Cardio-Metabolic Effects of Anti-Retroviral Treatment in the Cape Winelands region of South Africa

by

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March 2016

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Abstract (English)

Background: Although highly active antiretroviral therapy (HAART) has significantly improved the survival of human immunodeficiency virus (HIV)-infected patients there are increased concerns regarding the onset of co-morbidities (e.g. cardio-metabolic complications) and mortalities. Although South Africa is burdened with the highest number of HIV-infected individuals globally, there is a relative paucity of data regarding potential links between HIV infection, HAART and cardio-metabolic risk/onset.

Methods: This cross-sectional study therefore investigated the prevalence of cardio-metabolic risk factors in HIV-infected individuals within the Cape Winelands region of South Africa. Here we collected anthropometric, biochemical and lifestyle-related data for HIV-positive HAART naive (n =25) and HIV-positive individuals on HAART (n = 50) patients (20–55 years old) at the Worcester Community Day Centre (CDC) (Worcester, Western Cape, South Africa) during 2014 and 2015. Subjects on HAART were further divided into two sub-groupings, i.e. first line (n = 25) and second line treatments (n = 25).

Results: Our data reveal the relatively high prevalence of traditional, cardio-metabolic lifestyle risk factors in HIV-infected individuals. There was a relatively high prevalence of smoking, i.e. 88% for the HIV-positive HAART naive group and 27% for the HIV-positive group on HAART (P = 0.001), while more than half of the HIV-positive individuals exhibited a positive history of familial cardiovascular diseases (CVD). There were no significant differences for fasting blood glucose (FBG) and insulin levels between HIV-positive HAART naive and HIV-positive on HAART. Lipid metabolite analyses (Total cholesterol [TC], low-density lipoprotein [LDL], high density lipoprotein [HDL] and triglyceride [TG]) also did not reveal significant changes when comparing HIV-positive on HAART versus HIV-positive HAART naive groups. However, additional analyses (using established cut-off values for HDL, LDL) showed a significant difference in the proportion of individuals categorized with ''low HDL'' status, i.e. 68% for the HIV-positive HAART naive compared to 40% for the HIV-positive on HAART group (P = 0.022). HAART also enhanced anthropometric measures of obesity, with significant differences for

weight gain, triceps skin fold (TSF), biceps skin fold (BSF), waist circumference (WC) and mid-upper arm circumference (MUAC) between the naive and HAART groups. This applied similarly for first and second line treatments.

Conclusion: The study established the prevalence of several traditional lifestyle CVD risk factors in both HIV-positive naive and HIV-positive on HAART in the Cape Winelands region of South Africa. HAART enhanced several measure of weight gain and lipid profile, suggesting a restoration to health and well-being. However, there was a relatively high prevalence of obesity in the HIV-positive on HAART group (especially females) thus placing them at a greater risk for the onset of future cardio-metabolic complications. We are unable to distinguish whether this risk is due to HAART or lifestyle-related risk factors, and this question requires further investigation. The findings of this study indicate that clinicians should be attentive of lifestyle-related CVD risk factors in HIV-positive persons and make an effort to counsel patients to adopt improved lifestyle choices.

Abstract (Afrikaans)

Agtergrond: Alhoewel hoogs-aktiewe antriretrovirale terapie (HAART) die oorlewing van menslike immuniteitsgebrek virus (MIV)-geïnfekteerde pasiënte beduidend verbeter, is daar toenemende kommer aangaande die ontwikkeling van ko-morbiditeite (o.a. kardio-metaboliese komplikasies) en mortaliteit. Alhoewel Suid-Afrika gebuk gaan onder die grootste aantal MIV-geïnfekteerde individue wêreldwyd, is daar 'n relatiewe tekort aan data wat die potensiële verband tussen MIV infeksie, HAART en kardio-metaboliese risiko/aanvang betref.

Metodes: Hierdie dwarssnitstudie ondersoek dus die voorkoms van kardio-metaboliese risiko faktore in MIV-geïnfekteerde individue binne die Kaapse Wynland distrik van Suid-Afrika. Hier het ons antropometriese, biochemiese en lewensstyl-verwante data vir MIV-positiewe HAART naïewe (n = 25) en MIV-positiewe individue op HAART (n=50; 20-55 jaar oud) by die Worcester Gemeenskaps-dagsentrum (Worcester, Weskaap, Suid-Afrika) gedurende 2014 en 2015 versamel. Pasiënte op HAART is verder verdeel in twee sub-groepe, nl. eerste linie (n = 25) en tweede linie (n = 25) behandeling.

Resultate: Ons data het 'n relatiewe hoë voorkoms van tradisionele, kardio-metaboliese lewensstyl risiko faktore onder MIV-geïnfekteerde individue onthul. Daar was 'n relatiewe hoë voorkoms van rook, nl. 88% vir die MIV-positiewe HAART-naïewe groep en 27% vir die MIV-positiewe groep op HAART (P = 0.001), terwyl meer as helfte van die MIV-positiewe individue 'n positiewe geskiedenis van oorerflike kardiovaskulêre siektes (KVS) getoon het. Daar was geen beduidende verskille in vastende bloedglukose (FBG) en insulienvlakke tussen MIV-positiewe HAART naïewe en MIV-positiewe pasiënte op HAART behandeling nie. Lipied metaboliet analise (Totale cholesterol [TC], lae-digtheid lipoprotein [LDL], hoë-digtheid lipoprotein [HDL] en trigliseriede [TG]) het ook geen beduidende veranderinge onthul toe MIV-positiewe op HAART vs. MIV-positiewe HAART naïewe groepe vergelyk is nie. Additionele analise (deur gebruik te maak van afsny-waardes vir HDL, LDL) het egter beduidende verskille aangetoon in die proporsie individue gekategoriseer met "lae HDL" status, nl. 68% vir MIV-positiewe HAART naïewe pasiënte, in vergelyking met 40% vir MIV-positiewe pasiënte op HAART

(P = 0.022). HAART het ook die antropometriese mate van vetsugtigheid verbeter, met beduidende verskille in massa-toename, triseps velvou (TSF), biseps velvou (BSF), middelmaat (WC) en mid-boarm omtrek (MUAC) tussen die naïewe en HAART groepe. Hierdie was soortgelyk vir eerste en tweede linie behandeling met HAART.

Afleiding: Hierdie studie het die teenwoordigheid van verskeie tradisionele lewensstyl KVS risiko faktore in beide MIV-positiewe HAART-naïewe en MIV-positiewe op HAART bevestig in die Kaap Wynland distrik van Suid-Afrika. HAART het verskeie mates van massa toename en die lipied profiel verbeter, wat moontlike herstel van gesondheid en welwees aangedui het. Daar was egter 'n relatief hoë voorkoms van vetsug in die MIV-postiewe op HAART groep (veral vroulike pasiënte) wat hulle dus onder 'n verhoogde risiko vir die ontwikkeling van toekomstige kardio-metaboliese komplikasies plaas.

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List of Abbreviations

3TC	Lamivudine
ABC	Abacavir
ACS	Acute coronary syndrome
AIDS	Acquired immune deficiency syndrome
AIP	Atherogenic index of plasma
APV	Amprenavir
ART	Antiretroviral treatment
ARV	Antiretroviral
ATP III	Adult treatment panel III
ATV	Atazanavir
AZT	Zidovudine
BMI	Body mass index
BSF	Biceps skin fold
CCR5Is	Chemokine co-receptors inhibitors
CD4 T cell	Cluster of differentiation 4 T lymphocyte cell
CHD	Coronary heart disease
CMS	Cardio-metabolic syndrome
COBI	cobicistat
CRABP1	Cytoplasmic retinoic acid-binding protein type 1
CRP	C-reactive protein
CT	Computerized tomography
CVD	Cardiovascular diseases
d4T	Stavudine

DAD	Data Collection on Adverse Events of Anti-HIV Drugs
DBP	Diastolic blood pressure
ddc	Zalcitabine
ddi	Didanosine
DLV	Delavirdine
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
DTG	Dolutegravir
EFV	Efavirenz
ETV	Etravirine
EVG	Elvitegravir
FBG	Fasting blood glucose
FDA	Food and Drug Administration
FDC	Fixed dose combination
FFA	Free fatty acids
FMD	Flow-mediated vasodilation
fos-APV	fos-Amprenavir
FPG	Fasting plasma glucose
FTC	Emtricitabine
GLUT-4	Glucose transporter type 4
HAART	Highly active antiretroviral therapy
HC	Hip circumference
HDL	High density lipoprotein
HIV	Human immunodeficiency syndrome
HOMA	Homeostatic model assessment

HTN	Hypertension
ICAM	Intracellular adhesion molecule
IDF	International Diabetes Federation
IDV	Indinavir
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IL6	Interleukin 6
IMT	Intima–media thickness
IR	Insulin resistance
LA	Lipoatrophy
LD	Lipodystrophy
LDL	Low density lipoprotein
LDL-R	Low density lipoprotein receptor
LH	Lipohypertrophy
LPL	lipoprotein lipase
LPV	Lopinavir
LPV/r	Lopinavir/ritonavir
LRP1	LDL-receptor-related protein type 1
MetS	Metabolic syndrome
MI	Myocardial infarction
MRI	Magnetic resonance imaging
mtDNA	Mitochondrial deoxyribonucleic acid
mtRNA	Mitochondrial ribonucleic acid
MUAC	Mid-upper arm circumference
MVC	Maraviroc

NCEP	National Cholesterol Education Program
NFV	Nelfinavir
NNRTIs	Non-nucleoside reverse transcriptase inhibitors
NRTIs	Nucleoside reverse transcriptase inhibitors
NVP	Nevirapine
NVPXR	Nevirapine extended release
OGTT	Oral glucose tolerance test
PAI-1	Plasminogen activator-1
PIs	Protease inhibitors
PPAR-γ	Peroxisome proliferator-activated receptor gamma
RAS	Renin angiotensin system
RNA	Ribonucleic acid
RT	Reverse transcriptase
RTV	Ritonavir
RXR-PPARγ	Retinoid X receptor-peroxisome proliferator-activated receptor gamma
SAD	Sagittal abdominal diameter
SBP	Systolic blood pressure
SQV	Saquinavir
SREBP1	Sterol regulatory element binding protein 1
T2DM	Type 2 diabetes mellitus
TC	Total cholesterol
TDF	Tenofovir
TG	Triglyceride
TNFα	Tumor necrosis factor alpha
tPA	Tissue plasminogen activator

TSF	Triceps skin fold
UNAIDS	United Nations Program on HIV/AIDS
VAT	Visceral adipose tissue
VCAM	Vascular cell adhesion molecule
VLDL	Very low density lipoprotein
WC	Waist circumference
WHO	World Health Organization
WHR	Waist-to-hip ratio

CHAPTER ONE

LITERATURE REVIEW

1.0 Background

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1.0 Background

The introduction of highly active antiretroviral therapy (HAART) over the last few decades has significantly modified the devastating effects of the human immunodeficiency virus (HIV) by prolonging survival and improving patients' quality of life. In parallel, increasing cardio-metabolic complications have been documented since the introduction of HAART. Here early data established that HAART regimens (especially protease inhibitors [PIs]) are associated with increased metabolic (hyperlipidemia, insulin resistance [IR]) and somatic (lipodystrophy/lipoatrophy) (LD/LA) changes that are usually associated with a higher risk for cardiovascular diseases (CVD) (typically coronary and peripheral artery disease, strokes). Antiretroviral (ARV) drug regimens can be associated with metabolic side-effects that include dyslipidemia, impaired glucose metabolism and abnormal body fat distribution that all increase CVD risk. Compounding this problem is the HIV itself where persistent low-grade inflammation can also contribute to a higher incidence of cardio- and cerebrovascular events. Thus improved evaluations of metabolic status in HIV-positive individuals may assist with treatment modalities and enhance overall patient well-being by lowering co-morbidities such as CVD in this instance. Of concern, however, are rising lifestyle-related disease onset e.g. the cardio-metabolic syndrome (CMS) in sub-Saharan Africa that together with HIV constitutes a double burden of disease. This chapter introduces ARV-mediated metabolic dysregulation and its impact on CVD risk in HIV-positive individuals, with a particular focus on the African continent and the South African context.

1.1 HIV in Africa

HIV infects ~36.9 million people worldwide (UNAIDS 2014). The World Health Organization (WHO) estimated that acquired immune deficiency syndrome (AIDS) claimed about 2 million newly infected people at the end of 2014. Here sub-Saharan Africa remains the most severely affected with ~25.8 million persons saddled with HIV (Figure 1), representing ~70% of the globally afflicted population (UNAIDS 2014). For example, for three sub-Saharan countries (Nigeria, Uganda, South Africa) there were 1.4 million new HIV infections and 790, 000 deaths in 2014 due to AIDS-related causes (UNAIDS 2014). South Africa has the highest number HIV-positive individuals in the world, with ~6.19 million persons being infected. These alarming numbers require robust interventions to counter the devastating effects of HIV and this may for e.g. include:

a) Educational programs to decrease new HIV infections

b) Improvement of heath care systems in the region, providing ARV treatment and rehabilitating HIV-positive individuals

c) Support HIV patients to better cope with HIV /AIDS-related illness and death.

HIV type 1 and HIV type 2 are two distinct viruses, with type 1 the predominant one on a global scale. The HIV-1 strains can be classified into four groups (Hemelaar 2012), with ''M'' (for 'major') the most important group and greatly responsible for the global HIV epidemic. Within group M there are known to be at least nine genetically distinct subtypes of HIV-1, i.e. subtypes A, B, C, D, F, G, H, J and K. The dominant HIV subtype in the Americas, Western Europe and Australasia is subtype B. However, this subtype represents only ~12% of global HIV infections. By contrast, less research is available for subtype C although just under half of all persons living with HIV carry this particular subtype C. It is very common in the high prevalence countries of Southern Africa as well as in the African ''horn'' and countries such as India (Fox et al. 2010; Hemelaar 2012).
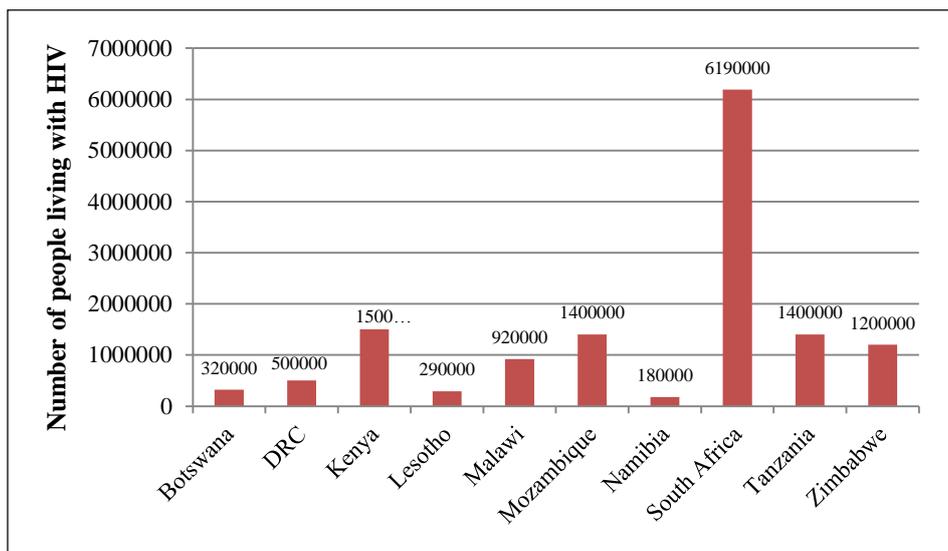
Figure 1: Persons living with HIV/AIDS in sub-Saharan Africa (UNAIDS 2014).

The advent of HAART changed the nature of AIDS from a terminal, fatal illness to a chronic disease (Mutimura et al. 2008). This is supported by recent statistics (UNAIDS 2014) that showed a significant decrease in mortality rates with ~1.2 million versus 2.3 million deaths worldwide in 2014 and 2005, respectively. This represents a 42% decline in the mortality rate, in sub-Saharan Africa with HAART clearly prolonging life expectancy.

1.2 South Africa's health care system and the issue of HIV

South Africa is considered as an upper-middle income, multi-ethnic country and has an estimated population of 54 million people, with 11 official languages (23% Zulu-speaking, 16% Xhosa-speaking, 14% Afrikaans-speaking, 9.6% English-speaking, 8% Setswana-speaking, 7.6% Sesotho-speaking, with the residual official languages less than 5%) (Statistics South Africa 2012; The Economist 2011). In South Africa there are two types of health care systems (public, private) with ~80% of the population dependent on the public health sector with the rest relying on private health care. Due to the HIV/AIDS epidemic in South Africa the government is making a concerted effort to roll out ARV treatment. Here ARV treatment roll-out increased from 1.2 million in 2011 to 2.6-million by 2014, making South Africa the largest country with programmed HIV treatment in the world (Media Club South Africa 2015).

1.2.1 HIV in South Africa

According to Statistics South Africa (2014; 2015), the total number of persons living with HIV increased from 4.02 million in 2002 to 6.19 million in 2015; this means that ~11.2% of the South African population is HIV-positive. KwaZulu-Natal is one of the most affected regions, with about a third of the population being HIV-positive. In addition, women at reproductive age are mostly affected, e.g. about one-fifth of South African women are HIV-positive. With ARV treatment the total number of deaths decreased from 3.6 million (2005) to 1.7 million (2014), while new HIV infections declined from 5.4 million (2004) to 3.7 million (2012). With the expansion of the ARV roll-out, ~2.2 million South Africans are currently receiving ARV treatment (Statistics South Africa 2014). Moreover, during December 2012 the Minister of Health announced the introduction of the fixed dose combination (FDC) of ARV triple therapy (to start April 2013). Drugs employed in the FDC (TDF, FTC/3TC and EFV) were selected in order to improve access to antiretroviral treatment (ART) and to also increase the adherence and retention of patients on treatment (National Department of Health 2014).

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1.2.2 HIV and AIDS treatment in South Africa

As South Africa has the largest ART roll-out globally (The South African Antiretroviral Treatment Guidelines 2013), the expected benefits of the program were:

- Increased life expectancy and quality of life for HIV patients

- Cost-efficient treatment and improved clinical outcomes

- Early HIV diagnosis by providing free testing

- Decreased HIV/AIDS-related deaths

- Prevention of new infections among children, adolescents, and adults.

According to Meintjes et al. (2014), ARV treatment should commence in all HIV-positive patients who display a cluster of differentiation 4 T lymphocyte (CD4 T cell) count of ≤ 500 cells/mm^3 and WHO clinical stages 3 or 4. There are three main treatment approaches:

The first line treatment includes the use of two nucleoside reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitors (NNRTIs) in different regimens in the following possible combinations:

- Tenofovir TDF + Lamivudine 3TC/Emtricitabine FTC + Efavirenz EFV/Nevirapine NVP

- Stavudine d4T + Lamivudine 3TC + EFV Efavirenz/Nevirapine NVP (for the new recommendation change d4T to TDF (no patient must be on d4T even if it is well tolerated)

- Zidovudine AZT + Lamivudine 3TC + EFV Efavirenz/Nevirapine NVP.

The recommended doses for first line treatment are indicated in Table 1.

However, if side-effects or resistance develop then **the second line of treatment** is initiated – where viral load persists above 1,000 copies/ml. A boosted PI plus two NRTIs are then recommended for the second line of treatment:

- Tenofovir TDF + Lamivudine 3TC/ Emtricitabine FTC + Lopinavir/ritonavir (LPV/r)

- Zidovudine AZT + Lamivudine 3TC + LPV/r

The recommended dose for LPV/r is LPV 200 mg/r 50mg given as 2 tablets twice daily (LPV 400 mg/r 100 mg).

Table 1: The recommended doses for first line treatment in South Africa.

Drug	Dose
Tenofovir (TDF)	300 mg daily
Lamivudine (3TC)	300 mg daily
Emtricitabine (FTC)	200 mg daily
Efavirenz (EFV)	600 mg at night
Nevirapine (NVP)	200 mg daily x 14 days, then 200 mg twice daily
Zidovudine (AZT)	300 mg twice daily
Stavudine (d4T)	30 mg twice daily (discontinued usage)

The third line of treatment (salvage) is usually employed when there is resistance and virological failure for NRTIs, NNRTIs and PIs classes. Recently a number of new drugs in third-line ART has emerged and these include: a) integrase inhibitors, b) second generation of NNRTIs and PIs and c) chemokine co-receptors antagonists (CCR5) (Meintjes et al. 2014).

1.3 The ABCs of ART

1.3.1 Development of treatment strategies

Since HIV was identified as the causative agent for AIDS a concerted effort has been made to develop medications that inhibit HIV replication. For example, the Food and Drug Administration (FDA) approved the first zidovudine (first ARV drug; NRTI) 28 years ago and this advance dramatically reduced the number of opportunistic AIDS related diseases and mortalities (UNAIDS 2011). Reverse-transcriptase inhibitors may also be non-nucleoside based and are referred to as NNRTIs. Both forms are commonly identified as reverse-transcriptase inhibitors (Batterham et al. 2001). Until the mid-1990s zidovudine and a number of other nucleoside analogues were the only available ART (Batterham et al. 2001). The NRTIs were initially employed as mono therapy, but later used as a dual combination. Although usage of such agents increased CD4 T cell counts, the first dose involved high drug toxicity. Furthermore, continued use triggered viral resistance and thus limited length of treatment and efficacy of such therapeutic agents (Barlett 1996). By the mid-1990s there was

widespread availability of viral load testing and the introduction of PIs, a new ARV drug class that inhibit the protease enzyme responsible for producing the mature virus (Lewin et al. 1997). LPV/r (Alluvia®) (Kaletra®) is a co-formulation of two structurally related PI ARV agents. LPV® is a highly potent and selective inhibitor of the HIV type 1 (HIV-1) protease, the enzyme responsible for production of the mature, infective virus. It acts by arresting maturation of HIV-1 and thereby blunting its capacity to infect and hence the main antiviral action of LPV is to prevent subsequent infections of susceptible cells (Cvetkovic & Goa 2003). Controlled trials of combinations containing PIs as one of three initial agents resulted in the recommendation that the standard HAART regimen should contain three ARV agents: two NRTIs plus PIs or two NRTIs plus an NNRTI (Carpenter et al. 1997) (refer Figure 2 for ARV time line). Recent data show that viral suppression following HAART results in decreased HIV transmission at individual (Cohen et al. 2011) and population levels (UNAIDS 2011). Moreover, HAART may provide protection against HIV infection when used consistently by HIV-uninfected persons (Grant et al. 2010; Baeten 2011; Thigpen et al. 2011). Together these developments have raised optimism regarding the HIV threat and translated into newly articulated visions of the "**beginning of the end of AIDS**" (Thompson et al. 2012:387).
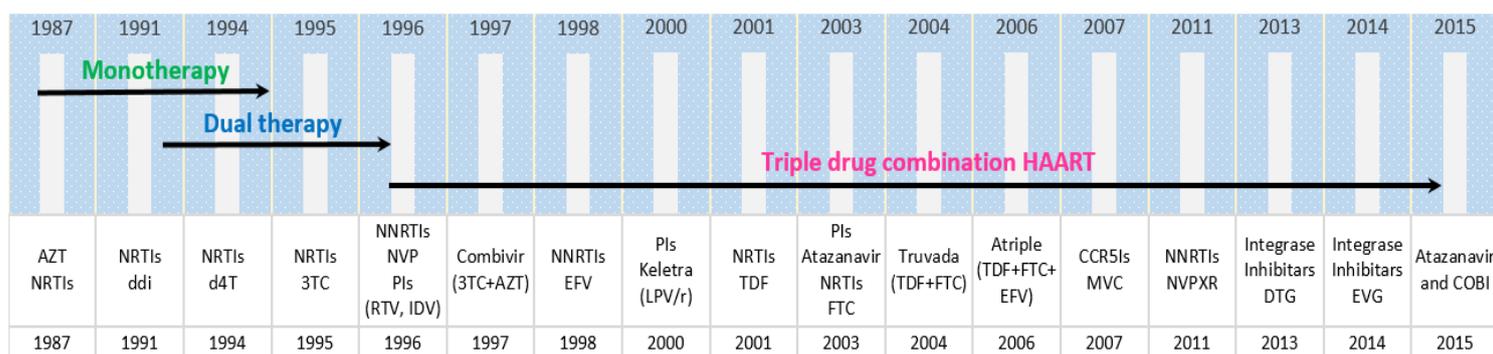
Figure 2: Time line for FDA approval and the development of ARV drugs. This reflects monumental efforts to produce most effective ARVs, to improve prognosis of persons living with HIV and to decrease AIDS-related mortalities and opportunistic infections. NRTIs were initially used as mono therapy and later employed in dual combination; the HAART regimen should contain three ARV agents: two NRTIs plus PIs or two NRTIs plus NNRTI. *NRTI,NNRTIs-nucleoside and non-nucleoside reverse transcriptase inhibitors, PIs-protease inhibitors, Chemokine co-receptors inhibitors (CCR5Is), Integrase inhibitors, AZT- Zidovudine, ddi-Didanosine, d4T-Stavudine, 3TC- Lamivudine, RTV- Ritonavir, IDV- Indinavir, NVP- Nevirapine, EFV-Efavirenz, Ritonavi-(r,RTV), TDF-Tenofovir, FTC- Emtricitabine, MVC-Maraviroc,NVPXR- Nevirapine extended release, DTG-Dolutegravir, EVG-Elvitegravir, ATV- Atazanavir, COBI- cobicistat.*

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1.3.2 ARVs - classes and characteristics

The aim of ARV treatment is to achieve and sustain a plasma viral load that is undetectable (Batterham et al. 2001). ARV treatments are classified according to their inhibitory effects in different stages of the HIV life cycle (Figure 3). The main classes of ARV therapy are:

1) NRTIs

2) NNRTIs

3) PIs

4) CCR5

5) Fusion inhibitors

6) Integrase inhibitors

7) Maturation inhibitors
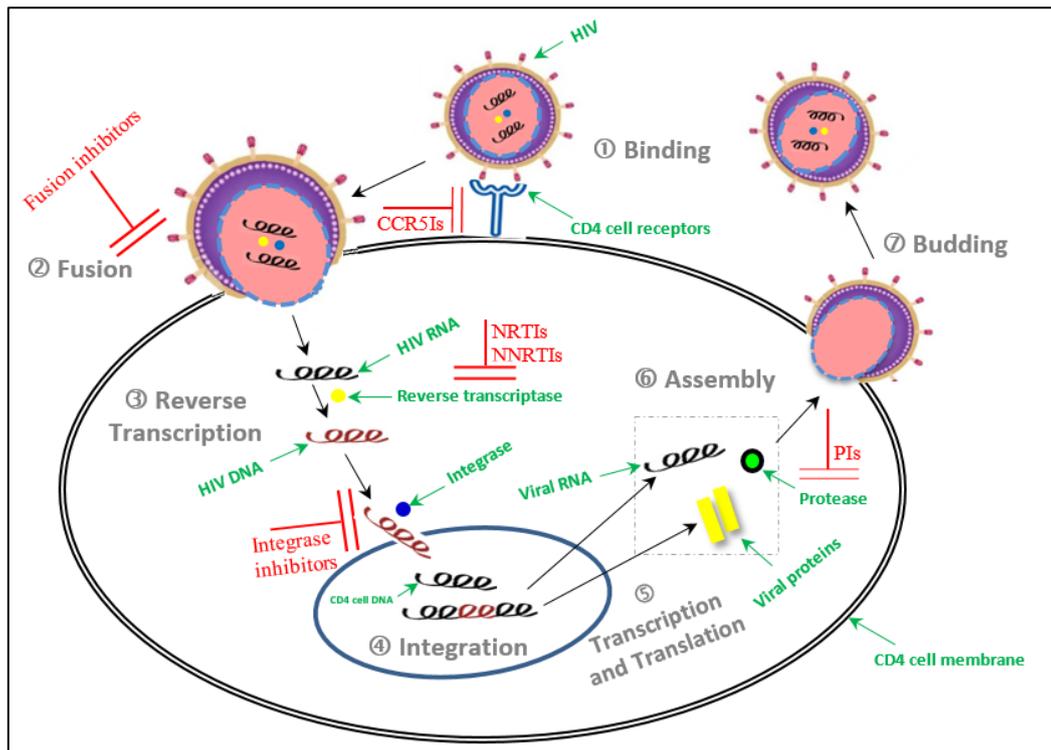
Figure 3: HIV life cycle and inhibitory action of ARVs. 1. Binding of HIV to receptors onto CD4 surface.   2. Fusion of HIV envelope to CD4 cell membrane. 3. Reverse Transcription of HIV genetic material; HIV-RNA is converted to DNA by reverse transcriptase. 4. Integration of HIV-DNA into host DNA by action of integrase. 5. Transcription and translation - use host cell machinery to synthesize long chains of HIV-proteins. 6. Assembly - HIV-protease enzyme cleaves long chains of HIV-proteins to smaller proteins that when combined with HIV-RNA form new virus. 7. Budding - the newly formed HIV pushes out or ''buds'' from the CD4 cell. HAART can inhibit at critical steps during HIV life cycle. *NRTI, NNRTIs-nucleoside and non-nucleoside reverse transcriptase inhibitors, PIs-protease inhibitors, Entry inhibitors (Chemokine co-receptors inhibitors (CCR5Is)), DNA-deoxyribonucleic acid, RNA-ribonucleic acid, HIV-human immunodeficiency virus.*

HAART regimens consist mainly of two or three drugs (from different classes) and typical regimens combine two NRTIs with NNRTI or PIs. Here treatment with triple combination regimens decreased AIDS-related morbidity and mortality (Hogg et al. 1998) (refer Table 2 for three main ARV classes).

Table 2: Three main ARV classes.

NRTIs	NNRTIs	PIs
Zidovudine® (ZDV, AZT)	Nevirapine (NVP)	Nelfinavir (NFV)
Lamivudine® (3TC)	Efavirenz (EFV, EFZ)	Ritonavir (RTV)
Stavudine® (d4T)	Delavirdine (DLV)	Saquinavir (SQV)
Didanosine (ddi)	Etravirine (ETV)	Indinavir (IDV)
Abacavir (ABC)		Lopinavir/ritonavir (LPV/r)
Tenofovir (TDF)		Atazanavir (ATV)
Emtricitabine (FTC)		Amprenavir (APV)
Zalcitabine (ddc)		fos-Amprenavir (fos-APV)

1. NRTIs and NNRTIs – mechanism of action

After fusion of the HIV envelope with the host cell membrane, the uncoating of viral core occurs followed by slow release of the viral genetic material (RNA genome) and associated enzymes into the host cell cytoplasm. Reverse transcriptase then copies the viral single-stranded RNA genome into a double-stranded viral DNA (reverse transcription and pro-viral DNA synthesis start). The viral DNA is then integrated into the host chromosomal DNA, which then allows the host cell to start both transcription and translation to produce new viruses. Reverse transcriptase (RT) was the first HIV enzyme that was targeted for ARV drug development. RT is a multifunctional enzyme with RNA-dependent DNA polymerase activities used to convert the single-stranded viral RNA to double-stranded DNA (Hu & Hughes 2011). RT is a target for two classes of ARV drugs, i.e. NRTIs and NNRTIs. NRTIs must be activated in the cell by addition of three phosphate groups to their deoxyribose end to form NRTI triphosphates that are then able to incorporate into the viral DNA. NRTIs block the function of reverse transcriptase and prevent completion of synthesis of double-stranded

viral DNA. This process is termed chain termination and stops the synthesis of viral DNA, thereby preventing HIV from multiplying. NNRTIs act by non- competitively binding to RT that leads to conformational changes that inhibit the catalytic activities of the enzyme, resulting in inhibition of transcription of the viral RNA and an attenuation of the replication rate (Imamichi 2004).

2. PIs – mechanism of action

HIV protease is responsible for a critical step during the HIV life cycle, i.e. the proteolytic cleavage of polypeptide precursors into mature enzymes and structural proteins. PIs are peptide-like chemicals that competitively inhibit the action of HIV protease. Such drugs inhibit proteolytic cleavage of HIV Gag and poly proteins which are important structural and enzymatic components of the new virus and thus prevents conversion of HIV particles into the mature virus (Debouck 1992; Imamichi 2004).

1.4 HAART and the onset of cardio-metabolic complications

HAART significantly improved outcomes of virological and the immunological tests (Hogg et al. 1998) and the quality of life of HIV-positive patients (Liu et al. 2006). However, HAART (especially PIs) can also be associated with unfavorable metabolic disturbances such as dyslipidemia, impaired glucose tolerance (IGT), body composition changes and hypertension (HTN) (Dolan et al. 2005). Such complications are of course well-known CVD risk factors in the general population and hence also puts HIV-positive individuals at risk (Assmann et al. 2007). Because of such side-effects there are ongoing research endeavors to gain improved insights into risk factors and its impact on the well-being of HIV-positive individuals, e.g. to ascertain whether these are similar or not compared to the general population (Mutimura et al. 2008). This is an ongoing and relatively recent process and here efforts are focusing on various metabolic, cardiovascular and anthropometric changes and how these intersect with more traditional risk factors (such as smoking, obesity) in HIV-positive persons (Figure 4).
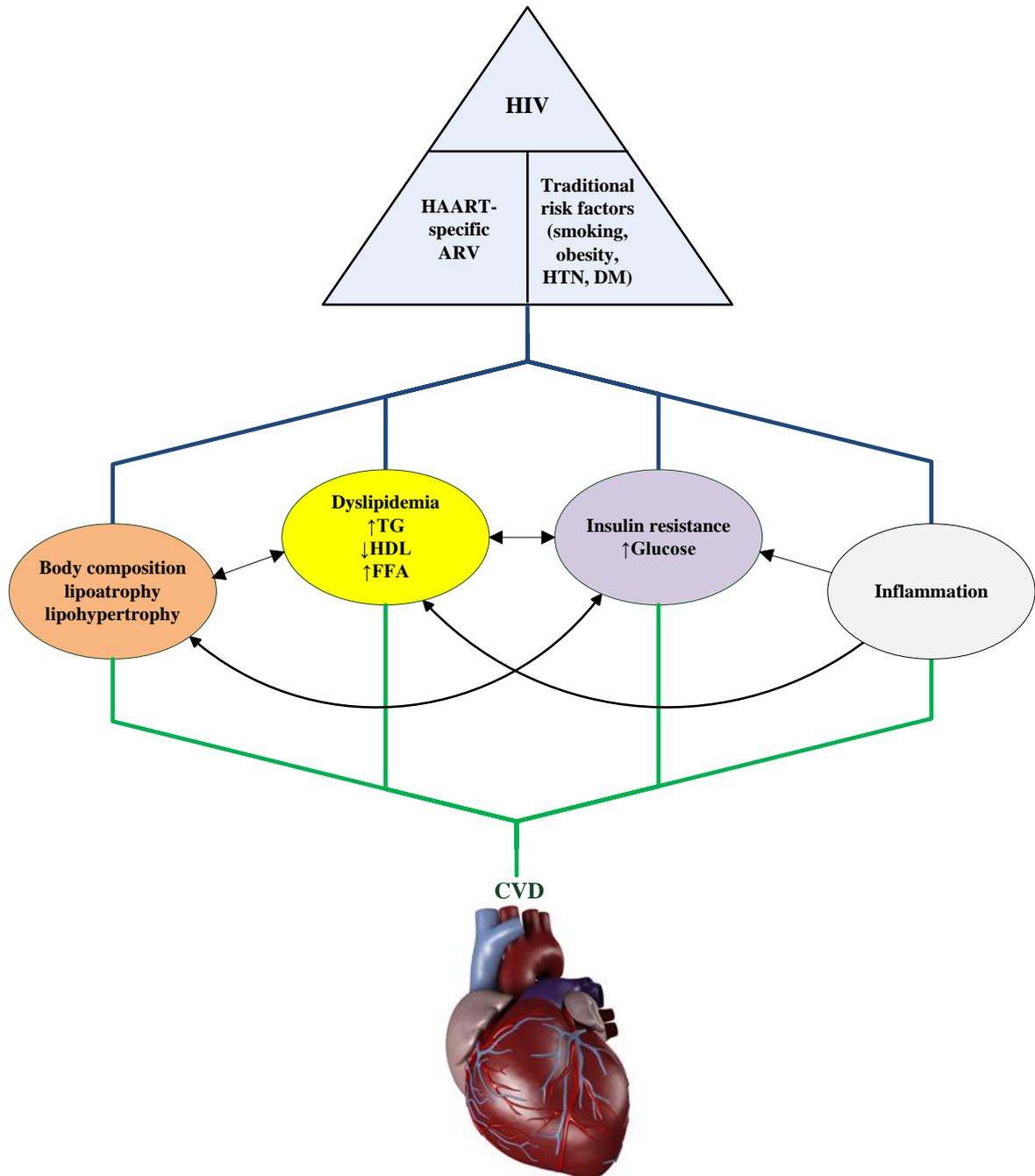
Figure 4: Summary of the effects of HIV, HAART and traditional risk factors on CVD risk. CVD linked to the metabolic, anthropometric, inflammatory changes caused by HIV infection and HAART and intersection of traditional risk factors. *TG- Triglyceride, HDL- High density lipoprotein, FFA- Free fatty acids, HTN-Hypertension, DM- Diabetes mellitus, CVD- Cardiovascular diseases.*

1.4.1 Metabolic complications

Effective treatment of HIV infection requires three or four drug regimens. However, as earlier discussed this can be associated with adverse side-effects (Schiller 2004) (Table 3).

Table 3: Major adverse effects of HAART by drug class and organ system.

System	PI	NRTI	NNRTI
Gastrointestinal	Diarrhoea, nausea, vomiting (NFV) (Drugs for HIV infection 2009; Dubé et al. 2003; Panel on Antiretroviral Guidelines for Adults and Adolescents 2011).	Pancreatitis, anorexia, nausea, vomiting, non-cirrhotic portal hypertension (ddI) (Drugs for HIV infection 2009; Panel on Antiretroviral Guidelines for Adults and Adolescents 2011).	Severe hepatotoxicity, liver failure (NVP) (Drugs for HIV infection 2009; Etravirine (Intelence) for HIV infection 2008; Panel on Antiretroviral Guidelines for Adults and Adolescents 2011).
Neurologic	Perioral paresthesia (fos-APV), circumoral and peripheral paresthesia (RTV) (Drugs for HIV infection 2009; Dubé et al. 2003; Panel on Antiretroviral Guidelines for Adults and Adolescents 2011).	Peripheral neuropathy, retinal changes, optic neuritis, (d4T, ddI) (Drugs for HIV infection 2009; Panel on Antiretroviral Guidelines for Adults and Adolescents 2011).	Dizziness, insomnia, headache, inability to concentrate, (EFV), peripheral neuropathy (ETV) (Drugs for HIV infection 2009; Etravirine (Intelence) for HIV infection 2008; Panel on Antiretroviral Guidelines for Adults and Adolescents 2011).
Metabolic	Lipohypertrophy, glucose intolerance or diabetes mellitus (DM), IR, lipid disorders and change in fat distribution (fat redistribution) all are not class-specific) (Drugs for HIV infection 2009; Dubé et al. 2003; Panel on Antiretroviral Guidelines for Adults and Adolescents 2011).	Lactic acidosis, hyperlipidemia; glucose intolerance; DM (d4t) (Drugs for HIV infection 2009; Panel on Antiretroviral Guidelines for Adults and Adolescents 2011).	Increased cholesterol and triglyceride levels (ETV), LA (EFV) when used with (d4T) (Drugs for HIV infection 2009; Etravirine (Intelence) for HIV infection 2008; Panel on Antiretroviral Guidelines for Adults and Adolescents 2011).
Renal	Kidney stones, renal insufficiency (IDV) (Drugs for HIV infection 2009; Dubé et al. 2003; Panel on Antiretroviral Guidelines for Adults and Adolescents 2011).	Renal toxicity, proteinuria, renal failure (TDF) (Drugs for HIV infection 2009; Panel on Antiretroviral Guidelines for Adults and Adolescents 2011).	-
Immunologic and dermatological	Rash (fos-APV, SQV), alopecia (IDV) (Drugs for HIV infection 2009; Dubé et al. 2003; Panel on Antiretroviral Guidelines for Adults and Adolescents 2011).	Hypersensitivity reaction (ABC) hyperpigmentation of oral mucosa and nail beds (ZDV) hyperpigmentation of palms and soles (FTC) (Drugs for HIV infection 2009; Panel on Antiretroviral Guidelines for Adults and Adolescents 2011).	Hypersensitivity reaction (ETV) (Drugs for HIV infection 2009; Etravirine (Intelence) for HIV infection 2008; Panel on Antiretroviral Guidelines for Adults and Adolescents 2011).

Abbreviations: ddI = didanosine, NVP = nevirapine, NFV = nelfinavir, d4T = stavudine, EFV = efavirenz, TDF = tenofovir disoproxil fumarate, IDV = indinavir, ABC = abacavir, fos-APV = fos-Amprenavir, SQV= Saquinavir, RTV = Ritonavir, ZDV = Zidovudine, FTC = Emtricitabine, ETV = Etravirine.

The prevalence of metabolic (DM, IR, dyslipidemia) and phenotypic (abnormal fat distribution or LD syndrome) changes in HIV patients on HAART are on the increase (Barbaro 2006) and characteristics of metabolic syndrome (MetS) (Biron et al. 2012). There is increasing concern that metabolic disturbances associated with HIV and HAART may lead to increased risk for CVD. In support, the prevalence of MetS in HIV patients range between 11-45% and associated with a higher risk of developing CVD (Biron et al. 2012; Feleke et al. 2012; Jacobson et al. 2006).

1.4.1.1 HAART and the MetS

MetS is defined as a cluster of risk factors that include central obesity, dyslipidemia (high levels of triglyceride [TG], low levels high density lipoprotein [HDL]), abnormal glucose tolerance, IR and HTN (Reaven & Banting 1988), all that directly increase the risk for CVD and type 2 DM (T2DM) onset. There are many international organizations and expert groups such as the WHO clinical criteria (WHO 1999), the National Cholesterol Education Program Adult Treatment Panel III (NCEP: ATP III) and the International Diabetes Federation (IDF) that have attempted to criteria that constitute the MetS. Here the basic premise is that the cluster of factors but an individual at even greater risk for CVD and T2DM onset compared to when such factors present independently (Bonora et al. 1998) (Table 4).

Table 4: The definition of the MetS by different criteria.

WHO clinical criteria	ATP III clinical criteria (NCEP Expert Panel 2001)		IDF clinical criteria	
The individual must present with IGT, or DM, and /or IR with two or more of the following: - IGT or diabetes -IR (under hyperinsulinemic euglycemic conditions, glucose uptake below lowest quartile for background population under investigation) - Raised arterial pressure $\geq$ 140/90 mm Hg - Raised plasma TG ($\geq$ 1.7 mmol/L; 150 mg/dL) and/or low HDL cholesterol (< 0.9 mmol/L, 35 mg/dL men; < 1.0 mmol/L, 39 mg/dL women) - Central obesity (males: WHR > 0.90; females: WHR > 0.85) and/or BMI > 30 kg/m^2 - Microalbuminuria (urinary albumin excretion rate $\geq$ 20g/min or albumin: creatinine ratio $\geq$ 30 mg/g)	**Risk factor**	**Defining level**	Individuals present with central obesity (with ethnicity-specific cut-off values, [Appendix 6]) plus two of the other factors	
	Central obesity	WC		
	• Men • Women	> 102 cm (> 40 in) > 88 cm (> 35 in)		
	TG	$\geq$ 150 mg/dL (1.7 mmol/L)	Increased TG levels	$\geq$ 150 mg/dL (1.7 mmol/L), or specific treatment for this lipid abnormality
	HDL cholesterol • Men • Women	< 40 mg/dL (1.03 mmol/L) < 50 mg/dL (1.29 mmol/L)	Reduced HDL cholesterol levels	< 40 mg/dL (1.03 mmol/L)* in males and < 50 mg/dL (1.29 mmol/L)* in females, or specific treatment for this lipid abnormality
	Blood pressure	$\geq$ 130/ $\geq$ 85 mm Hg	Raised blood pressure	systolic BP $\geq$ 130 or diastolic BP $\geq$ 85 mm Hg, or treatment of previously diagnosed hypertension
	FBG	$\geq$ 110 mg/dL (6.1 mmol/L)	Elevated fasting blood glucose (FBG) levels	(FBG) $\geq$ 100 mg/dL (5.6 mmol/L), or previously diagnosed T2DM
	Three or more of the above five risk factors		If above 5.6 mmol/L or 100 mg/dL, OGTT is strongly recommended but is not necessary to define presence of the syndrome. *These values have been updated from what were originally presented in order to ensure consistency with ATP III cut points (IDF 2006).	

Abbreviations: IGT = impaired glucose tolerance, DM = diabetes mellitus, IR = insulin resistance, TG = triglyceride, HDL = high density lipoprotein, WHR = waist-to-hip ratio, BMI = body mass index, WC = Waist circumference, FBG = fasting blood glucose, OGTT = oral glucose tolerance test.

- HAART and the prevalence of the MetS in Africa

Many studies have reported the occurrence of MetS in the context of HIV/ AIDS and HAART and that this phenomenon is more common than in HIV-negative individuals (Bruno et al. 2002; Gazzaruso et al. 2002; Palella et al. 2006) (Table 5).

Table 5: HIV/HAART and MetS: African studies.

Authors	Number of participants	HAART use	Outcomes
Sales et al. (2009) in Botswana.	402	No (naive)	The prevalence of MetS (7.7%) in this cohort is similar to other sub-Saharan African HIV-positive ones
(Mhlabi 2011) in Botswana.	190	Yes	Prevalence of MetS was 11% and the most significant risk factors were being on NRTIs and PIs containing regimens
Botha et al. (2013) in North-West province of South Africa	66 on HAART, 71 naive	Yes	Increase in pulse and systolic blood pressure together with an unfavorable lipid profile
Berhane et al. (2012) in South West Ethiopia	313	Yes	21.1% MetS prevalence rate LD affecting 12.1% of HIV-positive patients on HAART
Diouf, et al. (2012) in Senegal	242	Yes	High prevalence of DM and HTN in Senegalese HIV-positive patients receiving HAART
Muhammad et al. (2013) in Nigeria	200 (100 on HAART, 100 HAART‑naive)	Yes	Significantly higher prevalence of HTN, obesity and MetS among HIV-positive patients.
McLigeyo et al. (2012) in Kenya	265 patients	Yes	High prevalence of LD syndrome in HIV-infected patients (51.3%) on chronic HAART, main phenotype was LA (44%)

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Sales et al. (2009) determined the prevalence of MetS in a cohort of asymptomatic and ARV treatment- naive HIV-positive adults in Botswana and a prevalence of 7.7% was documented, together with high relatively percentages for obesity (11.6%) and HTN (31.9%). In support, another study in the same country (Mhlabi 2011) reported a similar prevalence of MetS (11%) and that the most significant risk factors were being on NRTIs and PIs therapeutic regimens. A study completed in the North-West province concluded that South Africans receiving HAART have a greater increase in pulse pressure, systolic blood pressure together with an unfavorable lipid profile versus normal controls (Botha, et al. 2013). However, whether long-term HAART will lead to increased arterial stiffness and/or accelerated atherosclerosis in this instance remains unknown. Similar trends were reported for the rest of the African continent. For example, others found a 21% prevalence of the MetS in South West Ethiopia with LD affecting ~12% of HIV-positive patients on HAART Berhane, et al. (2012). A similar scenario exists for Senegalese HIV-positive patients receiving HAART with high prevalence of DM and HTN in this population (Diouf, et al. 2012). This study confirms the association between the duration of HAART and DM and further highlights the need to implement preventative programs to reduce CVD risk factors in HIV-positive patients. Furthermore, Muhammad et al. (2013) reported that HAART is associated with a significantly higher prevalence of HTN, obesity and MetS among HIV-positive patients in Nigeria, while McLigeyo, et al. (2012) reported a high prevalence of LD syndrome (51%) in HIV-infected with LA the main phenotype.

1.4.1.2 Dyslipidemia associated with HAART

With prolonged HIV infection there are increased manifestation of metabolic side-effects such as hypertriglyceridemia (Feeney & Mallon 2011; Grunfeld et al. 1989). However, some may present at an earlier stage, e.g. dyslipidemia and IR can occur shortly after HAART initiation (Carr et al. 1998a; Feeney & Mallon 2011). Increased TG and low-density lipoprotein (LDL) cholesterol levels together with decreased HDL cholesterol levels are the most common lipid changes associated with

HIV/HAART (Fontas et al. 2004; Friis-Moller et al. 2003a; Friis-Moller et al. 2003b) and show overlap with the MetS (Grundy et al. 2004). In support, PIs lead to significant hypertriglyceridemia (Mulligan et al. 2000) and low HDL-C (Periard et al. 1999; Riddler et al. 2003), a strongly athrogenic pattern (Mercie et al. 2000). This is associated with increased risk for myocardial infarction (MI) in HIV-infected patients (Currier et al. 2003; Holmberg et al. 2002; Passalaris et al. 2000). HDL can inhibit the expression of E-selectin or other adhesion molecules by vascular endothelial cells exposed to cytokines and this results in decreased binding of inflammatory cells, which is consistent with functional inhibition of atherosclerosis (Moudry et al. 1997). Furthermore, supportive evidence for such HDL-mediated anti-atherogenic mechanisms is available from clinical studies where increased levels of adhesion molecules correlated with low HDL-cholesterol levels (Calabresi et al. 2002; Hernandez et al. 2003; Lupattelli et al. 2003). NNRTI-based regimens are also associated with decreased HDL levels (~40% of HIV-positive individuals) together with increased total cholesterol (TC), LDL-C and TG levels. For example, EFV or NVP (commonly prescribed NNRTIs) treatment or both used in combination together with two NRTIs (d4T and 3TC) resulted in higher TG levels in the EFV arm compared to the NVP one (49% versus 20% at 48 weeks) (van Leth et al. 2004). Some NRTIs agents such as d4T is more tightly associated with dyslipidemia than others such as TDF (Gallant et al. 2004). Of note, the nature of such increases are less pronounced compared to the effects of PIs (Haubrich et al. 2009).

- HAART-mediated dyslipidemia: pathophysiologic mechanisms

The pathogenesis of dyslipidemia in this instance is complicated and includes several factors such as the effects of different drug classes, HIV itself, genetic predisposition, immunological and hormonal influences (Fisher et al. 2006; Guardiola et al. 2006). In addition, traditional risk factors (e.g. male sex, age, increased BMI, family history of CVD) and sedentary lifestyle) also affect pathogenic mechanisms that can contribute to HAART-induced dyslipidemia. There are three possible causes for increased plasma TG-rich very low density lipoprotein (VLDL) levels: decreased catabolism of VLDL by HIV infection itself (Carpentier et al. 2005);

increased production of such particles by HAART (Carpentier et al. 2005), or decreased catabolism of VLDL as a result of PI effects (Shahmanesh et al. 2005). HAART treatment reduces activity of plasma and tissue lipases that impair hydrolysis of TG-rich lipoproteins (Purnell et al. 2000; Reeds et al. 2006; Sekhar et al. 2005), disrupts peripheral fatty acid (FA) uptake (van Wijk et al. 2005) and interferes with normal post-prandial lipoprotein and free fatty acid (FFA) catabolism (Reeds et al. 2006; van Wijk et al. 2005). Such detrimental effects may be mediated at the transcriptional level with transcriptional regulator sterol regulatory element binding protein 1 (SREBP1) a key regulator in this instance (Miserez et al. 2002).

PI treatment leads to increased and abnormal accumulation of intramyocellular fat (Torriani et al. 2006) causing IR, while this further increases plasma TG-rich lipoproteins and apolipoprotein B (Beatty et al. 2004). PIs inhibit the intracellular proteasomal degradation of apolipoprotein B in hepatocytes thereby increasing secretion of apolipoprotein B-containing lipoproteins (Liang et al. 2001). Moreover, PI-mediated LD is linked with decreased expression of the LDL receptor (Petit et al. 2002) and related receptors (Reeds et al. 2003) and this leads to increased plasma LDL levels.

Intracellular lipid metabolism changes are linked to the structural homology (about 60% at the amino acid level) between the catalytic region of HIV protease and both LDL-receptor-related protein type 1 (LRP1) and cytoplasmic retinoic acid-binding protein type 1 (CRABP1) (Carr et al. 1998b). CRABP1 contributes to the conversion of retinoic acid to cis-9-retinoic acid; this subsequently binds to the retinoid X receptor-peroxisome proliferator-activated receptor γ (RXR-PPARγ), thereby activating adipocyte differentiation and inhibiting apoptosis (Carr et al. 1998b). PIs may bind to CRABP1 that prevents the formation of cis-9-retinoic acid and resulting in decreased RXR-PPARγ activity and peripheral LA (especially gluteal region and the limbs) (Carr et al. 1998b). Ritonavir is a very strong inhibitor of the hepatic cytochrome P4503A4 enzyme (Acosta 2002) and this leads to a decrease in cis-9-retinoic acid formation, lowering of RXR-PPARγ activity and thereby resulting in more LA and aggravated dyslipidemia (Carr et al. 1998b; Penzak & Chuck 2000). TG and LDL-C levels

increased by 26% and 16%, respectively, following two weeks of ritonavir treatment (Shafran et al. 2005). Moreover, when LPV/r is administered to HIV-negative subjects (4 weeks period), serum TG increases by 83%, FFA by 30% and VLDL-C by 33% (Lee et al. 2004a).

PIs can also bind to LRP1 preventing formation of LRP1- lipoprotein lipase (LPL) complex and thus resulting in increased plasma TG levels and decrease storage capacity of adipose tissues (Zimmermann et al. 2001). LRP1 usually binds to LPL to inhibit its action (Hu et al. 2001). FFAs that cannot stored in adipocytes are taken up by the liver, stimulating hepatic synthesis of TG-rich lipoproteins and thereby causing more hypertriglyceridemia (Carr et al. 1999).

Another mechanism whereby HAART can contribute to dyslipidemia is mitochondrial dysfunction (Cossarizza et al. 2003), particularly with NRTIs in combination with PIs. This occurs as a result of their ability to inhibit mitochondrial DNA polymerase γ, the enzyme responsible for replication of mitochondrial deoxyribonucleic acid (mtDNA) (Cote 2005). This can cause the depletion of mtDNA (Nolan et al. 2003; Shikuma et al. 2001) and also lead to attenuated mitochondrial ribonucleic acid (mtRNA) expression (Mallon et al. 2008). Such changes can occur within a relatively short period of time e.g. after two weeks exposure (Mallon et al. 2005), leading to respiratory chain dysfunction and decreased energy production (Cossarizza et al. 2003). Mitochondrial respiratory chain dysfunction causes defects in many cell types, e.g. in adipocytes it can cause LA (Cossarizza et al. 2003) while in skeletal muscle it leads to IR and by secondary dyslipidemia (Pinti et al. 2006). Metabolic abnormalities may also be caused by interactions between cellular proteases and PIs thereby altering mitochondrial function (Zaera et al. 2001). The proposed pathophysiological mechanisms of ARV-associated dyslipidemia are summarized in Table 6.

Table 6: ARV-associated dyslipidemia: pathophysiologic mechanisms.

Mechanism	Effect
PIs	
• PIs bind to CRABP1 (60% homology between HIV-1 protease and CRABP1)	Prevent activation of retinoic acid, decreasing PPAR-γ activity
• PIs inhibit Cytochrome P4503A4	Decrease amount of activated retinoic acid synthesized
• PIs bind to the LDL-receptor-related protein-1 (LRP-1)	Prevent binding of LRP1 binding to endothelial lipoprotein lipase, resulting in increasing plasma triglyceride and FFA.
• Decreased lipid intake and increased lipolysis in adipocytes	Increased plasma TG levels
• PIs block the intracellular proteasome	Decreased degradation SREBP1 leading to increased hepatic production of lipoprotein.
• PIs lead to increased and abnormal accumulation of intramyocellular fat	Elevated levels of TG, IR and apolipoprotein B
• Inhibition of activated retinoic acid	Decreased PPAR-γ activity, inhibits adipocyte differentiation and increased apoptosis, FFA flux to the liver and skeletal muscle causing IR
NRTIs	
• Mitochondrial dysfunction	Inhibition of mitochondrial DNA polymerase γ, respiratory chain dysfunction, decreased energy production, LA and IR in skeletal muscle.

1.4.1.3 Insulin resistance, altered glucose tolerance and diabetes

IR occurs when target tissues fail to respond to insulin and hence higher insulin concentrations are required to achieve a normal metabolic response (Florescu & Kotler 2007). This leads to increased hepatic gluconeogenesis and decreased muscle glucose uptake, often resulting in high systemic glucose levels. The latter can occur even though there are relatively high insulin levels resulting in IGT followed by DM. An important method to measure IR and pancreatic β-cell function is the homeostatic model assessment (HOMA). This was initially defined in 1985 and is a mathematical calculation of β-cell function in response to glucose. However, the original model did not differentiate between peripheral and hepatic insulin sensitivity. An updated HOMA is currently employed to estimate IR and is based on fasting levels of plasma glucose and insulin, HOMA-IR can be calculated by using [Insulin (mU/L) × glucose (mmol/L)/22.5] (Henderson et al. 2011; Matthews

et al. 1985). Early diagnosis of IR is useful as it potentially allows for lifestyle change to be instituted in order to prevent development of T2DM (Matthews et al. 1985).

HIV-infected patients in the pre-HAART era displayed different alterations in glucose metabolism compared to the HAART era. Additionally, there were increased rates of insulin clearance and sensitivity of peripheral tissues to insulin in the pre-HAART era (Florescu & Kotler 2007; Hommes et al. 1991). Of note, the prevalence of IR, glucose intolerance and DM increased significantly following the introduction of HAART to HIV-positive individuals (Florescu & Kotler 2007). Here IR is very common; e.g. about 50% of PIs-treated patients experience IR compared to 25% for those on NRTIs therapy (DAD Study Group 2003). The definitions of impaired fasting glucose (IFG), IGT and DM are summarized in Table 7 (WHO 2006).

Table 7: WHO recommendations for the diagnostic criteria for DM and intermediate hyperglycemia.

IFG	FBG 6.1 to 6.9 mmol/l (110 mg/dl to 125mg/dl) and 2–h blood glucose < 7.8 mmol/l (140 mg/dl)
IGT	FBG < 7.0 mmol/l (126 mg/dl) and 2–h blood glucose ≥7.8 and < 11.1 mmol/l (140 mg/dl and 200 mg/dl)
DM	FBG ≥ 7.0 mmol/l (126 mg/dl) or 2–h blood glucose ≥11.1 mmol/l (200 mg/dl)

Abbreviations: IFG = impaired fasting glucose, IGT = impaired glucose tolerance, DM = diabetes mellitus, FBG = fasting blood glucose.

- Pathogenesis of HAART induced IR

The cause of IR, glucose intolerance and DM is multifactorial in HIV-infected patients (Florescu & Kotler 2007). Pathogenic factors may be related to the HIV infection itself, its treatment, treatment–associated weight gain or immune restoration (Hommes et al. 1991). However, other pathogenic processes share similarities with non-HIV-infected individuals, e.g. increased FFA concentrations, visceral fat accumulation, increased muscle and organ fat, hormonal alterations, chronic inflammation, genetic factors and co-morbidities (Hommes et

al. 1991). PIs were initially considered the most likely cause of such conditions, e.g. early after its introduction (1996) IR, DM and LD manifested in such treated individuals.

*1- PIs-associated IR***:** Ritonavir and LPV were the first HIV medications linked to the pathogenesis of glucose abnormalities among HIV-infected patients (Samaras 2009). Moreover, subsequent research demonstrated that the risk for DM is dose- (Taylor et al. 2010) and duration-dependent and individual PIs display different capacities to induce IR (Capeau et al. 2012). When the PI treatment is discontinued, glycemic dysregulation normalizes in most patients (Lee et al. 2004b). The available evidence indicates that PIs can disturb glucose metabolism through different mechanisms:

a) The down regulation of Glucose transporter type 4 (GLUT-4) that is responsible for the transport of glucose into fat cells, skeletal and cardiac muscle (Rudich et al. 2005).

b) Decreasing adipocyte differentiation due to inhibition of PPAR-γ (Lee et al. 2004b).

c) Saqunavir and ritonavir treatment lead to a 25-50% decrease in pancreatic β-cell function, although mechanisms remain poorly understood; thus patients with DM receiving such drugs should be treated with insulin therapy rather than insulin sensitizing drugs (Kim et al. 2011).

*2- NRTIs-associated mitochondrial toxicity***:** NRTIs can cause mitochondrial dysfunction as it also inhibits DNA polymerase γ, the DNA polymerase playing an active role in mitochondrial replication (Lewis et al. 2003). By contrast, NNRTIs do not inhibit DNA polymerase-γ and hence do not lead to mitochondrial dysfunction. Such mitochondrial perturbations can lead to organ dysfunction, e.g. leading to lactic acidosis and steatosis in the liver. Additional factors that can affect mitochondrial function include oxidative stress and inflammation. The end result following mitochondrial dysfunction (depending on the degree of) is cell death by apoptosis (Gerschenson & Brinkman 2004). Of note, two NRTIs (TDF and ABC) are weak inhibitors of DNA polymerase γ and hence less toxic to mitochondria (Birkus et al. 2002; Cihlar et al. 2002; Gerschenson & Brinkman 2004). Figure 5 displays a summary of whereby ART can contribute to dyslipidemia and IR.
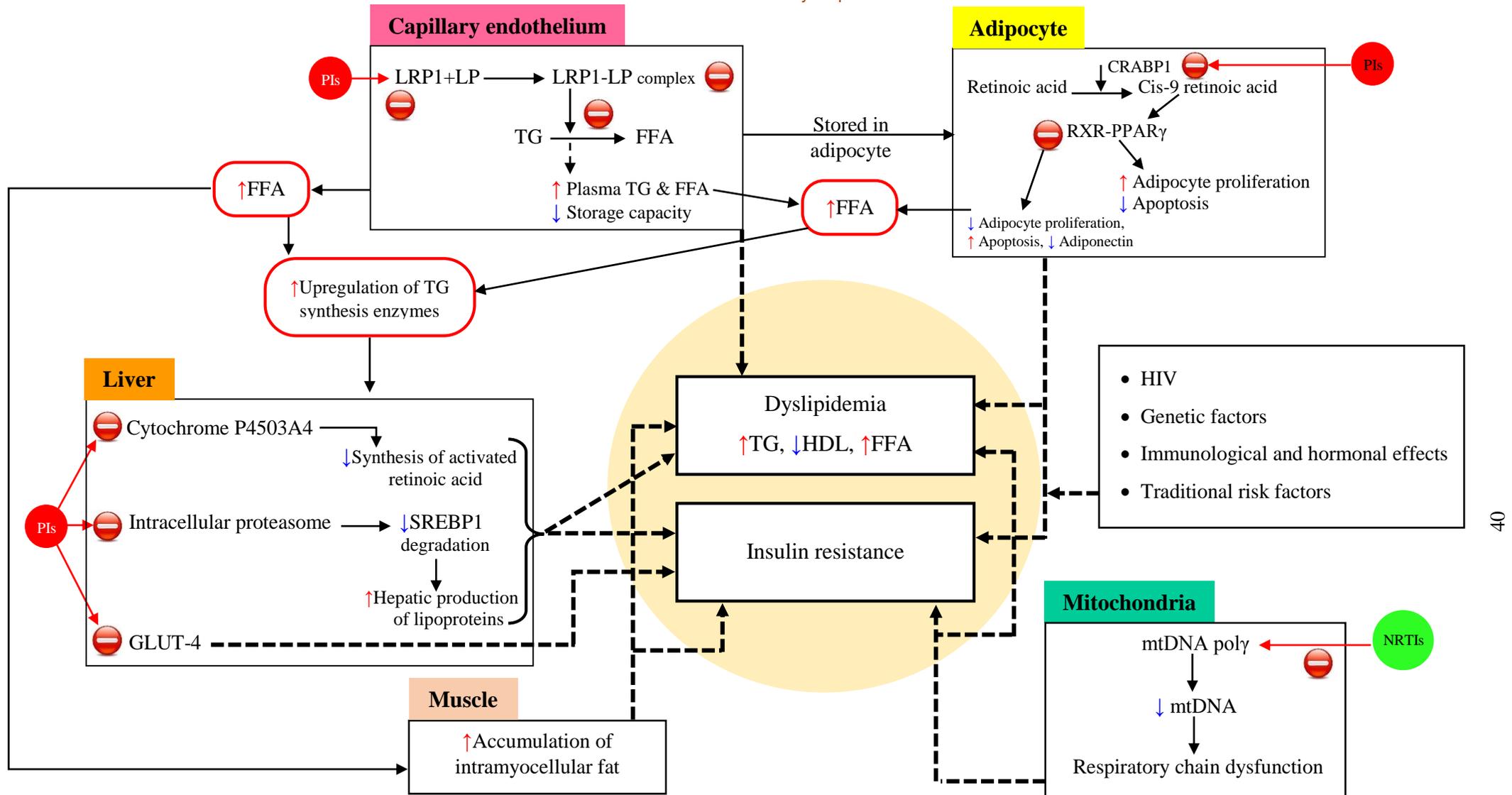
Figure 5: Pathophysiological mechanisms of ART-associated dyslipidemia and IR. The pathogenesis of dyslipidemia and IR is complex and includes effects of different drug classes, HIV itself, genetic predisposition, immunological and hormonal influences as well as traditional risk factors. PI class is mainly responsible for detrimental molecular changes underlying metabolic disturbances. PIs bind to CRABP1 preventing conversion of retinoic acid to cis-9-retinoic acid and decreased RXR-PPARγ activity leads to lowering of adipocyte differentiation and increasing apoptosis. In addition there is increased flux of FFA to the liver and skeletal muscle causing IR. PIs bind to the LRP-1 preventing binding of LRP1 to endothelial LPL and formation of the LRP1-LP complex resulting in increased plasma TG and FFA, decreased storage capacity of adipose tissues and stimulating hepatic synthesis of TG. PIs block the intracellular proteasome resulting in decreased degradation of SREBP1 and hence leading to increased hepatic production of lipoproteins. PIs inhibit Cytochrome P4503A4 resulting in decreased amounts of activated retinoic acid being synthesized. PIs inhibit GLUT4, leading to peripheral IR and impaired glucose tolerance. PIs lead to increased and abnormal accumulation of intramyocellular fat leading tolipidemia and IR. NRTIs can cause negative metabolic changes by their ability to inhibit mt DNA polγ leading to respiratory chain dysfunction, dyslipidemia and IR. *PIs-protease inhibitors, CRABP1- cytoplasmic retinoic acid-binding protein type 1, RXR-PPARγ- retinoid X receptor-peroxisome proliferator-activated receptor γ, FFA- free fatty acids, TG- triglyceride, LRP-1- LDL-receptor-related protein type 1, LPL- lipoprotein lipase, SREBP1- sterol regulatory element binding protein 1, GLUT4- glucose transporter 4, mt DNA polγ-mitochondrial DNA polymerase gamma, mit DNA- mitochondrial DNA.*

1.4.1.4 HAART-associated morphological alterations/body shape changes

The term LD (or fat redistribution syndrome) describes metabolic and stereotypical body disfiguring fat changes in HIV-infected patients receiving HAART. This syndrome is characterized by abnormal body fat redistribution and can present as a) peripheral fat wasting (LA) mainly in the face, limbs, and buttocks; and b) central fat accumulation in the abdomen, breast or posterior neck lipohypertrophy (LH); or a mixture of both. LD occurs in more than half of HIV-infected patients treated with HAART (Grunfeld et al. 2010; Jacobson et al. 2005) and here LA and LH are characterized by different risk factors that occur independently of each other despite the fact that such conditions occurs together (FRAM 2006). Many researchers found that NRTI drugs (especially d4T) associated mostly with LA and here the underlining mechanism is mitochondrial dysfunction (Mallon et al. 2005; Nolan 2003). PIs and NNRTIs can also contribute, e.g. PIs cause inhibition of adipocyte differentiation with subsequent LA (Kim et al. 2006; Zhang et al. 1999). The etiology of LH and ectopic fat accumulation is not clear but may related or caused either by high levels of inflammatory cytokines (Johnson et al. 2004) or elevated TG and FFA levels that cannot stored in atrophic subcutaneous fat tissues (Huang-Doran et al. 2010). A minority of HIV patients with LH also exhibit elevated serum and urine cortisol concentrations. However, there is appropriate suppression of cortisol levels in response to dexamethasone and normal diurnal variation in cortisol levels.

For HIV-infected patients with fat redistribution syndrome there is more metabolic disturbances especially dyslipidemia and IGT compared to negative controls (Hadigan et al. 2001). Interestingly, HIV-positive positive men with LD syndrome display the similar interleukin 6 (IL6), C-reactive protein (CRP), tumor necrosis factor α (TNFα) and adiponectin levels as for obese HIV-negative men (Samaras et al. 2009). Wohl et al. (2008) show that increased visceral fat and decreased subcutaneous leg fat are associated with elevated TG levels and attenuated HDL-C levels. Moreover, the Fat Redistribution and Metabolic Change in HIV (FRAM) cohort demonstrated that both peripheral LA and visceral LH are associated with an increased Framingham Risk Score (used to calculate absolute 10-year risk of MI or cardiac death) (Dube et al. 2003) and therefore predictive of MI (Lake et al. 2011). For both peripheral fat atrophy and visceral fat accumulation there is

evidence of subclinical atherosclerosis and studies confirmed that especially the latter is associated with progression of subclinical atherosclerosis (Guaraldi et al. 2012) as well as overall mortality (Scherzer et al. 2011) in HIV- infected patients. In addition, visceral adiposity (especially abdominal or central obesity) is associated with an increased risk of cardio-and cerebrovascular diseases as it is linked to numerous metabolic disturbances. For example, IR, dyslipidemia, IGT and HTN are all strong risk factors for CVD and premature death (Guzzaloni et al. 2009).

The best methods to assess the volume of visceral adipose tissue (VAT) are computerized tomography (CT) scan and magnetic resonance imaging (MRI). However, such techniques are not readily available in all clinics and are very expensive especially within a developing world context (Kullberg et al. 2007; Van der Kooy & Seidell 1993). Visceral adiposity can also be estimated by waist circumference (WC) or sagittal abdominal diameter (SAD) measurements, both simple anthropometric measurements that were proposed as alternatives for evaluating body fat distribution (Nordhamn et al. 2000; Sampaio et al. 2007; Turcato et al. 2000).

1.4.2 CVD

CVD and especially coronary heart disease (CHD) are a major cause of mortality death with HIV/AIDS (Mathers & Loncar 2006). Here HIV-infected patients display increased CVD rates that likely occurs due to HAART-mediated side-effects and also by systemic, chronic inflammation due to the HIV itself (Subramanian et al. 2012). In addition, endothelial injury can be triggered by HIV itself (Torre 2006), the effects of HAART (De Lorenzo et al. 2008) as well as increased traditional CVD risk factors such as dyslipidemia, DM, smoking and HTN (Hsue et al. 2012). Such risk factors may be associated with usage of specific ARV drugs (Amado & Ruiz 2007) and can all be predictors of cardiac events in such individuals (Ho & Hsue 2009) (Figure 6).

The Data Collection on Adverse Events of Anti-HIV Drugs (DAD) study (large prospective, multi-cohort study) confirmed an increased MI risk with ARV exposure (Friis-Moller et al. 2003a). Here there were a 26% increase in the MI rate per year of HAART exposure (Friis-Moller et al. 2003a). Moreover, others found that HIV-positive individuals receiving HAART exhibited atherogenic lipid profiles (high TC, total TG and low levels of HDL cholesterol) compared to

HAART naive HIV-infected patients (Fontas et al. 2004). Table 8 summarizes the relationship between CVD risk factors and MI risk in HIV patients (DAD Study Group et al. 2007).
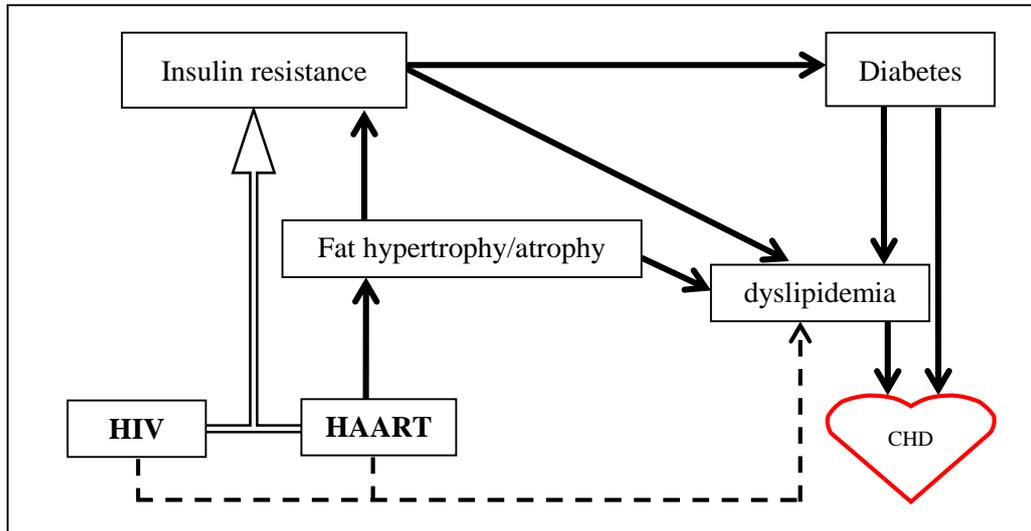
Figure 6: HIV/HAART induces lipodystrophy, dyslipidemia and IR and thus increases the risk of coronary heart disease and diabetes (adapted from Zou & Berglund 2007).

Table 8: Relationship between CVD risk factors and the rate of MI in HIV patients.

CVD risk factors	Relative risk of heart attack	P-value
TC	1.26 (1.19-1.35)	< 0.001
HDL	0.65 (0.48-0.88)	0.05
Diabetes	1.86 (1.31-2.65)	< 0.001
Current smoker	0.92 (2.04-4.18)	< 0.001
Former smoker	1.63 (1.07-2.48)	0.02
Hypertension	1.30 (0.99-1.72)	0.06
CVD family history	1.40 (0.92-1.91)	0.08
Previous CVD event	4.64 (3.22-6.69)	< 0.001
Age per additional 5 years	1.32 (1.23-1.41)	< 0.001
PIs use per additional year	1.10 (1.04-1.18)	0.002
BMI >30	1.34 (0.86-2.09)	0.19

1.4.2.1 CVD risk in HIV-positive individuals: contribution of metabolic dysfunction and traditional risk factors

The risk of CHD in HIV patients is significantly affected by traditional factors (Savès et al. 2003) while metabolic complications (mainly PI- and NRTI-mediated dyslipidemia and IR) also play a role (Ho & Hsue 2009). It is important to consider two issues when evaluating the evidence implicating ARV-induced metabolic complications as factors for increased CVD risk. Firstly, early studies were performed in HIV-infected patients receiving only single or dual treatment, while in the second instance the new HAART regimens make it challenging to discriminate between the distinct effects of various drugs making up the treatment cocktail (Grunfeld et al. 2008). HIV-infected patients with acute coronary syndrome (ACS) display specific features when compared to non-HIV individuals, e.g. this population develops ACS at a relatively younger age with high prevalence of smoking and relatively low HDL levels. Moreover, they also show no clear abnormalities in their coronary arteries (by angiography) and arterial lesions exhibit specific histological features, e.g. are diffuse and rapid progression of circumferential arterial pathology with proliferation of elastic fibers, smooth muscle cells and endoluminal protrusions (Subramanian et al. 2012).

1.4.2.2 HAART and HTN

According to the WHO and International Society of Hypertension (ISH) guidelines hypertension is defined and diagnosed as displaying a mean systolic blood pressure (SBP) $\geq$ 140 mmHg and a diastolic blood pressure (DBP) $\geq$ 90 mmHg. Alternatively, with two or more clinic visits individuals display elevated BP while others exhibit normal values but receiving antihypertensive drugs (Whitworth 2003). As HTN is a well-known as a risk factor for CVD, greater understanding of the link between HAART and HTN is of primary importance. For example, many studies reveal that HTN is common in HIV-positive patients (Chow et al. 2003; Gazzaruso et al. 2003), although it was rare before HAART introduction. Dillon et al. (2013) performed a systematic review and meta-analysis and found that individuals infected with HIV in sub-Saharan Africa displayed lower DBP and SBP than controls, regardless of ART status. Previous studies assessing the associations between HIV, ART and blood pressure have been inconsistent, with some suggesting increased risk of

hypertension with ART (Gazzaruso et al. 2003). By contrast, some reported no association with HIV or ART (Bergersen et al. 2003; Jerico et al. 2003) while others supported the findings of the meta-analysis highlighted above (Schutte et al. 2012).

There are numerous factors that may explain this phenomenon including a) an aging HIV-positive population and thus appearance of usual HTN risk factors for development of HTN and b) direct effects elicited by HIV itself or HAART. For example, the D:A:D study established that HTN in the HIV-positive population is related to traditional risk factors such as hyperlipidemia, age, black race, male gender, increased BMI and microalbuminuria (Friis-Moller et al. 2003b). However, further investigations are needed to better understand the link between ARV drugs and HTN as some mixed data have been generated thus far (Cattelan et al. 2001; Chow et al. 2003) (Table 9).

Table 9: HIV ± HAART and the risk for hypertension.

Author	Outcome
Medina-Torne et al. (2012)	High prevalence of HTN (31%) related to the duration, immune activation and endothelial changes caused by HIV
Boccara et al. (2010)	Adipocyte dysfunction and activation of renin angiotensin system (RAS) caused by PI treatment

For example, some found that HTN is common and correlated strongly with known risk factors but that it was not related to the use of HAART in HIV-positive patients (Medina-Torne et al. 2012). The authors indicated that the high prevalence of HTN is related to the duration of HIV-infection and that endothelial changes occurred due to the virus and immune activation. By contrast, Boccara et al. (2010) performed *in vitro* work on adipocytes incubated with LPV/r and found that such treatment induced adipocyte dysfunction and activation of renin angiotensin system (RAS). Moreover, others showed that duration of HAART and also PIs can increase the prevalence of HTN (Baekken et al. 2008; Seaberg et al. 2005). Here a link between LD and increased risk of HTN was consistently suggested (Baekken et al. 2008; Crane et al. 2009; Gazzaruso et al. 2003; Seaberg et al. 2005; Thiebaut et al. 2005). These conflicting data there indicate that additional studies are required to fully ascertain the link between HIV and/or HAART and the onset of HTN in HIV-positive

individuals. Here careful attention should be paid to different models employed (if pre-clinical work) and also the nature of clinical studies, e.g. with all the necessary control groups and sufficient recruits included.

1.4.2.3 Surrogate measures of atherosclerosis in HIV patient receiving HAART

Surrogate measures of early atherosclerosis are useful clinically and include endothelial dysfunction and carotid intima–media thickness (IMT) - sensitive measures and predictors of future CVD events. Studies focusing on IMT and endothelial dysfunction showed that the risk of CHD increases with PIs (Hsue et al. 2004; Maggi et al. 2000; Meng et al. 2002; Stein et al. 2001). The virus itself has also been implicated, e.g. HIV infection can cause endothelial dysfunction (Bonnet et al. 2004) and carotid IMT (Hsue et al. 2004). Here HIV stimulates inflammatory pathways and this lead to the production of TNFα and β, nuclear factor-κβ, IL-6, vascular cell adhesion molecule (VCAM)-1, intracellular adhesion molecule (ICAM)-1, and endothelial cell adhesion molecule-1 (Fisher et al. 2006). Subsequently, such chemokines can activate the atherosclerotic process and therefore increase the risk of an MI in this group of individuals (Fisher et al. 2006).

Endothelial dysfunction

Endothelial dysfunction is an early sign of atherosclerosis and it is a useful predictor of future CVD events (Davignon & Ganz 2004; Vita & Keaney 2002). There are many sensitive markers of endothelial dysfunction that include VCAM-1, ICAM-1, von Willebrand factor and thrombomodulin (Muller & Griesmacher 2000). These markers are usually elevated in persons with hyperlipidemia, HTN, DM, oxidative stress and a history of smoking (Cines et al. 1998). Markers for endothelial activation can be elevated in HIV-infected patients together with downstream effects such as the onset of a hypercoagulable state (Aukrust et al. 2000) (Cines et al. 1998). The latter can be monitored by measuring coagulation markers such as thrombin and anti-thrombin III. With thrombin production, activated endothelial cells produce adhesion molecules (Henn et al. 1998) and elicit an inflammatory reaction and atherosclerosis (Tsakiris et al. 1999).

HAART decreases (but does not normalize) immune activation and inflammatory cytokine levels in HIV-infected patients (Aukrust et al. 1999; Bisset et al. 1998), that can lead to a partial release of adhesion molecules (Moore et al. 1998; Sousa et al. 1999). The HAART-mediated decrease in HIV replication and viral load should improve T-cell function and decrease endothelial dysfunction. In agreement, others noted a significant decrease in VCAM-1 and ICAM-1 levels after the first months of HAART initiation. Such observations indicate that endothelial activation is mediated by HAART-mediated control of viral replication (Wolf et al. 2002). HAART may cause endothelial cell activation, e.g. HIV-positive persons treated with PIs or NNRTIs presented with higher levels of P-selectin, tissue plasminogen activator (tPA), and plasminogen activator-1 (PAI-1) than in HIV-infected naive subjects and there was a positive relationship between endothelial markers and lipid levels (De Gaetano et al. 2003). It is suggested that HAART should reduce endothelial damage by controlling HIV infection, but associated, deranged lipid and glucose metabolism can also contribute to endothelial activation. Furthermore, HAART seems to directly induce endothelial activation, e.g. ritonavir and indinavir can cause endothelial dysfunction together with mitochondrial DNA damage and cell death (independently of lipid profile) (Dube et al. 2002; Zhong et al. 2002). In addition, for a large cohort study of HIV-infected patients the serum concentrations of PAI-1 were high in PI- treated and untreated patients with MetS and its level associated with PI use, insulin levels, TG levels and BMI (Koppel et al. 2002). These results show that HAART may cause atherosclerosis both by direct effects on endothelial cells and by indirect effects via metabolic derangements.

Carotid intima-media thickness (IMT)

HIV-infected individuals have a higher prevalence of CHD which can be measured by non-invasive high-resolution ultrasound technique to assess IMT (representative of subclinical vascular disease and future CVD events) also found that HIV-infected persons display a higher CHD prevalence (Stein et al. 2008). Carotid IMT studies in the HIV-infected patients have yielded conflicting results, with high IMT caused by both HIV infection itself (Lorenz et al.

2008) and HAART related (Coll et al. 2006; Currier et al. 2007; Lebech et al. 2007; Lorenz et al. 2008). Some determined that IMT:IMT values were 24% more at the carotid bifurcation in HIV-infected individuals on HAART compared to controls and about 6% greater in the common carotid artery confirming that there are site-specific differences in IMT (Lorenz et al. 2008). Moreover, vascular risk was increased by 4% to 14% in HIV-positive persons (Lorenz et al. 2008). However, there were no differences in IMT in non-smoking HIV-infected HAART recipients, irrespective of TC levels (Lebech et al. 2007). Currier et al. (2007) reported that there were no differences in the rate of progression of IMT over three years between HIV-infected subjects receiving PIs, those on non-PIs HAART, and uninfected controls (all matched for age, gender, ethnicity, smoking status and blood pressure). There is also a three-fold increase in carotid IMT in HIV-infected patients on HAART with LD syndrome and also for IMT-related levels of circulating monocyte chemo-attractant protein-1 (Coll et al. 2006). There is also increased carotid artery IMT in HIV-infected patients on HAART with MetS (Mangili et al. 2007), while others with MetS also displayed significantly higher IMT and epicardial fat thickness (Iacobellis et al. 2007). In a three year prospective controlled study the progression of carotid IMT was linked to LDL-cholesterol and homocysteine levels but not to HIV-HAART (Currier et al. 2007). Carotid IMT has also been found to relate to epicardial fat thickness and visceral adipose tissue in HIV-infected patients on HAART. Metabolic changes are common in HIV infection/HAART have been associated with an increased IMT (Seminari et al. 2002). Together these studies demonstrate that it is important to establish the role of prolonged survival, age, traditional non-traditional risk factors with HIV/HAART in relation to surrogate markers of atherosclerosis.

1.5 Problem statement

Sub-Saharan African remains the most severely affected region with HIV/AIDS, with South Africa saddled with the highest number of HIV-positive positive individuals in the world. HAART has improved the prognosis of HIV-infected patients with clear decrease in the incidence of opportunistic

infections and transforming AIDS from an irreversible terminal illness to a chronic disease with improved quality of life. However, HAART regimens have been implicated in the development of metabolic complications (e.g. dyslipidemia, IR) and there is accumulating evidence of its link to elevated CVD onset. With the high prevalence of HIV/AIDS in South Africa, the extent and nature of cardio-metabolic complications in HIV-positive individuals remain relatively poorly understood.

1.6 Research question

To establish the prevalence of several cardio-metabolic complications in HIV-positive patients on HAART (first line versus second line) within the Cape Winelands region of South Africa. Such information should improve clinical management of HIV-positive patients within the region and help to enhance their overall health and wellbeing.

1.7 Study objectives

- To determine the prevalence of several cardio-metabolic risk factors in HIV patients within the Cape Winelands region (Community Day Centre (CDC) at Worcester Hospital, Worcester, South Africa)

- To determine the association between anthropometrical parameters, smoking status and cardio-metabolic risk factors in this population group.

CHAPTER TWO

METHODS

2.0 Introduction

2.1 Study design

2.2 Study setting, population and sampling

2.2.1 Eligibility criteria

2.3 Data collection procedures

2.3.1 Interviews

2.4 Data analysis

2.5 Ethical considerations

2.6 Study strategy

2.0 Introduction

This chapter discusses the methods employed to obtain and analyze data. In addition, there is also a description of the study setting, population, sampling and the criteria for participation. Ethical consideration/approvals are also discussed.

2.1 Study design

This study forms part of a larger longitudinal study namely the ''Cape Winelands HAART to HEART Study'' within the Cape Winelands region of the Western Cape (South Africa). This sub-study is of a cross-sectional nature and here a structured, validated lifestyle questionnaire and data collection forms were used to gather information regarding demographics, lifestyle, clinical and laboratory data (Appendix 3). We also collected blood samples to test for various biochemical markers, while blood pressure measurements (three readings per patient) and heart rates were also measured. In addition, selected anthropometric measurements were performed that included height, weight, BMI determination, WC, hip circumference (HC), waist-to-hip ratio (WHR), mid-upper arm circumference (MUAC), triceps skin fold (TSF) and biceps skin fold (BSF). Together such findings would allow for the evaluation of cardio-metabolic risk factors in this population.

2.2 Study setting, population and sampling

The study was conducted at Worcester CDC, located within the Worcester Hospital (Western Cape, South Africa). It is a public referral hospital and is also part of the metro and district health services. This CDC provides primary health care service and provides treatment/management of HIV-positive patients.

Written informed consent was obtained from all participants (Appendix 2) by verbal communication (in mother tongue language) explaining the purpose and the procedures of the study. Here translators assisted when this was required. This study received ethical approval from the Health Research Ethics Committee at Stellenbosch University and also by the Department of Health (Western Cape Government) (refer Section 2.5 for more details).

2.2.1 Eligibility criteria

All the patients in this study were enrolled at Worcester CDC and they have regular follow up. Recruitment was done by a part-time qualified nurse who assessed files in the clinic and then identified subject recruits for our study. The following is selection criteria that applied for the study population:

Inclusion:

- o HIV-positive or negative patients, both male and female and registered at Worcester CDC
- o Age range > 20 to 55 years
- o Residents from Worcester and surrounding areas
- o Signed informed consent

Exclusion:

- o Age < 20 and/or > 55 years
- o Not usually residing within the Cape Winelands region
- o Do not provide consent

2.3 Data collection procedures

Questionnaires were administered by the researcher and assisted by a qualified nurse. In addition, the researcher collected data from patient files. We determined blood pressure by using a Niscomed Blood Pressure Apparatus Clock Type Tabletop device (Niscomed Electro Devices Company, New Delhi, India). This was done in triplicate and an average value then recorded. The heart rate was measured by palpation method at the wrist (radial artery) and by counting the number of beats per one minute period. Blood samples were collected by qualified phlebotomists and immediately transported to the National Health Laboratory Service (NHLS) (Worcester) for analysis.

High TC was defined as > 6.2 mmol/L, high TG as > 2.2 mmol/L and low HDL as < 1 mmol/L, fasted blood glucose levels were measured using the photometric method. High levels of fasting blood glucose (FBG) defined as > 5.6 mmol/L (Appendix 4). Participants were defined as

diabetic if fasting glucose levels exceeded 7.0 mmol/L, if they reported a history of diabetes, or were currently receiving treatment for diabetes (insulin or oral hypoglycemic agents) insulin level determination was performed using the chemilluminescense non-competitive method. High insulin levels were defined as > 25 Mu/L, while IR was determined through the HOMA-IR mathematical calculation by using the formula: IR = Insulin mU/L× glucose mmol/L/22.5 and we defined IR as HOMA-IR cutoff level ≥ 2 on the basis of an approximation to the mean ± 1 SD value obtained in Ghanaians with normal FBG and blood pressure using the HOMA model (Amoah et al. 2003). Nonetheless, it should be noted that the mean HOMA-IR for normal individuals in most populations is between 2.1 and 2.7 (Lebovitz 2002). Specific cutoff points used for the definition of IR in some European and African populations range from 1.62 to 2.77 (Ascaso et al. 2003; Bonora et al. 1998; Ezenwaka et al. 1997; Ford and Giles 2003; Taniguchi et al. 1999). CRP levels were measured by the immuno turbidimetric method, high CRP was defined as > 10 mg/L (Okamura & Terada 1990). The Atherogenic Index of Plasma (AIP) for identifying high risk individuals for CAD was also calculated: AIP = log TG/HDL, low risk (AIP < 0.1), medium risk (AIP 0.1-0.24) and high risk (AIP > 0.24) (Dobiásová 2006).

A complete drug history was taken for all HIV-positive individuals receiving HAART. This information was accessed from patient files and typically included ARV type, treatment duration and adherence. We also recorded CVD family history and smoking status by using a validated demographic questionnaire. This also provided additional information, e.g. age, home language and levels of education. The anthropometrical part of the study included basic measurements included: height and weight were measured by PD300DHR digital weight and height scale (Model PD300DHR, DETECTO–division of cardinal scale manufacturing company, Webb City, MO, USA). The height was measured to the nearest 0.1 cm with the head of the patient in the Frankfort plane while standing in the anatomical position with feet together on a flat surface by a calibrated stadiometer, and weight which were measured (with minimal clothing and without shoes) to the nearest 0.01 kg. BMI was calculated by dividing weight (kilograms) by height in meter squared (BMI; kg/m^2) with specific categories (Appendix 5). Waist circumference (WC) was measured with a Lufkin® executive thin line tape measure (Lufkin W606PM) (Apex Tool Group, USA) at the

narrowest point between the lower costal border and the top of the iliac crest perpendicular to the long axis of the trunk (Stewart et al. 2011), while HC was determined at the greatest posterior protuberance of the gluteal muscles. The WHR was calculated by division of the previous two measurements. TSF and BSF were measured by calibrated caliper (Slim Guide Plastic Skinfold Caliper, brand name: CE, NexGen Ergonomics Co., Montreal, Canada), while MUAC was also assessed (Appendix 6).

2.3.1 Interviews

Interviews were conducted by the researcher on personal medical history and by focusing on HAART regimens (first or second line, naive) and a validated questionnaire was used to collect all the relevant information for traditional CVD risk factors (smoking, family history of CVD, HTN, DM).

2.4 Data analysis

Data collected was captured into Microsoft Excel (Microsoft Office 2013, USA) and an explanatory dictionary used to describe all the variables in more detail and thereafter data were exported to STATA v.13 (Stata: Release 13. Statistical Software. College Station, TX: StataCorp LP, USA). For analysis, all continuous variables were summarized using means and standard deviation. Categorical data were summarized using proportion and percentages, while comparison of continuous variables between the three groups (first line, second line and naive) were compared using two-way analysis of variance (ANOVA) or Kruskal-Wallis where appropriate. The comparison of continuous variables between the two groups (HIV-positive on HAART and HIV-positive HAART naive) was done using the Mann-Whitney test. Association between two categorical variables was tested using chi-squared or Fisher's exact tests. P-values of ≤ 0.05 were considered statistically significant.

2.5 Ethical considerations

Great effort and patience was used to confirm that procedures in conducting the study complied with the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines

for Research. Ethical approval for the research was sought from the Health Research Ethics Committee at Stellenbosch University (see Appendix 1) and also the Department of Health (Western Cape Government). All participants were given a consent form to read, understand and sign if they agreed to participate in the study. The aims of the study were also explained to all potential participants and that they were free to withdraw at any point, even if they previously had agreed to take part. The privacy and dignity of the participants were preserved and no personal identifiers (names or addresses) of patients were collated. Only a specific study code for each participant, their age and gender were recorded and subsequently used to match results obtained from laboratory analyses. Participants were also informed that some pain or discomfort might be felt for the collection of a blood sample. It was also further explained that blood was going to be drawn by a qualified and experienced nurse and that the samples would be used for study purposes only.

2.6 Study strategy

Figure 1 summarizes the overall study design and activities:
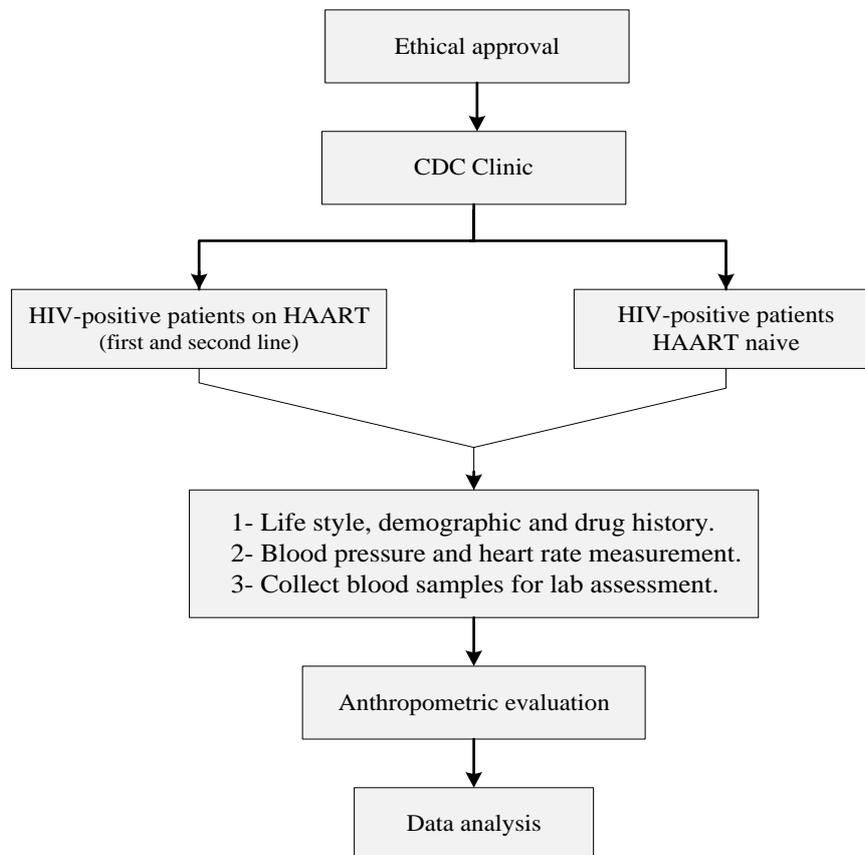
Figure 1: Diagrammatical representation of study strategy and assessments.

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CHAPTER THREE

RESULTS

3.0 Results

Socio-demographic characteristics of study participants

Seventy-five subjects were recruited and included in the final analysis; the study population was divided into two broad categories n = 50 were HIV-positive on HAART and the other n = 25 were HIV-positive HAART naive. Of the 50 participants in the first group, n = 9 were males (18%) and n = 41 females (82%), while for the second group n = 12 were males (48%) and n = 13 females (52%) (P = 0.006) (Figure 1). The ages of the participants in the HIV-positive on HAART group ranged from 20 to 55 years with a mean of 37.1 ±8.8 years. For the HIV-positive HAART naive group the ages ranged from 20 to 50 years with a mean of 35.3 ±8.48 years (P = 0.4183). The duration of HIV diagnosis ranged from 11 to 161 months for the HIV-positive HAART and ~96% were diagnosed more than one year ago. For those in the HIV-positive HAART naive group, the duration of HIV diagnosis was from 2 to124 months; ~16% were diagnosed less than a year and 84% more than one year ago (P = 0.003). The duration of HAART ranged from 7 to 144 months for HIV-positive individuals on HAART with high adherence (96%). Half of HIV-positive persons on HAART had a positive family history of CVD while this figure rose to 60% for HIV-positive HAART naive individuals. In terms of lifestyle variables, 27.3% of the HIV-positive (on HAART) participants reported current smoking, while this figure was much higher (88%) for HIV-positive HAART naive individuals (Figure 2). When evaluating HIV-positive individuals on HAART in terms of WHO clinical stages there were 52% in stage I, 12% in stage II, 22% in stage III and 14% in stage IV. For the HAART naive group the overwhelming majority (96% and 4%) were in stage I and II respectively, no participants were recorded in stage III and IV for HIV-positive HAART naive (Table 1).

Table 1: Socio-demographic characteristics of study participants.

Variables	HIV+ HAART naive	HIV+ on HAART	P-value
Age (years)			
Mean	35.36	37.1	P = 0.4183
SD	8.48	8.85	
Gender, n (%)			
Female	13 (52%)	41(82%)	**P = 0.006**
Male	12 (48%)	9 (18%)	
Smoking status, n (%)			
Yes	22 (88%)	19 (27.3%)	**P = 0.0001**
No	3 (12%)	31 (22.7%)	
HIV time (months)			
Median	35	72	**P = 0.003**
IQR	62	56	
HAART duration (months)			
Median	-	60	-
IQR	-	51	
WHO clinical stages, n (%)			
I	24 (96%)	26 (52%)	
II	1 (4%)	6 (12%)	-
III	0 (0%)	11 (22%)	
IV	0 (0%)	7 (14%)	

Abbreviations: *HIV+: HIV-positive, SD: Standard deviation, IQR: Interquartile range.*
P-values of ≤ 0.05 were considered statistically significant.
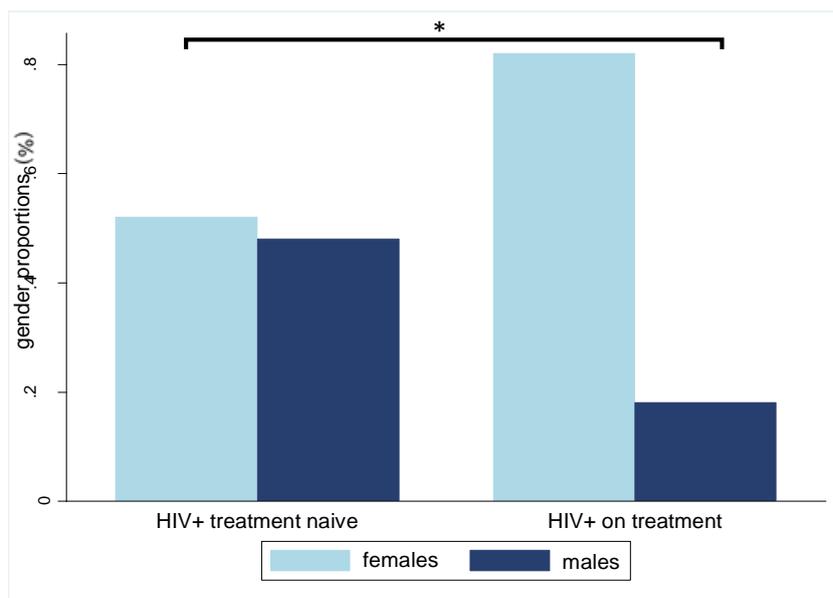
Figure 1: Gender proportion of HIV-positive HAART naive and HIV-positive (on HAART). Significant difference in gender: HIV-positive HAART naive versus HIV-positive on HAART (*P = 0.006).
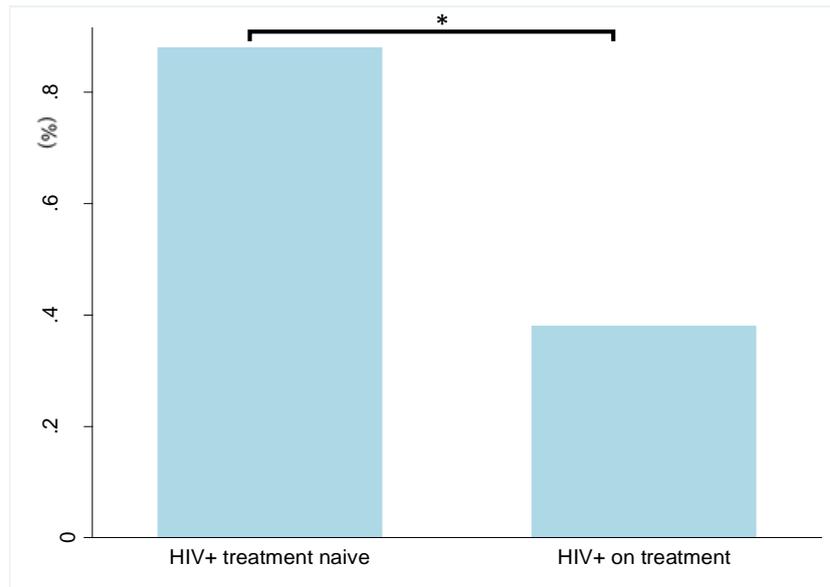
Figure 2: Smoking proportion of HIV-positive HAART naive and HIV-positive (on HAART). Significant difference in smoking proportion: HIV-positive HAART naive versus HIV-positive on HAART (*P = 0.0001).

All the subjects on HAART treatment were further divided into two sub-groupings i.e. first line (n = 25) and second line treatment (n = 25). These sub-groups were subsequently analyzed and here participants on first line treatment used a combination of HAART drugs with NRTIs and NNRTI provided as FDC: EFV+FTC+TDF (68%), TDF+3TC+EFV (16%), AZT+3TC+NVP (16%). Subjects on second line treatment used a combination of a boosted PI plus two NRTIs drugs: LPV/r+AZT+3TC (56%), LPV/r+3TC+TDF (44%) (Figure 3).
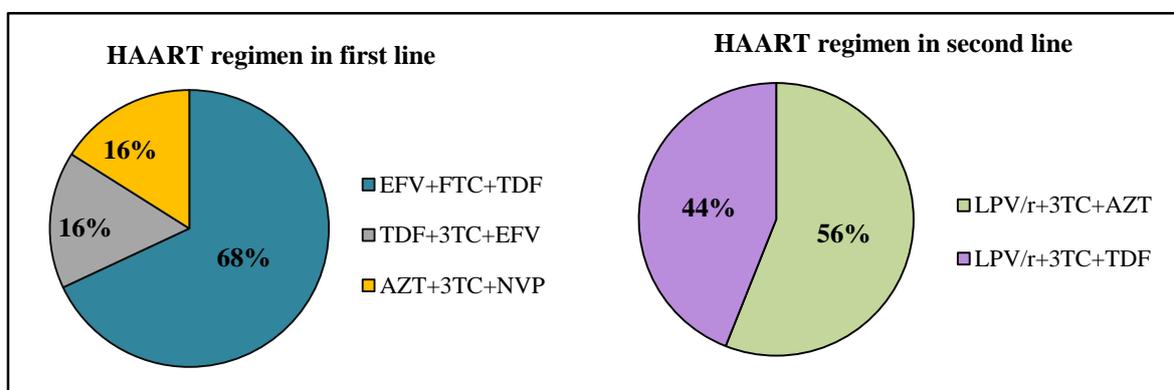
Figure 3: Percent distribution of types of HAART regimens in first versus second line. *EVF- Efavirenz, FTC- Emtricitabine, TDF- Tenofovir, 3TC- Lamivudine, AZT- Zidovudine, NVP- Nevirapine, LPV/r- Lopinavir/ritonavir.*

The socio-demographic characteristics of HIV-positive individuals on first and second line treatment is presented in Table 2, where 28% were males and the majority females for first line treatment. For second line treatment this was skewed even more towards females, i.e. 8% males versus 92% females. For the HIV-positive HAART naive group the pattern was different and here 48% were males and 52% females (P = 0.007) (Figure 4). Ages for first line treatment ranged from 20 to 53 years with a mean of 36.24 ± 8.65 years, while for the second line ages it ranged from 26 to 55 years with a mean of 37.96 ± 9.14 years. For the HIV-positive HAART naive group the ages ranged from 20 to 50 years with a mean of 35.36 ± 8.48 years (P = 0.931). For the HIV-positive group the duration of HIV diagnosis ranged from 11 to 147 months for first line and 34 to 161 months for second line treatment, for the HAART naive group it ranged between 2 to 124 months.

Table 2: Socio-demographic characteristics of first and second line treatments.

Variables	HIV+ HAART naive	First line	Second line	P-value
Age (years)				
Mean	35.36	36.24	37.96	P = 0.931
SD	8.48	8.65	9.14	
Gender, n (%)				
Female	13 (52%)	18 (72%)	23(92%)	**P = 0.007**
Male	12 (48%)	7 (28%)	2 (8%)	
Smoking status, n (%)				
Yes	22 (88%)	13 (52%)	6 (24%)	**P = 0.0001**
No	3 (12%)	12 (48%)	19 (76%)	
HIV time (months)				
Median	35	63	81	**P = 0.0008**
IQR	62	68.5	71	
HAART duration (months)				
Median	-	48	65	**P = 0.0182**
IQR		54	47	
Adherence on HAART, n (%)	-	25 (100%)	23 (92%)	P = 0.149
WHO clinical Stages, n (%)				
I	24 (96%)	16 (64%)	10 (40%)	
II	1 (4%)	1 (4%)	5 (20%)	-
III	0 (0%)	4 (16%)	7 (28%)	
IV	0 (0%)	4 (16%)	3 (12%)	

P-values of ≤ 0.05 were considered statistically significant.

The corresponding duration of HAART for first line and second line treatment ranged from 7-120 months and 25-144 months, respectively (P = 0.0182), with complete adherence for the first line (n = 25; 100%) and also relatively high for the second line (92%). A strong proportion of the naive group reported as current smokers (n = 22; 88%), with 52% and 24% for first and second line, respectively (P = 0.0001) (Figure 5). WHO clinical stages for the first line were: 64% in stage I, 4% in stage II and 16% for both stages III and IV, respectively. For second line treatment the data revealed: 40%, 20%, 28% and 12% in stages I, II, III and IV, respectively, compared to 96% in stage I and 4% in stage II for HIV-positive HAART naive.
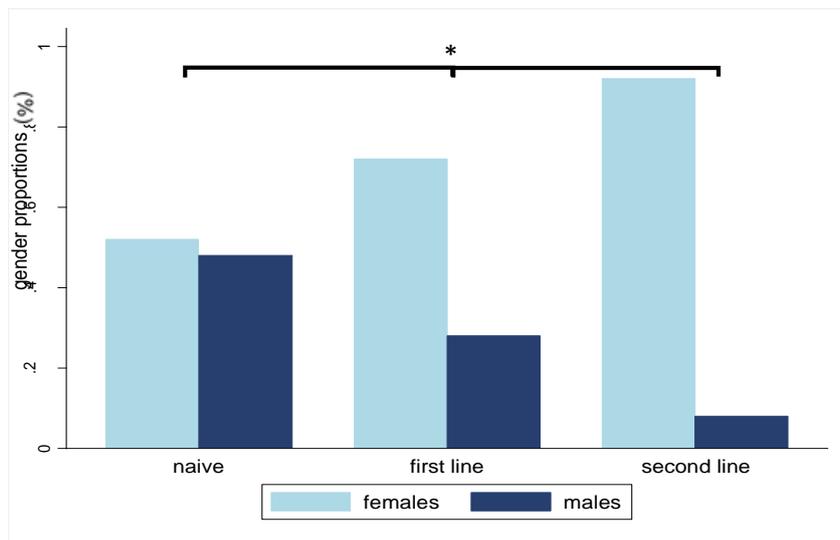
Figure 4: Gender proportion between HIV-positive HAART naive, first line and second line treatment. Significant difference in gender proportion between HIV-positive HAART naive, first line and second line persons (*P = 0.007).
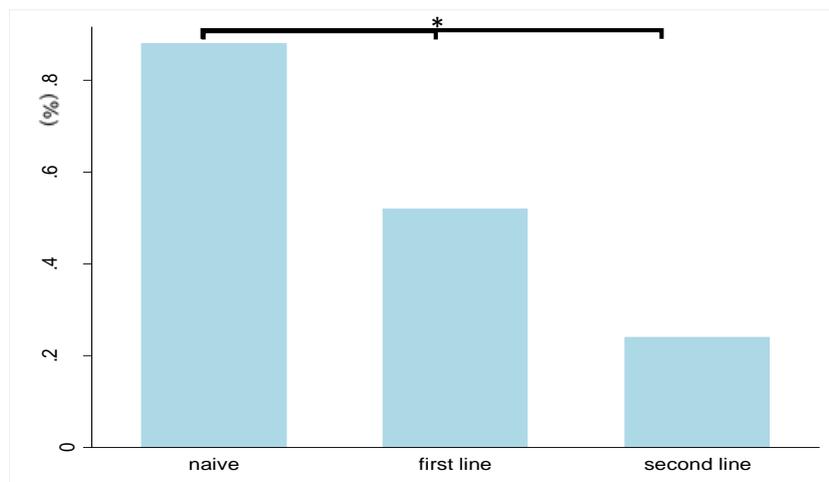
Figure 5: Smoking proportion between HIV-positive HAART naive, first line and second line treatment. Significant difference in smoking proportion between HIV-positive HAART naive against first line and second line persons (*P = 0.0001).

The characteristics of the study population are summarized in Table 3. For the HIV-positive group on HAART the following data emerged for BMI categories: 16% underweight, 36% healthy weight, 22% in class 1 obesity, 22% in class 2 and 4% in class 3 obesity (Appendix 5). For the HIV-positive HAART naive group 28% were underweight, 56% displayed healthy weight, 12% class 1 and 4% class 2 obesity (Figure 6). According to IDF ethnic-specific values for WC (Appendix 6), 58% females of the HIV-positive (on HAART) displayed WC ≥ 80 cm while only 2% males exhibited a WC ≥ 94 cm. This was significantly higher than that of the HIV-positive HAART naive where 8% females and 4% males displayed WC values above the stipulated thresholds (P = 0.0001). The median (IQR) for TSF in HIV-positive on HAART were higher than for the HIV-positive HAART naive group (P = 0.0038) (Figure 7). Moreover, the median (IQR) for BSF in the HIV-positive (on HAART) group was also higher than the HIV-positive HAART naive group (P = 0.0004) (Figure 8). A similar pattern was found for the MUAC data (P = 0.0417) (Figure 9). In addition, height, body weight and HC for the HIV-positive (on HAART) group were significantly different compared to the HIV-positive HAART naive group (P = 0.03, P = 0.04 and P = 0.0171, respectively) (Table 3).

(a)   (b)
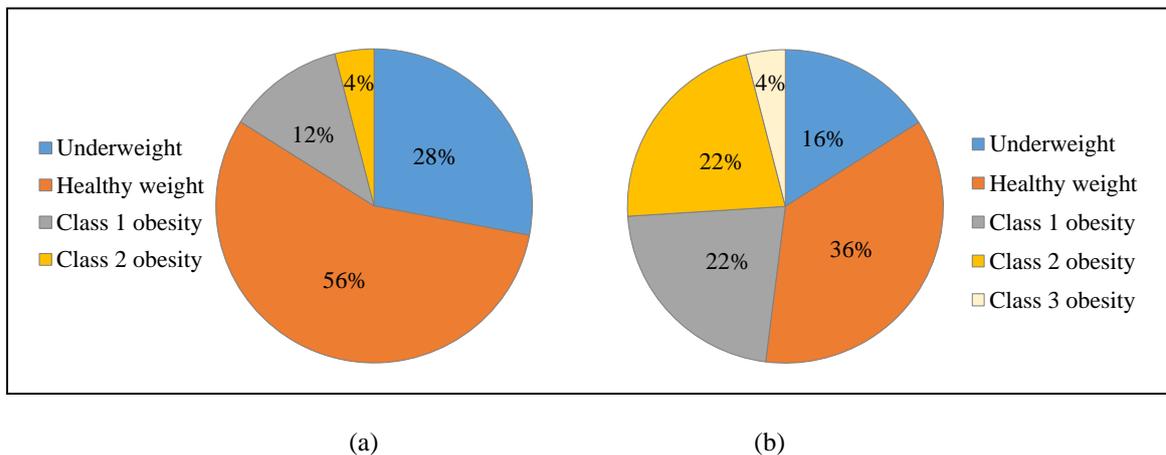
Figure 6: Proportion of BMI in (a) HIV-positive HAART naive and (b) HIV-positive on HAART.
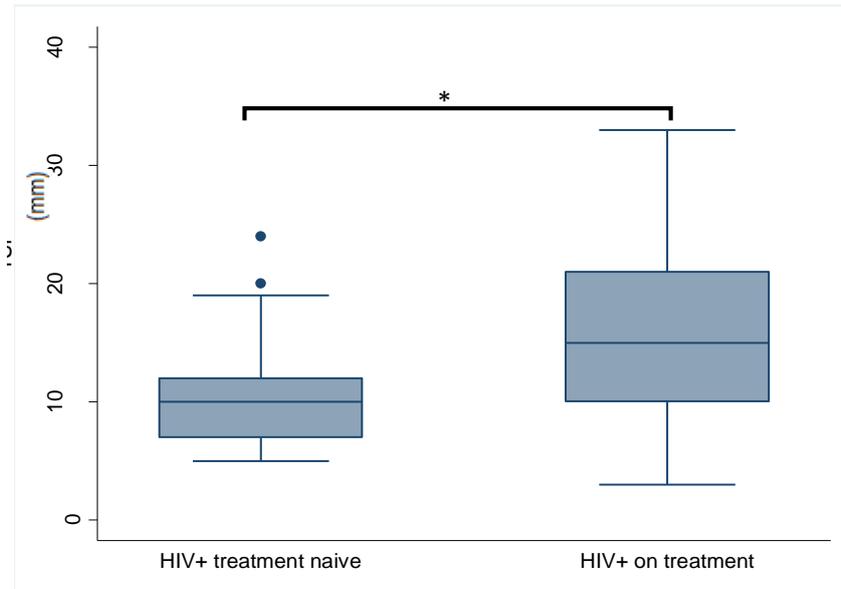
Figure 7: TSF analysis for HIV-positive HAART naive and HIV-positive on HAART. Significant difference in TSF between HIV-positive HAART naive versus HIV-positive on HAART (*P = 0.0038).
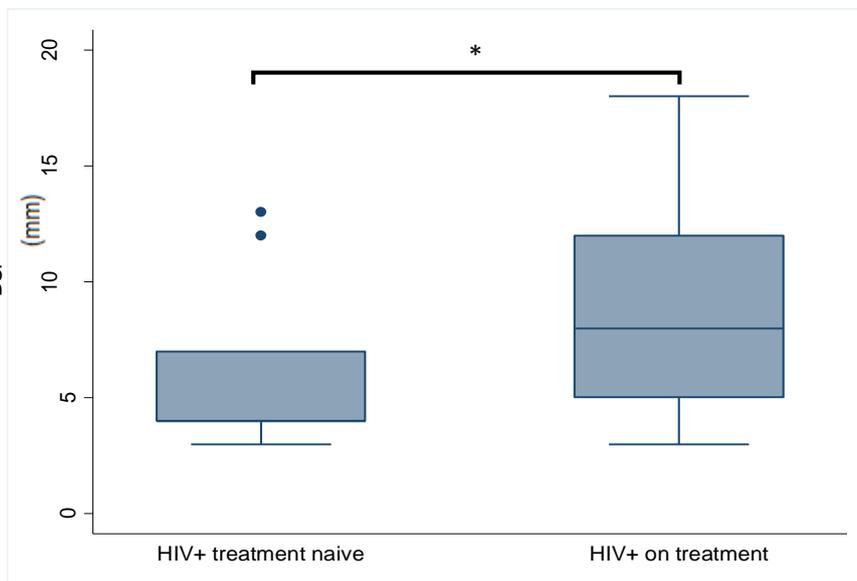
Figure 8: BSF analysis for HIV-positive HAART naive and HIV-positive on HAART. Significant difference in BSF between HIV-positive HAART naive against HIV-positive on HAART (*P = 0.0004).
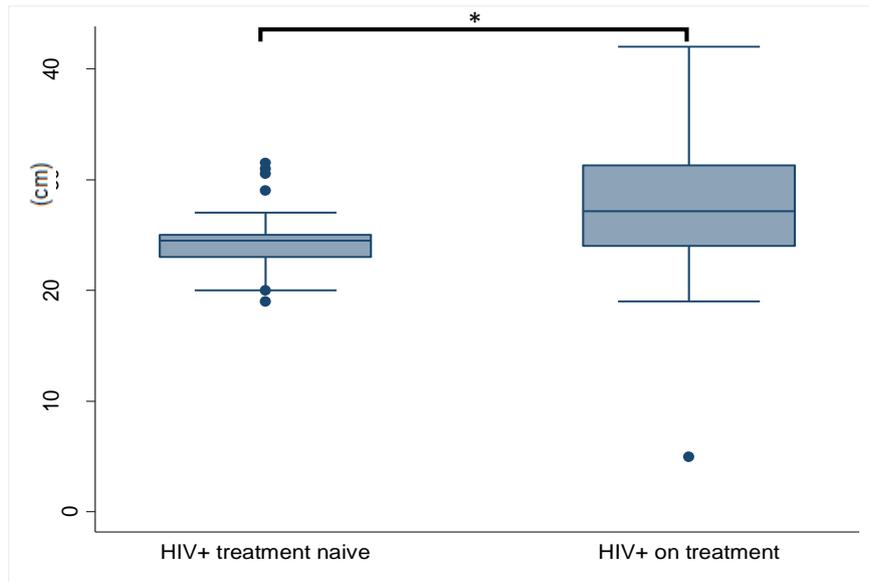
Figure 9: MUAC analysis for HIV-positive HAART naive and HIV-positive on HAART. Significant difference in MUAC between HIV-positive HAART against HIV-positive on HAART (*P = 0.0417).

Table 3: Anthropometric characteristics of HIV-positive individuals (on HAART) and HIV-positive HAART naive.

Variables	HIV+ HAART naive	HIV+ on HAART	P-value
Height (cm) mean ±SD	162.08 ±8.21	157.43 ±9.03	**P = 0.03**
Weight (kg) mean ±SD	54.77 ±10.5	62.96 ±18.4	**P = 0.04**
BMI categories (Appendix 5)			
Underweight, $< 18.5 Kg/m2$, n (%)	7 (28%)	8 (16%)	P = 0.09
Healthy weight, $18.5–24.9$ Kg/ m², n (%)	14 (56%)	18 (36%)	
Class 1 obesity, overweight, $25–29.9$ Kg/m², n (%)	3 (12%)	11 (22%)	
Class 2 obesity, obesity, $30–39.9$ Kg/m², n (%)	1 (4%)	11 (22%)	
Class 3 obesity, morbid obesity, > 40 Kg/m², n (%)	0 (0%)	2 (4%)	
WC (Appendix 6)			
Men ≥ 94 cm	1 (4%)	1 (2%)	**P = 0.0001**
Women ≥ 80 cm	2 (8%)	29 (58%)	
HC			**P = 0.0171**
Mean ±SD	92.02 ±8.55	99.84 ±14.82	
WHR, n (%) (Appendix 6)			
Excellent			
Male < 0.85	6 (24%)	2 (4%)	
Female < 0.75	1(4%)	2 (4%)	
Good			
Male 0.85-0.89	3 (12%)	3 (6%)	
Female 0.75–0.79	4 (16%)	7 (14%)	P = 0.109
Average			
Male 0.90–0.95	3 (12%)	3 (6%)	
Female 0.80–0.86	4 (16%)	14 (28%)	
At Risk			
Male > 0.95	0 (0%)	1 (2%)	
Female > 0.86	4 (16%)	18 (36%)	
TSF (mm)			
Median	10	15	**P = 0.0038**
IQR	5	11	
BSF (mm)			
Median	4	8	**P = 0.0004**
IQR	3	7	
MUAC (cm)			
Median	24.5	27.15	**P = 0.0417**
IQR	2	7.3	

Abbreviations: *BMI: Body mass index, WC: Waist circumference, HC: Hip circumference, WHR: Waist-to-hip ratio, TSF: Triceps skin fold, BSF: Biceps skin fold, MUAC: Mid-upper arm circumference.*
- P-values of ≤ 0.05 were considered statistically significant.

The characteristics of HIV-positive individuals on first and second line treatment compared to HIV-positive HAART naive are presented in Table 4. For BMI categories for the first line group there is 12% underweight, 40% healthy weight, 20% class 1 obesity and 28% class 2 obesity. For the second line 20% were underweight, 32% healthy weight, 24% class 1 obesity, 16% class 2 obesity and 8% class 3 obesity. For the HIV-positive HAART naive group 28% were underweight, 56% healthy weight, 12% class 1 obesity and 4% class 2 obesity (P = 0.117) (Appendix 5). Several of the anthropometric parameters (WC, WHR, TSF, BSF and MUAC) showed changes with first and second line treatment compared to HIV-positive HAART naive (Figure 10, 11 and 12).
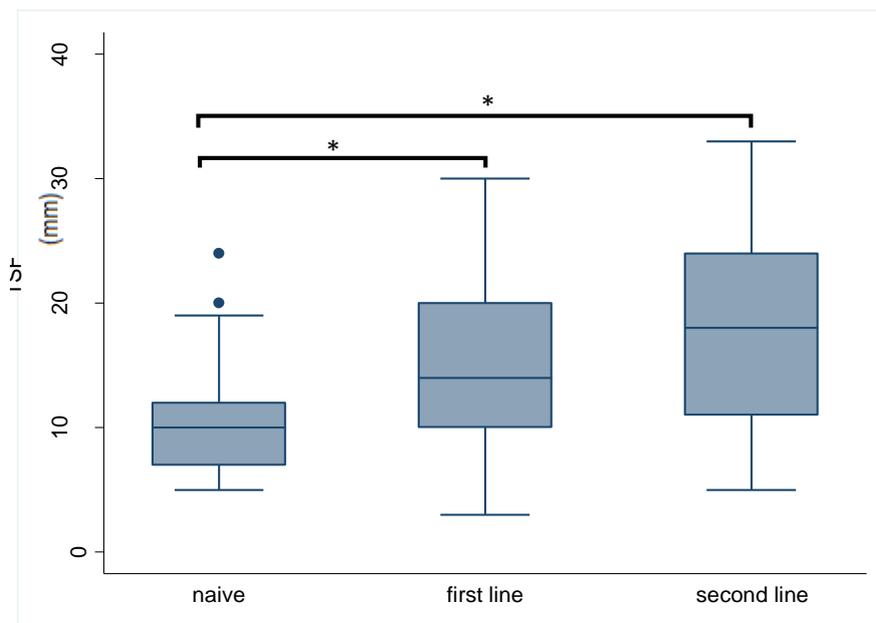
Figure 10: TSF analysis for HIV-positive HAART naive, first line and second line treatment. Significant difference for TSF between HIV-positive HAART naive versus first line (*P = 0.0222) and second line (*P = 0.0067) groups. There was no significant difference between first line and second line treatments (P = 0.3410).
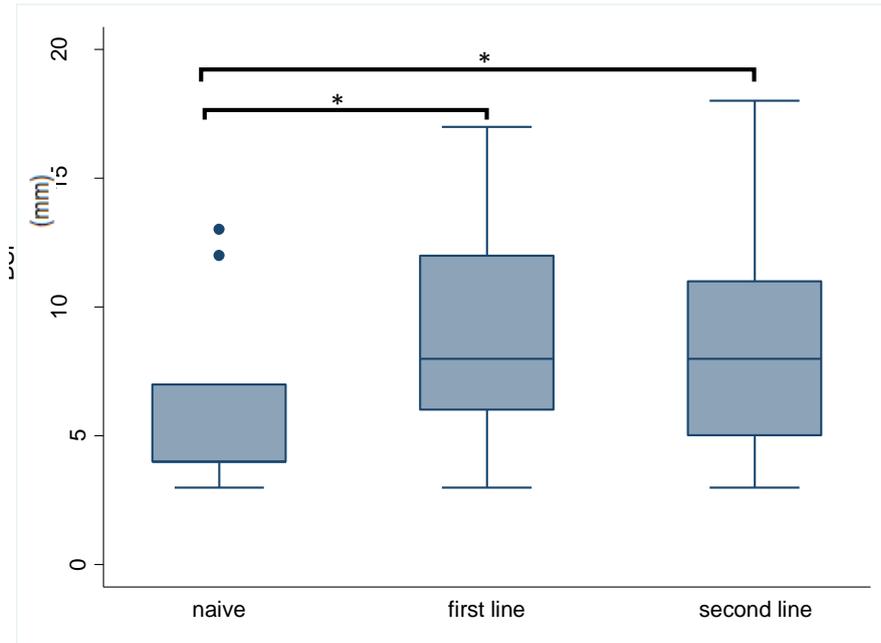
Figure 11: BSF analysis for HIV-positive HAART naive, first line and second line treatment. Significant difference for BSF between HIV-positive HAART naive versus first line (*P = 0.001) and second line (*P = 0.0042) groups. There was no significant difference between first line and second line treatments (P = 0.6404).
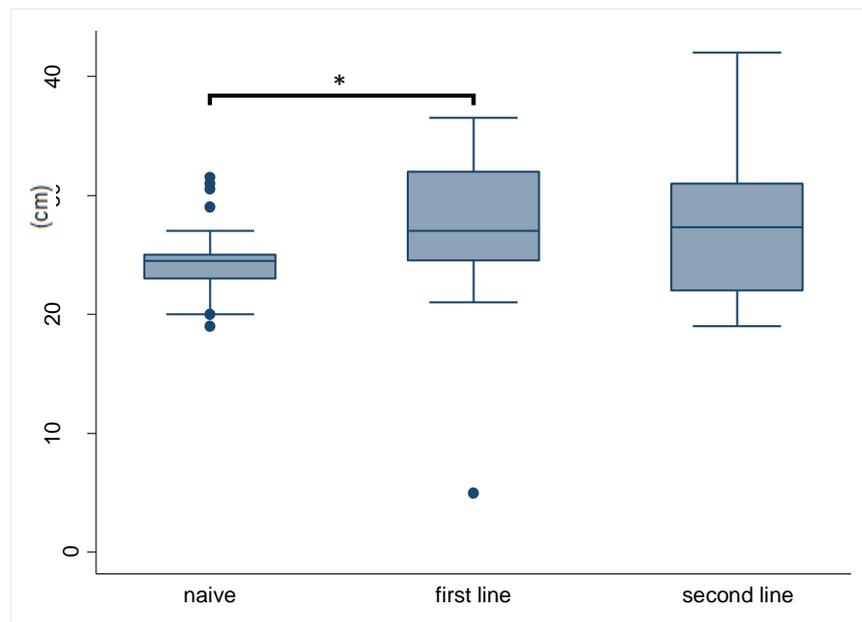
Figure 12: MUAC analysis for HIV-positive HAART naive, first line and second line treatment. Significant difference for MUAC between HIV-positive HAART naive versus first line persons (*P = 0.0077). There were no significant difference between HIV-positive HAART naive and second line treatment (P = 0.1588) and between first line and second line treatments (P = 0.5408).

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Table 4: Anthropometric characteristics for first and second line treatment groups versus the HIV-positive HAART naive group.

Variables	HIV+ HAART naive	First line	Second line	P-value
Height (cm) mean ±SD	162.08 ±8.21	158.03 ±9.59	156.82 ±8.58	P = 0.735
Weight (kg) mean ±SD	54.77 ±10.5	61.78 ±14.1	64.14 ±22.1	**P = 0.001**
BMI categories (Appendix 5)				
Underweight $< 18.5 Kg/m^2$, n (%)	7 (28%)	3 (12%)	5 (20%)	P = 0.117
Healthy weight 18.5–24.9 Kg/ m^2, n (%)	14 (56%)	10 (40%)	8 (32%)	
Class 1 obesity, overweight 25–29.9 Kg/m^2, n (%)	3 (12%)	5 (20%)	6 (24%)	
Class 2 obesity, obesity 30–39.9 Kg/m^2, n (%)	1 (4%)	7 (28%)	4 (16%)	
Class 3 obesity, morbid obesity > 40 Kg/m^2, n (%)	0 (0%)	0 (0%)	2 (8%)	
WC (Appendix 6)				**P = 0.0001**
Men ≥ 94 cm	1 (4%)	1 (4%)	0 (0%)	
Women ≥ 80 cm	2 (8%)	13 (52%)	16 (64%)	
HC				**P = 0.008**
Mean ±SD	92.02 ±8.55	97.93 ±12.87	101.76 ±16.57	
WHR, n (%) (Appendix 6)				P = 0.268
Excellent				
Male < 0.85	6 (24%)	2 (8%)	0 (0%)	
Female < 0.75	1 (4%)	1 (4%)	1 (4%)	
Good				
Male (0.85–0.89)	3 (12%)	1 (4%)	2 (8%)	
Female (0.75–0.79)	4 (16%)	3 (12%)	4 (16%)	
Average				
Male (0.90–0.95)	3 (12%)	3 (12%)	0 (0%)	
Female (0.80–0.86)	4 (16%)	5 (20%)	9 (36%)	
At Risk				
Male > 0.95	0 (0%)	1 (4%)	0 (0%)	
Female > 0.86	4 (16%)	9 (36%)	9 (36%)	
TSF (mm)				**P = 0.0114**
Median	10	14	18	
IQR	5	10	13	
BSF (mm)				**P = 0.0017**
Median	4	8	8	
IQR	3	6	6	
MUAC (cm)				**P = 0.044**
Median	24.5	27	27.3	
IQR	2	7.5	9	

There were no significant differences between groups for blood metabolites tested in this study (Appendix 4) except for low HDL in the HIV-positive HAART naive group (Figure 13) (Table 5). Figure 14 represents the proportional analysis of IR for the HIV-positive (on HAART) group (n = 18; 36%) versus HIV-positive HAART naive persons (n = 8; 32%). The median (IQR) values of blood metabolites for HIV-positive on HAART versus HIV-positive HAART naive are summarized in Appendix 7.

Table 5: Blood metabolite analysis for HIV-positive (on HAART) individuals versus HIV-positive HAART naïve (Appendix 4).

Variables	HIV+ HAART naive	HIV+ on HAART	P-value
TC, n (%)			
Desirable	21 (84%)	44 (88%)	P = 0.362
Borderline	3 (12%)	6 (12%)	
High	1 (4%)	0 (0%)	
LDL, n (%)			
Desirable	16 (64%)	26 (52%)	**P = 0.066**
Borderline	4 (16%)	20 (40%)	
High	5 (20%)	4 (8%)	
HDL, n (%)			
Desirable	8 (32%)	30 (60%)	**P = 0.022**
Low	17 (68%)	20 (40%)	
TG, n (%)			
Desirable	23 (92%)	45 (90%)	P = 0.429
High	2 (8%)	5 (10%)	
CRP, n (%)			
Normal	20 (80%)	39 (78%)	P = 0.842
High	5 (20%)	11 (22%)	
FBG, n (%)			
Normal	21 (84%)	37 (74%)	P = 0.145
High	4 (16%)	13 (26%)	
Insulin, n (%)			
Normal	21 (84%)	37 (74%)	P = 0.320
Low	3 (12%)	5 (10%)	
High	1 (4%)	8 (16%)	
IR HOMA index, n (%)			
Normal	17 (68%)	32 (64%)	P = 0.687
resistant	8 (32%)	18 (36%)	
AIP, n (%)			
Low risk (AIP: < 0.1)	21 (84%)	37 (74%)	P = 0.620
Medium risk (AIP: 0.1–0.24)	1 (4%)	3 (6%)	
High risk (AIP: > 0.24)	3 (12%)	10 (20%)	

There was strong significant difference in LDL percentages between HIV-positive persons on first and second line treatments and HIV-positive HAART naive persons (P = 0.006) (Table 6). However, we found no statistically significant differences for any of the other parameters here assessed (Appendix 4). For the first line group there was 36% with low HDL and 44% for the second line group, versus 68% for the HIV-positive HAART naive group (P = 0.065) (Figure 13). As before, proportional analysis showed the IR in the first line group (32%) and 44% with second line treatment (P = 0.510) (Figure 14). The proportional analysis also showed both first line and second line groups at higher risk for CVD; 20% for both first and second line groups (as per AIP categories) compared to the HIV-positive HAART naive group (12%) (Figure 16). The proportion of SBP and DBP for HIV-positive HAART naive versus HIV-positive (on HAART) groups is indicated in Figure 17. The median (IQR) values of blood metabolites for HIV-positive persons on first and second line treatments versus HIV-positive HAART naive persons are summarized in Appendix 8.
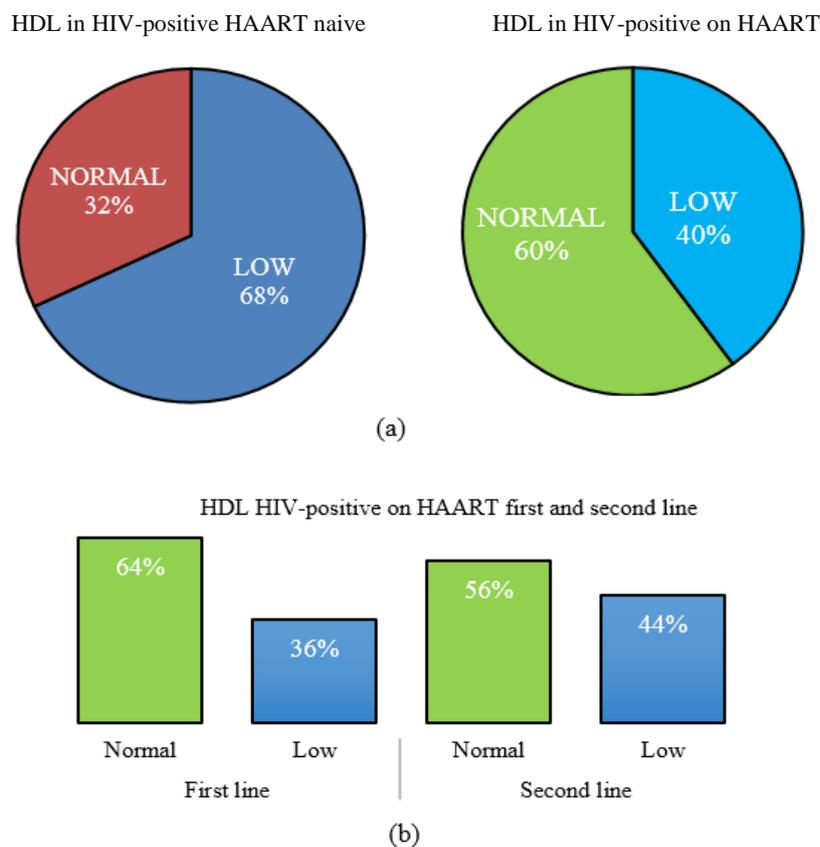
Figure 13: Distribution patterns of HDL (%). (a) HIV-positive HAART naive versus HIV-positive (on HAART), significant difference found (P = 0.022); (b) HDL distribution patterns between first line and second line treatments.

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Table 6: Blood metabolite analysis for HIV-positive persons on first and second line treatments versus HIV-positive HAART naive persons (Appendix 4).

Variables	HIV+ HAART naive	First line	Second line	P-value
TC, n (%)				
Desirable	21 (84%)	23 (92%)	21 (84%)	P = 0.594
Borderline	3 (12%)	2 (8%)	4 (16%)	
High	1 (4%)	0 (0%)	0 (0%)	
LDL, n (%)				
Desirable	16 (64%)	9 (36%)	17 (68%)	**P = 0.006**
Borderline	4 (16%)	15 (60%)	5 (20%)	
High	5 (20%)	1 (4%)	3 (12%)	
HDL, n (%)				
Desirable	8 (32%)	16 (64%)	14 (56%)	P = 0.062
Low	17 (68%)	9 (36%)	11 (44%)	
TG, n (%)				
Desirable	23 (92%)	23 (92%)	22 (88%)	P = 0.661
High	2 (8%)	2 (8%)	3 (12%)	
CRP, n (%)				
Normal	20 (80%)	18 (72%)	21 (84%)	P = 0.573
High	5 (20%)	7 (28%)	4 (16%)	
FBG, n (%)				
Normal	21 (84%)	18 (72%)	19 (76%)	P = 0.350
High	4 (16%)	7 (28%)	6 (24%)	
Insulin, n (%)				
Normal	21 (84%)	18 (72%)	19 (76%)	
Low	3 (12%)	3 (12%)	2 (8%)	P = 0.646
High	1 (4%)	4 (16%)	4 (16%)	
IR HOMA index				
Normal	17 (68%)	17 (68%)	14 (56%)	P = 0.510
resistant	8 (32%)	8 (32 %)	11 (44%)	
AIP, n (%)				
Low risk (AIP: < 0.1)	21 (84%)	19 (76%)	18 (72%)	P = 0.3542
Medium risk (AIP: 0.1–0.24)	1 (4%)	1 (4%)	2 (8%)	
High risk (AIP: > 0.24)	3 (12%)	5 (20%)	5 (20%)	

IR in HIV-positive HAART naive

IR in HIV-positive on HAART

(a)

IR in HIV-positive on HAART first and second lines
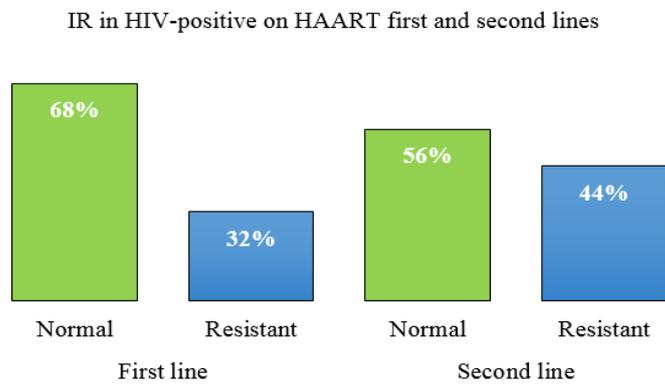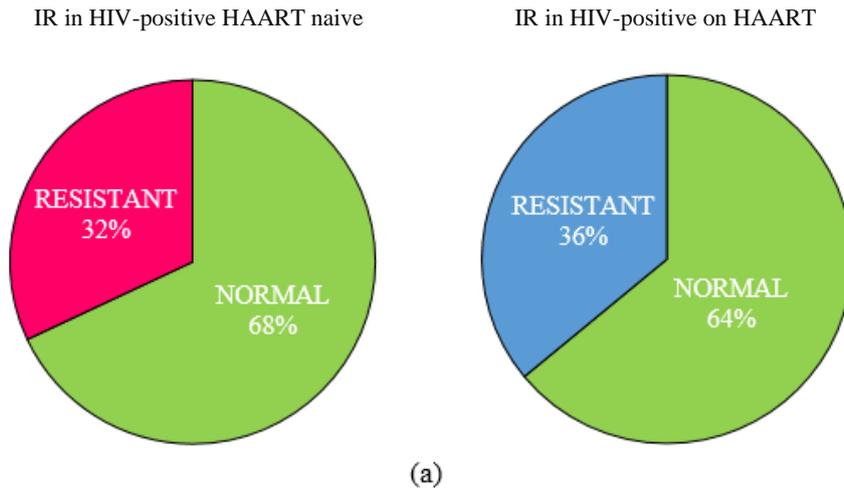
(b)

Figure 14: Distribution patterns of IR (%). (a) HIV-positive HAART naive versus HIV-positive (on HAART); and (b) IR distribution patterns between first line and second line treatments.
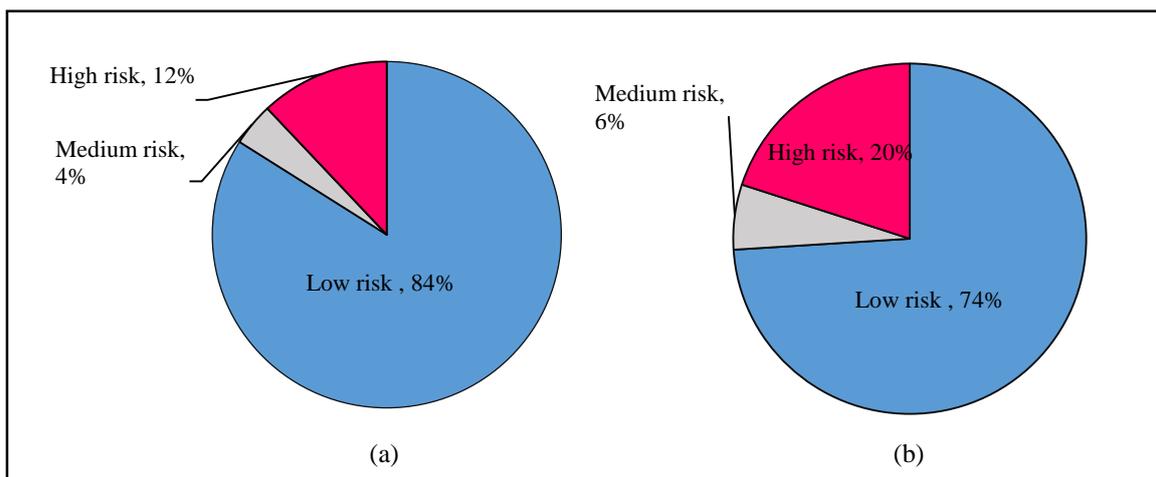
Figure 15: Proportion of AIP in (a) HIV-positive HAART naive and (b) HIV-positive (on HAART) groups.

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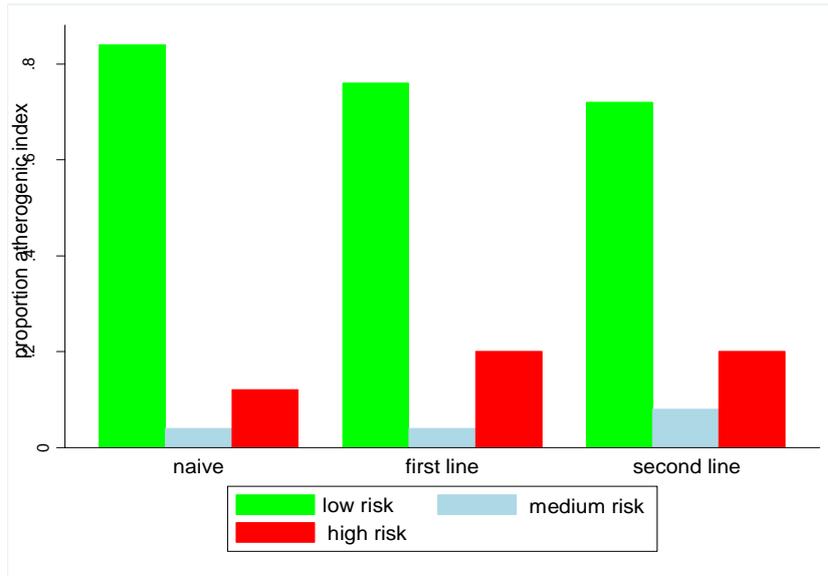
Figure 16: Proportion of AIP for first line and second line treatments versus HIV-positive HAART naive individuals (P = 0.3542).
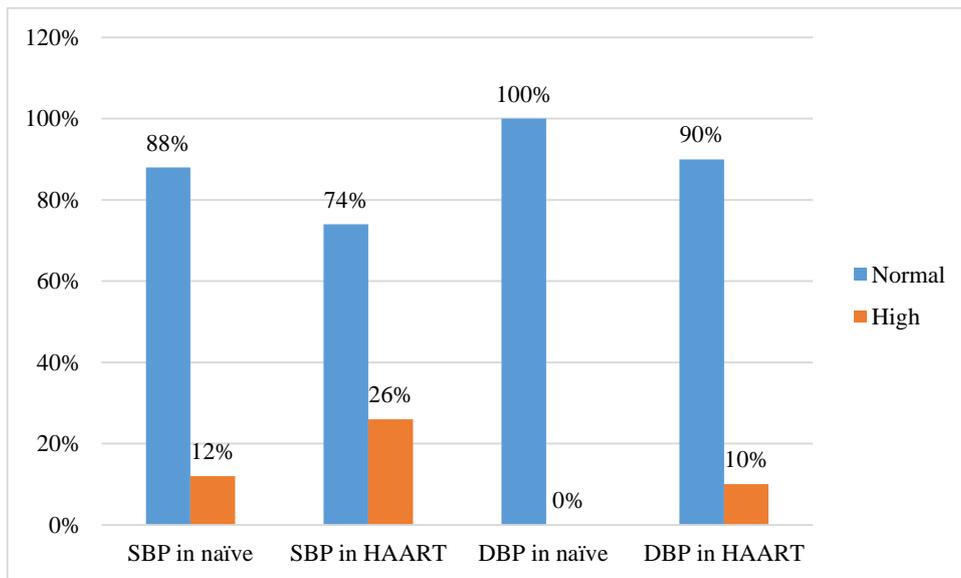
Figure 17: Proportion of SBP and DBP for HIV-positive HAART naive versus HIV-positive (on HAART) groups.

CHAPTER FOUR

DISCUSSION AND CONCLUSION

4.0 Discussion

4.1 Conclusion

4.0 Discussion

Although HAART has significantly improved the survival of HIV-infected patients, there are increased concerns regarding the onset of co-morbidities (e.g. cardio-metabolic complications) and mortalities in such individuals (Lohse et al. 2007; Sackoff et al. 2006). Although South Africa is burdened with the highest number of HIV-infected individuals globally, there is a relative paucity of data regarding potential links between HIV infection, HAART and cardio-metabolic risk/onset. This cross-sectional study therefore investigated the prevalence of cardio-metabolic risk factors in HIV-infected individuals on HAART within the Cape Winelands region of South Africa. Here we collected anthropometric, biochemical and lifestyle-related data for HIV-positive HAART naive (n = 25) and HIV-positive individuals on HAART (n = 50) patients (20 − 55 years old) at the Worcester CDC (Worcester, Western Cape, South Africa) during 2014 and 2015. Subjects on HAART were further divided into two sub-groupings, i.e. first line (n = 25) and second line treatments (n = 25). The main findings of this study are: a) the relatively high prevalence of traditional, cardio-metabolic lifestyle risk factors in HIV-infected individuals; b) a large proportion of HIV-positive HAART naive patients can be categorized into groups with relatively low HDL and high LDL levels, respectively; and c) HAART enhanced anthropometric measures of obesity.

Relatively high prevalence of traditional lifestyle risk factors
Relatively high prevalence of traditional lifestyle risk factors

Our data reveal a relatively high prevalence of smoking, i.e. 88% for the HIV-positive HAART naive group and 27.3% for the HIV-positive group on HAART. However, as we did not include a HIV-negative control group these data are difficult to interpret but our findings are in agreement with previous studies that established that HIV-infected persons displayed higher prevalence of smoking (Lifson & Lando 2012; Reynolds 2009). Additional studies with an HIV-negative control are required to assess whether this indeed the case for the Worcester population. There are relatively limited data regarding cigarette smoking prevalence among HIV-positive people in the Western Cape and in South Africa, making meaningful comparisons difficult. It also remains unclear why smoking prevalence is

so high in HIV-infected individuals in the Cape Winelands region and we speculate that this may be a combination of factors that likely include poor socio-economic backgrounds, a limited understanding of the damaging effects of smoking, and as a coping mechanism to deal with the stress when diagnosed as HIV-positive. Further studies are required to investigate this concerning phenomenon in HIV-positive individuals (versus negative controls) in the Cape Winelands region and the rest of South Africa.

We did not find any significant changes for HTN prevalence in HIV-infected individuals included in this study. Previous studies found a range of findings for HTN in HIV-positive patients with some reporting increased risk with ART (Gazzaruso et al. 2003) while others found no association (Bergersen et al. 2003; Jerico et al. 2005). Such differences may be influenced by a number of factors that can include differences in sample sizes, distinct presentation of lifestyle risk factors and methods employed to determine HTN in patients. Of note, the lifestyle questionnaire data established that more than half of the HIV-positive individuals indicated a prior family CVD history. The reasons for this phenomenon are unclear but it is likely that same arguments earlier put forward (for high smoking prevalence) may also apply in this instance. However, without an HIV-negative control group we cannot make any definitive conclusions in this instance. Compounding this picture is that the majority of cohort here studied (92%) falls into a relatively young age group (~45 years for men; ~55 years for women) and may potentially constitute a risk factor as such individuals will usually display an even higher risk as they continue to age (DAD Study Group 2003). This is in agreement with others that found that younger, HIV-infected persons are at greater risk of developing CVD than their peers of similar age (Carter 2010; Currier et al. 2008).

Relatively high proportion of HIV-positive HAART naive patients categorized into groups with relatively low HDL and high LDL levels, respectively

The lipid blood metabolite analyses (TC, LDL, HDL and TG) did not reveal significant changes when comparing HIV-positive on HAART versus HIV-positive HAART naive groups. Likewise, when such values were compared to established cut-off thresholds (NCEP Expert Panel 2001) there were no significant changes. However, in this case HDL levels in the HIV-positive HAART naive group were

lower than the HAART group although this did not reach statistical significance (P = 0.06). This suggests that untreated HIV-infected persons may potentially be at increased risk for CVD onset *before* treatment initiation and it is likely that such effects are mediated by the virus itself and/or lifestyle-related risk factors such as smoking. These findings are in agreement with others that found that untreated HIV individuals are at greater risk due to virus-induced effects such as chronic inflammation that may contribute to the onset of atherosclerosis (Riddler et al. 2003; Triant et al. 2007). This notion was echoed by additional analyses performed on our data set. When HDL and LDL data were placed into various, established sub-groups (NCEP Expert Panel 2001) then 68% of the naive individuals could be categorized as "low HDL". However, with HAART this situation improved with this number declining to 40% (P = 0.022). Of note, first line treatments decreased the low HDL numbers to 36% compared to 44% with second line treatment. This demonstrates that patients on second line treatment display an improved HDL profile compared to the naive group, although this decrease is not as robust as for the first line therapy. Such findings are in agreement with previous studies performed in the United States and other parts of sub-Saharan Africa that established that treatment-naive HIV-infected patients exhibit significantly lower levels HDL (Nguemaïm et al. 2010; Obirikorang et al. 2010; Shor-Posner et al. 1993).

Data from our study did not reveal any significant differences for CRP levels between the HIV-positive HAART naive and HIV-positive on HAART and likewise for comparisons between first and second line treatments. This indicates lack of an inflammatory response in HIV-infected persons within the Cape Winelands region. In support others reported the absence of opportunistic infections in HIV-positive individuals (Lawn et al. 2001), while Wilson et al. (2011) established normal CRP levels in a South African cohort (Wilson et al. 2011).

There were no significant difference when comparing FBG and insulin levels between HIV-positive HAART naive and HIV-positive on HAART. This also applied when additional tests were completed for first and second line treatments. Our findings are consistent with others (Muthumani et al. 2003) who found no significant changes in FBG levels in HIV-positive subjects compared to controls, while Hadigan et al. (2001) established that FBG levels remain within the normal range in most patients receiving potent ARV. Other studies reported the association of PI usage with impaired

FBG and DM (Carr & Cooper 2000; Palella et al. 1998), while Gadd (2006) found that high glucose levels are associated with low CD4 count in HIV-positive individuals. For our study we speculate that normal FBG levels may manifest due to the following possibilities: a) from the literature it is well documented that impaired glucose metabolism and IR are linked to defects in lipid metabolism (Magkos & Mantzoros 2011) while our study did not reveal dyslipidemia; b) the relatively small sample size; c) OGTT is a more sensitive test to detect glucose abnormalities in HIV-infected patients who display normal FBG levels (Gianotti et al. 2011); and d) lack of CD4 counts to assess disease progression.

We performed additional analyses to gain greater insights into our findings and calculated HOMA-IR values for the different groups investigated in this study. However, there were no significant changes/patterns between the naive and HAART groups indicating that IR is not a major factor in this population. By contrast, other South African studies (Kiage et al. 2013; Menezes et al. 2014) found that short-term ART (90 days treatment) was associated with a propensity towards IR development regardless of the ART employed. In support, some reported a higher risk for IR in ART patients versus naive HIV-infected patients (Idiculla et al. 2011). How do we explain our results in this regard? We are of the opinion that such differences may be due to the relatively small sample size of this study, the lack of a control group (HIV-uninfected subjects) to define more precise HOMA-IR cut-off points, and lack of CD4 count and viral load that are strongly associated with IR onset. For example, Squillace et al. (2009) found a significant association between a high CD4 count and IR, while El-Sadr et al. (2005) demonstrated an inverse relationship between CD4 count and IR. AIP is a simple and practical ratio for evaluation of CVD risk and may offer greater diagnostic utility than sole use of lipid profiles, especially for HIV-positive patients (Lemieux et al. 2001). Moreover, it correlates more closely with small LDL and HDL particles which are highly atherogenic (Dobiásová & Frohlich 2001). In light of this, we performed AIP analysis but this did not yield any significant differences. Thus the early data suggest that HIV-positive individuals in the Cape Winelands region are not at increased risk of atherogenesis. However, sample size number should be increased to confirm this and additional tests should be considered to improve quality of findings generated, e.g. employment of the high-sensitivity CRP test. In addition, functional assessments such as carotid IMT may further strengthen the conclusions derived, although costs and logistics are a serious consideration.

HAART improved anthropometric measures of obesity

The anthropometric data demonstrated significant improvements in weight gain with HAART and this applied equally for first and second line treatments employed. This is in agreement with others that found increased weight and improved survival with HAART (May et al. 2006). Our findings show significant differences for TSF, BSF, WC and MUAC between the naive and HAART groups and this applied for first and second line treatments. These data therefore indicate that with HAART there is improved well-being of patients and a general restoration to health (George et al. 2009).

Of concern, however, is that the HAART individuals displayed higher levels of obesity when assessed by established cut-off values. As there is a relatively large discrepancy in terms of recruitment numbers (males versus females) between the naive and HAART groups, this is a factor that should be taken into consideration. The higher female numbers in the HAART group may therefore help explain increased manifestation of obesity in this group. In agreement, Nell et al. (2015) showed that females on prolonged HAART also displayed increased prevalence of obesity. The reasons for this are unclear but it may relate to cultural or lifestyle choices (Hurley et al. 2011), e.g. factors such as poor nutritional intake and lack of exercise may be implicated. However, additional studies are required to pursue this interesting notion. According to WHO (2011) cut-off points and risk of metabolic complications, the higher levels of obesity (e.g. WHR $\geq$ 0.86 in HIV-positive [on HAART]) (Appendix 6) puts such individuals at risk for the onset of future cardio-metabolic complications (Rasheed et al. 2008; WHO 2011).

Limitations

The lack of an HIV uninfected control group limits our ability to attribute the changes observed to HAART, while the sample size was also relatively small with a strong gender bias for the HIV-positive (on HAART) group. This is generally due to males being the sole breadwinner and hence unable to take time off from work for medical care and/or contribute to research projects. This observation is in agreement with previous research work published by our group (Nell et al. 2015). In addition, we were unable to include CD4 count and viral load results in our study as there was a delay with processing of samples. Finally, we included various race groups – Coloureds and Blacks – in our research study and

this may have also impacted on our findings and conclusions. Future studies should recruit additional numbers and then sub-divide the groups into specific race groupings – this should enhance the quality of conclusions reached.

4.1 Conclusion

The study established the prevalence of several traditional CVD risk factors (smoking, family history, age) in both HIV-positive naive and HIV-positive on HAART in the Cape Winelands region of South Africa. HAART improved several measure of weight gain and the lipid profile, suggesting a restoration to health and well-being. However, there was a relatively high prevalence of obesity in the HIV-positive (on HAART) group and especially for females thus placing them at greater risk for future cardio-metabolic complications onset. We are unable to distinguish whether this risk is due to HAART or lifestyle-related risk factors and this question requires further investigations. In light of these findings we recommend that clinicians should be mindful of lifestyle-related CVD risk factors in HIV-positive individuals and make an effort to counsel patients to adopt improved lifestyle choices such as improved nutritional intake, smoking cessation and increased exercise. This can be monitored on a regular basis in follow-up sessions. Moreover, lipid profile (HDL, LDL) assessment may be useful to employ in the clinic to evaluate HIV-positive individuals (especially upon admission) and to be aware that such patients may be at a higher risk of cardiac complications.

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APPENDICES

Appendix 1: Health Research Ethics Committee approval.

Appendix 2: Participant information leaflet and consent form.

Appendix 3: Data sheet.

Appendix 4: List of reference values.

Appendix 5: International Classification of adult underweight, overweight and obesity according to BMI.

Appendix 6: IDF ethnic specific values for waist circumference.

Appendix 7: Median (IQR) values of blood metabolites HIV-positive on HAART persons versus HIV-positive HAART naive persons.

Appendix 8: Median (IQR) values of blood metabolites for HIV-positive persons on first and second line treatments versus HIV-positive HAART naive persons.

Appendix 1

Health Research Ethics Committee approval

Western Cape Government
Health

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REFERENCE: RP 090 /2013
ENQUIRIES: Ms Charlene Roderick

Private Bag X1
Matieland
7602

For attention: Prof. MF Essop, Dr Theo Nell, Dr E Calitz, Dr. C Gunst, Dr C Klussmann, Dr. E Teer, Dr F Abaid, Dr. M Lombard, Dr. R Essop

Re: A cross-sectional survey of the metabolic dysfunction and onset of cardiac diseases in HIV patients on HAART treatment within the Winelands region

Thank you for submitting your proposal to undertake the above-mentioned study. We are pleased to inform you that the department has granted you approval for your research.

Please contact to assist you with any further enquiries in accessing the following sites:

Cape Winelands	Ms S Neethling	Contact No. 023 348 8111
Paarl Hospital	Dr B Kruger	Contact No. 021 872 1711
Worcester Hospital	Dr W Marais	Contact No. 023 348 1101

Kindly ensure that the following are adhered to:

1. Arrangements can be made with managers, providing that normal activities at requested facilities are not interrupted.
2. Researchers, in accessing provincial health facilities, are expressing consent to provide the department with an electronic copy of the final report within six months of completion of research. This can be submitted to the provincial Research Co-ordinator (Health.Research@westerncape.gov.za).
3. The reference number above should be quoted in all future correspondence.

We look forward to hearing from you.

Yours sincerely
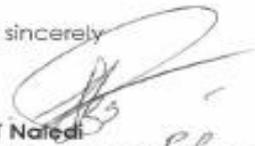
DR NT Naledi
DIRECTOR: HEALTH IMPACT ASSESSMENT
DATE: 30/10/2013
CC   DR L PHILLIPS   DIRECTOR: CAPE WINELANDS

Page 1 of 1

UNIVERSITEIT·STELLENBOSCH·UNIVERSITY
jou kennisvennoot · your knowledge partner

Ethics Letter

05-Aug-2015

Ethics Reference #: N12/12/086
Clinical Trial Reference #:
Title: Winelands HAART to HEART study (Prevalence)

Dear Prof Mogammad Essop,

At a meeting of HREC1 on 29 July 2015 the following progress report was approved:

Progress Report dated 8 July 2015

The approval of this project is extended for a further year.

Approval date: 29 July 2015

Expiry date: 29 July 2016

If you have any queries or need further assistance, please contact the HREC Office 0219399657.

Sincerely,

REC Coordinator
Franklin Weber
Health Research Ethics Committee 1

Appendix 2

Participant information leaflet and consent form

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

TITLE OF THE RESEARCH PROJECT:
WINELANDS HAART to HEART STUDY

REFERENCE NUMBER: N12/12/086

PRINCIPAL INVESTIGATOR: Prof MF Essop

ADDRESS:
Department of Physiological Sciences
Mike de Vries Building
Room 2005a
Stellenbosch University

CONTACT NUMBER: 021 8083146

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the **Health Research Ethics Committee at Stellenbosch University** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

What is this research study all about?

➢ *This study will only be done in the Stellenbosch district and surrounding areas. We will need approximately 300 Patients. We are trying to gather information on laboratory tests, body composition and patient questionnaires profiles of chronic HIV patients on ARV treatment (1st and 2nd line). By getting this information we would be able to assess the attenuation of metabolic function and contractail dysfunction caused by ARV treatment.*

> *As Africa adopts a more westernised lifestyle there is a higher incidence of HIV infection, diabetes and heart diseases.*

Your blood will be taken by a registered medical nurse. It will then be sent away to Pathcare (Stellenbosch) where metabolic-associated parameters will be measured. Other biochemical tests that will be done by Pathcare include C-reactive protein measurements. The remainder of your blood samples will be used to obtain genetic material (RNA).

The blood presser assessment, evaluation of cardiac function (ECG, echocardiography and carotid intima media thickness), full anthropometric evaluation and life style questionnaire will be done at the clinic

Why have you been invited to participate?

> *We are trying to gather information on laboratory tests, body composition and patient questionnaires profiles of chronic HIV patients on ARV treatment (1st and 2nd line). By donating blood to our study you will be helping us to determine these profiles and how we can relate them to the current diagnostic tests to investigate cardio-metabolic effects of ARV treatment. By getting this information we would be able to assess the attenuation of metabolic function and contractail dysfunction caused by ARV treatment. With your help we hope to identify the suitable therapeutic interventions that will blunt metabolic dysfunction, oxidative stress and enhance mitochondrial function under conditions where PIs are chronically administered.*

What will your responsibilities be?

> *As we need to examine the selected patients and then take the blood for laboratory tests and lifestyle questionnaire regarding your line of ARV treatment. A registered anthropometrist, Dr Theo Nel, will also perform anthropometric measurements that include waist circumference, waist: hip ratio, height and mass.*

Will you benefit from taking part in this research?

> *Although there may not be any direct benefits to me/the participant by participating at this stage, future generations may benefit if the researchers succeed in finding out more about how ARV treatment can lead to metabolic dysfunction and cardiac disease. If you choose to know the results of your blood tests we will make these available. However, you would have to discuss this information with your personal doctor, at your own cost, in order to assess your medical status with ARV treatment (metabolic and cardiac status).*

Are there in risks involved in your taking part in this research?

There are no more than minimal medical or physiological risks associated with this study.

> *I/the participant may feel some pain associated with having blood drawn from a vein and may experience some discomfort, bruising and/or slight bleeding at the site.*

If you do not agree to take part, what alternatives do you have?

> *It is your decision to participate or not and nothing will be done from the researchers' part to in any way to persuade you to take part.*

Who will have access to your medical records?

> *Only the principal researcher (Prof Faadiel Essop and the following masters student: Dr. Faten Abaid) will have access to your data and records. All information will be treated with respect and utmost confidentiality. Under no circumstances will your name or any form of identification be used in any publication, poster, lecture or thesis that results from this study. Professor Faadiel Essop and Dr. Faten Abaid will be the only authorised personnel who will have access to your results from this study as well as the lifestyle questionnaire and anthropometric measurements.*

What will happen in the unlikely event of some form injury occurring as a direct result of your taking part in this research study?

> *Not applicable here.*

Will you be paid to take part in this study and are there any costs involved?

> *No, you will not be paid to take part in the study. There will be no costs involved for you, if you do take part. Travelling costs in order to get to the Department of Physiological Sciences will not be covered by this study and you will need to cover this cost yourself.*

Would you like to know the results of your blood tests?

> Please indicate by marking the correct box with an X

> ☐ **YES**
>   ☐ **NO**

Is there anything else that you should know or do?

> You can contact Professor MF Essop on 021 808 3146 if you have any further queries or encounter any problems.

> You can contact the Health Research Ethics Committee at 021-938 9207 if you have any concerns or complaints that have not been adequately addressed by your study doctor.

> > You will receive a copy of this information and consent form for your own records.

Declaration by participant

By signing below, I …………………………………..………… agree to take part in a research study entitled **(Investigation of cardio-metabolic effects of ARV treatment in the winelands region).**

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.

- I have had a chance to ask questions and all my questions have been adequately answered.

- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.

- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.

- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Signed at (*place*) ....…………….. on (*date*) …………...………..

....
Signature of participant   **Signature of witness**

Declaration by investigator

I *(name)* ………………………………………….……… declare that:

- I explained the information in this document to …………………..

- I encouraged him/her to ask questions and took adequate time to answer them.

- I am satisfied that he/she adequately understands all aspects of the research, as discussed above

- I did/did not use a interpreter. (*If a interpreter is used then the interpreter must sign the declaration below.*

Signed at (*place*) ....….......…………….. on (*date*) …………...………..

....
Signature of investigator   **Signature of witness**

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Declaration by interpreter

I (*name*) …………………………………………..……… declare that:

- I assisted the investigator (*name*) ………………………………………… to

  explain the information in this document to (*name of participant*)

  ……………..………….. using the language medium of Tswana/English.

- We encouraged him/her to ask questions and took adequate time to answer them.

- I conveyed a factually correct version of what was related to me.

- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

Signed at (*place*) ....…….....………….. on (*date*) ……….……………..

....
Signature of interpreter   **Signature of witnes**

Appendix 3

DATA SHEET

_____

Project title: Cape Winelands HAART 2 HEART

Ethics approval nr: N12/12/086

IDENTIFICATION CODE: CWH2H_____			
Date of interview:			
Interviewer:			
Health District Site:			
	Worcester	TC Newman	Mbekweni	Stellenbosch
Age:			
Blood pressure (Sys/Dia):			
Heart rate (beats/min):			
HIV classification			
	Negative	Naive	1st line	2nd line
WHO stage:	1	2	3	4
Time since HIV diagnosis (months):			
HAART start date:			
Time on HAART:			
Current HAART regimen:			
Time on current HAART:			
Switch from regimen:			
Adherence:	YES	NO	
Viral load:			
Previous co-infections:			
Current co-infections:			
Drug history:			
Family history CVD:			
Smoking/snuff:			
Quantity per day:			
Frequency per week:			

DEMOGRAPHIC QUESTIONNAIRE

_____

Project title: Cape Winelands HAART to HEART study

Ethics approval nr: N12/12/086

IDENTIFICATION CODE: CWH2H_____							
Date of interview:							
Interviewer:							
Health District Site:	Worcester		TC Newman		Mbekweni		Stellenbosch
Household (Brick house, shack other dwelling)							
Total in household	**Male**				**Female**		
Children 0-6 yrs							
Children 7-12 yrs							
Children 13-18 yrs							
Adults 18-30							
Adults 31-45							
Adults 46-60							
Adults 61+							

LANGUAGE AND ACCULTURATION							
Home language of respondent	Afrikaans	Xhosa	Zulu	English	Sotho	Other	
Household head speaks	Home language only		Home language + Afr/Eng		English/Afrikaans only		
Can you read and understand a newspaper in your home language easily, difficult or not at all?	Easily		1				
	With difficulty		2				
	Not at all		3				
Can you read and understand a newspaper in the English language easily, difficult or not at all?	Easily		1				
	With difficulty		2				
	Not at all		3				

NEAREST CLINIC/HOSPITAL		
Name of clinic/hospital	**Walk (minutes)**	**Mode of transport** **Walk=1; Taxi=2; Own car=3; bicycle=4**

ANTHROPOMETRY	
Height (m):	
Weight (kg):	
BMI (kg/m²):	
Waist circumference (cm):	
Hip circumference (cm):	
W:H:	
Mid-Upper Arm Circumference (cm):	
Triceps skinfold:	
Biceps skinfold:	

Appendix 4

List of reference values

PARAMETER	REFERENCE RANGE	UNIT
∗TC	Desirable < 5.2 Borderline 5.2–6.2 High > 6.2	mmol/L
∗TG	Desirable 1.7–2.2 High > 2.2	mmol/L
∗HDL	Desirable > 1.2 Low < 1	mmol/L
∗LDL	Desirable < 2.6 Borderline 2.6–3.3 High > 3.3	mmol/L
CRP	Normal < 10	mg/L
◊FBG	Normal 3.9–5.5 High > 5.6	mmol/L
Insulin	Normal 3–25 High > 25	mu/L

* National Cholesterol Education Program (NCEP) and Adult Treatment Panel III (ATP III) guidelines (NCEP Expert Panel 2001).

◊ IDF clinical criteria (IDF 2006).

Appendix 5

International Classification of adult underweight, overweight and obesity according to BMI*

Classification	BMI(kg/m2): principal cut-off points
Underweight	< 18.5
Healthy weight	18.5–24.9
Class 1 obesity, overweight	25.0–29.9
Class 2 obesity, obesity	30.0–39.9
Class 3 obesity, morbid obesity	> 40.0

* Adapted from (WHO 1995; 2000; 2004).

Appendix 6

IDF ethnic specific values for waist circumference (IDF 2006)

Country/Ethnic groups	Waist circumference
Europids In the USA, the ATP III values (102 cm male; 88 cm female) are likely to continue to be used for clinical purposes	Male ≥ 94 cm Female ≥ 80 cm
South Asians Based on a Chinese, Malay and Asian-Indian population	Male ≥ 90 cm Female ≥ 80 cm
Sub-Saharan Africans	Use European data until more specific data are available
Chinese	Male ≥ 90 cm Female ≥ 80 cm
Japanese	Male ≥ 90 cm Female ≥ 80 cm
Ethnic South and Central Americans	Use South Asian recommendations until more specific data are available
Eastern Mediterranean and Middle East (Arab) populations	Use European data until more specific data are available

WHR risk categories*

Gender	Excellent	Good	Average	At risk
Males	< 0.85	0.85–0.89	0.90–0.95	> 0.95
Females	< 0.75	0.75–0.79	0.80–0.86	> 0.86

∗ World Health Organization cut-off points and risk of metabolic complications (WHO 2011).

Appendix 7

Median (IQR) values of blood metabolites HIV-positive (on HAART) persons versus HIV-positive HAART naive persons.

Variables	HIV+ HAART naive	HIV+ on HAART	P-value
FBG (mmol/l)			
median	4.6	4.9	P = 0.053
IQR	0.7	0.9	
TC (mmol/l)			
Median	3.9	4.3	P = 0.4429
IQR	1.22	0.97	
HDL (mmol/l)			
Median	0.99	1.29	P = 0.0620
IQR	0.52	0.4	
LDL (mmol/l)			
Median	2.32	2.46	P = 0.7419
IQR	1.37	0.9	
TG (mmol/l)			
Median	0.91	1	P = 0.3616
IQR	0.39	0.26	
CRP (mg/l)			
Median	2	3.2	P = 0.0585
IQR	6	7	
Insulin (mu/l)			
Median	6.9	6,8	P = 0.4821
IQR	6.1	8.7	
IR HOMA index			
Median	1.19	1.44	P = 0.4237
IQR	1.47	1.73	

Appendix 8

Median (IQR) values of blood metabolites for HIV-positive persons on first and second line treatments versus HIV-positive HAART naive persons.

Variables	HIV+ HAART naive	First line	Second line	P-value
FBG (mmol/l)				
median	4.6	4.85	4.9	P = 0.2209
IQR	0.7	0.95	0.7	
TC (mmol/l)				
Median	3.9	4.5	4.01	P = 0.2090
IQR	1.22	0.6	1.49	
HDL (mmol/l)				
Median	0.99	1.4	1.25	P = 0.1106
IQR	0.52	0.4	0.51	
LDL (mmol/l)				
Median	2.32	2.65	2.2	P = 0.2589
IQR	1.37	0.7	1.21	
TG (mmol/l)				
Median	0.91	0.9	1.01	P = 0.2428
IQR	0.39	0.4	0.44	
CRP (mg/l)				
Median	2	3	3.2	P = 0.1665
IQR	6	8.1	5	
Insulin (mu/l)				
Median	6.9	5.25	8.1	P = 0.1854
IQR	6.1	6.6	9.5	
IR HOMA index				
Median	1.19	1.12	1.69	P = 0.2046
IQR	1.47	1.38	1.96	