in the same place at the same time for 15 subjects/specimens. A two-way mixed-effects model assessing for absolute agreement was used to calculate intraclass correlations (ICCs): the ICC for intra-operator variability was 0.983 (95% CI 0.954-0.995) and for inter-operator variability was 0.997 (95% CI 0.990-0.999).

Laboratory performance evaluation was undertaken for measurement of HbA_{1c} concentration using the Abbot Architect i system: performance evaluation gave a score of 100% for acceptable results.

Analysis

Baseline characteristics of participants with and without hyperglycaemia, and with and without follow-up data, were compared using chi-squared tests. Descriptive frequency and percentage statistics were used to give the proportions of hyperglycaemic newly-diagnosed tuberculosis cases, measured separately by RBG, FBG and HbA_{1c}, who have diabetes mellitus rather than transient hyperglycaemia. To determine test accuracy, the test sensitivity, specificity, positive predictive value and negative predictive value were calculated for each index test, along with 95% confidence intervals. Participants with missing data on either the index or the reference standard test were excluded from the analysis.

Stratification by HIV status was specified *a priori* for subgroup analysis.

Additionally, for purposes of hypothesis generation, predictors of transient hyperglycaemia among all participants who were hyperglycaemic on any index test were explored using

unadjusted and adjusted logistic regression analysis. The potential predictors explored were age, sex, socioeconomic position, HIV status and body mass index.

Ethics

Each participant was required to give written informed consent. Ethics approval was granted from the London School of Hygiene and Tropical Medicine Ethics Committee and the University of Zambia Biomedical Research Ethics Committee.

Results

The case-control study linked to this study recruited 3,843 TB cases. All were assessed for eligibility to this diagnostic accuracy study. Of these, 490 were eligible for recruitment: 205 had a RBG <7.0mmol/L, 179 had a RBG 7.0-8.9mmol/L and 106 had a RBG ≥9.0mmol/L. The flow of study participants is shown in Figure 1. No participant had missing data for the index RBG test. 95 (19.4%) participants had missing data for the index FBG test, 86 (17.6%) participants had missing data for the index HbA_{1c} test and 248 (50.6%) participants had missing data for the 3-month follow-up reference standard test. We were able to obtain outcome data for 163 (65.7%) participants who were lost to follow-up for the reference standard test. Of these, 13 (8.0%) died, 9 (5.5%) defaulted on their TB treatment and 10 (6.1%) had their TB treatment transferred out to another clinic. Individuals who were lost to follow-up for the reference standard test were similar to participants with reference standard test data in terms of index test results and all baseline characteristics except for HIV status and community. Individuals who were infected with HIV and from community 1 were more likely to be lost to follow-up (supplementary Table 1).

426 (87.1%) participants were recruited on the same day as starting TB treatment. Index RBG tests were all measured at the time of recruitment. Index FBG tests were measured a median of 1 day after recruitment (interquartile range, IQR = 1-3 days). The mean length of fast prior to measurement of the index FBG concentration was 12.2 hours (standard deviation 2.7

hours). Samples for index HbA_{1c} tests were taken at the time of recruitment and processed in the laboratory a median of 3 days later (IQR = 1-6 days). Reference standard FBG tests were measured a median of 90 days after recruitment (IQR = 83-98 days). The mean length of fast prior to measurement of the reference standard FBG concentration was 12.3 hours (standard deviation 1.7 hours).

Table 1 shows the baseline characteristics of study participants, stratified by the absence or presence of hyperglycaemia on index tests and the reference standard test. Participants with and without hyperglycaemia on index tests were similar in terms of age, sex, education, smoking history, body mass index and HIV status. There was weak evidence that individuals with hyperglycaemia on index tests were of a higher socioeconomic position and had a higher body mass index than individuals without hyperglycaemia on index tests. There was evidence of a difference between communities. Participants with and without hyperglycaemia on the reference standard test had no evidence of a difference in baseline characteristics except for HIV status: there was evidence that individuals with hyperglycaemia were less likely to be HIV-infected than individuals without hyperglycaemia.

Tables 2a, 2b and 2c show cross tabulation of each of the index test results by the reference standard test results. The proportion of participants with hyperglycaemia at the time of TB diagnosis determined by RBG, FBG and HbA_{1c} tests respectively was 9.0%, 4.8% and 9.2%. The proportion of participants with hyperglycaemia after an average of 90 days on TB treatment, determined by the reference standard FBG test, was 3.3%. Persistent hyperglycaemia, and therefore diabetes, was found in 6 (33%) participants who had hyperglycaemia on the index RBG test, 4 (44%) participants who had hyperglycaemia on the index FBG test and 7 (39%) participants with hyperglycaemia on the index HbA_{1c} tests.

At the time of recruitment 12 (2.5%) participants had been given a prior diagnosis of DM. Of these, 6 were taking hypoglycaemic medication: 2 participants were taking oral hypoglycaemic agents (glibenclamide and metformin) and 4 participants were taking insulin. A further 2

participants were commenced on oral hypoglycaemic agents by local health care practitioners following the index test results. All participants on diabetes treatment who were followed up with a reference standard test had a result in the diabetes range.

The measures of test accuracy for each index test, overall and stratified by HIV status, are shown in Table 3. The specificity and negative predictive value were high (>93%) for all index tests, overall and within HIV strata. Overall, the sensitivity of index tests ranged from 57% to 88% but with wide confidence intervals for all tests due to the low number of individuals with hyperglycaemia in our study. Overall, the positive predictive value was low for all tests, ranging from 33% to 44%, again with wide confidence intervals. No test emerged as being more accurate than the others for diagnosing diabetes mellitus at the time of TB diagnosis; confidence intervals of the three tests overlapped for all measures of test accuracy.

When stratified by HIV, the point estimate of the positive predictive value (PPV) was lower among individuals infected with HIV compared to those uninfected for all index tests, though confidence intervals were wide and overlapping (Table 3).

There were 21 participants who had transient hyperglycaemia on at least one index test. On unadjusted logistic regression analysis of predictors of transient hyperglycaemia, there was evidence that being infected with HIV or being male were associated with transient rather than persistent hyperglycaemia (p=0.046 and 0.022 respectively). There was no evidence of association between age, socioeconomic position or body mass index and transient hyperglycaemia. On adjusted logistic regression analysis, the adjusted odds of transient hyperglycaemia was 12.87 (95% CI 0.63-264.91) times higher among those infected with HIV than those uninfected (p=0.061). The adjusted odds ratio for stress hyperglycaemia comparing women to men was 0.23 (95% CI 0.02-3.26; p=0.166). The main positive confounding factor for HIV as a risk factor was body mass index and the main negative confounding factor was socioeconomic position. The main positive confounding factor for sex as a risk factor for transient hyperglycaemia was HIV.

There were no adverse events from performing any of the index tests or the reference standard test.

Discussion

In our Zambian study setting we found that a low proportion of hyperglycaemia measured at the time of TB diagnosis was due to diabetes mellitus. Most hyperglycaemia was transient, which likely represents stress hyperglycaemia secondary to acute TB disease. Contrary to our expectation and to the pattern seen in individuals with acute myocardial infarction,[9, 11] glycated haemoglobin showed no greater test accuracy for diabetes diagnosis at the time of TB diagnosis than FBG or RBG tests. This could reflect prolonged hyperglycaemia among individuals in our population with stress hyperglycaemia prior to their TB diagnosis, in addition to prolonged hyperglycaemia among individuals with diabetes. This is in keeping with the typical onset of TB disease, which is slow and gradual, in contrast to the rapid onset of illness seen with acute myocardial infarction.

An alternative explanation of the transient hyperglycaemia seen is a previously described phenomenon with glucose similar to "white coat hypertension",[15] though this is less plausible than stress hyperglycaemia. Individuals with HIV had a greater proportion of transient hyperglycaemia and a lower test accuracy for diabetes than individuals without HIV. This is in keeping with the idea that transient hyperglycaemia represents stress hyperglycaemia, as individuals who are infected with HIV more commonly have severe TB[16-18] and therefore may be more likely to have stress hyperglycaemia than individuals who are uninfected with HIV. However, this study was not powered for sub-group analyses and so these analyses should be seen as hypothesis generating rather than hypothesis testing.

Our findings are in keeping with the few existing studies that have differentiated between transient and persistent hyperglycaemia in individuals with TB. In 1990 Oluboyo performed sequential OGTTs on 54 Nigerian patients with active pulmonary TB. Only one of eight patients with initial impaired glucose tolerance remained with persisting glucose intolerance after 3

months of TB treatment.[19] In a more recent larger cohort of Tanzanian TB patients, Boillat-Blanco et al found that a high proportion of hyperglycaemia was transient.[20] At the time of active TB diagnosis, 4.5%, 6.8% and 9.3% had hyperglycaemia measured by FBG, oral glucose tolerance test (OGTT) and HbA_{1c} respectively. After 5 months of receiving TB treatment and exclusion of patients with previously known DM, 75%, 64% and 71% of these initially hyperglycaemic participants were no longer hyperglycaemic on follow-up FBG, OGTT and HbA_{1c} tests respectively. A high proportion of transient hyperglycaemia in TB patients has similarly been described in Indonesia[21], Iran[22], Pakistan[23], Turkey[24] and India[25].

Our study was limited by the low proportion of participants with diabetes mellitus. This was despite recruiting participants from a pool of nearly 4,000 TB patients over 2 years, and reflects the low prevalence of diabetes in the general population in Lusaka. It is possible that our screening method of using a random blood glucose concentration to detect potential participants with hyperglycaemia has contributed to this low proportion as the method lacks sensitivity for the diagnosis of diabetes. As a result of the low proportion of participants with diabetes, estimates of sensitivity and positive predictive value have wide confidence intervals. Repeating this study in a population with a high prevalence of diabetes would be valuable to give more precise estimates of sensitivity and PPV.

To maximise follow-up of participants we opted to undertake only FBG as the follow-up test for diabetes and not to also measure repeat RBG concentration and HbA_{1c}. Consequently we needed to follow-up the participants just once after the recruitment procedures, and perform only a finger-prick blood test rather than undertake repeat venous blood sampling. The latter was much less acceptable to our participants. Even so, it was a challenge to follow-up some participants, even aside from those who had died, defaulted on TB treatment or been transferred out to another clinic for treatment. The main reason for this was the common practice of patients sending relatives to the TB clinic to collect medication on their behalf, coupled with a low proportion of participants having a personal mobile phone number that

remained unchanged during the study. It was therefore difficult to gain direct access to the participants to remind and encourage them to attend the clinic for follow-up. This was particularly a challenge in clinic 1 and has led to a loss of study power and precision. However, aside from clinic and HIV status, the participants who were lost to follow-up were similar to those who were followed-up in terms of baseline characteristics and index test results and so our low retention rate is unlikely to have caused systematic bias to our study findings. Any potential bias resulting from the disproportionate number of HIV infected participants lost to follow-up is mitigated by stratifying the results by HIV status. Other than the further loss of study power, it is unlikely that the lack of follow-up RBG and HbA_{1c} tests would have changed the study conclusions, as the comparison of like to like index FBG to follow-up FBG gave the same overall conclusions as comparing index RBG and HbA_{1c} tests to the follow-up FBG test.

In this study we used point of care tests using capillary blood samples for random and fasting blood glucose tests. As well as being more acceptable to participants than venous blood sampling, point of care tests are logistically less challenging and less expensive to undertake. Our assessment of intra- and inter-operator variability suggests that measurement of random blood glucose among cases in this study was consistent and valid. Additionally, we explored the possibility of undertaking laboratory validation of glucose measurements by comparing glucometer results to results from laboratory processed venous blood samples taken simultaneously. This proved to be not possible in our setting, as point-of-care glucose measurement is the principal method for measuring glycaemia, both in the community and centrally. The laboratory alternatives were therefore not equipped to offer a reliable benchmark.

For hypothesis generating purposes we explored predictors of transient hyperglycaemia. The number of participants with hyperglycaemia was small and confidence intervals were wide so no definitive predictors were identified. Further exploration of this with greater study power would be valuable.

The definition of TB used in this study is the same definition used clinically in the study setting and so the results are applicable to the clinical setting in Lusaka. As the participants were randomly selected the results are also likely generalizable to other settings in Zambia and surrounding southern African countries with a similar high prevalence of HIV.

Conclusions

In individuals with tuberculosis, a low proportion of hyperglycaemia measured at the time of tuberculosis diagnosis was persistent and therefore due to diabetes mellitus, particularly among individuals infected with HIV. The majority of hyperglycaemia measured at the time of tuberculosis diagnosis was transient and therefore most likely represents stress-induced hyperglycaemia rather than underlying diabetes mellitus. This was particularly the case for individuals co-infected with HIV. There was no evidence that glycated haemoglobin had greater test accuracy for diabetes diagnosis at the time of TB diagnosis than FBG or RBG. When testing for diabetes in individuals with tuberculosis, repeat testing after resolution of acute TB disease should be considered before a diagnosis of diabetes is made.

ACKNOWLEDGEMENTS

We thank all the members of all study teams who contributed to data collection. We thank the Zambian Ministry of Health, the Zambian National TB Programme, the Lusaka District Medical Officer and all health facility staff for allowing us to work in the TB clinics.

AUTHOR CONTRIBUTIONS

All authors contributed to initial study concept and study design. SLB and HA oversaw participant recruitment and data collection. SLB performed the data analysis. SLB wrote initial drafts and all authors contributed to final editing of the paper.

CONFLICTS OF INTEREST

We declare that we have no conflicts of interest.

FUNDING

SLB was supported by a Wellcome Trust Clinical PhD Fellowship (100141/Z/12/Z), and this study was funded by the fellowship. The contents of this manuscript are solely the responsibility of the authors and do not necessarily represent the official views of the Wellcome Trust.

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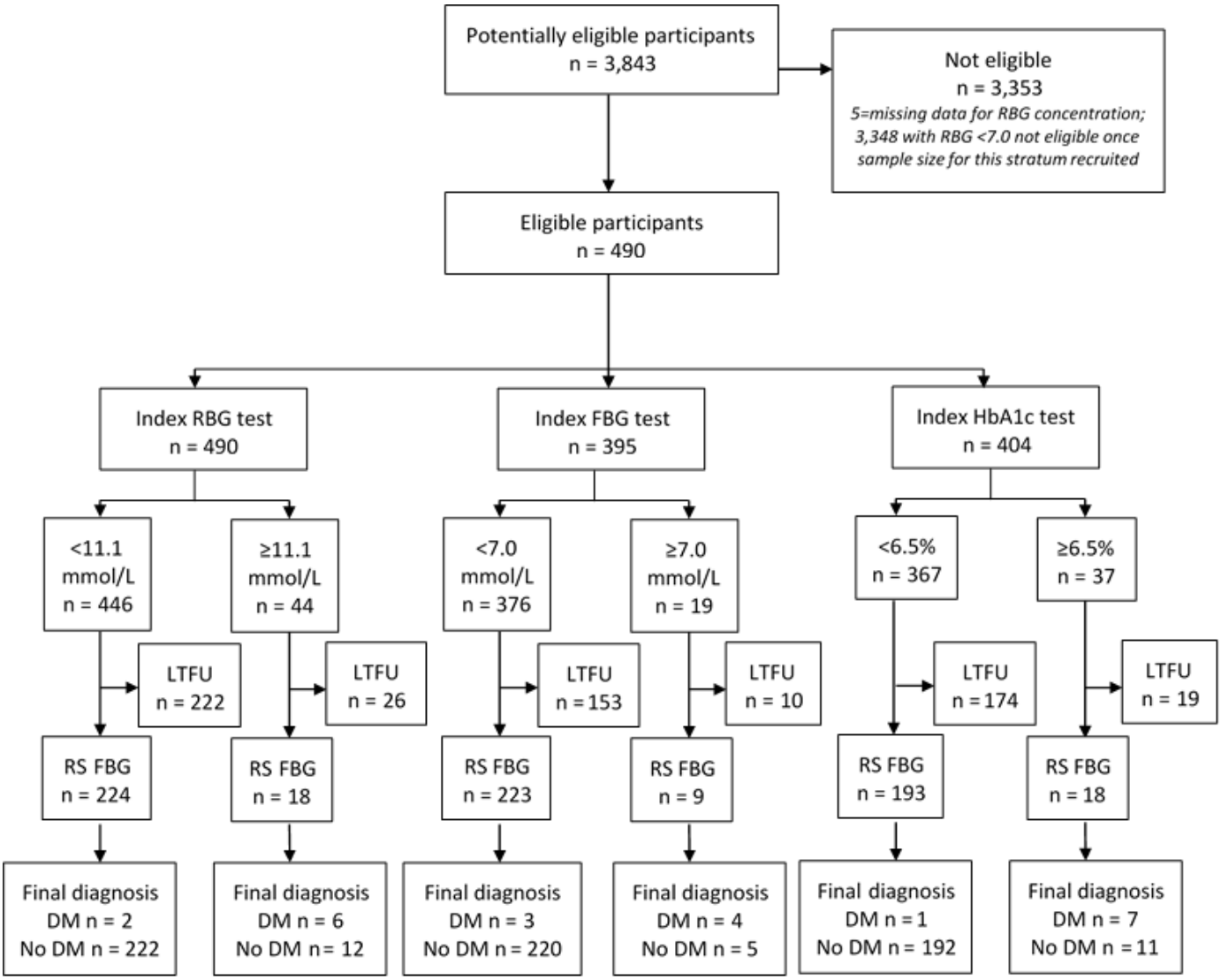
Figure 1: Flow of study participants



RBG=random blood glucose; FBG=fasting blood glucose; HbA_{1c}=glycated haemoglobin; RS=reference standard; DM=diabetes mellitus; LTFU=lost to follow-up

Table 1: Participant characteristics

Characteristic		Total n (%)	Hyper-glycaemia absent on all index tests[a] n (%)	Hyper-glycaemia present on at least one index test[b] n (%)	p-value	Hyper-glycaemia absent on reference standard test[c] n (%)	Hyper-glycaemia present on reference standard test[d] n (%)	p-value
Overall		490 (100.0)	419 (100.0)	71 (100.0)	-	234 (100.0)	8 (100.0)	-
Age (years)	18-24	58 (11.8)	51 (12.2)	7 (9.9)	0.139	27 (11.5)	2 (25.0)	0.290
	25-29	83 (16.9)	77 (18.4)	6 (8.5)		37 (15.8)	1 (12.5)	
	30-34	108 (22.0)	92 (22.0)	16 (22.5)		52 (22.2)	1 (12.5)	
	35-39	109 (22.2)	94 (22.4)	15 (21.1)		51 (21.8)	0 (0.0)	
	40-49	86 (17.6)	70 (16.7)	16 (22.5)		49 (20.9)	2 (25.0)	
	50+	46 (9.4)	35 (8.4)	11 (15.5)		18 (7.7)	2 (25.0)	
Sex	Male	337 (68.8)	290 (69.2)	47 (66.2)	0.612	163 (69.7)	4 (50.0)	0.237
	Female	153 (31.2)	129 (30.8)	24 (33.8)		71 (30.3)	4 (50.0)	
Household socio-economic position	Very low	78 (16.0)	70 (16.8)	8 (11.3)	0.058	38 (16.3)	1 (12.5)	0.899
	Low	212 (43.4)	185 (44.3)	27 (38.0)		101 (43.4)	3 (37.5)	
	Medium	164 (33.5)	138 (33.0)	26 (36.6)		79 (33.9)	3 (37.5)	
	High	35 (7.2)	25 (6.0)	10 (14.1)		15 (6.4)	1 (12.5)	
Highest level of education	None	39 (8.0)	34 (8.1)	5 (7.0)	0.877	13 (5.6)	0 (0.0)	0.191
	Grade 1-7	194 (39.7)	163 (39.0)	31 (43.7)		87 (37.3)	6 (75.0)	
	Grade 8-12	246 (50.3)	212 (50.7)	34 (47.9)		128 (54.9)	2 (25.0)	
	College/ University	10 (2.0)	9 (2.2)	1 (1.4)		5 (2.2)	0 (0.0)	
Smoking history	Never smoked	357 (72.9)	305 (72.8)	52 (73.2)	0.938	171 (73.1)	5 (62.5)	0.509
	Current or ex-smoker	133 (27.1)	114 (27.2)	19 (26.8)		63 (26.9)	3 (37.5)	
Body Mass Index (weight(kg) / height^2(m))	Healthy weight (18.5-24.9)	251 (51.3)	209 (50.0)	42 (59.2)	0.064	126 (54.1)	5 (62.5)	0.810
	Underweight (<18.5)	217 (44.4)	193 (46.2)	24 (33.8)		99 (42.5)	3 (37.5)	
	Overweight (25-29.9)	17 (3.5)	14 (3.4)	3 (4.2)		8 (3.4)	0 (0.0)	
	Obese (≥30)	4 (0.8)	2 (0.5)	2 (2.8)		0 (0.0)	0 (0.0)	
HIV status	Uninfected	141 (29.9)	119 (29.4)	22 (32.8)	0.567	73 (32.7)	6 (75.0)	0.013
	Infected	331 (70.1)	286 (70.6)	45 (67.2)		150 (67.3)	2 (25.0)	
TB clinic	1	217 (44.3)	175 (41.8)	42 (59.2)	0.013	78 (33.3)	4 (50.0)	0.578

	2	105 (21.4)	97 (23.2)	8 (11.3)		55 (23.5)	1 (12.5)	
	3	168 (34.3)	147 (35.1)	21 (29.6)		101 (43.2)	3 (37.5)	

[a]Index RBG <11.1mmol/L and index FBG <7.0mmol/L and index HbA_{1c} <6.5%; [b]Index RBG ≥11.1mmol/L or index FBG ≥7.0mmol/L or index HbA_{1c} ≥6.5%; [c]Reference standard FBG <7.0mmol/L; [d]Reference standard FBG ≥7.0mmol/L;

Table 2: Cross tabulation of the results of each index test by the reference standard results

(a)

		Reference standard: follow-up FBG		
		≥7.0 mmol/L	<7.0 mmol/L	Total
RBG at time of TB diagnosis	≥11.1 mmol/L	6	12	18
	<11.1 mmol/L	2	222	224
	Total	8	234	242

(b)

		Reference standard: follow-up FBG		
		≥7.0 mmol/L	<7.0 mmol/L	Total
FBG at time of TB diagnosis	≥7.0 mmol/L	4	5	9
	<7.0 mmol/L	3	220	223
	Total	7	225	232

(c)

		Reference standard: follow-up FBG		
		≥7.0 mmol/L	<7.0 mmol/L	Total
HbA_{1c} at time of TB diagnosis	≥6.5%	7	11	18
	<6.5%	1	192	193
	Total	8	203	211

RBG=random blood glucose; FBG=fasting blood glucose; HbA_{1c}=glycated haemoglobin; TB=tuberculosis

Table 3: Estimates of diagnostic accuracy and precision for each index test, overall and stratified by HIV status

Measure of test accuracy for diabetes diagnosis	RBG concentration	FBG concentration	HbA_{1c} concentration
All participants			
Sensitivity % (95% CI)	75.0 (34.9-96.8)	57.1 (18.4-90.1)	87.5 (47.4-99.7)
Specificity % (95% CI)	94.9 (91.2-97.3)	97.8 (94.9-99.3)	94.6 (90.5-97.3)
Positive predictive value % (95% CI)	33.3 (13.3-59.0)	44.4 (13.7-78.8)	38.9 (17.3-64.3)
Negative predictive value % (95% CI)	99.1 (96.8-100.0)	98.7 (96.1-99.7)	99.5 (97.2-100.0)
Participants uninfected with HIV			
Sensitivity % (95% CI)	83.3 (35.9-99.6)	60.0 (14.7-94.7)	83.3 (35.8-99.6)
Specificity % (95% CI)	93.2 (84.7-97.7)	100.0 (94.9-100.0)	96.8 (89.0-99.6)
Positive predictive value % (95% CI)	50.0 (18.7-81.3)	100.0 (29.2-100.0)	71.4 (29.0-96.3)
Negative predictive value % (95% CI)	98.6 (92.2-100.0)	97.3 (90.5-99.7)	98.4 (91.3-100.0)
Participants infected with HIV			
Sensitivity % (95% CI)	50.0 (1.3-98.7)	50.0 (1.3-98.7)	100.0 (15.8-100.0)
Specificity % (95% CI)	96.7 (92.4-98.9)	97.2 (93.0-99.2)	94.6 (89.2-97.8)
Positive predictive value % (95% CI)	16.7 (0.4-64.1)	20.0 (0.5-71.6)	22.2 (2.8-60.0)
Negative predictive value % (95% CI)	99.3 (96.2-100.0)	99.3 (96.1-100.0)	100.0 (97.1-100.0)

RBG=random blood glucose; FBG=fasting blood glucose; HbA_{1c}=glycated haemoglobin; CI=confidence interval

Supplementary Table 1: Baseline characteristics of participants, comparing individuals with and without reference standard test follow-up data

Characteristic		Participants with follow-up data n (%)	Participants without follow-up data n (%)	p-value
Overall		242 (100)	248 (100)	-
Index RBG	<11.1mmol/L	224 (92.6)	222 (89.5)	0.238
	≥11.1 mmol/L	18 (7.4)	26 (10.5)	
Index FBG	<7.0 mmol/L	223 (96.1)	153 (93.9)	0.302
	≥7.0 mmol/L	9 (3.9)	10 (6.1)	
Index HbA_{1c}	<6.5%	193 (91.5)	174 (90.2)	0.647
	≥6.5%	18 (8.5)	19 (9.8)	
Age (years)	18-24	29 (12.0)	29 (11.7)	0.445
	25-29	38 (15.7)	45 (18.2)	
	30-34	53 (21.9)	55 (22.2)	
	35-39	51 (21.1)	58 (23.4)	
	40-49	51 (21.1)	35 (14.1)	
	50+	20 (8.3)	26 (10.5)	
Sex	Male	167 (69.0)	170 (68.6)	0.913
	Female	75 (31.0)	78 (31.5)	
Household socioeconomic position	Very low	39 (16.2)	39 (15.7)	0.972
	Low	104 (43.2)	108 (43.6)	
	Medium	82 (34.0)	82 (33.1)	
	High	16 (6.6)	10 (7.7)	
Highest level of education	None	13 (5.4)	26 (10.5)	0.147
	Grade 1-7	93 (38.6)	101 (40.7)	
	Grade 8-12	130 (53.9)	116 (46.8)	
	College/ University	5 (2.1)	5 (2.0)	
Smoking history	Never smoked	176 (72.7)	181 (73.0)	0.949
	Current or ex-smoker	66 (27.3)	67 (27.0)	
Body Mass Index (weight(kg)/ $height^2$(m))	Healthy weight (18.5-24.9)	131 (54.4)	120 (48.4)	0.156
	Underweight (<18.5)	102 (42.3)	115 (46.4)	
	Overweight (25-29.9)	8 (3.3)	9 (3.6)	
	Obese (≥30)	0 (0.0)	4 (1.6)	
HIV status	Uninfected	79 (34.2)	62 (25.7)	0.044

	Infected	152 (65.8)	179 (74.3)	
TB clinic	1	82 (33.9)	135 (54.4)	<0.001
	2	56 (23.1)	49 (19.8)	
	3	104 (43.0)	64 (25.8)	

RBG=random blood glucose; FBG=fasting blood glucose; HbA_{1c} =glycated haemoglobin; p-values derived from chi-squared tests.

Discussion

Chapter 10: Overall discussion and conclusions

1 Summary of research findings

The research findings are presented in Chapters 7, 8 and 9, and so here I summarise the main findings relating to each PhD study question.

1.1 Study question 1: Does HIV modify the association between hyperglycaemia and active tuberculosis?

In our case-control study we found no evidence of an overall association between hyperglycaemia and active tuberculosis, though we did find evidence of effect modification by HIV for the association between hyperglycaemia and active TB: we found statistical evidence of a positive association among individuals infected with HIV, but no evidence of an association among individuals who were uninfected with HIV.

These findings could suggest that HIV and hyperglycaemia work synergistically to increase an individual's risk of active TB, or alternatively, the findings could be explained by an increased prevalence of transient stress hyperglycaemia among newly-diagnosed TB cases who are infected with HIV compared to individuals with newly-diagnosed TB but who are uninfected with HIV.

1.2 Study question 2: Is there a dose-response relationship between hyperglycaemia and tuberculosis treatment outcome?

In our cohort study we found no evidence of a dose-response relationship between glycaemia and tuberculosis treatment outcome, however, we found a low prevalence of hyperglycaemia in our study population and so our study was likely underpowered to detect any true association.

1.3 Study question 3: What proportion of hyperglycaemia among newly-diagnosed tuberculosis cases is due to diabetes mellitus rather than to stress-induced hyperglycaemia?

In our study of diagnostic accuracy, we found that a low proportion of hyperglycaemia among newly-diagnosed tuberculosis cases was due to diabetes. Of participants who had hyperglycaemia on the index random blood glucose, fasting blood glucose and glycated haemoglobin tests, 6 (33%), 4 (44%) and 7 (39%) participants respectively were found to have diabetes on the follow-up reference standard test. The remainder of the initial hyperglycaemia was found to be transient hyperglycaemia.

1.4 Study question 4: How accurate for the diagnosis of diabetes mellitus are measures of random blood glucose, fasting blood glucose and glycated haemoglobin concentrations measured at the time of tuberculosis diagnosis, compared to the reference standard of fasting blood glucose concentration measured after stabilisation of tuberculosis disease.

In our study of diagnostic accuracy, we found the sensitivity for diabetes diagnosis of random blood glucose, fasting blood glucose and glycated haemoglobin concentrations to be 75% (95% CI 35-97%), 57% (95% CI 18-90%) and 88% (95% CI 47-99%) respectively. We found the positive predictive value for diabetes diagnosis to be 33% (95% CI 13-59%), 44% (95% CI 14-79%) and 39% (95% CI 17-64%) respectively for RBG, FBG and HbA_{1c} concentrations. Confidence intervals were wide and overlapping for both these measures of accuracy, reflecting the low prevalence of hyperglycaemia and diabetes found in our study population. The specificity and negative predictive value were ≥95% with narrow confidence intervals for all index tests. We found no evidence that any one measure of glycaemia had greater test accuracy than the others for diabetes diagnosis at the time of tuberculosis diagnosis.

2 Study limitations

The limitations of each study are discussed in detail in Chapters 7, 8 and 9 and so here I focus on the overall limitations of this PhD work.

2.1 Low prevalence of hyperglycaemia

The main limitation of this work was the low prevalence of hyperglycaemia found in our study setting of Lusaka, Zambia. It was lower than the prevalence predicted from our preliminary background work, based on data from the ZAMSTAR study. The likely reason for this is the ZAMSTAR study was based in communities throughout Zambia, and so the prevalence of hyperglycaemia identified from the ZAMSTAR data was an average prevalence of hyperglycaemia among all the Zambian communities. Our PhD work was based only in Lusaka, in three low-income areas of the city. We found a lower prevalence of hyperglycaemia than we had anticipated. Whilst this is an important finding in itself, the consequence of low study power was seen in studies 2 and 3.

As a consequence of the low prevalence of hyperglycaemia, we chose to re-categorise our glycaemia categories in study 2, to allow us to compare TB treatment outcomes across a lower range of glycaemia. This enabled us to still explore a dose-response relationship between glycaemia and TB treatment outcome, but as we had so few participants with higher levels of glycaemia, we were unable to explore this dose-response relationship across higher levels of glycaemia. This may well account for why we found no evidence of an association between glycaemia and TB treatment outcome.

It is important to note that our own methods may have contributed to our finding of a low prevalence of hyperglycaemia, due to our use of a single random blood glucose level to screen for hyperglycaemia as discussed further in section 2.2.

2.2 Measurement of glycaemia

In these studies we focused on glycaemia as a continuous process in addition to a binary with/without disease entity, aiming to help us understand the shifts in glycaemic levels associated with TB (with or without HIV), whist simultaneously ensuring the studies were feasible to undertake. Ideally, if it was feasible and acceptable to participants, we would have had measures of oral glucose tolerance, fasting blood glucose and glycated haemoglobin at all

time points in all of our studies. But this would have been neither acceptable to participants nor feasible in just under 4,000 TB cases and 7,000 community controls. We therefore opted to choose the methods of glucose measurement that were feasible for each study, and then made certain that our subsequent interpretation of the findings reflected the methods used, and did not extrapolate to other methods of glucose measurement.

The major concern with this approach is the potential for underestimating the prevalence of hyperglycaemia. We know that measures of random blood glucose lack sensitivity for the detection of hyperglycaemia or diabetes. For the few participants who were tested with both a random blood glucose and HbA_{1c} in our study of diagnostic accuracy, we found that 12/80 participants (15%) had a RBG <7mmol/L but went on to have a HbA_{1c} ≥6.0%. It is likely therefore that for the many participants who only received a random blood glucose measurement we failed to detect some who were truly hyperglycaemic. We also found 42/60 participants (70%) had a RBG ≥7.0mmol/L but a HbA_{1c} <6.0%, which further highlights the failings of a single random blood glucose concentration as a measure of hyperglycaemia.

We also chose not to incorporate prior diagnoses of diabetes within our definition of hyperglycaemia, partly because we were explicitly focusing on hyperglycaemia rather than diabetes, partly because of the likelihood of misclassification due to a low level of understanding of diabetes within our study communities, and partly because the prevalence of well-controlled diabetes in our study setting is very low. It was however possible for individuals with previously diagnosed (known) diabetes mellitus who were on treatment to be normoglycaemic at the time of measuring glycaemia even though they may be hyperglycaemic at other times. This could have led to an underestimate of the association between long term hyperglycaemia and acute TB but did not affect the association between hyperglycaemia and TB treatment outcomes because for that study there were only two individuals who reported a prior diagnosis of diabetes and both were hyperglycaemic at baseline.

2.3 Missing data

We found a high proportion of missing data in our studies, particularly for follow-up data. A major cause of this was because we arranged to follow up participants in the TB clinics during routine appointments. However, we discovered during the studies that it is common practice in our study setting for TB patients to send relatives to represent them at the clinic, rather that the patients themselves attending. The relatives pick up medication and deliver any necessary specimens. We had neither the ethics approval nor the resources to visit participants in their homes to obtain follow-up data, and so we were unable to obtain high levels of follow-up data for all of our participants. We found no difference in baseline characteristics between individuals with and without missing data in our studies, so there is no reason to believe that this has introduced bias, but it did contribute to a lack of study power for our longitudinal studies.

We also experienced difficulties obtaining CD4 count results for many of our HIV-infected participants. In Ministry of Health establishments in Zambia, CD4 testing is a routine part of the management of newly-diagnosed TB patients who are infected with HIV. In Lusaka, many clinics have capabilities to process CD4 tests in the clinic laboratory. We therefore planned to obtain the routine CD4 count result for our participants, whenever possible, rather than duplicate the process again ourselves. However, we experienced great difficulties obtaining a timely result. We found that there was often a long delay between TB diagnosis and CD4 count testing, leaving us with a result that was not applicable to the time of TB diagnosis. However, a bigger difficulty was obtaining any result, regardless of timing. The clinic systems are mostly paper-based rather than computerised, so physically finding the result, if one was available, was challenging. Further, many participants simply did not have their CD4 count tested due to various reasons including breakdown of laboratory machines and a lack of reagents.

When planning these studies, we had intended to explore sub-group analyses for hypothesis generation purposes, including stratification of the case-control analysis by CD4 count. Given

the combined factors of a low prevalence of hyperglycaemia and a high proportion of missing data for CD4 count we were unable to explore this sub-group analysis. This was also the case for stratification by use of antiretroviral medication (as a proxy for viral load), though largely due to the low prevalence of hyperglycaemia rather than due to missing data.

3 Overall conclusions

The overall aim of this research was to determine how hyperglycaemia affects tuberculosis control in the sub-Saharan Africa context of high HIV prevalence and poor diabetes control. All studies conducted for this work found a low prevalence of hyperglycaemia, both in individuals with tuberculosis and in those without. We found no evidence of an overall association between hyperglycaemia and tuberculosis in our study population in Lusaka, Zambia. We found no evidence of a dose-response relationship between glycaemia and tuberculosis treatment outcome, and though our cohort study likely lacked power to determine any true association, it did so because of the low prevalence of hyperglycaemia found in our study population. Regardless, therefore, this suggests that hyperglycaemia is not a driver of poor tuberculosis treatment outcomes in our study setting. Combined, these studies suggest that hyperglycaemia is not a major threat to tuberculosis control in our study setting of Lusaka, Zambia.

However, we did find evidence of association between hyperglycaemia and active tuberculosis among individuals infected with HIV, and we later found that a high proportion of hyperglycaemia measured at the time of TB diagnosis was transient rather than persistent. It is possible that the association seen in our population between hyperglycaemia and active tuberculosis among individuals with HIV reflects a higher proportion of transient stress hyperglycaemia among individuals infected with HIV than in uninfected individuals. We were unable to test this hypothesis formally due to the low numbers of individuals with hyperglycaemia recruited to our studies and the consequent lack of power for subgroup analyses, but we did see a higher point prevalence of transient hyperglycaemia in participants

infected with HIV than in uninfected participants. This was true for all three measures of glycaemia.

We also found evidence of association between hyperglycaemia and active tuberculosis among individuals with sputum smear/Xpert-positive pulmonary TB. Transient hyperglycaemia could again be an explanation for this association, as it is plausible that the most unwell newly diagnosed pulmonary TB cases, and therefore the most likely to have stress hyperglycaemia, are found among individuals with smear-positive rather than smear-negative disease. Again, our studies were underpowered to test this hypothesis.

We were not able to identify any test that was accurate for diabetes diagnosis at the time of tuberculosis diagnosis, and so, when testing for diabetes in individuals with tuberculosis, repeat testing after resolution of acute tuberculosis disease should be considered before a diagnosis of diabetes is made, as any initial hyperglycaemia identified could reflect transient hyperglycaemia.

4 Future research

Future work following on from this research would be best located in a setting with equally high tuberculosis incidence and HIV prevalence, but with a higher prevalence of hyperglycaemia than we found in Lusaka. This would ensure that it is feasible to obtain adequate study power.

Further studies are needed to better understand the importance of transient hyperglycaemia in patients with tuberculosis.[1] Studies to determine the prevalence of transient hyperglycaemia among hyperglycaemic individuals with TB in other settings around the world would be valuable, as would studies among subgroups, including individuals infected and uninfected with HIV. Studies to better understand the nature of transient hyperglycaemia would also be valuable – does this reflect stress hyperglycaemia due to acute tuberculosis disease or are there other causes of transient hyperglycaemia in individuals with tuberculosis?

Further studies are needed to assess the relationship between increasing levels of glycaemia and TB treatment outcome. If adequately powered studies find a dose-response relationship between rising levels of glycaemia and poor tuberculosis treatment outcome, as did Boillat-Blanco *et al*'s study in Tanzania,[2] a trial of interventions to improve glucose control among individuals with tuberculosis would be of value. Urban Tanzania has high rates of TB, HIV and hyperglycaemia[2] and so would be ideal for such a trial.

5 References

1. Salman H. Sidiqi, S.R.-G., *MGIT Procedure Manual for Bactec MGIT 960 TB System.* 2006.
2. Kent, P.T. and G.P. Kubica, *Public Health mycobacteriology: a guide for the level III laboratory. Department of Health and Human Services, Centers for Disease Cntrol, Atlanta, Ga*. 1985.

Cover sheets for chapters based on research papers

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
www.lshtm.ac.uk

Registry
T: +44(0)20 7299 4646
F: +44(0)20 7299 4656
E: registry@lshtm.ac.uk

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RESEARCH PAPER COVER SHEET

PLEASE NOTE THAT A COVER SHEET MUST BE COMPLETED FOR EACH RESEARCH PAPER INCLUDED IN A THESIS.

SECTION A – Student Details

Student	Sarah Lou Bailey
Principal Supervisor	Helen Ayles
Thesis Title	Understanding the effect of hyperglycaemia on tuberculosis control in a southern African setting: the impact of HIV and diabetes control. Paper title: Association between diabetes mellitus and active tuberculosis in Africa and the effect of HIV

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

SECTION B – Paper already published

Please note the published version is not identical to the version presented in this thesis because the thesis version incorporates post-viva amendments

Where was the work published?	Tropical Medicine and International Health		
When was the work published?	March 2017		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	N/A		
Have you retained the copyright for the work?*	Yes Open-access CC-BY licence	Was the work subject to academic peer review?	Yes

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SECTION C – Prepared for publication, but not yet published

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

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I designed the study, conducted the literature review, drafted the manuscript, prepared the final version and responded to peer reviewers comments

Student Signature: [redacted] Date: 24/7/17

Supervisor Signature: [redacted] Date: 4/9/17

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
www.lshtm.ac.uk

Registry
T: +44(0)20 7299 4646
F: +44(0)20 7299 4656
E: registry@lshtm.ac.uk

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SECTION A – Student Details

Student	Sarah Lou Bailey
Principal Supervisor	Helen Ayles
Thesis Title	Understanding the effect of hyperglycaemia on tuberculosis control in a southern African setting: the impact of HIV and diabetes control Paper title: Diabetes mellitus in Zambia and the Western Cape province of South Africa: prevalence, risk factors, diagnosis and management

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

SECTION B – Paper already published

Where was the work published?	Diabetes Research and Clinical Practice		
When was the work published?	07 May 2016		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	N/A		
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Student Signature: [redacted] Date: 24/7/17

Supervisor Signature: [redacted] Date: 4/9/17

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
www.lshtm.ac.uk

Registry
T: +44(0)20 7299 4646
F: +44(0)20 7299 4656
E: registry@lshtm.ac.uk

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Student	Sarah Lou Bailey
Principal Supervisor	Helen Ayles
Thesis Title	Understanding the effect of hyperglycaemia on tuberculosis control in a southern African setting: the impact of HIV and diabetes control. Paper title: The association of hyperglycaemia with prevalent tuberculosis: a population-based cross-sectional study

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Where was the work published?	BMC Infectious Diseases		
When was the work published?	05 Dec 2016		
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Student Signature: [redacted] Date: 24/7/17

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London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
www.lshtm.ac.uk

Registry
T: +44(0)20 7299 4646
F: +44(0)20 7299 4656
E: registry@lshtm.ac.uk

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Principal Supervisor	Helen Ayles
Thesis Title	Understanding the effect of hyperglycaemia on tuberculosis control in a southern African setting: the impact of HIV and diabetes control. Paper title: The effect of HIV on the association between hyperglycaemia and active tuberculosis in Zambia, a case-control study

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

SECTION B – Paper already published

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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	Tropical Medicine and International Health
Please list the paper's authors in the intended authorship order:	Bailey SL, Floyd S, Yudkin JS, Godfrey-Faussett P, Ayles H
Stage of publication	**Not yet submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I designed the study, wrote the study protocol, managed a research team to undertake data collection, analysed the data, drafted the manuscript and prepared the final

	version.

Student Signature: [redacted] Date: 24/7/17

Supervisor Signature: [redacted] Date: 4/9/17

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
www.lshtm.ac.uk

Registry
T: +44(0)20 7299 4646
F: +44(0)20 7299 4656
E: registry@lshtm.ac.uk

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Principal Supervisor	Helen Ayles
Thesis Title	Understanding the effect of hyperglycaemia on tuberculosis control in a southern African setting: the impact of HIV and diabetes control. Paper title: The effect of hyperglycaemia on tuberculosis treatment outcome in Lusaka, Zambia; a cohort study

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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	International Journal of Tuberculosis and Lung Disease
Please list the paper's authors in the intended authorship order:	Bailey SL, Floyd S, Munkondya-Gondwe S, Mwanza W, Maluzi K, Cheeba-Lengwe M, Chiwele-Kangololo K, Kaluba-Milimo D, Kosloff B, Yudkin JS, Godfrey-Faussett P, Ayles H
Stage of publication	**Not yet submitted**

SECTION D – Multi-authored work

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Student Signature: [redacted] **Date:** 24/7/14

Supervisor Signature: [redacted] **Date:** 4/9/17

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
www.lshtm.ac.uk

Registry
T: +44(0)20 7299 4646
F: +44(0)20 7299 4656
E: registry@lshtm.ac.uk

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SECTION A – Student Details

Student	Sarah Lou Bailey
Principal Supervisor	Helen Ayles
Thesis Title	Understanding the effect of hyperglycaemia on tuberculosis control in a southern African setting: the impact of HIV and diabetes control. Paper title: The accuracy of measures of glycaemia for the diagnosis of diabetes mellitus in newly-diagnosed tuberculosis patients in Zambia, a study of diagnostic accuracy

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When was the work published?			
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
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Please list the paper's authors in the intended authorship order:	Bailey SL, Floyd S, Maluzi K, Cheeba-Lengwe M, Kosloff B, Yudkin JS, Godfrey-Faussett P, Ayles H
Stage of publication	**Not yet submitted**

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Student Signature: [redacted] **Date:** 24/7/17

Supervisor Signature: [redacted] **Date:** 4/9/17

Appendices

Appendix I: Ethics Approval and Permissions

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
United Kingdom
Switchboard: +44 (0)20 7636 8636
www.lshtm.ac.uk

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

Observational / Interventions Research Ethics Committee

Sarah Louise Bailey
Clinical Research Fellow, Wellcome Trust
Clinical PhD Programme, International Health
CR / ITD
LSHTM

19 March 2013

Dear Dr. Bailey,

Study Title: **Understanding the threat of diabetes mellitus to tuberculosis control in sub-Saharan Africa: the impact of HIV and diabetes control**
LSHTM ethics ref: **6368**

Thank you for your application of 18 February 2013 for the above research, which has now been considered by the Observational Committee.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
LSHTM ethics application	n/a	
Protocol including Information sheet and Consent form	V1	02/01/2013

After ethical review

Any subsequent changes to the application must be submitted to the Committee via an E2 amendment form. All studies are also required to notify the ethics committee of any serious adverse events which occur during the project via form E4. At the end of the study, please notify the committee via form E5.

Yours sincerely,

Professor Andrew J Hall
Chair
ethics@lshtm.ac.uk
http://intra.lshtm.ac.uk/management/committees/ethics/

THE UNIVERSITY OF ZAMBIA
BIOMEDICAL RESEARCH ETHICS COMMITTEE

Telephone: 260-1-256067
Telegrams: UNZA, LUSAKA
Telex: UNZALU ZA 44370
Fax: + 260-1-250753
E-mail: unzarec@unza.zm
Assurance No. FWA00000338
IRB00001131 of IORG0000774

Ridgeway Campus
P.O. Box 50110
Lusaka, Zambia

4th June, 2013

Your Ref: 007-04-13

Dr. Sarah Lou Bailey
ZAMBART Project
P.O. Box 50697
LUSAKA

Dear Dr. Bailey,

RE: RE-SUBMITTED RESEARCH PROPOSAL: **"UNDERSTANDING THE THREAT OF DIABETES MELLITUS TO TUBERCULOSIS CONTROL IN SUB-SAHARAN AFRICA: THE IMPACT OF HIV DIABETES CONTROL" (REF. NO.: 007-04-13)**

The above mentioned research proposal was re-submitted to the Biomedical Research Ethics Committee for ethical review on 29th May, 2013. The proposal is approved.

CONDITIONS:

- This approval is based strictly on your submitted proposal. Should there be need for you to modify or change the study design or methodology, you will need to seek clearance from the Research Ethics Committee.
- If you have need for further clarification please consult this office. Please note that it is mandatory that you submit a detailed progress report of your study to this Committee every six months and a final copy of your report at the end of the study.
- This waiver does not release you from the obligation of ensuring confidentiality.
- Please note that when your approval expires you may need to request for renewal. The request should be accompanied by a Progress Report (Progress Report Forms can be obtained from the Secretariat).
- **Ensure that a final copy of the results is submitted to this Committee.**

Yours sincerely,

[redacted]

Dr. J.C Munthali
CHAIRPERSON

Date of approval: 29 May, 2013 **Date of expiry**: 28 May, 2014

All Correspondence should be addressed to the Permanent Secretary
Telephone: +260 211 253040/5
Fax : +260 211 253344

REPUBLIC OF ZAMBIA

MINISTRY OF HEALTH

In reply please quote:
MH/101/17/6
No.....

NDEKE HOUSE
P. O. BOX 30205
LUSAKA

25th June, 2013

Dr. Sarah-Louise Bailey
Zambart Project
Department of Medicine
Ridgeway Campus
P.O. Box 50697
Lusaka
Zambia

Dear Dr. Bailey,

Re: Request for Authority to Conduct Research

The Ministry of Health is in receipt of your request authority to conduct a study titled, **"Understanding the threat of diabetes mellitus to tuberculosis control in the context of Sub-Saharan Africa: the impact of HIV and diabetes control".** I wish to inform you that following submission of your research proposal to my Ministry, our review of the same and in view of the ethical clearance, my Ministry has granted you authority to carry out the study on condition that:

1. The relevant Provincial and District Directors of Health where the study is being conducted are fully appraised;
2. Progress updates are provided to MoH quarterly from the date of commencement of the study;
3. The final study report is cleared by the MoH before any publication or dissemination within or outside the country;
4. After clearance for publication or dissemination by the MoH, the final study report is shared with all relevant Provincial and District Directors of Health where the study was being conducted, and all key respondents.

Yours sincerely,

[redacted]

Dr. P. Mwaba
Permanent Secretary
MINISTRY OF HEALTH

Telephone: (260) 211 235341
Fax (260) 211 235342

In reply please quote:

No.:.....

REPUBLIC OF ZAMBIA

MCDMCH/14/15/1

MINISTRY OF COMMUNITY DEVELOPMENT, MOTHER AND CHILD HEALTH

OFFICE OF THE PERMANENT SECRETARY
COMMUNITY HOUSE
SADZU ROAD
PRIVATE BAG W 252
LUSAKA

23rd July, 2013

Dr. Sarah Louise Bailey
Principal Lead Investigator
Zambart Project
Department of Medicine
Ridgeway Campus
LUSAKA

RE: REQUEST TO CARRY OUT A STUDY ON UNDERSTANDING THE THREAT OF DIABETES MELLITUS TO TUBERCULOSIS CONTROL IN THE CONTEXT OF SUB-SAHARAN AFRICA: THE IMPACT OF HIV AND DIABETES CONTROL

Reference is made to your letter concerning the above subject.

Please be informed that Ministry of Community Development, Mother and Child Health has no objection to your request to carry out a case-control study in Lusaka at George and Chawama Health Centres. However, be informed that permission to conduct the study will be subject to you providing ethical clearance from the Research Ethics Committee.

Kindly avail the Ministry with the report after completion of the study.

[redacted]

Prof. Elwyn M. Chomba
Permanent Secretary
MINISTRY OF COMMUNITY DEVELOPMENT, MOTHER AND CHILD HEALTH

REPUBLIC OF ZAMBIA

MINISTRY OF HEALTH
LUSAKA PROVINCE HEALTH OFFICE

Office of the Director, P.O. Box 32573, Lusaka, Zambia
Tel: 260 211 256815, Telefax: 260 211 256814

August 8, 2013

From: Provincial Medical Officer

To: District Medical Officer-Lusaka

RE: REQUEST FOR AUTHORITY TO CONDUCT A STUDY ON UNDERSTANDING THE THREAT OF DIABETIS MELLITUS TO TUBERCULOSIS CONTROL IN THE CONTEXT OF SUB-SAHARA AFRICA:THE IMPACTOF HIV AND DIABETIS CONTROL.

Dr. Sarah-Louise Baily is working with the Zambart Project and has been given permission by both the Ministries of Health and Community Development Mother and Child Health to conduct the above mentioned study. The study will be conducted in TB corners of Chawama and George Health centre.

The proposal has gone through Ministry of Health and Ethical clearance has been given. During the study the following conditions will apply

1. Produce the necessary identification and clearance documents to the District Medical Officer before starting the study.
2. The District and Provincial Medical Officers of Lusaka respectively will be appraised upon complexion of the study
3. Progress updates are provided to MoH quarterly from date of commencement of the study
4. The final study report is cleared by the MoH before any publication or dissemination within or outside the country.

Please accord the researcher all the necessary assistance she will need.

[redacted]

Dr Tackson Lambart

ZAMBART PROJECT

Department of Medicine
Ridgeway Campus
P. O. Box 50697
Lusaka
ZAMBIA

Tel/Fax: 211-25.47.10
Email: sarah-louise.bailey@lshtm.ac.uk

30th July 2013
Dr Masininga
Director
Lusaka District Health Office
Lusaka
Zambia

Permission granted. Kindly facilitate her study.

[redacted]

Dear Dr Masininga

Re: Understanding the threat of diabetes mellitus to tuberculosis control in the context of sub-Saharan Africa: the impact of HIV and diabetes control.

I would like to request your permission to conduct the above titled study, for which we have been granted ethical clearance and authority from both the Ministry of Health and the Ministry of Community Development, Mother and Child Health. We plan to conduct the data collection in the Tuberculosis corners of Chawama Health Centre and George Health Centre.

Hyperglycaemia is known to be associated with tuberculosis. The incidence of active tuberculosis is two to three times higher in those with high than normal blood glucose concentrations. The context of these associations in sub-Saharan Africa may differ from the rest of the world due to the high prevalence of HIV and the high prevalence of poorly controlled diabetes mellitus in this region.

Therefore, a case-control study (study 1) aims to determine if HIV modifies the association of hyperglycaemia with tuberculosis incidence. A subset of cases from study 1 will be recruited to a cohort study (study 2) to determine whether a dose-response relationship exists between hyperglycaemia and tuberculosis treatment outcome. Finally, a diagnostic accuracy study (study 3) will recruit a separate subset of cases from study 1 to distinguish between diabetes mellitus and stress-induced hyperglycaemia.

The anticipated duration of this project is 3 years.

I attach confirmation of ethical clearance from UNZA Biomedical Research Committee and permission from the Ministries of Health and Community Development, Mother and Child Health.

Thanking you in advance for your support.

Best Regards

[redacted]

Dr Sarah Lou Bailey
Principal lead Investigator "Understanding the threat of diabetes mellitus to tuberculosis control in the context of sub-Saharan Africa: the impact of HIV and diabetes control"

Appendix II: World Health Organization definition of a tuberculosis case

Taken from: Treatment of tuberculosis: guidelines – 4th edition. WHO/HTM/TB/2009.420. p23-25. http://whqlibdoc.who.int/publications/2010/9789241547833_eng.pdf

The TB case definitions below are based on the level of certainty of the diagnosis and on whether or not laboratory confirmation is available.

Definition of case of tuberculosis: A definite case of TB (defined below) or one in which a health worker (clinician or other medical practitioner) has diagnosed TB and has decided to treat the patient with a full course of TB treatment.

Note. Any person given treatment for TB should be [considered] a case. Incomplete "trial" TB treatment should not be [considered] as a method for diagnosis.

Definition of definite case of tuberculosis: A patient with *Mycobacterium tuberculosis* complex identified from a clinical specimen, either by culture or by a newer method such as molecular line probe assay. In countries that lack the laboratory capacity to routinely identify *M. tuberculosis*, a pulmonary case with one or more initial sputum smear examinations positive for acid-fast bacilli (AFB) is also considered to be a "definite" case, provided that there is a functional external quality assurance (EQA) system with blind rechecking.

Pulmonary tuberculosis (PTB) refers to a case of TB (defined above) involving the lung parenchyma. Miliary tuberculosis is classified as pulmonary TB because there are lesions in the lungs. Tuberculous intrathoracic lymphadenopathy (mediastinal and/or hilar) or tuberculous pleural effusion, without radiographic abnormalities in the lungs, constitutes a case of *extra*pulmonary TB. A patient with both pulmonary and extrapulmonary TB should be classified as a case of *pulmonary* TB.

Extrapulmonary tuberculosis (EPTB) refers to a case of TB (defined above) involving organs other than the lungs, e.g. pleura, lymph nodes, abdomen, genitourinary tract, skin, joints and bones, meninges. Diagnosis should be based on at least one specimen with confirmed *M. tuberculosis* or histological or strong clinical evidence consistent with active EPTB, followed by a decision by a clinician to treat with a full course of tuberculosis chemotherapy. The case definition of an EPTB case with several sites affected depends on the site representing the most severe form of disease. Unless a case of EPTB is confirmed by culture as caused by *M. tuberculosis*, it cannot meet the "definite case" definition given above.

Appendix III: World Health Organization criteria for diabetes mellitus diagnosis

Taken from: Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia. Report of a WHO/IDF consultation. WHO Document Production Services, Geneva, Switzerland. 2006. p3

The following Table summarises the 2006 WHO recommendations for the diagnostic criteria for diabetes and intermediate hyperglycaemia.

Diabetes	
Fasting plasma glucose	≥7.0mmol/l (126mg/dl)
OR	
2‑h plasma glucose*	≥11.1mmol/l (200mg/dl)

Impaired Glucose Tolerance (IGT)	
Fasting plasma glucose	<7.0mmol/l (126mg/dl)
AND	
2‑h plasma glucose*	≥7.8 and <11.1mmol/l (140mg/dl and 200mg/dl)

Impaired Fasting Glucose (IFG)	
Fasting plasma glucose	6.1 to 6.9mmol/l (110mg/dl to 125mg/dl)
AND (if measured)	
2‑h plasma glucose*	<7.8mmol/l (140mg/dl)

** Venous plasma glucose 2 ‑h after ingestion of 75g oral glucose load*
** If 2 ‑h plasma glucose is not measured, status is uncertain as diabetes or IGT cannot be excluded*

In 2011 an addendum to the diagnostic criteria published in the 2006 WHO/IDF report was published, following a WHO expert consultation (Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus. Report of a WHO Consultation. WHO Document Production Services, Geneva, Swizerland. 2011). The WHO consultation concluded that glycated haemoglobin can be used as a diagnostic test for diabetes, provided that stringent quality assurance tests are in place and assays are standardised to criteria aligned to the international reference values. An HbA1c of 6.5% is recommended as the cut point for diagnosing diabetes. There were no changes to the above 2006 recommendations on the use of plasma glucose measurements to diagnose diabetes.

Appendix IV: Participant Information Sheet

INFORMATION FOR POTENTIAL PARTICIPANTS OF THE DIABETES-TB CONTROL STUDY

You are invited to take part in a study to improve our understanding of how diabetes (sugar disease) affects tuberculosis and its control in Zambia. 2,000 people who are diagnosed with tuberculosis at ..... clinic (fill in clinic name) will take part in this study. Another 2,000 people who are diagnosed with tuberculosis in another clinic in Lusaka will also take part. Information on the study is supplied in this leaflet. A trained research nurse will be on hand to explain it and answer all your questions. Please check that you understand everything in this document. If you decide to take part, you will be asked to give written consent before you take part.

Who is doing the study?

This study is being performed by health workers from ZAMBART Project from the University of Zambia led by Dr Sarah Lou Bailey. Research assistants will be responsible for the day to day running of the study. ZAMBART Project is based at the Ridgeway Campus in Lusaka. The full contact details are: ZAMBART Project, P.O Box 50697, Lusaka Phone 021-1254710; Fax: 021-1257215 Email: Info@zambart.org.zm. Your Local Research Assistant can be contacted at:

……

.....(Name and Contact details of research assistant for each clinic here)

This research protocol has been approved by the University of Zambia Research Ethics Committee:

The Chairperson
Research Ethics Committee
University of Zambia
Ridgeway Campus
P.O Box 50110
Lusaka, Zambia
Tel/Fax 021-1250753
Email: unzarec@zamtel.zm

What is the purpose of the study?

The main aim of this study is to improve our understanding of how diabetes affects tuberculosis (TB) and its control in Zambia. We know from other studies that have been done in other countries around the world, that people who have diabetes are more likely to develop tuberculosis than people who don't have diabetes. We also know that once they have tuberculosis, the treatment for tuberculosis doesn't work as well as it does in people who don't have diabetes. But Zambia and other similar countries in Africa may be different to elsewhere in the world, and we want to know how diabetes affects tuberculosis here in

Zambia. This will in turn help to improve the way diabetes and tuberculosis are dealt with in our country.

Diabetes causes abnormal sugar levels in the blood. If untreated, this can cause a person to lose weight and become very thirsty. Over months and years, abnormal sugar levels in the blood can cause many different problems in the body, including poor eye sight and blindness, damage to the kidneys, heart disease and stroke. It also affects a person's ability to fight off infections, so a person with diabetes is more likely to get infections such as TB than a person without diabetes. To find out whether you have diabetes we will be asking you to provide a blood sample by means of a finger prick which will be screened immediately by a research nurse. The results can be available in just 5 minutes. You will be given your results and told what next step to take depending on the results.

TB is an infectious disease caused by bacteria (germs), which are spread through droplets (coughing). TB infection mainly affects the lungs, but can also affect other parts of the body, such as the lining of the brain (meningitis), and glands. In spite of a good programme for tracing and treating people with active TB infection, many people in Zambia still develop TB disease each year. If we can understand better why some people get TB more than others (such as people with diabetes) then this could help up to improve identifying people who have TB at an early stage of their disease.

We know that people with HIV, the virus that can cause AIDS, are also more likely to get TB than people without HIV. One thing that we're particularly interested in in this study is what happens to tuberculosis if someone has both diabetes and HIV. Therefore, if you haven't had a recent HIV test, we will be asking you to provide a blood sample which we use to test for HIV. The results of the HIV test can be made available in 20 minutes. A trained counsellor will give you pre-test and post-test counselling and you will be referred to the clinic if you need any care. If you prefer, you can have the test but choose not to receive the results.

We will ask you questions to get more details about yourself and your living conditions. This information will help us better understand how diabetes affects tuberculosis.

Taking part in this study is voluntary. You are free to withdraw from this study at any stage, without any consequences for you. No financial reward will be given to any persons taking part in this study.

Are there any risks for people who take part in this survey?
Taking part in this study does not pose any risks to you or your family. However, you may feel worried about the results of the tests done. We will refer you for care and we will be in your TB clinic every day Monday – Friday so that if you have any questions later you can come and discuss them with us. It is possible that you may experience some mild discomfort or bruising when we take a finger prick or other blood sample from you. We will do our best to minimise any discomfort. The following will be required of all those taking part:

1) You will be asked to sign two copies of a consent form after you have read and understood this information leaflet. You will be given an original copy of this leaflet and one of the copies of the consent form to keep.

2) You will be asked to answer questions about your health. The research nurse will ask you the questions and will record your answers on a computer copy of our questionnaire. She/he will write the answers directly onto a handheld computer.

3) We will measure your blood pressure, height, weight and the distance around your waist.

4) You will be asked to provide a finger prick blood samples for diabetes testing.

5) If you have not had a recent HIV test we will ask you if we can use some of this blood to also test for HIV.

6) If you are HIV positive we will ask if you've had a recent CD4 test. This is something that should be checked when you are diagnosed with TB, but if it hasn't been done then we can do it for you.

7) We will select some people in the study to follow-up to see how they get on over the first 2 months of their TB treatment. If we have selected you, we will ask you to cough up some phlegm once a week for two months, to see how quickly your tuberculosis is improving with treatment. We will also like to perform some more sophisticated tests for diabetes. One is called HbA_{1c}, for which we will need a small blood sample. The other is called fasting blood glucose, which involves us taking another finger prick blood sample. For this test you must not have eaten or drank anything except water for the previous 6 hours, so it's easiest if we do this first thing in the morning before you eat breakfast. As you won't have had breakfast we will give you a drink and a bun after the test. For this test, you may need to make an additional trip to the clinic, in which case we will pay for your travel to and from the clinic.

8) Your blood samples and TB cultures will be kept in a sample bank with only a bar code on (not your name). These samples can be used in future for further tests on diabetes or TB.

What is the benefit to you of taking part in this study?

By taking part in this study you will be checked for diabetes and HIV. If you have either of these conditions you will be referred for treatment so that you can be kept healthy. We will also give you a health information/education leaflet about diabetes. The information gained from this study will be used to provide suggestions for improving health services.

Blood Sugar and blood pressure Results

If your blood sugar results are abnormal, you will be given an information sheet on diabetes and referred to the health centre for further screening and advice. We will also refer you to the health centre if your blood pressure is persistently high.

What will happen if the HIV test is positive?

If your HIV results are positive, we will refer you to your local clinic for you to access appropriate care for HIV. We will also inform your TB clinic so that they can offer you the best treatment for your tuberculosis.

Quality assurance

As part of the process of collecting data, we will perform quality assurance on some of the data that will be collected. You may be asked some of the questions in the questionnaire a second time by a different member of the study team to check that we haven't made any mistakes.

Confidentiality of information and privacy of the participant
All personal information obtained during this study will remain strictly confidential. Your questionnaire answers will be transferred directly to a computer, but your name will not be included. You will be identified by a coded number only. No information about any of your results will be released to any other parties but the research team, without your further consent.

The information entered onto the computer will be stored on a central database which will be protected by a password. Your name and address will be stored on a separate database which will be protected by a separate password. No information regarding personal details which could identify individuals or individual households will be disclosed. However, there may be follow-up studies to this study. In this case personal details may be obtained only after further ethical approval and may only be made available to the principal investigator of any follow-up study to facilitate follow-up.

Thank you for reading this information sheet. If you have any questions, please ask them now. The research nurse will be pleased to answer them. If you wish to take part, please read and sign the consent form. Please keep this information sheet in a safe place.

Appendix V: Participant Consent Form

Participant study ID number

INFORMED CONSENT FORM FOR DIABETES-TB CONTROL STUDY (PERSONS AGED 18 YEARS AND OLDER)

1. I confirm that I have read the information sheet, and that the information and procedures involved in my taking part in this study have been explained to me.
2. I have been given time and opportunity to read the information carefully, to discuss it with others and to decide whether or not to take part in this study.
3. I understand that my blood and sputum specimens will be kept in a sample bank for up to 5 years with only a bar code on (not my name) and that these samples can be used in future for further tests relating to diabetes or TB.
4. I understand that the results of this study will be published in scientific journals but that my name will not be used.
5. I agree to take part in the study.

Subject's signature/fingerprint: _____ Date _____

Subject's name: _____ (please print)

The person who obtains the informed consent discussion must also sign and date this form.

Signature: _____Date _____

Name: _____ (please print)

Signature of witness (if applicable)

Signature of witness: _____Date_____

Witnessed by (print name): _____

Appendix VI: Flow Charts of Participant Activities

Flow Chart of Participant Activities:

Study 1 Participants

Today

- Read the information sheet
- Read and sign the consent form
- Answer some questions that we ask you about you and your health
- We will measure your blood pressure, height, weight and the distance around your waist
- We will take a finger-prick drop of blood for a sugar test
- (If you haven't had a recent HIV test we will also ask you if we can use some of this blood to test for HIV. If you have had a recent HIV test at the TB clinic then there's no need to repeat this. If you already know that you are HIV positive we will ask if you have had a recent CD4 test. This is something that should be checked when you are diagnosed with TB, but if it hasn't been done then we can do it for you.)

Flow Chart of Participant Activities:

Study 2 Participants

Today

- Read the information sheet
- Read and sign the consent form
- We will take a blood sample from you for a detailed sugar test (called HbA1c)
- Please give us a sputum sample by coughing up some phlegm into a collection pot
- You will need to fast overnight tonight to prepare for a sugar test tomorrow morning. Please don't eat or drink anything except water after you've gone to bed this evening

Tomorrow morning

- Please don't eat or drink anything except for water. Come straight to the clinic in the morning
- We will take a finger-prick drop of blood for the fasting sugar test before you collect your medicines. As soon as this is done you can eat and drink whatever you like. We will give you a drink and a bun to eat for you comfort

Next week and then weekly for a total of 8 weeks

- When you come to the clinic to collect your medicines each week, please give us another sputum sample

In three months

- We will repeat the fasting sugar test on a day that you are coming to the clinic to collect your medicines. When you wake up in the morning, again, please do not eat or drink anything except water. We will take another finger-prick drop of blood for the fasting sugar test. As soon as this is done you can eat and drink whatever you like. We will give you a drink and a bun to eat for your comfort
- We will also take another blood sample to repeat the detailed sugar test (HbA1c)

Flow Chart of Participant Activities:

Study 3 Participants

Today

- Read the information sheet
- Read and sign the consent form
- We will take a blood sample from you for a detailed sugar test (called HbA1c)
- You will need to fast overnight tonight to prepare for a sugar test tomorrow morning. Please don't eat or drink anything except water after you've gone to bed this evening

Tomorrow morning

- Please don't eat or drink anything except for water. Come straight to the clinic in the morning
- We will take a finger-prick drop of blood for the fasting sugar test before you collect your medicines. As soon as this is done you can eat and drink whatever you like. We will give you a drink and a bun to eat for you comfort

In three months

- We will repeat the fasting sugar test on a day that you are coming to the clinic to collect your medicines. When you wake up in the morning, again, please do not eat or drink anything except water. We will take another finger-prick drop of blood for the fasting sugar test. As soon as this is done you can eat and drink whatever you like. We will give you a drink and a bun to eat for your comfort

Appendix VII: Daily Task List for Investigators

For participants in studies 2 and 3 who were enrolled yesterday

1. Measure fasting blood glucose concentration

Initial procedures for study 1 participants

2. Identify newly diagnosed tuberculosis patients attending Kanyama, Chawama and George clinics through consultation with clinic staff and clinic records
3. Obtain individual written informed consent from those meeting inclusion criteria and willing to participate
4. Administer structured questionnaire
5. Measure blood pressure
6. Measure weight, height and waist circumference
7. Measure random blood glucose concentration
8. Record HIV status. If no current HIV status available, perform an HIV test with pre- and post-test counselling
9. For HIV +ve participants, record CD4 count. If no current CD4 count available, measure CD4 count
10. Give a diabetes fact sheet to each participant

Initial procedures for studies 2 and 3 participants

11. Identify sputum-smear positive participants within the three strata of RBG concentration
12. Take a venous blood sample for measurement of HbA_{1c} concentration
13. Collect a sputum specimen
14. Inform participants of procedures for fasting blood glucose measurement

Initial procedures for study 3 participants

15. Identify sputum-smear negative participants within the three strata of RBG concentration
16. Take a venous blood sample for measurement of HbA_{1c} concentration
17. Inform participants of procedures for fasting blood glucose measurement

For study 2 participants returning weekly for 8 weeks post-enrolment

18. Collect sputum specimens

For study 2 participants at 12 weeks post-enrolment

19. Repeat FBG measurement
20. Take a second venous blood sample for measurement of HbA_{1c} concentration

For study 3 participants at 12 weeks post-enrolment

21. Repeat FBG measurement

For all participants at 6 months post-enrolment (or earlier if appropriate)

22. Review TB clinic and laboratory records to determine if case has a microbiological confirmation of TB

For all specimens collected during day

23. Transport to ZAMBART lab for processing or storage

Appendix VIII: Participant Questionnaire

SECTION 1

ALL QUESTIONS IN THIS SECTION MUST BE ANSWERED

Q01_INC **Interviewer's code**

Q02_CLC **Clinic's code**

Chawama	1
George	2
Kanyama	3

Q03_DAT **Date today**

D	D	M	M	Y	Y	Y	Y

Q04_IND **Participant barcode number**

Q05_SEX **Sex**

M	F
1	2

Q06_AGE **Age**

Q07_DIS **Disability?**

No Disability	1
Sight(blind/ severe visual impairment)	2
Hearing (deaf/ profoundly hard of hearing)	3
Communication(speech impairment)	4
Physical(needs wheelchair/ crutches)	5
Mental disability	6
Unknown	7

Q08_CON **Consent**

No	Yes	Absent	Excluded
0	1	2	3

ONLY CONTINUE IF CONSENT IS GIVEN

SECTION 2 – FILL THIS AND SUBSEQUENT SECTIONS IN ONLY IF PERSON HAS GIVEN CONSENT

I would like to ask you some questions

Q09_DOB **Date of Birth** (01/01/1800 if unknown)
If not known, what was your age in years at **your last birthday?** (999 if unknown)

D	D	M	M	Y	Y	Y	Y

Q10_YLC How many years have you lived in this community?
Write down actual number, zero if less than one year

Q11_RAC What is your race?
Select only one option

Black	1
Coloured	2
Indian/Asian	3
White	4
Other	5

Q12_COB What is your country of birth?
(Drop down menu with SADC countries and few other Africa countries)

Q13_CMS What is your current marital Status?

Never married	1
Currently married or living as married	2
Divorced or Separated	3
Widowed	4

If Married, Divorced or widowed, continue. If never married go to Q15

Q14_AFM Age at first marriage? (years)

Q15_MOY What has been your main occupation during the past year?

Unemployed/working on own land	1
Occasional/seasonal employment	2
Employed (Formal employment or self employed making money)	3
Unable to work	4
Student	5
Housewife/ home-maker	6

I would like to ask you about your current drinking and smoking habits

Q16_HES Have you ever been a smoker?
If no, jump to Q17_CDH

No	Yes
0	1

Q16_1_HES How old were you when you first started regular cigarette smoking?

Age	Unk
	-1

Q16_2_HES If you have stopped smoking, how old were you when you stopped?

Age	Unk	Not stopped
	-1	-5

Q16_3_HES On average over the entire time that you smoke(d), about how many cigarettes do (did) you smoke?

Number per week	Unk
	-1

Q16_4_HES On average over the entire time that you smoke(d), do (did) you primarily smoke manufactured or hand rolled cigarettes?

Manufactured	1
Hand rolled	2

Q17_CDH How would you classify your drinking habits?

Have never drunk	1
Daily drinker	2
Occasional drinker	3
Ex-drinker	4

Now I will ask questions about your education

Q18_HEA What is the highest level of education you have attained?

No Formal Education	0
Grade 1-12(Indicate actual grade)Note Grade 8-12 is also Form 1 –form 5	
College	20
University	30

If has attended school, continue. If no formal education go to Q20

Q19_YES When was the last year you were enrolled in School/College/University? Enter 9999 if year is not known

Y	Y	Y	Y

Q20_OCC What was your main occupation at age 15 years?

Unemployed/ working on own land	1
Seasonal/Occasional employment	2
Employed (formal employment or self employed earning money)	3
Unable to work	4
Student	5
Housewife/home-maker	6
Can't remember	9

I would like to ask you about your health. (Current TB questions)

Q21_DTS **Date TB treatment started**

D	D	M	M	Y	Y	Y	Y

Q22_TTN **TB treatment Number**

Q23_CAT **Category of TB?**

Sputum smear Positive	1
Sputum smear Negative	2
Extrapulmonary	3
Unknown/not recorded	4

Questions about previous TB treatment

Previous TB treatment

Q24_TTB Have you ever been on TB treatment before?
If yes continue, if no go to Q26

No	Yes	Unk
0	1	9

Q25_HMT How many times?

Once	1
Twice	2
Three times	3
More than three times	4
Unknown	9

I would like to ask about your TB symptoms

Q26_CHC Do you currently have a cough?
If yes continue, if no go to Q28

No	Yes
0	1

Q27_WBC How many weeks have you been coughing?
Write number of weeks in box

Q28_CPS Do you currently produce sputum

No	Yes
0	1

Q29_CCB Do you currently cough up blood?

No	Yes
0	1

Q30_CCP Do you currently have chest pains?

No	Yes
0	1

Q31_CHF Do you currently have a fever?

No	Yes
0	1

Q32_DNS Do you currently have drenching night sweats?

No	Yes
0	1

Q33_LWU In the last month have you lost weight unintentionally?

No	Yes

0	1

Q34_DBB Do you currently have difficulty breathing or shortness of breath?

No	Yes
0	1

Now I will ask questions about Diabetes and HIV

Q35_THD Have you ever been told you have diabetes/blood sugar
If Yes continue, if No go to Q38

No	Yes
0	1

Q35_1_THD How long ago were you diagnosed with diabetes?

0-6 Months	1
7-12 Months	2
1-2 years	3
2-3 Years	4
>3 Years	5
Don't know/Refuse	-1

Q35_2_THD Was it diagnosed when you were a child?
If male, skip to Q36_CAT

No	Yes	Unk
0	1	9

Q35_3_THD Was it diagnosed during pregnancy?

No	Yes	Unk
0	1	9

Q35_4_THD Did it continue after pregnancy?

No	Yes	Unk
0	1	9

Q36_CAT Are you currently on any treatment for diabetes?
If yes continue. If no go to Q37_1_TON

No	Yes
0	1

Q37_TON What treatment are you on?

Dietary only	1
Tablets	2
Insulin injections	3

Q37_1_TON What tablets are you on?

Metformin	1
Glibenclamide	2
Other	3

Q37_2_TON If other, what medication?

Q37_3_TON Why are you not on treatment for diabetes?

Health facility too far	1
Long queues	2
No medicine at Health facility	3
Symptoms not too bad	4
Have no money	5
No transport	6
Too sick to go to Health facility	7
Other	8

Don't know/Refuse	-1

Q38_KHS	Do you know your HIV status?	No	Yes
		0	1

Q39_DHS	Are you willing to disclose your HIV status?	No	Yes
	If yes continue, if not willing to discuss go to Q43	0	1

Q40_HIV What was the result?

Negative	0
Positive	1

If HIV status is Positive, continue, if negative go to Q43

Q41_ART	Are you on Antiretroviral treatment(ART)	No	Yes
	If yes continue, if no go to Q43	0	1

Q42_LAR	How long have you been on ART? Write down actual number of months	

SECTION 3

Now I will ask some questions about your home.

Q43_HHH In your Household is there

(Check every option)

	No	Yes
Electricity	**0**	**1**
A radio/radio cassette	**0**	**1**
A television	**0**	**1**
A refrigerator/freezer	**0**	**1**
A bicycle	**0**	**1**
A motorcycle	**0**	**1**
A car	**0**	**1**
A domestic worker not related to household head	**0**	**1**
A mobile phone	**0**	**1**
A landline (non mobile telephone)	**0**	**1**

Q44_WOL **Do members of your household work on their or the family's agriculture land?**

No	Yes
0	1

Q45_WAT **What is the main source of DRINKING WATER for this household (check only one option)**

Piped water inside the residence	**1**
Piped water in the yard	**2**
Piped water from a public tap	**3**
Protected well	**4**
Unprotected shallow well	**5**
Traditional well	**6**
Bore hole	**7**
River, stream, lake etc	**8**
Other	**9**

Q46_TOI **What is the main type of TOILET facility for this household?**

(Check only one option)

Private flush toilet	**1**
Shared flush toilet	**2**
Pit Latrine without ventilation	**3**
VIP Latrine	**4**
None- use bush/field	**5**
Bucket system	**6**
Chemical	**7**
Other	**9**

Q47_DWE **Which of the following types best describes the main dwelling unit that this household occupies?**

House/brick structure on own stand (single unit)	**1**
Townhouse/cluster/semidetached house (multiunit residence)	**2**
Traditional dwelling/hut/structure made from traditional material	**3**
Flat in block of flats	**4**
Brick house/flat/room in backyard	**5**
Informal dwelling or shack in backyard	**6**
Informal dwelling or shack not in backyard (informal squatter settlement)	**7**
Caravan/Tent	**8**
Worker's hostel	**9**
Other	**99**

Q48_FLO **What is the main type of flooring for this household?**

(Check only one option)

Dirt/earth	**1**
Wood, plank	**2**
Parquet, lino	**3**
Cement	**4**
Tile flooring	**5**
Other	**9**

Q49_HEA **What type of fuel does your household mainly use to keep warm inside the house during winter?**

(Check only one option)

Nothing	**0**
Electricity	**1**
Liquefied Petroleum Gas	**2**
Kerosene/Paraffin	**3**
Charcoal	**4**
Wood	**5**
Other	**9**

Q50_FFC **What type of fuel does your household mainly use for cooking?**

(Check only one option)

No cooking is done	**0**
Electricity	**1**
Liquefied Petroleum Gas	**2**
Paraffin	**3**
Charcoal	**4**
Wood	**5**
Other	**9**

Q51_ TOS **What type of stove is usually used for cooking?** ____

Open fire	**1**
Surrounded fire	**2**
Stove with combustion chamber	**3**
Two or three pot stove	**4**
Sunken pot stove	**5**
Gas stove	**6**
Electric/gas range	**7**

Q52_WCH **Where does cooking mainly happen?**
(Check only one option)

Indoors in main house	**1**
Indoors separate building	**2**
Outdoors	**3**

Q53_RON **Did your household have to rely on any of the following in the last 18 months?**

(Each item must be answered)	**No**	**Yes**
Relief food, free food from government and other bodies	**0**	**1**
Reducing number of meals or food in-take	**0**	**1**
Borrowing cash (e.g. kaloba, borrowing from friends etc)	**0**	**1**
Sale of assets	**0**	**1**
Sending household members away	**0**	**1**

Q54_HUN **During the past three months, did it happen even once that you or any member of your family experienced hunger because you did not have any food to eat?**

No	**0**
Yes	**1**
Unk	**9**

SECTION 4

RECORD BODY HABITUS AND BLOOD PRESSURE MEASUREMENTS

Q55_ABP Blood pressure?
If not done, write 999/999 | | | | / | | | | mm/Hg

Q56_WEI Weight? Record weight in Kilograms (one decimal point)
If not done, write 999.9 | | | | . | | Kg

Q57_HEI Height? Record height in centimetres
If not done, write 999 | | | | cm

Q58_ABC Abdominal Circumference? Record in centimeters
If not done, write 999 | | | | cm

RECORD BLOOD SUGAR AND HIV RESULTS HERE

Q59_BLG Blood Glucose. Write actual Results below

		.	

Q60_LAG When did you last have anything to eat or drink (except water)? Write Number of hours ago

Q61_HIV_DET HIV Test result (Determine).

Negative	0
Positive	1
Not Done	9

Q62_HIV_UNI Confirmatory HIV Test result (Unigold)

Negative	0
Positive	1
Not Done	9

Q63_KHS Does study participant want to know his/her HIV Result?

No	Yes
0	1

Q64_GHB HIV test results given to study participant?

No	Yes
0	1

THANK YOU FOR YOUR HELP

Appendix IX: Diabetes Fact Sheet

What is diabetes?

Diabetes is a disease commonly known as sugar disease. A person suffering from diabetes has too much blood sugar.

What causes Diabetes?

There are two major types of diabetes:

Type 1: In type 1 (also called juvenile-onset or insulin-dependent) diabetes, the body completely stops producing any insulin, a substance in the blood which the body needs to use sugar. People with type 1 diabetes must take daily insulin injections to survive. This form of diabetes usually develops in children or young adults, but can occur at any age.

Type 2: Type 2 (also called adult-onset or non insulin-dependent) diabetes results when the body doesn't produce enough insulin and/or is unable to use insulin properly (insulin resistance). This form of diabetes usually occurs in people who are over 40, overweight, and have a family history of diabetes, although it is increasingly occurring in younger people, particularly adolescents.

How do people know if they have diabetes?

People with diabetes frequently experience certain symptoms. These include:

- being very thirsty and the mouth feels dry
- frequent urination which is more than normal
- weight loss
- increased hunger
- inability to see clearly
- itching around the genitals
- tingling or numbness in the hands or feet
- frequent skin, bladder or gum infections
- wounds that don't heal
- extreme unexplained fatigue

In some cases, there are no symptoms — this happens at times with type 2 diabetes. In this case, people can live for months, even years without knowing they have the disease. This form of diabetes comes on so gradually that symptoms may not even be recognized.

If you feel you have symptoms suggestive of diabetes, go to the health centre or Hospital to have your blood sugar checked

Who is at risk for getting diabetes?

Anyone, any age, anywhere can get diabetes. It is however more common in:

- People who are physically inactive
- People who are overweight;
- The elderly
- Those with a family history of diabetes.

Is there a cure for diabetes?

NO! But there is effective treatment, and knowing how to take care of yourself, you could lead an active healthy life.

What to do if you are found to have diabetes?

- Avoid eating food containing a lot of sugar
- Avoid eating food which contains a lot of fat
- Eat a lot of food that contains a lot of fibre such as beans, peas, roller meal and brown bread
- Eat a well balanced diet with a lot of fruits and vegetables
- Avoid alcoholic drinks
- Avoid smoking
- Avoid being overweight
- Exercise regularly
- Always remember to take medicines as recommended by the healthcare provider
- Go for regular checkups as recommended by the healthcare provider

What the complications of diabetes?

Immediate complications: There are two immediate complications and these are medical emergencies.

1. **High Blood sugar**

High blood sugar can occur if diabetes is inadequately treated or in persons who do not know that they are diabetic and are not on treatment.

Signs of high blood sugar include:

- Initially excessive urination, leading on to no urination due to severe dehydration
- Dehydration with a dry mouth
- Loss of consciousness

Follow instructions given by your healthcare provider about how to take your medicine. Go to a health facility if you are diabetic and you suspect that your blood sugar is high

2. **Low blood sugar**

Low blood sugar can occur in known diabetics if:

- They inject themselves with insulin or take tablets for diabetes without eating enough food
- If they exercise too much after injecting insulin or taking some types of tablets for diabetes

Signs of low blood sugar include:

- Feeling Shaky and Jittery
- Feeling Nervous
- Feeling Dizzy
- Feeling hungry
- Feeling the heart beating fast
- Sweatiness

If you experience any of these signs, immediately eat or drink something sweet such as sweets, a sugary drink or even a sugar solution

Can diabetes be prevented?

YES! Sometimes. There is a lot one can do to delay or prevent the onset of diabetes.

- Maintain a healthy body weight
- Exercise regularly
- If you have a relative with diabetes, go for regular checkups
- Eat a well balanced diet with a lot of fruits and vegetables

Appendix X: Diabetes Referral Letter

Referral letter – Blood sugar is abnormal

Name:.....

Date:.....

Your blood has been tested for the presence of sugar and the test results show that you have a sugar level of ……mmol/L in your blood. You had a second test to confirm whether or not you have a condition called diabetes (this test was a fasting blood sugar test) which showed a sugar level of .....mmol/L in your blood. These tests suggest that you do have diabetes.

We have therefore booked an appointment for you to see a clinic doctor at ..... Health Centre. The doctor will explain more about diabetes to you and you will be able to ask the doctor any questions that you may have about diabetes. The doctor will assess your health and arrange for any treatment or further management that may be needed. In the mean time please read our diabetes fact sheet to find out more about diabetes and if you have any concerns please speak to a member of the study team or visit your local health centre.

Your appointment is on ..... (date) at ..... (time).

PLEASE PRESENT THIS LETTER TO THE RECEPTION AT THE HEALTH FACILITY

Appendix XI: HIV Fact Sheet

What is HIV/AIDS?
Acquired Immune Deficiency Syndrome (AIDS) is the end stage of the infection caused the Human Immunodeficiency Virus (HIV). HIV is a virus that weakens the immune system.

How can one get HIV/AIDS?
HIV is transmitted through:

- Unprotected sex: you can become infected with HIV if you have unprotected vaginal, anal or oral sex with an infected person
- Exchanging body fluids such as blood transmission: HIV virus can be transmitted through blood transfusion if blood is not properly checked
- Sharing sharp utensils (e.g. razor blades and needles)
- The virus can also be transmitted from mother to child during pregnancy, at birth or during breast feeding

Signs and Symptoms HIV/AIDS
The symptoms of HIV/AIDS vary. You may remain symptom free for many years, but as the virus continues to multiply and destroy immune cells, you may develop chronic symptoms such as:

- Swollen lymph nodes- often one of the first signs of HIV infection.
- Diarrhoea
- Weight loss
- Fever
- A cough and shortness of breath

If you suspect you have contracted HIV go for VCT at any nearest clinic as soon as possible

HIV/AIDS treatment
HIV/ AIDS is not curable. Early testing and positively living is the key to the prevention of HIV infection.

Management of HIV/AIDS involves:

- Treatment of infections that come due to HIV infection
- Anti-retroviral Therapy (ART) to reduce HIV viral load. ARVs are to be taken throughout one's life
- ARVs, like any other drugs, have some side effects. If you experience any side effect you should consult your health care provider as soon as possible

How can HIV/AIDS be prevented?

- By avoiding multiple and unprotected sex
- By avoiding sharing utensils such as needles and razor blades
- By practicing safe motherhood. Pregnant mothers should attend antenatal clinics regularly

Appendix XII: HIV Referral Letter

Referral letter – HIV clinic

Name:.....

Date:.....

Your blood has been tested for the presence of the HIV virus. As has been discussed with you by the research staff, your HIV result is positive. You had a second test which confirmed the presence of the HIV virus in your blood.

Treatment is available for HIV to enable you to lead a long and healthy life. We have therefore booked an appointment for you to see a health care worker at ..... Health Centre. The health care worker will explain more about HIV to you and you will be able to ask them any questions that you may have about HIV. The health care worker will assess your health and arrange for appropriate treatment and/or any further management that may be needed. In the mean time please read our HIV fact sheet to find out more about HIV and if you have any concerns please speak to a member of the study team or visit your local health centre.

Your appointment is on ..... (date) at ..... (time).

PLEASE PRESENT THIS LETTER TO THE RECEPTION AT THE HEALTH FACILITY

Appendix XIII: Sputum collection poster

MUTIPASE CHIKOLALA

√ IMILILANI PAMWEKA NGATI MUKOSOMOLA CHIKOLALA.

√ MUSEGULE KABOTOLO KAMOZI.

√ MUONE KUTI KABOTOLO MULI CHIKOLALA NIKOVALISA MANINGI.

√ MUONE KUTI MWAPELEKA CHIKOLALA OSATI MATA.

DARTZ

Appendix XIV: Blood Glucose Testing Standard Operating Procedures

Instructions for blood glucose testing using a glucometer

In this study blood glucose measurement for both random and fasting blood glucose will be carried out using a glucometer through the collection of a capillary blood sample. The glucometer is a rapid method of determining the blood sugar level. This SOP outlines procedures for collection of a capillary blood sample, handling and discarding of the test strips, use of the glucometer and giving results back to the participants.

Materials for the test

- **Disposable gloves**
- **Sharps and biohazards disposable container**
- **Alcohol swabs**
- **Gauze/cotton pads**
- **Auto-lancets**
- **Glucose test strips**
- **Glucometer**
- **Batteries**

Performing the test

- Prepare a place where the test can be done. This should be a flat surface and there should be no clutter around.
- Wash your hands thoroughly. If water is not available, rub your hands with gel or with an alcohol swab.
- Use standard precautions – wear gloves.
- Confirm that the test strip is within the expiry date.
- Turn on the glucometer and place the test strip in the glucometer.
- Touch drop but not finger to test strip. Press the skin puncture site with dry cotton/gauze.
- Wait for the glucometer to read the test strip.
- Dispose of waste in biohazard waste container.
- Read the result given on the glucometer. Note whether the result falls outside the normal or reportable range.
- Record the result on the questionnaire (on the PDA) at the relevant position.

Quality control and maintenance policy

This should be performed whenever a new container of test strips are opened:

- Proceed in exactly the same way as when carrying out a blood glucose test.
- Remove control bottle cap.
- Wipe the tip of the bottle with a tissue.
- Hold the dropper horizontally with the tip pointed directly at the front edge of the yellow window of the test strip.
- Gently squeeze the bottle to form one small drop.
- Touch the drop to the front edge of the yellow window of the test strip.

- Allow the test strip to automatically draw in the control solution until you see the flashing eggcup in the display.
- Close the bottle tightly after use.
- A result appears on the display with a control bottle symbol and a flashing "L". Do not remove the test strip yet.
- Press the right arrow button once for a Level 1 control (low glucose control).
- Press the right arrow button a second time for a Level 2 control (high glucose control).
- Press the on/off button to set the level in the meter.
- "OK" and the result alternate in the display if the result is in range.
- "Err" and the result alternate in the display if the result is not in range.
- You can also compare the result with the permitted range given on the test strip container label.
- If the result obtained is within the permitted range, correct functioning of the system is assured. If the result obtained lies outside the range given or if "Err" is displayed, it is necessary to repeat the test. If the same applies to the second test, discuss with Dr. Bailey.

Giving results to the study participant

- Explain the results as given on the glucometer.
- If the random blood glucose level is above the normal range explain that a confirmatory test needs to be done the following morning. Explain to the participant that this test needs to be done early in the morning before breakfast or at least 6 hours after eating or drinking anything except water. Therefore it can be done the following morning when the participant returns to collect their TB medication.
- If the fasting blood glucose level is above the normal range explain that the participant needs to return to see a clinic doctor. Fill in a referral letter with them and arrange an appointment with the doctor at the earliest opportunity.
- Give all study participants diabetes information sheets.

Interpreting blood glucose results

Abnormal glucose levels if:

- Random blood glucose is ≥8.9mmol/L (or ≥160.2mg/dL)
- Fasting blood glucose is ≥7.0mmol/L

Any study participant with either an abnormal random or fasting blood glucose level should be referred to the clinic doctor for further screening.

Appendix XV: HIV Testing Standard Operating Procedures

Instructions for HIV testing using a Determine™ and UniGold™ HIV tests

1. Equipment required

- Test Kits (Abbot and Unigold)
- Self- retracting lancets
- Alcohol cotton swabs
- Cotton(dry)
- Sharps disposal containers
- Disposable gloves
- Pipettes
- Plastic bag waste bin for biohazards
- Markers
- Stationery
- Timer
- Kit bag
- Chase buffer

2. HIV Testing Kits

- Test kits should be stored between 2^{o}c and 30^{o}c, away from direct sunlight
- Allow test kits to come to room temperature prior to use.

3. Where test can be done

The HIV test should be carried out in the TB clinic or within the grounds of the health centre. Testing should only be carried inside a building and not outside. Ensure that you can have privacy whilst carrying out the test.

4. Doing the HIV test (Determine and UniGold)

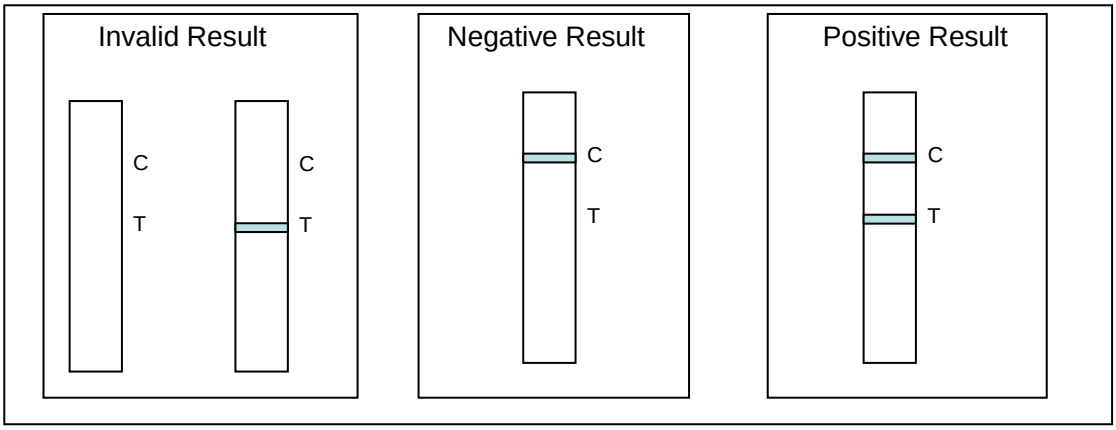
HIV testing should only be carried out after pre-test counselling has been done and the participant has accepted to have a test done.

- Prepare a place where the test can be done. This should be a flat surface and there should be no clutter around. There should be enough light to read the test.
- Wash your hands. If water is not available, rub your hands with gel or with an alcohol swab. Put on gloves.
- Ask the participant to rub his/her hands together.
- Select the middle finger of the right or left hand and massage it with your fingers
- Clean the selected finger with an alcohol swab
- Remove the lancet from its packaging
- Place the lancet off-centre on the fingertip. Firmly press the lancet against the finger and puncture the skin. Immediately discard the lancet in the sharps disposal container.
- Wipe off the first blood, and then massage the finger using up and down motions. Ensure that the finger is pointed down whilst you are doing this.

- When there is enough blood, place a drop of the blood onto the HIV testing strip and on the glucose testing strip (see blood glucose SOP for full details of the procedure). Press the finger stick site with dry cotton.
- Apply a drop of chase buffer on the testing strip
- Set your timer and wait 10-15 minutes before reading the results
- Throw away all other NON-SHARP biohazard stuff in a sealable plastic bag

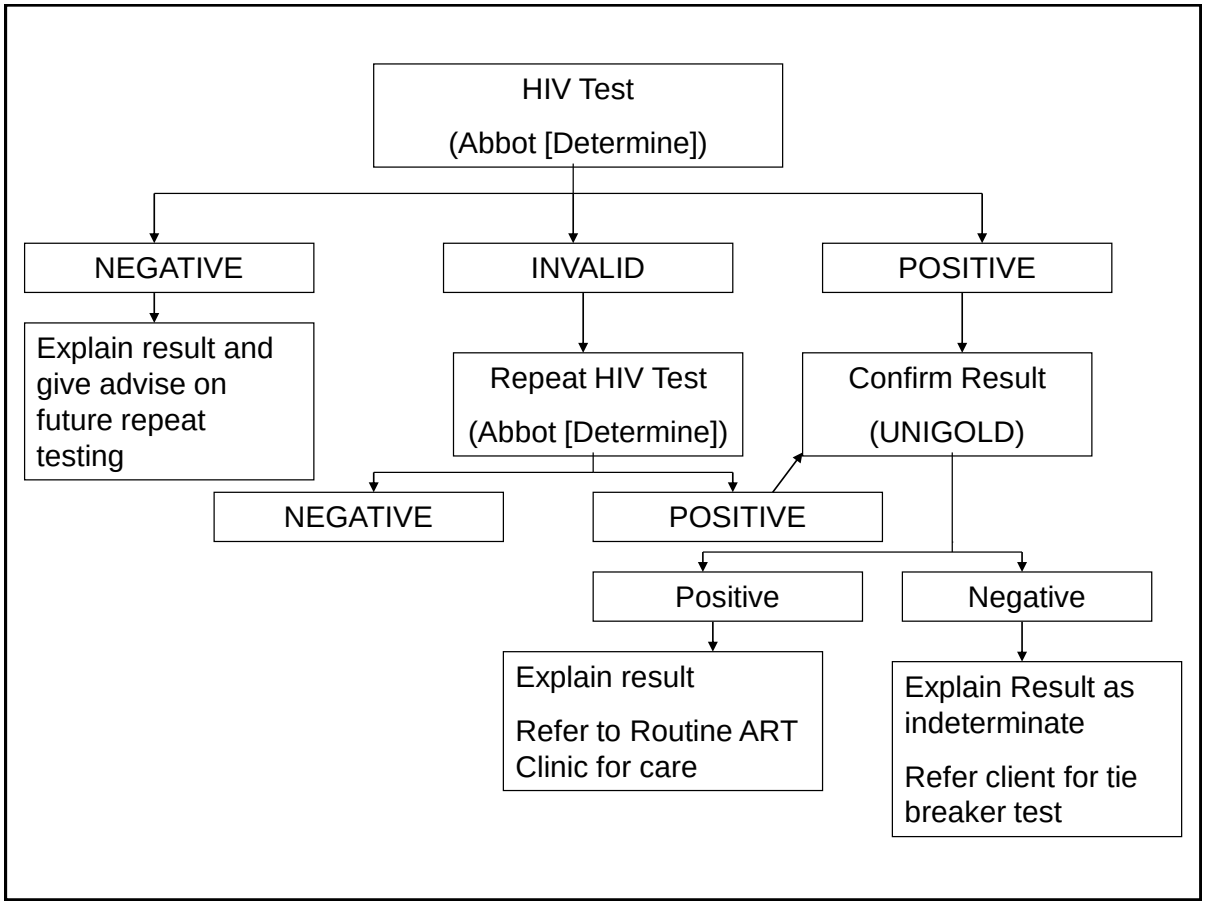
5. Reading the test results

- The results are ready between 10-15 minutes
- Check that the result is valid before reading and communicating the results



- Explain the result to the study participant. Follow the algorithm below to explain the results.
- If the result is invalid, explain this to the client and ask to repeat the test.
- If the test is negative, counsel the patient and follow the current guidelines for advising on repeat testing.
- If the test is positive, confirm results with Unigold, if confirmatory test is positive, give results as positive.
- If confirmatory test is negative, explain that results are inconclusive and refer patient to health centre for further testing.
- Enter results in appropriate section in the questionnaire (on the PDA).

Figure 1: HIV testing algorithm



Appendix XVI: CD4 Testing Standard Operating Procedures

1. Equipment

1. FACSCount machine
2. FACSCount Coring Station
3. FACSCount work station
4. FACSCount Software Protocol Diskette
5. FACSCount Thermal printer paper
6. Timer
7. Pipette capable of delivering 50 µl
8. Cleaning Tubes and Dispensing bottles

2. Reagents

1. The BD FACSCount Reagent kit
2. The BD FACSCount Control kit
3. System fluid (FACSflow)
4. Distilled water
5. Bleach (Sodium Hypochlorite)

3. Procedure

3.1 Collecting Patient Blood Samples

1. Blood must be collected in a 4mL K_3EDTA (liquid) VACUTAINER tube.
2. Blood should not be stored for more than 48 hours at room temperature (20-25^{o}C)

3.2 Sample preparation

1. Place the patient blood in the workstation.
2. Remove one reagent pair for each patient. Reseal the foil bag and return the unused reagent pairs to the refrigerator.
3. Label the tab of the reagent tube pair with the patient accession number or number that identifies the tube of blood.
4. Vortex the pair upside down for 5 seconds. *Set the vortex speed to a midrange setting.*
5. Vortex the pair upright for 5 seconds.
6. Open the reagent tubes with the coring station. Refer to manual for details.
7. Mix the patient whole blood by inverting the tube five times.
8. Pipette 50µl of the whole blood into each of the two reagent tubes. Change tips between tubes. (Discard tips in the provided sharps container).
9. Cap the tubes and vortex upright for 5 seconds.
10. Incubate the tubes for 60 to 120 minutes at room temperature (20-25^{o}C). *Do not expose the tubes to direct sunlight.*
11. Uncap the tubes and pipette 50µl of fixative solution into each reagent tube. Change tips between tubes. (Discard caps and tips in appropriate waste bags).
12. Recap the tubes and leave to stand until they are ready to be run.
13. Vortex upright for 5 seconds immediately before running.

14. Run the tubes on the FACSCount instrument within 48 hours after fixation. (Store samples at room temperature in the workstation until they are run on the instrument.

3.3 Preparing the FACSCount machine

3.3.1 Preparing the Fluidics

Before running controls or samples, prepare the fluidics by first filling the system fluid reservoir and then emptying the waste reservoir.

3.3.1.1 Filling and emptying the System Fluid Reservoirs

1. Open the fluidics compartment door to access the system fluid.
2. Disconnect the system fluid tubing and the system fluid detection probe connector from the fluidics panel.
3. Remove the fluid reservoir from the instrument and stand it on end before removing the cap.
4. Fill the reservoir with system fluid.
5. Recap the reservoir and replace in the instrument.
6. Reconnect the system fluid tubing and fluid detection probe connector.
7. Check to see that the system fluid filter is filled with fluid. If air is present in the filter, unclamp the plastic pinch clamp located on the air vent tubing to allow air to escape. Refer to manual for details.

Note: System fluidics reservoir shall be rinsed once every 2 months with bleach and distilled water.

3.3.1.2 Emptying the Waste Reservoir

1. Disconnect the waste tubing and the waste fluid detection probe connector from the fluidics panel.
2. Remove the waste reservoir from the instrument and stand it on end. *Do not dispose waste reservoir contents until at least 30 minutes after the completion of the last run, this helps inactivate the bio hazardous material before disposal.*
3. Empty the reservoir and rinse it with bleach and water.
4. Add 200mL of undiluted bleach to empty reservoir.
5. Recap the reservoir and replace in the instrument.
6. Reconnect the waste tubing and fluid detection probe detector.

3.3.1.3 Priming the System

1. Press [UTILITY] from the FACSCount screen to display the UTILITY screen.
2. Press [DRAIN].
3. Press [DRAIN] again.
4. When all the fluid has drained from the flow cell, press [STOP].
5. Press [MAIN] to return to the FACSCount screen.

3.3.1.4 Entering Patient and Reagent Information

1. From the FACSCount screen or the Control results screen, press [SAMPLE]
2. Enter or verify the reagent lot code and reference bead counts and press [CONFIRM]
3. Enter the patient accession number (up to 15 characters).

N.B. For further details refer to manual.

3.4. Running Patient Samples

1. After you enter sample information, the instrument prompts you for the CD4 tube (green top).
2. Vortex the reagent pair for 5 seconds.
3. Uncap the CD4 tube and set the cap aside. Place the reagent pair in the sample holder so the CD4 tube is in the run position.
4. Press [RUN].
5. Remove the reagent pair and recap the CD4 tube. Uncap the CD8 tube (clear top) and set the cap aside. Place the CD8 tube at the run position.
6. Press [RUN].
7. Remove the reagent pair and recap the CD8 tube.
8. The results are displayed on the screen of the machine and automatically printed per sample.
9. Check if the results are passed. If not repeat the whole process again. If the sample fails twice a new blood sample needs to be drawn.

N.B. If you need to run more samples you continue by entering patient and reagent information. Cleaning does not have to be done at this stage.

10. After all runs have been done clean the machine.
11. After cleaning leave a tube of distilled water on the instrument and bring the sample holder up.
12. Turn off the power of the instrument.

4.0 Report Results

If the test is valid the result of CD4, CD3 and CD8 appears on the print out. The results should be entered into the laboratory database. The result print out should be attached to the request form and filed appropriately and also recorded in a laboratory CD4 results book.

Appendix XVII: Mycobacteria Culture Standard Operating Procedures

ZAMBART PROJECT ZAMBART- Central Laboratory, P.O Box 50697, Ridgeway Campus Lusaka, Zambia **Standard Operating Procedures**	SOP No. DARTZ 01-TB
Title: MTB Culture Standard Operating Procedures	Page270 of 286

Effective Date: November 2013	**Version:**1	**Supersedes Version:** None

	Name /title	**Section**	**Signature**	**Date**
Compiled by	Sarah-Louise Bailey			
Compiled by	Winnie C. Mwanza			
Revised by				

Approved By (Title) *(Lab Supervisor/Lab Management /PI)*	**Signature**	**Date**

Reviewed By (Title)	**Reason for Review Annual/new document**	**Signature**	**Date**

DOCUMENT HISTORY

Version	**Effective Date**	**Changes/action taken**	**Section**
1	November 2013	New document	

1. Introduction

This document deals with the processing of sputum samples for culture and smears

1.1. Endpoints

For each sputum specimen received by the laboratory the following should be prepared:

I. Sputum archive
II. Inoculation on two MGIT tubes
III. Concentrated smear (stained, read and reported)-Auramine stained
IV. Capilia TB test

Safety Precautions

All sputum specimens should be treated with utmost care as they may be infectious. Universal safety precautions towards infectious materials should be followed. All processing must be done in a Bio-Safety cabinet.

This SOP is targeted at staff that are qualified to work in a TB culture laboratory

1.2. Abbreviations

AFB	Acid Fast Bacilli
AMGIT	Automated Mycobacteria Growth Indicator Tube
BA	Blood Agar
BSC	Bio-Safety Cabinet
MOTT	Mycobacteria other than tuberculosis
Mtb	Mycobacteria tuberculosis
NTM	Non Tuberculosis Mycobacteria
RT	Room temperature
SOP	Standard Operating Procedure
ZAMBART	Zambia AIDS Related Tuberculosis

2. Specimen Handling

2.1. Collection

Specimens must be collected into 50ml falcon tubes with a tight fitted lid. They should be placed in a leak proof plastic bag for transportation to the laboratory. One spot samples should be collected from each patient.

2.2. Transportation

Specimens should be transported to the laboratory as soon as possible after collection. Delays in transportation may increase the risk of contamination of cultures. Specimens should be transported in a cooler box with ice packs, in which temperature is maintained as low as possible.

Specimens must be packaged to withstand leakage of contents, shocks, pressure changes and other conditions incident to ordinary handling practices. Primary specimen container (containing actual specimen) must be placed first in simple plastic bag that is tied, and then placed in self-zipped bags which is then placed in secondary container.

2.3. Storage

Samples should be processed as soon possible after receipt. If samples are not processed on the same day of receipt, they should be refrigerated at 2-8^{o}C. **DO NOT FREEZE SPECIMENS**

All specimens must be processed within 4 days of submission.

3. Specimen Receipt and log in

Samples will be received at ZAMBART laboratory. All samples should arrive with a request form packed in the side pocket of the specimen transfer bag with a barcode stuck on the request form. The samples from one patient will be packed in one sample transfer bag.

3.1. Sample receipt

- Check for sample examination request form
- Remove from bag and check that barcode on request form matches that on samples
- Samples where barcodes are not matching: Field staff should be contacted for verification. Samples should be discarded where the mismatch cannot be verified or resolved
- Leaked out samples should be discarded if they cannot be salvaged
- Indicate number of samples received by ticking appropriately on the request form
- Sign request form to indicate that samples have been checked and received
- Place signed off request form in designated Box folder (pending registration)
- Place samples in appropriate drawer in the Fridge if they are not to be processed immediately
- Samples that have not been received and checked should be placed in the electric cool box until someone is available to receive them

Note: Samples that have not been received as described above should not be placed in the fridge. If samples cannot be appropriately received at the time of arrival, they should be placed in the electric cool box marked <u>UN RECEIVED</u> Samples.

3.2. Sample registration

- Remove sample examination request forms from folder
- Scan in barcode from request forms
- Enter date sample collected for each sample that is indicated on the request form
- Enter all samples to be processed on that day
- Once all samples are registered, print sample registration form
- Samples can now be moved from the fridge into BSC1
- After registration, request forms are placed in appropriately labeled box folder "Request forms, registered samples"

Note: Take care that only samples indicated as received are registered. Once all samples for processing on the day are registered, print sample registration form.

3.3. Unpacking samples from transfer bag

This should be done in BSC1

- Wipe BSC1 surface with appropriate disinfectant
- Place samples in BSC1 and carefully check samples for breakages or leakages before removing from bag
- Broken or leaked out samples should be recorded on the registration form and then discarded

Note: Samples that are not completely leaked out can be salvaged by transferring into another falcon tube

- Remove samples from the sample transfer bag and place on a rack
- Wipe down with disinfectant and place samples in centrifuge bucket
- Spin down samples for 1 minute
- Remove from centrifuge, wipe down with disinfectant
- Check and record volume and sample quality on the registration form
- Enter sample volume and quality into data base

3.4. Specimen rejection

- Sputum is leaked out completely and cannot be salvaged
- Sputum container is broken
- Sputum container is empty

3.5. Batch Creation

- Group samples into batches of maximum 26 (fewer if insufficient number of samples) with 2 control tubes included
- For each specimen place one blue top screw cap vial (sputum archive vial), one slide and two AMGIT tubes on a rack
- Label each MGIT tube with an individual barcode and another unique barcode. **Ensure not to cover the inbuilt barcode on the AMGIT tube**
- Label the sputum archive and slide with unique barcodes
- Place two extra MGIT tubes on the rack for negative and positive controls. These should have unique barcodes
    - Paste a red/pink sticker on top of the positive control tube and a green sticker on the negative control tube.
    - Write "PC" on the positive control tube and an "NC" on the negative control tube
- Take an empty MGIT box rack and paste a sticker and label with date of sample processing. All batches (samples) run on the same day use the same rack for incubation
- Create batch, enter batch number automatically
- Scan in individual barcode for sample followed by the unique MGIT tube barcodes, slide and sputum archive
- Scan in MGIT positive and negative control (every batch should have an accompanying positive and negative control tube)

Warnings!

- **From this point forward all techniques must be carried out in the biosafety lab[1, 2]**
- **No material may leave the lab unless it has been decontaminated or autoclaved**
- **Procedures that can cause the generation of an aerosol must be minimised and carried out in a bio-safety cabinet**
- **In order to minimize cross-contamination of cultures the following measures must be adhered to:**

♦ A single hood is dedicated to the isolation of mycobacteria from sputum. Fully-grown cultures (MGIT) are to be dealt with in a separate hood or later in the day after sample processing is complete. If a second hood is not available an interval of at least 30 minutes should be allowed between processing sputum samples and fully-grown cultures and handling of cultures should always be done after sputum samples have been processed and before to reduce cross contamination

♦ After shaking or spinning, aerosols are to be allowed to settle for 5 minutes.

4. Adding PANTA and OADC to each AMGIT tube

Note: All reagents should be at room temperature before use. PANTA/OADC mixture should be added to AMGIT tubes just before adding samples

- Reconstitute the PANTA with 15ml of OADC
- Mix by carefully shaking
- Put a sterile 10 ml syringe onto the multi-pipette
- Add 0.8ml of the PANTA/OADC mixture into each AMGIT tube
- Do not store MGIT tubes after addition of PANTA/OADC mix

Note: MGIT tubes to which PANTA/OADC has been added should be used within 30 minutes of adding PANTA/OADC

Left over PANTA/OADC should be used within 5 days of opening but should be kept at 4°C. ***Do not freeze****. Label the PANTA/OADC mixture with the date mixed*

6. Preparing positive and negative control

In BSC2

1. Label one empty falcon tube "NC" for negative control and "PC" for positive control
2. Add 3 ml of saline[1] to the negative control container, and add 2 ml of saline to the positive control container
3. Add 1ml of the positive control (H37Rv) culture broth from a pre-prepared vial to the positive control container
4. Nothing is added to the negative control container

NOTE: positive control samples are always placed at the end of the sample row and will only be opened if handling of the other clinical samples and negative control is done for the particular step of the protocol

7. Specimen Decontamination

- Processing should be done in batches. The Batch size is a maximum of 26 specimens plus 2 control tubes.
- For actual decontamination, the batch is split into 2 if the maximum number is reached so that only a maximum of 13 samples is processed for maximum batch size.

7.1. Materials and Methods

- Disposable 50ml plastic tubes (falcon tubes)
- Digestant (Sterile NaOH/Citrate/NALC (home made))
- Phospate buffer pH 6.8 (aliquoted into McCartney bottles)
- Graduated pasture pipettes
- Centrifuge
- Vortex mixer
- Timer
- Gloves
- Waste bags
- Media(2 AMGIT tubes)
- Normal saline

7.2. Prepare fresh digestant

1. Place the required number of Falcon tubes containing 0.25g pre-weighed NALC into BSC1.
2. Add sterile NaOH-Na-citrate upto the 50ml mark and mix well.

Note: Label the tubes with name of reagent, date and time activated. Keep refrigerated and use within 24 hours of activation.

7.3. Decontamination procedure

1. Place a batch of sputum samples into BSC 1
2. Place Negative and positive control tubes at the end of the rack
3. Add the digestant solution in equal volume to that of the specimen using a sterile pipette. Tighten the cap
4. Start timer when you have added the digestant to the first sample

[1] See Appendix…. For preparation of Normal saline

5. Put samples in orbital shaker and vortex the tube for 15-30 seconds and invert the tube to ensure that the mycoprep solution contacts all surfaces of the tube
6. Wait 20 minutes after adding the NaOH-NALC solution. Vortex lightly every 5-10 minutes or put tubes on a shaker and shake lightly during whole decontamination time
3. Make sure specimen is completely liquefied. If specimen is still mucoid, add a small quantity NALC-powder (30-35 grams) directly to the specimen tube, mix well
4. At the end of 20 minutes, add phosphate buffer (pH 6.8) up to the top ring 50ml mark on the centrifuge tube. Tighten tube. Mix well by light vortexing or inverting several times
5. Place tubes in centrifuge and close lid tightly. Centrifuge at 3800 X g for 20 minutes at 4-16^{o}C. Let the tube stand for 5 minutes after centrifuging to allow aerosol to settle but do not leave to stand for too long after centrifuge. If refrigerated centrifuge is not available use, use cold (2-6^{o}c buffer)
6. Remove tubes from centrifuge
7. Decant supernatant from tubes into bin containing mycobactericidal disinfectant, leaving only sample pellet in the tube
8. ***NB at this stage it is especially critical that only one specimen be open at a time and generation of aerosols be minimised to avoid cross-contamination of samples***
9. Re-suspend the pellet sediment with 2mls of phosphate buffer pH 6.8 using a sterile Pasteur pipette to achieve a final volume of 1- 3mls. Vortex the suspended sample
10. Wipe down the container containing the pellet, move the rack to BSC2
11. Spray the centrifuge buckets with 1% Virkon. Move to sink in readiness for washing
12. Clean the BSC with 1% Virkon

Note: Decontamination should be done on maximum sample volume of 5ml. Samples that are greater than 5ml should be aliquoted into another falcon tube to achieve volume of less or equal to 5 ml.

Note: Inoculation will be done in BSC2 only

8. Inoculation

For each specimen two MGIT cultures will be inoculated. Automated reading will be done

Materials and reagents

a. **AMGIT Media (7ml)**
b. **BACTEC MGIT 960 growth Supplement (enrichment)**
c. **BBL MGIT PANTA**

Note; for reconstitution of PANTA/OADC see section 4 above

8.1. Inoculation of MGIT media

1. Place specimens back in order with the corresponding AMGIT tubes, sputum archive vial and slide
2. Using a sterile transfer pipette;
    a. Add 0.5 ml of well mixed processed/concentrated specimen to the appropriately labeled 2 AMGIT tubes
    b. Add 1ml to the appropriately labeled sputum archive vial
    c. Add two drops to the appropriately labeled FM slide and spread into an oval area approximately 2 x 3cm.

Note: Wipe tubes and caps with a mycobactericidal (1% virkon) disinfectant and leave inoculated tubes at room temperature for 30 minutes before incubating

8.2. Incubation

All inoculated 7ml MGIT tubes should be entered into the BACTEC MGIT 960 instrument for incubation

1. Follow the instrument prompts for loading samples (please refer to the BACTEC MGIT 960 instrument manual for details)
2. Scan the inbuilt AMGIT barcode, then the unique barcode.
   NOTE: do not scan the individual barcode when loading samples in the instrument.
3. Place the tube on the automatically instrument allocated slot.
4. MGIT tubes should be incubated until the instrument flags them positive
5. If there is no growth in the tube after 6 weeks(42 days) the instrument flags the cultures as negative
6. Instrument positive cultures should be entered into the data base using the mass positive button.
7. Cultures that are flagged negative after 6 weeks must be inspected for any growth. Any showing any growth-cloudy, crumby particles etc must be captured as positive and entered as positive and worked up for positive samples
8. Instrument negative cultures are entered into the data base using the mass negative button if showing no visible growth
9. Mass negative cultures must be autoclaved before discarding. They should be discarded in the bag destined for incineration

9. Storage of archived sputum

- Place the sputum archive vials in a blue storage box (use one box that is labeled by week number and date)
- Store at -20^{o}C

10. Staining concentrated smears

Auramine O stain is used. See appendix 2 for FM staining procedure

Notes:

- **Remember not to leave smears exposed to UV light in the safety cabinet**
- **Do not leave stained slides exposed to light as this may cause them to fade**
- **Positive and Negative controls should be included when staining and reading a batch of slides**

11. Workup for cultures with growth

Note: ZN Stain work List should be available upon entering of positive cultures into the data base

11.1. Materials and reagents

- Slides
- Pasteur pipettes
- Sterile loops
- Methylene Blue -0.3%
- 3% Acid-Alcohol

- Carbol Fuchsin solution 0.3%
- 1% Bovine albumin
- Capilia(Tauns)TB test strips

11.2. AFB smear from MGIT tube with growth

1. Place a drop of 1% bovine albumin on the slide using a sterile pippette
2. Use a sterile pipette to remove an aliquot from the bottom of the tube
3. Place 1-2 drops on the slide and spread over a small area
4. Allow the smear to air dry
5. Heat fix using an electric warmer at 65^{o}C-70^{o}C for 2 hours to overnight

Note: Do not leave the smear openly exposed to the UV light of the safety cabinet. Heat fix smears that are air dry

6. Stain fixed slides with ZN
7. Place a drop of oil on the stained and completely dried smear and screen under a low power objective(x40) to locate bacteria. Switch to an oil immersion objective lens (x100) for detailed observation
8. Check for AFB presence and record smear results on the work sheet
9. Enter ZN stain results, scan in MGIT culture and MGIT DNA archives
10. Print Capilia TB work list
11. Capilia work list is printed after ZN stain results are captured

12. Capilia TB test (MPB64 antibody test)

1. ZN stain positive cultures are further identified to differentiate between ***Mtb*** and MOTT using the Capilia TB Test
2. Positive control culture should have a ZN stain done as well as Capilia test

12.1. Performing Capilia TB test MGIT isolates and archiving of isolates

- Apply 1 ml of culture broth to DNA archive tube
- Apply 0.5 ml of culture broth to MGIT culture archive tube(tube should have 0.5ml 20% glycerol)
- Apply 100µl of the positive media to the sample well on the slide directly without any manipulation
- Leave for 15minutes (*all results should be read within 1 hour after dispensing the sample into the sample well)*
- Check for the presence or absence of red to pink bands on both the test and control bands
- Enter results onto results onto the Capilia work sheet
- Enter Capilia TB test results
- Print results and send results to the PI

12.2. Interpretation of Capilia results

1. Positive for *Mycobacterium tuberculosis* complex

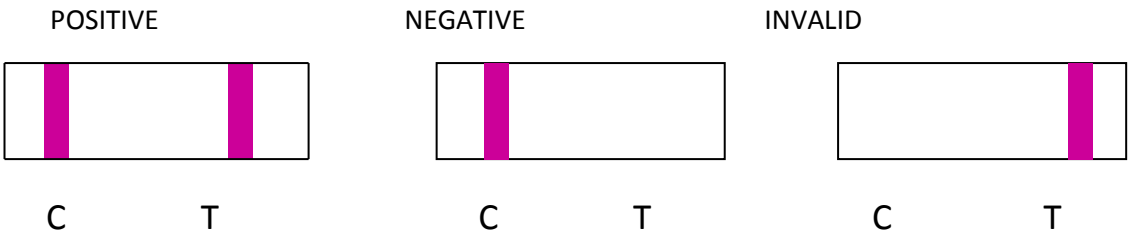
- Red-purple colour appears in both the T and C result windows. The result is read as positive if window T shows red-purple colour that is lighter than, the same as, or darker than the colour window C

2. Negative for *mycobacterium tuberculosis* complex

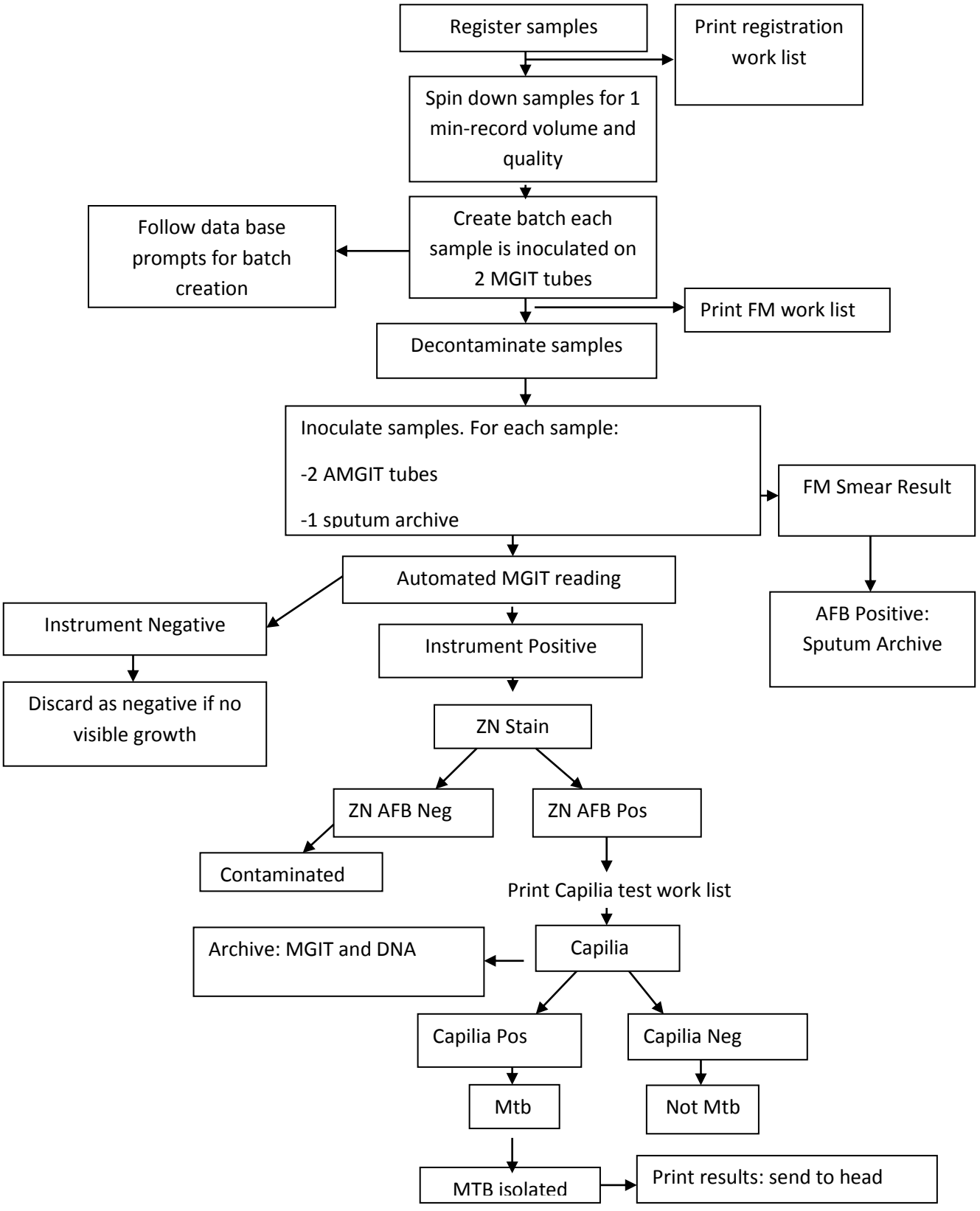
- Red-purple colour appears in results Window C but NOT in results window T

3. Invalid Test

- A test is invalid, if red-purple colour does not appear in result Window C
- If a test is invalid, report as invalid on results form. Repeat test using a new test Plate, preferably from a newly opened foil pouch



Appendix 1: Flow diagram culture process



Appendix 2: FM staining Procedure

Excerpt from SOP ZPLgen/001.
Details for preparing stains see SOP ZPLgen/001 or section
5.0 Smear preparation.
5.1 Materials

1. New frosted end clear glass slides
2. Pencils or barcodes
3. Pasture pipettes
4. Cotton wool
5. Disinfectant
6. Waste container

5.2 Procedure

1. Smear one drop of concentrated smear onto an appropriately labeled slide
2. Spread the specimen on the slide by smearing repeatedly in a coil like pattern, to an approximately 2 x 3 cm or 1 x2 cm oval shape with a thickness good enough to read a news print.
3. Use a separate pasture pipette for each specimen
4. Allow the smear to air-dry completely; do not dry smears under direct sunlight, slide warmer or over a flame
5. When dry, heat fix the smear by heating on a slide warmer for 2 to 3 hours
6. If fixed smears will not be stained immediately store in slide storage boxes in a cool dry place

6.0 Staining
6.1 Reagents/Equipment

1. Auramine O solution
2. Distilled water
3. 0.5% acid alcohol (Decolorizing solution)
4. Potassium Permanganate (Counter stain)
5. Timer

6.2 Procedure

1. Carefully place the fixed smears in a batch together with the positive and negative control slides on a staining rack **(smeared side up)**
2. Flood the smears with Auramine O solution and allow to stain for 15 minutes
3. Rinse the smears with **distilled water** and drain. Tap water contains chlorine which may interfere with florescence

4. Flood (decolorize) with 0.5% acid alcohol for 2 minutes
5. Rinse with **distilled water** and drain
6. Flood with potassium permanganate to counter stain for **2 minutes**. Counterstaining for longer time may quench florescence of the acid-fast bacilli
7. Rinse with **distilled water** and drain
8. Allow smears to **air dry**. Do not blot or dry using heat
9. Examine slides as soon as possible or otherwise keep slides in the dark to prevent fluorochrome from fading

7.0 Slide Examination and Recording of results

1. Use the X40 magnification fluorescent microscopy
2. The bacilli appear bright yellow against a dark background
3. Report results according to the guidelines in the table below

Fluorescent Microscopy Magnification	**Report**
400X	
0/100 fields or 1 length	No AFB seen
1-19/100 fields or 1 length	*Report exact number*
20-199AFB/100 fields or 1 length	*1+*
5-50/1 Field on average	*2+*
>50/1 field on average	*3+*

- *Record results directly onto the FM smear work sheet*
- *Use red ink for positive results*
- For a negative result report: "Acid-fast bacilli were not seen."
- For a positive result: report quantification of AFB seen. (It should not be assumed that all AFB are tubercle bacilli.)
- Never report "No TB" (or equivalent wording).

8.0 SOP DISTRIBUTION

Distributed to	**Number of copies**

9.0TRAINING RECORD FOR THIS SOP

The following personnel have been trained and do understand the contents of this SOP

Name of Personnel	Position	Signature	Date

Appendix XVIII: Height, Weight and Abdominal Circumference Standard Operating Procedures

INSTRUCTIONS FOR WEIGHT, HEIGHT AND ABDOMINAL CIRCUMFERENCE RECORDING

Step 1: Eligibility

Height, Weight and Abdominal circumference should only be measured in individuals who are eligible. The groups of people that are not eligible to have their measurements done are:

- Chair bound or bedbound participants
- Individuals who are unsteady on their feet
- Individuals who find it painful to stand straight
- Pregnant women
- Individuals who cannot stand without support
- Individuals with very high hair styles

Step 2: Site for taking measurements

It is preferable that weight and height are measured on a floor which is level and not carpeted (hard flat surface).

Step 3: Height Measurement

Equipment: Stadiometer- This is a portable collapsible device with a sliding head plate, a base plate and three contacting rods marked with a measuring scale.

The Protocol

1. Ask the participant to remove his/her shoes in order to obtain a measurement that is as accurate as possible.
2. Assemble the stadiometer and raise the head plate to allow sufficient room for the participant to stand underneath it. Double check that you have assembled the stadiometer correctly.
3. The participant should stand with his/her feet flat on the centre of the base plate, feet together and heels against the rod but not leaning on it. He/she should have his/her arms hanging loosely by their sides. He/she should be facing forwards.
4. Move the participant's head so that the Frankfort Plane is in a horizontal position (ie parallel to the floor). The Frankfort Plane is an imaginary line passing through the external ear canal and across the top of the lower bone of the eye socket, immediately under the eye. This position is important if an accurate reading is to be obtained. An additional check is to ensure that the measuring arm rests on the crown of the head, i.e. the top back half. To make sure that the Frankfort Plane is horizontal, you can use the Frankfort Plane Card to line up the bottom of the eye socket with the flap of skin

on the ear. The Frankfort Plane is horizontal when the card is parallel to the stadiometer arm.

5. Instruct the participant to keep his/her eyes focused on a point straight ahead, to breathe in deeply and to stretch to their fullest height. If after stretching up the participant's head is no longer horizontal, repeat the procedure. It can be difficult to determine whether the stadiometer head plate is resting on the participant's head. If so, ask the participant to tell you when she/he feels it touching their head.
6. Ask the participant to step forward. If the measurement has been done correctly the participant will be able to step off the stadiometer without ducking their head. Make sure that the head plate does not move when the participant does this.
7. Look at the bottom edge of the head plate cuff. There is a red arrowhead pointing to the measuring scale. Take the reading from this point and record the participant's height in centimetres and millimetres, at *Height* space in the Questionnaire (on the PDA).
8. Height must be recorded in centimetres and millimetres, e.g. 176.5 cms. If a measurement falls between two millimetres, it should be recorded to the nearest even millimetre. Eg. if an participant's height is between 176.4 and 176.5 cms, you should round it down to 176.4. Likewise, if the participant's height is between 176.5 and 176.6 cms, you should round it up to 176.6 cms.
9. Push the head plate high enough to avoid any member of the household hitting his/her head against it when getting ready to be measured.

Step 4: Weight Measurements

Equipment: Electronic bathroom scales

The protocol

1. Turn the display on by pressing firmly with your hand or foot on the top of the scales (the scales will turn themselves off after a short while). The readout should display 888.8 momentarily as a check for the operation. If this is not displayed check the batteries, if this is not the cause you may need to report the problem to the Principal Investigator. **While the scales read 888.8 do not attempt to weigh anyone.**
2. Ask the participant to remove shoes, heavy outer garments such as jackets and cardigans, heavy jewellery, loose change and keys.
3. Turn the scales on with your foot again. Wait for a display of 0.0 before the participant stands on the scales.
4. Ask the participant to stand with their feet together in the centre and their heels against the back edge of the scales. Arms should be hanging loosely at their sides and head facing forward. Ensure that they keep looking ahead - it may be tempting for the participant to look down at their weight reading. Ask them not to do this and assure them that you will tell them their weight afterwards if they want to know.
   The posture of the participant is important. If they stand to one side, look down, or do not otherwise have their weight evenly spread, it can affect the reading.

5. The scales will take a short while to stabilize and will read 'C' until they have done so. If the participant moves excessively while the scales are stabilizing you may get a false reading. If you think this is the case re-weigh the participant.
6. The scales have been calibrated in kilograms and 100 gram units (0.1 kg). Record the reading in the space provided for weight on the questionnaire.

WARNING

The maximum weight registering accurately on the scales is 130 kg (20 stone). If you think the participant exceeds this limit do not attempt to weigh them. Tick the box 'Weight exceeds scale maximum' on the Questionnaire.

Step 5: Abdominal circumference

Equipment: Insertion (SECA) measuring tape calibrated in mm, with a plastic buckle at the end.

The Protocol

1. Prepare the participant by asking him/her to;
    - Remove all outer clothing such as jackets, cardigans, waist coats, and heavy or baggy jumpers.
    - Remove shoes with high heels.
    - Remove tight garments that are intended to alter ones shape such as corsets or body suits.
    - Belts should be removed or loosened.
    - Pockets should be emptied.

If the study participant is not willing to remove bulky outer garments or tight garments and you are of the opinion that this will significantly affect the measurement, do not proceed with the measurement

2. Ask the participant to stand erect in a relaxed manner. The arms should be hanging loosely at their sides.
3. If possible, sit or kneel on a chair to the side of the participant.
4. Pass the tape around the body of the participant and insert the clip into the holder. Ensure that the tape is horizontal by peering around the participant.
5. Measure the abdominal circumference at the level of the navel (belly button)
6. Hold the buckle flat against the body and flatten the end of the tape to read the measurement from the outer edge of the buckle. Do not pull the tape towards you as this will lift away from the participant's body, affecting the measurement.
7. Record the measurement to the nearest centimetre on the questionnaire.