MORBIDITY FROM ANTIRETROVIRAL METABOLIC EFFECTS IN AFRICA: THE MAMA STUDY

by

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A dissertation submitted in partial fulfillment
of the requirements for the degree of
Master of Medical Science (Clinical Pharmacology)
at the University of Cape Town
2017

Declaration

I declare that "MORBIDITY FROM ANTIRETROVIRAL METABOLIC EFFECTS IN AFRICA: THE MAMA STUDY" is my own work and that it has not been submitted for any degree or examination in any other university and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

The work presented in this thesis is based on the following original publication in which I am the lead author:

S. Karamchand, R. Leisegang, M. Schomaker, G. Maartens, L. Walters, M. Hislop, J.A. Dave, N.S. Levitt, K. Cohen. Risk Factors for Incident Diabetes in a Cohort Taking First-Line Nonnucleoside Reverse Transcriptase Inhibitor-Based Antiretroviral Therapy. *Medicine (Baltimore)*. 2016;95(9):e2844. PMID: 26945366, DOI: https://dx.doi.org/10.1097/MD.0000000000002844 (See Appendix 1).

Dr. Sumanth Karamchand

Signed: Signed by candidate

Date: 28 September 2017

Acknowledgements

The following people are acknowledged for their contribution to the completion of this project:

Professor Gary Maartens (Supervisor)
Dr Karen Cohen (Supervisor)
Dr Rory Leisegang
Dr Michael Schomaker
Professor Naomi Levitt
Dr Joel Dave
Mr Michael Hislop
Mr Lourens Walters
Dr Leshern Karamchand

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List of Abbreviations

AfA: Aid for AIDS

AIC: Akaike Information Criterion

ART: Antiretroviral therapy

AZT: Zidovudine

BMI: Body mass index

CD4: Cluster of Differentiation 4

CHD: Coronary heart disease

d4T: Stavudine

DM: Diabetes Mellitus

EFV: Efavirenz

GLUT4: Glucose Transporter Type 4

HR: Hazard Ratio

HDL: High-density Lipoprotein

HIV: Human Immunodeficiency Virus

IFG: Impaired Fasting Glucose

IHD: Ischaemic heart disease

IQR: Interquartile Ratio

IRS-1: Insulin receptor substate-1

IRIS: Immune reconstitution inflammatory syndrome

LMICs: Low and middle-income countries

NVP: Nevirapine

NRTI: Nucleoside reverse transcriptase inhibitor

NNRTI: Non-nucleoside reverse transcriptase inhibitor

PI: Protease Inhibitor

PPAR-γ: Peroxisome proliferator-activated receptor gamma

PYFU: Patient-years of follow-up

RSAID: Republic of South Africa Identity

SREBP: Sterol regulatory element-binding protein

SSA: Sub-Saharan Africa

TNFα: Tumour necrosis factor

VL: Viral load

WHO: World Health Organization

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Abstract

Introduction: Combination antiretroviral therapy (ART) has considerably reduced both the morbidity and mortality of Human Immunodeficiency Virus (HIV) infection and its associated complications, thus effectively transforming a fatal disease into a manageable chronic condition. However, the chronic use of ART has been accompanied by the emergence of adverse metabolic abnormalities in HIV-infected patients, including dysglycaemia. There is, however, a paucity of data from sub-Saharan Africa on the incidence and risk factors associated with new onset diabetes mellitus in the HIV-infected population. Furthermore, efavirenz is the preferred nonnucleoside reverse transcriptase inhibitor (NNRTI) in first-line antiretroviral therapy (ART) regimens in low- and middle-income countries, where the prevalence of diabetes is increasing. Randomized control trials have shown mild increases in plasma glucose in participants in the efavirenz arms, but no association has been reported with overt diabetes. This study explores the risk factors and incidence of diabetes, and in particular the association between efavirenz exposure and diabetes, in a large Southern African cohort commencing NNRTI-based first-line ART.

Subjects and Methods: The study cohort included HIV-infected adults commencing NNRTI-based ART in a private sector HIV disease management programme from January 2002 to December 2011. Incident diabetes was identified by the initiation of diabetes treatment. Patients with prevalent diabetes were excluded. The incidence of diabetes in patients receiving efavirenz versus nevirapine containing regimens was compared with a Kaplan-Meier plot and a log-rank test. The association of efavirenz exposure with the hazard of developing diabetes was modelled using a multivariate

Cox-proportional hazards model. The following variables were adjusted for in the regression model: age, sex, baseline BMI, baseline CD4, baseline viral load, exposure to diabetogenic drugs, and nucleoside reverse transcriptase inhibitor (NRTI) exposure.

Results: Between January 2002 and June 2011, 62,467 patients commenced ART in the AfA program, of whom 56,298 patients met the inclusion and exclusion criteria and were included in the analysis. Median follow-up was 1.56 years (interquartile range (IQR): 0.71– 2.79 years), 21.7% of patients were followed up for 3 or more years. New onset diabetes was identified in 1500 (2.66%) patients over 113,297 patient-years of follow-up (PYFU), giving a crude incidence of 13.24 cases per 1000 PYFU. In the multivariate analysis treatment with efavirenz rather than nevirapine was associated with increased risk of developing diabetes (hazard ratio 1.27 (95% confidence interval: 1.10 - 1.46). Zidovudine and stavudine exposure, older baseline age, elevated baseline BMI, and exposure to diabetogenic medication were also associated with increased risk of diabetes. No association was found between baseline CD4 and an increased risk of diabetes. There was an association between the lowest stratum of baseline viral load and an increased relative risk for developing diabetes, but no association with higher viral load strata.

Conclusion: Treatment with efavirenz, as well as stavudine and zidovudine, increased the risk of incident diabetes. Interventions to detect and prevent diabetes should be implemented in ART programmes, and use of antiretrovirals with lower risk of metabolic complications should be encouraged.

Chapter 1: Introduction

Risk Factors for Incident Diabetes in a Cohort Taking First-Line Nonnucleoside Reverse Transcriptase Inhibitor-Based Antiretroviral Therapy

1.1 Background

Access to antiretroviral therapy (ART) in low- and middle-income country (LMIC) settings has considerably reduced both the morbidity and mortality associated with Human Immunodeficiency Virus (HIV) infection, thus transforming a fatal disease into a manageable chronic condition. However, the long-term use of ART has been accompanied by the emergence of adverse metabolic effects including insulin resistance and dysglycaemia, which are thought to be responsible for the association between long term ART and the risk of vascular disease.[1,2]

In accordance with World Health Organization (WHO) guidelines, HIV infected patients in low-middle income countries (LMICs) requiring ART are initiated on a triple therapy regimen comprising two nucleoside reverse transcriptase inhibitors (NRTIs), and a non-nucleoside reverse transcriptase inhibitor (NNRTI) (either efavirenz or nevirapine).[3] Protease inhibitors (PIs) (*e.g.* Lopinavir/ Ritonavir) were reserved as a second line therapy for adult patients experiencing treatment failure on first line agents. The decision of which specific NNRTI to use was determined largely by the respective side effect profile of the drugs. Efavirenz was thought to be teratogenic and therefore not advised for pregnant patients and its neuropsychiatric

adverse effects precluded its use in shift workers. Nevirapine demonstrated significant hepatotoxicity, dermatological adverse effects as well as Cytochrome P450 interactions. The use of Nevirapine was therefore not advised in patients undergoing tuberculosis (TB) chemotherapy. Despite their adverse effects, the NNRTIs exhibited potent viral suppression. However, recent studies have linked the emergence of dysglycaemia, insulin resistance and new onset diabetes mellitus to efavirenz use in addition to the NRTIs and PIs.[2,4]

Within Sub-Saharan Africa (SSA), the burden of diabetes is growing and this is paralleled by an increasing prevalence of obesity which includes HIV-positive patients.[5] Moreover, the impact of ART on the incidence of diabetes on a background of concomitantly increasing rates of obesity within SSA is undetermined. In addition, the recent trends of initiating ART at higher CD4 cell counts increases the lifetime exposure to these drugs thus making the likelihood of diabetes more incidental at younger age groups.[3]

At present, the added risk of diabetes mellitus (DM) in South African HIV patients on ART as compared to the general population is unknown. The findings from high income countries may not apply to the South African HIV infected population due to demographic and pharmacokinetic differences. The South African cohort of HIV infected patients comprises predominantly young black women as compared to middle-age men in developed countries.[4] Furthermore, the ethnic variability with the SA HIV infected cohort, may impart a varied metabolic response to ART in these patients. The recent trend of initiating ART at higher CD4 cell counts means that there is increased overall exposure time to ART and possibly a greater lifetime risk of

developing DM as compared to the general population. Currently SA is experiencing an increasing incidence of diabetes especially in younger age groups.[5,6] Risk factors include obesity, rapid urbanization, physical inactivity, nutrition transitions, and socioeconomic changes.[5,6] It is unascertained how ART modifies the incidence of diabetes and the concomitant coronary heart disease (CHD) risk. First line ART regimens in developed countries utilise PIs whereas these agents are reserved as second line therapy in South African ART guidelines.[3] It is also unknown whether the proposed metabolic abnormalities are dose dependant or develop due to increased time exposure.

The paucity of data emanating from SSA makes it difficult to determine the additional risk of developing diabetes mellitus in HIV infected patients using ART. The aim of this study is to investigate the association between first-line ART use and the incidence of diabetes mellitus in a South African cohort of HIV infected patients, controlling for the effects of other drugs associated with developing diabetes mellitus.

1.2 Literature review

1.2.1 Definition of Diabetes

Diabetes mellitus is regarded as a state of hyperglycaemic decompensation that ensues when pancreatic β-cell secretion is insufficient to respond to bodily insulin requirements.

Diabetes is defined as a fasting glucose >126 mg/dL (7.0 mmol/L), a plasma glucose level exceeding 200 mg/dL (11.1 mmol/L) 2 hours after a 75 g oral glucose load, or two random plasma glucose levels exceeding 200 mg/dL (11.1 mmol/L with symptoms of hyperglycaemia [thirst, polyuria, polydipsia, or recurrent infections]).[7]

1.2.2 Diabetes Burden

Diabetes affects about 336 million people worldwide.[5] Distinct ethnic susceptibilities are evident, particularly where there have been rapid transitions from traditional lifestyles to affluent lifestyles characterized by sedentariness and consumption of high energy-density foods. About 7 million people develop diabetes each year; diabetes is expected to affect 438 million people by 2030.[5]

According to the International Diabetes Federation, approximately 80% of people with diabetes live in low- and middle-income countries, where prevalence of diabetes is climbing.[5,6,8] Within Sub-Saharan, which bears the brunt of the HIV epidemic, the estimated overall prevalence of diabetes in 2010 was approximately 1.42%, with 1.9 million people living with the disease in South Africa alone.[5] The escalating burden of diabetes is paralleled by increasing rates of obesity in LMICs, with recent estimates

suggesting that the number of obese adults in certain regions of sub-Saharan Africa already exceeding that of their western counterparts.[8] Recent reports on the growing epidemic of obesity in HIV infected patients in LMICs are significant as obesity is an important risk factor for developing type II diabetes.[9-11] The inherent increase in non-communicable disease is expected to compound morbidity and mortality due to HIV associated infectious causes.[12] Diabetes in HIV infected patients could therefore have a compound effect on the development of vascular disease, and opportunistic infections like tuberculosis.[13,14]

1.2.3 Literature Search Strategy

A systematic review of electronic literature was conducted in accordance with Cochrane Collaboration guidelines with the aim of investigating the association between ART use and disorders of glucose metabolism: insulin resistance and new onset diabetes mellitus. The literature search was initiated on 10/05/2010 and continued until 30/12/2016, however the literature cited in this study is not restricted to this timeframe.

The search terms were; "anti-retroviral therapy AND diabetes OR dysglycaemia OR Insulin resistance," "highly active anti-retroviral therapy AND diabetes," "efavirenz AND diabetes," "nevirapine AND diabetes," "HIV AND diabetes," "human immuno-deficiency AND diabetes."

The following databases were searched for relevant articles; PubMed, EBSCO host, OVID, the Cochrane Controlled Trials Register in the Cochrane Library, ExcerptaMedica Database (EMBASE), and a clinical trials web site

(ClinicalTrials.gov). Included were articles in any language published from the beginning of each database until December 2016. For all included articles, a lateral search in PubMed by using the "related articles" link was performed.

No filter was employed to limit the search to developing country (resource-limited) settings. Randomised controlled trials, cohort and cross sectional studies that had been conducted in countries with a low or middle level of human development were included. Potentially relevant studies were included by reviewing titles and abstracts retrieved from bibliographic databases. Studies identified as potentially relevant were retrieved in full text and screened for inclusion. Of the eleven abstracts retrieved from the Pubmed search, only two were relevant to this study and were therefore included in the present review. Similarly, only 78 of the 114 papers retrieved from the literature search were relevant to this study and were therefore included in the present review.

1.2.4 Diabetes-conventional Risk Factors

1.2.4.1 Age

In the HIV seronegative population, age is a significant factor predisposing to the development of insulin resistance and type II diabetes mellitus.[12]

The Swiss HIV Cohort Study (SHCS), a prospective observational cohort, studied the influence of aging on the epidemiology of non-AIDS diseases in an HIV infected cohort categorized into 3 age groups: <50, 50–64, and ≥65 years of age. Seventy cases of diabetes were reported in the cohort of 8848 patients. The age stratified

incidence of diabetes was 2.09 per 1,000 person-years, 4.65 per 1,000 person-years and 8.56 per 1,000 person-years in the age groups <50, 50–64, and ≥65, respectively, suggesting that incidence of diabetes increases with advancing age.[15] The prospective collection of adverse events and the statistical power were significant strengths of the SHCS. The study was however limited by lack of a demographically comparable HIV-uninfected control group. The latter obscures the interpretation of advanced age and the incidence of diabetes, as the increased comorbidity of diabetes may be due solely to aging or due to attenuated immune recovery in elderly HIV infected patients.[15] The findings of the SHCS were however corroborated by Capeau *et al*, 2012, in the ANRS CO8 APROCO-COPILOTE Cohort Study.[16] This prospective study with a cohort of 1046 patients representing 7,846 PYFU evaluated the incidence of diabetes in combination ART treated individuals. A total of 111 patients developed diabetes, with an incidence of 14.1/1,000 PYFU. The investigators reported that the incidence of diabetes was associated with older age, specifically, an adjusted hazard ratio of 2.13 and 3.63 for the groups aged 40-49 years, and ≥50 years respectively.[16] Unlike the SHCS, the ANRS CO8 APROCO-COPILOTE Cohort Study was limited in power due to the number of patients included in the study. Furthermore, the ANRS CO8 APROCO-COPILOTE Cohort Study was initiated in 1997-1999, where the use of older antiretrovirals (stavudine and indinavir) now known to be strongly associated with the development of diabetes was commonly used.[2] The subsequent availability of less toxic antiretrovirals and drug policy changes resulted in a lower incidence rate of diabetes in follow up years. The effect of calendar year/date of inclusion was not observed in the SHCS. Despite the limitations noted, the ANRS CO8 APROCO-COPILOTE Cohort Study noted a significant association between short-term exposure to indinavir, stavudine and didanosine and

diabetes, which was not observed in patients exposed for a longer term to these drugs.[16] The relationship between age and the incidence of diabetes in HIV treated patients may not be a simple linear association. The findings of the SHCS and the ANRS CO8 APROCO-COPILOTE Cohort Study suggest that patients who are genetically predisposed to diabetes, develop the disease in the initial years of antiretroviral therapy whereas patients not prone to diabetes may tolerate long term ART without developing disorders of glucose metabolism.[16]

1.2.4.2 Sex

The current literature reflecting metabolic aberrations in HIV seropositive patients contain a predominance of male subjects. Despite this, most contemporary studies indicate that male sex poses a greater risk for the development of insulin resistance and overt diabetes.[4] There is however, in-homogeneity in the rates, risks and prevalence of gender associated diabetes reported in the studies published to date. The results of the Data collection on Adverse events of Anti-HIV Drugs (D:A:D) study[2], a large, prospective observational study, indicated that relative risk of diabetes was 60% greater for the male sex, whilst the French 10 year study by Capeau *et al*, 2012, reported incidence rates of 14.6/1,000 PYFU and 12.6/1,000 PFYU in men and women respectively.[16] The strength of the D:A:D study is the large number of study participants (over 22,000 patients) collated from 11 separate cohorts. However, as a limitation, the fragmented nature of the cohorts did not enable the investigators to fully evaluate the effects of treatment interruption and adherence.[2]

The prospective Women's Interagency HIV study investigated the incidence of diabetes in a cohort comprising 1,524 HIV infected and 564 HIV uninfected individuals.[17] In this cohort, diabetes developed in 116 HIV infected patients and 36

HIV uninfected women over 6,802 PYFU. The incidence rate of diabetes in the ART naïve group was 1.53/100 person-years as compared to an incidence rate of 2.50/100 person-years and 2.89/100 person-years in patients receiving PI-based and non-PI-based ART regimens, respectively.[17] These rates were however, paralleled by an incidence rate of 1.96/100 person-years in a group of HIV uninfected women in a study by Justman *et al,* 2003.[18] In the latter study, the HIV-seronegative group had higher BMIs than their sero-positive counterparts (33% versus 23%), however counter-intuitively, displayed half the rate of new onset diabetes.[18] The suggestion is that female ART-exposed patients have a greater likelihood of developing diabetes at relatively lower BMIs and adiposity levels. The literature to date indicates that ART exposed men have a greater risk of developing diabetes compared to women. However, women may develop dysglycaemia at lower adiposity levels.[15,19] This gender difference may reflect the variation in drug metabolism, with women inherently having lower rates of hepatic drug elimination.[16]

1.2.4.3 Central Obesity and Omental Adipose Tissue Accumulation

It is well established that obesity and visceral fat accumulation are integral to the pathogenesis of insulin resistance and the subsequent progression to diabetes mellitus.[20,21] The clinical measurement of waist to hip ratio has proven to be a better surrogate marker in the determination of the risk of developing diabetes. Elevated waist to hip ratios, rather than overall BMI, reflect on the type of adipose tissue distribution that subtends a greater risk of developing diabetes. This body habitus is similar to that of patients suffering from lipodystrophy/ lipoatrophy syndromes as a consequence of long term ART exposure.[15, 16]

The return to health, reconstitution of the immune system, and rapid weight gain observed in patients initiated on ART may compound the risk of developing diabetes inherent to HIV chemotherapy. The D:A:D study reported a fourfold increase in the rates of new onset diabetes in clinically obese patients as compared to controls with healthy BMIs.[2] Capeau *et al*, 2012, reported new onset diabetes hazard ratios of 1.91 and 2.85 for overweight and obese patients respectively, in 10-year follow-up period.[16]

1.2.4.4 Lipid Disturbances and the Metabolic Syndrome

The metabolic aberrations in diabetes are characterised by elevated plasma levels of triglycerides and lowered levels of high-density lipoprotein (HDL).[21] These, in combination with increased central visceral adiposity, inherent insulin resistance and elevated blood pressure are significant components of the metabolic syndrome. The D:A:D study investigated the characteristics of lipid dysregulation in HIV-seropositive patients and reported elevated triglycerides levels and lowered HDL levels, both of which were component predictors of new onset diabetes mellitus.[2] Specifically, triglyceride levels two times higher than normal translated into an 80% increased risk of developing diabetes, whereas higher HDL levels impart a protective effect against dysglycaemia. Elevated total cholesterol paralleled the predictive effect of elevated triglycerides. In this instance, lipid aberrations serve as a proxy marker in predicting the risk of developing diabetes.[2] An international cross sectional study by Samaras *et al*, 2007, reported that the prevalence of diabetes is approximately five to nine times higher in HIV seropositive individuals co-afflicted with the metabolic syndrome.[20] It is pertinent to note that this cohort emanated from large tertiary referral centres in high-income settings, and the results may not be generalizable to LMICs

such as SSA with lower levels of education, socio-economic status and suboptimal nutrition.

Results from the INITIO trial, an international, multicentre, randomized clinical trial suggests that patients with already present metabolic syndrome at the start of ART had a four times greater risk of developing diabetes.[22] The INITIO trial investigated the effect of three ART initiation strategies and the incidence of metabolic syndrome, cardiovascular disease and type 2 diabetes mellitus in treatment-naive HIV infected participants. The INITIO trial indicated that the onset of metabolic syndrome whilst on ART is associated with a higher risk of incident diabetes.[22] These findings reaffirm the contributory factors that lipid aberrations impart to the risk of developing diabetes mellitus. The study was however limited as there was departure from protocol-specified initial ART regimens. Most participants received didanosine and stavudine, agents that are strongly associated with diabetes and are no longer recommended as first line therapy, which may have also masked the effects of other drugs such as efavirenz and the development of metabolic syndrome.

1.2.4.5 Hepatitis C Co-infection

Chronic hepatitis C infection has a well-established association with development of peripheral insulin resistance.[23] The risk of subsequent diabetes development is debated. Retrospective studies have reported a diabetes prevalence twice as high in hepatitis C co-infected HIV positive patients, whereas other studies have reported no association.[16,24] The prevalence of hepatitis C is low in South Africa and is therefore a less significant factor in the development of insulin resistance.

1.2.4.6 Direct Viral Effects

Prior studies suggest that patients with untreated HIV infection have increased dyslipidaemia and elevated levels of bio-inflammatory markers predisposing to the development of insulin resistance.[25,26]

1.2.4.7 Duration of HIV Infection

There is a paucity of data reflecting the relationship between duration of HIV infection and the development of diabetes mellitus. Recent studies however, have suggested that there is no association between length of HIV infection and dysglycaemia.[16]

1.2.4.8 CD4 Nadir

There appears to be conflicting results with respect to nadir CD4 count and the development of insulin resistance. The Multicenter Aids Cohort Study (MACS) found that CD4 nadir less than 300 cells/mm^3 was associated with increased risk of insulin resistance, whereas the ANRS CO8 APROCO-COPILOTE cohort found no difference in the incidence of diabetes with nadir CD4 less than 200 cells/mm^3 compared to CD4 counts greater than 200 cells/mm^3.[16,19]

1.2.4.9 Body Fat Redistribution disorders

NRTIs and PIs have demonstrated variable effects on mitochondrial toxicity in muscle and adipose tissue.[27,28] More specifically there is a decrease in subcutaneous adipose tissue, increased visceral adipose tissue deposition and altered lipid metabolism (**Refer to Figure 1).** Lipodystrophy, the term that encompasses the

clinical manifestations of altered lipid and adipose tissue redistribution, appears to exacerbate insulin resistance.[29,30]

1.2.4.10 Diabetes Prevalence and Incidence in HIV Infected Patients

The occurrence of diabetes in HIV infected patients was relatively uncommon, with early studies indicating an incident rate of 2.0 – 2.6% in ART naïve patients.[31,32] The introduction of combination ART and the widespread use of PIs in particular have heralded a rapid increase in the incidence of new onset diabetes mellitus.[21,33,34] It was subsequently established that patients on ART had a higher predilection/propensity to develop glucose intolerance and overt DM than their counterpart controls.[20,35,36] The reports of incident diabetes vary widely due to the differential initiation of ART drugs *i.e.* PI based therapy versus NNRTI based therapy in accordance with differeing health policies. Eliciting the true prevalence and incidence of diabetes in context of HIV infected populations is complicated by confounding variables such as genetic differences in study populations and the differences and in-homogeneity in diagnostic and statistical methods used by investigators.

As part of the metabolic syndrome, hyperinsulinaemia is often accompanied by truncal adiposity, increased waist-to-hip ratios, 'buffalo hump' and loss of limb fat. In a small prospective cohort study, Hadigan *et al*, 2001, reported that in such patients there was a DM incidence of 7% compared with 0.5% in healthy controls.[37]

Cross sectional studies by Carr *et al*, 1998-1999, investigated ART associated lipodystrophy and found a 2% incidence of diabetes, which subsequently rose to 7% after a 14 month observation period and an overall 25% prevalence of all glucose

disorders.[29,30] Importantly, these studies were limited to the protease inhibitor class of drugs. Calza *et al*, 2011 have reported prevalence rates of diabetes and impaired fasting glucose rates of 4.5% and 9.1% respectively in a prospective cohort study including 755 HIV patients.[36] This study was limited by the number of patients enrolled, but did find significant associations between new onset diabetes and the NRTIs; stauvdine, didanosine and zidovudine.[38] Studies with a longer period of follow-up have demonstrated a fourfold increase in the relative risk of developing new onset diabetes (10% of ART patients over a four year follow-up period as compared to 3% in HIV-seronegative controls).[19] The D:A:D study reported a diabetes incidence rate of 5.72 per 1,000 person-years of follow-up (PFYU) having observed 33,389 HIV seropositive patients for an average of 3.8 years.[2] Conclusions from the D:A:D study suggest that new onset diabetes was strongly associated with use of the NRTI stavudine, and that exposure to the NRTIs; zidovudine and didanosine also impart an increased risk of developing diabetes, after controlling for potential diabetogenic risk factors.[2] In contrast, the PI, ritonavir, and the NNRTI, nevirapine, were associated with reduced risk of diabetes.[2]

The findings from the D:A:D study were corroborated by the Swiss HIV Cohort Study which reported an incidence of 3.12 cases per 1,000 PFYU.[15] Over a 3 year observation period of 8,444 participants, the prevalence of diabetes had increased from a baseline of 4% to 7%.[15] The prospective Multicenter Aids Cohort Study (MACS) reported the incidence of DM in a cohort of 411 HIV-infected men with HAART exposure was greater than 4 times that of HIV-seronegative men.[19] The rate of incident DM in the MACS was 4.7 cases per 100 person-years among HIV-infected men using ART compared with 1.4 cases per 100 person-years among HIV-seronegative men, after adjustment for age and body mass index in a 4 year

observation period.[19] This represented a 14% incidence of diabetes in HIV-infected men exposed to ART in comparison to HIV-seronegative controls.[19] The MACS trial, however, had several limitations; the end point determination of diabetes mellitus was based on a single fasting blood glucose level measurement, self-reported diagnosis of diabetes, not investigating the effect of hepatitis C on incident diabetes, and selection biases.

Capeau *et al*, 2012, investigated the ten-year diabetes incidence in 1,046 HIV-infected patients started on a combination antiretroviral treatment.[16] In this study cohort with 7,846 person-years of follow-up (PYFU), 111 patients developed diabetes, with an incidence of 14.1/1,000 PYFU. The incidence of diabetes was associated with older age, elevated body mass index, waist-to-hip ratio and exposure to indinavir, stavudine and didanosine.[16]

Polsky *et al*, 2011, studied the incidence of hyperglycaemia among older adults with or at risk for HIV infection.[39] In a prospective cohort comprising 377 participants followed up over an 18-month period with baseline rates of diabetes of 7% and pre-diabetes of 31%, the incidence of new onset diabetes was found to be 5%. Importantly, the authors concluded that neither HIV infection nor ART use were associated with increased risks of diabetes.[39] The strength of the study was endpoint determination by use of oral glucose tolerance tests, but was limited by the lack of confirmatory testing. This study was also limited by the small number of incident cases, which diminished the ability to detect associations between certain risk factors, and incident diabetes as described above.

The Women's Interagency HIV Study likewise investigated the incidence of diabetes mellitus in a cohort of HIV infected women (1,524 HIV infected subjects) as compared to a control group (564 HIV uninfected subjects).[40] ART-naïve HIV

infected patients had a DM incidence rate of 1.53/100 person-years; those reporting ART containing a protease inhibitor (PI) had a rate of 2.50/100 person-years and those reporting non-PI-containing ART regimen, a rate of 2.89/100 person-years. The HIV-uninfected control group had an incidence of 1.96/100 person-years.[40] The lower DM incident rate observed in the WIHS trial is less than that observed in the MACS trial, which may be indicative of a less sensitive case detection method.[19,40]

1.2.5 Diabetes and HIV in Africa

There are several small studies emanating from Sub-Saharan Africa providing age adjusted prevalence estimates for diabetes among the HIV infected population.

Dave *et al*, 2011, reported on South African cross sectional data where the prevalence of dysglycaemia in 406 ART-naïve patients and 443 patients on ART were 25.7% and 21.9% (P = 0.193), respectively.[4] However, there were higher levels of impaired fasting glucose (IFG) in the ART treated group (23.5%) as compared to the ART naïve group (18.5%).[4] The study was limited by the lack of an HIV negative control group, a short duration of ART exposure, and a relatively small number of male patients. The prevalence of impaired fasting glucose was 16-18% in a Rwandan cross sectional study of 571 patients receiving ART for more than 6 months.[41] Manuthu *et al*, 2008, reported a diabetes prevalence and IFG of 1.5% and 21.4%, respectively, in a Kenyan cross sectional study of 295 patients receiving ART for more than a year.[42] Zannou *et al*, 2009, reported a diabetes prevalence and IFG of 1.5% and 34-37%, respectively, in a prospective Benin cohort in 79 patients receiving ART for more than a year.[43] Most of the literature to date investigating HIV, ART and diabetes is limited by a lack of internal controls prohibiting the estimation of relative risk.

1.2.6 Pathogenesis of ART Related Insulin Resistance and Diabetes Mellitus

Whilst it is suggested that ART may influence the development of insulin resistance[2], the established traditional risk factors such as family history, elevated body mass index, smoking, and advanced age may be more significant factors in the pathogenesis of overt diabetes mellitus.

In summary, the proposed mechanisms that lead to the development of insulin resistance and diabetes in the context of HIV infection are attributed to either one or a combination of the following factors:[44,45]

- Antiretroviral agents
- Co-morbidities, such as chronic hepatitis C infection
- Opportunistic infections
- Altered fat distribution: visceral fat accumulation/ subcutaneous lipoatrophy
- Mitochondrial dysfunction
- Elevated circulating free fatty acids
- Increased muscle and organ fat
- Elevated pro-inflammatory cytokine release
- HIV direct viral effect
- Diet and lifestyle
- Genetic predisposition

1.2.7 Drug Exposures

Studies in resource-rich settings have shown an association between long-term ART and the risk of vascular disease, which is postulated to be due to adverse metabolic effects of antiretroviral drugs.[1] Dysglycaemia, insulin resistance, and diabetes mellitus

have been linked to a variety of antiretroviral drugs from three different classes: the nucleoside reverse transcriptase inhibitors (NRTIs), stavudine and zidovudine, the protease inhibitors (PIs), indinavir and ritonavir, and more recently, the non-nucleoside reverse transcriptase inhibitor (NNRTI), efavirenz.[4,46,47]

1.2.8 Antiretroviral Effects

Early reports and cases indicated that disorders of glucose and lipid metabolism were primarily due to PI therapy.[33] However, Brinkman *et al,* 1999, reported on data which suggested that NRTIs may be contributing factors in the pathogenesis of ART-associated insulin resistance and diabetes.[48] The pathogenic mechanism elucidated suggests that whilst NRTIs are potent inhibitors of HIV reverse transcriptase, the drugs also inhibit an integral mammalian enzyme, DNA polymerase-γ, responsible for mitochondrial replication.[48] The dysregulation of mitochondrial function in different compartments of the body results in various clinical manifestations of NRTI toxicity. The inhibition of mitochondrial replication in muscle manifests as insulin resistance, whereas mitochondrial dysregulation in the liver increases the propensity to develop lactic acidosis. Dysfunctional mitochondria in adipose tissue predispose to the development of lipoatrophy, and peripheral neuropathy results from dysfunctional neuronal mitochondria. Brown *et al*, 2005, found that exposure to NRTIs most strongly correlated with insulin resistance after controlling for other risk factors; age, BMI, and CD4 count.[35] The D:A:D study also found that cumulative exposure to NRTIs increased the risk of insulin resistance, with the highest risk attributed to stavudine followed by zidovudine, and lamivudine.[2] Studies indicate that HIV infection itself as well as ART may be responsible for altered pancreatic β-cell responses to plasma glucose, and hence inadequate insulin secretion.[49-51]

1.2.9 Insulin Resistance Associated with Protease Inhibitor Use

Within the PI class, insulin resistance has been most commonly observed with ritonavir, indinavir and nelfinavir.[52] Ritonavir has been reported to impair beta cell function with a concomitant reduction of insulin synthesis of between 25 to 30%.[53] Noor *et al*, 2004, demonstrated that insulin resistance was induced in healthy subjects after administration of lopinavir/ritonavir for 5-10 days of treatment.[54] Indinavir, and lopinavir/ritonavir to a lesser extent, inhibits the rate-limiting step in glucose uptake into muscle and adipose tissue; *i.e.* insulin stimulated glucose transport mediated by Glucose Transporter Type 4 (GLUT4).[46] Other proposed mechanisms by which PIs lead to insulin resistance include inhibition of peroxisome proliferator-activated receptor gamma (PPAR-γ) and sterol regulatory element-binding protein (SREBP). PPAR-γ is involved in insulin resistance by regulating the maturation and metabolic activity of adipocytes. The products of adipocyte metabolism include adipokines, such as adiponectin and leptin, which mediate the activity of insulin in peripheral tissues.[52] Other studies suggest that insulin resistance, and overt diabetes associated with PIs may be a result of immune reconstitution, a subsequent increase in body mass index[55], and induction of lipodystrophy and dyslipidaemia.[56]

1.2.10 Insulin Resistance Associated with NNRTI Use

There are limited studies investigating the effects of NNRTIs, and the development of insulin resistance and diabetes. The available data has indicated that nevirapine-based ART may be protective against insulin resistance relative to efavirenz-based therapy, and that efavirenz may be associated with overt dysglycaemia.[4,47]

A prior cross sectional study found an increased risk of dysglycaemia in South African patients taking efavirenz compared with those taking nevirapine, but there

were insufficient numbers of cases of diabetes for analysis.[4] A small case–control study from Botswana suggested an association between efavirenz use and diabetes.[57] Randomized controlled trials showed significantly higher serum glucose concentrations in participants in the efavirenz arms than the following comparator antiretroviral drugs: nevirapine,[58] abacavir,[58] atazanavir,[59] atazanavir–ritonavir,[60] and raltegravir.[61]

The mechanism by which efavirenz mediates insulin resistance and diabetes is unknown. Possible mechanisms include mitochondrial toxicity[62], and toxic effects on adipocytes and increased rates of lipoatrophy.[63,64] Efavirenz causes hepatic mitochondrial toxicity[62], and induces hepatocyte endoplasmic reticulum stress leading to activation of the unfolded protein response, and apoptosis.[39,40] Efavirenz mediates mitochondrial toxicity *via* various mechanisms. Firstly, efavirenz directly inhibits Complex I of the electron transport chain, resulting in a markedly reduced mitochondrial transmembrane potential, thus compromising oxidative phosphorylation and ATP generation.[65-67] Secondly, efavirenz reduces complex IV (COIV) mRNA (a marker gene of mitochondrial function), and impairs mitochondrial function in adipocytes.[68] Furthermore, efavirenz associated mitochondrial dysregulation in adipose tissue causes impaired adipogenesis, increased lipolysis, and release of free fatty acids and inflammatory cytokines.[68] The increased release of fatty acids due to adipocyte mitochondrial toxicity are thought to impair muscle and liver insulin sensitivity, leading to insulin resistance and diabetes mellitus.[45,69-74] In addition to its mitochondrial toxicity, efavirenz has been shown to reduce the secretion of adiponectin (an insulin-sensitizing, anti-diabetic adipokine) by adipocytes.[68] It is therefore hypothesized that impairment of mitochondrial bioenergetics and toxicity to adipocytes contributes to the development of diabetes in

patients on efavirenz. By contrast, nevirapine does not appear to exert mitochondrial toxicity.[63,68]

Prior studies have demonstrated a positive correlation between plasma efavirenz concentrations, and both fasting and 2-hour glucose concentrations after oral glucose tolerance tests in South African patients.[75] People of African origin are more likely to be genotypic "slow metabolizers" of efavirenz, which results in elevated efavirenz plasma concentrations, than people of European descent (20% and 3%, respectively).[76] Therefore efavirenz may have a larger diabetogenic effect in Africans, which may explain why studies from high-income countries have not found an association between efavirenz and diabetes.

1.2.11 Non-Antiretroviral Effects: Epidemiological Evidence and Mechanisms

In essence, it is difficult to delineate the metabolic effects of ART from those of HIV. There are few studies that describe the prevalence of diabetes in ART-naïve HIV infected patients. El Sadr *et al*, 2005, investigated the effects of HIV infection on lipid, glucose and insulin levels in 419 ART-naïve patients and found a 2.6% baseline prevalence of diabetes in their cohort.[31]

Immunological studies have indicated that HIV infection is associated with elevated plasma levels of tumour necrosis factor (TNFα) and other cytokines that may alter glucose metabolism.[77] Certain HIV expressed proteins (Tat and Vpr) have a propensity to directly induce the development of insulin resistance. In particular, Vpr inhibits PPAR-γ activity and dysregulates the transcriptional activity of insulin.[78] Tat activates Nuclear Factor-kappa B, which in turn increases TNFα secretion. There is subsequent decreased uptake of free fatty acids by adipocytes, suppression of insulin

receptor and insulin signalling events, and decreased GLUT4 translocation and phosphorylation of the insulin receptor substate-1 (IRS-1).[79-81] Immune reconstitution following ART initiation may result in an increased and paradoxical inflammatory response, thus promoting insulin resistance. Brown *et al*, 2010, reported that the initiation of ART was followed by elevated TNFα levels at 48 weeks with associated incident diabetes mellitus.[82]

1.2.12 Prevalence of the Metabolic Syndrome

Hyper-insulinaemia and impaired glucose tolerance are components of the metabolic syndrome. It is therefore imperative to assess HIV infected individuals, whether treated or not, for development of the metabolic syndrome, as each individual component precludes the development of another.

Estrada *et al*, 2006, studied the prevalence of lipodystrophy and the metabolic syndrome in HIV-infected patients on ART and concluded that the prevalence in the ART group was 15.8% as compared to 3.2% in the HIV seronegative healthy controls.[36] This study was limited by a lack of fat distribution measurements for the entire study population and in general, a lack of an objective criterion of the diagnosis of lipodystrophy. In the Women's Interagency Study, the HIV-seropositive group had a 33% prevalence of the metabolic syndrome whilst the HIV-seronegative controls had a prevalence of 22%.[40]

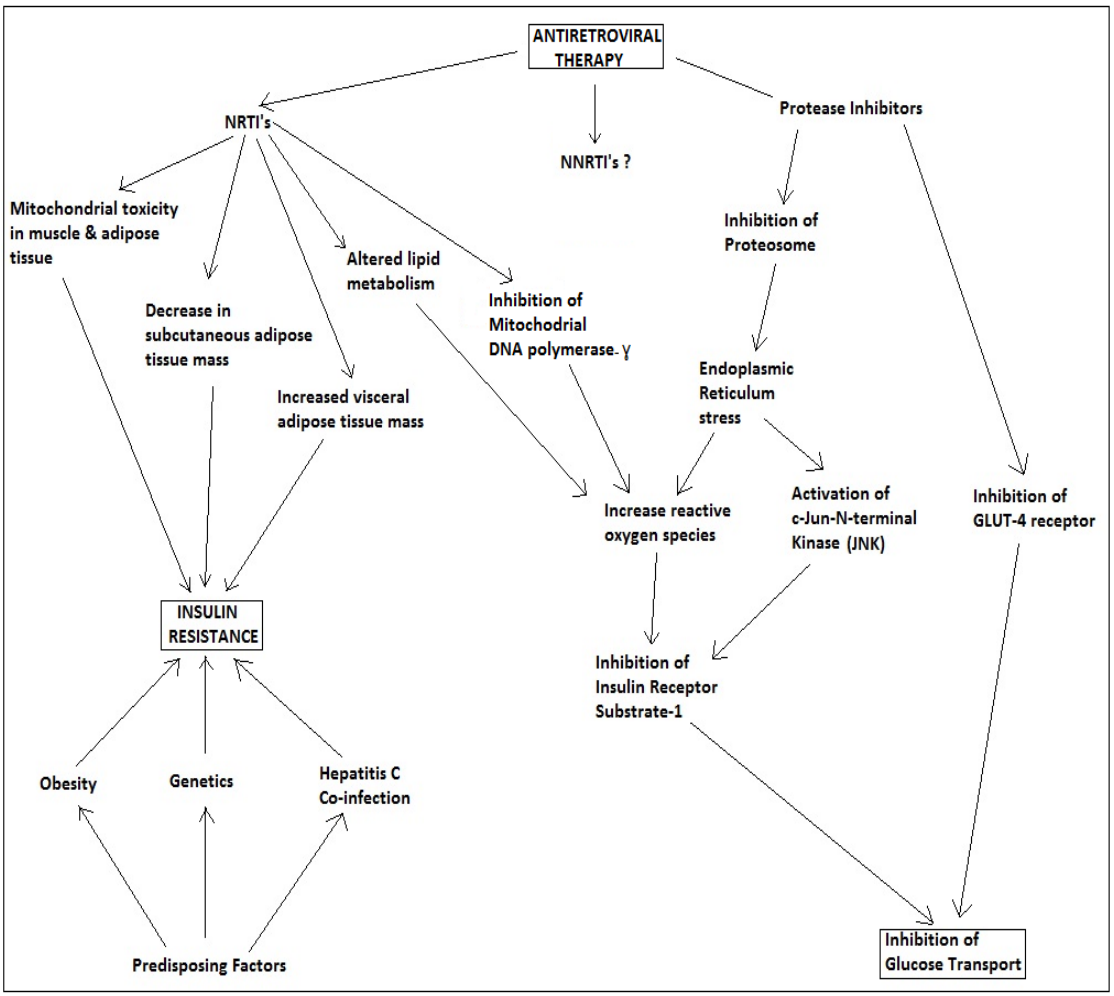

Figure 1. The potential pathways and mechanisms of ART induced insulin resistance and diabetes (ART; Antiretroviral Therapy).[42,44,46]

1.2.13 Separating the Effects (Drugs versus Virus)

Studies have suggested that untreated HIV infection and high viraemia levels are independent risk factors for endothelial damage.[83] The subsequent inflammation is linked to accelerated atherosclerosis and the inherent risk of cardio/cerebrovascular ischaemic events.[83] The viraemia associated inflammatory response may also be related to pancreatic β-cell dysfunction, which upon a background of elevated BMI, traditional risk factors and lipodystrophy may lead to insulin resistance and overt diabetes mellitus.[83] The dilemma in evaluating and analysing treated HIV seropositive patients is the inability to separate the effects of high viral loads, and ART. It is

therefore uncertain whether disorders of glucose metabolism and vascular disease are due to untreated viraemia, ART or a combination of both.[44] What is known is that ART rapidly lowers viral loads with a concomitant rise in CD4 cells. The development of insulin resistance and diabetes may then be more likely due to ART effects, a rapid return to health and increasing BMI upon a background of traditional diabetes risk factors and advancing age.[40]

Furthermore, the effects of ART and the development of immune reconstitution inflammatory syndrome (IRIS) soon after the initiation of therapy are well established.[84] It is plausible that the generalised inflammatory state may contribute to the pathogenesis of insulin resistance and diabetes early on in therapy. The observed metabolic complications observed in HIV treated patients may, however, be due to either toxic effects of long term ART, chronic inflammation associated with HIV infection, lifestyle related factors and advancing age.[15]

Current literature poorly reflects the influence of HIV infection and ART on a population with a background of age-related β-cell dysfunction. It is uncertain whether HIV and ART exacerbate the genetic susceptibility in the general population, making family history a pertinent clinical consideration/factor, or by directly impacting on the pathogenesis of insulin resistance and diabetes.

1.2.14 Diabetic and Vascular Complications in HIV

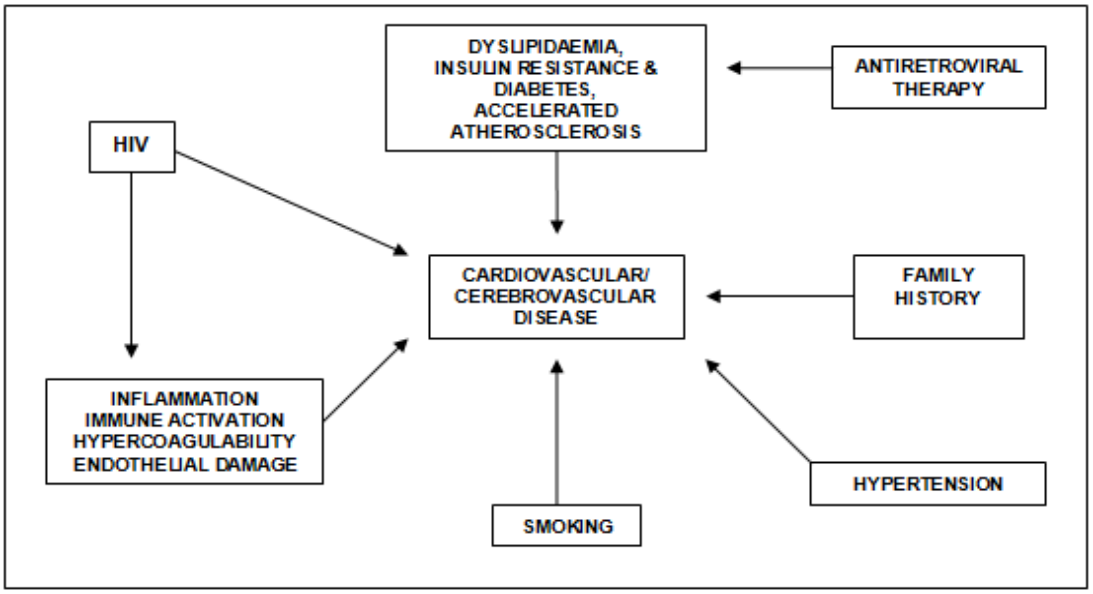

Figure 2. Schematic diagram depicting the possible implications and consequences of long-term ART.[1,2, 121]

1.2.15 Consequences of Diabetes Mellitus

The long-term consequences of diabetes mellitus have implications for multi-organ disease and can be attributed to both micro and macro-vascular complications. Several pathologies become manifest and significant morbidity and mortality is attributed to the development of diabetic retinopathy and nephropathy. In the context of cardiovascular disease, there have been reports of myocardial infarctions and related mortality in the younger HIV seropositive patients on ART.[85] This link is important as diabetes is considered a coronary heart disease equivalent in the general population. It is undetermined whether the development of insulin resistance and subsequent diabetes mellitus is accelerated by concurrent HIV infection. The D:A:D study reported a myocardial infarction incidence of 7.6% in patients with pre-existing diabetes but without pre-existing coronary heart disease, and 31.1% for those with pre-existing coronary heart disease without concurrent diabetes.[86] The suggestion is

that within this cohort, diabetes is not a coronary heart disease equivalent in HIV seropositive patients, despite the fact that diabetes remains a significant risk factor for the development of CHD.

1.2.16 Risk Profiling

The clinical relevance of the emergent cohort data suggesting an association between ART use and diabetes has enabled the development of population specific risk profiles that aid in predicting the short-term risk of diabetes in HIV-positive patients.[87] Several models that predict the risk of diabetes in the HIV seronegative population have been described.[88-91] Commonly included variables include age, sex, weight, glucose, blood pressure, HDL-C, triglyceride and family history of diabetes. Whilst these models have been proven to be adequate predictors of diabetes in the HIV seronegative population, their utility has not been validated in HIV infected patients, and in particular those patients within the SSA region.

The D:A:D study group developed and compared a predictive model to the well-reported Framingham equation.[91] The investigators found that the factors most likely to predict diabetes included elevated blood glucose levels, higher BMI, elevated triglyceride levels and older age.[87] Factors predictive of diabetes within the HIV context included recent CD4 counts <200 cells/μL and lipodystrophy. The authors concluded that the Framingham equation over-estimated the likelihood of diabetic incident events in comparison to the D:A:D equation with reference to the parameters of blood glucose levels, triglycerides and BMI levels below 25kg/m^2.[87]

1.2.17 Non-communicable Disease Patterns in Sub-Saharan Africa

The prevalence of diabetes within SSA is determined by several dynamics; demographic changes, ageing populations, urbanization, and an increasing burden of obesity and physical inactivity.[92]

Hall *et al*, 2011, reported that type II diabetes mellitus accounts for more than 90% of the diabetes burden in SSA.[93] The population prevalence proportions ranged from 1% in rural Uganda to 12% in urban Kenya.[93] The proportion of diabetic patients suffering diabetic complications ranged from 7-63% for retinopathy, 27-66% for neuropathy, and 10-83% for microalbuminuria.[93] The prevalence of diabetes associated coronary heart disease ranges from 4% to 23% for the black and white population, respectively, in a South African study.[94] Diabetes induced immuno-suppression is also likely to increase the risk of mortality from several infections including; tuberculosis, pneumonia and sepsis. The prevalence of obesity and insulin resistance within the SSA region is also influenced by the use of ART. Within the SSA region, the five-year mortality proportions of patients with diabetes ranges from 4 – 57%.[93]

South Africa has a large and growing burden of non-communicable diseases, especially diabetes, obesity, hypertension and cardiovascular disease.[95,96] The World Health Organization (WHO) estimated that in 2004, 28% of the total burden of disease in South Africa was attributable to non-communicable disease.[97] South Africans of lower socioeconomic status residing in urban environments are worst affected and have increased demands for chronic disease care. [98,99]

The relationship between diabetes and cardiovascular disease is inextricable, and in the current South African context, cardiovascular morbidity is still characterised by the persistence of rheumatic heart disease, idiopathic cardiomyopathies, and hypertensive disease.[96] There is, however, an emergence of obesity and diabetes and a likely concomitant increase in ischaemic heart disease (IHD) incidence in South African rural communities.[96,100]

There are several gaps in knowledge regarding diabetes and HIV infected patients in SSA. It is unknown whether there is an increase in new onset diabetes mellitus disease among HIV infected South African patients as compared to the uninfected population with similar characteristics. It is unknown if the incidence of diabetes mellitus increased in HIV infected South African patients on ART. Furthermore, do the NRTIs, NNRTIs and PIs exert a class effect risk of diabetes, or are there specific drugs associated with increased risk of diabetes? There is no data on the characteristics of HIV infected patients in the South African population who are at greatest risk for diabetes. This study aims to address some of these questions.

1.3 Aims and Objectives

1.3.1 Objectives

To investigate the association between ART exposure and the incidence of new onset diabetes mellitus in a cohort Southern African HIV infected adults.

1.3.2 Specific aims

In South African HIV infected adults:

1. To assess the incidence of diabetes mellitus in patients on ART (as per initiation of anti-hypoglycaemic agents and statin therapy), and to determine if there is a duration-dependent increase in risk associated with exposure to ART, or any ARV drug class;
2. To determine the risk factors associated with new onset diabetes mellitus in patients on ART.

Chapter 2: Methodology

2.1 Methods

2.1.1 Study design

Retrospective cohort study.

2.1.2 Study Population and Data Source

The study population comprises South African HIV infected adults enrolled in a private sector HIV disease management program, Aid for Aids (AfA), a company that was contracted to oversee the HIV-related care in members from a number of private medical insurance schemes in Southern Africa. HIV-related care was undertaken by private care physician or general practitioner registered with AfA, and members were accepted into the program if the diagnosis of HIV infection was confirmed, or they had been exposed to HIV, and require post exposure prophylaxis. AfA has been operating since June 1998, and maintained a database with information captured on registration, and on an ongoing basis as part of their founding directive. The AfA program collects demographic, laboratory, and clinical data on individuals who registered for HIV benefits. Claim data were captured by AfA from the medical insurance fund claim database. These include laboratory, hospitalization, pharmacy, and medical practitioner claims which were submitted to the scheme for processing either: at the time of the service by the provider (*e.g.* pharmacy, hospitalization) for direct reimbursement, or after the service date by the member where the member had already paid the claim. Reimbursement was subject to established AfA protocols, including protocols for ART initiation, change of ART regimen, and the treatment of

certain opportunistic infections. No co-payment was required for ART, viral load (VL) and CD4 monitoring, and doctor visits.

Despite being a private sector program, AfA standardized guidelines for HIV management, are similar to the WHO guidelines for LMICs.[101] Patients were eligible for ART initiation if their CD4 cell count was below 350 cells/mm^3, or they had WHO stage 3 or 4 illness irrespective of the CD4 count. The recommended initial regimen was a combination of 2 NRTIs and an NNRTI. VL and CD4 counts were monitored every 6 months.

Data linkage to the South Africa death registry allowed ascertainment of deaths and date of death,.[102,103] Civil identification numbers, Republic of South Africa Identity (RSAID) number, were cross-checked in the national death registry to confirm or ascertain dates of death for those with registered deaths. All data were de-identified prior to analysis. Linkage to the death registry was essential in performing a competing risk analysis.

2.1.3 Data collation

Data was pooled from separate medical aid schemes (Medscheme, Bonitas and GEMS) and collated using Microsoft SQL Server® 2008 **(Fig. 3, pg. 41).** Patients were identified by RSAID numbers and then assigned an internal cohort identifier. Patients were matched across schemes *via* use of a protocol that detailed their each unique RSAID numbers, name, surname, date of birth, and sex. Patients with duplicate entries were excluded. The data extract was then exported to the statistical program STATA® version 13. The raw data was then inspected for invalid characters, outliers and inspected with frequency plots.

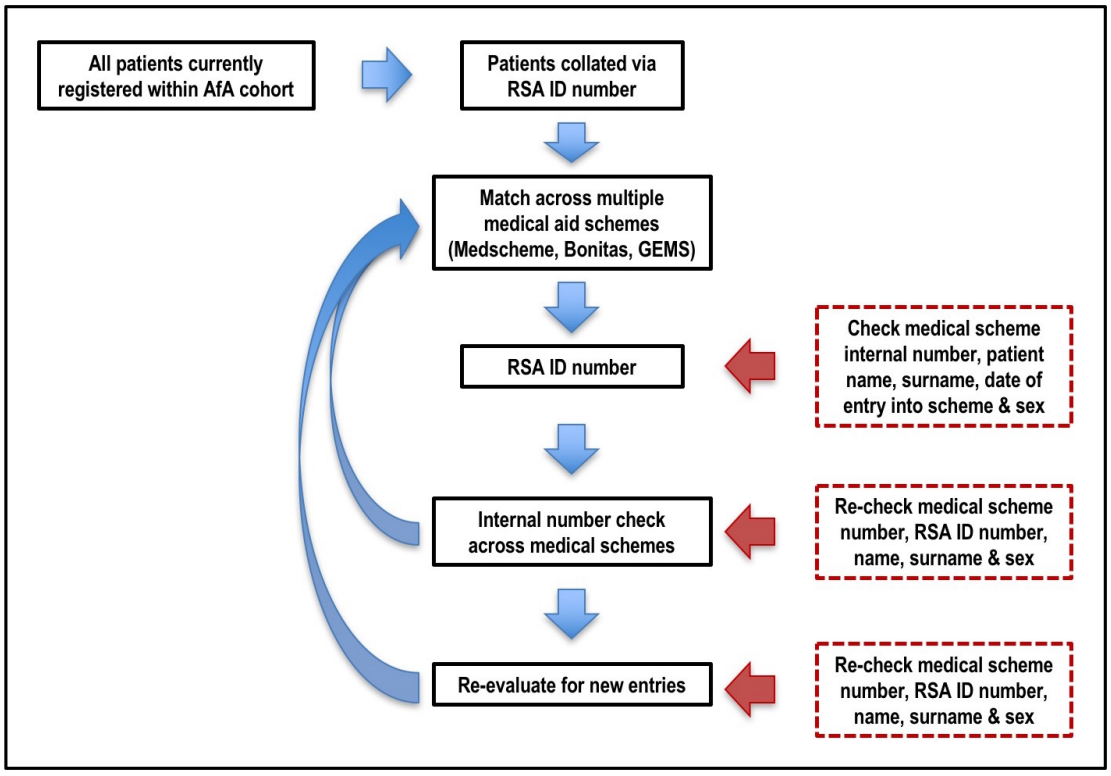

Figure 3. Algorithm for patient collation across medical aid schemes and entry into study cohort

2.1.4 Data Cleaning and Preparation

All variables to be included in the uni- and multivariate analysis were identified and cleaned as appropriate. All categorical variables were tabulated to identify missing values as determined by the size of the final cohort. Continuous variables were plotted on histograms to analyse the distribution of values and outliers were identified using box and whisker plots and the STATA Version 13 (StataCorp LP, College Station, TX) "Hilo" function. Any data entries identified as outliers (*e.g.* age = "o", Height >2.3 m, Weight >200 kg), were excluded from the final cohort at this stage. Variables with missing data were identified and earmarked for imputation. Baseline CD4 count, VL, weight and height were collated. These variables were defined as values captured within one year (365 days) of starting ART. Baseline BMI was calculated from individual weight and height.

2.1.5 Cohort description

The cohort, for the purpose of this study, is defined as all patients registered with AfA from the period 01/01/2002 to 31/12/2011. The patient must have initiated ART with AfA and been a member of a medical aid scheme in this time period **(Fig. 4, below).**

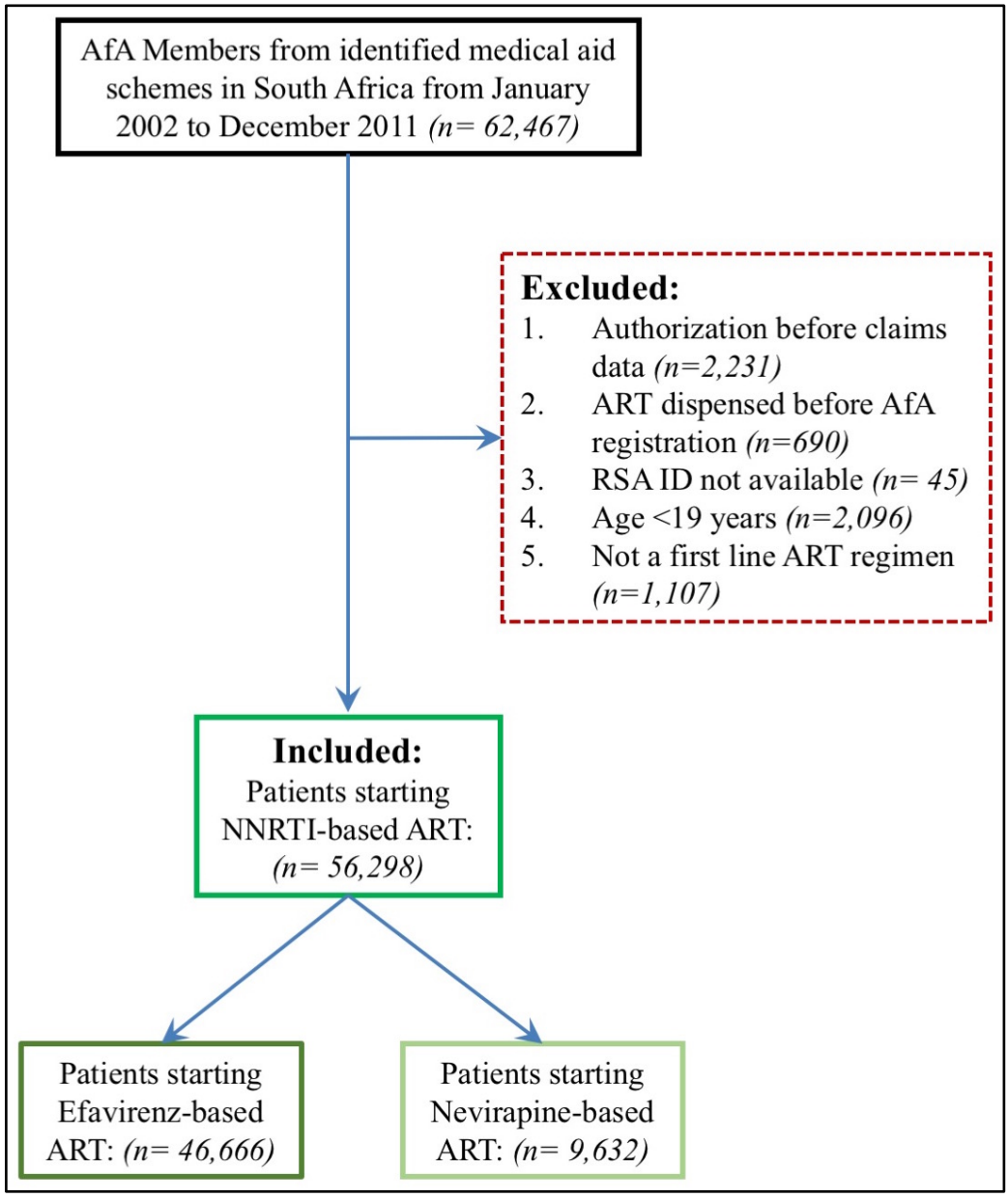

Figure 4. Participant selection and exclusion criteria.

2.1.6 Variables and Definitions

The following variables were extracted from AfA database; sex, date of birth, weight, height, Republic of South Africa Identity Number, and date of joining the AfA program.

Longitudinal results for CD4 count and VL, and all medication claims for antiretrovirals, and concomitant medicines were extracted. A list of diabetogenic drugs was created using a pharmacology reference textbook[104] and a review[105] **(Refer to Table 1, pg. 44).** Patients were categorized as exposed to diabetogenic drugs if they submitted claims for a diabetogenic drug on two or more occasions.

The ART start date was defined as the date on which antiretroviral drugs were first dispensed in the AfA program. The ART starting regimen was the regimen dispensed on this date. The baseline CD4 count, VL, and weight were the values measured closest to the date of ART initiation, within the 12-month window before the ART start date. The primary exposure variable of interest was the NNRTI component of the first-line antiretroviral regimen.

Table 1. Table of Diabetogenic Drug Classes. [104,105]

1. Antidepressants Fluvoxamine, Venlafaxine, Amitriptyline, Paroxetine
2. Atypical antipsychotics Clozapine, Olanzapine, Quetiapine, Risperidone
3. Phenothiazines Chlorpromazine, Trifluoperazine, Promethazine
4. Glucocorticoids and Immunosuppressants Glucocorticoids, Ciclosporin/Tacrolimus
5. Antihypertensives Thiazide diuretics, β-blockers, Ca^{2+} - Channel blockers
6. Antiretrovirals ***6.1 Protease Inhibitors*** Ritonavir, Lopinavir/Ritonavir, Amprenavir, Nelfinavir, Indinavir/Saquinavir, Atazanavir ***6.2 Nucleoside Reverse Transcriptase Inhibitors*** Stavudine, Didanosine
7. Oral contraceptives
8. Anti-epileptics Sodium Valproate, Phenytoin
9. HMG Co-A Reductase Inhibitors

2.1.7 Inclusion and Exclusion Criteria

For this study, all AfA-registered patients were included if they initiated a first-line NNRTI-containing ART regimen from January 2002 to December 2011, and were 19 years or older when starting ART. Patients excluded from the study were those who

were already on antidiabetic medication before starting ART, and if they did not have a recorded South Africa identification number (as the identification number was used to determine if death occurred by linkage with the South African death registry). **(Refer to Fig. 4, pg. 42).**

2.1.8 Endpoint

Incident diabetes was defined, using claims data, as the date on which any of the antidiabetic agents available in South Africa (insulins, metformin, sulfonylureas, alpha glucosidase inhibitors, thiazolidinediones, dipeptidyl peptidase-IV inhibitors, glucagon-like-peptide-1 receptor agonists and meglitinides) were initiated. Patients who started and stopped antidiabetic medication in the months around a pregnancy (from 6 months before delivery until 1 month after delivery) were assumed to have diabetes in pregnancy, and were therefore not included as incident diabetes.

2.1.9 Imputation of Missing Data

Some data were missing for baseline CD4 count, baseline VL, baseline weight and height **(Refer to Table 2, pg. 49 in Results section)**. Five datasets were imputed with the multiple imputation by chained equations (MICE) module in STATA version 13. The imputation model included the following variables: sex, baseline weight, height, age, CD4 count, and VL, death, incident diabetes, and exposure time. CD4 count and VL were actively imputed, and body mass index (BMI) was passively imputed (using actively imputed baseline weight and height). The results of the imputation model were checked by comparing the imputed data with the actual data.[106,107]

2.1.10 Analysis

The data for statistical analysis were collated and prepared using a relational database (Microsoft SQL Server 2008). The statistical analyses were performed using STATA Version 13 (StataCorp LP, College Station, TX) and R version 3.2.1 (R Development Core Team). The incidence of diabetes in patients receiving efavirenz-containing regimens versus nevirapine-containing regimens was compared with a Kaplan–Meier plot and a log-rank test.

A multivariate Cox-proportional hazards model was conducted to explore the association of efavirenz exposure with the hazard of developing diabetes. The following variables were adjusted for in the regression model: age, sex, baseline BMI, baseline CD4 count, baseline VL, exposure to diabetogenic drugs. For the primary analysis, ART was included in the model as time-updated NRTI backbone (AZT-containing, d4T-containing, or other NRTI combination), and time-updated NNRTI (efavirenz or nevirapine); and patients were censored when they died, left the medical insurance scheme, switched to PI-based ART, or reached the end of the study period. A secondary analysis was conducted to explore incident diabetes within the first ART regimen only. For this analysis, two additional reasons for censoring were included: NRTI or NNRTI substitution. The effect of including calendar year was explored in the multivariate model. The following sensitivity analyses were performed: (i) controlling for the competing risk of death, and (ii) a model that excluded patients who were virologically-suppressed at baseline.

The proportional hazards assumption was verified by testing interaction effects of analysis time with baseline variables (α=0.05), and graphically in each imputed dataset via log–log plots, amongst others. Model selection was performed using the Akaike Information Criterion (AIC) after multiple imputation.[106,108-110]

2.1.11 Ethics

The study protocol was reviewed and approved by the University of Cape Town Faculty of Health Sciences Human Research Ethics committee (HREC reference number: 282/2011). A copy of the research ethics approval and renewal documents are included in Appendix 2.

Chapter 3: Results

Between January 2002 and June 2011, 62,467 patients commenced ART in the AfA program, of whom 56,298 patients met the inclusion and exclusion criteria **(Fig. 4, pg. 42)**, and were included in the analysis. The demographic and clinical characteristics of patients are given in **Table 2, pg. 49**. Results from the multiple imputations for missing covariates are shown in **Table 3, pg. 50**. Median follow-up was 1.56 years (interquartile range (IQR): 0.71– 2.79 years); 21.7% of patients were followed up for 3 or more years.

New onset diabetes was identified in 1,500 (2.66%) patients over 113,297 patient-years of follow-up (PYFU), giving a crude incidence of 13.24 cases per 1000 PYFU **(Table 2, pg. 49)**. There were 17 pregnancy-associated diabetic events, which were not included as cases of incident diabetes. Exposure to diabetogenic medicines occurred in 19,137 (41.01%) of patients taking efavirenz and 3,643 (37.82%) of patients taking nevirapine. The Kaplan Meier analysis of incident diabetes in efavirenz versus nevirapine-containing regimens **(Fig. 5, pg. 52)** demonstrates a higher percentage of new onset diabetes in patients receiving efavirenz-containing ART; log rank test p value <0.001. The results of the Cox proportional hazard regression analyses, including univariate and multivariate analyses and model selection, are shown in **Table 4, pg. 51**. Efavirenz-containing ART was associated with a higher risk of developing new-onset diabetes than nevirapine-containing ART, adjusted hazard ratio (HR) 1.27 (95% confidence interval (CI): 1.10–1.46). Zidovudine and stavudine-containing NRTI backbones, older age at baseline, elevated baseline BMI, and exposure to diabetogenic medication were also associated with an increased risk of developing diabetes **(Table 4, pg. 51)**.

Table 2. Cohort Description.

Variable	Category	Whole cohort	Efavirenz-containing ART	Nevirapine-containing ART
Number of patients		56,298	46,666	9,632
Age (years)	Median (IQR)	38.14 (33.15 to 44.26)	39.1 (34.1 to 45.17)	34.05 (29.99 to 38.65)
Sex	Male	20,224 (35.92)	18,822 (40.33%)	1,402 (14.55%)
	Female	36,074 (64.08)	27,844 (59.67%)	8,230 (85.44%)
Race	Asian	148 (0.26%)	126 (0.27%)	22 (0.23%)
	Black	53,270 (94.62%)	44,179 (94.67%)	9,091 (94.38%)
	Mixed	768 (1.36%)	628 (1.35%)	140 (1.45%)
	White	989 (1.76%)	825 (1.77%)	164 (1.7%)
	Not Reported	1,123 (1.99%)	908 (1.95%)	215 (2.23%)
Nucleoside Reverse Transcriptase Inhibitor (NNRTI) (initial regimen containing)	Zidovudine	26,917 (47.81%)	25,329 (54.28%)	1,588 (16.49%)
	Stavudine	22,465 (39.9%)	16,002 (34.29%)	6,463 (67.1%)
	Other	6,916 (12.28%)	5,335 (11.43%)	1,581 (16.41%)
Exposure to other Diabetogenic Drugs		22,780 (40.46%)	19,137 (41.01%)	3,643 (37.82%)
Baseline Height (m)	Median (IQR)	165 (160 to 170)	165 (160 to 170)	163 (158 to 168)
	Missing	19,033 (33.81%)	15,390 (32.98%)	3,643 (37.82%)
Baseline Weight (kg)	Median (IQR)	68 (60 to 79)	68 (60 to 79)	70 (62 to 81)
	Missing	15,042 (26.72%)	12,349 (26.46%)	2,693 (27.96%)
Baseline Body Mass Index (kg/m^2)	Median (IQR)	25.06 (21.97 to 29.00)	24.82 (21.80 to 28.69)	26.52 (23.14 to 30.49)
	Missing	23,178 (41.17%)	18,776 (40.23%)	4,402 (45.7%)
Baseline CD4 Count (cells/µl)	Median (IQR)	181 (88 to 280)	176 (81 to 277)	201 (121 to 306)
	Missing	948 (1.68%)	769 (1.65%)	179 (1.86%)
Baseline Log Viral Load (copies/ml)	Median (IQR)	4.78 (3.66 to 5.37)	4.87 (3.86 to 5.43)	4.35 (2.59 to 5.03)
	Missing	3,464 (6.15%)	2,845 (6.1%)	619 (6.43%)
Follow up time (years)	Median (IQR)	1.56 (0.71 to 2.79)	1.50 (0.67 to 2.69)	1.90 (0.93 to 3.34)
Patient years		113,297	89,915	23,382
3 or more years of NNRTI exposure		21.70%	29.86%	20.66%
Reason for censoring	Diabetes	1,500 (2.66%)	1,257 (2.69%)	243 (2.52%)
	Switch to PI	3,706 (6.58%)	2,778 (5.95%)	928 (9.63%)
	Study end	40,785 (72.44%)	34,186 (73.26%)	6,599 (68.51%)
	Death	1774 (3.15%)	1488 (3.19%)	286 (2.97%)
	Left scheme	8533 (15.16%)	6957 (14.91%)	1576 (16.36%)
Crude incidence	Events/1000 patient years	13.24	13.98	10.39

ART = antiretroviral therapy, IQR = interquartile range, NNRTI = non-nucleoside reverse transcriptase inhibitor, PI = protease inhibitor.

Table 3: Results of imputation for baseline variables with missing data.

Variable		Total cohort (n = 56,298)	Efavirenz-containing ART (n = 46,666)	Nevirapine-containing ART (n = 9,632)
		Mean (95% CI)	Mean (95% CI)	Mean (95% CI)
Height (m)	Before imputation	164.93 (164.84 to 165.03)	165.25 (165.18 to 165.31)	163.14 (162.92 to 163.35)
	After imputation	164.74 (164.64 to 164.85)	165.14 (165.05 to 165.23)	163.07 (162.88 to 163.26)
Weight (kg)	Before imputation	70.34 (70.18 to 70.51)	69.88 (69.72 to 70.03)	72.81 (72.39 to 73.23)
	After imputation	70.14 (69.97 to 70.30)	69.80 (69.62 to 69.97)	71.59 (71.18 to 72.00)
CD4 Count (cells/µl)	Before imputation	215.82 (214.27 to 217.38)	208.77 (207.64 to 209.90)	254.44 (251.66 to 257.22)
	After imputation	215.39 (214.41 to 216.38)	207.18 (206.12 to 208.25)	250.26 (247.83 to 252.69)
Viral Load (copies/ml)	Before imputation	4.31 (4.30 to 4.33)	4.39 (4.38 to 4.40)	3.88 (3.86 to 3.90)
	After imputation	4.32 (4.31 to 4.33)	4.38 (4.37 to 4.39)	3.92 (3.90 to 3.94)
Body Mass Index (kg/m^2)	Before imputation	25.95 (25.89 to 26.01)	25.67 (25.62 to 25.72)	27.44 (27.31 to 27.56)
	After imputation	25.93 (25.87 to 25.99)	25.68 (25.61 to 25.75)	27.00 (26.86 to 27.14)

ART = antiretroviral therapy, CI = confidence interval

Table 4. Univariate, Multivariate, and Model selection results for the Cox regression model of associations with incident diabetes. Drug switches within first line regimen included in the model, with censoring at switch to second line therapy. All results are based on multiple imputation.

Variable	Category	Univariate		Multivariate		Model Selection (AIC)
		HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)
Non-Nucleoside Reverse Transcriptase Inhibitor	Efavirenz	1.40 (1.22 - 1.60)	<0.001	1.27 (1.10 - 1.47)	0.001	1.27 (1.10 - 1.46)
	Nevirapine	Referent		Referent		Referent
Nucleoside Reverse Transcriptase Inhibitor	Zidovudine	1.30 (1.15 - 1.46)	<0.001	1.35 (1.19 - 1.52)	<0.001	1.37 (1.21 - 1.54)
	Stavudine	1.53 (1.32 - 1.78)	<0.001	1.60 (1.38 - 1.87)	<0.001	1.64 (1.41 - 1.91)
	Other	Referent		Referent		Referent
Exposure to other Diabetogenic Drugs		1.68 (1.51 - 1.86)	<0.001	1.53 (1.37 - 1.70)	<0.001	1.53 (1.38 - 1.71)
Baseline Age (years)	19–24	0.35 (0.20 - 0.60)	<0.001	0.47 (0.27 - 0.81)	0.007	0.46 (0.27 - 0.80)
	25–34	0.64 (0.56 - 0.73)	<0.001	0.71 (0.62 - 0.82)	<0.001	0.71 (0.62 - 0.81)
	35–44	Referent		Referent		Referent
	45–54	1.50 (1.33 - 1.70)	<0.001	1.38 (1.21 - 1.56)	<0.001	1.36 (1.20 - 1.54)
	≥55	1.86 (1.50 - 2.31)	<0.001	1.64 (1.32 - 2.04)	<0.001	1.57 (1.26 - 1.95)
Sex	male	1.41 (1.27 - 1.56)	<0.001	1.47 (1.32 - 1.64)	<0.001	1.44 (1.29 - 1.61)
	female	Referent		Referent		Referent
Baseline Body Mass Index (BMI) Quartile (kg/m²)	10–17	0.38 (0.23 - 0.63)	0.001	0.33 (0.19 - 0.56)	0.001	0.32 (0.19 - 0.55)
	18–24	0.65 (0.58 - 0.74)	<0.001	0.61 (0.53 - 0.69)	<0.001	0.60 (0.53 - 0.69)
	25–34	Referent		Referent		Referent
	35+	1.45 (1.16 - 1.81)	0.002	1.58 (1.26 - 1.97)	<0.001	1.58 (1.27 - 1.97)
Baseline CD4 Count (cells/µl)	0–200	1.04 (0.92 - 1.17)	0.534	1.08 (0.95 - 1.23)	0.220	
	200–349	Referent		Referent		Excluded by AIC
	350+	1.25 (1.06 - 1.47)	0.007	1.10 (0.91 - 1.34)	0.324	
Baseline Viral Load (copies/ml)	0–999	1.31 (1.14 - 1.51)	<0.001	1.24 (1.05 - 1.47)	0.011	1.28 (1.11 - 1.47)
	1,000–99,999	Referent		Referent		Referent
	100,000–999,999	1.07 (0.95 - 1.21)	0.261	1.03 (0.91 - 1.16)	0.674	1.03 (0.91 - 1.17)
	≥1,000,000	1.15 (0.89 - 1.48)	0.285	1.15 (0.89 - 1.49)	0.278	1.14 (0.89 - 1.48)

Drug switches within first-line regimen included in the model, with censoring at switch to second line therapy. All results are based on multiple imputations.
AIC = Akaike Information Criterion, CI = confidence interval, HR = hazard ratio.

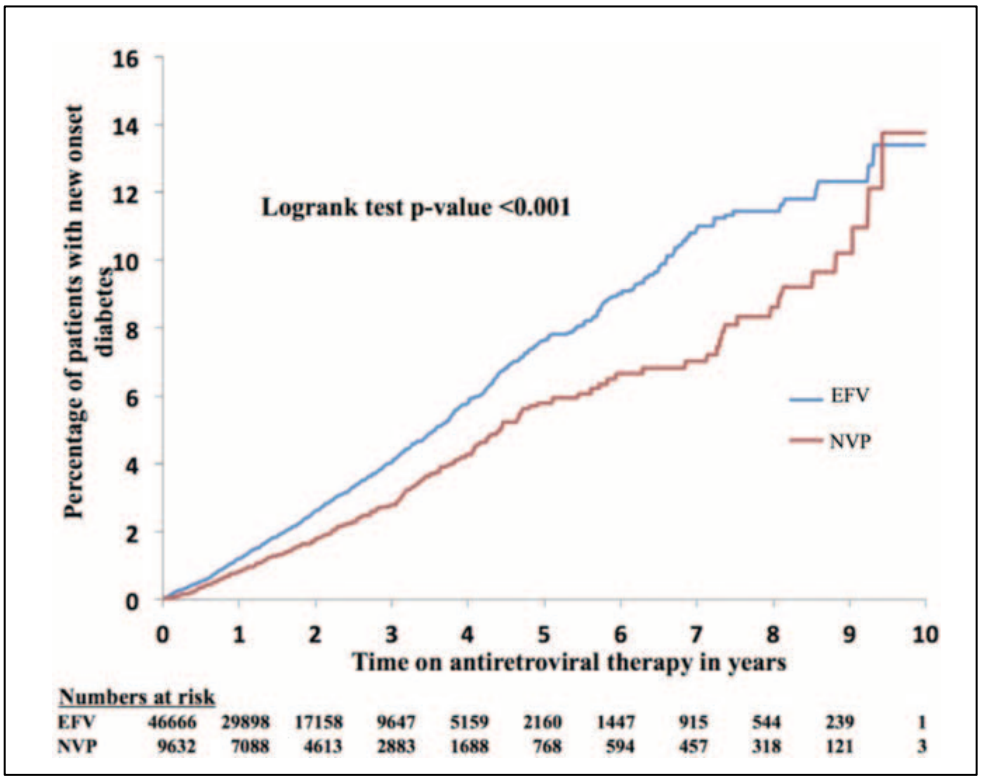

Figure 5. Kaplan Meier analysis of incident diabetes.

No association was found between baseline CD4 and an increased risk of diabetes. There was an association between the lowest stratum of baseline VL and an increased relative risk for developing diabetes, but no association with higher VL strata.

The results of the Cox proportional hazard regression analyses where patients were censored at the time of first drug switch are shown in **Table 5, pg. 53**; reasons for censoring for this analysis are shown in **Table 6, pg. 54**. The estimated HRs of the variables remained similar after model averaging; confirming the stability of the findings.[111] These findings did not differ from the regression model with updated regimen (censoring at the point of change to second line regimens). Adding calendar year to the model did not change the associations observed **(Table 7, pg. 55)**. Findings did not change when accounting for competing risk **(Table 8, pg. 56)**. Excluding patients with suppressed baseline VLs attenuated the effect of efavirenz on incident diabetes somewhat: adjusted HR 1.16; 95% CI: 0.99–1.36. **(Table 9, pg. 57)**.

Table 5. Univariate, Multivariate, and Model selection results for the Cox regression model of associations with incident diabetes (censored at first ever drug switch). All results are based on multiple imputation.

Variable	Category	Univariate		Multivariate (Rubins Rule)		Model Selection (AIC)
		HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)
Non-Nucleoside Reverse Transcriptase Inhibitor	Efavirenz	1.38 (1.18 - 1.60)	<0.001	1.26 (1.07 - 1.49)	0.005	1.33 (1.13 - 1.56)
	Nevirapine	Referent		Referent		Referent
Nucleoside Reverse Transcriptase Inhibitor	Zidovudine	1.34 (1.17 - 1.54)	<0.001	1.39 (1.21 - 1.60)	<0.001	1.57 (1.37 - 1.80)
	Stavudine	1.62 (1.37 - 1.90)	<0.001	1.67 (1.41 - 1.98)	<0.001	1.96 (1.66 - 2.32)
	Other	Referent		Referent		Referent
Exposure to other Diabetogenic Drugs		1.68 (1.50 - 1.88)	<0.001	1.52 (1.36 - 1.71)	<0.001	1.50 (1.34 - 1.69)
Baseline Age (years)	19–24	0.39 (0.22 - 0.69)	0.001	0.53 (0.30 - 0.94)	0.031	0.52 (0.29 - 0.93)
	25–34	0.63 (0.55 - 0.73)	<0.001	0.71 (0.61 - 0.82)	<0.001	0.70 (0.60 - 0.81)
	35–44	Referent		Referent		Referent
	45–54	1.49 (1.30 - 1.70)	<0.001	1.37 (1.20 - 1.57)	<0.001	1.36 (1.19 - 1.56)
	≥55	1.89 (1.50 - 2.37)	<0.001	1.69 (1.34 - 2.13)	<0.001	1.61 (1.28 - 2.03)
Sex	male	1.40 (1.25 - 1.56)	<0.001	1.48 (1.31 - 1.67)	<0.001	1.47 (1.30 - 1.66)
	female	Referent		Referent		Referent
Baseline Body Mass Index (kg/m²)	10–17	0.40 (0.23 - 0.71)	0.004	0.35 (0.19 - 0.63)	0.002	0.35 (0.19 - 0.62)
	18–24	0.65 (0.53 - 0.81)	0.001	0.61 (0.48 - 0.76)	0.001	0.61 (0.48 - 0.76)
	25–34	Referent		Referent		Referent
	35+	1.44 (1.14 - 1.82)	0.004	1.57 (1.24 - 1.98)	<0.001	1.58 (1.25 - 1.99)
Baseline CD4 Count (cells/µl)	0–200	1.06 (0.93 - 1.21)	0.415	1.11 (0.97 - 1.27)	0.136	
	200–349	Referent		Referent		Excluded by AIC
	350+	1.32 (1.11 - 1.57)	0.001	1.11 (0.91 - 1.36)	0.307	
Baseline Viral Load (copies/ml)	0–999	1.35 (1.17 - 1.57)	<0.001	1.27 (1.06 - 1.53)	0.010	1.27 (1.09 - 1.48)
	1,000–99,999	Referent		Referent		Referent
	100,000–999,999	1.03 (0.90 - 1.18)	0.653	0.99 (0.86 - 1.13)	0.841	1.00 (0.87 - 1.14)
	≥1,000,000	1.15 (0.88 - 1.50)	0.311	1.15 (0.88 - 1.50)	0.320	1.15 (0.88 - 1.50)

AIC = Akaike Information Criterion, CI = confidence interval, HR = hazard ratio

Table 6. Follow up time and censoring reasons when censoring participants at the time of first drug switch.

Variable	Category	Whole cohort	Efavirenz-containing ART	Nevirapine-containing ART
Number of patients		56,298	46,666	9,632
Follow up time (years)	Median (IQR)	1.32 (0.56 - 2.43)	1.30 (0.56 - 2.36)	1.48 (0.62 - 2.78)
Patient years		97,667	78,693	18,974
3 or more years of follow-up		17.05%	21.86%	16.05%
Reason for censoring	Diabetes	1272 (2.26%)	1078 (2.31%)	194 (2.01%)
	Drug switch within 1st line	8712 (15.47%)	6410 (13.74%)	2302 (23.9%)
	Switch to PI	2891 (5.14%)	2209 (4.73%)	682 (7.08%)
	Study end	34457 (61.2%)	29494 (63.2%)	4963 (51.53%)
	Death	1551 (2.75%)	1323 (2.84%)	228 (2.37%)
	Left scheme	7415 (13.17%)	6152 (13.18%)	1263 (13.11%)
Crude diabetes incidence	Events/1000 patient years	13.02	13.70	10.22

IQR = Interquartile Range, PI = Protease Inhibitor

Table 7. Multivariate Cox regression model of associations with incident diabetes including calendar year. Drug switches within first line regimen included in the model, with censoring at switch to second line therapy. All results are based on multiple imputation.

Variable	Category	HR (95% CI)
Non-Nucleoside Reverse Transcriptase Inhibitor	Efavirenz	1.28 (1.10 - 1.48)
	Nevirapine	Referent
Nucleoside Reverse Transcriptase Inhibitor	Zidovudine	1.32 (1.16 - 1.50)
	Stavudine	1.56 (1.32 - 1.84)
	Other	Referent
Exposure to other Diabetogenic Drugs		1.51 (1.36 - 1.69)
Baseline Age (years)	19–24	0.46 (0.27 - 0.80)
	25–34	0.71 (0.62 - 0.81)
	35–44	Referent
	45–54	1.38 (1.21 - 1.56)
	≥55	1.67 (1.34 - 2.08)
Sex	male	1.48 (1.32 - 1.66)
	female	Referent
Baseline Body Mass Index (kg/m²)	10–17	0.31 (0.20 - 0.49)
	18–24	0.60 (0.51 - 0.70)
	25–34	Referent
	35+	1.61 (1.30 - 1.98)
Baseline CD4 Count (cells/µl)	0–200	1.08 (0.93 - 1.24)
	200–349	Referent
	350+	1.12 (0.93 - 1.36)
Baseline Viral Load (copies/ml)	0–999	1.23 (1.03 - 1.46)
	1,000–99,999	Referent
	100,000–999,999	1.03 (0.91 - 1.17)
	≥1,000,000	1.18 (0.91 - 1.54)
Calendar year	2002	Referent
	2003	0.97 (0.71 - 1.33)
	2004	1.06 (0.77 - 1.46)
	2005	0.83 (0.59 - 1.16)
	2006	0.89 (0.64 - 1.24)
	2007	0.87 (0.66 - 1.14)
	2008	1.03 (0.78 - 1.36)
	2009	1.00 (0.75 - 1.33)
	2010	0.82 (0.61 - 1.10)
	2011	0.65 (0.44 - 0.96)
CI = Confidence Interval, HR = Hazard Ratio		

Table 8. Cox proportionate multivariate model censored at first ever drug switch, including competing risk. Results are based on multiple imputation.

Variable	Category	Multivariate HR (95% CI)
Non-Nucleoside Reverse Transcriptase Inhibitor	Efavirenz	1.26 (1.07 - 1.49)
	Nevirapine	Referent
Nucleoside Reverse Transcriptase Inhibitor	Zidovudine	1.39 (1.21 - 1.60)
	Stavudine	1.67 (1.41 - 1.97)
	Other	Referent
Exposure to other Diabetogenic Drugs		1.54 (1.37 - 1.73)
Baseline Age (years)	19–24	0.52 (0.29 - 0.93)
	25–34	0.71 (0.61 - 0.82)
	35–44	Referent
	45–54	1.36 (1.19 - 1.56)
	≥55	1.66 (1.31 - 2.10)
Sex	male	1.47 (1.31 - 1.66)
	female	Referent
Baseline Body Mass Index (kg/m^2)	10–17	0.34 (0.19 - 0.61)
	18–24	0.60 (0.48 - 0.76)
	25–34	Referent
	35+	1.57 (1.24 - 1.98)
Baseline CD4 Count (cells/µl)	0–200	1.11 (0.96 - 1.27)
	200–349	Referent
	350+	1.11 (0.91 - 1.35)
Baseline Viral Load (copies/ml)	0–999	1.28 (1.07 - 1.53)
	1000–99,999	Referent
	100,000–999,999	0.98 (0.86 - 1.13)
	≥1,000,000	1.14 (0.87 - 1.50)
CI = Confidence Interval, HR = Hazard Ratio		

Table 9. Univariate, Multivariate, and Model selection results for the Cox regression model of association with incident diabetes, excluding virally suppressed patients (VL <400 copies/ml). Drug switches within first line regimen included in the model, with censoring at switch to second line therapy. All results are based on multiple imputation.

Variable	Category	Univariate HR (95% CI)	Multivariate HR (95% CI)	Model Selection (AIC) HR (95% CI)
Non-Nucleoside Reverse Transcriptase Inhibitor	Efavirenz	1.30 (1.12 - 1.52)	1.15 (0.98 - 1.35)	1.16 (0.99 - 1.36)
	Nevirapine	Referent	Referent	Referent
Nucleoside Reverse Transcriptase Inhibitor	Zidovudine	1.34 (1.17 - 1.53)	1.40 (1.22 - 1.61)	1.42 (1.23 - 1.63)
	Stavudine	1.52 (1.28 - 1.80)	1.59 (1.33 - 1.89)	1.62 (1.36 - 1.93)
	Other	Referent	Referent	Referent
Exposure to other Diabetogenic Drugs		1.81 (1.60 - 2.04)	1.61 (1.42 - 1.82)	1.62 (1.43 - 1.83)
Baseline Age (years)	19–24	0.33 (0.18 - 0.60)	0.42 (0.23 - 0.77)	0.42 (0.23 - 0.77)
	25–34	0.65 (0.56 - 0.75)	0.71 (0.62 - 0.83)	0.71 (0.61 - 0.83)
	35–44	Referent	Referent	Referent
	45–54	1.47 (1.27 - 1.69)	1.32 (1.15 - 1.53)	1.31 (1.13 - 1.51)
	≥55	1.61 (1.24 - 2.08)	1.42 (1.09 - 1.84)	1.34 (1.04 - 1.74)
Sex	male	1.42 (1.27 - 1.59)	1.48 (1.30 - 1.69)	1.46 (1.28 - 1.66)
	female	Referent	Referent	Referent
Baseline Body Mass Index (BMI) Quartile (kg/m²)	10–17	0.40 (0.24 - 0.67)	0.33 (0.19 - 0.56)	0.33 (0.19 - 0.55)
	18–24	0.69 (0.57 - 0.85)	0.62 (0.50 - 0.78)	0.61 (0.50 - 0.75)
	25–34	Referent	Referent	Referent
	35+	1.48 (1.17 - 1.88)	1.64 (1.30 - 2.06)	1.63 (1.30 - 2.04)
Baseline CD4 Count (cells/µl)	0–200	1.05 (0.92 - 1.20)	1.06 (0.92 - 1.22)	
	200–349	Referent	Referent	Excluded by AIC
	350+	0.86 (0.62 - 1.18)	0.90 (0.65 - 1.24)	
Baseline Viral Load (copies/ml)	0–999	0.73 (0.41 - 1.33)	0.74 (0.40 - 1.34)	0.76 (0.36 - 1.64)
	1,000–99,999	Referent	Referent	Referent
	100,000–999,999	1.10 (0.97 - 1.24)	1.07 (0.94 - 1.22)	1.05 (0.90 - 1.24)
	≥1,000,000	1.16 (0.89 - 1.50)	1.21 (0.93 - 1.58)	1.14 (0.78 - 1.67)

AIC = Akaike Information Criterion, CI = confidence interval, HR = hazard ratio

Chapter 4: Discussion

In this retrospective analysis of 56,298 patients with a median follow up of 1.56 years, the crude incidence of diabetes was 13.24 cases per 1000 PYFU, which is at the upper end of the range reported from cohort studies in high income countries (4.2–14.1 per 1000 PYFU).[2,16,35,87]. Consistent with prior literature, the NRTIs stavudine and zidovudine were associated with an increased incidence of diabetes. In addition, use of efavirenz was associated with a significantly higher incidence of diabetes than nevirapine in this large cohort of South African patients. To the best of my knowledge, this is the first cohort study that shows an increased risk of diabetes from efavirenz use in first-line ART.

These findings have important implications for LMICs, which are facing a burgeoning diabetes epidemic, as efavirenz is the preferred NNRTI in first-line ART, zidovudine is recommended in second line ART, and many people are still taking stavudine, even though it is no longer recommended by the WHO.[101]

HIV-infected patients have an estimated 4-fold greater relative risk of developing diabetes than the HIV-uninfected population.[35] Factors contributing to the increased risk of diabetes in people with HIV include, insulin resistance due to the chronic inflammatory response to HIV infection,[112] which persists despite effective ART[112,113] and the effects of certain antiretroviral drugs. The finding that the NRTIs stavudine and zidovudine were both associated with an increased incidence of diabetes has previously been reported.[2,19] NRTIs inhibit the enzyme DNA polymerase-γ, responsible for mitochondrial replication. The dysregulation of mitochondrial function in different compartments of the body results in various clinical manifestations of NRTI toxicity, including insulin resistance and diabetes.[28,114,115] In a

prior cross sectional study, Dave *et al*, 2011, found an increased risk of dysglyacemia in South African patients taking efavirenz compared with those taking nevirapine, but there were insufficient numbers of cases of diabetes for analysis.[4] A small case–control study from Botswana suggested an association between efavirenz use and diabetes.[57] Randomized controlled trials showed significantly higher serum glucose concentrations in participants in the efavirenz arms than the following comparator antiretroviral drugs: nevirapine,[58] abacavir,[58] atazanavir,[59] atazanavir–ritonavir,[60] and raltegravir.[61]

The mechanism by which efavirenz mediates insulin resistance and diabetes is unknown. Possible mechanisms include mitochondrial toxicity[62] and toxic effects on adipocytes and increased rates of lipoatrophy.[63,64] Efavirenz causes hepatic mitochondrial toxicity[62] and induces hepatocyte endoplasmic reticulum stress leading to activation of the unfolded protein response, and apoptosis.[116,117] Efavirenz mediates mitochondrial toxicity via various mechanisms. Firstly, efavirenz directly inhibits Complex I of the electron transport chain, resulting in a markedly reduced mitochondrial transmembrane potential, thus compromising oxidative phosphorylation and ATP generation.[65-67] Secondly, efavirenz reduces complex IV (COIV) mRNA (a marker gene of mitochondrial function), and impairs mitochondrial function in adipocytes.[68] Furthermore, efavirenz associated mitochondrial dysregulation in adipose tissue causes impaired adipogenesis, increased lipolysis, and release of free fatty acids and inflammatory cytokines.[68] The increased release of fatty acids due to adipocyte mitochondrial toxicity are thought to impair muscle and liver insulin sensitivity, leading to insulin resistance and diabetes mellitus.[45,69-74] In addition to its mitochondrial toxicity, efavirenz has been shown to reduce the secretion of adiponectin (an insulin-sensitizing, antidiabetic adipokine) by

adipocytes.[68] It is therefore hypothesized that impairment of mitochondrial bioenergetics and toxicity to adipocytes contributes to the development of diabetes in patients on efavirenz. By contrast, nevirapine does not appear to exert mitochondrial toxicity.[63,68]

A prior cross sectional study demonstrated a positive correlation between plasma efavirenz concentrations and both fasting and 2-hour glucose concentrations after oral glucose tolerance tests in South African patients.[118] People of African origin are more likely to be genotypic ‘‘slow metabolizers’’ of efavirenz, which results in elevated efavirenz plasma concentrations, than people of European descent (20% and 3%, respectively).[76] Therefore, efavirenz may have a larger diabetogenic effect in Africans, which may explain why, in contrast with the findings of this analysis, studies from high-income countries have not found an association between efavirenz and diabetes.

In this analysis, increasing age, and male sex were associated with an increased risk of diabetes, which is consistent with findings of other studies.[2,15,31,40,112,119-121] There was no demonstrable association between baseline CD4 count and diabetes, which is similar to that reported by a French cohort,[16] but other studies have found an increased risk of diabetes with lower CD4 counts.[112,119]

The relationship between CD4 count and incident diabetes remains unresolved. Some studies suggest that activated CD4 cells are integral in non-insulin mediated glucose disposal.[122] The association of dysglycaemia and low CD4 count may therefore be explained by impaired non-insulin mediated glucose disposal attributable to a reduced reserve of CD4 cells and elevated levels of systemic inflammation.[122,123] Other studies suggest that autoimmune diabetes occurs in patients post ART initiation where it has been demonstrated that antibodies to glutamic acid decarboxylase develop, coinciding

with rapid increases in CD4 count, inducing a form of type 1 diabetes.[124,125] Alternatively, there may be defective glucose disposal at high CD4 counts due to post-ART recovery of CD4 cells, that are functionally unable to utilize glucose efficiently because of low GLUT-1 or interleukin 7 receptor expression.[122,126] The relationship between CD4 count and dysglycaemia may also be complicated by ethnic influences on varying responses to immune reconstitution and the subsequent risk of developing diabetes.[122] The associations between CD4 and incident diabetes are tenuous are require further exploration.

In this study, there was an association between the lowest stratum of baseline VL and an increased relative risk for developing diabetes, but no association with higher VL strata. The French cohort reported no association between VL and diabetes.[16] In contrast, other studies have found an association between high VLs and diabetes.[15,112] The association observed between low baseline VLs and increased risk of diabetes may be attributed to the inclusion of patients already on undisclosed ART on entry to the AfA program.

This study has several limitations. A minority of patients in South Africa (approximately 18 percent) have access to medical aid schemes, especially amongst the HIV-infected population. Patients within these medical aid schemes may represent a population with higher socioeconomic status and characteristics not generalizable to patients treated in the public sector. Patients receiving care from funded schemes may have had closer/ less close follow up with different rates of loss-to –follow up than their public sector counter parts. Adherence may differ from public sector patients. However, it is important to note that patients in the medical schemes were treated in accordance WHO guidelines for resource limited settings, with regimens and

monitoring that is similar to patients in the public sector. Only baseline data was used for this analysis, as no follow up data was available. It was therefore not possible to adjust for weight changes during follow-up. This is a significant limitation as trends in weight gain post-ART initiation are important factors in the development of insulin resistance and diabetes mellitus. Similarly, there was no follow up CD4 and VL data, which potentially serve as adherence markers. As such, there was no reliable indicator of adherence in this study. Furthermore, there was a significant amount of missing baseline data, notably of weight and height, 26.7% and 33.8% respectively. However, missing data was imputed, which is known to be superior to using complete case analysis.[127,128] Notably, ART exposure may have occurred before commencing ART within the AfA program and there were 18.8% of patients with a suppressed VL at baseline, which is likely due to undisclosed ART use. In a sub-analysis, excluding virally suppressed patients at baseline (VL <400copies/ml) diminishes the effect of efavirenz on incident diabetes; adjusted HR 1.16; 95% CI: 0.99 -1.36 **(Table 9, pg. 57)**. The inclusion of virally suppressed patients in the analysis reflects a critical limitation of the cohort and that the relationship between efavirenz and incident diabetes is rather modest and elucidated due to the large sample size in this study. The determination of incident diabetes was based on initiation of diabetes therapy, as the results of plasma glucose or glycated haemoglobin are not captured in the database. It is therefore possible that cases of diabetes that were only treated with lifestyle modifications were missed. As patients were not routinely screened for diabetes at the time of ART initiation, some patients may have entered the cohort with undiagnosed diabetes. A further limitation, in common with other studies, was the lack of an internal control (HIV-uninfected population) prohibiting the estimation of the relative risk of diabetes. A further limitation is that a propensity score was not

utilized to adjust for changes in drug regimen prescribing trends that occurred in accordance with health policy changes over the study period.

Future studies with longer follow-up utilizing a control group, time-updated BMI, CD4 and VL, will allow better characterization of risk factors associated with diabetes in the ART treated population. Studies with internal controls may better investigate the effect of ART and disorders of glucose metabolism by controlling for the effect of age, a major risk factor for diabetes. Further studies are required to investigate the effect of PI based therapy (currently reserved as second line agents) and the incidence of diabetes in the SSA population.

A strength of this study is that the use of diabetogenic medication was adjusted for in the multivariate model, and was associated with a 53% increase in the relative risk of diabetes.

In conclusion, exposure to efavirenz, stavudine, and zidovudine was associated with an increased incidence of diabetes. Although the increased risk of diabetes with these antiretrovirals was relatively modest, the large African patient population exposed to ART for prolonged periods means that the findings of this study has important public health implications. While screening for diabetes should be increased in people on long-term ART, consideration should be given to using antiretrovirals with less risk of metabolic complications. Further studies to confirm the association of efavirenz and risk of diabetes should be conducted in LMICs, and the molecular mechanisms of efavirenz-induced dysglycaemia need further investigation.

Chapter 5: Conclusions

In this retrospective analysis of a cohort, with a median 1.56-year period of follow-up, there appears to be a significant risk for the development of diabetes whilst on ART. This risk is greater than the general population. There appears to be added risk of developing diabetes whilst on efavirenz-containing ART. The main factors contributing to the development of diabetes appears to be advancing age and increased time exposure to ART. The above has pertinent implications for the continued management of HIV infected patients, as efavirenz is the preferred NNRTI component due to its lower potential for hepatotoxicity, drug interaction profile, cost and patient compliance. Further investigation and expansion of the regression models evaluating the efavirenz-diabetes association are however warranted.

References

1. Friis-Moller N, Thiebaut R, Reiss P, et al. Predicting the risk of cardiovascular disease in HIV-infected patients: the data collection on adverse effects of anti-HIV drugs study. *European journal of cardiovascular prevention and rehabilitation : official journal of the European Society of Cardiology, Working Groups on Epidemiology & Prevention and Cardiac Rehabilitation and Exercise Physiology.* 2010;17(5):491-501.
2. De Wit S, Sabin CA, Weber R, et al. Incidence and risk factors for new-onset diabetes in HIV-infected patients: the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study. *Diabetes care.* 2008;31(6):1224-1229.
3. World Health Organization WHO. Scaling Up Antiretroviral Therapy in Resource-Limited Settings: Treatment Guidelines for a Public Health Approach. 2004.
4. Dave JA, Lambert EV, Badri M, West S, Maartens G, Levitt NS. Effect of nonnucleoside reverse transcriptase inhibitor-based antiretroviral therapy on dysglycemia and insulin sensitivity in South African HIV-infected patients. *Journal of acquired immune deficiency syndromes.* 2011;57(4):284-289.
5. Diabetes Atlas fecNuc-c. *International Diabetes Federation.* 2011;5th edition.
6. Mbanya JC, Motala AA, Sobngwi E, Assah FK, Enoru ST. Diabetes in sub-Saharan Africa. *Lancet.* 2010;375(9733):2254-2266.
7. Association AD. Diagnosis and classification of diabetes mellitus. . *Diabetes care.* 2012;35:S64-71.
8. Jamison DT, Shahid-Salles SA, Jamison J, Lawn JE, Zupan J. Incorporating Deaths Near the Time of Birth into Estimates of the Global Burden of Disease. In: Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJL, eds. *Global Burden of Disease and Risk Factors*. Washington (DC)2006.
9. Maia Leite LH, De Mattos Marinho Sampaio AB. Progression to overweight, obesity and associated factors after antiretroviral therapy initiation among Brazilian persons with HIV/AIDS. *Nutricion hospitalaria.* 2010;25(4):635-640.
10. Hossain P, Kawar B, El Nahas M. Obesity and diabetes in the developing world--a growing challenge. *The New England journal of medicine.* 2007;356(3):213-215.
11. Bray GA. Risks of obesity. *Endocrinology and metabolism clinics of North America.* 2003;32(4):787-804, viii.
12. Levitt NS, Steyn K, Dave J, Bradshaw D. Chronic noncommunicable diseases and HIV-AIDS on a collision course: relevance for health care delivery, particularly in low-resource settings--insights from South Africa. *The American journal of clinical nutrition.* 2011;94(6):1690S-1696S.
13. Harries AD, Lin Y, Satyanarayana S, et al. The looming epidemic of diabetes-associated tuberculosis: learning lessons from HIV-associated tuberculosis. *The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease.* 2011;15(11):1436-1444, i.
14. Evans SR, Ellis RJ, Chen H, et al. Peripheral neuropathy in HIV: prevalence and risk factors. *Aids.* 2011;25(7):919-928.
15. Ledergerber B, Furrer H, Rickenbach M, et al. Factors associated with the incidence of type 2 diabetes mellitus in HIV-infected participants in the Swiss

HIV Cohort Study. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2007;45(1):111-119.
16. Capeau J, Bouteloup V, Katlama C, et al. Ten-year diabetes incidence in 1046 HIV-infected patients started on a combination antiretroviral treatment. *Aids.* 2012;26(3):303-314.
17. Barkan SE, Melnick SL, Preston-Martin S, et al. The Women's Interagency HIV Study. WIHS Collaborative Study Group. *Epidemiology.* 1998;9(2):117-125.
18. Justman JE, Benning L, Danoff A, et al. Protease inhibitor use and the incidence of diabetes mellitus in a large cohort of HIV-infected women. *Journal of acquired immune deficiency syndromes.* 2003;32(3):298-302.
19. Brown TT, Cole SR, Li X, et al. Antiretroviral therapy and the prevalence and incidence of diabetes mellitus in the multicenter AIDS cohort study. *Archives of internal medicine.* 2005;165(10):1179-1184.
20. Samaras K, Wand H, Law M, Emery S, Cooper D, Carr A. Prevalence of metabolic syndrome in HIV-infected patients receiving highly active antiretroviral therapy using International Diabetes Foundation and Adult Treatment Panel III criteria: associations with insulin resistance, disturbed body fat compartmentalization, elevated C-reactive protein, and [corrected] hypoadiponectinemia. *Diabetes care.* 2007;30(1):113-119.
21. Dube MP, Johnson DL, Currier JS, Leedom JM. Protease inhibitor-associated hyperglycaemia. *Lancet.* 1997;350(9079):713-714.
22. Wand H, Calmy A, Carey DL, et al. Metabolic syndrome, cardiovascular disease and type 2 diabetes mellitus after initiation of antiretroviral therapy in HIV infection. *Aids.* 2007;21(18):2445-2453.
23. Milner KL, van der Poorten D, Trenell M, et al. Chronic hepatitis C is associated with peripheral rather than hepatic insulin resistance. *Gastroenterology.* 2010;138(3):932-941 e931-933.
24. Mehta SH, Moore RD, Thomas DL, Chaisson RE, Sulkowski MS. The effect of HAART and HCV infection on the development of hyperglycemia among HIV-infected persons. *Journal of acquired immune deficiency syndromes.* 2003;33(5):577-584.
25. Grunfeld C, Kotler DP, Hamadeh R, Tierney A, Wang J, Pierson RN. Hypertriglyceridemia in the acquired immunodeficiency syndrome. *The American journal of medicine.* 1989;86(1):27-31.
26. Grunfeld C, Kotler DP, Shigenaga JK, et al. Circulating interferon-alpha levels and hypertriglyceridemia in the acquired immunodeficiency syndrome. *The American journal of medicine.* 1991;90(2):154-162.
27. Murata H, Hruz PW, Mueckler M. Indinavir inhibits the glucose transporter isoform Glut4 at physiologic concentrations. *Aids.* 2002;16(6):859-863.
28. Brinkman K, Kakuda TN. Mitochondrial toxicity of nucleoside analogue reverse transcriptase inhibitors: a looming obstacle for long-term antiretroviral therapy? *Current opinion in infectious diseases.* 2000;13(1):5-11.
29. Carr A, Samaras K, Burton S, et al. A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. *Aids.* 1998;12(7):F51-58.
30. Carr A, Samaras K, Thorisdottir A, Kaufmann GR, Chisholm DJ, Cooper DA. Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor-associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study. *Lancet.* 1999;353(9170):2093-2099.

31. El-Sadr WM, Mullin CM, Carr A, et al. Effects of HIV disease on lipid, glucose and insulin levels: results from a large antiretroviral-naive cohort. *HIV medicine.* 2005;6(2):114-121.
32. Kilby JM, Tabereaux PB. Severe hyperglycemia in an HIV clinic: preexisting versus drug-associated diabetes mellitus. *Journal of acquired immune deficiency syndromes and human retrovirology : official publication of the International Retrovirology Association.* 1998;17(1):46-50.
33. Visnegarwala F, Krause KL, Musher DM. Severe diabetes associated with protease inhibitor therapy. *Annals of internal medicine.* 1997;127(10):947.
34. Eastone JA, Decker CF. New-onset diabetes mellitus associated with use of protease inhibitor. *Annals of internal medicine.* 1997;127(10):948.
35. Brown TT, Li X, Cole SR, et al. Cumulative exposure to nucleoside analogue reverse transcriptase inhibitors is associated with insulin resistance markers in the Multicenter AIDS Cohort Study. *Aids.* 2005;19(13):1375-1383.
36. Estrada V, Martinez-Larrad MT, Gonzalez-Sanchez JL, et al. Lipodystrophy and metabolic syndrome in HIV-infected patients treated with antiretroviral therapy. *Metabolism: clinical and experimental.* 2006;55(7):940-945.
37. Hadigan C, Meigs JB, Corcoran C, et al. Metabolic abnormalities and cardiovascular disease risk factors in adults with human immunodeficiency virus infection and lipodystrophy. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2001;32(1):130-139.
38. Calza L, Masetti G, Piergentili B, et al. Prevalence of diabetes mellitus, hyperinsulinaemia and metabolic syndrome among 755 adult patients with HIV-1 infection. *International journal of STD & AIDS.* 2011;22(1):43-45.
39. Polsky S, Floris-Moore M, Schoenbaum EE, Klein RS, Arnsten JH, Howard AA. Incident hyperglycaemia among older adults with or at-risk for HIV infection. *Antiviral therapy.* 2011;16(2):181-188.
40. Tien PC, Schneider MF, Cole SR, et al. Antiretroviral therapy exposure and incidence of diabetes mellitus in the Women's Interagency HIV Study. *Aids.* 2007;21(13):1739-1745.
41. Mutimura E, Stewart A, Rheeder P, Crowther NJ. Metabolic function and the prevalence of lipodystrophy in a population of HIV-infected African subjects receiving highly active antiretroviral therapy. *Journal of acquired immune deficiency syndromes.* 2007;46(4):451-455.
42. Manuthu EM, Joshi MD, Lule GN, Karari E. Prevalence of dyslipidemia and dysglycaemia in HIV infected patients. *East African medical journal.* 2008;85(1):10-17.
43. Zannou DM, Denoeud L, Lacombe K, et al. Incidence of lipodystrophy and metabolic disorders in patients starting non-nucleoside reverse transcriptase inhibitors in Benin. *Antiviral therapy.* 2009;14(3):371-380.
44. Paik IJ, Kotler DP. The prevalence and pathogenesis of diabetes mellitus in treated HIV-infection. *Best practice & research. Clinical endocrinology & metabolism.* 2011;25(3):469-478.
45. Hruz PW. Molecular Mechanisms for Altered Glucose Homeostasis in HIV Infection. *American journal of infectious diseases.* 2006;2(3):187-192.
46. Murata H, Hruz PW, Mueckler M. The mechanism of insulin resistance caused by HIV protease inhibitor therapy. *The Journal of biological chemistry.* 2000;275(27):20251-20254.

47. Shahmanesh M, Das S, Stolinski M. Insulin resistance and apolipoprotein B kinetics: a comparison of protease inhibitor-, Efavirenz-, or Nevirapine-containing antiretrviral regimens. 11th Conference on retroviruses and Opportunistic Infections. 8-11 february 2004, San Francisco. Abstract 704. . 2004.
48. Brinkman K, Smeitink JA, Romijn JA, Reiss P. Mitochondrial toxicity induced by nucleoside-analogue reverse-transcriptase inhibitors is a key factor in the pathogenesis of antiretroviral-therapy-related lipodystrophy. *Lancet.* 1999;354(9184):1112-1115.
49. Muthumani K, Choo AY, Hwang DS, et al. Mechanism of HIV-1 viral protein R-induced apoptosis. *Biochemical and biophysical research communications.* 2003;304(3):583-592.
50. Arnoult D, Petit F, Lelievre JD, Estaquier J. Mitochondria in HIV-1-induced apoptosis. *Biochemical and biophysical research communications.* 2003;304(3):561-574.
51. Caron M, Auclair M, Lagathu C, et al. The HIV-1 nucleoside reverse transcriptase inhibitors stavudine and zidovudine alter adipocyte functions in vitro. *Aids.* 2004;18(16):2127-2136.
52. Rudich A, Vanounou S, Riesenberg K, et al. The HIV protease inhibitor nelfinavir induces insulin resistance and increases basal lipolysis in 3T3-L1 adipocytes. *Diabetes.* 2001;50(6):1425-1431.
53. Behrens G, Dejam A, Schmidt H, et al. Impaired glucose tolerance, beta cell function and lipid metabolism in HIV patients under treatment with protease inhibitors. *Aids.* 1999;13(10):F63-70.
54. Noor MA, Parker RA, O'Mara E, et al. The effects of HIV protease inhibitors atazanavir and lopinavir/ritonavir on insulin sensitivity in HIV-seronegative healthy adults. *Aids.* 2004;18(16):2137-2144.
55. Dube MP, Qian D, Edmondson-Melancon H, et al. Prospective, intensive study of metabolic changes associated with 48 weeks of amprenavir-based antiretroviral therapy. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2002;35(4):475-481.
56. Noor MA, Lo JC, Mulligan K, et al. Metabolic effects of indinavir in healthy HIV-seronegative men. *Aids.* 2001;15(7):F11-18.
57. Moyo D, Tanthuma G, Mushisha O, et al. Diabetes mellitus in HIV-infected patients receiving antiretroviral therapy. *South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde.* 2013;104(1):37-39.
58. Martinez E, Arnaiz JA, Podzamczer D, et al. Substitution of nevirapine, efavirenz, or abacavir for protease inhibitors in patients with human immunodeficiency virus infection. *The New England journal of medicine.* 2003;349(11):1036-1046.
59. Jemsek JG, Arathoon E, Arlotti M, et al. Body fat and other metabolic effects of atazanavir and efavirenz, each administered in combination with zidovudine plus lamivudine, in antiretroviral-naive HIV-infected patients. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2006;42(2):273-280.
60. Erlandson KM, Kitch D, Tierney C, et al. Impact of randomized antiretroviral therapy initiation on glucose metabolism. *AIDS.* 2014;28(10):1451-1461.
61. Lennox JL, Dejesus E, Berger DS, et al. Raltegravir versus Efavirenz regimens in treatment-naive HIV-1-infected patients: 96-week efficacy,

durability, subgroup, safety, and metabolic analyses. *Journal of acquired immune deficiency syndromes.* 2010;55(1):39-48.
62. Blas-Garcia A, Apostolova N, Ballesteros D, et al. Inhibition of mitochondrial function by efavirenz increases lipid content in hepatic cells. *Hepatology.* 2010;52(1):115-125.
63. El Hadri K, Glorian M, Monsempes C, et al. In vitro suppression of the lipogenic pathway by the nonnucleoside reverse transcriptase inhibitor efavirenz in 3T3 and human preadipocytes or adipocytes. *The Journal of biological chemistry.* 2004;279(15):15130-15141.
64. Perez-Molina JA, Domingo P, Martinez E, Moreno S. The role of efavirenz compared with protease inhibitors in the body fat changes associated with highly active antiretroviral therapy. *The Journal of antimicrobial chemotherapy.* 2008;62(2):234-245.
65. Apostolova N, Gomez-Sucerquia LJ, Moran A, Alvarez A, Blas-Garcia A, Esplugues JV. Enhanced oxidative stress and increased mitochondrial mass during efavirenz-induced apoptosis in human hepatic cells. *British journal of pharmacology.* 2010;160(8):2069-2084.
66. Karamchand L, Dawood H, Chuturgoon AA. Lymphocyte mitochondrial depolarization and apoptosis in HIV-1-infected HAART patients. *Journal of acquired immune deficiency syndromes.* 2008;48(4):381-388.
67. Pilon AA, Lum JJ, Sanchez-Dardon J, Phenix BN, Douglas R, Badley AD. Induction of apoptosis by a nonnucleoside human immunodeficiency virus type 1 reverse transcriptase inhibitor. *Antimicrobial agents and chemotherapy.* 2002;46(8):2687-2691.
68. Gallego-Escuredo JM, Del Mar Gutierrez M, Diaz-Delfin J, et al. Differential effects of efavirenz and lopinavir/ritonavir on human adipocyte differentiation, gene expression and release of adipokines and pro-inflammatory cytokines. *Current HIV research.* 2010;8(7):545-553.
69. Arner P. Insulin resistance in type 2 diabetes: role of fatty acids. *Diabetes/metabolism research and reviews.* 2002;18 Suppl 2:S5-9.
70. Hadigan C, Borgonha S, Rabe J, Young V, Grinspoon S. Increased rates of lipolysis among human immunodeficiency virus-infected men receiving highly active antiretroviral therapy. *Metabolism: clinical and experimental.* 2002;51(9):1143-1147.
71. Johnson JA, Albu JB, Engelson ES, et al. Increased systemic and adipose tissue cytokines in patients with HIV-associated lipodystrophy. *American journal of physiology. Endocrinology and metabolism.* 2004;286(2):E261-271.
72. Kim JA, Wei Y, Sowers JR. Role of mitochondrial dysfunction in insulin resistance. *Circulation research.* 2008;102(4):401-414.
73. Krebs M, Roden M. Molecular mechanisms of lipid-induced insulin resistance in muscle, liver and vasculature. *Diabetes, obesity & metabolism.* 2005;7(6):621-632.
74. Sivitz WI, Yorek MA. Mitochondrial dysfunction in diabetes: from molecular mechanisms to functional significance and therapeutic opportunities. *Antioxidants & redox signaling.* 2010;12(4):537-577.
75. Sinxadi P, McIlleron H, Dave JA, Smith P, Levitt N, Maartens G. Hign Efavirenz concentrations are associated with increases in plasma lipids and glucose. *Basic & Clin Pharmacol Toxicol.* 2014(115).

76. Haas DW, Ribaudo HJ, Kim RB, et al. Pharmacogenetics of efavirenz and central nervous system side effects: an Adult AIDS Clinical Trials Group study. *AIDS.* 2004;18(18):2391-2400.
77. Limone P, Biglino A, Valle M, et al. Insulin resistance in HIV-infected patients: relationship with pro-inflammatory cytokines released by peripheral leukocytes. *The Journal of infection.* 2003;47(1):52-58.
78. Shrivastav S, Kino T, Chrousos G. HIV-1 Vpr binds and inhibits PPAR-gamma: implications for HIV associated insulin resistance and lipodystrophy. . 2000.
79. Kino T, Gragerov A, Pavlakis G, Chrousos G. HIV-1 accessory protein Vpr antagonizes insulin's effect on the cytosolic translocation of FKHR and promotes its transcriptional activity: potential contribution of Vpr to insulin resistance of AIDS patients. Program of the 83rd Annual Meeting of the Endocrine Society, Denver, CO, 2001, p 95 (Abstract OR20-4). . 2001.
80. Hotamisligil GS, Murray DL, Choy LN, Spiegelman BM. Tumor necrosis factor alpha inhibits signaling from the insulin receptor. *Proceedings of the National Academy of Sciences of the United States of America.* 1994;91(11):4854-4858.
81. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science.* 1996;271(5249):665-668.
82. Brown TT, Tassiopoulos K, Bosch RJ, Shikuma C, McComsey GA. Association between systemic inflammation and incident diabetes in HIV-infected patients after initiation of antiretroviral therapy. *Diabetes care.* 2010;33(10):2244-2249.
83. Gutierrez AD, Balasubramanyam A. Dysregulation of glucose metabolism in HIV patients: epidemiology, mechanisms, and management. *Endocrine.* 2012;41(1):1-10.
84. Murdoch DM, Venter WD, Van Rie A, Feldman C. Immune reconstitution inflammatory syndrome (IRIS): review of common infectious manifestations and treatment options. *AIDS research and therapy.* 2007;4:9.
85. Henry K, Melroe H, Huebsch J, et al. Severe premature coronary artery disease with protease inhibitors. *Lancet.* 1998;351(9112):1328.
86. Worm SW, De Wit S, Weber R, et al. Diabetes mellitus, preexisting coronary heart disease, and the risk of subsequent coronary heart disease events in patients infected with human immunodeficiency virus: the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D Study). *Circulation.* 2009;119(6):805-811.
87. Petoumenos K, Worm SW, Fontas E, et al. Predicting the short-term risk of diabetes in HIV-positive patients: the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study. *Journal of the International AIDS Society.* 2012;15(2):17426.
88. Stern MP, Williams K, Haffner SM. Identification of persons at high risk for type 2 diabetes mellitus: do we need the oral glucose tolerance test? *Annals of internal medicine.* 2002;136(8):575-581.
89. Wilson PW, Meigs JB, Sullivan L, Fox CS, Nathan DM, D'Agostino RB, Sr. Prediction of incident diabetes mellitus in middle-aged adults: the Framingham Offspring Study. *Archives of internal medicine.* 2007;167(10):1068-1074.

90. Griffin SJ, Little PS, Hales CN, Kinmonth AL, Wareham NJ. Diabetes risk score: towards earlier detection of type 2 diabetes in general practice. *Diabetes/metabolism research and reviews.* 2000;16(3):164-171.
91. D'Agostino RB, Sr., Grundy S, Sullivan LM, Wilson P, Group CHDRP. Validation of the Framingham coronary heart disease prediction scores: results of a multiple ethnic groups investigation. *Jama.* 2001;286(2):180-187.
92. Peer N, Steyn K, Lombard C, Lambert EV, Vythilingum B, Levitt NS. Rising diabetes prevalence among urban-dwelling black South Africans. *PloS one.* 2012;7(9):e43336.
93. Hall V, Thomsen R. Diabetes in Sub Saharan Africa 1999-2011: epidemiology and public health implications. A systematic review. BMC Public Health 11:564. 2011.
94. Kalk WJ, Joffe BI. The metabolic syndrome, insulin resistance, and its surrogates in African and white subjects with type 2 diabetes in South Africa. *Metabolic syndrome and related disorders.* 2008;6(4):247-255.
95. Abegunde DO, Mathers CD, Adam T, Ortegon M, Strong K. The burden and costs of chronic diseases in low-income and middle-income countries. *Lancet.* 2007;370(9603):1929-1938.
96. Mayosi BM, Flisher AJ, Lalloo UG, Sitas F, Tollman SM, Bradshaw D. The burden of non-communicable diseases in South Africa. *Lancet.* 2009;374(9693):934-947.
97. World Health Organisation (WHO). The Global Burden of Disease. 2008.
98. Tollman SM, Kahn K, Sartorius B, Collinson MA, Clark SJ, Garenne ML. Implications of mortality transition for primary health care in rural South Africa: a population-based surveillance study. *Lancet.* 2008;372(9642):893-901.
99. Groenewald P, Bradshaw D, Daniels J. Cause of death and premature mortality in Cape Town, 2001-2006. Cape Town: South African Medical Research Council, 2008. . 2008.
100. Sliwa K, Wilkinson D, Hansen C, et al. Spectrum of heart disease and risk factors in a black urban population in South Africa (the Heart of Soweto Study): a cohort study. *Lancet.* 2008;371(9616):915-922.
101. *Consolidated Guidelines on The Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Recommendations for a Public Health Approach.* Geneva, Switzerland: World Health Organization (WHO);2013. ISBN 978 92 4 150572 7.
102. Boulle A, Van Cutsem G, Hilderbrand K, et al. Seven-year experience of a primary care antiretroviral treatment programme in Khayelitsha, South Africa. *AIDS.* 2010;24(4):563-572.
103. Schomaker M, Gsponer T, Estill J, Fox M, Boulle A. Non-ignorable loss to follow-up: correcting mortality estimates based on additional outcome ascertainment. *Stat Med.* 2014;33(1):129 -142.
104. Davis S. Diabetogenic drugs: Treating chronic conditions to minimise new onset diabetes. *SAPJ.* 2010:22-27.
105. *Goodman & Gilman's The Pharmacological Basis of Therapeutics.* 11th ed. New York.: McGraw-Hill; 2005.
106. Schomaker M, Heuman C. Model selection and model averaging after multiple imputation. *Computational Statistics & Data Analysis.* 2014;71:758 - 770.

107. Honaker J, King G, Blackwell M. Amelia II: A Program for Missing Data. *J Stat Softw.* 2011;45(7):1-47.
108. Bozdogan H. Model selection and Akaike's Information Criterion (AIC): The general theory and its analytical extensions. *Psychometrika.* 1987;52(3):345-370.
109. Schomaker M, Wan ATK, Heumann C. Frequentist model averaging with missing observations. *Computational Statistics & Data Analysis.* 2010;54. :3336 - 3347.
110. Volinsky CT, Madigan D, Raftery AE, Kronmal RA. Bayesian model averaging in proportional hazard models. Assessing the risk of a stroke. *Applied Statistics- Journal of the Royal Statistical Society Series.* 1997;46:433 - 448.
111. Burnham K, Anderson D. *Model selection and multimodel inference. A practical information-theoretic approach.* Springer, New York; 2002.
112. Brown TT, Patil SP, Jacobson LP, et al. Association between systemic inflammation and obstructive sleep apnea in men with or at risk for HIV infection from the Multicenter AIDS Cohort Study. *Antiviral therapy.* 2009(14):(Suppl. 2): A19.
113. Brigham EP, Patil SP, Jacobson LP, et al. Association between systemic inflammation and obstructive sleep apnea in men with or at risk for HIV infection. *Antiviral therapy.* 2014;19(8):725-733.
114. Blumer RM, van Vonderen MG, Sutinen J, et al. Zidovudine/lamivudine contributes to insulin resistance within 3 months of starting combination antiretroviral therapy. *AIDS.* 2008;22(2):227-236.
115. Kakuda TN. Pharmacology of nucleoside and nucleotide reverse transcriptase inhibitor-induced mitochondrial toxicity. *Clinical therapeutics.* 2000;22(6):685-708.
116. Apostolova N, Gomez-Sucerquia LJ, Alegre F, et al. ER stress in human hepatic cells treated with Efavirenz: mitochondria again. *Journal of hepatology.* 2013;59(4):780-789.
117. Polo M, Alegre F, Funes HA, et al. Mitochondrial (dys)function - a factor underlying the variability of efavirenz-induced hepatotoxicity? *British journal of pharmacology.* 2015;172(7):1713-1727.
118. Sinxadi PZ, McIlleron HM, Dave JA, et al. Plasma Efavirenz Concentrations Are Associated With Lipid and Glucose Concentrations. *Medicine.* 2016;95(2):e2385.
119. Galli L, Salpietro S, Pellicciotta G, et al. Risk of type 2 diabetes among HIV-infected and healthy subjects in Italy. *European journal of epidemiology.* 2012;27(8):657-665.
120. Brambilla AM, Novati R, Calori G, et al. Stavudine or indinavir-containing regimens are associated with an increased risk of diabetes mellitus in HIV-infected individuals. *AIDS.* 2003;17(13):1993-1995.
121. Butt AA, McGinnis K, Rodriguez-Barradas MC, et al. HIV infection and the risk of diabetes mellitus. *AIDS.* 2009;23(10):1227-1234.
122. Misra R, Chandra P, Riechman SE, et al. Relationship of ethnicity and CD4 Count with glucose metabolism among HIV patients on Highly-Active Antiretroviral Therapy (HAART). *BMC endocrine disorders.* 2013;13:13.
123. Jones RG, Thompson CB. Revving the engine: signal transduction fuels T cell activation. *Immunity.* 2007;27(2):173-178.

124. Kalra S, Kalra B, Agrawal N, Unnikrishnan A. Understanding diabetes in patients with HIV/AIDS. *Diabetology & metabolic syndrome.* 2011;3(1):2.
125. Takarabe D, Rokukawa Y, Takahashi Y, et al. Autoimmune diabetes in HIV-infected patients on highly active antiretroviral therapy. *The Journal of clinical endocrinology and metabolism.* 2010;95(8):4056-4060.
126. Kalev I, Oselin K, Parlist P, et al. CC-chemokine receptor CCR5-del32 mutation as a modifying pathogenetic factor in type I diabetes. *Journal of diabetes and its complications.* 2003;17(6):387-391.
127. Rubin DB. Multiple imputation after 18+ years. . *Journal of the American Statistical Association* 1996;91:473 - 489.
128. van der Heijden GJ, Donders AR, Stijnen T, Moons KG. Imputation of missing values is superior to complete case analysis and the missing-indicator method in multivariable diagnostic research: a clinical example. *Journal of clinical epidemiology.* 2006;59(10):1102-1109.

Appendix 1

Published Article

Herewith attached is the following article in its original, published format:

S. Karamchand, R. Leisegang, M. Schomaker, G. Maartens, L. Walters, M. Hislop, J.A. Dave, N.S. Levitt, K. Cohen. Risk Factors for Incident Diabetes in a Cohort Taking First-Line Nonnucleoside Reverse Transcriptase Inhibitor-Based Antiretroviral Therapy. *Medicine (Baltimore)*. 2016;95(9):e2844. PMID: 26945366, DOI: https://dx.doi.org/10.1097/MD.0000000000002844

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Risk Factors for Incident Diabetes in a Cohort Taking First-Line Nonnucleoside Reverse Transcriptase Inhibitor-Based Antiretroviral Therapy

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Abstract: Efavirenz is the preferred nonnucleoside reverse transcriptase inhibitor (NNRTI) in first-line antiretroviral therapy (ART) regimens in low- and middle-income countries, where the prevalence of diabetes is increasing. Randomized control trials have shown mild increases in plasma glucose in participants in the efavirenz arms, but no association has been reported with overt diabetes. We explored the association between efavirenz exposure and incident diabetes in a large Southern African cohort commencing NNRTI-based first-line ART.

Our cohort included HIV-infected adults starting NNRTI-based ART in a private sector HIV disease management program from January 2002 to December 2011. Incident diabetes was identified by the initiation of diabetes treatment. Patients with prevalent diabetes were excluded.

We included 56,298 patients with 113,297 patient-years of follow-up (PYFU) on first-line ART. The crude incidence of diabetes was 13.24 per 1000 PYFU. Treatment with efavirenz rather than nevirapine was associated with increased risk of developing diabetes (hazard ratio 1.27 (95% confidence interval (CI): 1.10–1.46)) in a multivariate analysis adjusting for age, sex, body mass index, baseline CD4 count, viral load, NRTI backbone, and exposure to other diabetogenic medicines. Zidovudine and stavudine exposure were also associated with an increased risk of developing diabetes.

We found that treatment with efavirenz, as well as stavudine and zidovudine, increased the risk of incident diabetes. Interventions to detect and prevent diabetes should be implemented in ART programs, and use of antiretrovirals with lower risk of metabolic complications should be encouraged.

Editor: Giuseppe Lapadula.
Received: July 29, 2015; revised: December 29, 2015; accepted: January 25, 2016.
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This study was supported by the South African National Research Foundation.
The authors have no conflicts of interest to disclose.
Supplemental Digital Content is available for this article.

ISSN: 0025-7974
DOI: 10.1097/MD.0000000000002844

(*Medicine* 95(9):e2844)

Abbreviations: AfA = Aid for AIDS, AIC = Akaike Information Criterion, ART = antiretroviral therapy, AZT = zidovudine, BMI = body mass index, d4T = stavudine, EFV = efavirenz, HIV = human immunodeficiency virus, HR = hazard ratio, IQR = interquartile range, LMICs = low- and middle-income countries, NNRTI = nonnucleoside reverse transcriptase inhibitor, NRTIs = nucleoside reverse transcriptase inhibitors, NVP = nevirapine, PI = protease inhibitor, PYFU = patient-years of follow-up, VL = viral load, WHO = World Health Organization.

INTRODUCTION

Access to antiretroviral therapy (ART) has considerably reduced morbidity and mortality associated with human immunodeficiency virus (HIV) infection. However, long-term ART is associated with adverse metabolic effects including dysglycemia and new onset diabetes mellitus.[1,2] With the prevalence of noncommunicable diseases, including diabetes, increasing in low- and middle-income countries (LMICs),[3] patients on ART in LMICs face a dual burden of disease.[4]

A number of antiretroviral drugs are known to cause diabetes, including the nucleoside reverse transcriptase inhibitors (NRTIs) stavudine (d4T) and zidovudine (AZT),[2] and the older protease inhibitors (PIs) indinavir[5] and ritonavir.[6,7] Efavirenz, which is now the preferred nonnucleoside reverse transcriptase inhibitor (NNRTI) for first-line ART in LMICs,[8] is associated with slight increases in blood glucose in randomized controlled trials,[9–13] and, in one study conducted by our group.[14] However, there is no good evidence that efavirenz is associated with an increased risk of developing diabetes.

The aim of our study was to investigate the association between efavirenz use and the incidence of diabetes mellitus in a South African cohort of patients on first-line ART.

METHODS

Study Population and Data Source

The study population comprises South African HIV-infected adults enrolled in a private sector HIV disease management program, Aid for AIDS (AfA). The AfA program collects demographic, laboratory, and clinical data on individuals who registered for HIV benefits. Claim data were captured by AfA from the medical insurance fund claim database. These include laboratory, hospitalization, pharmacy, and medical practitioner claims which were submitted to the scheme for processing either: at the time of the service by the provider (eg, pharmacy,

hospitalization) for direct reimbursement or after the service date by the member where the member had already paid the claim. Reimbursement was subject to established AfA protocols, including protocols for ART initiation, change of ART regimen, and the treatment of certain opportunistic infections. No copayment was required for ART, viral load (VL) and CD4 monitoring, and doctor visits.

Despite being a private sector program, AfA standardized guidelines for HIV management, are similar to the World Health Organization (WHO) guidelines for LMICs.[8] Patients were eligible for ART initiation if their CD4 cell count was below 350 cells/μl or they had WHO stage 3 or 4 illness irrespective of the CD4 count. The recommended initial regimen was a combination of 2 NRTIs and an NNRTI. VL and CD4 counts were monitored every 6 months.

Data linkage to the South Africa death registry allowed ascertainment of deaths and date of death, as previously described.[15,16]

Variables and Definitions

We extracted sex, date of birth, weight, height, Republic of South Africa Identity Number, and date of joining the AfA program from the form completed by the doctor on registering the patient with AfA.

We extracted longitudinal results for CD4 count and VL, and all medication claims for antiretrovirals and concomitant medicines. We created a list of diabetogenic drugs using a pharmacology reference textbook[17] and a review[18] (see Appendix 1, http://links.lww.com/MD/A735). We categorized patients as exposed to diabetogenic drugs if they submitted claims for a diabetogenic drug on 2 or more occasions.

We defined the ART start date as the date on which antiretroviral drugs were first dispensed in the AfA program. The ART starting regimen was the regimen dispensed on this date. The baseline CD4 count, VL, and weight were the values measured closest to the date of ART initiation, within the 12-month window before the ART start date. The primary exposure variable of interest was the NNRTI component of the first-line antiretroviral regimen.

Inclusion and Exclusion Criteria

For this study we included AfA-registered patients who initiated a first-line NNRTI-containing ART regimen from January 2002 to December 2011 and were 19 years or older when starting ART. We excluded patients already on antidiabetic medication before starting ART, and patients with missing South Africa identification numbers (as the identification number was used to determine if death occurred by linkage with the South African death registry).

Endpoint

Incident diabetes was defined, using claims data, as the date on which any of the antidiabetic agents available in South Africa (insulins, metformin, sulfonylureas, alpha glucosidase inhibitors, thiazolidinediones, dipeptidyl peptidase-IV inhibitors, glucagon-like-peptide-1 receptor agonists and meglitinides) were initiated. Patients who started and stopped antidiabetic medication in the months around a pregnancy (from 6 months before delivery until 1 month after delivery) were assumed to have diabetes in pregnancy and were therefore not included as incident diabetes.

Imputation of Missing Data

Some data were missing for baseline CD4 count, baseline VL, baseline weight and height (see Table 1). We imputed 5 datasets with the multiple imputation by chained equations (MICE) module in STATA version 13. The imputation model included the following variables: sex, baseline weight, height, age, CD4 count, and VL, death, incident diabetes, and exposure time. CD4 count and VL were actively imputed, and body mass index (BMI) was passively imputed (using actively imputed baseline weight and height). We checked the results of the imputation model by comparing the imputed data with the actual data.[19,20]

Analysis

We collated and prepared the data for statistical analysis using a relational database (Microsoft SQL Server 2008). We used STATA Version 13 (StataCorp LP, College Station, TX) and R version 3.2.1 (R Development Core Team) for statistical analyses.[21] We compared the incidence of diabetes in patients receiving efavirenz-containing regimens versus nevirapine-containing regimens with a Kaplan–Meier plot and a log-rank test.

We explored the association of efavirenz exposure with the hazard of developing diabetes using a multivariate Cox-proportional hazards model. We adjusted for the following variables: age, sex, baseline BMI, baseline CD4 count, baseline VL, exposure to diabetogenic drugs. For the primary analysis ART was included in the model as time-updated NRTI backbone (AZT-containing, d4T-containing, or other NRTI combination) and time-updated NNRTI (efavirenz or nevirapine); and patients were censored when they died, left the medical insurance scheme, switched to PI-based ART, or reached the end of the study period. We performed a secondary analysis exploring incident diabetes within the first ART regimen only. For this analysis we included 2 additional reasons for censoring: NRTI or NNRTI substitution. We explored the effect of including calendar year in the multivariate model. We performed the following sensitivity analyses: we controlled for the competing risk of death, we constructed a model excluding patients virologically suppressed at baseline.

The proportional hazards assumption was verified by testing interaction effects of analysis time with baseline variables ($\alpha = 0.05$), and graphically in each imputed dataset via log–log plots, amongst others. We performed model selection using the Akaike Information Criterion (AIC) after multiple imputation.[19,22–24]

Ethics

The study protocol was reviewed and approved by the University of Cape Town Faculty of Health Sciences Human Research Ethics committee.

RESULTS

Between January 2002 and June 2011, 62,467 patients commenced ART in the AfA program, of whom 56,298 patients met our inclusion and exclusion criteria (Figure 1) and were included in the analysis. The demographic and clinical characteristics of patients are given in Table 1. Results from the multiple imputations for missing covariates are shown in Table 2. Median follow-up was 1.56 years (interquartile range (IQR): 0.71–2.79 years), 21.7% of patients were followed up for 3 or more years.

We identified new onset diabetes in 1500 (2.66%) patients over 113,297 patient-years of follow-up (PYFU), giving a crude incidence of 13.24 cases per 1000 PYFU (Figure 2). There were 17 pregnancy-associated diabetic events, which were not included as cases of incident diabetes. Exposure to diabetogenic

TABLE 1. Cohort Description

	Whole Cohort	Efavirenz-Containing ART	Nevirapine-Containing ART
Number of patients	56,298	46,666	9632
Age (yr)			
Median (IQR)	38.14 (33.15–44.26)	39.1 (34.1–45.17)	34.05 (29.99–38.65)
Sex			
Male	20,224 (35.92)	18,822 (40.33%)	1402 (14.55%)
Female	36,074 (64.08)	27,844 (59.67%)	8230 (85.44%)
Race			
Asian	148 (0.26%)	126 (0.27%)	22 (0.23%)
Black	53,270 (94.62%)	44,179 (94.67%)	9091 (94.38%)
Mixed	768 (1.36%)	628 (1.35%)	140 (1.45%)
White	989 (1.76%)	825 (1.77%)	164 (1.7%)
Not reported	1123 (1.99%)	908 (1.95%)	215 (2.23%)
Nucleoside reverse transcriptase inhibitor (initial regimen containing)			
Zidovudine	26,917 (47.81%)	25,329 (54.28%)	1588 (16.49%)
Stavudine	22,465 (39.9%)	16,002 (34.29%)	6463 (67.1%)
Other	6916 (12.28%)	5335 (11.43%)	1581 (16.41%)
Exposure to other diabetogenic drugs	22,780 (40.46%)	19,137 (41.01%)	3643 (37.82%)
Baseline height (m)			
Median (IQR)	165 (160–170)	165 (160–170)	163 (158–168)
Missing	19,033 (33.81%)	15,390 (32.98%)	3643 (37.82%)
Baseline weight (kg)			
Median (IQR)	68 (60–79)	68 (60–79)	70 (62–81)
Missing	15,042 (26.72%)	12,349 (26.46%)	2693 (27.96%)
Baseline body mass index (kg/m^2)			
Median (IQR)	25.06 (21.97–29.00)	24.82 (21.80–28.69)	26.52 (23.14–30.49)
Missing	23,178 (41.17%)	18,776 (40.23%)	4402 (45.7%)
Baseline CD4 count (cells/μl)			
Median (IQR)	181 (88–280)	176 (81–277)	201 (121–306)
Missing	948 (1.68%)	769 (1.65%)	179 (1.86%)
Baseline log viral load (copies/ml)			
Median (IQR)	4.78 (3.66–5.37)	4.87 (3.86–5.43)	4.35 (2.59–5.03)
Missing	3464 (6.15%)	2845 (6.1%)	619 (6.43%)
Follow-up time (yr)			
Median (IQR)	1.56 (0.71–2.79)	1.50 (0.67–2.69)	1.90 (0.93–3.34)
Patient years	113,297	89,915	23,382
3 or more years of NNRTI exposure	21.70%	29.86%	20.66%
Reason for censoring			
Diabetes	1500 (2.66%)	1257 (2.69%)	243 (2.52%)
Switch to PI	3706 (6.58%)	2778 (5.95%)	928 (9.63%)
Study end	40,785 (72.44%)	34,186 (73.26%)	6599 (68.51%)
Death	1774 (3.15%)	1488 (3.19%)	286 (2.97%)
Left scheme	8533 (15.16%)	6957 (14.91%)	1576 (16.36%)
Crude diabetes incidence			
Events/1000 patient years	13.24	13.98	10.39

ART = antiretroviral therapy, IQR = interquartile range, NNRTI = nonnucleoside reverse transcriptase inhibitor, PI = protease inhibitor.

medicines occurred in 19,137 (41.01%) of patients taking efavirenz and 3643 (37.82%) of patients taking nevirapine. The Kaplan Meier analysis of incident diabetes in efavirenz versus nevirapine-containing regimens is shown in Figure 2.

The results of the Cox proportional hazard regression analyses, including univariate and multivariate analyses and model selection, are shown in Table 3. Efavirenz-containing ART was associated with a higher risk of developing new-onset diabetes than nevirapine-containing ART, adjusted hazard ratio (HR) 1.27 (95% confidence interval (CI): 1.10–1.46). Zidovudine and stavudine-containing NRTI backbones, older age at baseline, elevated baseline BMI, and exposure to diabetogenic medication were also associated with an increased risk of developing diabetes. We found no association between baseline CD4 and an increased risk of diabetes.

The results of the Cox proportional hazard regression analyses where we censored patients at the time of first drug switch are shown in Table 4; reasons for censoring for this analysis are shown in Supplementary Table 1, http://links.lww. com/MD/A736. The estimated HRs of the variables remained similar after model averaging, confirming the stability of our findings.[25] These findings did not differ from the

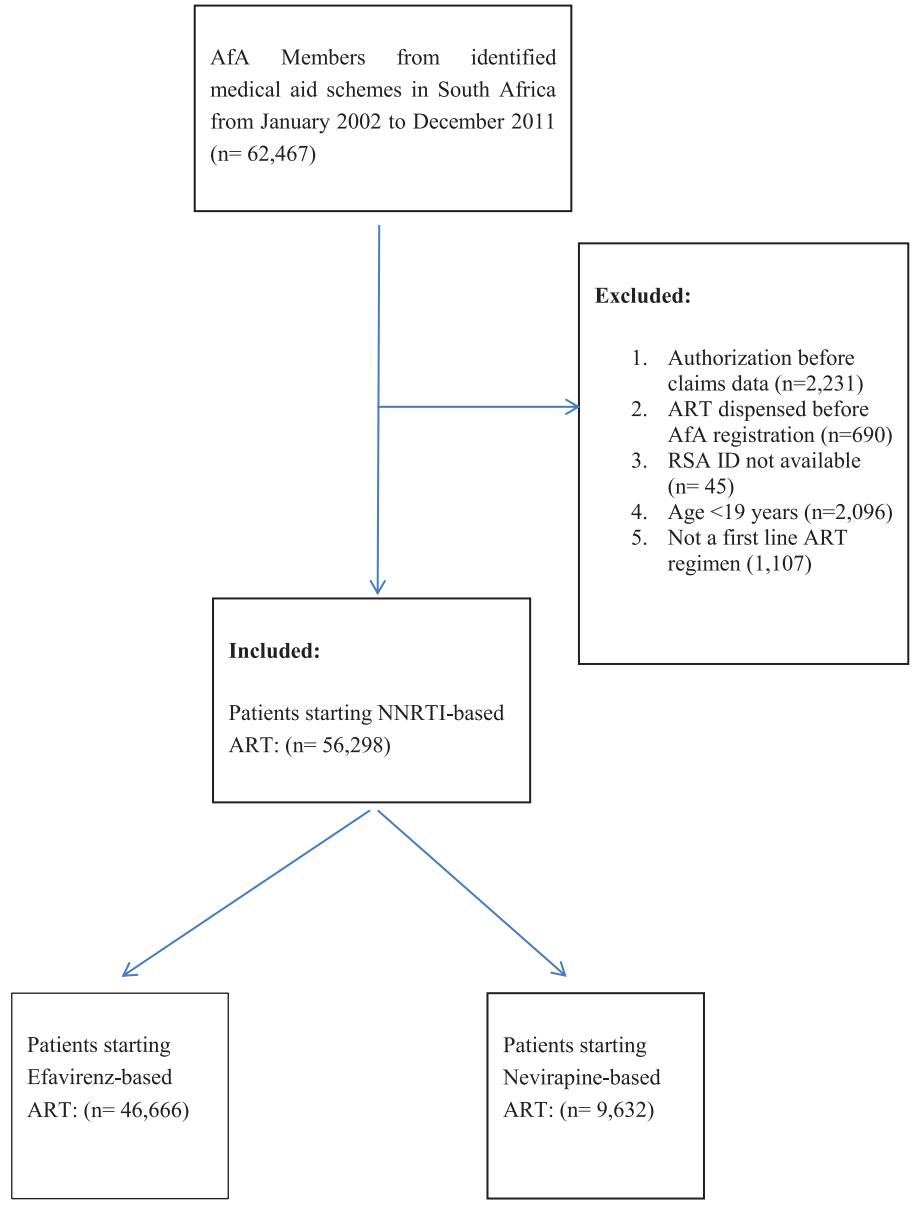

FIGURE 1. Participant selection and exclusion criteria.

regression model with updated regimen (censoring at the point of change to second line regimens). Adding calendar year to the model did not change the associations observed (Supplementary Table 2, http://links.lww.com/MD/A736). Findings did not change when we accounted for competing risk (Supplementary Table 3, http://links.lww.com/MD/A736). Excluding patients with suppressed baseline VLs attenuated the effect of efavirenz on incident diabetes somewhat: adjusted HR 1.16; 95% CI: 0.99–1.36 (Supplementary Table 4, http://links.lww.com/MD/A736).

DISCUSSION

We found efavirenz use to be associated with a significantly higher incidence of diabetes than nevirapine in a large cohort of South African patients. To the best of our knowledge, this is the first cohort study to show an increased risk of diabetes from efavirenz use in first-line ART. We also found that the NRTIs stavudine and zidovudine were associated with an increased incidence of diabetes. These findings have important implications for LMICs, which are facing a burgeoning diabetes epidemic, as efavirenz is the preferred NNRTI in first-line ART, zidovudine is recommended in second line ART, and many people are still taking stavudine, even though it is no longer recommended by the WHO.[8]

HIV-infected patients have an estimated 4-fold greater relative risk of developing diabetes than the HIV-uninfected population.[26] We found a crude incidence of diabetes of 13.24 per 1000 PYFU, which is at the upper end of the range reported from cohort studies in high income countries (4.2–14.1 per 1000 PYFU).[2,26–28] Factors contributing to the increased risk of diabetes in people with HIV include insulin resistance due to

TABLE 2. Results of Imputation for Baseline Variables With Missing Data

	Total Cohort (n = 56,298)	Efavirenz-Containing ART (n = 46,666)	Nevirapine-Containing ART (n = 9632)
Variable	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**
Height (m)			
Before imputation	164.93 (164.84–165.03)	165.25 (165.18–165.31)	163.14 (162.92–163.35)
After imputation	164.74 (164.64–164.85)	165.14 (165.05–165.23)	163.07 (162.88–163.26)
Weight (kg)			
Before imputation	70.34 (70.18–70.51)	69.88 (69.72–70.03)	72.81 (72.39–73.23)
After imputation	70.14 (69.97–70.30)	69.80 (69.62–69.97)	71.59 (71.18–72.00)
CD4 count (cells/μl)			
Before imputation	215.82 (214.27–217.38)	208.77 (207.64–209.90)	254.44 (251.66–257.22)
After imputation	215.39 (214.41–216.38)	207.18 (206.12–208.25)	250.26 (247.83–252.69)
Viral load (copies/ml)			
Before imputation	4.31 (4.30–4.33)	4.39 (4.38–4.40)	3.88 (3.86–3.90)
After imputation	4.32 (4.31–4.33)	4.38 (4.37–4.39)	3.92 (3.90–3.94)
Body mass index (kg/m^2)			
Before imputation	25.95 (25.89–26.01)	25.67 (25.62–25.72)	27.44 (27.31–27.56)
After imputation	25.93 (25.87–25.99)	25.68 (25.61–25.75)	27.00 (26.86–27.14)

ART = antiretroviral therapy, CI = confidence interval.

the chronic inflammatory response to HIV infection,[29] which persists despite effective ART[29,30] and the effects of certain antiretroviral drugs. Our finding that the NRTIs stavudine and zidovudine were both associated with an increased incidence of diabetes has previously been reported.[2,31] NRTI's inhibit the enzyme DNA polymerase-γ, responsible for mitochondrial replication. The dysregulation of mitochondrial function in different compartments of the body results in various clinical manifestations of NRTI toxicity, including insulin resistance and diabetes.[32–34] In a prior cross sectional study we found an increased risk of dysglycemia in South African patients taking efavirenz compared with those taking nevirapine, but there were insufficient numbers of cases of diabetes for analysis.[14] A small case–control study from Botswana suggested an association between efavirenz use and diabetes.[35] Randomized controlled trials showed significantly higher serum glucose concentrations in participants in the efavirenz arms than the following comparator antiretroviral drugs: nevirapine,[13] abacavir,[13] atazanavir,[11] atazanavir–ritonavir,[9] and raltegravir.[12]

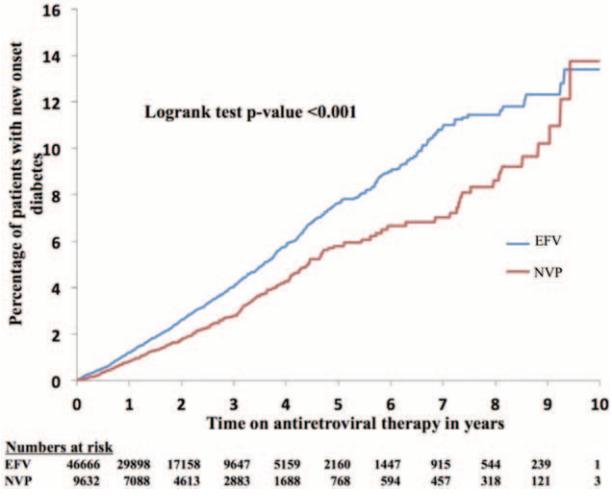

FIGURE 2. Kaplan–Meier analysis of incident diabetes.

The mechanism by which efavirenz mediates insulin resistance and diabetes is unknown. Possible mechanisms include mitochondrial toxicity[36] and toxic effects on adipocytes and increased rates of lipoatrophy.[37,38] Efavirenz causes hepatic mitochondrial toxicity[36] and induces hepatocyte endoplasmic reticulum stress leading to activation of the unfolded protein response, and apoptosis.[39,40] Efavirenz mediates mitochondrial toxicity via various mechanisms. Firstly, efavirenz directly inhibits Complex I of the electron transport chain, resulting in a markedly reduced mitochondrial transmembrane potential, thus compromising oxidative phosphorylation and ATP generation.[41–43] Secondly, efavirenz reduces complex IV (COIV) mRNA (a marker gene of mitochondrial function), and impairs mitochondrial function in adipocytes.[44] Furthermore, efavirenz-associated mitochondrial dysregulation in adipose tissue causes impaired adipogenesis, increased lipolysis, and release of free fatty acids and inflammatory cytokines.[44] The increased release of fatty acids due to adipocyte mitochondrial toxicity are thought to impair muscle and liver insulin sensitivity, leading to insulin resistance and diabetes mellitus.[45–51] In addition to its mitochondrial toxicity, efavirenz has been shown to reduce the secretion of adiponectin (an insulin-sensitizing, antidiabetic adipokine) by adipocytes.[44] We hypothesize that impairment of mitochondrial bioenergetics and toxicity to adipocytes contributes to the development of diabetes in patients on efavirenz. By contrast, nevirapine does not appear to exert mitochondrial toxicity.[37,44]

Our group have demonstrated a positive correlation between plasma efavirenz concentrations and both fasting and 2-hour glucose concentrations after oral glucose tolerance tests in South African patients.[52] People of African origin are more likely to be genotypic ''slow metabolizers'' of efavirenz, which results in elevated efavirenz plasma concentrations, than people of European descent (20% and 3%, respectively).[53] Therefore efavirenz may have a larger diabetogenic effect in

TABLE 3. Univariate, Multivariate, and Model Selection Results for the Cox Regression Model of Associations With Incident Diabetes

		Univariate		Multivariate		
Variable	**Category**	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***	**Model Selection (AIC)**
Nonnucleoside reverse transcriptase inhibitor	Efavirenz	1.40 (1.22–1.60)	<0.001	1.27 (1.10–1.47)	0.001	1.27 (1.10–1.46)
	Nevirapine	Referent		Referent		Referent
Nucleoside reverse transcriptase inhibitor	Zidovudine	1.30 (1.15–1.46)	<0.001	1.35 (1.19–1.52)	<0.001	1.37 (1.21–1.54)
	Stavudine	1.53 (1.32–1.78)	<0.001	1.60 (1.38–1.87)	<0.001	1.64 (1.41–1.91)
	Other	Referent		Referent		Referent
Exposure to other diabetogenic drugs		1.68 (1.51–1.86)	<0.001	1.53 (1.37–1.70)	<0.001	1.53 (1.38–1.71)
Baseline age (yr)	19–24	0.35 (0.20–0.60)	<0.001	0.47 (0.27–0.81)	0.007	0.46 (0.27–0.80)
	25–34	0.64 (0.56–0.73)	<0.001	0.71 (0.62–0.82)	<0.001	0.71 (0.62–0.81)
	35–44	Referent		Referent		Referent
	45–54	1.50 (1.33–1.70)	<0.001	1.38 (1.21–1.56)	<0.001	1.36 (1.20–1.54)
	≥55	1.86 (1.50–2.31)	<0.001	1.64 (1.32–2.04)	<0.001	1.57 (1.26–1.95)
Sex	Male	1.41 (1.27–1.56)	<0.001	1.47 (1.32–1.64)	<0.001	1.44 (1.29–1.61)
	Female	Referent		Referent		Referent
Baseline body mass index (BMI) quartile (kg/m/m)	10–17	0.38 (0.23–0.63)	0.001	0.33 (0.19–0.56)	0.001	0.32 (0.19–0.55)
	18–24	0.65 (0.58–0.74)	<0.001	0.61 (0.53–0.69)	<0.001	0.60 (0.53–0.69)
	25–34	Referent		Referent		Referent
	35+	1.45 (1.16–1.81)	0.002	1.58 (1.26–1.97)	<0.001	1.58 (1.27–1.97)
Baseline CD4 count (cells/μl)	0–199	1.04 (0.92–1.17)	0.534	1.08 (0.95–1.23)	0.220	
	200–349	Referent		Referent		Excluded by AIC
	350+	1.25 (1.06–1.47)	0.007	1.10 (0.91–1.34)	0.324	
Baseline viral load (copies/ml)	0–999	1.31 (1.14–1.51)	<0.001	1.24 (1.05–1.47)	0.011	1.28 (1.11–1.47)
	1000–99,999	Referent		Referent		Referent
	100,000–999,999	1.07 (0.95–1.21)	0.261	1.03 (0.91–1.16)	0.674	1.03 (0.91–1.17)
	≥1,000,000	1.15 (0.89–1.48)	0.285	1.15 (0.89–1.49)	0.278	1.14 (0.89–1.48)

Drug switches within first-line regimen included in the model, with censoring at switch to second line therapy. All results are based on multiple imputations.

AIC = Akaike Information Criterion, CI = confidence interval, HR = hazard ratio.

Africans, which may explain why, in contrast with our findings, studies from high income countries have not found an association between efavirenz and diabetes.

Our finding that increasing age, and male sex were associated with an increased risk of diabetes is consistent with findings of other studies.[2,29,54–59] We could not show any association between baseline CD4 count and diabetes, which is similar to that reported by a French cohort,[27] but other studies have found an increased risk of diabetes with lower CD4 counts.[29,54] We found an association between the lowest stratum of baseline VL and an increased relative risk for developing diabetes, but no association with higher VL strata. The French cohort reported no association between VL and diabetes.[27] In contrast, other studies have found an association between high VLs and diabetes.[29,58] The association we observed between low baseline VLs and increased risk of diabetes may be attributed to the inclusion of patients already on undisclosed ART on entry to the AfA program.

Our study has limitations. We had missing baseline data, notably of BMI. However, we imputed missing data, which are known to be superior to using complete case analysis.[60,61] ART exposure may have occurred before commencing ART within the AfA program and there were 18.8% of patients with a suppressed VL at baseline, which is likely due to undisclosed ART use. We identified incident diabetes based on initiation of diabetes therapy as the results of plasma glucose or glycated hemoglobin are not captured in the database. We will therefore have missed cases of diabetes that were only treated with lifestyle modifications. As patients were not routinely screened for diabetes at the time of ART initiation, some patients may have entered the cohort with undiagnosed diabetes. We could not adjust for weight changes during follow-up in our analyses, as weight is only recorded at baseline. A strength of our study is that we adjusted for the concurrent use of diabetogenic medication in the multivariate model, which was associated with a 53% increase in the relative risk of diabetes.

In conclusion, we found that exposure to efavirenz, stavudine, and zidovudine was associated with an increased incidence of diabetes. Although the increased risk of diabetes with these antiretrovirals was relatively modest, the large African patient population exposed to ART for prolonged periods means that our finding has important public health implications. While screening for diabetes should be increased in people on long-term ART, consideration should be given to using antiretrovirals with less risk of metabolic complications. Further studies to confirm the association of efavirenz and risk of diabetes should be conducted in LMICs, and the molecular mechanisms of efavirenz-induced dysglycemia need further investigation.

TABLE 4. Univariate, Multivariate, and Model Selection Results for the Cox Regression Model of Associations With Incident Diabetes (Censored at First Ever Drug Switch)

		Univariate		Multivariate (Rubins Rule)		Model Selection (AIC)
Variable	**Category**	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**
Nonnucleoside reverse transcriptase inhibitor	Efavirenz	1.38 (1.18–1.60)	<0.001	1.26 (1.07–1.49)	0.005	1.33 (1.13–1.56)
	Nevirapine	Referent		Referent		Referent
Nucleoside reverse transcriptase inhibitor	Zidovudine	1.34 (1.17–1.54)	<0.001	1.39 (1.21–1.60)	<0.001	1.57 (1.37–1.80)
	Stavudine	1.62 (1.37–1.90)	<0.001	1.67 (1.41–1.98)	<0.001	1.96 (1.66–2.32)
	Other	Referent		Referent		Referent
Exposure to other diabetogenic drugs		1.68 (1.50–1.88)	<0.001	1.52 (1.36–1.71)	<0.001	1.50 (1.34–1.69)
Baseline age (yr)	19–24	0.39 (0.22–0.69)	0.001	0.53 (0.30–0.94)	0.031	0.52 (0.29–0.93)
	25–34	0.63 (0.55–0.73)	<0.001	0.71 (0.61–0.82)	<0.001	0.70 (0.60–0.81)
	35–44	Referent		Referent		Referent
	45–54	1.49 (1.30–1.70)	<0.001	1.37 (1.20–1.57)	<0.001	1.36 (1.19–1.56)
	≥55	1.89 (1.50–2.37)	<0.001	1.69 (1.34–2.13)	<0.001	1.61 (1.28–2.03)
Sex	Male	1.40 (1.25–1.56)	<0.001	1.48 (1.31–1.67)	<0.001	1.47 (1.30–1.66)
	Female	Referent		Referent		Referent
Baseline body mass index (kg/m^2)	10–17	0.40 (0.23–0.71)	0.004	0.35 (0.19–0.63)	0.002	0.35 (0.19–0.62)
	18–24	0.65 (0.53–0.81)	0.001	0.61 (0.48–0.76)	0.001	0.61 (0.48–0.76)
	25–34	Referent		Referent		Referent
	35+	1.44 (1.14–1.82)	0.004	1.57 (1.24–1.98)	<0.001	1.58 (1.25–1.99)
						Excluded by AIC
Baseline CD4 count (cells/μl)	0–199	1.06 (0.93–1.21)	0.415	1.11 (0.97–1.27)	0.136	
	200–349	Referent		Referent		
	350+	1.32 (1.11–1.57)	0.001	1.11 (0.91–1.36)	0.307	
Baseline viral load (copies/ml)	0–999	1.35 (1.17–1.57)	<0.001	1.27 (1.06–1.53)	0.010	1.27 (1.09–1.48)
	1000–99,999	Referent		Referent		Referent
	100,000–999,999	1.03 (0.90–1.18)	0.653	0.99 (0.86–1.13)	0.841	1.00 (0.87–1.14)
	≥1,000,000	1.15 (0.88–1.50)	0.311	1.15 (0.88–1.50)	0.320	1.15 (0.88–1.50)

All results are based on multiple imputations.
AIC = Akaike Information Criterion, CI = confidence interval, HR = hazard ratio.

REFERENCES

1. Samaras K. Prevalence and pathogenesis of diabetes mellitus in HIV-1 infection treated with combined antiretroviral therapy. *J Acquir Immune Defic Syndr.* 2009;50:499–505.
2. De Wit S, Sabin CA, Weber R, et al. Incidence and risk factors for new-onset diabetes in HIV-infected patients: the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study. *Diabetes Care.* 2008;31:1224–1229.
3. Mbanya JC, Motala AA, Sobngwi E, et al. Diabetes in sub-Saharan Africa. *Lancet.* 2010;375:2254–2266.
4. Levitt NS, Steyn K, Dave J, et al. Chronic noncommunicable diseases and HIV-AIDS on a collision course: relevance for health care delivery, particularly in low-resource settings—insights from South Africa. *Am J Clin Nutr.* 2011;94:1690S–1696S.
5. Murata H, Hruz PW, Mueckler M. Indinavir inhibits the glucose transporter isoform Glut4 at physiologic concentrations. *AIDS.* 2002;16:859–863.
6. Taylor SA, Lee GA, Pao VY, et al. Boosting dose ritonavir does not alter peripheral insulin sensitivity in healthy HIV-seronegative volunteers. *J Acquir Immune Defic Syndr.* 2010;55:361–364.
7. Vyas AK, Koster JC, Tzekov A, et al. Effects of the HIV protease inhibitor ritonavir on GLUT4 knock-out mice. *J Biol Chem.* 2010;285:36395–36400.
8. Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Recommendations for a Public Health Approach. Geneva, Switzerland: World Health Organization (WHO); 2013. ISBN 9789241505727.
9. Erlandson KM, Kitch D, Tierney C, et al. Impact of randomized antiretroviral therapy initiation on glucose metabolism. *AIDS.* 2014;28:1451–1461.
10. Fisac C, Fumero E, Crespo M, et al. Metabolic benefits 24 months after replacing a protease inhibitor with abacavir, efavirenz or nevirapine. *AIDS.* 2005;19:917–925.
11. Jemsek JG, Arathoon E, Arlotti M, et al. Body fat and other metabolic effects of atazanavir and efavirenz, each administered in combination with zidovudine plus lamivudine, in antiretroviral-naive HIV-infected patients. *Clin Infect Dis.* 2006;42:273–280.
12. Lennox JL, Dejesus E, Berger DS, et al. Raltegravir versus efavirenz regimens in treatment-naive HIV-1-infected patients: 96-week efficacy, durability, subgroup, safety, and metabolic analyses. *J Acquir Immune Defic Syndr.* 2010;55:39–48.
13. Martinez E, Arnaiz JA, Podzamczer D, et al. Substitution of nevirapine, efavirenz, or abacavir for protease inhibitors in patients with human immunodeficiency virus infection. *N Engl J Med.* 2003;349:1036–1046.
14. Dave JA, Lambert EV, Badri M, et al. Effect of nonnucleoside reverse transcriptase inhibitor-based antiretroviral therapy on

dysglycemia and insulin sensitivity in South African HIV-infected patients. *J Acquir Immune Defic Syndr.* 2011;57:284–289.

15. Boulle A, Van Cutsem G, Hilderbrand K, et al. Seven-year experience of a primary care antiretroviral treatment programme in Khayelitsha, South Africa. *AIDS.* 2010;24:563–572.
16. Schomaker M, Gsponer T, Estill J, et al. Non-ignorable loss to follow-up: correcting mortality estimates based on additional outcome ascertainment. *Stat Med.* 2014;33:129–142.
17. Davis S. Diabetogenic drugs: treating chronic conditions to minimise new onset diabetes. *SAPJ.* 2010:22–27.
18. Brunton L, Lazo J, Parker K (Eds): *Goodman & Gilman's the Pharmacological Basis of Therapeutics*. New York: McGraw-Hill; 2005.
19. Schomaker M, Heumann C. Model selection and model averaging after multiple imputation. *Comput Stat Data An.* 2014;71:758–770.
20. Honaker J, King G, Blackwell M. Amelia II: a program for missing data. *J Stat Softw.* 2011;45:1–47.
21. R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.
22. Bozdogan H. Model selection and Akaike's Information Criterion (AIC): the general theory and its analytical extensions. *Psychometrika.* 1987;52:345–370.
23. Schomaker M, Wan ATK, Heumann C. Frequentist model averaging with missing observations. *Comput Stat Data An.* 2010;54:3336–3347.
24. Volinsky CT, Madigan D, Raftery AE, et al. Bayesian model averaging in proportional hazard models. Assessing the risk of a stroke. *Appl Stat.* 1997;46:433–448.
25. Burnham K, Anderson D. Model Selection and Multimodel Inference. A Practical Information-Theoretic Approach. New York: Springer; 2002
26. Brown TT, Li X, Cole SR, et al. Cumulative exposure to nucleoside analogue reverse transcriptase inhibitors is associated with insulin resistance markers in the Multicenter AIDS Cohort Study. *AIDS.* 2005;19:1375–1383.
27. Capeau J, Bouteloup V, Katlama C, et al. Ten-year diabetes incidence in 1046 HIV-infected patients started on a combination antiretroviral treatment. *AIDS.* 2012;26:303–314.
28. Petoumenos K, Worm SW, Fontas E, et al. Predicting the short-term risk of diabetes in HIV-positive patients: the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study. *J Int AIDS Soc.* 2012;15:17426.
29. Brown TT, Tassiopoulos K, Bosch RJ, et al. Association between systemic inflammation and incident diabetes in HIV-infected patients after initiation of antiretroviral therapy. *Diabetes Care.* 2010;33:2244–2249.
30. Brigham EP, Patil SP, Jacobson LP, et al. Association between systemic inflammation and obstructive sleep apnea in men with or at risk for HIV infection. *Antivir Ther.* 2014;19:725–733.
31. Brown TT, Cole SR, Li X, et al. Antiretroviral therapy and the prevalence and incidence of diabetes mellitus in the multicenter AIDS cohort study. *Archiv Intern Med.* 2005;165:1179–1184.
32. Blumer RM, van Vonderen MG, Sutinen J, et al. Zidovudine/lamivudine contributes to insulin resistance within 3 months of starting combination antiretroviral therapy. *AIDS.* 2008;22:227–236.
33. Brinkman K, Kakuda TN. Mitochondrial toxicity of nucleoside analogue reverse transcriptase inhibitors: a looming obstacle for long-term antiretroviral therapy? *Curr Opin Infect Dis.* 2000;13:5–11.
34. Kakuda TN. Pharmacology of nucleoside and nucleotide reverse transcriptase inhibitor-induced mitochondrial toxicity. *Clin Ther.* 2000;22:685–708.
35. Moyo D, Tanthuma G, Cary MS, et al. Cohort study of diabetes in HIV-infected adult patients: evaluating the effect of diabetes mellitus on immune reconstitution. *Diabetes Res Clin Pract.* 2014;103:e34–e36.
36. Blas-Garcia A, Apostolova N, Ballesteros D, et al. Inhibition of mitochondrial function by efavirenz increases lipid content in hepatic cells. *Hepatology.* 2010;52:115–125.
37. El Hadri K, Glorian M, Monsempes C, et al. In vitro suppression of the lipogenic pathway by the nonnucleoside reverse transcriptase inhibitor efavirenz in 3T3 and human preadipocytes or adipocytes. *J Biol Chem.* 2004;279:15130–15141.
38. Perez-Molina JA, Domingo P, Martinez E, et al. The role of efavirenz compared with protease inhibitors in the body fat changes associated with highly active antiretroviral therapy. *J Antimicrob Chemother.* 2008;62:234–245.
39. Apostolova N, Gomez-Sucerquia LJ, Alegre F, et al. ER stress in human hepatic cells treated with efavirenz: mitochondria again. *J Hepatol.* 2013;59:780–789.
40. Polo M, Alegre F, Funes HA, et al. Mitochondrial (dys)function—a factor underlying the variability of efavirenz-induced hepatotoxicity? *Br J Pharmacol.* 2015;172:1713–1727.
41. Apostolova N, Gomez-Sucerquia LJ, Moran A, et al. Enhanced oxidative stress and increased mitochondrial mass during efavirenz-induced apoptosis in human hepatic cells. *Br J Pharmacol.* 2010;160:2069–2084.
42. Karamchand L, Dawood H, Chuturgoon AA. Lymphocyte mitochondrial depolarization and apoptosis in HIV-1-infected HAART patients. *J Acquir Immune Defic Syndr.* 2008;48:381–388.
43. Pilon AA, Lum JJ, Sanchez-Dardon J, et al. Induction of apoptosis by a nonnucleoside human immunodeficiency virus type 1 reverse transcriptase inhibitor. *Antimicrob Agents Chemother.* 2002;46:2687–2691.
44. Gallego-Escuredo JM, Del Mar Gutierrez M, Diaz-Delfin J, et al. Differential effects of efavirenz and lopinavir/ritonavir on human adipocyte differentiation, gene expression and release of adipokines and pro-inflammatory cytokines. *Curr HIV Res.* 2010;8:545–553.
45. Arner P. Insulin resistance in type 2 diabetes: role of fatty acids. *Diabetes Metab Res Rev.* 2002;18(Suppl. 2):S5–S9.
46. Hadigan C, Borgonha S, Rabe J, et al. Increased rates of lipolysis among human immunodeficiency virus-infected men receiving highly active antiretroviral therapy. *Metab Clin Exp.* 2002;51:1143–1147.
47. Hruz PW. Molecular mechanisms for altered glucose homeostasis in HIV infection. *Am J Infect Dis.* 2006;2:187–192.
48. Johnson JA, Albu JB, Engelson ES, et al. Increased systemic and adipose tissue cytokines in patients with HIV-associated lipodystrophy. *Am J Physiol Endocrinol Metab.* 2004;286:E261–E271.
49. Kim JA, Wei Y, Sowers JR. Role of mitochondrial dysfunction in insulin resistance. *Circ Res.* 2008;102:401–414.
50. Krebs M, Roden M. Molecular mechanisms of lipid-induced insulin resistance in muscle, liver and vasculature. *Diabetes Obes Metab.* 2005;7:621–632.
51. Sivitz WI, Yorek MA. Mitochondrial dysfunction in diabetes: from molecular mechanisms to functional significance and therapeutic opportunities. *Antioxid Redox Signal.* 2010;12:537–577.
52. Sinxadi P, McIlleron H, Dave JA. Plasma Efavirenz Concentrations are associated with Lipid and Glucose Concentrations. *Medicine (Baltimore).* 2016;95:e2385.
53. Haas DW, Ribaudo HJ, Kim RB, et al. Pharmacogenetics of efavirenz and central nervous system side effects: an Adult AIDS Clinical Trials Group study. *AIDS.* 2004;18:2391–2400.
54. Galli L, Salpietro S, Pellicciotta G, et al. Risk of type 2 diabetes among HIV-infected and healthy subjects in Italy. *Eur J Epidemiol.* 2012;27:657–665.

55. Brambilla AM, Novati R, Calori G, et al. Stavudine or indinavir-containing regimens are associated with an increased risk of diabetes mellitus in HIV-infected individuals. *AIDS.* 2003;17: 1993–1995.

56. Butt AA, McGinnis K, Rodriguez-Barradas MC, et al. HIV infection and the risk of diabetes mellitus. *AIDS.* 2009;23:1227–1234.

57. Tien PC, Schneider MF, Cole SR, et al. Antiretroviral therapy exposure and incidence of diabetes mellitus in the Women's Interagency HIV Study. *AIDS.* 2007;21:1739–1745.

58. Ledergerber B, Furrer H, Rickenbach M, et al. Factors associated with the incidence of type 2 diabetes mellitus in HIV-infected participants in the Swiss HIV Cohort Study. *Clin Infect Dis.* 2007;45:111–119.

59. El-Sadr WM, Mullin CM, Carr A, et al. Effects of HIV disease on lipid, glucose and insulin levels: results from a large antiretroviral-naive cohort. *HIV Med.* 2005;6:114–121.

60. Rubin DB. Multiple imputation after 18+ years. *J Am Stat Assoc.* 1996;91:473–489.

61. van der Heijden GJ, Donders AR, Stijnen T, et al. Imputation of missing values is superior to complete case analysis and the missing-indicator method in multivariable diagnostic research: a clinical example. *J Clin Epidemiol.* 2006;59:1102–1109.

Appendix 2

Research Ethics Documents

Herewith attached are the relevant ethical approval documents provided for this study by the Human Research Ethics Committee of the University of Cape Town.

UNIVERSITY OF CAPE TOWN

Faculty of Health Sciences
Human Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6626 • Facsimile [021] 406 6411
e-mail: shuretta.thomas@uct.ac.za

21 June 2011

Sent via Internal mail & Email

HREC REF: 282/2011

DR K COHEN,
Division of Clinical Pharmacology
K-Floor
OMB
Fax: 0214481989

Dear DR COHEN,

PROTOCOL NUMBER 282/2011

PROJECT TITLE: MORBIDITY FROM ANTIRETROVIRAL METABOLIC EFFECTS IN AFRICA: THE MAMA STUDY

Thank you for submitting your new study to the Faculty of Health Sciences Human Research Ethics Committee

It is a pleasure to inform you that the Ethics Committee has formally approved the above-mentioned study.

Approval is granted until 28 June 2012

Please submit an annual progress report (FHS016) if the research continues beyond the expiry date. Please submit a brief summary of findings if you complete the study within the approval period so that we can close our file.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC. REF in all your correspondence.

Yours sincerely

A/PROF MARC BLOCKMAN
CHAIRPERSON, FHS HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.

The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS017: Annual Progress Report / Renewal

Record Reviews/Audits/Collection of Biological Specimens/Repositories/Databases/Registries

HREC office use only (FWA00001637; IRB00001938)			
This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30.6.2017
☐ Not approved	See attached comments		
		Date Signed	27/6/16

Principal Investigator to complet

1. Protocol information

Date (when submitting this form)	22 June 2016		
HREC REF Number	282/2011	Current Ethics Approval was granted until	30.6.2016
Protocol title	Morbidity from antiretroviral metabolic effects in Africa, The MAMA study		
Principal Investigator	Dr. Karen Cohen		
Department / Office Internal Mail Address	Division of Clinical Pharmacology, Department of Medicine, K- Floor Rm. K45, Old Main Building, Groote Schuur Hospital		
1.1 Does this protocol receive US Federal funding?		☐ Yes	☐ ✓ No

2. Protocol status (tick ✓)

☐	Research-related activities are ongoing
☐	Data collection is complete, data analysis only
Please indicate (in the block below) the titles and HREC reference numbers of any projects currently making use of the Database/registry/repository	

3. Protocol summary

Total number of records or specimens collected, reviewed or stored since the original approval		56298
Total number of records or specimens collected, reviewed or stored since last progress report		56298
Have any research-related outputs (e.g. publications, abstracts, conference presentations) resulted from this research? If yes, please list and attach with this report.	☐ Yes ✓	☐ No

4. Signature

Signature of PI		Date	22 June 2016

(Note: Please complete the Closure form (FHS019) if the study is completed within the approval period)

3.4 Is this protocol for degree purposes? (tick √)	
☐ No	☐ Yes √
If yes, please specify: MSc Medicine (Pharmacology)	
Type of degree	Masters of Science in Medicine -Pharmacology
Student's name and e-mail	Sumanth Karamchand (krmsum001@uct.ac.za)
Supervisor's name and e-mail	Dr Karen Cohen (karen.cohen@uct.ac.za) Professor Gary Maartens (gary.maartens@uct.ac.za)
Department and University	Division of Pharmacology, Department of Medicine, University of Cape Town

3.5 Does this protocol comply with the Helsinki Declaration of 2008? (tick √)	
☐ No	☐ Yes √
If no, please explain with full justification	

3.6 Does the protocol provide insurance for research-related adverse events (tick √)		
☐ NA (e.g. minimal risk research, medical record review) √	☐ No	☐ Yes
If yes, please describe:		
ABPI-compliant corporate insurance policy		
UCT's no-fault insurance policy		
Other. Please specify		

3.7 Does the protocol comply with UCT's intellectual property rights policy? (tick √)	
☐ Yes √	☐ No
If no, please justify	

4. Funding and grant information

4.1 Funding source (tick √ at least one)	
No funding/sponsor → skip to Q. 5	√
Agency-funded (e.g. MRC, NRF, Wellcome Trust, Bill and Melinda Gates Foundation)	
US Federal funding (e.g. NIH, CDC)	
UCT (e.g. departmental funding)	
Other. Please specify:	