DYSLIPIDEMIA AND DYSGLYCEMIA IN HIV–INFECTED ADULTS RECEIVING CARE AT THE MOI TEACHING AND REFERRAL HOSPITAL, ELDORET, KENYA

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Declaration

Student's Declaration

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Dedication

I would like to dedicate this Research thesis to my grandmother Tabutany Leitich Nyigei, my late small brother Dennis Kipng'etich Kirui and my entire family. Your words of wisdom and constant encouragement through the years have contributed significantly to who I am today. You desired for me what you did not get an opportunity to have. Thank you.

Title: Dyslipidemia and dysglycemia in HIV infected adults receiving care at the Moi Teaching and Referral Hospital, Eldoret, Kenya

Abstract

Background: HIV infected patients exhibit a number of metabolic complications that include dyslipidemia and dysglycemia. These metabolic complications are among the traditional risk factors for cardiovascular diseases. However, the prevalence of these complications among HIV infected patients is not known at the Moi Teaching and Referral Hospital (MTRH).

Objective: To determine the association between the prevalence of newly diagnosed dyslipidemia and dysglycemia with ART use among HIV infected adults receiving care at MTRH.

Methods: This was a comparative cross-sectional study conducted at MTRH. The study population was all HIV infected adults receiving care at MTRH. HIV positive adults were grouped into ART naïve and ART experienced arms, with each arm having a sample of 150 study participants. An interviewer administered structured questionnaire was used to collect socio-demographic and clinical data. Presence of dyslipidemia and dysglycemia was determined by measuring the fasting lipid profiles and fasting blood sugars respectively. Data was dually entered into Epidata software and validated. Data analysis was performed using STATA version 13 special edition. A p-value of <0.05 was considered to be statistically significant.

Results: Out of 300 participants who were enrolled, 69% were female. ART naïve and ART experienced participants were comparable in terms of social & demographic characteristics (p>0.05), body mass index (p=0.094) and blood pressure (p=0.658). However, ART experienced participants were younger (p<0.001). The prevalence of newly-diagnosed dyslipidemia and dysglycemia was 70% and 15.3%, respectively. There was no statistically significant difference in the prevalence of dyslipidemia (p=0.603) and dysglycemia (p=0.055) between the ART naïve and ART experienced participants. The prevalence of elevated total cholesterol, elevated low density lipoprotein cholesterol, elevated triglycerides and low high density lipoprotein cholesterol among all study participants was 7.7%, 53%, 15.8% and 19.8%, respectively. The prevalence of diabetes mellitus and impaired fasting glucose among all study participants was 2.3% and 12%, respectively. There was no association between ART use and prevalence of dyslipidemia and dysglycemia.

Conclusion: Irrespective of ART use, the prevalence of dyslipidemia was high and that of dysglycemia was also significant. ART use was not associated with the prevalence of either dyslipidemia or dysglycemia.

Recommendations: The clinical suspicion of dyslipidemia should be raised among all HIV-infected patients receiving care at MTRH irrespective of ART use.

Table of Contents

List of Tables

List of Figures

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I would like to sincerely thank my parents, siblings and my fiancée for their constant support and encouragement through this labor intensive process. Finally, I would like to thank my research assistant Edmond Ngetich and Alfred Koskei who helped me collect the data reported in this thesis.

List of abbreviations

ADA	-	American Diabetes Association
AMPATH	-	Academic Model Providing Access to Healthcare
ART	-	Antiretroviral therapy
ARVs	-	Antiretroviral drugs
CVD	-	Cardiovascular disease
DM	-	Diabetes mellitus
D4T	-	Stavudine
HDL-C	-	High Density Lipoprotein – Cholesterol
IREC	-	Institutional Research and Ethics Committee
KAIS	-	Kenya AIDS Indicator Survey
KNH	-	Kenyatta National Hospital
LDL-C	-	Low Density Lipoprotein – Cholesterol
LMIC	-	Low and Middle Income Countries
MTRH	-	Moi Teaching and Referral Hospital
MUSOM	-	Moi University School of Medicine
NRTI	-	Nucleoside reverse transcriptase inhibitors
NNRTI	-	Non – nucleoside reverse transcriptase inhibitors
PI	-	Protease inhibitor
SSA	-	Sub-Saharan Africa
WHO	-	World Health Organization

Definition of Terms

Dysglycemia

The American Diabetes Association (ADA) defines dysglycemia as the presence of diabetes mellitus (fasting plasma glucose > 7.0mmol/L or glycated hemoglobin >=6.5% or 2-hour plasma glucose >= 11.1mmol/L during an oral glucose tolerance test [OGGT]) and impaired fasting glucose or impaired glucose tolerance (fasting blood sugar $\geq$ 5.6 mmol/L but $\leq$ 6.9 mmol/L or glycated hemoglobin 5.6%-6.4%).

Dyslipidemia

The Third Report of The National Cholesterol Education Program Expert panel on Detection, Evaluation and Treatment of High Blood Cholesterol In Adults (NCEP Adult Treatment Panel III) defines dyslipidemia as presence of any of the following: total cholesterol > 6.2 mmol/l, LDL-cholesterol > 2.6 mmol/L, HDL-cholesterol < 0.9 mmol/l, and/or triglycerides > 2.3 mmol/l.

Body composition

Body composition is used to describe the percentages of fat, bone, water and muscle in human bodies.

Body fat percentage

Body fat percentage is the amount of body fat as a proportion of the body weight.

CHAPTER ONE: INTRODUCTION

1.1 Background

Human immunodeficiency virus (HIV), the virus that causes Acquired Immunodeficiency Syndrome (AIDS), has been one of the most devastating epidemics in human history. Sub-Saharan Africa (SSA) bears the greatest burden of disease. In the 2010 World Health Organization (WHO) global report, 22.5 m (20.9 – 24.2m) adults and children were HIV-infected in SSA. The adult HIV prevalence in this population was 5.0% (WHO, 2010).

In 2014, the Kenya National AIDS and Sexually Transmitted Infections Control Program (NASCOP) estimates showed the adult national HIV infection prevalence to be 6.0% (NASCOP, 2014). HIV prevalence rates vary throughout the country depending on the social, cultural and economic circumstances. The western region of Kenya has a HIV prevalence ranging between 6.3% and 14.9%, (Nyanza – 14.9%; Western - 5.4%; and Rift Valley - 6.3%) (NASCOP, 2007).

HIV infection is now considered a chronic illness due to the use of antiretroviral therapy (ART) (Carpenter et al., 2000; Mocroft et al., 1998; Palella et al., 1998). More people are receiving ART in all regions of the world than at any previous time in the history of the epidemic (WHO, 2010). Patients receiving long-term ART exhibit a number of metabolic complications, including lipid abnormalities, dysregulation of glucose metabolism, body-fat redistribution, mitochondrial abnormalities, and bone abnormalities. In addition, they manifest with the sequelae of these metabolic

complications that include atherosclerosis, coronary artery disease, myocardial infarction, ischemic heart disease and peripheral arterial disease (Brown, Li, et al., 2005). Most of these metabolic complications are part of the traditional risk factors for cardiovascular diseases and may increase the patient's risk for these diseases (S. Grinspoon & Carr, 2005).

Cardiovascular diseases (CVD) are widely recognized as a complication of HIV infection (Sklar & Masur, 2003). These CVDs include atherosclerosis, coronary heart disease (CHD), myocardial infarctions (MI), atrial fibrillation, heart failure and peripheral arterial disease (Bloomfield et al., 2011; Butt et al., 2011; Hsu et al., 2013; Tseng et al., 2012). HIV infection itself has been associated with increased risk of cardiovascular disease and heart failure (Butt et al., 2011). There is a significant and graded association between HIV severity and atrial fibrillation (Hsu et al., 2013). Cardiovascular abnormalities that include dilated cardiomyopathy, pulmonary hypertension and prolonged QTc intervals have been associated with sudden cardiac deaths in HIV-infected persons (Tseng et al., 2012).

Dyslipidemia and insulin resistance manifested by impaired glucose tolerance and diabetes mellitus (DM) are thought to enhance atherosclerosis explaining the link between ART and CVD (Friis-Moller et al., 2007; Kiage et al., 2013). There is a direct relationship between levels of Low Density Lipoprotein cholesterol (LDL-C) and the rate of new onset coronary heart disease as demonstrated by the Framingham heart study, (Wilson et al., 1998) Multiple risk factor intervention study, (Stamler,

Wentworth, & Neaton, 1986) and Lipids research clinics Trial (I, 1984; II, 1984). Estimates from the WHO on ischemic heart disease predict that it will be among the first three most common causes of death in the HIV population in Low and Middle Income Countries (LMICs) by 2030 (Mathers & Loncar, 2006). The synergistic impact of HIV and CVD will be a major health concern in the near future (S. K. Grinspoon et al., 2008). HIV infected patients have the classical CV risk factors superimposed to different extents on the impact of HIV associated events that include HIV viremia, ART, immunosuppression and opportunistic infections (Blanco et al., 2010). The care for HIV infected patients should include both identification and management of associated cardiovascular risk factors (Bloomfield et al., 2011).

The mechanisms underlying HIV associated dyslipidemia remain unclear but include factors leading to increased lipoprotein synthesis as well as those causing decreased clearance (Shor-Posner et al., 1993). A large cross-sectional study in 2003 showed an increased prevalence of elevated total cholesterol ($\geq$ 6.2 mmol/L) in 27% of participants receiving ART that included a protease inhibitor (PI), 23% receiving a non-nucleoside reverse transcriptase inhibitor (NNRTI), and 10% receiving only a nucleoside reverse transcriptase inhibitor (NRTI), as compared with 8% of previously untreated patients (Friis-Moller et al., 2003). Dillon *et al.,* in systematic review and meta-analysis in SSA in 2013 showed that HIV infection was associated with higher triglycerides, lower LDL and lower HDL. It also showed that ART use was associated with higher HDL and LDL cholesterol (Dillon et al., 2013).

The incidence of DM in HIV-infected men with ART exposure in the Multicenter AIDS cohort study was four times greater than that of HIV-seronegative men (Brown, Cole, et al., 2005). A study at Kenyatta National Hospital (KNH) in 2006 showed that the overall prevalence of dyslipidemia was 63.1% and that of dysglycemia was 20.7% among HIV positive patients at the hospital (Manuthu, Joshi, Lule, & Karari, 2008). High total cholesterol occurred in 39.2% of patients on ART compared to 10% in ART naïve patients ($p<0.0001$, OR 5.18, CI 3.11 to 10.86). Though these studies vary in results and methodology, they show important problems of dyslipidemia and dysglycemia in HIV infected patients.

Drugs within each class of ART have different effects on lipid profiles (Riddler et al., 2003). Higher levels of serum hyperlipidemia have been associated with use of PI's (fosamprenavir/ritonavir, lopinavir/ritonavir) and efavirenz (Hill, Sawyer, & Gazzard, 2009). Patients on abacavir/lamivudine, zidovudine/lamivudine or stavudine/lamivudine combination therapy have also showed significantly higher levels of lipid parameters (Hill et al., 2009). Two main classes of ART have been associated with insulin resistance and hyperinsulinemia: NRTIs and PIs (Brown, Li, et al., 2005). PIs increase hepatic triglyceride synthesis and thus higher plasma triglyceride levels (Lenhard, Croom, Weiel, & Winegar, 2000). Cumulative exposure to NNRTI's (nevirapine and efavirenz) has not been associated with markers of insulin resistance (Brown, Li, et al., 2005).

The care of HIV infected patients has changed rapidly over the past decade with the introduction of more efficacious, durable and tolerable HIV care and treatment options. Longevity has been shown to predispose to some of the traditional risk factors of CVD. Knowledge of the effect of HIV and the changing ART medications on dyslipidemia and dysglycemia is currently unknown in our setting. The aim of this study was therefore to determine the association between the prevalence of newly diagnosed dyslipidemia and dysglycemia with ART use and body composition among HIV infected adults receiving care the Academic Model Providing Access to Healthcare (AMPATH) HIV clinic at the Moi Teaching and Referral Hospital.

1.2 Problem statement

Dyslipidemia and insulin resistance are important risk factors of atherosclerosis. Although atherogenesis is a multifactorial process, abnormalities in lipid metabolism lead to dyslipidemia which is one of the key factors representing about 50% of all population – attributable risk of developing CVD (Millan et al., 2009). There is a direct relationship between levels of LDL-C and the rate of new onset coronary heart disease (I, 1984; II, 1984; Stamler et al., 1986; Wilson et al., 1998). LDL-C particles are small and dense and are highly atherogenic as they are more likely to form oxidized LDL and thus are less readily cleared and more readily adhere to and subsequently invade the arterial wall (Nesto, 2005). Insulin resistance leads to accelerated atherosclerosis with its associated clinical sequelae of coronary artery disease, myocardial infarction, ischemic heart disease and peripheral arterial disease (Toth, 2013).

There is evidence showing that cardiovascular diseases are increasing among HIV infected patients (Blanco et al., 2010; S. Grinspoon & Carr, 2005; Guaraldi et al., 2009; Sklar & Masur, 2003), and the cardiovascular risk factors of dyslipidemia and dysglycemia have been established among these patients (Adewole et al., 2010; Buchacz et al., 2008; Guaraldi et al., 2009). This has been attributed to the chronic inflammation due to the HIV infection itself and the metabolic complications due to prolonged use of ART. In addition, HIV infected patients are growing older and these risk factors could be a feature of the normal aging process. As HIV infected patients live longer due to use of ART, CVDs and cardiovascular risk factors will increase and will require additional attention and interventions. This is particularly important in SSA where there is an epidemiological transition from infectious diseases to chronic non-communicable diseases (including CVD's). The global burden of disease estimates show that by 2030, ischemic heart disease will be among the first three most common causes of death in HIV infected patients in LMICs including Kenya (Mathers & Loncar, 2006). Manuthu *et al.,* at KNH in 2006 a high prevalence of dyslipidemia among HIV infected patients (Manuthu et al., 2008). This study was done about a decade ago and most patients on ART were on stavudine based therapy. In addition, Njoroge *et al*., in Nairobi also found a high prevalence on dyslipidemia in 2014. However, despite the large number of patients on care at AMPATH, there are no studies on the prevalence's' of dyslipidemia and dysglycemia which are two important cardiovascular risk factors.

1.3 Study justification

HIV infection continues to be a public health problem in Kenya and SSA. This means that ART use will continue into the near future. The long duration of the HIV infection with the associated use of ART will therefore predispose and possibly lead to important CVDs. These CVDs will be an additional morbidity among HIV infected patients who already have the burden of infectious opportunistic diseases and malignancies. Long term clinical sequelae of dyslipidemia and dysglycemia which are modifiable cardiovascular risk factors are devastating leading to atherosclerosis, myocardial infarction, coronary heart diseases and peripheral arterial diseases.

The AMPATH program in western Kenya provides care to a large number of HIV infected patients over 30,000 (over 14,000 on ART) patients' currently receiving care at the AMPATH clinic at MTRH. With the global burden of disease estimates showing that ischemic heart disease will be among the first three most common causes of death among HIV infected patients globally by 2030, this problem will also be seen among patients receiving care at AMPATH. CVDs are chronic diseases with modifiable risk factors whose timely and appropriate interventions can alter the disease progression.

Early and effective intervention measures to control and mitigate these CVDs among HIV infected patients receiving care at AMPATH will only be possible through identification and management of important modifiable cardiovascular risk factors that include dyslipidemia and dysglycemia. International HIV treatment guidelines have recommended routine screening for dyslipidemia and dysglycemia among HIV

infected patients. These guidelines have however not been effected at AMPATH due to the high cost and lack of awareness on the extent of the problem. This study therefore sought to determine the magnitude of the problem of dyslipidemia and dysglycemia which are two modifiable cardiovascular risk factors and their possible association with ART. This data will be used to inform clinicians and policy makers to institute timely and appropriate interventions where necessary that will be feasible and cost effective to prevent long term morbidity and mortality of CVDs. The aim of this study was therefore to determine the association between the prevalence of newly diagnosed dyslipidemia and dysglycemia with ART use and body composition among HIV infected adults receiving care at MTRH, Eldoret, Kenya.

1.4 Research question

What is the association between the prevalence of newly diagnosed dyslipidemia and dysglycemia with ART use and body composition among HIV infected adults receiving care at the AMPATH HIV clinic at MTRH?

1.5 Research objectives

1.5.1 Broad objective

To determine the association between the prevalence of newly diagnosed dyslipidemia and dysglycemia with ART use and body composition among HIV infected adults receiving care at the AMPATH HIV clinic at MTRH.

1.5.1 Specific objectives

1. To compare the prevalence of newly diagnosed dyslipidemia and dysglycemia among ART naïve and ART experienced HIV infected adults receiving care MTRH.

2. To determine the association between the prevalence of newly diagnosed dyslipidemia and dysglycemia and ART use.

3. To determine the association between the prevalence of newly diagnosed dyslipidemia and dysglycemia and body composition.

CHAPTER TWO: LITERATURE REVIEW

2.1 Epidemiology

In 2010, the World Health Organization (WHO) global AIDs report showed that 22.5million (20.9 – 24.2m) adults and children in SSA were HIV infected. The adult HIV prevalence in this population was 5.0% (WHO, 2010). In 2014, the Kenya National AIDS and Sexually Transmitted Infections Control Program (NASCOP) estimates showed the adult national HIV infection prevalence to be 6.0% (NASCOP, 2014). HIV prevalence rates vary throughout the country depending on the social, cultural and economic circumstances. The western region of Kenya has a HIV prevalence ranging between 6.3% and 14.9%, (Nyanza – 14.9%; Western - 5.4%; and Rift Valley - 6.3%) (NASCOP, 2007).

2.2 HIV and lipid abnormalities

Dyslipidemia is defined by an increase in serum triglyceride levels of varying and sometimes major intensity, either isolated or combined with a more moderate increase in low density lipoprotein cholesterol (LDL-C), while high density lipoprotein cholesterol (HDL-C) levels may decrease or remain unchanged.(Chanu & Valensi, 2005) The mechanisms underlying HIV associated dyslipidemia remain unclear but include factors leading to increased lipoprotein synthesis as well as those causing decreased clearance (Shor-Posner et al., 1993).

Early studies before the availability of ART in persons infected with HIV type 1 demonstrated lipid abnormalities (Riddler et al., 2003). These abnormalities were

hypertriglyceridemia in association with low HDL and LDL (Grunfeld et al., 1992). Shor-Posner *et al*., in 1993 demonstrated significantly lower levels of total, high-density, and low-density lipoprotein cholesterol in HIV-1-seropositive men, relative to sero-negative controls ($p < 0.05$), with 40% of the HIV-1-infected group demonstrating hypocholesterolemia (less than 150 mg/dL). Low values of total, high-density, and low-density cholesterol were associated with elevated levels of beta 2-microglobulin in HIV-1-seropositive men. No difference between the groups was noted for serum triglycerides (Shor-Posner et al., 1993).

The mechanism of hypocholesterolemia in HIV and other infections is not known (Grunfeld et al., 1991). Contributing factors may include increased apo-lipoprotein E levels, increased hepatic synthesis of very-low-density lipoprotein, and decreased clearance of triglycerides (Grunfeld et al., 1997; Hellerstein et al., 1993). Dyslipidemia may also be due in part to the effects of viral infection, acute-phase reactants, and circulating cytokines, including interferon-*alpha* (Christeff, Melchior, de Truchis, Perronne, & Gougeon, 2002). Interferon-*alpha* has been shown to modulate triglyceride metabolism *in vitro* and *in vivo* (Feingold et al., 1989).

Friis-Moller *et al.,* in a large cross-sectional study in 2003 showed an increased prevalence of elevated total cholesterol ($\geq$ 6.2 mmol/L) in 27% of participants receiving ART including a protease inhibitor (PI), 23% receiving a non-nucleoside reverse transcriptase inhibitor (NNRTI), and 10% receiving only a nucleoside reverse transcriptase inhibitor (NRTI), as compared with 8% of previously untreated patients

(Friis-Moller et al., 2003). The corresponding percentages of hypertriglyceridemia were 40%, 32% and 23%, as compared with 15% among previously untreated patients. Low levels of HDL-C were reported in 27%, 19% and 25% respectively, as compared with 26% who were previously untreated. Dillon *et al.,* in systematic review and meta-analysis in SSA in 2013 showed that HIV infection was associated with higher triglycerides, lower LDL and lower HDL. It also showed that ART use was associated with higher HDL and LDL cholesterol (Dillon et al., 2013).

Drugs within each class of ART have different effects on lipid values (Riddler et al., 2003). Buchacz *et al.*, in Uganda demonstrated changes in lipid profiles of patients on stavudine based first line regimen in 2008 (Buchacz et al., 2008). Stavudine related toxicities that included peripheral neuropathy, lactic acidosis & symptomatic hyperlactatemia and lipodystrophy were demonstrated in several regions of SSA (Forna et al., 2007; Menezes, Maskew, Sanne, Crowther, & Raal, 2011; van Griensven et al., 2007; van Griensven et al., 2010; van Oosterhout et al., 2012). Higher serum levels of dyslipidemia have been associated with use of fosamprenavir/ritonavir, lopinavir/ritonavir and efavirenz when these drugs are combined with NRTI's other than Tenofovir (Hill et al., 2009). Patients treated with efavirenz showed similar rises in total cholesterol and LDL-C compared with the PIs (fosamprenavir/ritonavir, lopinavir/ritonavir) (Gotti et al., 2012; Hill et al., 2009). In addition, patients treated with abacavir/lamivudine, zidovudine/lamivudine, or stavudine/lamivudine showed elevations in lipid parameters (Hill et al., 2009).

Use of PI's has been associated with the most common pattern of dyslipidemia (Behrens et al., 1999; Berthold et al., 1999). These drugs increase hepatic triglyceride synthesis and plasma triglyceride levels (Lenhard et al., 2000). They also tend to increase total cholesterol levels, an effect which varies with the individual drugs in this class (Periard et al., 1999). Carr *et al*., showed that PI treatment leads to unspecified interactions with two proteins (cytoplasmic- acid binding protein type 1 and low density lipoprotein-related protein) that regulate lipid metabolism. This inhibition results in hyperlipidemia (Carr, Samaras, Chisholm, & Cooper, 1998). In-vitro studies have shown that PI's can inhibit lipogenesis, and that indinavir may do this via altered retinoid acid signaling (Zhang et al., 1999). Patients receiving combination therapy (NRTI and PI) also have alterations in apolipoprotein B and have an increase in small, dense LDL 2; an increase in apolipoprotein B and a shift towards triglyceride-rich very-low-density lipoprotein (Schmitz et al., 2001). PI's have also been shown to decrease proteasomal degradation of nascent lipoprotein B in vitro (Liang et al., 2001).

2.3 HIV and dysglycemia

Dysregulation of glucose metabolism manifests itself as insulin resistance and hyperinsulinemia. Hyperinsulinemia is a surrogate marker of insulin resistance. Studies have shown that insulin resistance and hyperinsulinemia results from the HIV infection itself and the toxicity due to the various agents used for treatment of HIV (Brown, Li, et al., 2005; Gelato, 2003).

Insulin resistance and hyperinsulinemia presents itself in a broad spectrum of impaired glucose metabolism from glucose intolerance to frank DM (Gelato, 2003). Several studies have demonstrated insulin resistance due to HIV infection itself. First, Todd *et al*., in a prospective cohort of 533 HIV-infected and 755 HIV-sero-negative men in the Multicenter AIDS Cohort Study evaluated at 6-month intervals between 1999 and 2003 showed that the presence of HIV infection, regardless of ART treatment status, was associated with decreased insulin sensitivity as well as an increased risk of fasting hyperinsulinemia (Brown, Tassiopoulos, Bosch, Shikuma, & McComsey, 2010). Second, in a cohort of 75 HIV-infected women (age, 25–46 years), and 30 healthy weight-matched premenopausal control subjects evaluated in 1994-1997, Hadigan *et al*., demonstrated significant fasting hyperinsulinemia and an increased insulin-to-glucose ratio in HIV infected women. The mean insulin level was more than twice the level of weight-matched control subjects and above the reference range of the assay in 30% of HIV-infected subjects (Hadigan et al., 1999).

It has been postulated that chronic HIV infection and the resulting cytokine elaboration may contribute to hyperglycemia (Gelato, 2003). Pro-inflammatory cytokines, such as tumor necrosis factor *alpha* (TNF-), may induce insulin resistance by binding to insulin-responsive elements in skeletal muscle (Gelato, 2003; Hotamisligil et al., 1996). In a nested case-control study, comparing 55 previously ART-naive individuals who developed diabetes 48 weeks after ART initiation (case subjects), with 55 individuals who did not develop diabetes during a comparable follow-up (control subjects), inflammatory markers 48 weeks after ART initiation were associated with

increased risk of diabetes. TNF- was a significant predictor of incident diabetes in adjusted analyses (Brown et al., 2010).

There are two main classes of ART that have been associated with insulin resistance and hyperinsulinemia: NRTIs and PIs. Cumulative exposure to NNRTI's; nevirapine and efavirenz, has not been associated with markers of insulin resistance (Brown, Li, et al., 2005). The strength of association between insulin resistance and the various ART regimens varies in different studies.

In the DAD study, (Data collection on Adverse events of anti-HIV Drugs), all ART regimens were associated with an increased risk of diabetes when compared with ART naïve patients. The DAD study was an observational study formed by the collaboration of previously established HIV cohorts in Europe, America & Australia and enrolled 17 852 patients (Friis-Moller et al., 2003). Ledergerber *et al.,* in the Swiss HIV Cohort Study showed that treatment with PI– and NRTI–containing regimens was associated with an increased risk of developing Type 2 Diabetes mellitus (T2 DM). A total of 123 of 6513 persons experienced diabetes mellitus during 27,798 person-years of follow-up (PYFU), resulting in an incidence of 4.4 cases per 1000 PYFU (95% confidence interval [CI], 3.7–5.3 cases per 1000 PYFU) (Ledergerber et al., 2007). In the Multicenter AIDS cohort study 57 (14%) of the 411 HIV-infected men using ART at the baseline visit had prevalent DM, compared with 33 (5%) of the 711 HIV-seronegative men (prevalence ratio=4.6; 95% CI, 3.0-7.1). The rate of incident DM was 4.7 cases per 100 person-years among HIV-infected men using HAART compared

with 1.4 cases per 100 person-years among HIV sero-negative men (rate ratio=4.11; 95% CI, 1.85-9.16, adjusted for age and body mass index), during the 4-year observation period, based on a median follow-up of 2.3 years (Brown et al., 2010). In contrast to the above findings, a large systematic review and meta-analysis in SSA in 2013 did not show any association between HIV infection and fasting blood glucose or glycated hemoglobin (Dillon et al., 2013).

NRTI are a common component of all ART regimens (Carpenter et al., 2000). Cumulative exposure to NRTI, particularly lamivudine (3TC) and stavudine (d4T) has the strongest association of any drug class with fasting markers of insulin resistance. The mechanisms underlying this association have not been well clarified (Shaw, Sicree, & Zimmet, 2010). Brambilla *et al.,* found that d4T exposure was associated with increased incidence of DM in an Italian cohort. DM was diagnosed in 16 out of 1011 patients with a median follow-up of 289 days [person year incidence 2.06, 95% CI, 1.18–3.33]. Use of d4T was associated with a significantly higher risk of developing DM, hazard ratio 16.0 [95% CI 3.03–83.8, P=0.001] (Brambilla et al., 2003).

A prospective randomized study of sixteen healthy participants demonstrated that short-term administration of d4T reduced insulin sensitivity in healthy subjects. In addition, use of d4T resulted in 52% reduction in muscle mitochondrial DNA within treated subjects (Fleischman et al., 2007). Mitochondrial dysfunction precedes the development of diabetes in insulin-resistant offspring of patients with T2 DM

(Petersen, Dufour, Befroy, Garcia, & Shulman, 2004). Brinkman *et al*., postulated that the mitochondrial toxicity due to NRTI is a result of inhibition of mitochondrial DNA polymerase-gamma with impairment of hepatic glycogen and fat oxidation, leading to increased oxidation of peripheral energy stores and to lipoatrophy (Brinkman, Smeitink, Romijn, & Reiss, 1999). A study by Gerschenson *et al*., published in 2009 showed that mitochondrial function, morphology and metabolic profiles improved after switching from a d4T regimen to a tenofovir based regimen. At entry into the above study, when patients were on d4T containing regimen, mitochondria appeared abnormal with poorly defined inner membrane structures and often lysed outer membranes. However, 48 weeks after substituting with tenofovir, mitochondria appeared healthier, with well-defined cristae architecture with intact double outer membranes (Gerschenson et al., 2009).

Todd *et al*., showed that HIV infected patients taking PI-containing ART regimens in the 6 months prior to assessment appeared to have the most insulin resistance compared to non-PI regimens. However, cumulative exposure to PI drugs as a class showed no increased risk of elevated insulin resistance markers (Brown, Li, et al., 2005). A cross-sectional study of 67 patients showed that treatment with PI is associated with peripheral insulin resistance leading to impaired or diabetic oral glucose tolerance in some of the patients. 61% of the patients in this study had peripheral insulin resistance (Walli et al., 1998).

The mechanism by which PI causes insulin resistance is not well understood (Ismail, King, Anwar, & Pillay, 2013), but several mechanisms have been proposed. First, the catalytic region of HIV-1 protease, to which PIs bind, has approximately 60% homology to regions within two proteins that regulate lipid metabolism; cytoplasmic retinoic-acid binding protein type 1 (CRABP-1) and low density lipoprotein-receptor-related protein (LRP). Carr et al. hypothesized that PI inhibit CRABP-1-modified, and cytochrome P450 3A-mediated synthesis of cis-9-retinoic acid, a key activator of the retinoid X receptor; and peroxisome proliferator activated receptor type gamma (PPAR-gamma) heterodimer, an adipocyte receptor that regulates peripheral adipocyte differentiation and apoptosis (Carr et al., 1998). PI binding to LRP would impair hepatic chylomicron uptake and triglyceride clearance by the endothelial LRP-lipoprotein lipase complex. The resulting hyperlipidaemia contributes to central fat deposition, insulin resistance, and, in susceptible individuals, T2 DM.

Second, some PIs (ritonavir and nelfinavir) have been shown to impair -cell function. Ritonavir and nelfinavir decrease calcium [Ca2+] channels leading to reduction of glucose-induced insulin secretion. The effects of ritonavir on [Ca2+] channels and insulin secretion are not mediated by an influence on Potassium adenosine triphosphate (KATP) channels or [Ca2+] channels, the predominant channels in -cell stimulus-secretion coupling or by interference with the endoplasmic reticulum Ca2+ store (Dufer, Neye, Krippeit-Drews, & Drews, 2004).

PI's have also been shown to induce insulin resistance in vitro by reducing glucose transport mediated by glucose transporter 4 (Glut-4) (Marshall, Murata, Hresko, & Mueckler, 1993; Murata, Hruz, & Mueckler, 2000). Glut-4 is the principal transporter isoform mediating insulin stimulated glucose uptake in the skeletal, cardiac and fat cells (James, Strube, & Mueckler, 1989). The inhibitory effect of PI's on Glut-4 is therefore likely the direct cause of insulin resistance observed in patients receiving this class of drugs (Murata et al., 2000).

2.4 HIV infection, dyslipidemia and dysglycemia as cardiovascular disease risk factors

Dyslipidemia and insulin resistance manifested by impaired glucose tolerance and diabetes mellitus are thought to enhance atherosclerosis (Kiage et al., 2013). LDL-C particles are small and dense and are highly atherogenic as they are more likely to form oxidized LDL and thus are less readily cleared (Nesto, 2005). They also more readily adhere to and subsequently invade the arterial wall. The atherogenicity of LDL-C may also be enhanced by non-enzymatic glycation (Krentz, 2003). There is a direct relationship between levels of LDL-C and the rate of new onset coronary heart disease (I, 1984; II, 1984; Stamler et al., 1986; Wilson et al., 1998). Hypertriglyceridemia is a strong predictor of coronary heart disease (Nesto, 2005).

Insulin resistance leads to high levels of very low-density lipoprotein (VLDL), which contain a high concentration of triglycerides, resulting in high serum triglyceride levels and low serum HDL-C levels (Nesto, 2005). Patients with insulin resistance are highly

vulnerable to the development of accelerated atherosclerosis with its clinical sequelae of coronary artery disease and myocardial infarction, carotid artery disease, ischemic stroke and peripheral arterial disease (Toth, 2013).

HIV infection itself has been associated with increased risk of cardiovascular disease and heart failure (Butt et al., 2011). There is a significant and graded association between HIV severity and atrial fibrillation (Hsu et al., 2013). Cardiovascular abnormalities that include dilated cardiomyopathy, pulmonary hypertension and prolonged QTc intervals have been associated with sudden cardiac deaths in HIV-infected persons (Tseng et al., 2012).

Global burden of disease estimates from the WHO for 2030 predict that ischemic heart disease will be among the first three most common causes of death in HIV infected patients in LMICs (Mathers & Loncar, 2006). The synergistic impact of HIV and CVD must be viewed as a major health concern in the aging population (S. K. Grinspoon et al., 2008). Classical CV risk factors may be superimposed to different extents on the impact of HIV infection, its associated morbidities and the ART used to control the HIV infection (Blanco et al., 2010). Therefore, identification and management of cardiovascular risk factors should be considered in the care of HIV infected patients (Bloomfield et al., 2011).

CHAPTER THREE: RESEARCH METHODOLOGY

3.1 Study setting

The study was conducted at the AMPATH HIV clinics at the Moi Teaching and Referral Hospital (MTRH). MTRH serves as the teaching hospital for Moi University School of Medicine (MUSOM) and is the second largest tertiary referral center in Kenya. It serves a population of 16 million people (40% of Kenya's population) in western Kenya and is the primary care site for the 300,000 urban population of Eldoret town.

The hospital has a total bed capacity of 720 with 140 beds in two adult general medical wards. There are four AMPATH HIV clinics, dubbed modules, one of which is for pediatric patients. The AMPATH clinics offer a comprehensive HIV care program with various services ranging from Prevention of Mother-to-Child transmission of HIV, ART delivery, Multidrug Resistance – Tuberculosis care, food equity, microfinance, Orphans and Vulnerable Children and Women's shelter. The AMPATH Centre at MTRH has over 30,000 (over 14,000 on ART) HIV-infected patients actively enrolled into care. AMPATH is also working to improve primary health care services throughout the western region of Kenya utilizing its HIV care model.

3.2 Study Population

The study population was adult HIV-infected patients enrolled into care in the AMPATH clinic. The participants were grouped into two groups based on ART use: ART naïve and ART experienced study groups. Approximately 80 adult patients are

seen daily in each module at AMPATH. Approximately 50% of patients on follow up at AMPATH clinics are on ART.

3.3 Eligibility Criteria

3.3.1 Inclusion criteria

1. HIV-infected adults receiving care in the AMPATH HIV clinic. ART experienced participants must have been on ART for more than one year at the time of recruitment. There was no limitation in the duration of follow-up for the ART naïve participants.

2. Patients aged 18 years and older.

3.3.2 Exclusion criteria

1. Pregnant women. In pregnancy, there are many physiological changes due to hormonal changes. These changes are associated with altered basal metabolic processes that revert back to normal at the end of the pregnancy period.

2. Patients with known diabetes mellitus and dyslipidemia by either patient report or chart review.

3. Patients with known history of renal, heart & hepatic diseases and malignancies. These are chronic conditions which are known to alter the metabolic processes and therefore predispose to dysregulation of lipid and glucose metabolism.

3.4 Study design

This was a cross-sectional comparative study.

3.5 Sample size

In order to be 95% sure that the true difference between the proportion of HIV infected patients who were on ART with dysglycemia and the proportion of HIV infected patients who were not on ART with dysglycemia was at least 15% with a probability of 80%, a sample size to compare two proportions was computed using the following formula.(Hulley, 2007)

$$n = \left[\frac{z_{1-\frac{r}{2}}\sqrt{(p_1+p_2)(1-p_1+1-p_2)\big/2} + z_{1-s}\sqrt{p_1(1-p_2)+p_2(1-p_2)}}{p_1-p_2} \right]^2$$

Where:

p_1 was the proportion of HIV infected patients not on ART with dysglycemia.

p_2 was the proportion of HIV infected patients on ART with dysglycemia.

$z_{1-\frac{r}{2}}$ was the $100\left(1-\frac{r}{2}\right)$ percentile of the standard normal distribution under type I error while z_{1-s} was the $100(1-s)$ percentile of the standard normal distribution under type II error.

p_1-p_2 gave the effect size called the true difference herein.

The proportion of HIV infected patients not on ART with dysglycemia at Kenyatta National Hospital was found to be 21%.(Manuthu et al., 2008) This study intended to find a true difference of at least 15% between the two groups of study participants.

The probability of wrongfully rejecting the null hypothesis when there is no difference between the proportions of study participants with dysglycemia was set to be 5%. The study was powered with 80% chance of being able to detect the existence of a true difference in the two study groups.

The study assumed an effect size to be the difference between the two proportions. This effect size was taken as the smallest effect that would be important to detect and any effect smaller that this would not be of clinical significance. The test is two tailed, therefore an effect in either direction would be interpreted.

With these conditions the study required a sample size of 141 participants in each study group, giving a total of 282 participants, in order to detect the true difference between the two groups.

A sample size to detect a true difference of 15% between HIV positive patients on ART with dyslipidemia to the proportion of HIV positive patients not on ART with dyslipidemia with a probability of 80% gave us a sample size of 100 patients per arm to give a total of 200 participants. The proportion of HIV infected patients not on ART with dyslipidemia was taken as 10% from the study at KNH in 2006.(Manuthu et al., 2008) This sample size was smaller compared to that required to determine the true difference between the HIV infected patients on ART with dysglycemia and HIV infected patients not on ART with dysglycemia. Since the intention of the study was to

determine the difference in the two characteristics, we therefore took the larger sample size of 282.

3.6 Sampling technique

Systematic random sampling technique was used to sample the participants meeting the inclusion criteria. Simple random sampling was used to identify the first study participant in any randomly selected module out of the first five patients who arrived at the clinic on any given day. This was made from the records available in the clinic (nurse station). Every fifth patient who reported to the clinic (nurse station) was subsequently approached by the principal investigator or the research assistant and requested to take part in the study. If the fifth patient was not eligible to participate in the study, the next patient was approached until an eligible participant was recruited. Every fifth interval was arrived at by considering that an average of eighty patients was seen on a daily basis in each module and the study target was to recruit ten participants each day. Eighty divided by 10 gives an interval of eight. This interval was adjusted downwards to adjust for possible non-response and exclusions, hence arriving at every fifth patient. This sampling interval assumed that only 50% of the patients approached would meet the inclusion criteria and would consent to participate in the study.

Once a patient gave consent to participate in the study, their ART exposure status was determined and the patient was grouped into the ART naïve or the ART experienced study group. Each of the two study groups had equal number of study participants. Recruitment was done until the desired sample size was achieved in each of the two groups.

3.7 Data variables

3.7.1 Primary outcome variables

1. Dysglycemia was defined as the presence of diabetes mellitus or impaired fasting glucose.(Diagnosis & Mellitus, 2003)

    i. Pre-existing diabetes mellitus – this was be based on patient's self-report i.e any person who had been told they have DM in the past by a clinician and was taking medication for it or had taken medication for it in the past.

    ii. Diagnosis of DM and glucose intolerance was based on the criteria of the American Diabetes Association (ADA) 2013 (Association, 2013). DM was defined as a FBS $\geq$ 7.0mmol/L, or classic features of DM that included polyuria, polydipsia & polyphagia and hyperglycemia and a random blood glucose $\geq$ 11.1mmol/L. Impaired fasting glucose was defined as FBS $\geq$ 5.6 mmol/L but $\leq$ 6.9 mmol/L.

2. Dyslipidemia was defined according to the Third Report of The National Cholesterol Education Program Expert panel on Detection, Evaluation and Treatment of High Blood Cholesterol In Adults (NCEP Adult Treatment Panel III) as presence of any of the following: total cholesterol > 6.2 mmol/l, LDL-cholesterol > 2.6 mmol/L, HDL-cholesterol < 0.9 mmol/l, and/or triglycerides > 2.3 mmol/l (National Cholesterol Education Program Expert Panel on Detection & Treatment of High Blood Cholesterol in, 2002).

3.7.2 Other data variables that were collected

1. CD4 count

2. Viral load

3. WHO clinical staging of HIV infection

4. Family, prior and current history of diabetes mellitus and hypertension

5. Alcohol intake

6. Cigarette smoking

7. Body mass index

8. Body composition measurement by bio impedance analysis (BIA) using a body composition analyzer was done – body fat mass and percentage, total body water and percentage, fat free mass and muscle mass, metabolic age, visceral fat rating and ideal body weight.

9. Blood pressure

3.8 Study procedure

The study was conducted within the HIV clinic at the AMPATH centre. Recruitment of participants into the study was done in the triage room and at the waiting bay as they waited to see the clinicians. The purpose of the study and potential benefits was explained to the participants individually in a language that they understood and all their questions were answered. Those who met the inclusion criteria and consented to participate in the study were enrolled after signing informed consent forms. Thereafter, the ART exposure status of the study participant was determined. The participants were then recruited either into the ART naïve or the ART experienced study group. The two

study groups had equal number of participants (141 participants in each group).
Recruitment was done until the desired sample size was achieved in each of the two
study groups.

The informed consent process explained the purpose of the study and the measures that
were taken to ensure the safety and the confidentiality of the participants' data. It also
emphasized that their participation in the study was voluntary. In addition, the
participants were allowed to change their mind later and quit before the end of the
study. The decision not to participate, or to quit the study, did not affect the health care
services they received in the clinic. The participants were also free to decline to answer
any particular question they do not wish to answer for any reason. The informed
consent forms are attached in Appendix II & III.

Participants were first taken through the study questionnaire (Appendix I).
Subsequently they had their anthropometric measurements (height – Appendix VIII,
body weight – Appendix IX and waist circumference – X) and blood pressure taken
(Appendix VI). Blood samples were taken for a RBS (Appendix V). RBS was done
with a point of care Abbot Optium exceed glucose meter (Appendix VII). All
participants were then requested to return for their next clinic visit early in the morning
in a fasting state to have a confirmatory FBS and fasting lipid profile (COBAS
INTEGRA Roche automatic analyzer) done. Fasting lipid profile was done on a venous
blood sample (Appendix IV). Body composition measurements by bio impedance
analysis using a body composition analyzer BC-420MA III were also done on the

return visit (Appendix XI). The body analyzer measures body composition using a constant current source with a high frequency current. Fat within the body allows almost no electricity to pass through, while electricity passes easily through water much of which is found in muscle. The body fat percentage and other body constituents is inferred from the resistance with which electricity passes through the body constituent (Tanita).

During recruitment into the study, participants' return to clinic dates and phone numbers were recorded in a diary. The participants were called by the Investigator/ Research assistant one day before their next clinic visit reminding them to come to the clinic in a fasting state. After blood had been drawn for FBS and fasting lipid profile, participants were offered breakfast of a cup of tea and a snack (mandazi). Those diagnosed with DM, pre-diabetes, hypertension, dyslipidemia, overweight and obesity were referred to the respective clinics for education and appropriate care. The same information was communicated to the attending clinicians. The algorithm for the study procedure is summarized in Figure 1 below.

Figure 1: Algorithm of study procedure
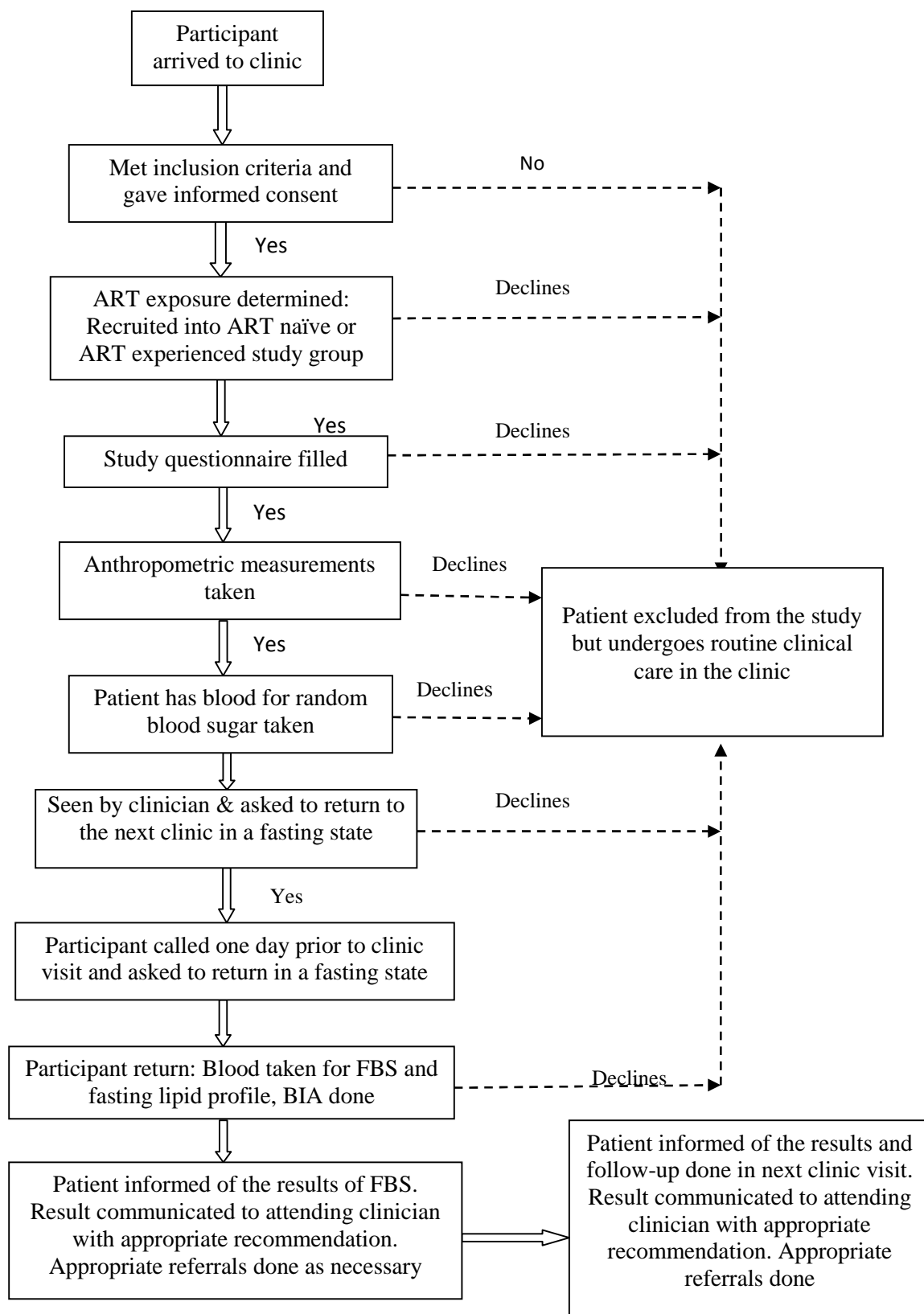
3.9 Data collection and management

3.9.1 Data collection

An interviewer administered structured questionnaire was used to collect the study participants' demographic, socioeconomic and clinical data. Anthropometric and laboratory measurements were recorded on a pro-forma. Data were dually entered into Epidata software and validated.

3.9.2 Data analysis

Data analysis was performed using STATA version 13 special edition. Categorical variables were summarized as frequencies and corresponding percentages while continuous variables that assumed the Gaussian distribution were summarized as mean and the corresponding standard deviation (SD). Continuous variables that violated the Gaussian assumptions were summarized as the median and the corresponding inter quartile range (IQR).

Normality assumption was assessed using Shapiro Wilks test. Association between categorical variables was assessed using Pearson's Chi Square test. We used the wald test for differences in the proportions to assess the differences in the proportions. The test for differences between continuous variables was done using the two sample t-test if the continuous variables assumed the Gaussian assumption otherwise we used Wilcoxon two sample test (a.k.a Mann Whitney U-test). We used logistic regression model to assess the strength of the association and direction of estimates between the variables. We reported the prevalence and the corresponding 95% confidence limits

(95% CL). Similarly, we reported the odds ratio (OR) and the corresponding 95% CL. The association between continuous variables and the categorical variables was assessed using Wilcoxon-rank sum test for skewed continuous variables and two sample t-tests for the continuous variables that assumed the Gaussian distribution. A p-value < 0.05 was considered statistically significant.

3.10 Ethical considerations

1. Ethics review and approval to conduct the study were sought and received from the Moi Teaching and Referral Hospital/Moi University Institutional Research and Ethics Committee before commencement of this study.

2. Permission to conduct the study was obtained from the management of MTRH and AMPATH.

3. Written informed consent was obtained from every participant before participating in the study.

4. All patient information was kept confidential with only the study results of participants with dyslipidemia and dysglycemia being shared with the attending clinicians.

5. The Study Investigators did not have any conflict of interest to declare.

6. No payment was made to the participants. However, all participants who returned to the clinic for a FBS and fasting lipid profile were offered breakfast of tea and mandazi to avoid keeping them hungry for the whole morning while in the clinic.

7. The clinicians directly managing the patients did not take part in recruitment or consenting of the participants and therefore there was a very minimal risk of coercion.

8. Study participants who were found to have deranged RBS and FBS were linked to care in the Diabetes clinic within the hospital. Participants who had deranged fasting lipid profiles were informed of the results. The attending clinicians were informed of any deranged RBS, FBS and fasting lipid profiles to facilitate follow-up of the patients in the subsequent clinics.

CHAPTER 4: RESULTS

4.1 Screening and enrollment

A total of 795 HIV infected patients at the MTRH HIV clinics were screened for the study between January and June 2014. Of these, 461 (192 ART naïve and 269 ART experienced) fulfilled the study inclusion criteria, and were enrolled. Of those enrolled, 300/461 (65%) returned for fasting blood sugar and lipid profile and were included in the analysis. Figure 2 shows the recruitment schema for the study. During the study period, AMPATH care policy was changed and all patients with a CD4 count < 500 cells/mm^{3}were being initiated on ART. ART naïve patients were therefore being given an earlier return appointment date to the clinic and could explain the fewer number of non-responders in the ART naïve arm compared to the ART experienced arm.
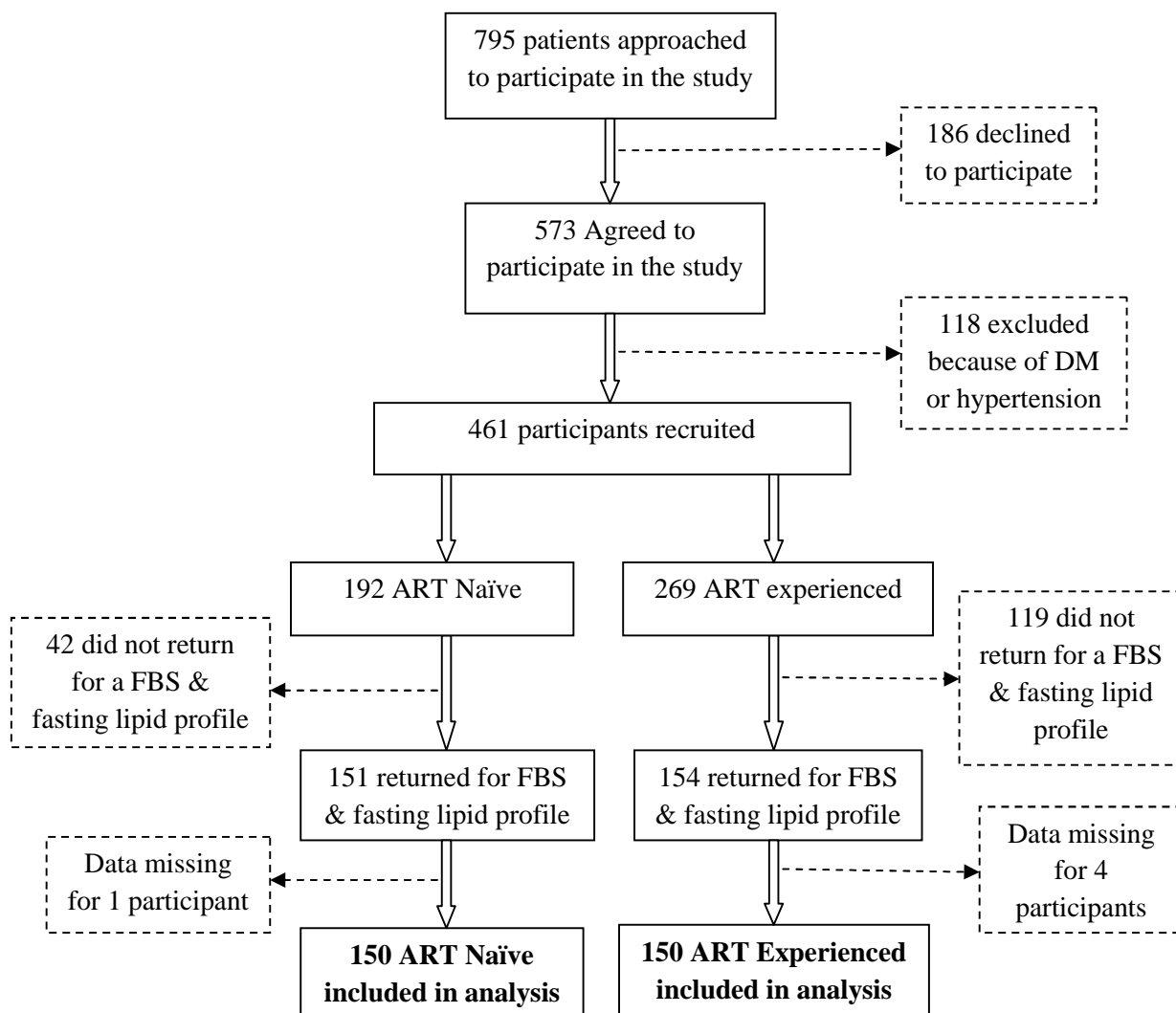
Figure 2: Recruitment Schema

4.2 Social and demographic characteristics

In comparison to non-respondent participants, the participants who returned for FBS and fasting lipid profiles were younger (p=0.045) and majority were female (p=0.001). Table 1 show the social and demographic characteristics of the participants who returned for fasting lipid & blood sugars and non-responders. Comparison of socio-demographic characteristics of non-responders and those who returned for FBS & fasting lipid profiles in the ART experienced group did not reveal any significant differences except for sex with more non-responders being males(p=0.003).

Table 1: Social and demographic characteristics

Variable	Loss to follow-up (n=161) n(%)/Median (IQR)/Mean(SD)	Included (n=300) n(%)/Median (IQR)/Mean(SD)	p-value
Sex (Female)	85 (53%)	209 (69%)	0.001
Age	41 (35 – 49)	39 (33 – 46)	0.045
Smoking (Yes)	21 (13%)	23 (8)	0.061
Alcohol use (Yes)	48 (30%)	95 (32%)	0.682
Family member with DM (Yes)	25 (16%)	33 (11%)	0.162
Family member with hypertension (Yes)	29 (18%)	38 (13%)	0.121
Marital status (n= 366)			
Single	16 (24%)	42 (14%)	
Married	38 (56%)	178 (60%)	
Divorced	0	5 (2%)	0.389[f]
Widowed	6 (9%)	30 (10%)	
Separated	8 (12%)	43 (14%)	
Occupation (n= 366)			
Farmer/casual	28 (41%)	106 (36%)	
laborer	11 (16%)	34 (11%)	
Employed/Business	6 (9%)	72 (24%)	0.045
Unemployed	23 (34%)	86 (29%)	
Education (n = 366)			
None	8 (12%)	10 (3%)	
Primary	31 (46%)	145 (49%)	0.063[f]
Secondary	23 (34%)	111 (37%)	
Tertiary	6 (9%)	32 (11%)	

4.3 Baseline clinical characteristics

Table 2: Baseline clinical characteristics

Variable	ART Naïve (n=150) Median (IQR)/ Mean (SD)/ n (%)	ART Experienced (n=150) Median (IQR)/ Mean (SD)/ n (%)	Test for differences p-value
Age (years)	37 (31-43)	41(36-48)	<0.0001
BMI (Kgs/m^2)	23.6 (19.9-27.1)	24.4(21.4-28.6)	0.034
BMI Categories			
<18.5	23 (15%)	14 (9%)	
18.5-25	73 (49%)	71 (47%)	
25-30	40 (27%)	38 (25%)	0.094
>30	14 (9%)	27 (18%)	
SBP (mmHg)	110 (110-120)	110 (110-120)	0.221
SBP Categories			
<100	1(0.7%)	0	
100-119	97(64.7%)	83(55.3%)	0.227^f
120-140	50(33.3%)	65(43.3%)	
>140	2(1.3%)	2(2.3%)	
DBP (mmHg)	70(60-80)	70(67-80)	0.211
DBP Categories			
<70	49(32.7%)	39(26%)	
70-80	97(64.7%)	103(68.7%)	0.192^f
80-90	2(1.3%)	7(4.5%)	
>90	2(1.3%)	1(0.7%)	
Hypertension			
SBP>140 | DBP>90	3(2%)	3(2%)	0.658^f
CD4 count (cells/mm^3) (n=254)	481 (354-637)	398 (262-524)	0.0003
WHO clinical stage			
Stage 1	106 (71%)	60 (40%)	
Stage 2	23 (15%)	28 (19%)	<0.0001
Stage 3	18 (12%)	48 (32%)	
Stage 4	3 (2%)	14 (9%)	

* *- Variables with mean and standard deviation reported*
"f" – Fishers Exact P value reported because some cells have expected cell counts less than 5.

ART naïve participants were younger (p<0.0001), lower BMI (p=0.034) and most were
in the WHO clinical stage 1 and 2 (p<0.0001). They also had higher CD4 count
(p=0.0003).

4.4 HIV Treatment status

The study participants' had known their HIV status for a median duration of 226 (IQR: 70 – 399) weeks with those on ART having a median of 323 (IQR: 182 – 439) weeks since diagnosis of HIV. Half (50%) of the study participants had been on ART for a median duration of 280 weeks (IQR: 148 – 387).

The ART regimens the participants were on included: Zidovudine, Lamivudine & Nevirapine 33/150 (22%), Zidovudine, Lamivudine & Efervirenz 14/150 (9%), Tenofovir, Lamivudine & Nevirapine 53/150 (35%), Tenofovir, Lamivudine & Efervirenz 30/150 (20%), Stavudine, lamivudine & Nevirapine/ Efervirenz 3/150 (2%), and Lopinavir/ritonavir, lamivudine & Tenofovir/Nevirapine/Zidovudine 11/150 (7%). Five (3%) participants were on second line ART which were Lopinavir/ritonavir based therapy. Almost all the participants, 296/300 (99%), were on septrin for pneumocystis carinii pneumonia prophylaxis while the rest were on dapsone.

4.5 Prevalence of dyslipidemia and dysglycemia in ART naïve and experienced patients

Table 3: Prevalence of dyslipidemia and dysglycemia

Variable	ART Naïve (N=150)		ART Experienced (N=150)		Total (N=300)	
	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
Dyslipidemia	103	68.7 (60.6, 76.0)	105	71.4 (63.4, 78.7)	208	70.0 (64.5, 75.2)
Dysglycemia	17	11.6 (6.9, 17.9)	29	19.0 (13.1, 26.1)	46	15.3 (11.4, 19.9)
Dyslipidemia & Dysglycemia	14	9.3 (5.2, 15.2)	25	17.0 (11.3, 24.1)	39	13.1 (9.5, 17.5)

The overall prevalence of dyslipidemia (total cholesterol > 6.2 mmol/L, LDL-cholesterol > 2.6 mmol/L, HDL-cholesterol < 0.9 mmol/L, and/or triglycerides > 2.3 mmol/L) was 208/300 (70.0%, 95% CI: 64.5, 75.2) among the study participants. Among the ART naïve participants the prevalence was 103/150 (68.7%, 95% CI: 60.6, 76.0) as compared to ART experienced participants which was 105/150 (71.4%, 95% CI: 63.4, 78.7) (p=0.603).

Elevated total cholesterol was present in 23/300 (7.7%, 95% CI: 5.0, 11.4) of the participants. The prevalence's of elevated LDL-C, low HDL-C and elevated triglycerides in the study are shown in Table 3. Among the ART naïve participants the prevalence of elevated total cholesterol was 3/150 (2%, 95% CI: 0.4, 5.7) as compared to the ART experienced which was 20/150 (13.5%, 95% CI: 8.5, 20.1) (p<0.001). ART naïve participants had a higher prevalence of low HDL (p<0.001) while ART

experienced participants had a higher prevalence of both elevated LDL (p=0.048) and elevated triglycerides (p=0.014).

Dysglycemia was present in 17/150 (11.6%, 95% CI: 6.9, 17.9) of the ART naïve participants and 29/150 (19.0%, 95% CI: 13.1, 26.1) of the ART experienced participants (p=0.055). The overall prevalence of diabetes mellitus was 7/300 (2.3%, 95% CI: 0.9, 4.7) with no difference in prevalence between the ART naïve and ART experienced participants (p=1.000). Impaired fasting glucose was present in 39/300 (13.0%) of all study participants.

Table 4: Sub-group analysis – Prevalence of parameters of dyslipidemia and dysglycemia

Variable	ART Naïve n (%)	On ART n (%)	Total n (%)	p-value
Elevated Total cholesterol (n=298)	3(2%)	20 (13.5%)	23 (7.7%)	<0.001[f]
High LDL (n=298)	71(47.3%)	87 (58.8%)	158 (53%)	0.048
Low HDL (n=298)	45 (30.0%)	14 (9.5%)	59 (19.8%)	<0.001
Elevated Triglycerides (n=297)	16 (10.7%)	31 (21.1%)	47 (15.8%)	0.014
Diabetes Mellitus (n=300)	3 (2%)	4 (2.7%)	7 (2.3%)	1.000[f]
Impaired fasting glucose (n=300)	14 (9.3%)	25 (16.7%)	39 (13.0%)	0.085[f]

[f] – *Fisher's exact P-value reported because the expected cell count for at least one cell was less than 5.*

Compared to those not on ART there is a significantly high prevalence of elevated total cholesterol, 20(13.5%) vs. 3(2%), and elevated triglycerides, 31(21.1%) vs. 16(10.7%), among the participants on treatment, P<0.001, and 0.014 respectively.

However, there is a significantly high prevalence of low HDL among the participants not on ART, 45(30.0%) vs. 14(9.5%), P<0.001.

Table 5: Cardiovascular risk categories (TC: HDL-C ratio)

TC:HDL-C ratio	ART naive	ART experienced	Total	p-value
High risk[*]	45 (30%)	29 (20%)	74 (25%)	
Intermediate risk[**]	21 (14%)	17 (11%)	38 (13%)	0.061
Low risk[***]	84 (56%)	102 (69%)	186 (62%)	
Median(IQR)	3.90 (3.12-4.90)	3.42 (2.78-4.43)	3.72 (2.99-4.61)	0.010

[*] *(TC: HDL-C ratio) Male > 5.0, Female > 4.5*   [**] *(TC: HDL-C ratio) Male 4.5 – 5.0, Female 4.0 – 4.5*
[***] *(TC: HDL-C ratio) Male < 4.5, Female < 4.0*

Participants not on ART had a higher median TC: HDL-C ratio compared to those on ART (p=0.01), with 30% of all participants not on ART having a high risk of CVD.

4.6 Comparison of dyslipidemia and dysglycemia between ART naïve and experienced patients

The difference between the prevalence of dyslipidemia in the ART naïve and ART experienced participants was 2.8% (95% CI: 13.2%, -7.7%), (p=0.603). Therefore there was no sufficient evidence against the null hypothesis that the ART naïve and the ART experienced participants had similar prevalence of dyslipidemia.

The difference in prevalence of dysglycemia between the ART naïve and ART experienced participants was 8.0% (95% CI: 16.1%, 0.1%), (p=0.055). There was no sufficient evidence against the null hypothesis that the ART naïve and ART experienced participants had a similar prevalence of dysglycemia.

4.7 Association between type and duration of antiretroviral therapy use and dyslipidemia and dysglycemia

Nevirapine and Zidovudine use were independently associated with more than twice increased risk of dysglycemia, OR 2.12 (95% CI: 1.12, 4.03), and 2.21 (95% CI: 1.09, 4.49), respectively. The ART regimen of Zidovudine, Lamivudine & Nevirapine was associated with more than twice increased risk of dysglycemia, OR: 2.33 (95% CI: 1.01, 5.41). The ART regimens of Zidovudine, Lamivudine & Efervirenz, and Tenofovir, Lamivudine & Nevirapine, were not associated with an increased risk of dysglycemia, OR: 2.33 (95% CI: 0.70, 7.75), and 1.59 (95% CI: 0.75, 3.37) respectively.

The ART regimen of Tenofovir, Lamivudine & Efervirenz was not associated with a change in risk of dyglycemia, OR: 0.59 (95% CI: 0.17, 2.01). Overall ART use was not associated with dysglycemia, OR: 1.88 (95% CI: 0.98, 3.58).

There was no association between use of any ART medication and dyslipidemia, OR: 1.14 (95% CI: 0.69, 1.88). Similarly, there was no association between duration of ART use and either dyslipidemia or dysglycemia OR: 1.00 (95% CI: 0.99, 1.00) & OR: 0.99 (95% CI: 0.99, 1.00) respectively. For the five patients on second line ART who were on Lopinavir/ritonavir combination, three had dyslipidemia and one had dysglycemia. However no associations were calculated because of the small number of patients in this category.

4.8 Association between body composition and either dyslipidemia or dysglycemia

Table 6: Body composition

Variable	ART naive (n=150) Median (IQR)/ Mean (SD)/ n (%)	ART experienced (n=150) Median (IQR)/ Mean (SD)/ n (%)	All (n=300) Median (IQR)/ Mean (SD)/ n (%)	p-value
BMI (Kgs/m^2)	23.6 (19.9-27.1)	24.4 (21.4-28.6)	24.0 (20.5-27.7)	0.034
BMI levels				
<18.5 (Kgs/m^2)	23 (15%)	14 (9%)	37 (12.3%)	
18.5-25 (Kgs/m^2)	73 (49%)	71 (47%)	144 (48%)	0.094
25-30 (Kgs/m^2)	40 (27%)	38 (25%)	78 (26%)	
>30 (Kgs/m^2)	14 (9%)	27 (18%)	41 (13.7%)	
Waist circumference(cm)	81 (81-90)	87 (81-96.5)	84 (81-94)	0.001
Body fat percent (%)	31.1 (19.5-38.7)	31.1 (20.2-40.8)	31.1 (20.1-39.7)	0.430
Body fat mass (Kg)	20.1 (11.2-27.3)	21.3 (13.5-31.5)	20.5 (11.9-29.1)	0.111
Free fat mass (Kg)	44.1 (40.8-48.8)	46.5 (43.4-53.8)	45.1 (41.7-51.9)	0.0001
Muscle mass (Kg)	41.9 (38.7-46.3)	43.9 (41.2-51.1)	42.7 (39.6-49.4)	0.0001
Total body water (Kg)	31.1 (28.3-34.6)	33.1 (30.3-37.8)	32 (29.2-36.4)	0.0002
Total body water percent (%)	48.7 (43.2-55.4)	48.4 (42.0-55.0)	48.5 (42.8-55.0)	0.491
Bone mass (Kg)	2.2 (2.1-2.5)	2.4 (2.2-2.7)	2.3 (2.1-2.6)	0.0002
Basal metabolic rate (Kcal)	1358 (1241-1471)	1413.5 (1313.0-1593.0)	1385.5 (1273-1549)	0.0006
Metabolic age	38 (25-49)	45 (31-55)	42 (29-53)	0.0009
Ideal Weight (Kg)	60.6 (57.0-63.6)	62.1 (58.5-66.6)	61.4 (57.7-65.8)	0.019
Degree of obesity (%)	7.1 (-9.0-23.4)	11 (-2.7-29.5)	9.2 (-6.6-26.1)	0.040

The median body mass index (BMI) was 24.0 (20.5-27.7) kilograms per square meter. Close to half of the participants, 144/300 (48%) had ideal BMI. The distribution of the other BMI categories is shown in Table 7.

Other body composition parameters that were assessed included body fat percentage, body fat mass, free fat mass, muscle mass, total body water (TBW), TBW percent,

bone mass, basal metabolic rate in Kilo joules, basal metabolic rate in Kilocalories, metabolic age, ideal body weight and degree of obesity. The results of these parameters are shown in Table 7.
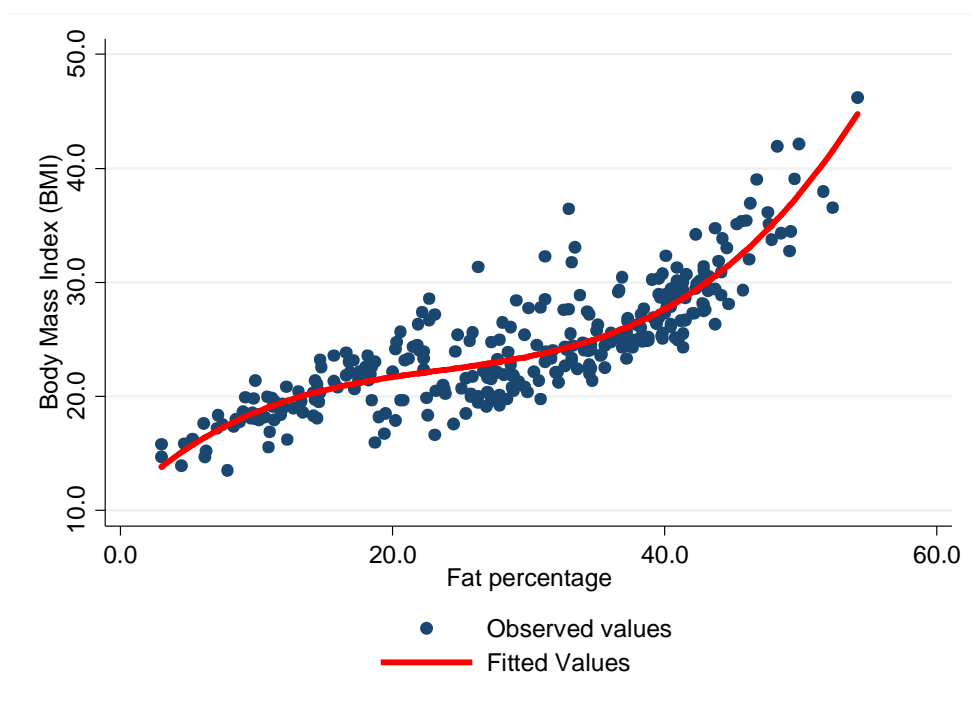
Figure 3: BMI and body fat percentage

The relationship between BMI and body fat percentage is shown in Figure 3. BMI had a curvilinear relationship with body fat percentage. Body fat percentage of 20 – 40% was associated with a minimal change in the BMI, with most BMI values in this range being within the ideal measures of 18.5 – 25 Kg/m^2. There was a big change in BMI with body fat percentage above 40% and a slight change in BMI for body fat percentage below 20%.
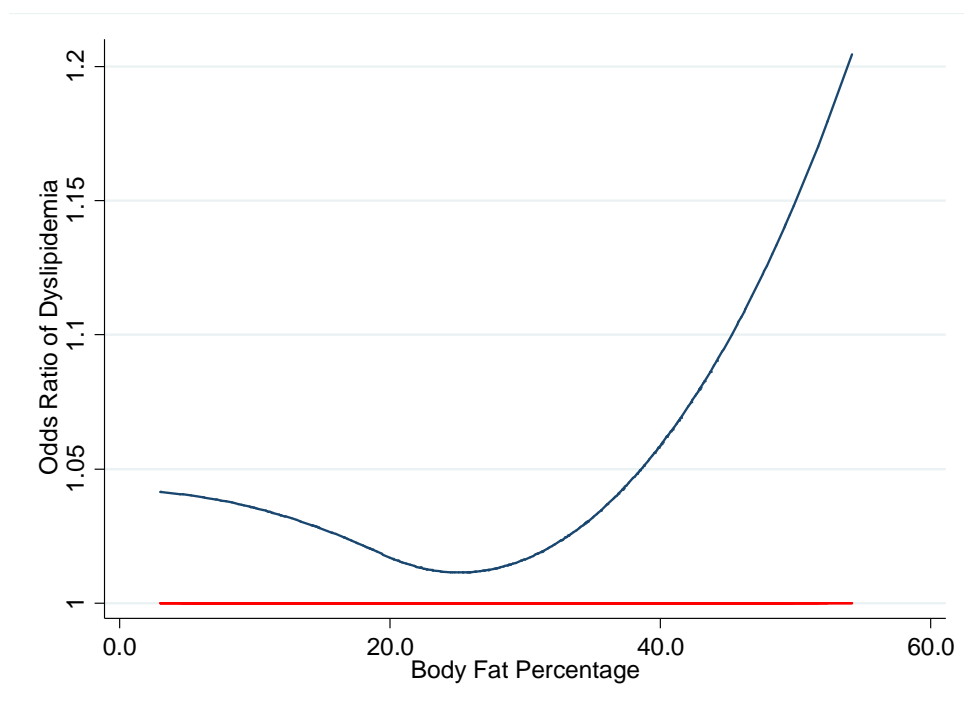
Figure 4: Dyslipidemia and body fat percentage

Figure 4 shows that there was a curvilinear relationship between the risk of dyslipidemia and body fat percentage. There was exponential increase in the risk of dyslipidemia for body fat percentage above 40% with a low baseline risk of dyslipidemia for all the study participants.

Figure 5: Dysglycemia and body fat percentage

Figure 5 shows that there was a curvilinear relationship between the risk dysglycemia

and body fat percentage. The graph shows that there was an exponential increase in the

risk of dysglycemia for body fat percentage above 40%.

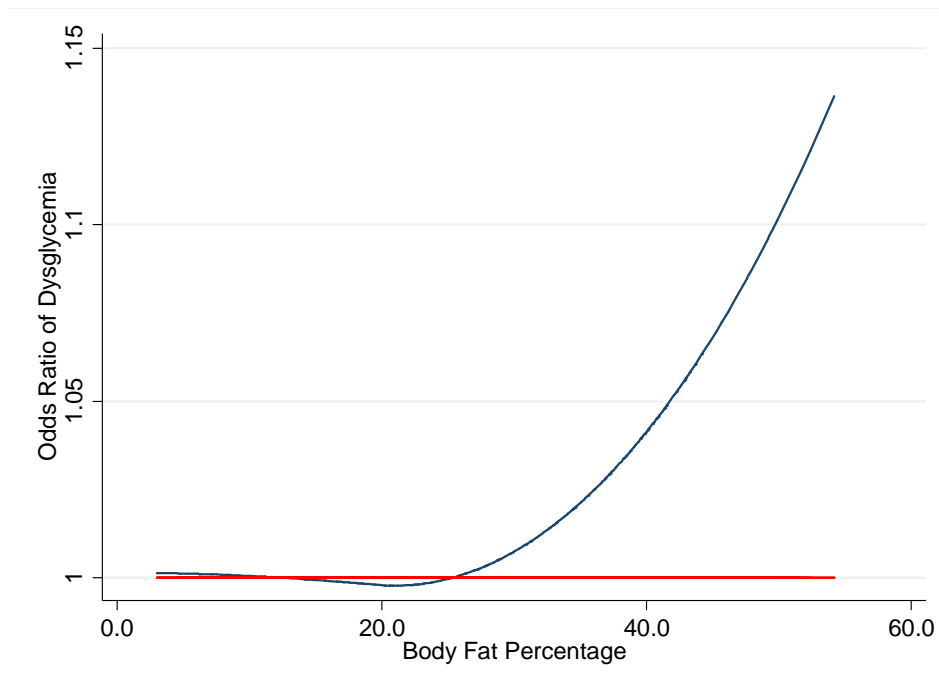# CHAPTER FIVE: DISCUSSION

5.1 Socio-demographic and clinical characteristics

Socio-demographic characteristics are important health features that affect the development and prevalence of cardiovascular diseases including dyslipidemia and dysglycemia in both the general population and among patients with HIV/AIDS. Various studies on dyslipidemia and dysglycemia among HIV infected adults in Africa included patients who were relatively young with a mean age ranging from 30 – 39 years. A study done in KNH in 2006 by Manuthu *et al*., found that most participants had a mean age of 36 years in the ART naïve group & 36.5 years in the ART experienced group (Manuthu et al., 2008). In Zambia, a study by Kiage *et al*., found a mean age of 35 years (Kiage et al., 2013) and Adewole *et al*., found a mean age of 30 – 39 years in Nigeria (Adewole et al., 2010). Both studies were among HIV infected adults. This is consistent with our study in which the participants had a median age of 39 (IQR: 33 – 46) years. In addition, the NASCOP report in 2012 showed that the national HIV prevalence was higher in the younger population.(NASCOP, 2012)

The NASCOP report 2012 also showed that sixty percent of all HIV infected adults in Kenya are females.(NASCOP, 2012) Our study found that seventy percent of all participants were female, with equal proportions among the ART naïve and ART experienced patients. This higher proportion of females could also be due to the differences in the health seeking behavior among the sexes, with females' health seeking behavior being better than males.

Alcohol use has been associated with cardiovascular disease, though it may have a plausible relationship with the prevalence of dyslipidemia and dyglycemia. Bloomfield *et al.,* in a study on cardiovascular risk factors in a peri-urban population based study in Bungoma East District among 4037 adults with a median age of 35 (IQR: 26 – 50) years in Western Kenya showed that 7% of the population used tobacco while 16% of the population reported alcohol use (Bloomfield et al., 2013). The findings in the study in Western Kenya are similar in terms of smoking rates to those of the present study at 9% but differ in terms of alcohol use since 30% of the participants in the present study had a history of alcohol intake. A study similar to the present study done in KNH in 2006 found that 9% of the patients had history of smoking (Manuthu et al., 2008).

A study on cardio-metabolic risk factors among HIV infected patients by Kiage *et al*. in Malawi showed that 30% of the study participants were underweight, 64% were of normal BMI and 6.8% were overweight (Kiage et al., 2013). These results by Kiage *et al*., were different from the findings in our study with 12% of the participants being underweight, 50% had a normal BMI, 25% were overweight and 14% were obese. The study results in the present study were comparable to those of a population-based survey in an urban slum population in Nairobi that showed that 30% of the participants were overweight while 16% were obese (Ayah et al., 2013). A similar population based study in Togo showed that 25% of the study population was obese which was higher than this study finding (Baragou et al., 2012).

Hypertension is one of the common cardiovascular risk factors among HIV infected patients and a better immune function being related to higher blood pressures (Arruda JE, 2010). Findings in this study showed that there was a small prevalence of newly diagnosed hypertension (2%) among all the study participants. The low prevalence could be due to a number of reasons; the study population was young (median age 39 years) and did not have the main risk factor for hypertension, which is advanced age. Secondly, HIV patients attend clinic regularly and those with pre-existing hypertension would probably have had the diagnosis made earlier and referred to care and subsequent initiation of appropriate management. The studies on hypertension among HIV infected patients have not mentioned the prevalence of newly diagnosed hypertension among the patients they studied and therefore no comparisons were made with this study.

5.2 Prevalence and associations of dyslipidemia in context of ART use

Although atherogenesis is a multifactorial process, abnormalities in lipid metabolism leading to dyslipidemia is one of the key factors representing about 50% of all population – attributable risk of developing CVD (Millan et al., 2009). There was a high prevalence of dyslipidemia in this study with no significant difference in the prevalence of dyslipidemia between the ART naïve and ART experienced patients (p=0.603). Other studies in Kenya have found a similarly high prevalence of dyslipidemia among HIV infected patients. Manuthu *et al.,* in a study in KNH in 2006, among 295 HIV infected adults with a mean age 36.5 years in the ART naïve group

and 39.4 years in the ART experienced group, found an overall prevalence of dyslipidemia of 63.1% (Manuthu et al., 2008). In the study by Manuthu *et al.,* 52 % of the study participants were on ART with 82% of them being on a stavudine based regimen (Manuthu et al., 2008). Unpublished data by Njoroge *et al.,* in a study among sero-discordant couples in Nairobi in 2014 with a median age of 32 (IQR: 23 – 41) years found a prevalence of dyslipidemia of 83.8% among HIV infected participants and 78.4% among HIV uninfected participants. Njoroge *et al.* in their study used stored samples collected from 196 participants between the years 2007 and 2008. Though the three studies among the HIV infected patients used different methodologies at different time periods, they all reported high prevalence's of dyslipidemia. Dyslipidemia could therefore be considered a potential problem among HIV infected patients in Kenya. Another possible explanation of the high prevalence's of dyslipidemia among HIV infected patients could be a relatively high baseline prevalence of dyslipidemia in the general Kenyan population. Two studies on dyslipidemia in the general adult population in Kenya have shown different results. Mathenge *et al.,* in a study done in the general urban population in Nakuru in 2008 that sampled 4396 participants found a prevalence of elevated total cholesterol of 21.1% (Mathenge, Foster, & Kuper, 2010). In 2014, the Africa Middle East Cardiovascular Epidemiological (ACE) study, in which data from Kenya was included, that assessed cardiovascular risk factors among 4378 patients in rural and urban cohorts attending general practice clinics found the prevalence of dyslipidemia to be 70% (Alsheikh-Ali et al., 2014). The study by Mathenge et al. is more comparable to our study unlike the ACE that included data

from other regions without mention of the prevalence of dyslipidemia in the different countries from which data was sampled.

It is known that LDL-C is an atherogenic lipid marker which increases with increased total cholesterol (Millan et al., 2009). In this study, the prevalence of dyslipidemia was different between the ART naïve and ART experienced participants. Low HDL-C was more prevalent in ART naïve participants compared to the ART experienced participants (30% vs 9.5%). High total cholesterol (13.5% vs 2%) and LDL-C (58.8% vs 47.3%) was more prevalent in the ART experienced participants compared to the ART naïve participants. These findings, therefore demonstrated that HIV infection itself, not necessarily the ART, could also contribute to the high prevalence of dyslipidemia among HIV infected patients with or without ART.

The most significant lipid abnormality among ART naïve HIV infected patients is low HDL cholesterol that has been demonstrated in several studies (Grunfeld et al., 1992; Riddler et al., 2003; Shor-Posner et al., 1993). Low HDL-C was present in 19.8% of the study participants in our study which was much lower than the study by Shor-Poser *et al*., in which low HDL was present in 40% of HIV 1 seropositive men. The prevalence in our study was also much lower compared to a study in the UK that showed a prevalence of 27% of low HDL in a HIV infected cohort (Elgalib et al., 2011) and a local study at KNH, Kenya showed that the prevalence of low HDL was 51.3% among ART naïve patients. The results on low HDL-C are not universal and conclusive and could vary widely depending on the population phenotype and the

different time periods when the studies were done. In addition, other factors such as nutritional status and the predominant diet in a particular population cohort could contribute to the lower prevalence of low HDL in this study.

Use of ART among HIV infected patients leads to sustained viral suppression lowering the level of risk for cardiovascular disease, including decreased level of hypocholesterolemia (low HDL-C) (Phillips, Neaton, & Lundgren, 2008). ART use also leads to higher levels of atherogenic cholesterol particles predominantly elevated total cholesterol and elevated LDL-C (Millan et al., 2009). ART experienced patients in the present study had a higher prevalence of LDL-C compared to the ART naïve participants with ART experienced patients having higher mean LDL-C levels by 0.2mmol/L (p=0.001). These findings are similar to the study by Manuthu *et al*., that showed that the median LDL-C values were higher by 0.7mmol/L in patients on ART which could be due to the use of stavudine by majority of patients in his study (Manuthu et al., 2008). The study by Manuthu *et al*., done in KNH however found a relatively lower prevalence of LDL-C (40.8%) compared to this study (53%), and the difference could be due to variations in progression of HIV disease, differences in ART adherence, and nutritional status of the study participants.

There is no universal accepted standard on how information on total cholesterol, HDL-C, LDL-C and triglycerides should be used and interpreted but several consensus statements have shown that the TC/HDL-C ratio has a better correlation with CVD and therefore the better predictor of CVD than simple lipid parameters (Lemieux et al.,

2001; Millan et al., 2009; Wilson, Abbott, & Castelli, 1988). TC/HDL-C ratio of > 5.0 in men and > 4.5 in women represents a high atherogenic CVD risk (Millan et al., 2009), irrespective of the cause of the derangement of the individual lipid parameters owing to the imbalance between cholesterol carried by atherogenic and protective lipoproteins (Criqui & Golomb, 1998). High atherogenic risk indicates a $> 20\%$ risk of coronary heart disease in 10 year (National Cholesterol Education Program Expert Panel on Detection & Treatment of High Blood Cholesterol in, 2002). In this study, 25% of all participants (20% on ART Vs 30% ART naive) had a high atherogenic risk of CVD and therefore a $> 20\%$ risk of a CHD over the next ten years. ART naïve patients also had a higher median TC/HDL-C ratio compared to the ART experienced participants (p=0.01). This is attributed to by the lower HDL-C among participants not on ART compared to those on ART (p<0.001). HDL is the protective form of cholesterol and is important in protection of the vascular endothelium (National Cholesterol Education Program Expert Panel on Detection & Treatment of High Blood Cholesterol in, 2002). Increase in HDL-C is more prevalently associated with plaque regression in addition to slowed plaque progression, while a decrease in LDL-C is associated with slowed down plaque progression (Millan et al., 2009). It has been shown that the concentration of HDL-C correlates inversely with CVD risk. Furthermore, high HDL-C has been shown to be cardio-protective through its main anti-atherogenic functions that include reverse cholesterol transport, antioxidant, anti-inflammatory, anti-thrombotic & anti-apoptotic properties. HDL-C also has endothelial stabilizing and repair properties (Castelli et al., 1986; Lemieux et al., 2001; Reiner, Muacevic-Katanec, Katanec, & Tedeschi-Reiner, 2011).

5.3 Prevalence and associations of dysglycemia in context of ART use

The present study found an overall prevalence of dysglycemia of 15.3% and did not establish any association between dysglycemia and ART use (p=1.000). The prevalence of dysglycemia in our study could have been underestimated because oral glucose tolerance test and glycated hemoglobin tests were not done. Other studies among HIV infected patients in Kenya have found higher prevalence's of dysglycemia. Manuthu *et al*., in a study at KNH in 2006 while utilizing both fasting blood sugar and oral glucose tolerance tests found a prevalence of dysglycemia of 20.7% among HIV infected patients irrespective of ART use (Manuthu et al., 2008). When stratified by ART use, Manuthu *et al*., found a prevalence of dysglycemia of 17.9% in the ART naïve group and 22.9% in the ART experienced group (p=0.284). In addition, most patients (>82%) in the Manuthu *et al.*, study were on a stavudine based ART regimen which has been shown to cause more metabolic complications due to mitochondrial toxicity.

A study by Dave *et al*., in South Africa published in 2011 among 406 ART naïve patients and 443 ART experienced patients found a prevalence of dysglycemia of 21.9% and 25.7% respectively, but this difference was not statistically significant (Dave et al., 2011). The study in South Africa utilized oral glucose tolerance tests with additional assessment of insulin sensitivity and – cell function with 66.8% of the patients being on a stavudine based ART regimen.

The lack of association between ART use and dysglycemia established in the present study and in the studies by Manuthu *et al*., in KNH and Dave *et al*., in South Africa are consistent with findings of a systematic review done in 2013 in SSA which did not find any association between HIV infection, ART use and fasting blood glucose or HbA1c (Dillon et al., 2013). The systematic review in SSA comprised of 49 published and 3 unpublished studies (with data from 25,755 individuals) of which 23 studies (15,073 individuals) had been conducted in East Africa. This contrasts the results of a Multicenter AIDS Cohort Study (MACS) that showed that ART use among HIV infected patients was associated with decreased insulin sensitivity as well as an increased risk of fasting hyperinsulinemia compared to those not on ART (Brown, Li, et al., 2005). The MACS recruited 5622 homosexual and bisexual men in several centers in the United States between 1984 and 1991 and therefore comprised a different population compared to the patients whose data was analyzed in the systematic review in SSA.

Newly diagnosed diabetes mellitus was found in 2.3% of all participants and this could be attributed to the fact that this was a low risk hospital population with regular access to medical services and possible early diagnosis of DM in addition to the study recruitment criteria. It has been shown that the incidence of newly diagnosed DM among HIV infected patients' increases with cumulative exposure to ART but this varies depending on use of different ART regimen (Brown, Cole, et al., 2005). Stavudine and Zidovudine use have been significantly associated with increased risk of DM after adjustment for traditional risk factors for DM (De Wit et al., 2008). This is

similar to the findings in this study which found that Zidovudine use was associated with more than two-fold increase in dysglycemia risk (OR 2.21, 1.09 – 4.49). Tien *et al*., in the Women's HIV Interagency study (WHIS) showed that Nevirapine use was associated with reduced risk of DM (De Wit et al., 2008; Tien et al., 2007), findings that contrast with our study that showed that Nevirapine use was associated with more than two-fold increase risk of dysglycemia (OR 2.12, 1.12 – 4.03). The WHIS was a large prospective cohort study (1524 HIV infected women and 564 HIV uninfected women) conducted from 2000 to 2006 and diagnosis of DM was done based on fasting blood sugar tests.

5.4 Body composition and dyslipidemia & dysglycemia

Body mass index is the most widely used method to evaluate nutritional status in clinical practice (Funghetto et al., 2015). This method is used to determine obesity and overweight and therefore could predict the risk of cardiovascular disease (Harrington & Lee-Chiong, 2009). Fungetto *et al*., in a study in Brazil comparing percentage body fat and BMI for the prediction of inflammatory and atherogenic lipid profiles in elderly women showed that obese individuals exhibit a higher atherogenic lipid risk profile than do normal weight individuals (Funghetto et al., 2015). Two other studies have shown that increased BMI is related to chronic inflammation with increased serum inflammatory markers such as Interleukin 6, Interleukin 1 and C-reactive protein that have been demonstrated as cardiovascular risk factors (Bruunsgaard, Skinhoj, Pedersen, Schroll, & Pedersen, 2000; Moleres et al., 2009). In this study, ART experienced patients had a higher median BMI compared to ART naïve participants

(p=0.034) and a higher prevalence of obesity (18% vs 9%). This could be attributed to good adherence to ART and other complementary supportive services such as nutrition. In addition, HIV infection itself leads to increased inflammatory markers and varied opportunistic infections that cause malnutrition and lower BMI.

The main assumption of BMI guidelines is that BMI is closely related to body fat percentage and consequent morbidity and mortality (Bray, 1996). This study showed that the assumption of the BMI guidelines could be wrong as body fat percentage above 40% led to significantly increased odds of dyslipidemia and dysglycemia, irrespective of the BMI status. This could be due to different dynamics in interaction of body fat percentage, BMI and obesity (Gallagher et al., 2000). There has been no consensus on how body fat is linked with morbidity and mortality because of the absence of appropriate prospective studies (Gallagher et al., 2000). This finding was consistent with the study by Fungetto *et al*., among elderly women in Brazil which found that classification of body fat percentage along with biochemical tests were more reliable predictors for identifying obesity, systemic inflammation and atherogenic lipid profile than BMI (Funghetto et al., 2015). The relationship between BMI and body fat percentage in this study was curvilinear with persons having a BMI > 40 having higher body fat percentage. This study further showed that the odds of both dyslipidemia and dysglycemia increased exponentially with body fat percentage more than 40%. This demonstrates the possible contribution of body composition and specifically increased body fat percentage in the risk of dyslipidemia and dysglycemia and therefore increased cardiovascular risk.

5.5 Limitations of the study

The following were the limitations of this study:

1. The inability to do oral glucose tolerance test or glycated hemoglobin levels for the study participants due to limited funding for the study may have led to the underestimation of prevalence of dysglycemia.

2. Inability of all the study participants to return for fasting blood sugars and fasting lipid profiles. During the study period, all patients not on ART with a CD4 count < 500 cells/µL were being started on ART. ART naïve patients were therefore being given an earlier return date and could explain the fewer number of non-responders on the ART naïve arm compared to the ART experienced arm.

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

1. This study found a high prevalence of dyslipidemia (70%) and a low but significant prevalence of dysglycemia (15.3%) among ART naïve and HIV infected patients.

2. ART use was not associated with dyslipidemia and dysglycemia.

3. The odds of dyslipidemia and dysglycemia were increased significantly for body fat percentage above 40%.

6.2 RECOMMENDATIONS

1. A high risk of dyslipidemia and a low but significant risk of dysglycemia were found among HIV infected patients receiving care at the MTRH HIV clinics and appropriate routine screening considered. Clinicians should also establish the type of dyslipidemia present among HIV infected patients and initiate appropriate management.

2. Further studies should be done to show the effect of early initiation of ART on hypocholesterolemia due to low HDL-C among ART naïve HIV infected patients.

3. Further studies should be done to establish the possible contribution of body fat percentage to increased risk of dyslipidemia and dysglycemia and therefore increased cardiovascular risk.

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8. APPENDICES

Appendix I: Questionnaire and Data collection form

DYSLIPIDEMIA AND DYSGLYCEMIA IN HIV INFECTED PATIENTS IN THE

AMPATH CLINIC AT THE MOI TEACHING AND REFERRAL HOSPITAL,

ELDORET KENYA

<u>Instructions</u>

1. To be filled by investigator/research assistant once the client consents to the study.
2. Please fill all sections.
3. If the response is a date and the participant does not remember the exact put the approximate year if still cannot remember the year write **00/0000**
4. Please write legibly and clearly.
5. Follow the instructions in each of the sections.

Study Number __ __/__ __ __

Date (MUST FILL): dd/mm/year __ __/__ __/__ __ __ __

Module: _____

AMRS # (MUST FILL!)   __ __ __ __ __ __ __ __ __ __ __

Participant name _____

Mobile phone # _____

Home phone # _____

BIODATA
1. Date of birth: __ __/__ __/__ __ __ __ **(dd/mm/year)**
2. If date of birth unknown, age at last birthday: __ __ __ years
3. Gender (**Tick appropriate response**)
  ☐ Male   ☐ Female

MEDICAL HISTORY
4. Have you ever been told you have diabetes? (**Tick appropriate response**)
  ☐ Yes   ☐ No
5. When were you told you have diabetes? (**Give at least month & year**)
  __ __/__ __/__ __ __ __ (**dd/mm/year**)
6. Have you ever been told you have high blood pressure? (**Tick appropriate response**)
  ☐ Yes   ☐ No

7. When were you told you have high blood pressure? (**Give at least month & year**)
  __ __/__ __/__ __ __ __ (**dd/mm/year**)

If either of the above is YES, Exclude from the rest of the study

8. When were you told you are HIV infected? (**Give at least month & year**)
  __ __/__ __/__ __ __ __ (**dd/mm/year**)
9. Do you take any medication for your HIV infection?
  ☐ Yes   ☐ No (**If No skip to Q.17**)
10. Which HIV medication (ARVs) do you take for your HIV infection? (**Tick all appropriate**)
  ☐ Stavudine   ☐ Lamivudine
  ☐ Zidovudine   ☐ Tenofovir
  ☐ Nevirapine   ☐ Efervirenz
  ☐ Lopinavir/ritonavir ☐
11. When did you start taking your HIV medication? (**Give at least month & year**)
  __ __/__ __/__ __ __ __ (**dd/mm/year**)
12. Have you ever had your HIV medication changed/stopped?
  ☐ Yes   ☐ No (**If No skip to Q.17**)
13. When was your HIV medication changed/stopped? (**Give at least month & year**)
  __ __/__ __/__ __ __ __ (**dd/mm/year**)
14. Which HIV medication (ARVs) are you now taking for your HIV infection? (**Tick all appropriate**)
  ☐ Stavudine   ☐ Lamivudine
  ☐ Zidovudine   ☐ Tenofovir
  ☐ Nevirapine   ☐ Efervirenz
  ☐ Lopinavir/ritonavir ☐ None

15. Do you smoke or have you ever smoked cigarettes?
   ☐ Yes   ☐ No
16. Do you drink or have you ever drunk alcohol?
   ☐ Yes   ☐ No
17. Does any of your immediate family members have diabetes? (**Tick all appropriate**)
   ☐ Father   ☐ Mother
   ☐ Sister   ☐ Brother
   ☐ Grandmother   ☐ Grandfather
18. Does any of your immediate family members have high blood pressure? (**Tick all appropriate**)
   ☐ Father   ☐ Mother
   ☐ Sister   ☐ Brother
   ☐ Grandmother   ☐ Grandfather

ANTHROPOMETRIC MEASUREMENTS
   1. Height (cm) _____
   2. Weight (Kg) _____
   3. BMI _____
   4. Waist circumference _____
   5. Blood pressure (mmHg) _____

OPPORTUNISTIC INFECTIONS (From chart)
WHO Clinical staging _____ (Appendix XI)

	Opportunistic infection	Date diagnosed
1.		
2.		
3.		
4.		
5.		

LABARATORY VALUES (From chart)

	Narration		Date (dd/mm/year)	Lab. value
1.	Viral load	Highest value (in chart)		
		Lowest value (in chart)		
		Other value 1		
		Other value 2		
	Narration		Date (dd/mm/year)	Lab. value
2.	CD4 count	Highest value (in chart)		
		Lowest value (in chart)		
		Other value 1		
		Other value 2		
		Other value 3		

LABARATORY MEASUREMENTS

	Narration	Date (dd/mm/year)	Laboratory value
1.	Random blood sugar (mmol/l)		
2.	FBS (mmol/l)		
3.	Total cholesterol (mmol/l)		
4.	Triglycerides (mmol/l)		
5.	LDL – cholesterol (mmol/l)		
6.	HDL – cholesterol (mmol/l)		

Appendix II: Consent Form – English

<u>CONSENT FOR PARTICIPATION</u>

You are being invited to participate in a research study on dyslipidemia and dysglycemia in HIV infected patients in the AMPATH clinic at Moi Teaching and Referral Hospital. This study is being conducted by Dr. Nicholas Kirui, a student at the Department of Medicine, Moi University School of Medicine.

The study shall involve an initial interview of about 10-15 minutes. Subsequently you shall have your weight, height and waist circumference taken. A random blood sugar shall then be taken. You shall thereafter be requested to come for your next clinic visit without having taken breakfast (including tea or milk). This shall enable us to take blood for a fasting blood sugar and a fasting lipid profile. However breakfast (a cup of tea and mandazi) shall be provided on that day. **In addition we shall take your body composition by bio impedance analysis on the return visit.** Though the study shall be associated with some pain you shall benefit directly as a participant because you shall know if you have or do not have diabetes mellitus, pre-diabetes and dyslipidemia. Early diagnosis of diabetes and dyslipidemia shall enable you to start treatment and prevent long term complications. The results from this study shall enable us to know if diabetes and dyslipidemia is a problem in our patients with HIV infection. There are no costs to you for participating in the study.

The results of the study will be stored in a database that is password protected and only accessible by those conducting the study. No one will be able to identify you or your results. Should the data be published, no individual information will be disclosed. Your participation in this study is voluntary. If you decide to participate, you can change your mind later and quit the study before the end of the study. If you decide not to participate, or if you quit the study, it will not affect health care services you receive. By signing this document, you are voluntarily agreeing to participate. You are free to decline to answer any particular question you do not wish to answer for any reason.

If you have any questions about the study, please contact Dr. Nicholas Kirui at AMPATH, Moi Teaching and Referral Hospital. The Institutional Review and Ethics Committee of Moi University and Moi teaching & Referral Hospital has reviewed and approved our request to conduct this study.

I have read this informed consent and authorization form. ALL OF MY QUESTIONS HAVE BEEN SATISFACTORILY ANSWERED, AND I WANT TO TAKE PART IN THIS RESEARCH STUDY. By signing below, I give my permission to participate in this research study and for the described uses and releases of information.

Signature of the participant _____ **Date_____**

Signature of person giving consent_____ **Date_____**

Appendix III: Consent Form – Kiswahili

IDHINI YA KUHUSISHWA

Unaalikwa kushiriki kwenye utafiti juu ya hali ya kukosekana kwa urari wa viwango vya chembechembe za mafuta na chembechembe za sukari kwa damu miongoni mwa watu waliona virusi vya ukimwi kwenye Kliniki yaAMPATH, Hospitali ya Rufaa na Mafunzo ya Moi, Eldoret, Kenya. Utafiti huu unafanywa na Dkt. Nicholas Kirui, msomi wa Uzamili kwenye Idara ya Afya ya Watu Wazima, Chuo Kikuu cha Moi.

Uchunguzi huu itahusisha udadisi wa awali wa dakika10 hadi 15. Kisha, utapimwa kilo, urefu na mzingo wa kiuno. Kiwango cha sukari kwa damu kisha kitapimwa. Kisha utaagizwa kuja kliniki itakayofuata kabla hujala kiamsha kinywa (ikiwemo chai wala maziwa). Hii itatuwezesha kukadiria kiwango cha sukari na kuchukua vipimo vya chembechembe vya mafuta wakati wa mfungo.Hatahivyo, utapewa kiamsha kinywa baadaye (kikombe cha chai na mandazi). **Pia uzani wa mafuta na maji mwilini itachukuliwa utakaporejea wakati wa pili.** Ingawa utafiti huu utahusisha kiwwango kidogo cha uchungu, utafaidika moja kwa moja maana utapata kujua kama uanougonjwa wa kisukari au la, na vile vile ikiwa unao haliunaoishia kuwa kisukari ama hali ya kukosekana kwa urari wa viwango vya chembechembe za mafuta kwa damu. Kugunduliwa mapema kwa matatizo haya yatakuwezesha kuanza matibabu ya mapema itakayozuia madhara ya muda mrefu. Matokeo ya utafiti huu utatuwezesha kutathmini ikiwa haliya kukosekana kwa urari wa viwango vya chembechembe za mafuta nakisukari ni shida ya mara kwa mara inayowakumba wagonjwa walio na virusi vya Ukimwi. Utafiti huu hautakugharimu malipo yoyote.

Matokeo ya utafiti huu yatawekwa siri kwenye orodha iliyo na ulinzi kamilifu na ambao unaweza kufikiwa na watafiti peke yao. Hamna yeyote mwingine atakayeweza kufikia matokeo ya vipimo yako. Ikiwa matokeo yatachapishwa, habari zozote za kibinafsi hazitachapishwa. Uhusishwaji wako kwenye utafiti huuni wa hiari. Ukipenda kushirikishwa, unaweza kubadili nia baadaye na kujiondoa kabla ya utafiti kumalizika. Ikiwa hungependa kushirikishwa ama ukijiondoa, huo hautaadhiri matibabu utakayopewa. Kwa kutia sahihi hati hii unatoaidhini ya kushirikishwa kwa hiari yako. Pia una uhuru wa kutojibu swali lolote utakalopenda kutojibu kwa sababu yoyote ile.

Kwa maswali yoyote juu ya utafiti huu, tafadhali wasiliana na Dkt. Nicholas Kiru au Kamati ya Maadiliya Tathmini na Utafitiya Chuo Kikuu cha Moi na Hospitali ya Rufaa na Mafunzo ya Moi imetathmini ombi letu la kuendeleza utafitihuu.

Nimesoma na kuelewa hati hii ya idhini ya hiari na kutoa ridhaa. MASWALI YANGU YOTE YAMEJIBIWA KWA NJIA INAYORIDHISHA. Kwa kutia sahihi hapa, naidhinisha kuhusishwa kwangu kwenye utafiti huu pamoja na kuchapishwa na kutumiwa kwa matokeo yake.

Sahihi ya mshiriki _____ **Tarehe**_____

Sahihi ya mtu anayepana idhini_____**Tarehe**_____

Appendix IV: Procedure for drawing venous blood

Venous blood will be drawn for fasting lipid profile. The procedure will be explained to the participant and verbal consent obtained. Universal safety procedures shall be observed. Venous blood draw will be from the median cubital vein (in the antecubital fossa) of the less dominant upper limb.

Below is an overview of the steps that will be followed:

1. Arm is selected and a tourniquet is placed on the arm above the draw site. The median cubital vein is selected.
2. Site is cleansed with a sterile alcohol/methylated spirit preparation pad.
3. A needle is inserted into the vein and the collection tube is engaged.
4. Two milliliters of blood is collected into a Vacutainer (plain) blood collection bottle.
5. Tourniquet is removed once the quantity of blood desired has been obtained.
6. A small gauze pad and Band-aid are placed on the venous blood draw site.
7. The blood collection tube is labeled with the patient's information.
8. Blood collection tubes batched until five samples obtained before being taken to the laboratory for analysis.

Appendix V: Procedure for drawing finger stick blood

Finger stick blood will be drawn for random and fasting blood sugar. The procedure will be explained to the participant and verbal consent obtained. Universal safety procedures will be observed. Finger stick blood will be drawn from the second or third finger of the less dominant hand.

Below is an overview of the steps that will be followed:

1. Finger and site selected.

2. Finger cleaned with alcohol/methylated spirit and allowed to air dry to decrease hemolysis and not alter glucose results.

3. Spring activated lancet applied to finger and a little puncture is made.

4. First drop of blood wiped away.

5. Approximately 4 drops of blood (35µL) is collected using a capillary tube.

6. An adhesive bandage is placed on the small puncture.

7. Using the capillary tube, the whole blood is transferred into blood glucose electrode of the Optium exceed glucose meter.

Appendix VI: Procedure for measuring Blood pressure

Blood pressure will be taken using an Omron M2 compact upper arm blood pressure (BP) monitor (Omron Healthcare, Inc., 1200 Lakeside Drive, Bannockburn, Illinois 60015). The patients will be required to have had at least 15 minutes rest in a quiet place and in a relaxed sitting position with no tight fitting clothing on the upper arm, or any thick clothing such as a sweater.

Below is an overview of the steps that will be followed:

1. Participant will be seated upright with the back straight and right arm placed on the table so that the cuff will be on the same level as the heart.

2. Cuff will be wrapped on the arm so that the bottom of the cuff will be at least 1cm above the elbow. The cuff will then be fastened snugly.

3. Start button will then be pressed and the cuff will automatically inflate to take the blood pressure reading.

4. Blood pressure and pulse rate results will be displayed on the on the screen of the machine.

5. Blood pressure readings will be recorded on the pro-forma.

6. Should an error occur during the process, the cuff will be deflated and the process repeated.

7. High blood pressure readings (Systolic BP >140mmHg and Diastolic BP >90mmHg) will be confirmed manually using a mercury sphygmomanometer.

Appendix VII: Procedure for measuring blood glucose

Random and fasting blood sugar will be done using an Abbot Optium exceed glucose meter.

Below are the steps that will be followed in measuring finger stick blood glucose:

1. Finger stick blood will be drawn according to the procedure shown in Appendix V above.

2. Remove the blood glucose electrode from the foil packet.

3. Insert the three black lines at the end of the electrode into the electrode port.

4. Push the electrode in until it stops. The monitor turns on automatically.

5. Apply blood message appears on the screen of glucose meter (tells one that the monitor is ready for the application of blood to the blood glucose electrode).

6. Whole blood in the capillary tube (obtained according to procedure in Appendix V) is transferred into blood glucose electrode. The blood drop is applied to the white area at the end of the electrode. The blood is drawn into the electrode.

7. Continue to touch the blood drop to the end of the electrode until the monitor begins the test.

8. The blood glucose result shows on the display window.

9. Blood sugar readings will be recorded on the participant's pro-forma.

Appendix VII: Procedure for measuring height

The height of participants will be taken to help calculate the body mass index (BMI), which is the weight relative to the height. The height will be measured with a Mechanical roll-up measuring tape (Seca 260) with wall attachment in the nursing station.

Below are the steps that will be followed in measuring height:

1. The participant will be asked to remove their footwear (shoes, slippers, sandals, etc) and head gear (hat, cap, hair bows, comb, ribbons, etc). However, those with a scarf or veil shall not be asked to remove them (measurement may be taken over light fabric).

2. Participant will be asked stand next to the measuring board/wall facing the research assistant/ investigator.

3. Participant will be asked to stand with: feet together, heels against the measuring board/wall, knees straight.

4. Participant will be asked to look straight ahead and not tilt their head up.

5. The research assistant/ investigator will make sure eyes are the same level as the ears.

6. The measure arm will be moved gently down onto the head of the participant and the participant asked to breathe in and stand tall.

7. The height will be read in centimeters at the exact point.

8. The participant will be asked to step away from the measuring board/wall.

9. The height measurement in centimeters will be recorded in the participant's pro-forma.

Appendix IX: Procedure for measuring body weight

The weight of participants will be taken to help calculate the body mass index (BMI), which is the weight relative to the height. The weight will be measured with a 762 Dial Bathroom Floor Scale at the nursing station.

Below are the steps that will be followed in measuring weight:

1. The scale will be placed on a firm, flat surface.

2. Participant will be asked to remove their footwear (shoes, slippers, sandals, etc).

3. The participant will be asked to step onto scale with one foot on each side of the scale.

4. Participant will be asked to: stand still, face forward, place arms on the side and wait until they will be asked to step off.

5. The weight in kilograms will be recorded on the participant's pro-forma.

6. The participant will be asked to step off the scale.

Appendix X: Procedure for measuring waist circumference

Waist circumference measurements will be taken to provide additional information on overweight and obesity. A constant tension tape (Figure Finder Tape Measure) will be used to take this measurement. A private area will be necessary for this procedure.

Below are the steps that will be followed in measuring waist circumference:

1. The measurement will be taken over light clothing.

2. Standing to the side of the participant, the investigator/ research assistant will locate the last palpable rib and the top of the hip bone. The participant will be asked to assist in locating these points on their body.

3. The participant will be asked to wrap the tension tape around them and then position the tape at the midpoint of the last palpable rib and the top of the hip bone, making sure to wrap the tape over the same spot on the opposite side.

4. The research assistant/investigator will check that the tape is horizontal across the back and front of the participant and as parallel with the floor as possible.

5. Measurement should be taken: at the end of a normal expiration, with the arms relaxed at the sides, at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest (hip bone).

6. The waist circumference will then be measured. The measurement shall be read at the level of the tape to the nearest 0.1 cm, making sure to keep the measuring tape snug but not tight enough to cause compression of the skin.

7. The measurement will then be recorded on the participant's pro-forma.

Appendix XI: Body composition measurement by bio impedance analysis (BIA)

Remote display version

Control Box

Printer cover

Display

Operating key

Accessories

☑ Instruction manual (This manual)
☐ Operation guide
☐ AC adapter
☐ AC cord

☐ Syringe (1 item)

☐ Printer paper
(Ordinary thermal paper, roll
diameter: 55mm, roll length:
approx. 34m)
* Please contact the agent from
which you have purchased
the product for details.

Platform

Level gauge

Electrode
(4 positions)

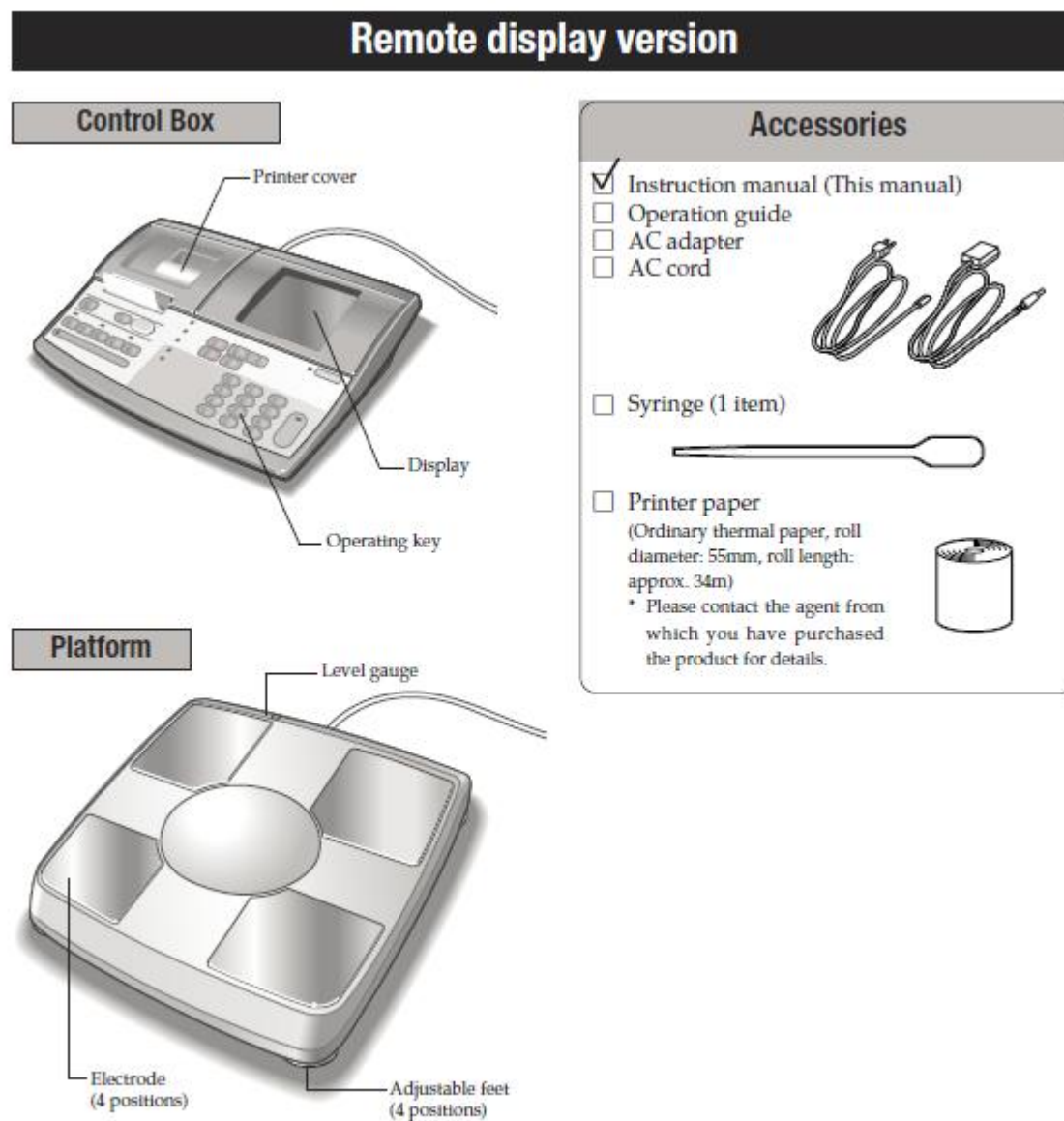Adjustable feet
(4 positions)

Figure 6: Tanita body composition analyzer BC-420MA III

Principles of body composition measurement

BIA is a means of measuring body composition by measuring bioelectrical impedance in the body. Fat within the body allows almost no electricity to pass through, while

electricity passes rather easily through water, much of which is found in muscles. The degree of difficulty with which electricity passes through a substance is known as the electrical resistance, and the percentage of fat and other body constituents can be inferred from measurements of this resistance.

The Tanita Body Composition Analyzer measures body composition using a constant current source with a high frequency current (50kHz, 90μA). The 8 electrodes are positioned so that electric current is supplied from the electrodes on the tips of the toes of both feet, and voltage is measured on the heel of both feet. The current flows into the upper limbs or lower limbs, depending on the body part(s) to be measured.

Procedure for measuring body composition by BIA

1. The Tanita body composition analyzer BC-420MA III (shown above) is turned on.

2. Select the body composition monitor and input the clothes weight (0.5kgs), then press enter/next. Patient is asked to remove any heavy clothing – jackets, sweater and shawls.

3. Input the height, then press enter/next.

4. Ask the patient to step on the electrodes on the platform with bare feet.

5. The display goes off sequentially as the measurement of the body composition is taken.

6. The measurement result and the body fat percentage are displayed.

7. The result is automatically printed out.

Appendix XII: WHO clinical staging of HIV/AIDS in adults and adolescents
WHO Clinical stage 1

1. Asymptomatic

2. Persistent generalized lymphadenopathy (PGL)

WHO Clinical stage 2

1. Moderate unexplained weight loss (<10% of presumed or measured body weight)

2. Minor mucocutaneous manifestations (seborrheic dermatitis, popular pruritic eruptions, fungal nail infections, recurrent oral ulcerations, angular cheilitis)

3. Herpes zoster

4. Recurrent upper respiratory tract infections (sinusitis, tonsillitis, bronchitis, otitis media, pharyngitis)

WHO Clinical stage 3

1. Unexplained severe weight loss (over 10% of presumed or measured body weight)

2. Unexplained chronic diarrhoea for longer than one month

3. Unexplained persistent fever (intermittent or constant for longer than one month)

4. Persistent oral candidiasis

5. Oral hairy leukoplakia

6. Pulmonary tuberculosis

7. Severe bacterial infections (e.g. pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteraemia)

8. Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis

1. Unexplained anaemia (below 8 g/dl), neutropenia (below 0.5 x 109/l) and/or chronic thrombocytopenia (below 50 x 109 /l)

WHO Clinical stage 4

Conditions where a presumptive diagnosis can be made using clinical signs or simple investigations*:*

1. HIV wasting syndrome

2. Pneumocystis jiroveci pneumonia (PCP)

3. Recurrent severe bacterial pneumonia (≥2 episodes within 1 year)

4. Cryptococcal meningitis

5. Toxoplasmosis of the brain

6. Chronic orolabial, genital or ano-rectal herpes simplex infection for >1 month

7. Kaposi sarcoma (KS)

8. HIV encephalopathy

9. Extra pulmonary tuberculosis (EPTB)

Conditions where confirmatory diagnostic testing is necessary*:*

1. Cryptosporidiosis, with diarrhoea >1 month

2. Isosporiasis

3. Cryptococcosis (extra pulmonary)

4. Disseminated non-tuberculous mycobacterial infection

5. Cytomegalovirus (CMV) retinitis or infection of the organs (other than liver, spleen, or lymph nodes)

6. Progressive multifocal leucoencephalopathy (PML)

7. Any disseminated mycosis (e.g. histoplasmosis, coccidiomycosis)

8. Candidiasis of the oesophagus or airways

9. Non-typhoid salmonella (NTS) septicaemia

10. Lymphoma cerebral or B cell Non Hodgkin's Lymphoma

11. Invasive cervical cancer

10. Visceral leishmaniasis

11. Symptomatic HIV-associated nephropathy or HIV-associated cardiomyopathy

Appendix XIII: MU – MTRH IREC Approval

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471/2/3
Reference: IREC/2013/129
Approval Number: 0001041

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET
26th August, 2013

Dr. Kirui Nicholas Kiplangat,
Moi University,
School Medicine
P.O. Box 4606 - 30100,
ELDORET-KENYA.

Dear Dr. Kirui,

RE: FORMAL APPROVAL

The Institutional Research and Ethics Committee have reviewed your research proposal titled:-

"Dyslipidemia and Dysglycemia in adult HIV-Infected Patients at the Moi Teaching and Referral Hospital, Eldoret"

Your proposal has been granted a Formal Approval Number: *FAN: IREC 1041* on 26th August, 2013. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 25th August, 2014. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc   Director-   MTRH   Dean -   SOM   Dean -   SON
      Principal-   CHS   Dean -   SPH   Dean -   SOD

Appendix XIV: MTRH Permission to conduct study

MOI TEACHING AND REFERRAL HOSPITAL

Telephone: 2033471/2/3/4
Fax: 61749
Email: director@mtrh.or.ke

P. O. Box 3
ELDORET

Ref: ELD/MTRH/R.6/VOL.II/2008

1st November 2013

Dr.Kirui Nicholas Kiplangat
Moi University
School of Medicine
P.O. Box 4606-30100,
ELDORET-KENYA.

RE: APPROVAL TO CONDUCT RESEARCH AT MTRH

Upon obtaining approval from the Institutional Research and Ethics Committee (IREC) to conduct your research proposal titled:

"Dyslipidemia and Dysglycemia in adult HIV-Infected Patients at the Moi Teaching and Referral Hospital, Eldoret".

You are hereby permitted to commence your investigation at Moi Teaching and Referral Hospital.

DR.J. KIBOSIA
DIRECTOR
MOI TEACHING AND REFERRAL HOSPITAL

CC   -   Deputy Director (CS)

  -      Chief Nurse

  -      HOD, HRISM

Appendix XV: AMPATH Permission to conduct study

Academic Model Providing Access To Healthcare
Telephone: 254 53 2033471/2 P.O. BOX 4606, ELDORET Fax: 254 53 2060727
RESEARCH CENTRE

Ref: RES/STUD/18/2013   November 4, 2013

To: In Charges, AMPATH Module 1, 2 Clinics

RE: PERMISSION TO CONDUCT RESEARCH AT AMPATH

This is to kindly inform you that **Dr. Nicholas Kiplangat Kirui,** a postgraduate student at the School of Medicine, College of Health Sciences, Moi University has been granted permission to conduct research at AMPATH MTRH Module 1, 2. His study; *"Dyslipidemia and dysglycemia in Adult HIV infected patient at Moi teaching and Referral Hospital, Eldoret"* has been reviewed by IREC and reviewed by the AMPATH Research Program Office.

His research activities should not in any way interfere with the care of patients. This approval does not support access to AMRS data at AMPATH.

The researcher is to submit a final report of their findings to the AMPATH Research Program Office.

Should the researchers wish to publish their findings, permission has to be sort from AMPATH Publications Committee. Please contact the AMPATH Research Office in case of any enquiry regarding this matter.

Thank you,

Prof. Nyandiko,
Deputy Chief of Party, Research and Training