CARDIOVASCULAR RISK FACTORS IN AN HIV INFECTED RURAL POPULATION OF LIMPOPO PROVINCE, SOUTH AFRICA.

By

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THESIS

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DEDICATION

I dedicate this thesis to my late mother, *Winnie Samhere* who has always wanted to see me achieve great things in life. I also dedicate this thesis to my husband and kids for their unconditional love and support during my study period. To the almighty, let all the glory be unto you.

DECLARATION

I declare that the thesis**,** Cardiovascular risk factors in an HIV infected rural population of Limpopo Province, South Africa hereby submitted to the University of Limpopo, for the degree of Doctor of Philosophy in Medical Science is my own work and has not previously been submitted by me for a degree at this or any other institution. Furthermore, I declare that the sources that I have quoted have been indicated and acknowledged by means of complete references.

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Felistas Mashinya **(**Mrs**)** Date

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ABSTRACT

BACKGROUND AND AIM

Human immunodeficiency virus (HIV) and/or antiretroviral therapy (ART) through direct or indirect mechanisms may induce risk factors for developing cardiovascular disease (CVD) such as diabetes mellitus, dyslipidaemia, hypertension, lipodystrophy and endothelial dysfunction. Furthermore, some traditional risk factors such as low physical activity, low intake of fruit and vegetables, increased body mass index (BMI), smoking, genetic predisposition and age still have important roles in the development of metabolic risk factors for CVD in HIV infected people. Studies have suggested increased CVD risk factors in HIV infected when compared to uninfected people. The present study assessed the CVD risk factors, the estimated 5-year Data Collection on Adverse Effects of Anti-HIV Drugs (D.A.D) risk score and the 10-year Framingham risk score in persons with HIV infection in a rural area in South Africa.

STUDY DESIGN AND SETTING

The present study comprised of two phases. The first phase was a cross-sectional sub-study of the project on “Prevention, Control and Management of Chronic diseases in a rural population, South Africa”, conducted in the Dikgale Health and Demographic Surveillance System (HDSS) Centre between August 2011 and February 2012. The second phase was a cross-sectional study conducted in the Primary Health Care clinics, Seobi-Dikgale, Sebayeng and Dikgale that are situated within the Dikgale HDSS site. The Dikgale HDSS site is a rural area situated 20 to 40 km from University of Limpopo, in Capricorn District, Limpopo Province

STUDY POPULATION

Eight hundred and fifteen randomly selected people participated in the main project. Those who tested positive for HIV following pre-counselling and were not on ART (89 people) and their age and gender matched HIV negative (178 people) randomly selected from those who tested negative for HIV were included in Phase 1 of the present study. In

Phase 2 of the study, a non random sample of 214 people on ART participated. The study excluded pregnant women and non-residence in Dikgale HDSS site.

OUTCOME MEASURES

Data on demographic status, lifestyle, chronic disease, blood pressure and anthropometric measurements were collected using the World Health Organization stepwise approach to surveillance (STEPS) questionnaire. Blood pressure and anthropometric measurements were done using standard procedures.

Biochemical analysis were performed using ILab 300 Plus Chemistry (Instrumentation Laboratory Company, Italy) and IMMAGE Immunochemistry System (Beckman Coulter, United States of America). Lipoprotein subclasses were analysed using polyacrylamide gradient gel electrophoresis (PAGGE). HIV screening was performed using Elisa based kits, HIV ½ Ag/Ab Combo and DoubleCheckGold Ultra HIV1/2 kits, both supplied by Inverness Medical, Japan. The CD4 count was determined using the PIMA analyser (Inverness Medical, Tokyo, Japan). Viral load testing, using the branched deoxyribonucleic acid (DNA) technique (Siemens, South Africa) was outsourced to Toga Molecular Biology and Pathology medical laboratories, South Africa. Estimates of 5 and 10-year CVD risk were calculated using online tools.

RESULTS

ART naïve HIV infected people had a mean age of 49.7 ± 16.8 years. Their median viral load was 3536 (50-18860) copies/ml and CD4 count was 377 ± 192 cells/mm^3. A comparison of CVD risk factors between ART naïve HIV infected and HIV negative people showed a similar prevalence. However, the prevalence of low HDL-C was higher (62.4% vs 41.6%, p value 0.001) and hypercholesterolaemia was lower (17.4% vs 38.2%, p value 0.001) in ART naïve HIV infected than in uninfected people. The proportion of large LDL-particles was lower (79.9 ± 5.4% vs 81.3 ± 4.7%, p value 0.048) and the proportion of small LDL-particles was higher (20.1 ± 5.4% vs 18.7 ± 4.7%, p value 0.04) in ART naïve HIV infected than in uninfected people. The proportion of large and small HDL-particles were not different between ART naïve HIV infected and uninfected people.

The study showed that tobacco use was higher, while the prevalence of obesity and abdominal obesity was lower in males than in females, irrespective of HIV status. A higher proportion of ART naïve HIV infected than uninfected females used alcohol (25.4% vs 11.9%, p value 0.02).

Among ART naïve HIV infected people, an age more than 50 years increased the likelihood of being physically inactive (OR: 3.27, p value <0.05) compared to an age less than 50 years. In addition people more than 50 years of age were more likely to be hypertensive (OR: 4.05, p value <0.05) and diabetic (OR: 1.17, p value <0.05) than people less than 50 years of age. Males were more physically active than females and were more likely to use tobacco (OR: 6.78, p value <0.05) than females. The use of tobacco was more likely to be associated with diabetes mellitus (OR: 7.95, p value <0.05) than non-tobacco use. A viral load of more than 50 copies/ml was more likely to be associated with a low HDL-C concentration (OR: 7.37, p value <0.05), a high TC/HDL-C ratio (OR: 2.51, p value <0.05), a high ApoB/ApoA ratio (OR: 2.45, p value <0.05) and a high TG/HDL-C ratio (OR: 1.58, p value 0.05) than a viral load of less than 50 copies/ml. The presence of abdominal obesity was more likely to be associated with hypertension (OR: 10.95, p value<0.05), a high TC level (OR: 7.67, p value <0.05) and a high TG level (OR: 3.44, p value <0.05) compared to an absence of abdominal obesity. People with a CD4 count of below 300 cells/mm^3 were less likely to be hypertensive (OR: 0.16, p value <0.05) and hypercholesterolaemic (OR: 0.04, p value 0.05).The CD4 count was positively associated with large HDL-particles and negatively associated with small HDL-particles. Abdominal obesity was positively associated with large HDL-particles and negatively associated with small HDL-particles. A positive association was observed between abdominal obesity and large LDL-particles, while a negative association was observed with small LDL-particles.

People on ART had a mean age of 44.8 ± 11.8 years, with a median viral load of <50 copies/ml and a mean CD4 count of 461.9 ± 235.3 cells/mm^3. The majority of people were on the 1st line non-nucleoside reverse transcriptase inhibitor (NNRTI)-based ART. The prevalence of tobacco use (p value 0.001), alcohol use (p value 0.001), hypertriglyceridaemia (p value 0.001), a high TC/HDL-C ratio (p value 0.01), a high TG/HDL-C ratio (p value 0.002) and physical activity (p value 0.002) was higher in males

than in females. The proportions of small and large LDL and HDL particles were similar in males and females. Males on ART were more likely to be physically active than females. In addition, males were more likely to use tobacco (OR: 2.45, p value <0.05), alcohol (OR: 3.31, p value <0.05) and to have a high TC/HDL-C ratio (OR: 2.94, p value <0.05) than females. Married people were more likely to use tobacco (OR: 3.13, p value <0.05) than unmarried people. However, married people with secondary education and above were less likely to use tobacco (OR: 0.17, p value=0.05) than married people with primary education. People older than 50 years of age were more likely to have hypertension (OR: 4.67, p value <0.05), diabetes mellitus (OR: 5.66, p value <0.05), metabolic syndrome (OR: 1.10, p value <0.05), a low HDL-C concentration (OR: 2.27, p value <0.05), a high TG concentration (OR: 2.91, p value <0.05) and a high TC/HDL-C ratio (OR: 3.29, p value <0.05) than people less or equal to 50 years of age. People with a viral load of more than 50 copies/ml were more likely to have a low HDL-C concentration (OR: 3.82, p value <0.05) and a high ApoB/ApoA ratio (OR: 3.83, p value <0.05) but were less likely to have hypertension (OR: 0.08, p value <0.05) than people with a viral load of less than 50 copies/ml. People who had been on ART for less than 60 months were less likely to have a high TC/HDL-C ratio (OR: 0.29, p value <0.05) than those on ART for more than 60 months. Individuals with abdominal obesity were more likely to have a high TC level (OR: 2.10, p value <0.05) and a high TG/HDL-C ratio (OR: 2.98, p value <0.05) than those without abdominal obesity. A low intake of fruit and vegetables was associated with high levels of triglycerides. The CD4 count was positively associated with large HDL-particles (beta 0.18, p value 0.02) and negatively with small HDL-particles (beta -0.19, p value 0.01). Abdominal obesity was negatively associated with large HDL-particles (beta -0.16, p value 0.03) and positively with small HDL-particles (beta 0.20, p value 0.01). Furthermore, a high TG/HDL-C ratio was negatively associated with large HDL-particles (beta -0.20, p value 0.01) and positively with small HDL-particles (beta 0.17, p value 0.02). ApoB/ApoA ratio was positively associated with large LDL-particles (beta 0.19, p value 0.01) and negatively with small LDL-particles (beta -0.27, p value 0.0001). In addition, metabolic syndrome was positively associated with small LDL-particles (beta 0.14, p value 0.04).

Overall, the prevalence of CVD risk factors was higher in ART naïve HIV infected people than in people on ART. The findings showed that the prevalence of low HDL-C and physical inactivity was lower in those on ART than in ART naïve HIV infected people. In addition, the prevalence of obesity, hypertension, diabetes mellitus and metabolic syndrome was twice as high in ART naïve HIV infected people as in people on ART. The proportions of the atheroprotective large LDL and HDL particles were higher in those on ART than in ART naïve HIV infected people. According to Framingham risk estimation, 5.6% of ART naïve HIV infected people had a high risk and 9.2% had a moderate risk of having a CVD event in the next 10 years, while none of the people on ART had a high risk and 6.7% had a moderate risk of having a CVD event in the next 10 years. However, the DAD risk equation showed that 31.1% of people on ART had a moderate to high 5-year risk of developing a CVD event. The level of agreement between the DAD and the Framingham risk equations was 73.8% (k=0.23; 95% CI: 0.10-0.35; p value-0.001).

CONCLUSION

The prevalence of CVD risk factors was similar in ART naive HIV infected and HIV negative people. Except for the prevalence of hypercholesterolaemia, the prevalence of most CVD risk factors was lower in people on ART than in those naïve to ART. While 5.6% of ART naïve HIV infected people had a high risk of developing CVD in the next 10-years, none of the people on ART had such a high 10- year CVD risk, according to the Framingham equation. However, the high proportion of people on ART with a moderate to high 5-year CVD risk, observed with the DAD risk equation, represents a considerable health burden that can be reduced by educational programs on CVD prevention for people on ART. There is however a need to develop and evaluate a race/ethnicity-specific CVD risk estimation tool for HIV infected Africans.

PUBLICATIONS

The following papers were written from this thesis:

Review paper

Published

1. **Mashinya, F**., Alberts, M., Colebunders, R. & Van Geertryden, J.P. (2014). Lipoprotein and its subclasses in HIV infected individuals: A Review of the Literature. *African Journal for Physical, Health Education, Recreation and Dance* 20 (3-1): 886-913.

Original papers

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2. **Mashinya F**, Alberts M, Colebunders R, Van Geertruyden JP. Cardiovascular risk factors in a treatment naïve Human immunodeficiency virus infected rural population in Dikgale, South Africa. *South African Family Practice*. 2014; 56(3): 190-195.

3. **Mashinya F**., Alberts, M., Van Geertruyden, JP., Colebunders, R. & Choma SSR. (2015). Tobacco use among ARV treated HIV infected rural South Africans: prevalence and its determinants. *African Journal for Physical, Health Education, Recreation and Dance* 21(3:1): 768-776.

4. **Mashinya F**, Alberts M, Colebunders R, Van Geertruyden JP. Assessment of cardiovascular risk factors in people with HIV infection treated with ART in rural South Africa. *AIDS Research and Therapy*. 2015, 12:42

PRESENTATIONS AT CONFERENCES

1. Presented on “Risk factors for cardiovascular disease in a treatment naïve HIV infected rural population in Dikgale, South Africa” at the Annual Rural Health Conference Hosted by RUDASA, RUReSA and PACASA at St Lucia, KZN on the 8-10th August 2013.

2. Presented on “Risk factors for cardiovascular disease in a treatment naïve HIV infected rural population in Dikgale, South Africa” at the Annual University of Limpopo Research Day at MEDUNSA, on 20-21th August 2013.

3. Presented on “Assessment of CVD risk factors in an ARV treated HIV infected rural population, Dikgale, South Africa” at the first Annual University of Limpopo, Turfloop Campus, School of Health Science Research Day, 0n 16-17 September 2014.

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LIST OF ABBREVIATIONS

Ab	Antibody
ABC	Abacavir
ABC-A1	Adinosine triphosphate binding cassette transporter A1.
ADP	Adinosine diphosphate
Ag	Antigen
AIDS	Acquired immunodeficiecincy syndrome
Apo A	Apolipoprotein A
Apo B	Apolipoprotein B
ART	Antiretroviral therapy
ARV	Antiretroviral
ATP	Adinosine triphosphate
ATP III	Adult Treatment Panel III
ATV	Atazanavir
AZT	Zidovudine
BMI	Body mass index
BMPR2	Bone morphogenetic protein receptor type II
BP	Blood pressure
CAD	Coronary artery disease
CARDIA	Coronary Artery Risk Development in Young Adults study
CDC	Centre for Disease Control
CDIA	Chronic Disease Initiative for Africa
CETP	Cholesterol ester transfer protein
CHD	Coronary heart disease
CREATE-1	Cardiovascular Risk Evaluation and Antiretroviral therapy-1 study
CRF	Circulating recombinant forms
CV	Coefficient of variations
CVD	Cardiovascular disease
DAD	Data Collection on Adverse Effects of Anti-HIV Drugs
D4T	Stavudine

DBP	Diastolic blood pressure
ddI	Didanosine
DHDSS	Dikgale Health Demographic Surveillance Site centre
DLV	Delavirdine
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
DNTPs	Deoxynucleotide triphosphates
DRV	Duranavir
DTG	Dolutegravir
EDTA	Ethylene diamine tetraacetic acid
EFV	Efavirenz
ELISA	Enzyme linked immunosobent assay
eNOS	Endothelial nitric oxide synthetase
ER	Endoplasmic reticulum
ETR	Etravirine
FDA	Food and drug association
FFA	Free fatty acid
FH	Familial hypercholesterolaemia
FI	Fusion inhibitor
FPV	Fosamprenavir
FTC	Emtricitabine
GCKR	Glucokinase regulator
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon like peptide
gp	Glycoprotein
HAART	Highly active antiretroviral therapy
HC	Hip circumference
HCV	Hepatitis C virus
HDL-C	High density lipoprotein cholesterol.
HDL-p	High density lipoprotein-particle
HEART	Hyperlipidaemia, Education and Atherosclerosis Research Trust

HIV	Human immunodeficiency virus
HPAEC	Human pulmonary artery endothelial cells
HR	Hepta repeat, region of glycoprotein 41
hsCRP	High sensitivity C-reactive protein
HTN	Hypertension
ICAM-1	Intercellular adhesion molecule-1
IDF	International Diabetes Federation
IDV	Indinavir
IGF-1	Insulin like growth factor
IKK	IkB kinase
IL	Interleukin
INF	Interferon
INI	Integrase inhibitors
IR	Insulin resistance
IRS-1	Insulin receptor substrate 1
IRS-2	Insulin receptor substrate 2
ISO	International Standards Association
JNK	Jun kinase
KZN	KwaZulu Natal
LDL-C	Low density lipoprotein cholesterol
LDL-p	Low density lipoprotein particle
Lp (a)	Lipoprotein (a)
LPV	Lopinavir
LRP1	Low density lipoprotein-receptor related protein type 1
MAPK	Mitogen-activated protein kinase
MEDUNSA	Medical University of Southern Africa
MetS	Metabolic syndrome
MI	Myocardial infarction
MRC	Medical Research Council
MREC	Medical University of Southern Africa Reaserch Ethics Committee
MRFIT	Multiple Risk Factor Intervention Trial

mRNA	Messenger ribonucleic acid
MSM	Men who have sex with men
mtDNA	Mitochondrial deoxyribonucleic acid
MVC	Maraviroc
NCEP-ATPIII	National Cholesterol Education Programe: Adult Treatment Panel III III
NF-kB	Nuclear factor -kB
NFV	Nelfinavir
NNIBP	Non-nucleside inhibitor binding pocket
NNRTI	Non-nucleoside reverse transcrptase inhibitor
NO	Nitric oxide
NRTI	Nucleoside reverse transcriptase inhibitor
NtRTI	Nucleotide reverse transcriptase inhibitor
NVP	Nevirapine
PAGGE	Polyacrylamide gradient gel electrophoresis
PAH	Pulmonary artery hypertension
PI	Protease inhibitor
PKC	Protein kinase C
PLWHIV	People living with human immunodeficiency virus
PPARγ	Peroxisome proliferator activated receptor-γ
RAL	Raltegravir
RBP4	Retinol-binding protein 4
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RPV	Rilpivirine
RT	Reverse transcriptase
RTV	Ritonavir
SANAS	South African National Accreditation System
SAT	Subcutaneous adipose tissue
SBP	Systolic blood pressure
SCORE	Systematic COronary Risk Evaluation
SD	Standard deviation

SNS	Sympathetic nervous system
SOC-3	Suppressor of cytokine signaling -3.
SQV	Saquinavir
STEPS	Stepwise approach to surveillance
T-20	Enfuvirtide
3TC	Lamivudine
TC	Total cholesterol
TDF	Tenofovir
TG	Triglycerides
TNF	Tumor necrosis factor
TPV	Tipranavir
UK	United Kingdom
UNAIDS	United Nations Programme on HIV/AIDS
URF	Unique recombinant forms
USA	United States of America
VAT	Visceral adipose tissue
VLDL	Very low density lipoprotein
VLIR-OUS	Vlaamse Interuniversitaire Raad-University Development Cooperation
WC	Waist circumference
WHO	World Health Organisation
WHR	Waist hip ratio

CHAPTER 1

ORIENTATION TO THE STUDY

1.1 INTRODUCTION

Cardiovascular disease (CVD) is currently considered the second most common cause of mortality after cancer in HIV infected people (Giannarelli et al., 2011). With the number of people living with HIV infection risen to 35.3 million worldwide and 25 million in sub-Saharan Africa (UNAIDS 2013:1), the burden of CVD and its mortality may continue to rise in the future.

CVD risk factors which include dyslipidaemia, diabetes mellitus, hypertension, obesity, abdominal obesity, smoking and metabolic syndrome (Fedele et al., 2011) are common in HIV infected people (Malvestutto and Aberg 2010, Gandhi et al., 2012). In untreated HIV infected people, as reported from developed countries, dyslipidaemia is characterised by decreased concentration of total cholesterol, low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) (Feingold et al., 1993, Rose et al., 2008, Baker et al., 2010). This is similar to the findings observed in sub-Saharan Africa (Dillon et al., 2013, Daniyam and Iroezindu 2013, Ngatchou et al., 2013). Similarly, Fourie et al. (2010) observed reduced serum lipid concentrations, except triglyceride concentration among ART naïve HIV infected compared to uninfected South Africans. As HIV infection progresses to AIDS, the plasma concentrations of TC, HDL-C and LDL-C continue to decrease while triglyceride (TG) concentrations increase (Feingold et al., 1993). In addition, the lipoprotein subclasses that are regarded as accurate predictors of CVD risk, undergo changes resulting in a predominance of small dense LDL-particles (Feingold et al., 1993), giving rise to the atherogenic lipoprotein phenotype B associated with increased risk of CVD.

While highly active antiretroviral treatment (HAART) has rendered HIV infection a manageable disease, prolonging the lives of HIV infected people, treatment has been

associated with metabolic disorders including hyperlipidaemia, diabetes, lipodystrophy, and metabolic syndrome (Palios et al., 2012). The severity of these metabolic disorders differ among antiretroviral (ARV) classes with protease inhibitors (PIs) associated with more metabolic alterations than non-PIs and HIV itself (Grispoon et al., 2008, Blanco et al., 2010). Studies from developed countries (Badiou et al., 2003, Riddler et al., 2008, Tien et al., 2010) and Africa (Yone et al., 2011) reported dyslipidaemia characterised by higher concentrations of TC, LDL-C, TG and HDL-C in people on ART compared to ART naïve HIV infected people. In addition, ART was associated with an increase in LDL-particles, particularly small dense LDL-p and an increase in HDL-particles, though not to pre-infection levels (Stein et al., 2008).

Other CVD risk factors (smoking, metabolic syndrome, C-reactive protein, diabetes mellitus), reported from developed countries were more prevalent among ART naïve HIV infected compared to uninfected people, except for hypertension and abdominal obesity (Bonfanti 2007, Hilleman et al., 2014). A similar CVD risk profile was observed among ART naïve HIV infected and uninfected Africans (Ngatchou et al., 2013), except for smoking where the prevalence was similar between the two groups (Fourie et al., 2010, Daniyam and Iroezindu 2013). On the other hand, people from developed, African and Asian countries on ART had higher CVD risk factors compared to those naïve to treatment (Bonfanti 2007, Edward et al., 2013, Carey et al., 2013, Kagaruki et al., 2014).

Studies on CVD risk factors among HIV infected people are however scarce in South Africa. A study conducted in rural KwaZulu-Natal by Malaza et al. (2012), comparing hypertension and obesity among people living with HIV (combined treated and untreated) and HIV negative people found hypertension and obesity more prevalent among HIV negative than HIV positive people. A study from North-West Province by Fourie et al (2010), reported higher levels of C-reactive protein, but similar prevalence of smoking and metabolic syndrome among ART naïve HIV infected compared to uninfected people. A recent attempt to describe the CVD risk in rural people on ART from Mpumalanga was made (Clark et al., 2015), but the use of ART was self-reported and according to the

authors, the prevalence of CVD risk factors in that rural population on ART may be underestimated since there remains considerable stigma associated with HIV.
In South Africa, the intersection of an epidemiological transition (Houle et al., 2014), a high number of people living with HIV (van Rooyen et al., 2014) and a widespread adult treatment coverage that was close to 80% by mid-2011 (Johnson et al., 2012), presents a high risk of CVD among people infected with HIV and needs to be investigated. While CVDs are preventable, little is known regarding CVD risk factors in HIV infected rural South Africans on ART (Clark et al., 2015).

1.2 RESEARCH PROBLEM

Cardiovascular disease is currently the second most common cause of death after cancer in HIV infected people. The increased rates of CVD in HIV infected people both naive to ART and those on ART pose a worldwide health problem. In South Africa, the coincidence of epidemiological transition, an ever increasing number of HIV infected people and a wide coverage of ART could worsen the CVD risk among HIV infected people. Furthermore, as a result of continued rollout of ART, people infected with HIV are living longer and could experience age related CVD risk. Currently there is paucity of data on the CVD risk factor profile among HIV infected rural South Africans on ART. The results of this study would help to understand and quantify the challenge that is faced by the health care system. Furthermore the findings would guide in making informed interventions targeting the most prevalent risk factors in HIV infected people.

1.3 PURPOSE OF STUDY

1.3.1 Research questions

What is the CVD risk factor profile in HIV infected rural black South Africans naïve to ART and those on ART?
What is the estimated future risk of CVD among HIV infected rural black South Africans naïve to ART and those on ART?

1.3.2 Hypothesis

The prevalence of CVD risk factors is increased in ART naïve HIV infected compared to uninfected people, but lower when compared to people on ART.

1.3.3 Aim

The main aim of the study was to determine the prevalence of CVD risk factors in HIV infected individuals naïve to ART and those on ART and estimate the future CVD risk.

1.3.4 Objectives

To accomplish the aim, the research objectives were:

- To collect data on fruit and vegetable intake and physical activity.
- To measure blood pressure, weight and height (for calculation of BMI), waist and hip circumference (for calculation of waist to hip ratio).
- To test for HIV and determine CD4 counts and viral load in participants.
- To measure the concentration of serum lipids (triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol), apolipoprotein B-100 (apo B-100), apolipoprotein A-1 (apo A-1), Lipoprotein (a) (Lp a), high sensitivity C-reactive protein and glucose in serum samples.
- To determine the lipid and lipoprotein subclass patterns in ART naive HIV infected people and those on ART.
- To determine prevalence of risk factors for cardiovascular disease in ART naïve HIV infected people and those on ART.
- To calculate the future risk of CVD in HIV infected both naïve to treatment and those on treatment.

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CHAPTER 2

LITERATURE REVIEW

2.1 HUMAN IMMUNODEFICIENCY VIRUS INFECTION

2.1.1 Human Immunodeficiency Virus structure and Replication Cycle

Human immunodeficiency virus (HIV) is a Lentivirus belonging to the family Retroviridae. Structurally, HIV comprises of an inner matrix consisting of protein 17 (p17) and an outer sphere-shaped lipid bilayer envelope consisting of viral glycoprotein (gp) 120 that mediates attachment of virus to CD4+ receptors and co-receptors and gp 41 that anchors the viral envelop (Hedestam et al., 2008). The cone shaped capsid protein 24 (p24) encloses the two positive sense, single-stranded ribonucleic acid (RNA), proteins and enzymes responsible for viral replication and maturation (Hedestam et al., 2008).

Human immunodeficiency virus utilizes nine genes comprising three structural genes (gag, pol and env), two regulatory genes and four accessory genes. The pol gene is responsible for the production of the three enzymes, (reverse transcriptase, protease and integrase) while the env and gag genes are responsible for production of structural glycoproteins and core proteins respectively. The regulatory genes contain important information for the production of proteins that control viral replication and accessory genes encode for proteins that interact and inactivate the antiviral effect of host defence cells (Romani et al., 2009).

HIV uses the host cell machinery to produce multiple copies of new HIV. The HIV life cycle is initiated by the attachment of the viral surface glycoprotein gp 120 to the CD4+ cell receptors and subsequent interactions with the core receptors CCR5 or CXCR4, resulting in the fusion of viral membrane and host cell membrane. The viral RNA is transcribed into DNA by its reverse transcription enzyme. The viral DNA enters the host cell nucleus and integrates into host DNA aided by viral protein integrase and host DNA

repair enzymes (Simon et al., 2006). Spliced mRNA is transported into the cytoplasm and translated into enzymes, regulatory and structural viral proteins while unspliced mRNA is exported into cytoplasm forming the HIV-1 RNA. The viral proteins and HIV RNA assemble near the cell membrane and form new viruses by budding. The new viruses undergo maturation process involving cutting of long HIV proteins into small functional units by HIV protease. The proteins are reassembled into a mature virus (Abbas and Herbein 2012, Jayappa et al., 2012, NIAID) (Fig 2.1).

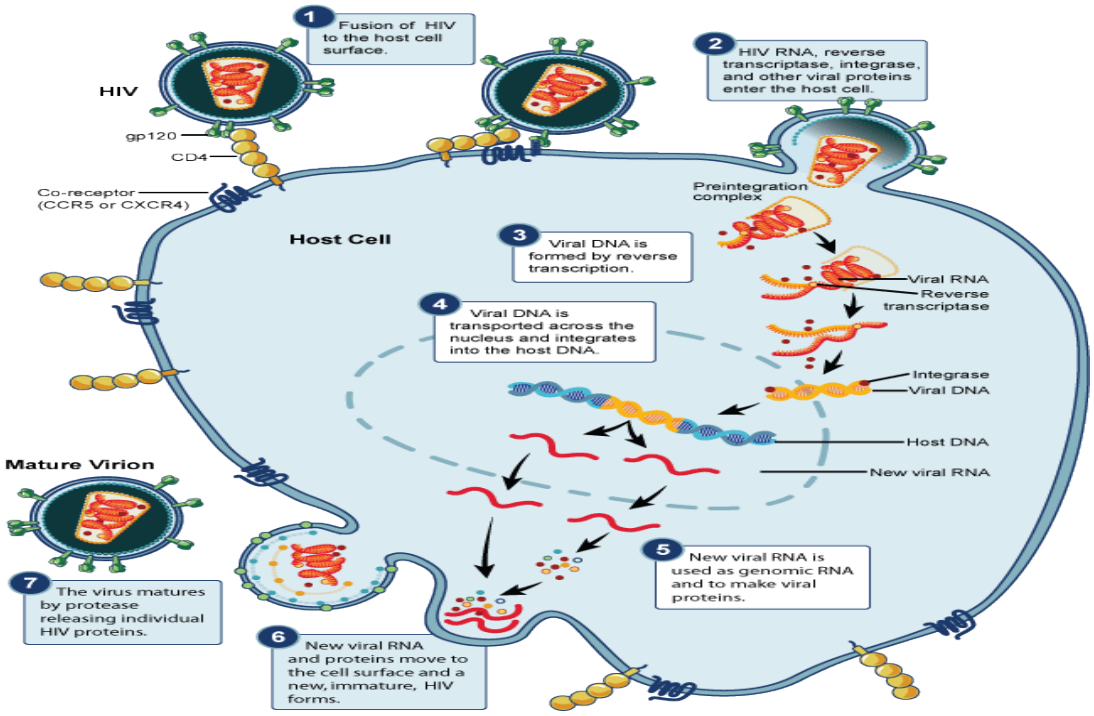

Figure 2. 1: HIV Replication cycle
http://www.niaid.nih.gov/topics/HIVAIDS/Understanding/Biology/pages/hivreplicationcycle.aspx

2.1.2 Pathogenesis of HIV infection

HIV infection and disease progression involve changes in cytokine production and in T-cells which can lead to increase in virus production and hinder immune response (Reuter et al., 2012). The full course of untreated HIV infection involves three stages: acute, chronic and late stage HIV infection (AIDS) (Fig 2.2).

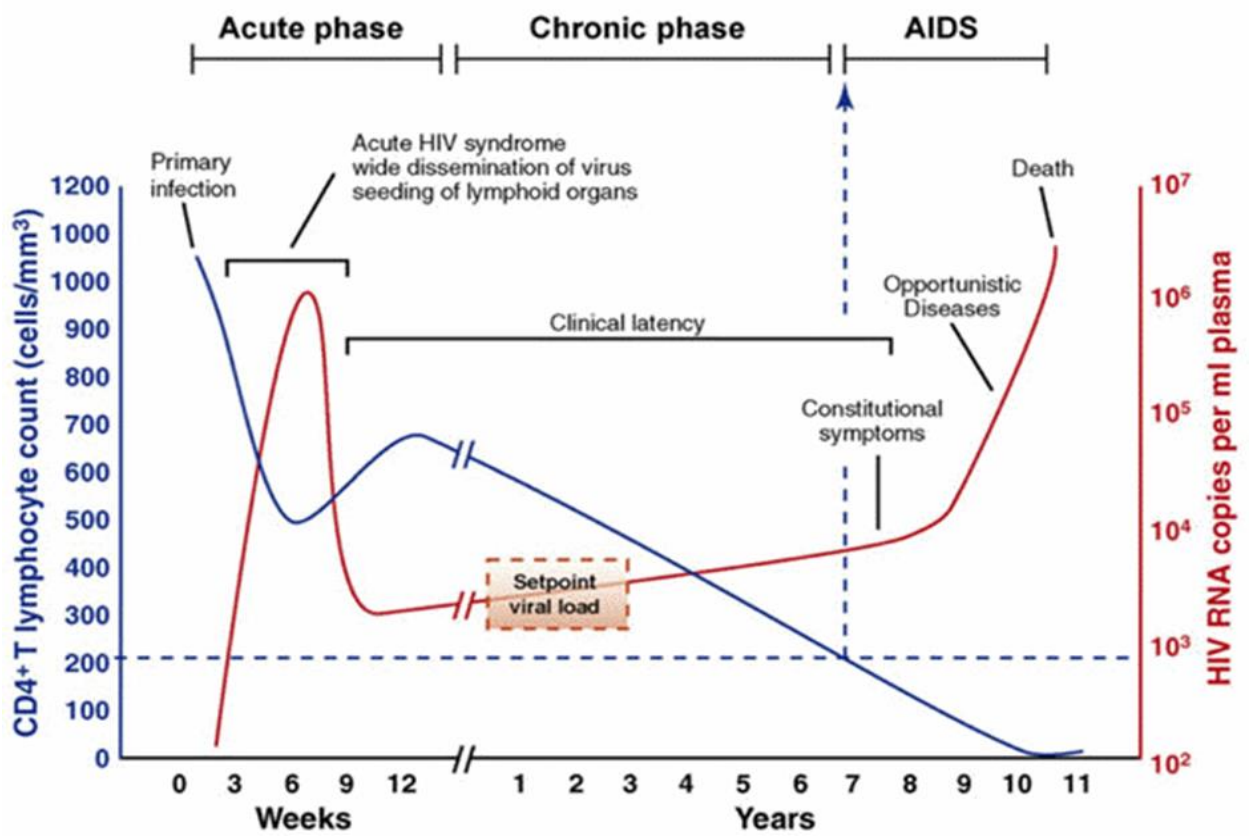

Figure 2. 2: Time course of typical HIV infection.
CD 4⁺ T cell decline and viraemia patterns differ among individuals. (Adapted from Fauci et al., 1996).

The acute HIV infection stage is characterized by production of pro-inflammatory cytokines (IL-1, IL-2, IL-6, TNF-α and INF-γ) and anti-inflammatory cytokines (IL-4, IL-10 and IL-13) (Llano and Este 2005, Roberts et al., 2010). The increased pro-inflammatory cytokines activate CD4⁺ T-lymphocytes and make them more susceptible to HIV infection with subsequent increases in viral load and increased viral set point, while the anti-inflammatory cytokines may reduce the target cells resulting in decreased viral set point (Katsikis et al., 2011). During the acute HIV infection phase, rapid virus replication in the activated lymphocytes in regional lymph nodes is followed by a burst of virus infected cells into the blood stream (Weber 2001). The virus spreads throughout the body and is deposited in lymphoid organs (Kahn and Walker 1998). High plasma levels of replicating virus and high infectivity characterize this period. The viraemic peak that is attained during this phase is a result of the absence of early immune response and the presence of large numbers of activated CD4⁺ T-cells used for viral replication resulting in transient decrease

of $CD4^+$ T-cells (Coffin and Swanstrom 2013). Seroconversion symptoms consisting of fever, rash, headache, pharyngitis, gastrointestinal distress and enlarged lymph nodes may appear or the phase may be clinically asymptomatic (Weber 2001, Coffin and Swanstrom 2013). At the end of the acute HIV infection phase, the viraemic peak declines to a set 'steady state' due to virus-specific immune responses that control viral replication and exhaustion of activated target cells (Weber 2001, Coffin and Swanstrom 2013). The proliferation of cytotoxic T-cells ($CD8^+$ T-lymphocytes) mediated by IL-2 and INF-γ appear as the first effective anti-viral response (Kahn and Walker 1998, Llano and Este 2005).

In the chronic HIV infection stage, $CD4^+$ T-lymphocyte numbers usually rise to near normal levels but remain lower than pre-infection levels. Thereafter the $CD4^+$ T-lymphocytes steadily decrease over time in a linear manner (Weber 2001). As the disease progresses, there is a reduction of T-helper 1 cell activity which decreased production of IL-2 and INF-γ resulting in reduced cellular immunity. Simultaneously, there is an increase of T-helper 2 cell activity which increased production of IL-4, IL-6, IL-10 and IL-13 resulting in reduced cellular response accompanied by increased B cell activity. This shift in T-helper cells' activity creates a change in cytokine balance that would favor the development of AIDS (Clerici and Shearer 1993). During the chronic phase people are asymptomatic and sometimes unaware of the infection. However viral replication persists with large numbers of $CD4^+$T cells infected and dying every day (Coffin and Swanstrom 2013).

In the late stage of HIV infection (AIDS), the rate of $CD4^+$ T lymphocytes decline is increased coupled with reduction in $CD8^+$ T lymphocyte response, while the virus in blood and lymph nodes increase to high levels. The loss in cell-mediated immunity results in infections with a variety of opportunistic microbes (Coffin and Swanstrom 2013).

2.1.3 Epidemiology of HIV-1 infection

2.1.3.1 HIV-1 subtypes

HIV exists in two types namely HIV type 1 (HIV-1) and HIV type 2 (HIV-2). HIV-2 is less virulent, less transmissible and geographically limited to western Africa (Sharp and Hahn 2011). HIV-1 shows a high degree of genetic variation resulting from its rapid replication cycle, high error rate of reverse transcriptase, and the recombination of two RNA genomes from two separate subtypes within a dually infected person (Buonaguro et al., 2007, Kiwanuka et al., 2008). HIV-1 strains are classified into three groups namely group M (major), group O (outlier) and group N (non-M/ non-O) (Buonaguro et al., 2007, Taylor et al., 2008). Recently a new group was identified in Cameroon and was designated as group P (Plantier et al., 2009, Vallari et al., 2011). HIV-1 group M is the most widely circulating group and consists of subtypes (A, B, C, D, F, G, H, J, K) and sub-subtypes (A1, A2, A3, A4, A5, F1, F2) (Taylor et al., 2008, Lihana et al., 2012). The sequencing of the full HIV genome led to identification of circulating recombinant forms (CRFs) (recombinant progeny isolated in three or more people with no direct epidemiological linkage) and unique recombinant forms (URFs) (recombinant progeny identified in three or more people with direct epidemiological linkage (Taylor et al., 2008, Lihana et al., 2012). The distribution of HIV-1 subtypes and recombinant forms vary in different regions and is shown in Fig. 2.3.

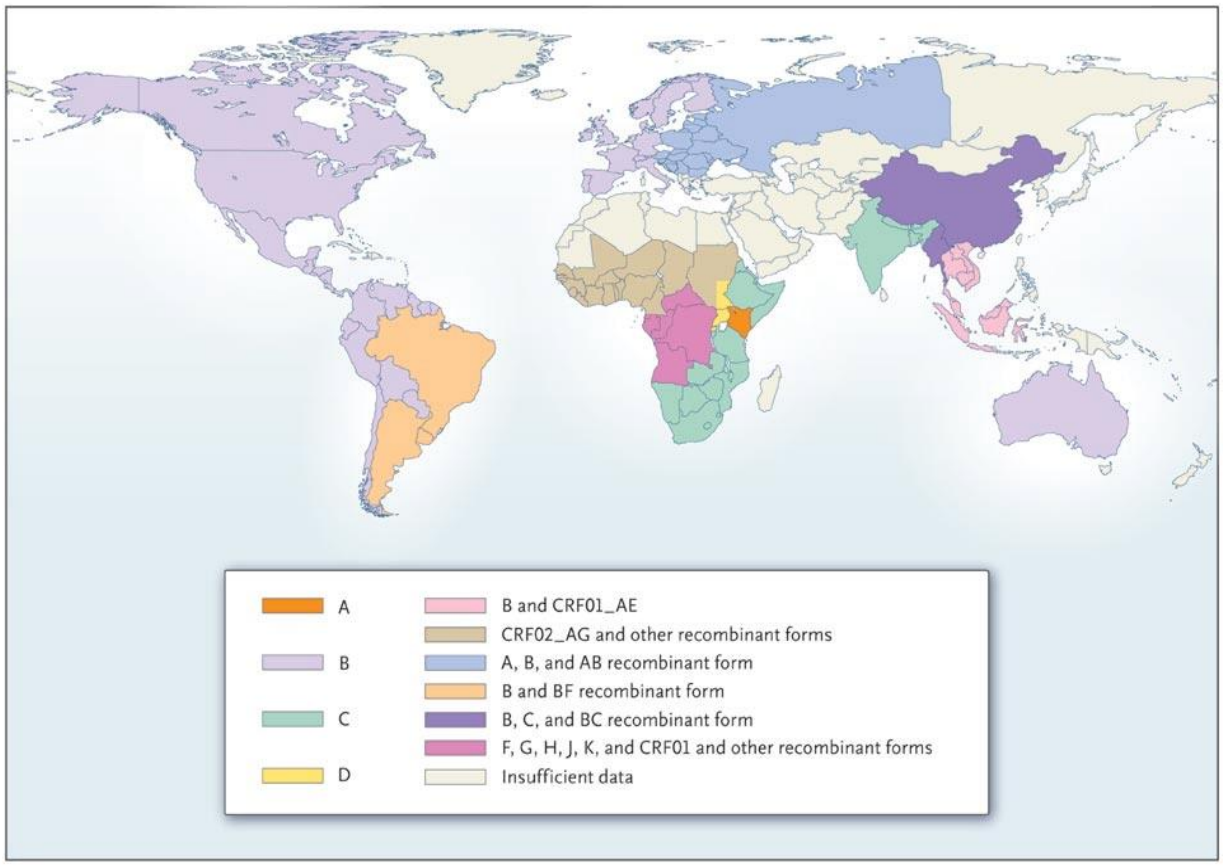

Figure 2. 3: Global distribution of HIV-1 Subtypes and Recombinant forms.
(Adapted from Taylor et al., 2008).

A recent investigation into subtype distribution of HIV-1 in Europe and Israel showed subtype B (66.1%) as the predominant subtype, followed by sub-subtype A1 (6.9%), subtype C (6.8%) and CRF 02_AG (4.7%) (Abecasis et al., 2013). Immigrant participants in this study had subtypes known epidemiologically to be prevalent in their countries of origin. CRF 01_ AE was prevalent in immigrants from South and South-East Asia, while immigrants from sub-Saharan Africa had all subtypes with subtype C dominant and immigrants from north Africa and middle east had subtypes B (58.3%) and C (16,7%) (Abecasis et al., 2013).

The distribution of HIV subtypes in three geographic regions of Africa (Eastern and southern, western and central, and northern) over a decade (2000-2011), was shown to be broadly stable, although unique, circulating recombinant subtypes have emerged over

the years (Lihana et al., 2012). Subtypes A and D have been stable in east Africa, subtypes A,G,CRF 02_AG and CRF 06_cpx in western Africa, subtypes B and CRF 02_AG in northern Africa subtype while subtype C in predominant southern Africa (Lihana et al., 2012). In South Africa HIV subtype C accounts for most of HIV infections (Rao et al., 2013). The continued monitoring of these subtypes will enable countries to deal with complex forms of subtypes or recombinants that may evolve or form over time.

The HIV-1 subtypes were shown to exert varying impact on the disease progression in the absence of ARV therapy, with subtype D being the most aggressive with a higher rate of CD4 count decline when compared with subtypes A, B, C, and CRF02_AG whose CD4 count declines were similar (Easterbrook et al., 2010). In two separate studies, HIV-1 subtype D was associated with faster disease progression when compared to subtype A (Baeten et al., 2007, Kiwanuka et al., 2008).

2.1.3.2 HIV-1 modes of transmission

Vertical transmission is the primary means by which infants become infected with HIV-1 either in the womb, during delivery or by breast feeding. Mother to child transmission rates of HIV-1 in African countries vary from 25-42% (Chakraborty et al., 2008). HIV-1 infection is predominantly spread through unprotected heterosexual behaviours in adults, accounting for over 90% of new HIV infections in some sub-Saharan African countries (Wamai et al., 2011). Some of the HIV infections occurring in eastern and southern Africa have been attributed to unsafe traditional practices which include circumcision procedures performed outside the formal healthcare setting by traditional practitioners using unsterilized instruments (Brewer et al., 2007). In central and western Europe the predominant mode of HIV transmission is sex between men followed by heterosexual transmission, while in eastern Europe HIV is mostly transmitted through heterosexual followed by injecting drug use (ECDC, 2011). In sub-Saharan Africa, little is known regarding HIV prevalence in people who inject drugs (Asher et al., 2013) probably due to laws that prohibit this behaviour in most sub-Saharan countries. However, socio-economic hardships, and that many countries in the region are being increasingly used

for transit of illicit drugs into Europe, warrants monitoring of injecting drug use as an additional potential route of HIV transmission in sub-Saharan Africa (Mathers et al., 2008). The contribution made by men who have sex with men (MSM) towards HIV prevalence in sub-Saharan Africa is not known, as this practice is criminalized in most countries in sub-Saharan Africa. However, the prevalence deduced from studies conducted in Malawi, Namibia and Botswana was approximately 17.4% (Baral et al., 2008) while a study conducted in South Africa, based on self-reported HIV status among MSM reported a prevalence of 14.1% (Sandfort et al., 2008).

2.1.3.3 HIV-1 infection trends: Global, sub-Saharan Africa and South Africa

While HIV-1 infection remains amongst the leading public health challenges worldwide, some milestones have been achieved with regards to roll-out of antiretroviral drugs (ARV) to reach most infected people. According to UNAIDS report of 2013, there has been worldwide and regional increase in the number of people living with HIV-infection owing to widespread availability of ARV drugs, while the numbers of new infections and death from HIV infection have decreased since 2007 (Table 2.1) (UNAIDS 2013:1).

Table 2. 1: Global and sub-Saharan Africa figures on HIV infection.

	Global figures (millions)		sub-Saharan Africa figures (millions)	
	2007	2012	2007	2012
Total number of people living with HIV	33.2 (30.6-36.1)	35.3 (32.2-38.8)	22.5 (20.9-24.3)	25.0 (23.5-26.6)
Total number of people newly infected with HIV	2.5 (1.8- 4.1)	2.3 (1.9 - 2.7)	1.7 (1.4 - 2.4)	1.6 (1.4-1.8)
Total number of deaths due to AIDS	2.1 (1.9 - 2.4)	1.6 (1.4 - 1.9)	1.6 (1.5 – 2.0)	1.2 (1.1-1.3)

Within sub-Saharan Africa, South Africa habours the highest number of people with HIV infection (approximately 5.26 million) (Statistics South Africa 2013). HIV infection trend in South Africa (Table 2.2), shows an increase in number of people living with HIV infection, probably due to availability of ARV treatment. The incidence among the 15-49 year old has decreased over the years probably reflecting the uptake of preventative strategies and campaigns launched on prevention of HIV infection (Statistics South Africa 2013).

Table 2. 2: HIV prevalence estimates and the number of people living with HIV in South Africa from 2002-2013.

Year	Prevalence (%)				Incidence Adult 15-49	HIV population (millions)
	Women 15-49	Adult 15-49	Youth 15-24	Total population		
2002	15.9	15.1	13.6	8.7	1.26	4.00
2003	16.0	15.1	12.8	8.9	1.25	4.10
2004	16.1	15.1	12.0	8.9	1.28	4.18
2005	16.2	15.1	11.4	9.0	1.32	4.25
2006	16.4	15.2	10.9	9.1	1.29	4.34
2007	16.5	15.3	10.5	9.2	1.21	4.46
2008	16.7	15.4	10.1	9.3	1.12	4.59
2009	16.9	15.5	9.7	9.5	1.03	4.74
2010	17.1	15.6	9.3	9.6	0.98	4.88
2011	17.2	15.7	9.0	9.8	0.95	5.01
2012	17.3	15.8	8.7	9.9	0.87	5.13
2013	17.4	15.9	8.5	10.0	0.85	5.26

Adopted from Statistics South Africa 2013.

2.1.3.4 Treatment options

Since 1987, over 20 drugs have been approved by the Food and Drug Association (FDA) for the clinical use against HIV infection (FDA 2013). Effective antiretroviral therapy (ART) can achieve persistent viral suppression and good immunological recovery (Thaker and Snow, 2003). There are six classes of antiretroviral drugs that are currently being used in

the treatment of infections caused by HIV. These include nucleoside /nucleotide analogue reverse transcriptase inhibitors (NRTIs/NtRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase inhibitors (INIs), fusion inhibitors (FIs) and entry inhibitors (FDA 2013). The ARV classes and respective drugs are presented Table 2.3 below.

Table 2. 3: ARV classes and approved drug generic name (acronyms)

NRTI/ NtRTI	NNRTI	PI	Integrase inhibitor	Fusion inhibitor	Entry inhibitor
Abacavir (Abacavir, ABC)	Delavirdine (delavirdine mesylate, DLV)	Atazanavir (Atazanavir sulfate, ATV)	Dolutegravir (DTG)	Enfuvirtide (T-20)	Maraviroc (MVC)
Didanosine (ddI)	Efavirenz (EFV)	Duranavir (Duranavir ethanolate,DRV)	Raltegravir (RAL)		
Emtricitabine (FTC)	Etravirine (ETR)	Fosamprenavir (Fosamprenavir calcium, FPV)			
Lamivudine (3TC)	Nevirapine (NVP)	Indinavir (Indinavir sulfate, IDV)			
Stavudine (d4T)	Rilpivirine (rilpivirine hydrochloride (RPV)	Nelfinavir (Nelfinavir mesylate, NFV)			
Tenofovir disoproxil fumerate (TDF)		Ritonavir (RTV)			
Zidovudine (Azidothymidine, AZT, ZDV)		Saquinavir (saquinavir mesylate, SQV)			
		Tipranavir (TPV)			
		Lopinavir (LPV)			

Source: Adopted from FDA approved HIV medicines 2013, (FDA 2013).

a) Nucleoside and nucleotide reverse transcriptase inhibitors (NRTI and NtRTI)

The NRTI interferes with the activities of the HIV reverse transcriptase enzyme. They are structurally similar to the nucleoside building blocks of nucleic acids (RNA, DNA), but devoid of the hydroxyl (-OH) group in the 3` position making them unable to form the 5` to 3` phosphodiester bond essential for DNA synthesis (Warnke et al., 2007). To apply their antiviral activity, the NRTIs are first intracellularly phosphorylated by cellular kinases to their active 5` triphosphate forms. The activated 5` triphosphate competes with the natural deoxynucleotide triphosphates (dNTPs) for the incorporation by reverse transcriptase (RT) into the growing primer. Once the 5` triphosphate form is incorporated, it results in the termination of the elongation and synthesis of DNA, due to their lack of 3` hydroxyl group (Wanke et al., 2007, Maga et al., 2010, Esposito et al., 2012). The HIV reverse transcriptase recognizes the NRTIs substrate with a higher affinity than the cellular DNA polymerases, thus the NRTIs do not interfere with cellular polymerases (Esposito et al., 2012).

The NtRTIs are compounds that already possess a phosphate molecule in their structure and only requires two phosphorylation steps to be converted to their active triphosphate forms. Similarly to NRTI, NtRTIs active forms compete with natural dNTPs for incorporation by RT into the growing primer, thereby terminating the viral DNA synthesis (Esposito et al., 2012). Currently tenofovir is the available NtRTI compound.

b) Nonnucleoside reverse transcriptase inhibitors (NNRTIs).

NNRTIs are diverse in their structure and chemical composition. They act by binding non-competitively to the same hydrophobic pocket (nonnucleoside inhibitor binding pocket, NNIBP) that is situated close to the polymerase catalytic active site within the palm domain of the p66 subunit of the RT (Sluis-Cremer and Tachedjian 2008, Maga et al., 2010). The NNRTIs do not interfere with dNTP binding but alter the conformation and mobility of RT resulting in unproductive complexes (Maga et al., 2010). Unlike the NRTIs, NNRTIs do not require the involvement of cellular enzymes to apply their antiviral activity

and are not effective against HIV-2 (Wanke et al., 2007, Esposito et al., 2012). Some NNRTIs also inhibit the late stages of HIV-1 life cycle by interfering with the Gag-Pol polyprotein processing. However, higher concentrations are needed to affect the late stage of HIV-1 replication, when compared to the concentrations that are required to block RT (Sluis-Cremer and Tachedjian 2008).

c) Protease inhibitors (PIs).

PIs interfere with the proteolytic maturation, a stage that is vital for the production of infectious HIV virus particles. The PIs inhibit the activity of the protease subsequently blocking the cleavage of the large precursor polypeptide chain into smaller functional proteins. This results in the production of non-infectious virus particles (Warnke et al., 2007, Adamson 2012).

d) Integrase inhibitors (INIs).

The integrase inhibitors interfere with the catalytic functions of HIV integrase during the strand transfer stages of viral integration. Thus the transfer of virally coded DNA into host chromosome is blocked (Temesgen and Siraj 2008, Hicks and Gulick 2009).

e) Fusion inhibitors (FIs)

Fusion inhibitors are polypeptides that are homologous to the C-terminal hepta repeat (HR) region of glycoprotein 41 (gp 41). The fusion inhibitors act by competing with the C-terminal for binding to the N-terminal hepta repeat region of gp 41. Once the fusion inhibitor binds to the N-terminal it prevents the formation of the hairpin-like structure that is responsible for the fusion of viral and cell membranes. Thus the viral genome is prevented from entering the CD4+ cells (De-Clercq 2009, Matos et al., 2010).

f) Entry Inhibitors

The entry inhibitors (CCR5 antagonist) block the gp 120-CCR5 interaction by binding to the co-receptor (CCR5) and changing its shape such that gp120 cannot recognize it. The viral genome is prevented from entering the CD4+ cells (Britz et al 2006, De Clercq 2009).

g) Recommended treatment regimen

The current treatment of HIV infection consists of a combination regimen referred to as highly active antiretroviral therapy (HAART). The HAART regimen combines two NRTIs with either NNRTI or PI (Clarke and Mousa 2009). The HAART regimen is aimed at obtaining synergy between compounds targeting different stages of the HIV life cycle, lowering the individual drug doses in order to reduce their toxic side effects and combating the development of resistance (Klimas et al., 2008, Nguyen and Holodniy 2008). Some HAART regimens are now available in the form of a single tablet (Atripla, manufactured 2006) (FDA 2013) making it easier for patients to take it daily (De Clercq 2009).

The administration of HAART is guided by the World Health Organisation (WHO). In 2013, the WHO recommended initiation of HIV treatment to individuals with CD4+ cell count of 500cells/cubic millimeter (mm^3) or less, individuals co-infected with HIV and active tuberculosis irrespective of the CD4+ cell count, HIV infected people co-infected with hepatitis B virus with severe chronic liver disease, serodiscordant HIV positive partners, pregnant women, breastfeeding women and children younger than five years of age (WHO 2013: 1). The WHO 2013 recommended first and second line ARV regimens are shown in Table 2.4.

Table 2. 4: Recommended first and second line ARV regimens (WHO 2013).

		1st line ARV	2nd line ARV
Adults and adolescents	Preferred	TDF + 3TC (or FTC) + EFV	→ AZT + 3TC + ATV/r (or LPV/r)
	Option	AZT + 3TC + EFV / NVP TDF + 3TC (or FTC) + NVP	→TDF+3TC (or FTC)+ATV/r (or LPV/r)
Pregnant and breast Feeding women	Preferred	TDF + 3TC (or FTC) + EFV	→ AZT + 3TC + EFV
Children 3-10 years	Preferred	ABC + 3TC + EFV (or NVP)	→ AZT + 3TC + EFV (or NVP)
	Option	AZT(or TDF)+3TC(or FTC) + EFV (or NVP)	→ ABC + 3TC (or FTC) + EFV (or NVP)
Children < 3 years	Preferred	ABC (or AZT) + 3TC+ LPV/r	Remain on 1st line and improve adherence
	Option	ABC (or AZT) + 3TC + NVP	

TDF tenofovir, 3TC lamivudine, FTC emtricitabine, EFV Efavirenz, AZT zidovudine, NVP Nevirapine, ABC abacavir, ATV/r atazanavir/ritonavir, LPV/r lopinavir/ritonavir. Stavudine (d4T) was phased out from 1st line regimen for adults and adolescents. Adopted from WHO (2013:1).

In South Africa, the switching of HIV treatment from three separate ARV drugs to the single fixed dose combination (FDC) tablet containing tenofovir (TDF), emtricitabine (FTC) and Efavirenz (EFV) has taken a gradual phased approach with the following groups being given priority:

Group 1: All HIV positive patients newly starting ART.

Group 2: HIV positive pregnant and breastfeeding mothers currently stable on 3TC, TDF and EFV.

Group 3: Patients on Stavudine (d4T) based regimen with normal renal function.

Group 4: Patients stable on individual TDF, 3TC, and EFV and are co-infected with tuberculosis.

Group 5: Patients stable on individual TDF, 3TC, and EFV and have co-mobidities such as diabetes, hypertension.

Group 6: Patients receiving individual TDF, 3TC, and EFV who wish to switch to FDC treatment.
Group 7: Patients receiving individual TDF, 3TC and EFV and after counselling agree to switch to FDC treatment (Davies 2013).

While HAART has changed the HIV infection into a manageable chronic disease and making people live longer and healthier lives, HAART has not been without side effects. HAART and HIV infection itself have largely been blamed for the development of metabolic disorders which include dyslipidaemia, lipodystrophy, insulin resistance, and diabetes mellitus which predisposes the HIV infected people to high risk of developing cardiovascular disease (CVD) (Palios et al., 2012).

2.2 CARDIOVASCOLAR RISK FACTORS.

2.2.1 Traditional Risk factors

According to Centre for Disease Control (CDC), 'A risk factor is an aspect of personal behaviour or lifestyle, an environmental exposure, or a hereditary characteristic that is associated with an increase in the occurance of a particular disease, injury or other health conditions (CDC 2013). Traditional and well-established risk factors for CVD include obesity, insulin resistance (IR), diabetes mellitus, hypertension, dyslipidaemia, advanced age, gender, smoking and physical inactivity (Fedele et al., 2011). The integration of some of these risk factors into conventional risk prediction models such as the Framingham Risk Score provides a quantitative prediction of future risk of coronary heart disease (Mozaffarian et al., 2008). Traditional risk factors are either non modifiable or modifiable.

2.2.1.1. Non modifiable risk factors.

Non modifiable risk factors are unpreventable, uncontrollable and cannot be changed by intervention. These include age, gender, family history of CVD and ethnicity (WHO 2009, CDC 2013).

Age increases the risk of CVD and this relationship is partly explained by the presence of major cardiovascular risk factors in older people (Jousilahti et al., 1999). Men are at an increased risk of coronary heart disease (CHD) compared to women of premenopausal age. The difference has been attributed to the hormonal protective effect in premenopausal women (Fedele et al., 2011).

The risk of developing CVD is higher in people with a family history of CVD. In the Physicians Health Study, the relative risk of developing myocardial infarction was higher in people with either paternal or maternal or both paternal and maternal history of MI compared to people with no parental history of MI (Sesso et al., 2001). A recent Dutch cohort study reported a hazard ratio range of 1.1 to 2.2 for people with a parental history of MI. Furthermore a maternal history of MI before age 60 was the strongest predictor of CVD incidence (van Dis et al., 2011). In the Interheart study, the odds ratio for MI in the presence of parental history of MI was 1.67 to 6.56 after adjusting for hypertension, diabetes mellitus, lipids, tobacco use, alcohol use, physical activity, waist to hip ratio, psychological risk factors and fruit and vegetables intake (Chow et al., 2011).

CVD mortality rates differ among different ethnic groups (WHO 2009). Higher CVD deaths were noted among South Asians and American blacks compared with whites (WHO 2009). The Multiple Risk Factor Intervention Trial (MRFIT) conducted in the United States of America reported higher CVD mortality rates among black men compared to white men (Thomas et al., 2005). The ethnic difference in CVD mortality rates was largely mediated by other risk factors and income (Thomas et al., 2005). In South Africa, the highest CVD death rates are found in Indian people, followed by coloured people, with whites and blacks having lowest and similar rates (MRC 2007). Even though the CVD death rates for whites and blacks are similar, the whites mainly die from heart attacks while black African people die from stroke, diseases of heart muscle and high blood pressure (MRC 2007).

2.2.1.2. Modifiable risk factors

Modifiable risk factors are preventable, controllable and treatable (WHO 2009) and include lifestyle risk factors (dietary habits, physical inactivity and smoking) and metabolic risk factors (obesity, insulin resistance, diabetes mellitus, dyslipidaemia, hypertension, inflammation and metabolic syndrome) (Mozaffarian et al., 2008, Spring et al., 2013). Having a high number of risk factors (smoking, poor diet quality, physical inactivity, high body mass index (BMI), blood pressure, blood cholesterol and fasting blood glucose) is associated with high rates of CVD (Folsom et al., 2011).

a) Lifestyle risk factors

The lifestyle risk factors have a profound effect on the development of the metabolic risk factors and subsequent CVD complications (Fig. 2.4) (Mozaffarian et al., 2008). The elimination of health risk behaviours (smoking, physical inactivity, and unhealthy diets) may result in the prevention of at least 80% of heart disease, stroke and type 2 diabetes mellitus and about 40% of cancers (Spring et al., 2013). The Coronary Artery Risk Development in Young Adults (CARDIA) study demonstrated that it was never too late for a behaviour change and regardless of when a healthy lifestyle was adopted, cardiovascular health would still be improved. However, most benefits were derived if healthy lifestyle was adopted from young adulthood and maintained through to middle age (Liu et al., 2012). The improvement of cardiovascular health at individual or community level would therefore warrant concerted interventions on the lifestyle risk factors.

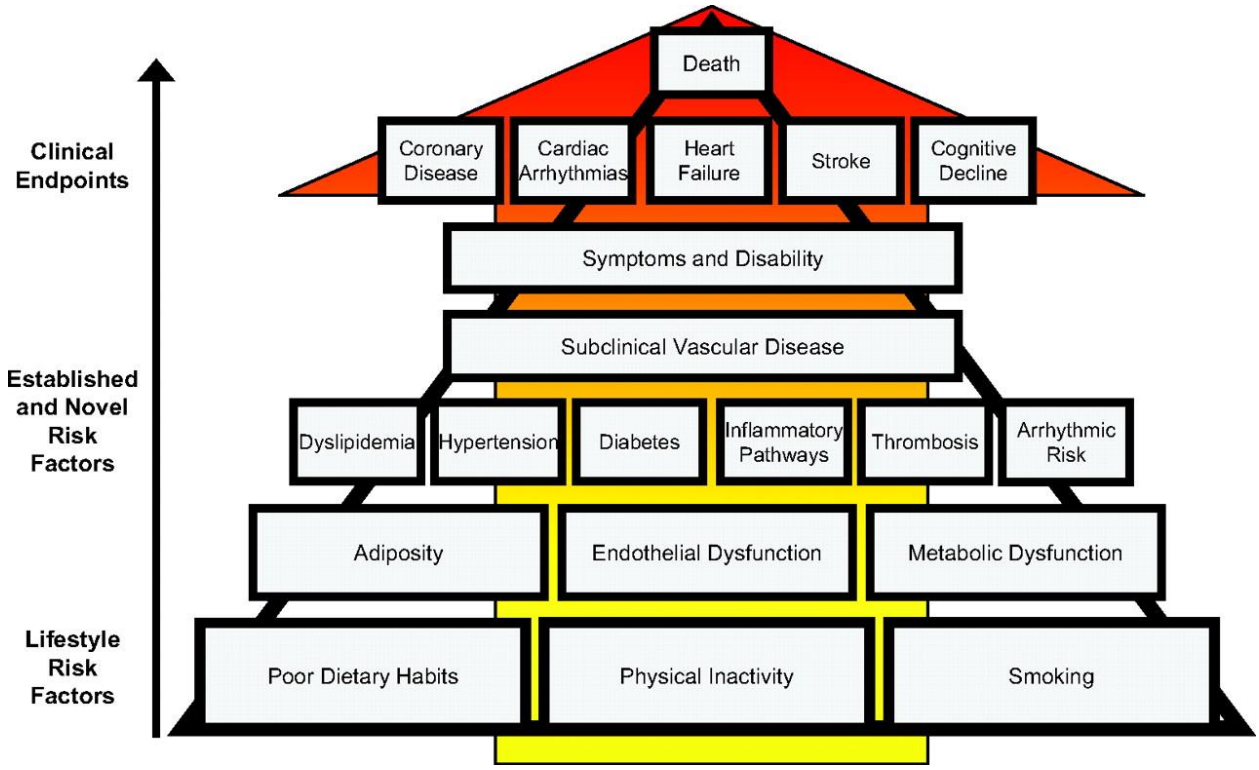

Figure 2. 4: The relation of lifestyle, established and novel risk factors and CVD. (Adapted from Mozaffarian et al., 2008).

In America, strategies aimed at fostering lifestyle behaviour changes by 2020 included counselling following the 5 'A' comprehensive, validated treatment algorithm and a sound healthcare system (Spring et al., 2013). The '5' A treatment algorithm involves 5 counselling steps namely assessment of the risk behaviour, advise on change, agree on goals and an action plan, assist with treatment and arrange follow-up, while a sound healthcare system would provide the practical tools required to assess health behaviour (Spring et al., 2013).

The South Africa 2013-2017 strategic plan for the prevention and control of non-communicable diseases encompasses three main areas which include healthy lifestyle promotion, health system strengthening and monitoring cases and risk factors (South Africa Health News Service 2013). The suggested tools to be use in fostering behaviour change include combined lifestyle assessment form, 5 'A' prompt, motivational interviewing (MI) tools and road 2 health card for adults (CDIA 2013).

b) Metabolic risk factors

i) Obesity and overweight

Obesity and overweight are defined as excessive build-up of fat in the body and are measured by the body mass index (a calculated value obtained by dividing weight in kilograms (kg) by the square of height in metres (m^2) (WHO 2013: 2). A body mass index of 25.00 to 29.99kg/m^2 and greater or equal to 30kg/m^2 defines overweight and obesity respectively (WHO 2013: 2). Obesity, once regarded as a problem of developed nations is becoming an increasing problem in developing countries undergoing epidemiological transition (Ogunbanjo 2013). Globally by 2008, more than 1.4 billion adults 20 years and older were either overweight or obese (WHO 2013: 2).

In South Africa the prevalence of obesity or overweight was 29% in men and 56% in women (Goedecke et al., 2007). Obesity is caused by environmental factors involving over nutrition and sedentary lifestyle (Misra and Khurana 2008). However, a recent report has largely blamed the quality of diets, particularly diets loaded with carbohydrates as the primary driver of the current obesity epidemic (Bowden 2013). Genetically, rare single gene defects in regions that code for leptin and leptin receptor, proopiomelanocortin peptide and receptor, and melanocortin receptors that control key components of appetite in the hypothalamus, increase an individual's susceptibility to becoming obese (Fig. 2.5) (McCormack and Grant 2013, Murphy et al., 2013). Rare causes of obesity include endocrine disorders, medications and psychiatric illness (Ogunbanjo 2013).

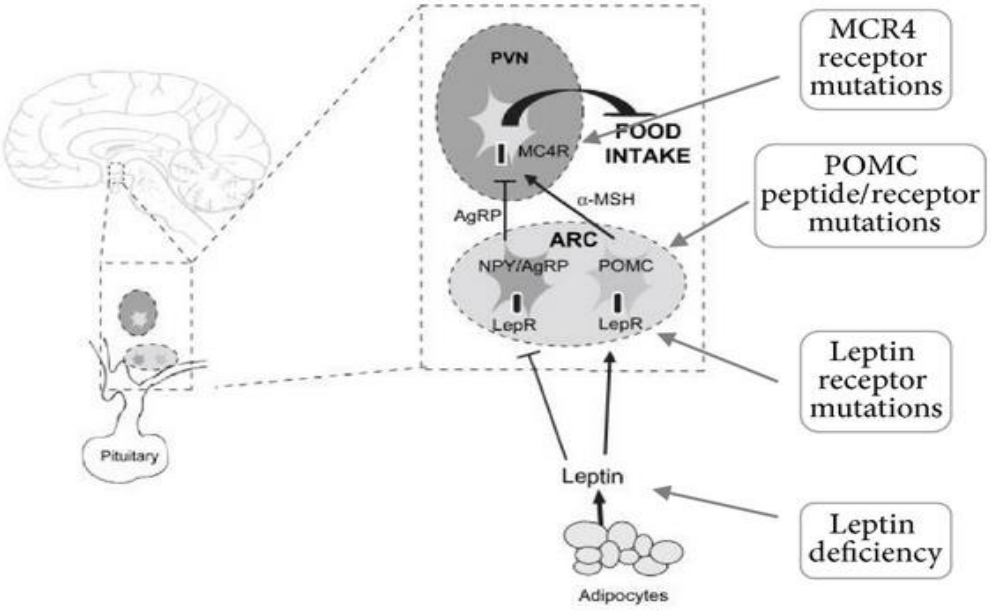

Figure 2. 5: The sites of known monogenic causes of obesity which affect the central regulators of appetite. Adapted from Murphy et al. (2013**).**

Obesity, particularly visceral adiposity is a primary driver of insulin resistance, hypertension, type 2 diabetes mellitus, inflammation and is integral to initiation and progression to CVD (Tchernof and Després 2013). Furthermore obesity may also result in musculoskeletal disorders (osteoarthritis) and cancers (endometrial, breasts, colon) (WHO 2013: 2). While the prevention and management of obesity demand adoption of healthier diets and increased physical activity (Ogunbanjo 2013), challenges in the form of low education and high unemployment, may hinder efforts of reducing the epidemic (Ntsoane et al., 2012).

ii) Insulin resistance

Insulin resistance (IR) refers to reduced response of target cells (liver, muscle and adipocytes) to insulin (Defronzo and Tripathy 2009). IR may be caused by single mutations in genes that code for insulin receptors (Murphy et al., 2013). If the defective insulin receptors are on the muscle cells, the uptake of glucose by muscle cells is impaired and would increase uptake of glucose in adipocytes and promote formation of

triglycerides in the adipocytes resulting in obesity, whereas defective insulin receptors on adipocytes favors leanness (Wilcox 2005). Inversely, IR may be driven by obesity through some complex interplay involving endocrine, inflammatory, neural and cell intrinsic mechanisms (Qatanani and Lazar 2007). In obesity, several adipokine concentrations are altered. Adiponectin concentration decreases while resistin, interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α), retinol-binding protein 4 (RBP4) and fatty acids (FAs) concentrations increase and these changes interfere with insulin signaling resulting in IR (Rabe et al., 2008, Li et al., 2013). Decreases in adiponectin promotes hepatic glucose output and reduces FA oxidation in liver, promotes influx of FA and reduces insulin sensitivity (Bastard et al., 2006). Increased resistin and IL-6 triggers the expression of Suppressor of Cytokine signaling-3 (SOC-3) which interferes with insulin receptor substrate-1(IRS-1) and IRS-2 tyrosine phosphorylation or target IRS-1 and IRS-2 for proteosomal degradation blocking insulin signaling. An increase in FA and TNF-α activates Protein kinase C (PKC), Jun kinase (JNK), nuclear factor-kB (NF-kB), IkB kinase (IKK), p38, mitogen-activated protein kinase (MAPK) that promote serine phosphorylation of IRS-1 and IRS-2 making them poor substrates for insulin receptor activating kinases and increasing their degradation (Bastard et al., 2006). The increased RBP4 impairs the insulin stimulated uptake of glucose in muscles and elevates hepatic glucose production (Qatanani and Lazar 2007). Cellular mechanisms that interfere with insulin signaling in obesity include oxidative stress, endoplasmic reticulum (ER) stress and mitochondrial dysfunction (Qatanani and Lazar 2007). Oxidative stress and ER stress activate the kinases (IKK, JNK, NF-kB) which increase serine phosphorylation of IRS-1 and IRS-2 and interfering with insulin signaling. Mitochondrial dysfunction resulting from a decrease in the expression of nuclear encoded genes such as PPARγ co-activator 1 (PGC-1), that regulate mitochondrial biogenesis, results in accumulation of fat in muscle and liver which impairs insulin signaling through the activation of protein kinases by increased FA (Qatanani and Lazar 2007). While it may not be clear, which one comes first obesity or insulin resistance the two are closely linked and tend to co-exist in obese individuals (Morton and Schwartz 2011).

iii) Diabetes mellitus type 2

Diabetes mellitus type 2 is a metabolic disorder characterized by increased concentration of glucose in the blood (Hinnen 2013). According to the International Diabetes Federation (IDF) at least 382 million people worldwide are living with diabetes and the number is expected to reach 592 million by the year 2035 (IDF 2013). About 80% of the total number of people living with diabetes are from low to middle income countries (IDF 2013). Type 2 diabetes mellitus is associated with complications including retinopathy, neuropathy, nephropathy and CVD. People with type 2 diabetes mellitus have a 2- to 3-fold increase in risk of having stroke or myocardial infarction (Hinnen 2013). Factors known to predispose people to the risk of type 2 diabetes mellitus include abdominal obesity, IR, excessive alcohol intake, poor dietary habits, physical inactivity, age and a genetic predisposition (Hu 2011). More than forty (40) genetic variants associated with susceptibility to type 2 diabetes mellitus have been identified (Hu 2011). Most of these genetic variants are associated with impaired β-cell function, peroxisome proliferator activated receptor-γ (PPAR-γ), insulin receptor substrate 1 (IRS-1), insulin-like growth factor (IGF-1) and glucokinase (hexokinase 4) regulator (GCKR) genes (Spellman 2010, Hu 2011).

The elevated concentration of glucose present in type 2 diabetes mellitus is a result of an interplay of several mechanisms. Insulin resistance in muscle and liver cells results in reduced uptake of glucose and elevated glucose concentration in plasma. The consistently elevated glucose concentration exert pressure on β-cells to produce more insulin and this leads to progressive β-cell damage and failure to produce insulin (DeFronzo et al., 2013). The damaged β-cells become nonresponsive to the stimulatory effects of the incretin hormones, glucagon-like peptide (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) resulting in reduced insulin secretion (Knop et al., 2007). Additionally, adipocyte resistance to insulin anti-lipolytic effect results in increased plasma free fatty acids (FFA), which through oxidation will provide energy to fuel glucose production in the liver through gluconeogenesis (Groop et al., 1989). Glucose production in the liver is enhanced following increased glucagon secretion by the α-cells when GLP-

1 secretion is reduced in the intestines which is associated with an enhanced hepatic sensitivity to glucagon (Spellman 2010). The increased reabsorption of glucose by the kidney in type 2 diabetes mellitus contributes to the maintenance of an elevated plasma glucose concentration (Hinnen 2013). The central nervous system contributes to the elevated glucose concentration by resisting the effect of insulin and altered neurosynaptic hormone secretion resulting in appetite dysregulation (Morton and Schwartz 2011). The incidence of type 2 diabetes mellitus may however be reduced by increasing physical activity and preventing obesity (Hu 2011).

iv) Hypertension

Hypertension (HTN) or persistently raised blood pressure (BP) defined as a systolic blood pressure (SBP) above 140mmHg and/or a diastolic blood pressure (DBP) above 90mmHg (WHO 2009), can be categorized into secondary hypertension resulting from a specific cause and primary hypertension resulting from some underlying pathophysiological alterations (Leisman et al., 2014). Secondary hypertension has a low prevalence affecting between 2% to 5%, while primary hypertension affects more than 95 % of hypertensive people (Beevers et al., 2001). Persistent hypertension may lead to complications including kidney disease and CVD (Kaur and Khannab 2012). In the year 2000, about 972 million people had HTN worldwide, of which 333 were from developed countries and 639 million people were from low to middle income countries. By 2025, about 1.5 billion people worldwide, an increase of 60% will have HTN (Kearney et al., 2005).

Primary hypertension probably results from an interplay of genetic factors, age, environmental factors including alcohol consumption, smoking, low potassium intake, high sodium intake, poor dietary habits and physical inactivity giving rise to obesity and insulin resistance (Oparil et al., 2003). Genetic variants in 14 loci have been associated with susceptibility to hypertension with significant risk of hypertension conferred when genes act conjointly (Khullar 2010). The independent loci were located in or near genes encoding enzymes (kinases and cytochrome), solute channels, transcription factors,

growth factor, cell signaling proteins and structural proteins (Khullar 2010). As people age, the risk of hypertension is increased due to reduced elasticity and increased stiffness of the large arteries (Kaur and Khannab 2012). Smoking also contributes to stiffness of arteries causing increased systolic blood pressure (Landberg 2013). Excessive sodium intake in diet, excessive alcohol consumption and high levels of insulin and leptin present in obesity and insulin resistance tend to activate the sympathetic nervous system (SNS) (Makaritsis et al., 2000, Kaur and Khannab 2012, Landberg et al., 2013). The activation of SNS stimulates the heart, vasculature and the renin angiotensin aldosterone system resulting in increased heart rate, vascular resistance and fluid retention following sodium reabsorption, respectively (Oparil et al., 2003). These changes when combined together may contribute to the development of high blood pressure and to the maintenance of hypertension.

Since hypertension is related to obesity (Landberg et al., 2013), it is expected that the prevalence of hypertension may increase following the current obesity epidemic. Strategies to reverse obesity and insulin resistance would indirectly resolve hypertension in most people. However, a multiple approach involving lifestyle changes as well as treatment targeting various systems that are deranged in hypertension maybe appropriate (Gupta and Guptha 2010).

v) Dyslipidaemia

Dyslipidaemias are generally defined by abnormal amounts of lipids and lipoproteins in the blood. Most dyslipidaemias are characterized by increased plasma total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and triglyceride (TG) concentrations, and decreased plasma high density lipoprotein cholesterol (HDL-C) concentration (Duval et al., 2007). Dyslipidaemias maybe classified as primary or secondary, and both predispose to atherosclerosis which may subsequently lead to CVD.

The primary causes include a variety of genetic factors. The most important is the familial hypercholesterolaemia (FH) characterized by elevations of plasma cholesterol (Palacio

et al., 2011). A predominance of small, dense LDL particles, elevated apolipoprotein B and decreased HDL-C levels has been commonly reported in members of FH families (Juo et al., 1998). Familial hypercholesterolaemia is caused by a mutation in the LDL receptor gene which is located on chromosome 19, and results in a lack of expression of receptors or defective receptors for LDL (Juo et al., 1998). Other genetic disorders include familial hypertriglyceridemia resulting from possibly multiple defects (Tullu et al., 2008), familial defective apo B-100 due to LDL receptor–binding region defect (García-Álvarez et al., 2003) and familial HDL deficiency resulting from defects in the ATP-binding cassette transporter A1 (ABC A1) gene (Pisciotta et al., 2004).

The secondary causes which include conditions such as obesity, diabetes mellitus, renal diseases, insulin resistance and HIV infection contribute to most cases of secondary dyslipidaemia (Tovar 2006, Tsimihodimos and Elisaf 2011). Insulin resistance is central to the development of secondary dyslipidaemia present in conditions such as diabetes mellitus type 2 and obesity (Mooradian 2009). The characteristic features of this dyslipidaemia are high TG concentration, low HDL cholesterol concentration and high small dense LDL concentration (Mooradian 2009, Klop et al., 2013). This triad of plasma lipid abnormalities is also referred to as Atherogenic Lipoprotein Phenotype B (Musunuru 2010).

The increased influx of free fatty acids from dietary intake, intravascular lipolysis and adipose tissue resistance to the anti-lipolytic effects of insulin leads to the increased synthesis of TG in the presence of adequate glycogen in the liver (Klop et al., 2013). Hypertriglyceridaemia in the liver triggers the secretion of apolipoprotein B and very low density lipoprotein (VLDL) cholesterol. The TG carried in the VLDL particles is exchanged for cholesterol ester carried by HDL via the action of Cholesterol Ester Transfer Protein (CETP).The TG-rich HDL is then hydrolysed by hepatic lipase or lipoprotein lipase giving rise to reduced size HDL from which apolipoprotein A1 is liberated. The released apolipoprotein A1 is filtered by renal glomeruli followed by degradation in renal tubular cells resulting decreased levels of HDL cholesterol (Mooradian 2009). In addition, CETP exchanges TG from VLDL particles for cholesterol ester from LDL-particles giving rise to

TG-rich LDL which is hydrolysed by hepatic lipase or lipoprotein lipase in the liver resulting in increased lipid-depleted small dense LDL-particles (Mooradian 2009).

Dyslipidaemia is one of the main risk factors for CVD (Tsimihodimos et al., 2011). While drugs such as statins, fibrates and nicotinic acid can be used in management of secondary dyslipidaemia (Mooradian 2009, Mesquita et al., 2010), treatment may partially be directed to the underlying conditions (Tsimihodimos and Elisaf 2011).

vi) Metabolic Syndrome

Metabolic syndrome (MetS) is a condition in which multiple interrelated CVD risk factors co-exist in an individual (Alberti et al., 2009). Metabolic syndrome has been defined in a variety of ways by different organisations. According to National Cholesterol Education Program ATP III, MetS was defined using any three risk factors from five which include abdominal obesity (circumference: males>102cm, women>88), high TG concentration≥ 1.7mmol/l, low HDL cholesterol concentration (men<1.03mmol/l, women<1.29mmol/l), high BP (systolic BP≥ 130mmHg or diastolic≥85mmHg or both) and raised fasting plasma glucose concentration (≥6.1mmol/l) (NCEP ATP III). The International Diabetes Federation (IDF) defined MetS as a combination of central obesity (circumference: males>94cm, women>80; with ethnicity specific values for other groups) and a choice of two risk factors from high TG concentration ≥ 1.7mmol/l, low HDL cholesterol concentration (men<1.03mmol/l, women<1.29mmol/l), high BP (systolic BP≥ 130mmHg or diastolic≥85mmHg or both) and raised fasting plasma glucose concentration (≥5.6mmol/l). In 2009, these organisations unified and agreed on the definition of MetS as any 3 of the 5 risk factors and abdominal obesity seized to be a pre-requisite for defining MetS (Alberti et al., 2009).

High prevalences of MetS have been reported from rural and urban Brazil, 29.6% (de Carvalho Vidigal et al., 2013), rural South Africa, 22.1% (Motala et al., 2011) and urban India, 19.52% (Sawant et al., 2011). The high prevalence of MetS, may be related largely to the increase in obesity and sedentary lifestyle (Alberti et al., 2009).

2.2.2 Pathophysiology of HIV and ARV related CVD risk factors

HIV infected people may have increased CVD risk through direct or indirect mechanisms from HIV or antiretroviral treatment, which induce risk factors such as DM, dyslipidaemia, hypertension, lipodystrophy, and endothelial dysfunction (Manfredi and Calza 2009). However, some traditional risk factors such as genetic predisposition, increased BMI, smoking, positive family history and increasing age may still have a crucial role in the development of metabolic risk factors in HIV infected people (Paik and Kotler 2011). Furthermore, three possible scenarios of patients with metabolic disorders and infected with HIV may be identified, firstly; patients with pre-existing known metabolic disorders who then contract HIV, secondly; those who are diagnosed with metabolic disorder at onset of HIV infection, and thirdly; those who develop metabolic disorders after initiating antiretroviral treatment (Kalra et al., 2011). Thus the effects of HIV infection, HAART, environmental and genetic factors may be difficult to separate in an individual.

2.2.2.1 Insulin resistance (IR) and Diabetes mellitus type 2 (DM)

HIV induced IR is mediated through the attenuation of PPAR-γ activity by HIV accessory protein Vpr leading to inhibition of adipocyte differentiation, reduced uptake of free fatty acid (FFA) and inhibition of transcriptional activity of insulin (Shrivastav et al., 2008). HIV protein Tat, activates NF-Kb, which triggers the secretion of TNF-α, the blockage of FFA uptake by adipocytes and suppression of insulin signalling due to reduction of insulin receptor substrate-1 (IRS-1) (Paik and Kotler 2011). HIV infected people on treatment tend to experience improvement in their general health, which is associated with improved appetite, increased food intake and weight gain. This may lead to overweight or obesity, conditions which predispose to insulin resistance and type 2 diabetes mellitus (Reid 2012).

Antiretroviral treatment, particularly protease inhibitors (PIs) (Indinavir and ritonavir) inhibits the activity of PPAR- γ (Caron et al., 2009, Reid 2012), and promotes down regulation of GLUT-4, a transporter of glucose into fat cells, cardiac and muscle cells,

thus maintaining high concentration of glucose in circulation (Hresko and Hruz 2011). Protease inhibitors especially ritonavir and nelfinavir but not Indinavir interfere with the secretion of insulin by β-cells giving rise to glucose intolerance which can progress to type 2 DM (Neye et al., 2006, Flint et al., 2009). Nucleoside reverse transcriptase inhibitors (NRTIs) are toxic to the mitochondrial organelle. They inhibit the mitochondrial DNA polymerase resulting in defective mitochondrial DNA replication. This gives rise to mitochondrial dysfunction (Paik and Kotler 2011). Mitochondrial dysfunction leads to decreased β-oxidation of FFA and ATP production, while reactive oxygen species (ROS) production increases. The accumulation of FFA in plasma due to reduced β-oxidation interferes with insulin signalling resulting in IR which can induce type 2 DM (Kim et al., 2008). The increased ROS, may result in oxidative damage of small LDL in the subendothelial space, triggering an inflammatory state that recruits macrophages resulting in formation of foam cells marking the initial stages of atherosclerosis (Masiά et al., 2007).

2.2.2.2 Obesity and abdominal obesity

The obesity and abdominal obesity problem is evident among the HIV infected people at diagnosis (Crum-Ciaflone et al., 2010) and probably reflects on the current prevailing obesity problem among the HIV negative people in the communities. The aetiology of obesity in ARV naïve HIV infected, as with HIV negative may involve the imbalance between energy intake and energy expenditure caused by factors such as quality and quantity of dietary intake, environmental and genetic factors (Ali and Crowthers 2010).
The use of ART has reduced the prevalence rates of wasting in HIV infected people, probably due to improved health and calorie intake (appetite) (Silva et al., 1998), but has been associated with increases in BMI of equal or greater than 25kg/m^2 (overweight and obesity) (Reid et al., 2012, Akinboro et al., 2013). However, findings on association of ART treatment and BMI have been inconsistent, with studies reporting an increase in BMI with ART use (Akinboro et al., 2013), absence of association between obesity and antiretroviral therapy (Crum-Ciaflone 2008) and a peak BMI increase after ART initiation followed by a decline in weight due to lipoatrophy (Silva et al., 1998). These differences

have partly been ascribed to differences in duration of treatment among the different studies (Crum-Ciaflone et al., 2008). In addition, the ART has been associated with visceral lipohypertrophy giving rise to abdominal obesity (Reid et al., 2012).

2.2.2.3 Lipodystrophy

Lipodystrophy in HIV infected people is marked by decreased subcutaneous fat in upper and lower extremities with prominent veins (lipoatrophy) or fat accumulation around the neck, the dorsocervical region as a “buffalo hump”, the abdomen and the trunk (lipohypertrophy) or both lipoatrophy and lipohypertrophy (Palios et al., 2012).

Lipoatrophy in HIV infected treatment naïve people has been associated with chronic low grade inflammation at systemic and adipocyte levels. Systemic inflammation promotes movement of monocytes across the vascular endothelium into the fat tissue where they differentiate into activated macrophages which enhances the local inflammation at adipocyte level, increasing the levels of TNF-α. Increased TNF-α, HIV proteins, Vpr and Nef, interfere with adipocyte function and differentiation through inhibition of PPAR-γ leading to limited lipoatrophy (Caron-Debarle et al., 2010) (Fig 2.6).

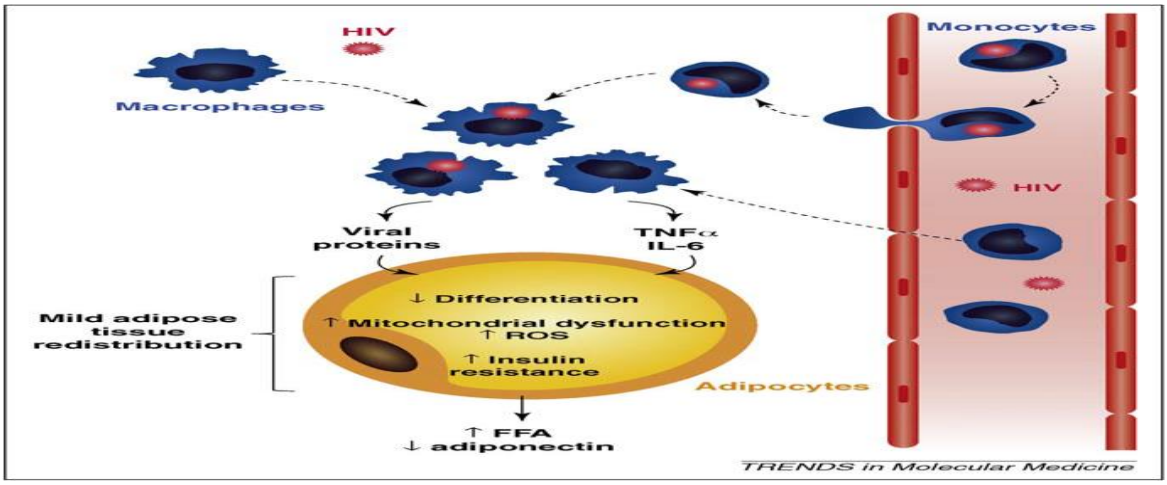

Figure 2. 6: Possible HIV mechanisms in adipose tissue leading to lipoatrophy Caron-Debarle et al. (2010).

Treatment with thymidine nucleoside reverse transcriptase inhibitors (tNRTI), stavudine and zidovudine account for the development of lipoatrophy (Reid et al., 2012). The low mitochondria content of subcutaneous adipose tissue (SAT) makes it more prone to

lipoatrophy than visceral adipose tissue (VAT) through increased mitochondrial dysfunction resulting from inhibition of mitochondrial DNA (mtDNA) polymerase γ, oxidative mtDNA damage and inhibition of Adinosine diphosphate (ADP)/ Adinosine triphosphate (ATP) translocase. Additionally, NRTI increases the expression of genes involved in oxidative stress and apoptosis (Martinez 2011). On the other hand, the higher mitochondrial content of VAT makes it initially less sensitive to drug and virus induced mitochondrial damage giving rise to a mild mitochondrial dysfunction. Slight damage to mitochondria permits increased production of ROS which drives mitochondrial biogenesis, and adipogenesis resulting in lipohypertrophy (Caron-Debarle et al., 2010). Moreover, unlike SAT, hypertrophied VAT has no impairment of adipogenic gene expression (Caron-Debarle et al., 2010). However, long term exposure to NRTI may result in severe damage to mitochondria which can lead to inhibition of adipogenesis, lipogenesis and increased apoptosis leading to lipoatrophy (Caron-Debarle et al., 2010). Lipohypertrophy may probably be modified through lifestyle changes involving low caloric high fibre diets and exercising but the same may not be applicable in lipoatrophy as it may worsen the fat loss (Leyes et al., 2008).

2.2.2.4 Dyslipidaemia

Dyslipidaemia in treatment naïve HIV infected people is characterized by decreases in TC, LDL-C, HDL-C and increases in TG concentrations (Feingold et al., 1993, Riddler et al., 2008, Rose et al., 2008, Fourie et al., 2010). As uncontrolled HIV infection progresses to AIDS, the TC, LDL-C and HDL-C concentrations continue to decline while TG concentration continues to increase (Feingold et al., 1993). The lipoprotein subclass (small LDL-p, large LDL-p, small HDL-p and large HDL-p) concentrations decrease in treatment naïve HIV infected compared to uninfected people (Stein et al., 2008, Baker et al., 2010). The initiation of ARV therapy tends to raise the lipids to pre-infection levels and above (Riddler et al., 2008, Tien et al., 2010). Some HAART regimens, especially PI-based regimens have been associated with severe dyslipidaemia characterised by high concentrations of TC, LDL-C, TG and low concentration of HDL-C (Riddler et al., 2003; Rose et al., 2006) coupled with increased concentration of total LDL-p, small dense LDL-

p and low concentration of HDL-p (Swanson et al., 2009). Some NNRTI-based HAART result in smaller increase in TC, LDL-C, and TG concentrations with noticeable increase in HDL-C and HDL-p concentrations (Minnaar and van der Merwe 2008; Swanson et al., 2009). ARV dyslipidaemia, particularly induced by PIs may resemble the Atherogenic Lipoprotein Phenotype B, which is associated with increased risk of atherosclerosis and CVD (Riddler et al., 2008).

The down regulation and up regulation of lipoproteins is a result of a combination of mechanisms (Figure 2.7). The HIV virus via its accessory protein Nef, impairs the Adenosine Triphosphate (ATP)-binding cassette transporter subfamily A, member 1 (ABC A1) system resulting in decreased cholesterol efflux from macrophages and decreased HDL-C (Mujawar et al., 2006). Inflammation triggers release of cytokines TNF-α and IL-6. TNF-α attenuates the ability of insulin to suppress lipolysis from fat cells and thus interferes with free fatty acid metabolism promoting hypertriglyceridemia (Oh and Hegele 2007). IL-6 decreases lipoprotein lipase activity and increases phospholipase A2 and endothelial lipase activity resulting in the accumulation of TG-rich lipoproteins and an increase in catabolism of HDL (Rader 2006).

Protease inhibitor containing regimens are associated with abnormal accumulation of intramyocellular fat, leading to insulin resistance, which increases plasma apolipoprotein-B containing and TG-rich lipoprotein. Furthermore PI regimen impairs hydrolysis of triglyceride-rich lipoproteins by plasma and tissue lipases, resulting in high levels of TG (Purnell et al., 2000, Torriani et al., 2006). Structural homology between LDL-receptor related protein type 1 (LRP 1) and HIV protease may promote binding of PI to LRP 1. LRP 1 normally binds to lipoprotein lipase on capillary endothelium, which hydrolyses free fatty acids (FFA) from triglycerides promoting their accumulation in adipocytes. Thus PI binding to LRP1 would interfere with LRP1-lipoprotein lipase complex formation, reducing adipose storage capacity and increasing plasma triglyceride-rich lipoproteins (Carr et al., 1998).

The contribution of other metabolic conditions such as diabetes, insulin resistance and metabolic syndrome that normally cause similar lipoprotein abnormalities to HAART should be born in mind when managing dyslipidaemia in HIV infected people. In addition to lipid lowering drugs, dietary intake and physical activity remain a priority. Evidence from Calza et al. (2005) showed that the lipid lowering agents (pravastatin and bezafibrate) are more effective in improving the dyslipidaemia induced by ARV rather than ARV switching to drugs with less atherogenic effect. Thus pravastatin with minimal interactions with ARV drugs could be a preferred statin in HIV infected people (Dau and Holodniy 2008).

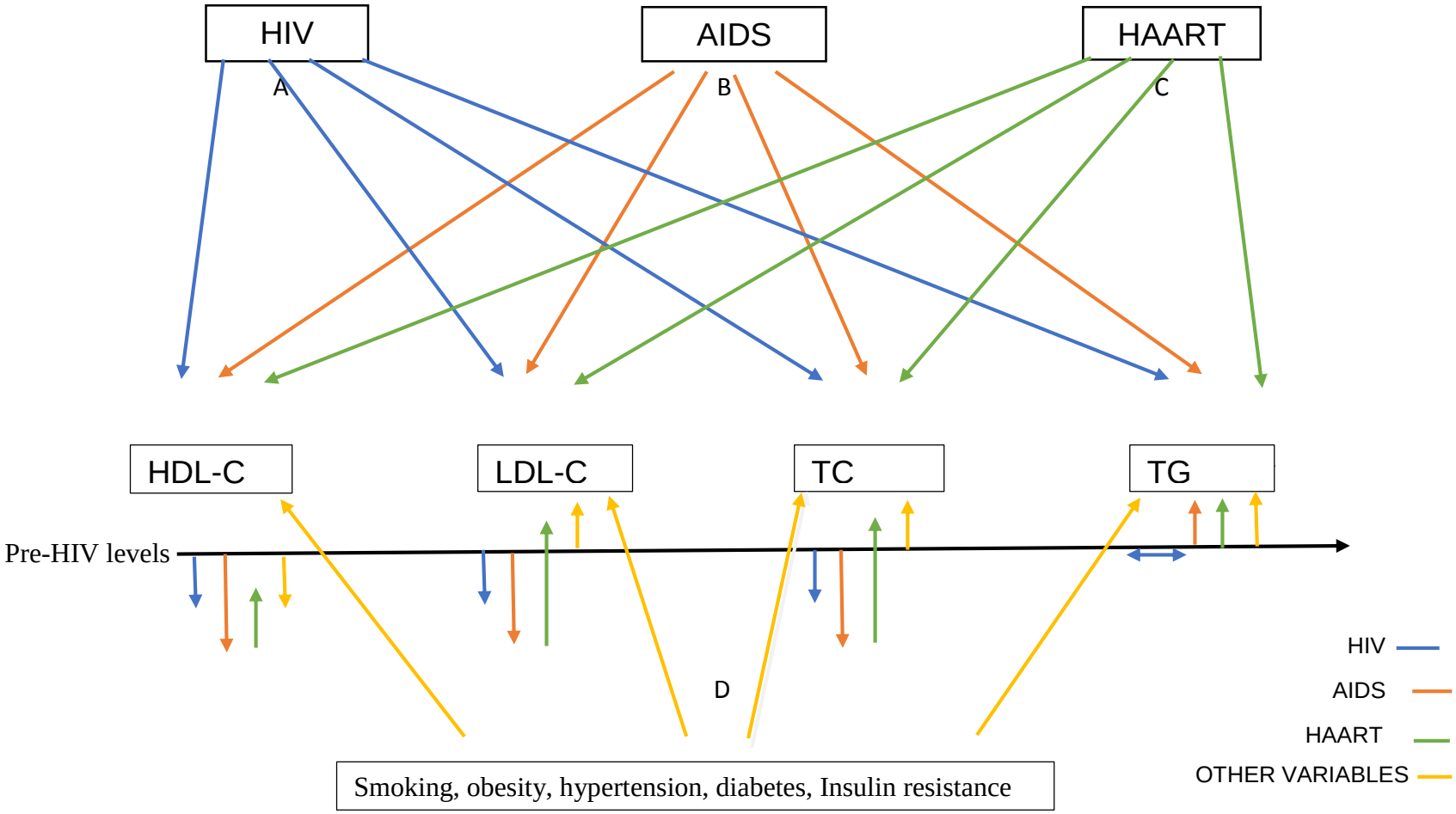

Figure 2. 7: Schematic diagram representing the down regulation and up regulation of lipoproteins by HIV, AIDS, HAART intervention and other variables
Schematic diagram representing the down regulation and up regulation of lipoproteins by HIV, AIDS, HAART intervention and other variables that may be present during HIV infection and its treatment. HIV Nef protein impairs ABC A1 system resulting in decreased HDL-C (A). Chronic inflammation (A, B,C) triggers release of TNF-α which attenuates insulin ability to suppress lipolysis resulting in hypertriglyceridemia and release of IL-6 which decreases lipoprotein lipase activity and increases phospholipase A2 and endothelial lipase activity resulting in the accumulation of TG-rich lipoproteins and an increase in catabolism of HDL. PI regimen impairs hydrolysis of triglyceride-rich lipoproteins by plasma and tissue lipases, resulting in high levels of TG (C). The other variables cause dyslipidaemia through the mediation of adiponectin (D). Adopted from Mashinya et al. (2014).

2.3 PREVALENCE OF CVD RISK FACTORS IN HIV INFECTED PEOPLE.

Studies that have reported on CVD risk in HIV infected people, have utilized different study designs. Most studies were cross sectional without comparative groups, while other studies compared the CVD risk factors between HIV infected and uninfected people, HIV infected people on ARV and HIV infected treatment naïve people and lastly between HIV infected males and females. Moreover, different risk factors were analyzed in each study (Table 2.5).

2.3.1 Smoking

Studies that compared CVD risk factors between HIV infected (combined treated and untreated) and HIV uninfected people reported significantly higher proportions of smokers in the HIV infected groups (38-68%) compared to HIV negative groups (18-37%) (Triant et al., 2007, Kwiatkowska et al., 2011, Malaza et al., 2012, Kakinami et al., 2013). In a recent study by Hilleman et al. (2014) the prevalence of smoking remained higher in ART naïve HIV infected compared to HIV negative people. In the Cardiovascular Risk Evaluation and Antiretroviral therapy (CREATE-1) study from United Kingdom (UK), HIV infected people were divided by gender and compared to HIV uninfected gender counterparts from the HEART-UK study. Significantly higher proportion of HIV infected males and females were smokers compared to their HIV uninfected counterparts. However, despite being infected with HIV and a high prevalence of smoking, the 10-year CVD risk in the CREATE-1 cohort was lower than the HEART-UK cohort (Aboud et al., 2010). The older age of the HEART-UK cohort could have contributed to the higher 10-year CVD risk compared to the CREATE-1 cohort.

Cross sectional studies that compared CVD risk factors in ARV treated HIV infected and HIV infected treatment naïve people observed a similar prevalence of smoking in the two groups (Manfredi 2009, Muhammad et al., 2013, Edward et al., 2013, Kagaruki et al., 2014). However, studies have observed a higher prevalence of smoking in HIV infected males than in females (Aboud et al., 2010, Edward et al., 2013), probably contributing to the high CVD risk associated with male gender.

2.3.2 Obesity, abdominal obesity and Hypertension

In a South African study, hypertension (27.9% vs 19.5%) and obesity (24.5% vs 20.0%) were significantly higher in HIV uninfected than in HIV infected people (Malaza et al., 2012). In contrast, a study conducted in Poland by Kwiatkowska et al. (2011) observed a similar prevalence of hypertension and obesity among HIV infected and uninfected people, while two studies conducted in United States of America (USA) reported a significantly higher prevalence of hypertension ($p<0.001$) in HIV infected compared to uninfected people (Triant et al., 2007, Kakinami et al., 2013). A comparison of HIV infected people from the CREATE-1 study and HIV uninfected from the HEART-UK study, showed that a significantly higher proportion of HIV infected than uninfected women had abdominal obesity, while HIV infected and uninfected men had a similar prevalence of abdominal obesity (Aboud et al., 2010).

Some studies reported a non-significant difference in the prevalence of obesity and hypertension among ART treated HIV infected and treatment naïve HIV infected people (Manfredi 2009, Edward et al., 2013, Carey et al., 2013). Contrastingly, a cross-sectional study from urban Nigeria reported a significant difference in the prevalence of obesity (11% vs 2%, $p=0.018$) and hypertension (17% vs 2%, $p=0.001$) between ARV treated HIV infected and treatment naïve people (Muhammad et al., 2013). The high prevalence of hypertension among ARV treated people was attributed to the duration of ARV treatment and high BMI in these people (Muhammad et al., 2013). High BMI in ARV treated people is associated with a state of 'return to health', in which most ARV treated people gain appetite and take more calories resulting in weight gain (Reid 2012). Similarly, significantly higher prevalence of obesity, abdominal obesity, and hypertension was observed among Tanzanians on ART compared to those naïve to treatment (Kagaruki et al., 2014).

A cross-sectional study conducted in Nigeria, comparing CVD risk factors in HIV infected men and women reported significantly higher proportions of obesity and abdominal obesity in HIV infected females than HIV infected males (Edward et al., 2013). A similar study conducted in Kenya reported a higher prevalence of obesity in HIV infected females

than in HIV infected males, however the level of significance was not indicated (Bloomfield et al., 2011). Similarly, studies conducted in the South African general population have reported a higher prevalence of obesity in females when compared to males (Malhotra et al., 2008, Morris et al., 2011).

Studies conducted in Ethiopia (Berhane et al., 2012) and Senegal (Diouf et al., 2012) among people on ART only, found a high prevalence of hypertension, while the prevalence of hypertension among South Africans on ART was low (19.1%) (Julius et al., 2011).

2.3.3 Diabetes mellitus (DM) and metabolic syndrome

Two USA studies on the prevalence of DM were discordant where the study by Triant et al. 2007 reported a significant difference while the study by Kakinami et al. (2013) reported a similar prevalence of DM among HIV infected and uninfected people. However, when the prevalence of DM was compared between HIV infected people on ART and those naïve to ART, no significant difference was observed (Manfredi 2009, Edward et al., 2013, Carey et al., 2013, Muhammad et al., 2013, Kagaruki et al., 2014). In the study by Muhammad et al. (2013) the prevalence of metabolic syndrome was significantly higher in ARV treated than untreated HIV infected people.

2.3.4 Dyslipidaemia

Studies that compared lipid profiles of HIV infected and uninfected people have unanimously found decreased TC, HDL-C, LDL-C concentrations and increased TG concentration in HIV infected treatment naïve compared to uninfected people (Feingold et al., 1993, Rose et al., 2008). Despite the low LDL-C concentration in HIV infection, the proportions of atherogenic small dense LDL-C particles may be increased posing high risk of atherosclerosis (Feingold et al., 1993). Some studies that compared lipid profiles of ARV treated HIV infected and uninfected people reported significantly higher concentration of TG and lower HDL-C concentration in the ARV treated HIV infected compared to uninfected people (Riddler et al., 2008, Tien et al., 2010).

The prevalence of dyslipidaemia was significantly higher in HIV infected compared to uninfected people (Triant et al., 2007). The prevalence of hypertriglyceridaemia and hypercholesterolaemia was significantly higher, while the prevalence of low HDL-C was significantly lower in ARV HIV treated than in HIV infected treatment naïve people (Carey et al., 2013, Muhammad et al., 2013, Abebe et al., 2014, Kagaruki et al., 2014). A study from Nigeria found no significant difference in the prevalence of hypertriglyceridaemia, hypercholesterolaemia and low HDL-C among ARV treated and ARV untreated people (Edward et al., 2013). When HIV infected males and females were compared, the prevalence of low HDL-C was significantly higher among HIV infected females than HIV infected males. However, the prevalence of low, moderate and high 10-year CVD risk was similar between the two groups (Edward et al., 2013).

Cross sectional studies involving people on ART only, that were conducted in Ethiopia (Berhane et al., 2012) and South Africa (Julius et al., 2011) found a high prevalence of low HDL-C. In addition the prevalence of high TG was high among Ethiopians, while a low prevalence of high TG was observed among the South Africans.

2.3.5 Lipoprotein subclasses

The distribution of lipoprotein subclasses in HIV infected people with CVD was determined and compared to the distribution of HIV infected without CVD in the Strategies for Management of Antiretroviral Therapy (SMART) study. There were no differences in total LDL-p, small LDL-p and total VLDL-p between CVD cases and controls. However, differences were observed in total HDL-p ($p<0.0001$), large HDL-p ($p<0.006$) and small HDL-p ($p<0.01$) between CVD cases and controls. The reduced HDL-p in CVD cases could have contributed to the reduced cholesterol transportation back to the liver, hence more cholesterol retention in the vessels increasing the probability of plaque formation. The evidence suggests that a low HDL-p concentration, is a more important risk factor for CVD in HIV infected people than traditional LDL-p concentration in particular small dense LDL-p concentration (Duprez et al., 2009; Baker et al., 2010).

2.3.6 CVD estimation equations

Several CVD risk prediction models that include the Systematic Coronary Risk Estimation (SCORE) (Conroy et al., 2003), Prospective Cardiovascular Mu¨nster Study (PROCAM) (Assmann et al., 2002), Framingham risk equation (D'Agostino et al., 2001) and the Data Collection on Adverse Effects of Anti-HIV Drugs (D.A.D) (Friis-Moller et al., 2010) have been developed and are used to estimate the future CVD risk in various populations. The DAD is the only risk estimation equation to date that was developed specifically for HIV infected people, while the Framingham equation is the most widely used conventional CVD risk estimation equation. Literature suggests that the Framingham risk estimation equation tends to overestimate CVD risk in Europeans (De Visser et al., 2003, Cooper et al., 2005) and underestimates CVD risk in black South Africans (Klug et al., 2015). Although an agreement has been reported between Framingham and DAD risk estimation equations (Nery et al., 2013), findings on the use of the Framingham equation in HIV infected people are conflicting (Law et al., 2006, Nery et al., 2013). Law et al. (2006) found the Framingham equation to underestimate while Nery et al. (2013) found the Framingham equation to overestimate the 10-year CVD risk in people infected with HIV and on ART. These differences may be due to age, an important factor in determining risk for CVD using the Framingham equation (Cooney et al., 2010). Therefore studies with young people may have low risk score irrespective of their risk factor levels.

Table 2. 5: Cardiovascular risk factor prevalences (%) in HIV infected people.

Author/ study design	Study population	Smoking	Obesity	overweight	Abdominal obesity	DM	HTN	Dyslipidaemia		
								High TC	High TG	Low HDLC
Triant et al., 2007 / Retrospective - Case-control USA	HIV infected – 3851 people On ARV (40.9%) Males- 69% Mean age- 38 (32-44). AA – 23.5%, C-54.1%, H-7.1%	38	-	-	-	11.5	21.2	Dyslipidaemia- 23.3		-
	HIV uninfected- 1 044 589 people Males 40.9% Mean age- 39 (29-54) AA - 6.7%, C - 66.1%, H – 7.1%	18	-	-	-	6.6	15.9	Dyslipidaemia-17.6		
	p-values	-	-	-	-	<0.001	<0.001	<0.001		-
Kakinami et al., 2013/ Cross-sectional Case-control USA	HIV infected - 239 people On ARV-79% Males-69% Mean age -44.5±9.5 years AA – 37%, C – 52%, H – 11%	43	-	-	-	7	38	-	-	-
	HIV uninfected – 717 people Males – 69% Mean age- 44.5 ± 9.5 years AA – 37%, C – 52%, H – 11%	27	-	-	-	8	19	-	-	-
	p- values	<0.001	-	-	-	0.94	<0.001	-	-	-
Kwiatkowska et al., 2011/ Cross sectional Case control Poland	HIV infected – 72 people On ARV – 94.4% Males – 67% Mean age – 39.4 ± 8.9 years	63.8	8.3	-	-	2.7	34.2	-	-	-
	HIV uninfected – 27 people Males – 67% Mean age – 39.3 ± 10.9 years	37	18.5	-	-	3.7	33.3	-	-	-
	p - values	0.04	NS	-	-	NS	NS	-		-

Author/ study design	Study population	Smoking	Obesity	overweight	Abdominal obesity	DM	HTN	Dyslipidaemia		
								High TC	High TG	Low HDLC
Malaza et al., 2012/ Case control South Africa	HIV infected – 2513 people Males and females	-	20	45.6	-	-	19.5	-	-	-
	HIV uninfected – 27 people Males and females	-	24.5	46.0	-	-	-27.9	-	-	-
	p-values	-	<0.001	0.76	-	-	<0.001	-	-	-
Aboud et al., 2010/ Cross sectional UK	*CREATE-1 study:* HIV infected – 1021 people On ARV – 73.1 % Males – 76% Mean age – 40 (35-46) years AA – 33.3%, C – 50.8%, others- 15.9%	37	24	-	-	3	12	35.6	-	33
	DAD study: HIV infected – 23 468 people Males – 75% Mean age – 39(34-47) years	51	-	-	-	2.5	8.5	-	-	-
	p - values	<0.05	-	-	-	NS	<0.05	-	-	-
	CREATE -1 study: HIV males infected – 737 people Mean age – 41.2 ± 9.2 years C – 65%	45	-	-	24	2	12	-	-	-
	Heart –UK study: HIV uninfected males – 27 776 Mean age – 51.5 ± 16.2* C – not available	13.4	-	-	25	4	13	-	-	-
	p-values	<0.05			ns	ns	ns			

Aboud et al., 2010/ Cross sectional UK	*CREATE -1 study:* HIV infected females – 253 Mean age – 38.8 ± 8.8 years C – 9 %	16	-	-	64	2	9	-	-	-
	Heart –UK study: HIV uninfected females – 43261 Mean age – 52.1 ± 15.4* C – not available	12.7	-	-	40.1	3	13	-	-	-
	p - values	<0.05	-	-	<0.05	NS	NS	-	-	-
Manfredi 2009/ Cross-sectional Italy	Total HIV infected – 27 people Males 70% Mean age – 44 ± 13 years									
	HIV infected ARV naïve – 11 people	45.4	-	-	-	-	0	Lipodystrophy - 0		
	HIV infected on ARV – 16 people	43.7	-	-	-	-	12.5	Lipodystrophy -50		
	p - values	NS	-	-	-	-	NS	0.021		-
Muhammad et al., 2013/ Cross sectional Urban Nigeria	Total HIV infected – 200 people Males – 47 % Mean age – 32.5 ± 7.55 years	9	6.5	31	15.5	3	9.5	19	16	68.5
	HIV infected ARV naïve– 100 people Males – 48% Mean age – 32.36 ± 7.50 years	8	2	28	21	3	2	7	13	76
	HIV infected on ARV – 100 people Males – 46% Mean age – 32.8 ± 7.63 years	10	11	34	10	3	17	31	19	61
	p-values (between ARV and ARV naïve)	0.621	0.018	0.359	0.032	1.0	0.001	0.001	0.247	0.022
Edwards et al., 2013/ Cross-sectional Nigeria	HIV infected ARV naïve– 51 people Males – 35 % Mean age – 36.8 ± 8.7 years	2.0	3.9	25.5	13.7	9.8	7.8	31.4	13.7	56.7
	HIV infected on ARV – 214 people Males – 32 % Mean age – 39.1 ± 8.8 years	1.9	7.5	24.2	19.2	10.7	12.1	34.1	12.6	47.2
	p-values	0.97	0.55	0.55	0.37	0.84	0.38	0.71	0.83	0.22

Author/ study design	Study population	Smoking	Obesity	overweight	Abdominal obesity	DM	HTN	High TC	High TG	Low HDLC
Edwards et al., 2013/ Cross-sectional Nigeria	HIV infected males – 86 Mean age – 41.7 ± 8.4 years	4.7	1.2	26.7	2.3	11.6	12.8	32.6	12.8	36.0
	HIV infected females – 179 Mean age – 37.2 ± 8.5 years	0.6	9.5	25.7	25.7	10.1	10.6	34.1	12.8	55.3
	p-values	0.04	0.02	NS	0.001	NS	0.6	0.81	0.99	0.003
	All HIV infected (ARV treated and ARV naïve)—265 people Males – 32.4%	1.9	6.8	26.0	13.1	10.6	11.3	33.6	12.8	49.1
Carey et al., 2013/ Cross sectional South India	HIV infected on ARV -59 people Males – 57.63 % Mean age – 39.2 ± 9.1 years	-	23.6	-	35	34	17.3	36[c]	48[c]	54[c]
	HIV infected ARV naive -66 people Males – 34.85 % Mean age – 33.4 ± 6.0 years	-	54.7	-	36.5	20.7	12.1	6.9	17.2	86.2
	HIV uninfected infected -75 people Males – 69.33 % Mean age – 37.8 ± 8.7 years	-	38.6	-	46.7	31.2	36[a]	20[a,b]	20[b]	78.4[b]
Kagaruki et al., 2014/Cross sectional study Tanzania	HIV infected on ARV –354 people Males- 32.2 %	38.5	61.1		61.7	3.7	30	76.6	67	43.6
	HIV infected ARV naïve –317 people Males- 26.5 %	61.5	38.9		38.3	4.7	21.9	28.4	33	56.4
	p-values	0.99	=0.010		<0.001	-	=0.010	<0.001	=0.001	<0.001
Abebe et al., 2014/ Cross sectional Ethiopia	HIV infected ARV naïve –126people Males- 23.8% Mean age – 33.4±10.2years							11.1	31	73
	HIV infected on ARV –126people Males- 31.7 % Mean age – 37.1 ±9.8years							42.1	46.8	50.8
	p-values							<0.0001	<0.01	<0.0001

Author/ study design	Study population	Smoking	Obesity	overweight	Abdominal obesity	DM	HTN	Dyslipidaemia		
								High TC	High TG	Low HDLC
Bloomfield et al., 2011/ Retrospective Cross sectional Kenya	HIV infected males (ARV treated and ARV naïve) – 4293 Mean age – 43 (37-49) years	-	11	-	-	-	11	-	-	-
	HIV infected females (ARV treated and ARV naïve) – 7901 Mean age – 40(34-47) years	-	23	-	-	-	7	-	-	-
Hilleman et al., 2014/Cohort USA	HIV infected ARV naive -47 people Males – 70% Mean age – 40[25-50]years AA –70%, C –28%, others-2 %	72	-	-	-	-	-	-	-	-
	HIV uninfected infected -41 people Males – 68 % Mean age – 37[25-49] years AA –66%, C –34%, others-0 %	15	-	-	-	-	-	-	-	-
	p-values	0.001								
Berhane et al., 2012/Cross sectional Ethiopia	HIV infected on ARV –313people Males- 34.8 %	-	-	-	Males-0 Females-9.3	-	35.1		39	Male-36.7 Female-53.4
Julius et al., 2011/Cross sectional study South Africa	HIV infected on ARV –304people Males- 34.8 %	-	-	-	-	1.3	19.1	32.2	15.8	45.7
Diouf et al., 2012/ Cross-sectional Senegal	HIV infected on ARV –242 people Median age-46 years	-	-		-	14.5	28.1	-	-	-

AA African American, C causations, H Hispanic, ARV antiretroviral, HIV human immunodeficiency virus, TC total cholesterol, TG triglyceride, HDL- high density lipoprotein cholesterol, DM diabetes mellitus type 2, HTN hypertension, NS non-significant, [a] $p<0.05$ vs ARV naïve, [b]$p<0.05$ vs HIV ARV treated, [c]$p<0.05$ vs ARV naïve *$p<0.05$,- not-reported '– '.

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CHAPTER 3

METHODOLOGY

3.1 INTRODUCTION

The present study comprised of two phases. The first phase was a cross-sectional sub-study of the project “Prevention, Control and Management of Chronic diseases in a rural population, South Africa” in which a random sample of 815 people participated. Participants who tested HIV positive following pre-counselling and were not on ARV treatment according to a questionnaire formed the cases. Participants who tested HIV negative formed the controls. The second phase was a cross-sectional study conducted in the Primary Health Care clinics, Seobi-Dikgale, Sebayeng and Dikgale situated within the Dikgale HDSS site. The study was approved by the MEDUNSA Research Ethics Committee (MREC) (Appendix I and Appendix II), the Department of Health Provincial office (Appendix III) and Capricorn district office (Appendix IV).

3.2 STUDY AREA

The study was conducted in Dikgale Health and Demographic Surveillance System (HDSS) site, a rural area located in the Capricorn District, Limpopo Province. Dikgale HDSS is situated 20-40km from University of Limpopo and about 70 kilometers to the Northeast of Polokwane, the capital city of Limpopo Province. The site consists of 15 villages which have population of approximately 35000 people. Dwellings in Dikgale HDSS are a mix of shacks, conventional brick houses and traditional mud huts. In addition to Dikgale HDSS site, the following active Primary Health Care clinics, within Dikgale HDSS site, Seobi-Dikgale, Sebayeng and Dikgale were included in the study.

3.3 SAMPLE SIZE CALCULATION

Phase 1 of study

The sample size for Phase 1 was determined using the formular for difference in proportions between cases and controls as given below:

$$n = \left(\frac{r+1}{r}\right)\frac{(\bar{P})(1-\bar{P})(Z_{\beta}+Z_{\alpha/2})^2}{(P_1-P_2)^2}$$

Where: n= sample size in the case group

r= ratio of controls to cases

$\bar{p}$ =mean proportion

Z_{β} = Required power normally 0.84 for 80%.

$Z_{\alpha/2}$ = Desired level of statistical significance, normally 1.96.

Therefore considering the prevalence of one of CVD risk factors such as hypercholesterolemia in the general population to be 20% and the prevalence of hypercholesterolemia in ARV naïve HIV infected people to be 6.9% (Carey et al., 2013), the required sample size to show a difference between the cases and controls with a power of 80%, 5% level of significance and a ratio of one ARV naïve HIV infected (case) to two HIV negative (controls) people was calculated as:

Number of cases = $1.5\ \frac{(0.1345)(0.8655)(7.84)}{(0.131)^2}$

= 80 cases

A 10% allowance was considered and the number of cases required was 88 cases. The number of cases obtained from screening of population was 89 and all were included in the study. The corresponding controls included in the analysis were 178 people.

Phase 2 of study

The sample size for Phase 2 was obtained using the formular for proportion in a cross-sectional study as given below:

n = $\frac{Z^2\ P\ (1-P)}{d^2}$ (Naing et al., 2006)

Where:

n= sample size.

Z= a statistic for 95% confidence interval is 1.96.

P= expected proportion.

d= the maximum permissible difference between sample proportion and population proportion calculated as 20/100 x P (Naing et al., 2006).

According to literature the proportion of hypercholesterolemia in ARV treated ranges from 31.0 % to 36.0% (Muhammad et al., 2013, Edward et al., 2013, Carey et al., 2013). Considering a proportion of 36%, and a corresponding maximum margin of error of 7%, sample size was calculated as:

Sample size = $\frac{(1.96)^2\ 0.36\ (1-0.36)}{(0.07)^2}$

= 181 people

A 10% allowance was considered and the expected sample size was 200 people. A total of 214 people participated in the study.

3.4 STUDY PHASE ONE

3.4.1 Study design and settings

This was a cross sectional study in which the treatment naïve HIV infected and the HIV negative people were compared. The study utilised laboratory based methods for producing quantitative data and a standardised STEP questionnaire for collection of data on risk factors of CVD. The study was conducted in Dikgale Health and Demographic Surveillance System (HDSS) site.

3.4.2 Study participants

The study participants from the project on “Prevention, Control and Management of Chronic disease project in a rural population, South Africa” who tested HIV positive

following pre-counselling and were not on ARV treatment according to a questionnaire formed cases (89). The controls (178) matched for gender and age (± 2 years) were randomly selected from those who tested negative for HIV. Pregnant women were excluded from the study. A sub-group of non-diabetic treatment naïve HIV infected people (70) and corresponding age (± 2years) and gender matched controls (140) were included in the 10-year CVD risk estimation.

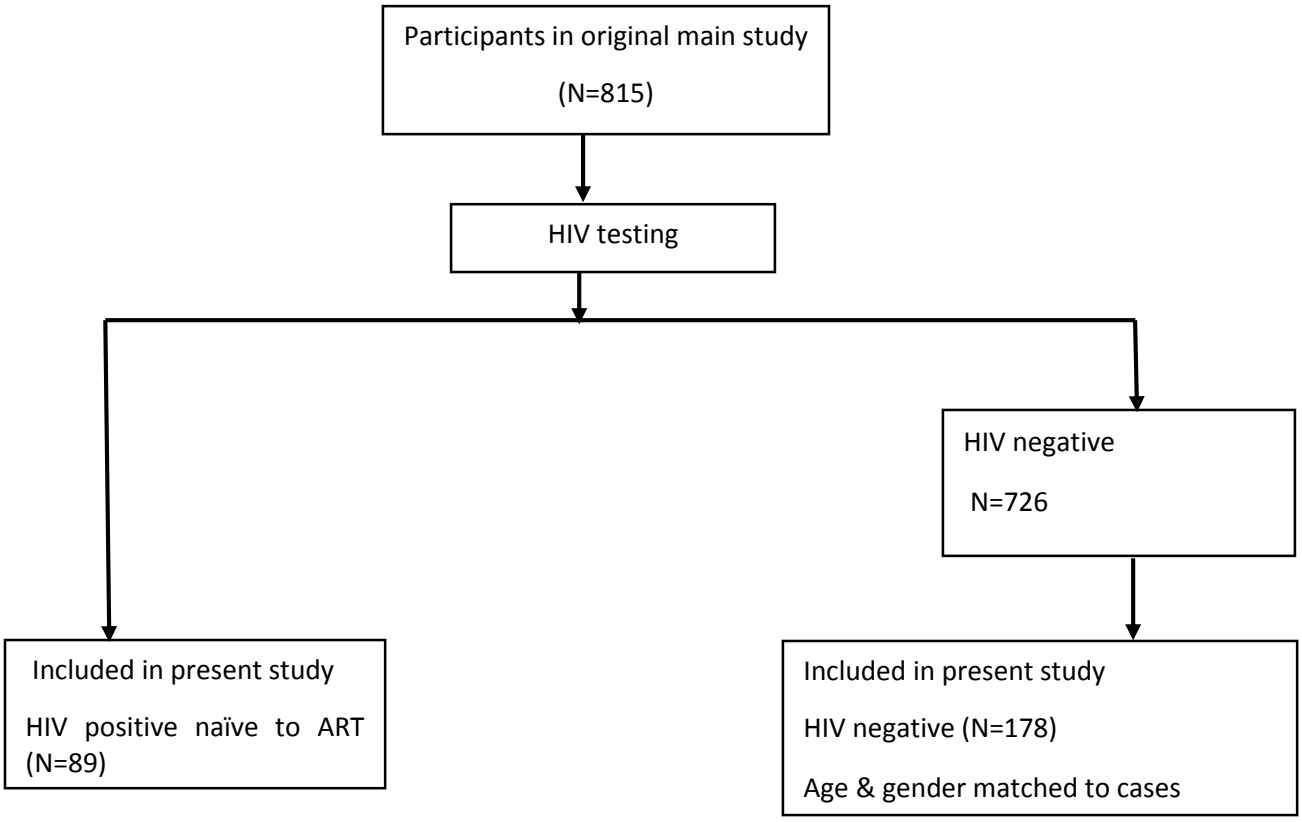

Figure 3. 1: Sample Flow Chart

Written informed consent was obtained from all participants and guardians of minors (<18years) prior to the study commencement (Appendix V). The study participants were aged 15 years and above, both males and females.

3.4.3 Experimental protocol

The study was conducted from August 2011 to February 2012. Data for the treatment naïve HIV infected and HIV negative people was extracted from the main study database. This included fruit and vegetable intake, physical activity and demographic data, anthropometric measurements, blood pressure measurements and biochemical test results.

3.5 STUDY PHASE TWO

3.5.1 Study design and settings

The study was cross sectional and utilised laboratory based methods for producing quantitative data and standardised STEP questionnaire for collection of data on risk factors of CVD. The study was conducted in the Dikgale HDSS site active Primary Health Care clinics namely Seobi-Dikgale, Sebayeng and Dikgale.

3.5.2 Study participants

The study participants were recruited to participate in the study by health professional nurses who had received prior information regarding the study (Appendix VI). At least 74 people with HIV infection are treated at Seobi-Dikgale clinic, while 373 people and 377 people with HIV infection are treated at Sebayeng and Dikgale clinics respectively. Based on these databases, 20 people were recruited from Seobi-Dikgale, 96 people from Sebayeng and 98 from Dikgale clinics. A total of 214 people on ART participated in the study.

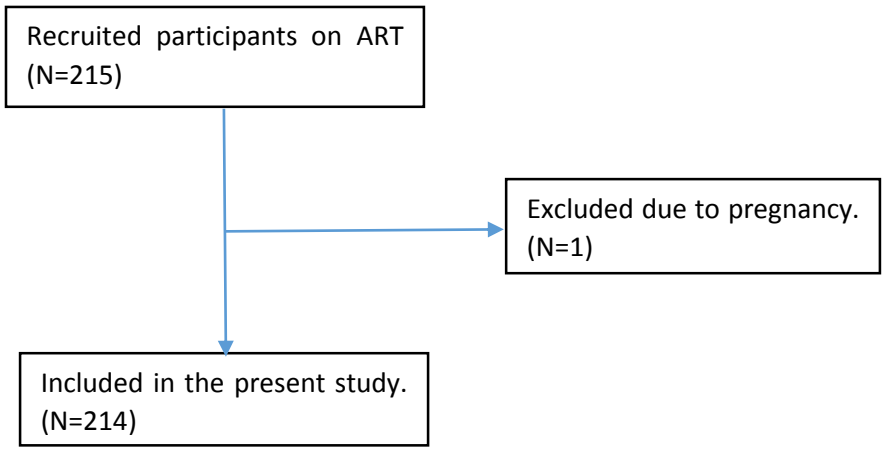

Figure 3. 2: Sample Flow Chart

Written informed consent was obtained from all participants and guardians of minors (<18years) prior to the study commencement (Appendix V). The study participants were aged 15 years and above, both males and females.

3.5.3 Experimental protocol

The study was conducted between November 2013 and April 2014. HIV infected people on ART, received information regarding the study, from the healthcare professional nurses. During one month all HIV infected people who came to collect their ART were asked to participate in the study. Those willing to participate were advised on the participation dates, and time (7:00am) at their clinic, a week in advance. Participants were also advised to fast overnight in order to provide a fasting blood sample. On the scheduled date, participants and guardians of minors (<18years) received information regarding the study from the researcher (Appendix VI) and were requested to complete consent forms (Appendix V). Consenting participants went through completion of questionnaire, blood pressure measurements, anthropometric measurements and blood collection. Individual results were sent back to the Primary Health Care clinic. Participants who could not come on the scheduled date were visited in their homes on an arranged date and participated.

3.6 INCLUSION AND EXCLUSION CRITERIA

HIV infected people on ART, ART naïve HIV infected people and HIV negative people matched for gender and age were included in the study. Pregnant woman were excluded from the study. Based on demographic information, (provided in patient information file) people from urban areas attending the ARV clinics within Dikgale HDSS site were excluded. People with diabetes mellitus were excluded from the analysis of lipoprotein subclasses. In addition people who were less than 20 years old, had a history of cardiovascular and diabetes were excluded from Framingham score estimation.

3.7 DATA COLLECTION

Uniform data collection instruments, blood collection procedures and laboratory testing methods were used in both phase 1 and 2 of the study.

3.7.1 WHO STEP Questionnaire

Data on medical history, physical activity, fruit and vegetable intake, alcohol and tobacco use was collected using the standardized WHO STEP questionnaire (WHO) (Appendix VII). TB and HIV information was obtained using additional questionnaire (Appendix VIII).

3.7.2 Anthropometric measures and blood pressure

Weight was measured using Omron BF 400 (Omron Healthcare, Japan). Subjects were required to take their shoes and heavy coats off. The weight was measured to the nearest 0.1 kg. Height was measured with a stadiometer. The subjects were requested to take their shoes off and to stand in an upright position. The height was measured to the nearest 0.1cm. Height and weight were used to determine body mass index (BMI). BMI was calculated as weight (kg)/ height (m^2). BMI was considered as normal (18.50-24.99), overweight (25.00-29.99) and obese (≥30.00) (WHO 1995).

Waist and hip circumferences were measured using a measuring tape. Waist circumference was measured around the area between the last rib and the hip bone, while the hip circumference was measured around the widest part in the gluteal area. Both parameters were measured to the nearest 0.1cm and were used to calculate the waist to hip ratio.

Blood pressure was measured using the Omron M5-1 (Omron Healthcare, Japan).The subjects were requested to relax for five minutes before the first measurement was taken and were also restricted from talking during measurement procedure. Three measurements were taken with few minutes break in between measurements. Pulse rate for the corresponding blood pressure reading was also recorded. The mean of the last two values were calculated for systolic blood pressure, diastolic blood pressure and pulse rate. High blood pressure was defined as a systolic blood pressure (SBP) equal/or above 140mmHg and/or a diastolic blood pressure (DBP) equal/or above 90mmHg (Mancia et al., 2013).

3.7.3 Blood collection

Fasting venous blood samples were drawn by registered nurses. Whole blood was used to measure CD4 counts on the day of collection. Serum from clotted blood and plasma from whole blood were separated through centrifugation at 2000rpm for 15minutes. HIV status and glucose were analysed soon after centrifugation using plasma from an EDTA and sodium fluoride tubes, respectively. The remaining samples were stored at -80^{o}C until analysis.

3.7.4 Laboratory Procedures

3.7.4.1 Biochemical parameters were analysed using standard biochemical methods (Appendix IX).

3.7.4.2. Determination of Lipoprotein subclasses in plasma

Lipoprotein subclass particle sizes and proportions were determined using Polyacrylamide Gradient Gel Electrophoresis. The PAGGE method is not standard and different studies have used gels of varying sizes, concentrations and conditions to separate lipoproteins subclasses (Pascot et al., 2002, Fonda et al., 2003, Goedecke et al., 2010, Rosenbaum et al., 2012). Therefore the estimated number of LDL and HDL subfractions is method dependent. The current study used pre-cast gels of 4-16% and used the running conditions as stated in manufacturer's instructions.

The method is based on the electrophoretic mobility of lipoprotein subclasses which is a function of macromolecular charge, size and shape. In the 4-16% Native PAGE Novex Bis-Tris Gel System (Invitrogen, United States of America), lipoproteins of different sizes are sieved by the acrylamide gel with uniform electric strength, and the mobility of lipoproteins is inversely proportional to the size. In the Native PAGE Novex Bis-Tris Gel (Invitrogen, United States of America), lipoproteins migrate from lower to the higher concentration of a polyacrylamide gradient in a slab gel. As the gel pores decrease in size, the migration rate of lipoproteins also decreases. The lipoproteins reach their respective pore limits determined by their molecular sizes and form sharp bands.

Polyacrylamide Gradient Gel Electrophoresis (PAGGE) was performed using a NativePAGE Novex 4-16% Bis-Tris gel, NativePAGE running buffer, NativePAGE sample buffer (4X), NativePAGE standard according to the manufacturer instructions (Invitrogen, United States of America). The gel was run on the X-Cell SureLock Mini Vertical cell system (Invitrogen, United States of America). The NativePAGE standard was run parallel to samples and internal serum control. The standard comprised of protein markers of known diameters. These markers included IgM Pentamers (30nm) (Cheesbro et al., 1968), apoferitin (13nm) (Henderson and McMullan 2013,), B-phycoerythrin (10.1nm) (Grant 1969), lactate dehydrogenase (8.16nm) and bovine serum albumin (7.1nm) (Rosenbaum et al., 2012), soybean trypsin inhibitor (3.5nm) (Sweet et al., 1974).

Five microlitres of thawed serum and protein standard were each mixed with 2.5µl of Native PAGE Sample Buffer (4X) and 2.5µl of distilled water. The running buffer was prepared by mixing 50ml Native PAGE 20X running buffer with 950ml of distilled water. Using a pair of scissors, the 10well 4-16% Native PAGE gel was removed from gel cassette pouch. The tap at bottom of gel cassette and the comb were removed from gel cassette. The gel wells were rinsed by filling wells with running buffer (1X Native PAGE), inverting the gel and shaking the buffer out of the wells. The wells were rinsed twice and all air bubbles were removed. The 4-16% Native PAGE gel was mounted onto the X-Cell SureLock Mini Cell system. Ten microlitres protein standard and sample preparations were loaded into respective gel wells using a 10-100 µl Thermo Scientific finnpipette and gel loading tips. The wells were filled to the top with buffer. The gel was made to seat on the bottom of the cassette facing inwards towards buffer core and was locked into place using gel tension wedge. The upper (inner) buffer chamber was filled with ~200ml running buffer and checked for tightness of seal. The buffer level had to exceed the level of the wells. The lower (outer) buffer chamber was filled with ~600ml of running buffer. The (-) and (+) electrodes were properly aligned and the lid pressed down to firmly close the Mini-Cell system. The electrode cords were connected to the power supply and the power was turned on. The gel was run at 4^{o}C with initial voltage of 150 V constant for 60 minutes, then the voltage was increased to 250 V constant for 30 minutes. After electrophoresis, the power was switched off and gel cassette removed from the mini cell system. The gel was removed from gel cassette by breaking the bonds around cassette using gel knife. The gel portion containing the protein standard bands was separated from the sample containing gel portion by cutting the gel using the gel knife. The protein standard gel portion was fixed using 100ml of fixing solution (40% methanol, 10% acetic acid) and microwaving on high (950watts) for 45seconds. After microwaving, the gel was shaken on orbital shaker for 15 minutes at room temperature. The fixing procedure was repeated once. The fixing solution was decanted and the gel was stained overnight using Coomassie G-250 stain. The sample gel portion from electrophoresis was immediately placed in the Sudan black B staining solution (0.5g Sudan black B in 20ml acetone, 15ml acetic acid and 85ml distilled water), and stained overnight. The protein standard gel portion was destained in 8% acetic acid for 5 minutes on orbital shaker. The Sudan black

B stained gel was destained in three changes of destain solution (15ml acetic acid, 20ml acetone and 65ml distilled water). The two gel portions were placed side by side on scanner and were scanned.

For quantification, the ImageScanner III (GE Healthcare, Sweden) was first calibrated using the Step Tablet serial number 56112885 (GE Healthcare, Sweden). Scanning was performed on ImageScanner III at 632nm using the Labscan 6.0 software comprising of an IQTL (ImageQuant TL) converter (GE Healthcare, Sweden). The scanned images were converted to an IQTL file and analysed by 1D IQTL software. The particle sizes were derived from standard curve and particle proportions calculated as area under the curve (AUC) using IQTL software. The AUC for both small-HDL-p and large-HDL-p was determined using the cut-off particle size of 8.7nm, while the AUC for large-LDL-p was between 26-30nm while small-LDL-p was less than 26 nm. The inter-assay CV for the four lipoprotein subclass proportion ranged between 4.9% -10.0 %, while the intra- assay CV was from 0.78 %-7.3 %. A scanned gel image is shown in Figure 3.1.

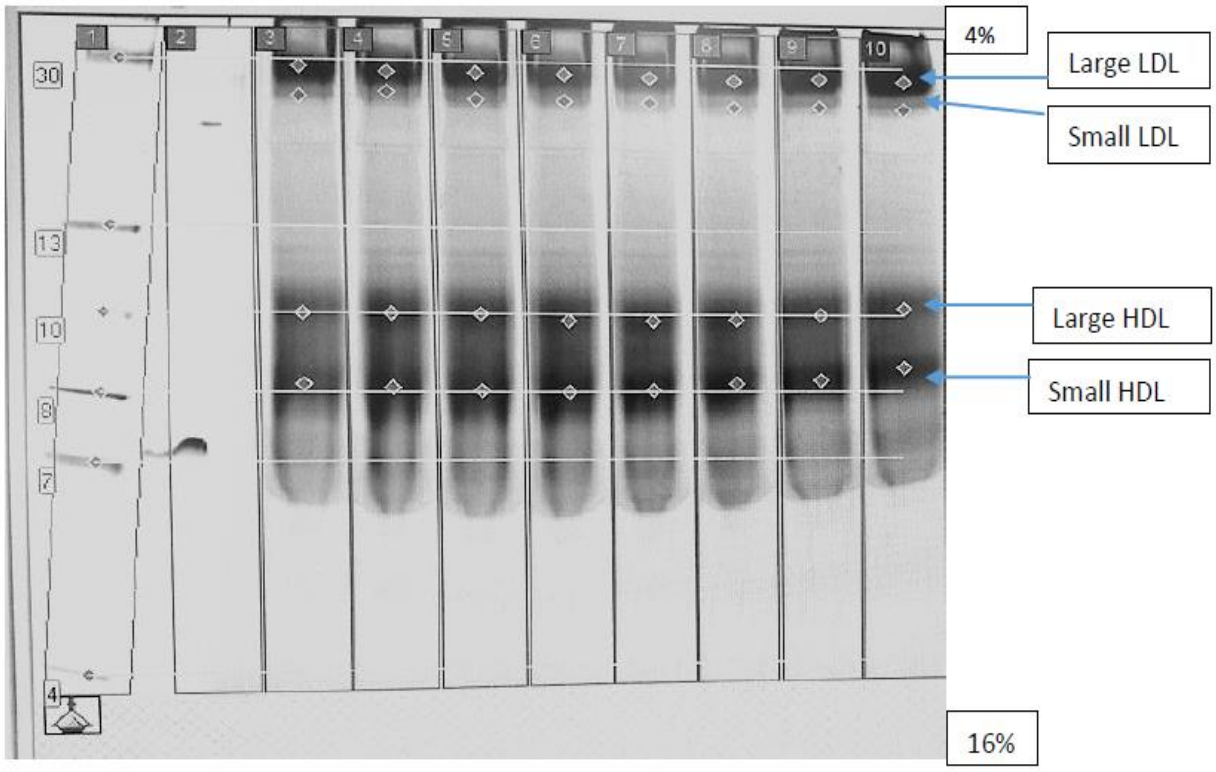

Figure 3.3: Image showing the bands for the LDL (large and small) and HDL (large and small) particles on a 4-16% polyacrylamide gradient gel.

Lane 1: protein standard, Lane 2: empty, Lane 3: control serum; Lane 4-10 serum samples

3.8 RELIABILITY AND VALIDITY

In all the procedures listed above, normal and abnormal controls were run pararell to test samples to ensure reliability of the results. The ILab 300, IMMAGE, and Access machines were calibrated prior to sample analysis. An HIV kit included a positive control. The PIMA analyser software checked the sample volume and expiry date of test catridges. The low CD4 count and normal CD4 count catridges were used as controls. The image scanner III for quantification of lipoprotein subclasses was calibrated using the Step Tablet serial number 56112885 (GE Healthcare, Sweden). The protein standards with diameter size ranging from 3.5nm to 30nm were ran pararell to samples on the Native PAGE gel to ensure that separation of lipoprotein subclasses occurs and for determination of diameter size of lipoprotein subclasses.

The intra-assay coefficient of variation (CV) was obtained by measuring one sample three times in one run, while the inter-assay CV was obtained by measuring one sample three times on separate runs. Coefficient of variation is calculated as follows:

CV = SD x 100 / mean – where SD is standard deviation.

A CV of less than 10% is acceptable. Intra and inter assay CVs are presented in Table 3.1.

Table 3. 1: Coefficient of variation (CV) intra and inter assays

Variables	Intra-assay CV (%)	Inter-assay CV (%)
TC	1.9	4.0
HDL-C	1.6	2.8
TG	1.3	2.9
ApoB	0.7	0.8
ApoA	1.1	3.1
HsCRP	1.1	4.9
Glucose	0.2	0.6
Lp (a)	2.0	3.4
CD4 count	0.4	1.3
Lipoprotein subclasses	0.78-7.3	4.9-10.0

TC total cholesterol, HDL-C high density lipoprotein cholesterol, TG triglycerides, Apo apolipoprotein, HsCRP high sensitivity C-reactive protein, Lp (a) lipoprotein (a)

Interpretation of variable values was based on the reference ranges given in Table 3.2.

Table 3. 2: Reference ranges (RR) for variables

Variables	**Below RR**	**Normal RR**	**Above RR**
SBP (mmHg)		< 140	
DBP (mmHg)		< 90	
WC (cm)		females ≤ 88 males ≤ 102	
BMI (kg/m^2)	≤ 18.49	18.50 – 24.99	≥ 25
TC (mmol/l)		< 5	≥ 5
HDL-C (mmol/l)	females ≤ 1.3 Males ≤ 1.1	females >1.3 males > 1.1	
LDL-C (mmol/l)		< 3	≥ 3
TG (mmol/l)		< 1.7	≥ 1.7
TC/HDL-C		< 5	≥ 5
TG/HDL-C		<1.49	≥1.49
Apo A (mg/dl)		females 107-214 males 90-170	
ApoB (mg/dl)	females <51 males < 56	females 51-171 males 56-162	females >171 males >162
ApoB/ApoA		< 0.68	≥ 0.68
Lp(a) (mg/dl)		< 30	≥ 30
Glucose (mmol/l)		< 7.0	≥ 7
HsCRP (mg/l)		< 3	≥3
CD4 count (cells/mm^3)	< 500	500-1000	>1000

SBP systolic blood pressure, DBP diastolic blood pressure, WC waist circumference, BMI body mass index, TC total cholesterol, HDL-C high density lipoprotein cholesterol, LDL-C low density lipoprotein cholesterol, TG triglycerides, Apo A apolipoprotein A, Apo B apolipoprotein B, Lp(a) lipoprotein (a), hsCRP high sensitivity CRP,

3.9 STATISTICAL ANALYSIS

Statistical analysis was performed with Statistical Package for Social Science version 22 software. Data set was checked for incorrectly entered values and outliers by sorting each variable values in ascending order. Variables were tested for normality using frequency

histograms and line graphs. Data not normally distributed was logarithmically transformed for analysis.

The Chi-squared test was used for categorical variables. Data was presented in cross-tabs as percentages. Comparison of anthropometric measurements and biochemical parameters was performed using the independent Student t-test. Normally distributed data is presented as mean ± standard deviation, while data not normally distributed is presented as median [interquartile range].

Bivariate correlation was used to determine associations of HIV markers (CD4 count and viral load) with factors associated with cardiovascular disease. Data is presented as correlation coefficient (r) and p-value.

Univariate logistic regression analysis was used to determine possible candidate predictors of CVD risk factors (categorical risk factors). Covariates that were significant at p≤0.25 (Soboka et al., 2014) in univariate models were considered as candidates for inclusion in the 1st multivariate model. Two way interactions that were significant at p≤0.05 were included in the 1st multivariate model. Covariates that were significant at p≤ 0.25 and interactions that were significant at p≤0.05 from 1st model were included in the 2nd model. Covariates that were significant at p≤0.25 in the 2nd model were included in the 3rd model. The model with the highest classification was chosen to explain the association of variables with the outcome variable.

Univariate linear regression analysis was used to determine possible candidate predictors of CVD risk factors (continuous risk factors). Covariates that were significant at p≤0.25 (Soboka et al., 2014) in univariate models were considered as candidates for inclusion in the multivariate linear backward modelling. The first and the last models are reported. The level of significance for statistical analysis was set at p value less than 0.05.

Framingham 10-year CVD risk estimation was calculated for each participant over the age of 20 years having no heart disease or diabetes, by entering the following variables: age, gender, TC, HDL-C, SBP, smoking status and current treatment for high blood pressure, as required by the Framingham risk model tool (NIH 2013). Participants were regarded as low risk, moderate risk, or high risk when the risk score for developing CVD

in 10 years was <10%, 10-20% or >20% respectively (Reinsch et al., 2011). Variables included in the 5-year DAD risk estimation tool were age, sex, SBP, TC, HDL-C, diabetes mellitus, smoking status, family history of CVD, current use of abacavir, indinavir, or lopinavir and duration on indinavir and lopinavir [HIVPV 2007]. The risk of developing coronary heart disease in the next 5-years was regarded as low (<1%), moderate (1 to 5%), high (5 to 10%), or very high (>10%) [Friis-Møller 2010]. The level of agreement between DAD and Framingham risk equations was determined using Cohen's Kappa coefficient with 95% CI. For comparison with Framingham, participants with high and very high scores according to the DAD equation were combined and considered as high risk group. Kappa coeffient was interpreted as poor agreement (<0), slight agreement (0.0-0.20), fair agreement (0.21-0.40), moderate agreement (0.41-0.60), substantial agreement (0.61-0.80) and perfect agreement (0.81-1.00) [Landis and Koch 1977].

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CHAPTER 4

RESULTS

4.1. INTRODUCTION

This chapter is divided into three sections as follows:

An overview of descriptive data and CVD risk factors among the HIV negative, ART naïve HIV positive and HIV positive participants on ART .

Results from Phase 1 of the study.
In this section, results of socio-demographic data, anthropometric measurements, biochemical parameters and cardiovascular risk factors in ARV naïve HIV infected participants are presented. In addition the 10-year CVD risk was estimated in 54 ARV naïve HIV infected participants who fulfilled the Framingham criteria. Results for age and gender matched HIV negative controls are included. Data is presented as numbers (percentages), normally distributed data is presented as mean ± standard deviation, and not normally distributed is presented as median (interquartile range).

Results from Phase 2 of the study.
This section presents results of socio-demographic data, anthropometric measurements, biochemical parameters and cardiovascular risk factors in ARV treated HIV infected participants. In addition the 10-year CVD risk was estimated in 164 ARV treated HIV infected participants who fulfilled the Framingham criteria. Data is presented as numbers (percentages), normally distributed data is presented as mean ± standard deviation, and not normally distributed is presented as median (interquartile range).

4.2 OVERVIEW OF STUDY RESULTS

An overview of characteristics for HIV negative, ART naïve and ART HIV infected participants is presented is presented in Table 4.1.

Table 4. 1: Characteristics of study participants from the Phase 1 and Phase 2 of the study

	Variables	**HIV Negative participants N=178**	**ART naïve HIV infected participants N= 89**	**ART HIV infected participants N= 214**
Mean age (years±SD)		49.7±16.6	49.7±16.8	44.8±11.8
Marital status n (%)	Not married	87 (48.9)	51 (57.3)	164 (76.6)
	Married	91 (51.1)	38 (42.7)	50 (23.4)
Education level n (%)	Primary	119 (66.9)	55 (61.8)	114 (53.3)
	Secondary	49 (27.5)	33 (37.1)	98 (45.8)
	University	10 (5.6)	1 (1.1)	2 (0.9)
Work status n (%)	Unemployed	129 (72.5)	68 (76.4)	149 (69.6)
	Retired	30 (16.9)	11 (12.4)	17 (7.9)
	Employed	19 (10.6)	10 (11.2)	48 (22.4)
Tobacco use n (%)	No	151 (84.8)	73 (82.0)	169 (79.0)
	Yes	27 (15.2)	16 (18.0)	45 (21.0)
Alcohol use n (%)	No	143 (80.3)	63 (70.8)	167 (78.0)
	Yes	35 (19.7)	26 (29.2)	47 (22.0)
Physical activity (%)	Normal≥ 600MET-min	44 (24.7)	23 (25.8)	119 (75.8)
	Low <600MET-min	134 (75.3)	66 (74.2)	38 (24.2)
Fruit & Vegetable intake (%)	≥ 5 servings/day	33 (18.5)	14 (15.7)	7 (4.5)
	< 5 servings/day	145 (81.5)	75 (84.3)	150 (95.5)

ARV antiretroviral, MET-min metabolic equivalent of task-minutes

In the present study, people on ART were younger than the ART naïve HIV infected people (44.8±11.8 vs 49.7±16.8 years). The socio-demographic factors were similar among the HIV negative people, ART naïve HIV infected people and those on ART. However, the prevalence of HIV negative and ART naïve HIV infected married people was nearly double that of people on ART. The prevalence of employed people was twice

as high in those on ART as in ART naïve HIV infected and in uninfected people. The prevalence of physically active people was three times higher in people on ART than in ART naïve HIV infected and in uninfected people (Table 4.1).

An overview of CVD risk factors in HIV negative, ART naïve and ART HIV infected participants is presented in Figure 4.1.

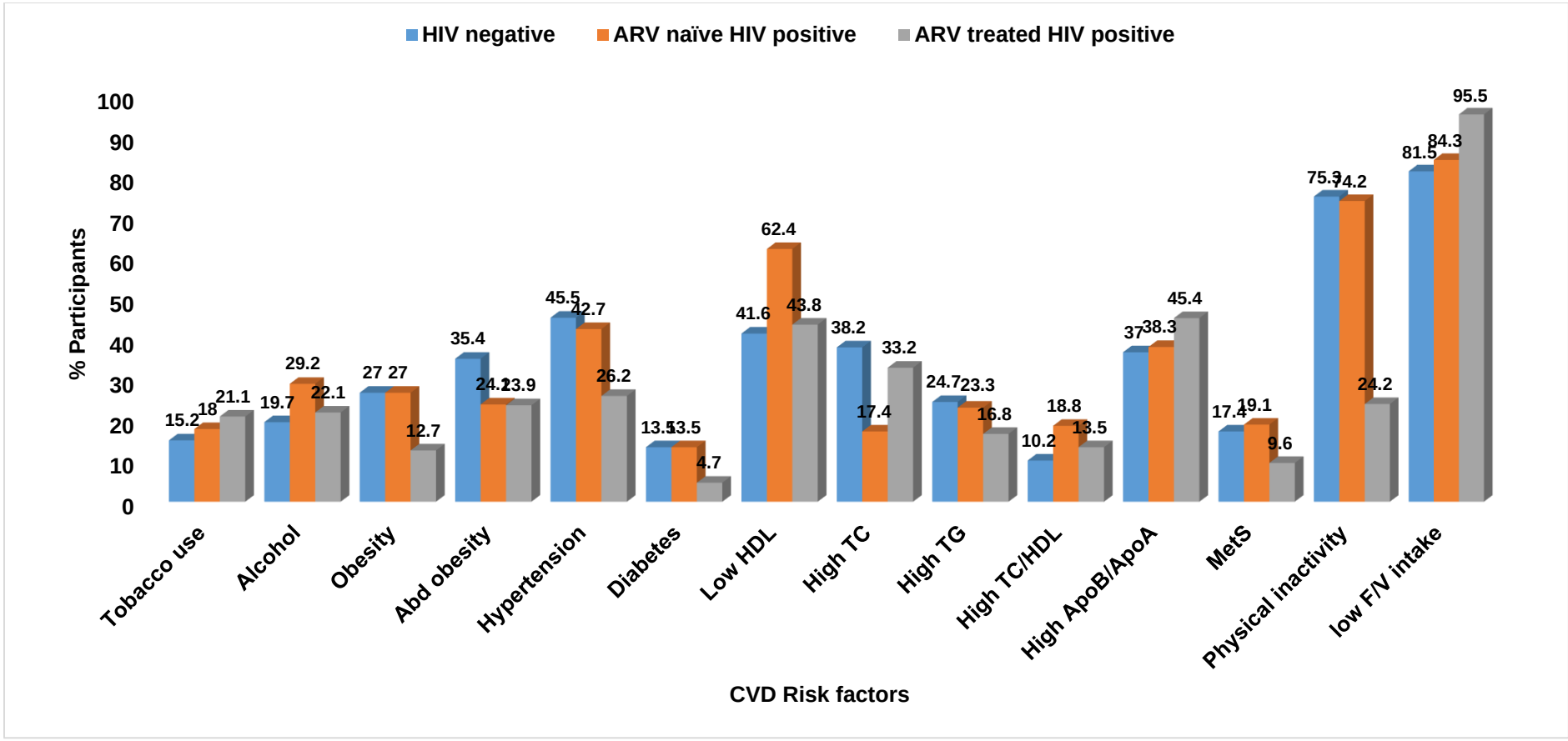

Figure 4. 1: CVD risk factors for HIV negative, ART naïve and ART HIV infected participants

An overview of Lipoprotein subclasses in HIV negative, ART naïve and ART HIV infected participants is presented in Figure 4.2.

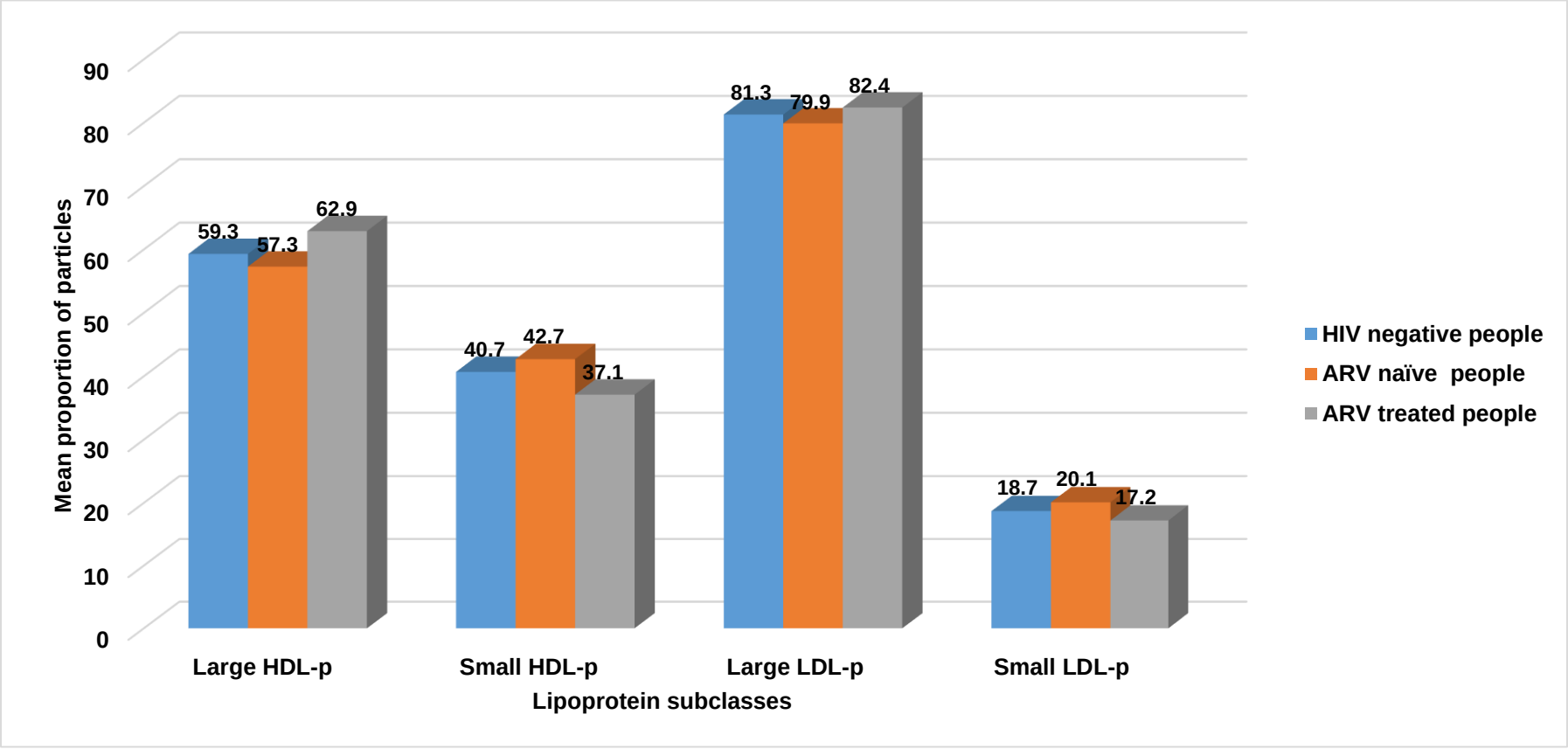

Figure 4. 2: The distribution of lipoprotein subclasses in HIV negative, ART naïve HIV infected and ART treated HIV infected people

The prevalence of tobacco and alcohol use, hypertriglyceridaemia, a high TC/HDL-C ratio, a high ApoB/ApoA ratio and low fruit and vegetable intake was similar among HIV negative people, ART naïve HIV infected people and people on ART. The prevalence of low HDL-C concentration and hypercholesterolaemia was similar between HIV negative people and people on ART, while the prevalence of low HDL-C concentration was higher and hypercholesterolaemia was lower in ART naïve HIV infected people compared to the other two groups. The prevalence of obesity, abdominal obesity, hypertension, metabolic syndrome and physical inactivity was higher in both ART naïve HIV infected and uninfected people compared to those on ART (Figure 4.1).

The proportion of large and small LDL and HDL particles was similar in HIV negative and in people on ART. ART naïve HIV infected people had a lower proportion of the large LDL and HDL particles and had a higher proportion of the small LDL and HDL particles compared to both HIV negative and those on ART (Figure 4.2).

4.3 PHASE 1 RESULTS

4.3.1 Socio-demographic data

Socio-demographic characteristics of ART naïve HIV infected and HIV negative participants are presented in Table 4.2.

Table 4. 2: Socio-demographic characteristics in ART naïve HIV infected and HIV negative participants by gender

Variables		**HIV Negative participants N=178**	**ART naïve HIV infected participants N= 89**	**P-value**	**Females**			**Males**		
					HIV Negative N=126	**ART naïve HIV infected N=63**	**p-value**	**HIV Negative N=52**	**ART naïve HIV infected N=26**	**p-value**
Mean age (years ± SD**)**		49.7 ± 16.6	49.7 ± 16.8	0.98	47.7±15.5	47.6 ± 15.6	0.96	54.5 ± 18.3	55.0 ±18.8	0.91
Marital status n (%)	Not married	87(48.9)	51 (57.3)	0.24	60 (47.6)	40 (63.5)	**0.045**	27 (51.9)	11 (42.3)	0.48
	Married	91 (51.1)	38 (42.7)		66 (52.4)	23 (36.5)		25 (48.1)	15 (57.7)	
Education level n (%)	Primary	118 (66.3)	55 (61.8)	0.94	80 (63.4)	37 (58.7)	0.93	38 (73.1)	18 (69.2)	0.80
	Secondary	49 (27.5)	32 (36.0)		39 (31)	26 (41.3)		10 (19.2)	6 (23.1)	
	University	11(6.2)	2 (2.2)		7 (5.6)	0 (0)		4 (7.7)	2 (7.7)	
Work status n (%)	Unemployed	128(71.9)	67(75.3)	0.76	95 (75.4)	50 (79.4)	0.81	33 (63.5)	17 (65.4)	0.84
	Retired	29(16.3)	11(12.4)		19 (15.1)	6 (9.5)		10 (19.2)	5 (19.2)	
	Employed	21(11.8)	11(12.3)		12 (9.5)	7 (11.1)		9 (17.3)	4 (15.4)	
Tobacco use n (%)	No	151(84.8)	73(82.0)	0.60	116 (92.1)	55 (87.3)	0.30	35 (67.3)	18 (69.2)	1.00
	Yes	27(15.2)	16(18.0)		10 (7.9)	8 (12.7)		17 (32.7)*	8 (30.8) [#]	
Alcohol use n (%)	No	143(80.3)	63(70.8)	0.09	111 (88.1)	47 (74.6)	**0.02**	32 (61.5)	16 (61.5)	1.00
	Yes	35(19.7)	26(29.2)		15 (11.9)	16 (25.4)		20 (38.5) *	10 (38.5)	
Physical Activity n (%)	>600 MET-min	44 (24.7)	23 (25.8)	0.88	28 (22.2)	14 (22.2)	1.00	16 (30.8)	9 (34.6)	0.80
	Low <600 MET-min	134 (75.3)	66 (74.2)		98 (77.8)	49 (77.8)		36 (69.2)	17 (65.4)	
Fruit & Vegetable n (%)	> 5 servings /day	33 (18.5)	14 (15.7)	0.61	20 (15.9)	8 (12.7)	0.67	13 (25.0)	6 (23.1)	
	< 5 servings /day	145 (81.5)	75 (84.3)		106 (84.1)	55 (87.3)		39 (75.0)	20 (76.9)	1.00

*p-value<0.05 vs HIV negative females (N=126), [#]p-value <0.05 vs ARV naïve HIV infected females. Data is presented as number (percentage), HIV human immunodeficiency virus, ART antiretroviral therapy, MET-min metabolic equivalent of task-minute.

The prevalence of socio-demographic factors such as tobacco use, alcohol consumption, physical inactivity, low fruit and vegetable intake, being unmarried, primary education and unemployment were similar in HIV negative and ART naïve HIV infected participants. Similarly, no difference in socio-demographic factors was observed between HIV negative and ART naïve HIV infected males. However, in contrast a significantly higher proportion of ART naïve HIV infected than HIV negative females were unmarried (63.5% vs 47.6%, p=0.045) and consumed alcohol (25.4% vs 11.9%, p=0.02).

Of the 89 ART naïve HIV infected participants, 26 (29.2%) were males and 63 (70.8%) were females. The mean age of ART naïve HIV infected participants was 49.7±16.8 years. More than half of the ART naïve HIV infected participants (57.3%) were not married and 40 (78.4%) were females. Further analysis of HIV infection and marital status, showed a similar proportion of married and unmarried males with HIV infection, while a higher proportion of unmarried than married (63.5% vs 36.5%, p=0.045) females was infected with HIV. The majority of ART naïve HIV infected participants had only primary education (61.8%) and were unemployed (75.3%). Among the ART naïve HIV infected participants, 18.0% used tobacco and 29.2% consumed alcohol. When stratified by gender, tobacco use was higher in ART naïve HIV infected males than in ART naïve HIV infected females (30.8% vs 12.7%, p<0.05). The proportions of ART naïve HIV infected males and females who were physically inactive, had low fruit and vegetable intake and consumed alcohol were similar.

In the HIV negative control group, 48.9 % of participants were not married. Most of the participants had only primary education (66.9%) and were unemployed (71.9%). The prevalence of tobacco use was 15.2% and alcohol consumption was 19.7%. When HIV negative males and females were considered separately, the proportions of HIV negative males and females who were physically inactive, had low fruit and vegetable intake, consumed alcohol, unemployed, uneducated, and not married were similar. Tobacco use (32.7% vs 7.9%, p=0.001) and alcohol consumption (38.5% vs 11.9%, p=0.001) were higher in males than in females.

4.3.2 Anthropometric measurements and blood pressure.

A comparison of anthropometric measurements in HIV negative and ART naïve HIV infected people by gender is presented in Table 4.3.

Table 4. 3: Anthropometric measurements among ART naive HIV infected and HIV negative participants by gender

	All Participants		**P-value**	**Females**		**P-value**	**Males**		**P-value**
	HIV negative N=178	**ART naïve HIV infected N=89**		**HIV negative N=126**	**ART naïve HIV infected N=63**		**HIV negative N=52**	**ART naïve HIV infected N=26**	
SBP (mmHg)	129.5 ± 21.6	128.2 ± 24.8	0.68	126.8 ± 20.9	127.5 ± 24.3	0.83	136.0 ± 22.1#	129.8 ± 26.4	0.31
DBP (mmHg)	81.8 ± 12.3	81.2 ± 15.8	0.72	81.3 ± 12.5	81.6 ± 16.8	0.91	83.1 ± 11.7	80.0 ± 13.2	0.33
Weight (kg)	68.3 ± 15.7	69.2 ± 17.9	0.70	69.1 ± 15.8	70.6 ± 19.9	0.62	66.4 ± 15.3	65.9 ± 11.0	0.86
Height (cm)	1.60 ± 0.09	1.62 ±0. 09	0.07	1.58 ± 0.08	1.60 ± 0.08	0.10	1.66 ± 0.09	1.68 ± 0.09	0.29
WC (cm)	88.1 ± 14.4	85.4 ± 16.5	0.20	90.0 ± 14.3	87.3 ± 18.1	0.29	83.5 ± 13.5#	80.9 ± 10.7*	0.64
HC (cm)	102.3 ± 14.4	100.7 ± 13.6	0.40	105.8 ±14.1	102.1 ± 15.2	0.11	93.8 ± 11.4	97.3 ± 7.4	0.11
WHR	0.87 ± 0.12	0.84 ± 0.09	**0.03**	0.85 ± 0.11	0.84 ± 0.09	0.36	0.90 ± 0.15	0.83 ± 0.08	**0.02**
BMI(kg/m^2)	26.2 ± 6.5	25.9 ± 7.3	0.71	27.2 ± 6.7	27.0 ± 7.7	0.84	23.7 ±5.4#	23.1 ± 5.2*	0.38

*p-value < 0.05 vs ARV naïve HIV positive females (N=63), # p<0.05 vs HIV negative females (N=126), mean ± standard deviation, HIV human immunodeficiency virus, ART antiretroviral therapy, WC waist circumference, HC hip circumference, WHR waist hip ratio, SBP systolic blood pressure, DBP diastolic blood pressure.

There was no significant difference in mean systolic and diastolic blood pressure, WC, and BMI between ART naïve HIV infected and HIV negative participants, but the WHR was higher ($p<0.05$) in HIV negative (0.87±0.12) than in ART naïve HIV infected participants (0.84 ± 0.09), and remained significantly higher in HIV negative than in ART naïve HIV infected males (Table 4.3).

ART naïve HIV infected males and females had similar mean systolic and diastolic blood pressure. ART naïve HIV infected males had lower body mass index (23.1±5.2 vs 27.0±7.7, $p<0.05$) and waist circumference (80.9±10.7 vs 87.3±18.1, $p<0.05$) than ART naïve HIV infected females (Table 4.3).

The mean systolic blood pressure was significantly higher in HIV negative males than in females (136.0±22.1 vs 126.8±20.9, $p<0.01$), while the mean BMI (23.7±5.4 vs 27.2±6.7, $p< 0.05$) and waist circumference (83.5±13.5 vs 90.0±14.3, $p<0.05$) was lower in HIV negative males than in females (Table 4.3).

4.3.3 Biochemical parameters

Table 4. 4: Biochemical parameters in ART naïve HIV infected people by gender

	All ART naïve HIV infected Participants. N=89	**ART naïve HIV infected females. N=63**	**ART naïve HIV infected males. N=26**	**p-value**
TC (mmol/l)	4.16 ± 1.27	4.27 ± 1.35	3.92 ± 1.02	0.19
HDL-C (mmol/l)	1.17 ± 0.44	1.21 ± 0.46	1.07 ± 0.36	0.15
LDL-C (mmol/l)	2.37 ± 1.10	2.47 ± 1.13	2.15 ± 1.03	0.21
TG (mmol/l)	1.12 [0.78-1.67]	1.15 [0.78-1.52]	1.02 [0.74-2.15]	0.54
TC/HDL-C	3.84 ± 1.34	3.76 ± 1.19	4.02 ± 1.64	0.46
TG/HDL-C	1.06 [0.62-1.62]	1.04 [0.62-1.56]	1.20 [0.82-2.15]	0.26
Apo A (mg/dl)	133.4 ± 33.4	138.5 ± 35.2	121.9 ± 25.8	**0.02**
Apo B (mg/dl)	83.9 ± 24.3	86.3 ± 23.0	78.8 ± 26.7	0.22
ApoB/ApoA	0.67 ± 0.26	0.66 ± 0.26	0.67 ± 0.27	0.87
Lp(a) mg/dl)	27.80 [13.70-54.50]	31.30 [14.53 – 61.03]	19.70 [11.95 – 37.25]	0.17
Glucose (mmol/l)	5.23 ± 1.63	5.11 ± 1.38	5.51 ± 2.13	0.39
hsCRP (mg/l)	1.87 [0.68-5.64]	1.58 [0.61-5.23]	3.79 [0.93-10.03]	0.07
Large LDL-p (%)	79.9 ± 5.4	79.6 ± 5.5	80.4 ± 5.0	0.53
Small LDL-p (%)	20.1 ± 5.4	20.4 ± 5.5	19.6 ± 5.1	0.52
Large HDL-p (%)	57.3 ± 15.5	58.9 ± 14.5	53.5 ± 17.3	0.17
Small HDL-p (%)	42.7 ± 15.5	41.1 ± 14.5	46.5 ± 17.3	0.17
CD4 count (cells/mm^3)	397 ± 192	406 ± 184	365 ± 227	0.59
Viral load (copies/ ml)	3536 [50-18860]	2314 [50-14779]	6728 [818-54878]	0.07

Data expressed as mean ± standard deviation, median [interquartile range]. N total number of participants, HIV human immunodeficiency virus, ART antiretroviral therapy, SBP systolic blood pressure, DBP diastolic blood pressure, TC total cholesterol, HDL-C high density lipoprotein cholesterol, LDL-C low density lipoprotein cholesterol, TG triglycerides, Apo A apolipoprotein A, Apo B apolipoprotein B, Lp (a) lipoprotein (a), hsCRP high sensitivity C-reactive protein, LDL-p low density lipoprotein particle, HDL-p high density lipoprotein particle.

The biochemical parameters of ART naïve HIV infected participants stratified by gender are presented in Table 4.4.

Except for the mean value of apolipoprotein A that was significantly higher in females than in males, all the other parameters were similar in ART naïve HIV infected males and females (Table 4.4).

Table 4. 5: Biochemical parameters in HIV negative and ART naïve HIV infected participants by gender

	All Participants			Females			Males		
	HIV negative N=178	**ART naïve HIV infected. N=89**	**P-value**	**HIV negative N=126**	**ART naïve HIV infected. N=63**	**P-value**	**HIV negative N=52**	**ART naïve HIV infected. N=26**	**P-value**
Age (years)	49.7 ± 16.6	49.7 ± 16.8	0.98	47.7 ± 15.5	47.6 ± 15.6	0.96	54.5 ± 18.3	55.0 ±18.8	0.91
TC (mmol/l)	4.75 ± 1.15	4.16 ± 1.27	**0.001**	4.75 ± 1.21	4.27 ± 1.35	**0.02**	4.74 ± 1.00	3.92 ± 1.02	**0.001**
HDL-C (mmol/l)	1.37 ± 0.35	1.17 ± 0.44	**0.001**	1.36 ± 0.36	1.21 ± 0.46	**0.03**	1.39 ± 0.33	1.07 ± 0.36	**0.001**
LDL-C (mmol/l)	2.76 ± 0.95	2.37 ± 1.10	**0.01**	2.78 ± 0.99	2.47 ± 1.12	0.07	2.69 ± 0.85	2.15 ± 1.03	**0.03**
TG (mmol/l)	1.18 [0.83-1.70]	1.12 [0.78-1.67]	0.88	1.14 [0.79-1.70]	1.15[0.78-1.52]	0.98	1.29[0.88-1.74]	1.03[0.74-2.15]	0.75
TC/HDL-C	3.61 ± 0.99	3.84 ± 1.34	0.17	3.63 ± 1.00	3.76 ± 1.19	0.48	3.56 ± 0.99	4.02 ± 1.64	0.20
TG/HDL-C	0.91 [0.56-1.46]	1.06 [0.62-1.62]	**0.04**	0.95 [0.54-1.46]	1.04 [0.62-1.56]	0.13	0.87 [0.61-1.47]	1.20 [0.82-2.15]	0.16
Apo A (mg/dl)	150.5 ± 32.0	133.4 ± 33.4	**0.001**	149.3 ± 31.4	138.4 ± 35.2	**0.047**	153.4 ± 33.3	121.8 ± 25.8	**0.001**
Apo B (mg/dl)	89.0 ± 27.9	83.9 ± 24.3	0.14	90.3 ± 28.5	86.3 ± 22.9	0.31	85.8 ± 26.3	78.8 ± 26.7	0.28
Apo B/ Apo A	0.61 ± 0.22	0.67 ± 0.26	0.12	0.63 ± 0.22	0.66 ± 0.26	0.35	0.59 ± 0.22	0.67 ± 0.27	0.16
Lp (a) (mg/dl)	37.2[19.3-79.6]	27.8[13.7-54.5]	**0.002**	39.6[20.0-81.1]	31.3[14.5-61.0]	**0.037**	32.2[18.8-74.5]	19.7[12.0-37.3]	**0.029**
hsCRP (mg/l)	2.24 [0.87-5.85]	1.87 [0.68-5.64]	0.90	2.81[0.88-6.99]	1.58[0.61-5.23]	0.14	1.85[0.87-3.17]	3.79[0.93-10.03]	**0.047**
Glucose (mmol/l)	5.79 ± 2.78	5.23 ± 1.63	**0.04**	6.00 ± 3.22	5.11 ± 1.38	**0.01**	5.29 ± 0.93	5.51 ± 2.13	0.62
Large LDL-p (%)	81.3 ± 4.7	79.9 ± 5.4	**0.048**	81.2 ± 4.9	79.6 ± 5.5	0.05	81.4 ± 4.3	80.4 ± 5.0	0.41
Small LDL-p (%)	18.7 ± 4.7	20.1 ± 5.4	**0.04**	18.8 ± 4.9	20.4 ± 5.5	0.05	18.6 ± 4.3	19.6 ± 5.1	0.43
Large HDL-p (%)	59.3 ± 17.2	57.3 ± 15.5	0.34	58.5 ± 17.0	58.9 ± 14.5	0.86	61.5 ± 17.5	53.5 ± 17.3	0.07
Small HDL-p (%)	40.7 ± 17.2	42.7 ± 15.5	0.34	41.5 ± 17.0	41.1 ± 14.5	0.86	38.5 ± 17.5	46.5 ± 17.3	0.07

Data expressed as mean ± standard deviation, median [interquartile range]. N total number of participants, HIV human immunodeficiency virus, ART antiretroviral therapy, SBP systolic blood pressure, DBP diastolic blood pressure, TC total cholesterol, HDL-C high density lipoprotein cholesterol, LDL-C low density lipoprotein cholesterol, TG triglycerides, Apo A apolipoprotein A, Apo B apolipoprotein B, Lp (a) lipoprotein (a), hsCRP high sensitivity C-reactive protein, LDL-p low density lipoprotein particle, HDL-p high density lipoprotein particle.

Comparison of biochemical parameters between HIV negative and ART naïve HIV infected participants, by gender is presented in Table 4.5.

Mean concentrations of TC (p=0.001), LDL-C (p=0.01), HDL-C (p=0.001), Apo A (p=0.001), Lp (a) (p=0.002) and glucose (p=0.04) were lower in ART naïve HIV infected than in HIV negative participants, while the ratio of TG/HDL-C (p=0.04) was higher in ART naïve HIV infected than in HIV negative participants . The proportion of large LDL-particles was lower (p=0.048) and the proportion of small LDL-particles was higher (p=0.04) in ART naïve HIV infected than in HIV negative participants. The proportion of HDL-particles was not significantly different in ART naïve HIV infected and HIV negative participants (Table 4.5).

ART naïve HIV infected females had lower concentrations of TC, (p=0.02), HDL-C (p=0.03), Apo A (p=0.047), Lp (a) (p=0.037) and glucose (p=0.01) than HIV negative females. The percentage of small LDL-particles (LDL-p) was marginally higher in ART naïve HIV infected females than in HIV negative females (p=0.05), while the large LDL, large and small HDL-particles were similar in the two groups.

Lower concentrations of TC (p=0.001), LDL-C (0.03), Apo A (0.001), Lp (a) (p=0.029) and HDL-C (p=0.001) were present in ART naïve HIV infected males than in HIV negative males. ART naïve HIV infected males had higher median concentration of high sensitivity CRP than HIV negative males (p=0.047). The percentages of large and small LDL and HDL particles was similar in ART naïve HIV infected males and HIV negative males (Table 4.5).

4.3.4 Prevalence of cardiovascular risk factors

Table 4. 6: Prevalence (%) of CVD risk factors in ART naïve HIV infected participants by gender

	All ART naïve HIV infected Participants N=89 n (%)	**ART naïve HIV infected females. N=63 n (%)**	**ART naïve HIV infected males. N=26 n (%)**	**p-value**
Tobacco use	16 (18.0)	8 (12.7)	8 (30.8)	**0.045**
Alcohol consumption	26 (29.2)	16 (25.4)	10 (38.5)	0.31
Obesity	24 (27.0)	21 (33.3)	3 (11.5)	**0.04**
Abdominal obesity	21 (24.1)	19 (30.6)	2 (8.0)	**0.02**
Hypertension	38 (42.7)	28 (44.4)	10 (38.5)	0.65
Diabetes	12 (13.5)	7 (11.1)	5 (19.2)	0.32
Low HDL-C	53 (62.4)	39 (66.1)	14 (53.8)	0.34
Hypercholesterolemia	15 (17.4)	12 (20.0)	3 (11.5)	0.54
Hypertriglyceridaemia	20 (23.3)	11 (18.3)	9 (34.6)	0.16
High TC/HDL-C	16 (18.0)	9 (15.3)	7 (26.9)	0.24
High TG/HDL-C	23 (27.1)	15 (25.4)	8 (30.8)	0.61
High ApoB/ApoA	31 (38.3)	20 (36.4)	11 (42.3)	0.63
Metabolic Syndrome	17 (19.1)	13 (20.6)	4 (15.4)	0.77

ART antiretroviral therapy, HIV human immunodeficiency virus, CVD cardiovascular disease, ApoB apolipoprotein B, apo A apolipoprotein A, TC total cholesterol, HDL-C high density lipoprotein cholesterol. Diabetes – glucose > 7mmol/l and/or history of diabetes, hypertension- systolic blood pressure >140 and/or diastolic blood pressure >90 or history of high blood pressure, abdominal obesity- waist circumference - > 80cm for females and > 94 cm for males, obesity- body mass index ≥ 30 kg/m^2 , low HDL- ≤1.3 for females and ≤1.1 for males, hypercholesterolemia- TC ≥5mmol/l, hypertriglyceridemia- TG≥ 1.7mmol/l, high TC/HDL-C >5, high ApoB/ApoA >0.68, high TG/HDL ≥1.49, Met Syndrome -any 3 from abdominal obesity high TG, low HDL cholesterol, high blood pressure and raised fasting plasma glucose.

The prevalence of cardiovascular risk factors in ART naïve HIV infected participants stratified by gender is given in Table 4. 6.

The most common cardiovascular risk factors among the ART naïve HIV infected participants were hypertension (42.7%), low HDL-C (62.4%) and a high ratio of ApoB/ApoA (38.3%).

A significantly higher proportion of ART naïve HIV infected males (30.8%) than females (12.7%) used tobacco. Obesity (33.3% vs 11.5%, p=0.04) and abdominal obesity (30.6% vs 8.0%, p=0.02) were more common in ART naïve HIV infected females than in males. The prevalence of alcohol consumption, diabetes, hypertension, low HDL-C, hypercholesterolaemia, hypertriglyceridaemia, metabolic syndrome, high TC/HDL-C, TG/HDL-C and ApoB/ApoA ratios was similar in ART naïve HIV infected males and females (Table 4.6).

Table 4. 7: Comparison of the prevalence (%) of CVD risk factors between HIV negative and ART naïve HIV infected participants, by gender.

	All Participants			Females			Males		
	HIV negative N=178	**ART naïve HIV infected N=89**	**P-value**	**HIV negative N=126**	**ART naïve HIV infected N=63**	**P-value**	**HIV negative N=52**	**ART naïve HIV infected N=26**	**P-value**
Tobacco use	27 (15.2)	16 (18.0)	0.60	10 (7.9)	8 (12.7)	0.30	17 (32.7)	8 (30.8)	1.00
Alcohol	35 (19.7)	26 (29.2)	0.09	15 (11.9)	16 (25.4)	**0.02**	20 (38.5)	10 (38.5)	1.00
Obesity	48 (27.0)	24 (27.0)	1.00	41 (32.5)	21 (33.3)	1.00	7 (13.5)	3 (11.5)	1.00
Abdominal obesity	63 (35.4)	21 (24.1)	0.07	52 (41.3)	19 (30.6)	0.20	11 (21.2)	2 (8.0)	0.20
Hypertension	81 (45.5)	38 (42.7)	0.70	52(41.3)	28 (44.4)	0.76	29 (55.8)	10 (38.5)	0.23
Diabetes	24 (13.5)	12 (13.5)	1.00	18 (14.3)	7 (11.1)	0.65	6 (11.5)	5 (19.2)	0.49
Low HDL	74 (41.6)	53 (62.4)	**0.001**	64 (50.8)	39 (66.1)	0.06	10 (19.2)	14 (53.8)	**0.001**
Hypercholesterolemia	68 (38.2)	15 (17.4)	**0.001**	46 (36.5)	12 (20.0)	**0.03**	22 (42.3)	3 (11.5)	**0.01**
Hypertriglyceridaemia	44 (24.7)	20 (23.3)	0.88	31 (24.6)	11 (18.3)	0.45	13 (25.0)	9 (34.6)	0.43
TC/HDL (≥5)	18 (10.2)	16 (18.0)	0.08	13 (10.4)	9 (15.3)	0.34	5 (9.6)	7 (26.9)	0.09
High TG/HDL (≥1.49)	42(23.7)	23 (27.1)	0.65	29 (23.2)	15 (25.4)	0.85	13 (25.0)	8 (30.8)	0.60
ApoB/ApoA (≥ 0.68)	64 (37.0)	31 (38.3)	0.89	46 (37.4)	20 (36.4)	1.00	18 (36.0)	11 (42.3)	0.63
Metabolic Syndrome	31 (17.4)	17 (19.1)	0.74	24 (19.0)	13 (20.6)	0.85	7 (13.5)	4 (15.4)	1.00

ART antiretroviral therapy, HIV human immunodeficiency virus, CVD cardiovascular disease, ApoB apolipoprotein B, apo A apolipoprotein A, TC total cholesterol, HDL-C high density lipoprotein cholesterol. Diabetes – glucose > 7mmol/l and/or history of diabetes, hypertension- systolic blood pressure >140 and/or diastolic blood pressure >90 or history of high blood pressure, abdominal obesity- waist circumference - > 80cm for females and > 94 cm for males, obesity- body mass index ≥ 30 kg/m^2 , low HDL- ≤1.3 for females and ≤1.1 for males, hypercholesterolemia- TC ≥5mmol/l, hypertriglyceridemia- TG≥ 1.7mmol/l, high TC/HDL-C >5, high ApoB/ApoA >0.68, high TG/HDL-C ≥1.49, Metabolic Syndrome -any 3 from abdominal obesity, high TG, low HDL-cholesterol, high blood pressure and raised fasting plasma glucose.

A Comparison of CVD risk factors between HIV negative and ART naïve HIV infected stratified by gender is presented in Table 4.7.

A significantly higher proportion of HIV negative participants than ART naïve HIV infected participants had hypercholesterolaemia (38.2% vs 17.4%, p=0.001). The prevalence of low HDL-C concentration was higher in ART naïve HIV infected than in HIV negative participants (62.4% vs 41.6%, p=0.001). The prevalence of tobacco use, alcohol consumption, diabetes mellitus (DM), hypertension, hypertriglyceridaemia, obesity, abdominal obesity, metabolic syndrome, high TC/HDL-C ratio, high ApoB/ApoA ratio and high TG/HDL-C ratio was not significantly different between ART naïve HIV infected and HIV negative participants.

When males and females were considered separately (Table 4.7) the prevalence of hypercholesterolaemia (36.5% vs 20.0%, p=0.03) was higher in HIV negative than ART naïve HIV infected females. A higher proportion of HIV infected than HIV negative females consumed alcohol (25.4% vs 11.9%, p=0.02). The prevalence of low HDL-C concentration was lower (19.2% vs 53.8%, p=0.00), while the prevalence of hypercholesterolaemia was higher (42.3% vs 11.5%, p=0.01) in HIV negative than in ART naïve HIV infected males respectively. The prevalence of other risk factors was similar in HIV negative and ART naïve HIV infected males and females (Table 4.7).

4.3.5 Partial correlation of CVD risk factors with viral load and CD4 count after controlling for age and gender.

Table 4. 8: Partial correlation of viral load and CD4 count with cardiovascular disease risk factors after controlling for age and gender, in ART naïve participants.

		Alcohol Use	**Tobacco use**	**SBP**	**DBP**	**WC**	**BMI**	**Glucose**	**hsCRP**	**TC**	**HDL**	**TG**	**ApoB/ Apo A**	**TC/ HDL**	**TG/HDL-C**
CD4 count	r	-0.91	-0.09	0.17	-0.41*	0.08	0.36*	0.27	-0.04	0.33*	0.01	0.11	0.39*	0.31*	0.12
	p	0.56	0.59	0.26	**0.01**	0.63	**0.02**	0.08	0.79	**0.03**	0.94	0.46	**0.01**	**0.04**	0.43
Viral load	r	0.15	-0.12	-0.07	0.04	0.10	0.01	-0.15	0.28	-0.38*	-0.60*	-0.18	0.23	0.35*	0.24
	p	0.32	0.44	0.66	0.79	0.53	0.96	0.34	0.07	**0.01**	**0.00**	0.24	0.14	**0.02**	**0.01**

r- correlation coefficient, p- level of significance, SBP systolic blood pressure, DBP diastolic blood pressure, WC waist circumference, BMI body mass index, hsCRP high sensitivity CRP, TC total cholesterol, HDL high density lipoprotein cholesterol, TG triglycerides, ApoB apolipoprotein B, Apo A apolipoprotein A, Lp (a) lipoprotein (a). Met Syndrome -any 3 from abdominal obesity, high TG, low HDL cholesterol, high blood pressure and raised fasting plasma glucose, ART antiretroviral therapy.

The partial correlation of CVD risk factors with viral load and CD4 count after controlling for age and gender is presented in Table 4.8.

Viral load correlated negatively with TC (r=-0.38, p=0.01) and HDL-C (r=-0.60, p=0.001), and positively with TC/HDL-C ratio (r=0.35, p=0.02) and TG/HDL-C ratio (r=0.24, p=0.01). CD4 count correlated positively with body mass index (BMI) (r=0.36, p=0.02), TC (r=0.33, p=0.03), TC/HDL-C ratio (r=0.31, p=0.04) and apolipoprotein B/apolipoprotein A ratio (r=0.39, p=0.01), but negatively with diastolic blood pressure (r=-0.41, p=0.01) after controlling for age and gender (Table 4.8).

4.3.6 Regression analysis to determine predictors of CVD risk factor in ART naïve HIV infected people.

The univariate regression analysis was used to determine which factors to include in multivariate regression analysis. Univariate regression analysis is presented in Tables 4.9, 4.11, and 4.13.
Factors that were significant at p-value ≤ 0.25 in univariate analysis were considered as candidates in multivariate modelling. Two-way interactions that were significant at p-value < 0.05 were included in the modelling. In multivariate logistic regression, the model with the highest classification accuracy was chosen to explain the association of factors with outcome variable and in multivariate backward linear modelling, the significant factors were obtained from the last model.

Predictors of behavioural CVD risk factors among ART naïve HIV infected people is presented in Table 4.10

Table 4. 9: Univariate logistic regression to examine the association of covariates with behavioural CVD risk factors among ART naive HIV infected people.

Variables		Behavioural CVD risk factors			
		Tobacco use	Alcohol consumption	Physical inactivity	Low fruit and vegetable intake
Age (years):	≤50	1 [ref]	1 [ref]	1 [ref]	1[ref]
	>50	**1.02** *	**3.42***	**2.74***	0.66
Gender:	females	1 [ref]	1 [ref]	1 [ref]	1 [ref]
	males	**3.06***	**1.84***	**0.54***	**0.49***
Educational status:	primary	1 [ref]	1 [ref]	1 [ref]	1 [ref]
	Secondary and above	**0.48***	**0.20***	0.74	0.79
Marital status:	Unmarried	[ref]	1 [ref]	1 [ref]	1 [ref]
	Married	0.55	1.52	**2.67***	**2.07***
Work status:	Unemployed	1 [ref]	1 [ref]	1 [ref]	1 [ref]
	Employed	1.02	0.50	**0.36***	2.00

Values are reported as Unadjusted Odds ratio. *p value ≤ 0.25 are candidates for multivariate modelling. CVD-cardiovascular disease.

Table 4. 10: Predictors of behavioural CVD risk factors among ART naive HIV infected people.

Variables		Behavioural CVD risk factors			
		Tobacco use	**Alcohol consumption**	**Physical inactivity**	**Low fruit and vegetable intake**
Age (years):	≤50	1 [ref]	1 [ref]	1 [ref]	
	>50	0.89	2.16	**3.27 ***	___
Gender:	Females	1 [ref]	1 [ref]	1 [ref]	1 [ref]
	Males	**6.78***	1.44	**0.33**[#]	0.39
Educational status:	primary	1 [ref]	1 [ref]		
	Secondary and above	1.43	**0.27 ***	___	___
Marital status:	Unmarried			1 [ref]	1 [ref]
	Married	___	___	2.66	2.55
Work status:	: Unemployed			1 [ref]	
	Employed	___	___	0.48	___
Chi-square; p-value		11.48; 0.02	11.87; 0.01	12.07; 0.02	3.57; 0.17
Nagelkerke		19.8%	17.8%	18.6%	6.8%
Hosmer and Lemeshow		0.30	0.23	0.99	0.73
-2 Log likelihood		72.37	95.66	89.64	73.89
Classification accuracy		82.0%	75.3%	77.5%	84.3

Values are reported as Adjusted Odds ratio. *p-value is significant at <0.05. [#]p value=0.05 is marginally significant. ART antiretroviral therapy

People more than 50 years of age were 3.27 times (p-value 0.04) more likely to be physically inactive than people of less than 50 years of age.
Males were more physically active than females (p value 0.05) and were 6.78 times more likely to use tobacco than females.
People with secondary education and above used less alcohol than people with primary education (p value 0.04).
None of the examined socio-demographic factors showed an association with low fruit and vegetable intake (Table 4.10).

Predictors of metabolic CVD risk factors among ARV naïve HIV infected people are presented in table 4.12.

Table 4. 11: Univariate logistic regression to examine association of covariates with Metabolic CVD risk factors among ART naive HIV infected people.

Predictor variable		Metabolic CVD risk factors							
		HTN	**Diabetes**	**Low HDL-C**	**High TC**	**High TG**	**High TC/ HDL-C**	**High Apo B/ Apo A**	**High TG / HDL-C**
Age (years):	≤50	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1[ref]
	>50	**1.63**[2]	**15.47**[1]	**0.49**[2]	1.83	0.67	1.09	0.80	0.92
Gender:	females	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1[ref]
	males	0.78	1.91	0.60	0.52	**2.36**[2]	**2.05**[2]	1.28	1.31
Alcohol :	No	1 [ref]	1[ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1[ref]
	Yes	**1.90**[2]	**4.27**[1]	**0.34**[1]	1.83	1.44	**0.29**[2]	0.62	0.81
Tobacco use:	No	1[ref]	1[ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1[ref]
	Yes	**2.68**[2]	**4.29**[1]	**0.33**[2]	1.23	1.87	0.62	0.68	0.72
VL(copies/ml):	<50	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1[ref]
	>50	1.08	0.47	**6.03**[1]	**0.32**[2]	1.23	**2.28**[1]	**5.06**[1]	**2.32**[2]
Obesity:	No	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1[ref]
	Yes	**3.91**[1]	0.89	1.08	**2.29**[2]	**2.48**[2]	0.94	0.75	**2.67**[2]
Abdominal Obesity:	No	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1[ref]
	Yes	**3.50**[1]	1.98	1.69	**3.44**[1]	**2.13**[2]	0.63	**1.91**[2]	**1.81**[2]
CD 4 count:	>500	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1[ref]
(cells/mm^3)	301-500	**0.46**[2]	0.65	0.92	**0.24**[2]	0.92	0.92	**0.39**[2]	1.64
	≤300	**0.24**[2]	0.00	0.50	**0.10**[1]	0.92	0.26	**0.31**[2]	0.90
PA:	>600MET- min/week	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1[ref]
	<600 MET-min/week	1.22	1.88	0.93	1.01	0.81	1.06	1.08	0.71
F/V:	>5servings/day	1[ref]	1[ref]	1[ref]	1[ref]	1[ref]	1[ref]	1[ref]	1[ref]
	<5servings/day	**0.50**[2]	0.92	0.23	3.14	0.71	0.82	1.29	0.85

Values are reported as Unadjusted Odds ratio. [1]p-value <0.05 and [2]p-value is from 0.05 to 0.25 candidates for multivariate modelling. VL-viral load, PA-physical activity, F/V-fruit and vegetable intake, HDL-C high density lipoprotein cholesterol, LDL-C low density lipoprotein cholesterol, TC total cholesterol, TG triglycerides, Apo B apolipoprotein B, Apo A apolipoprotein A, Met S metabolic syndrome, HTN hypertension, ART antiretroviral therapy, MET-min metabolic equivalent of task-minute.

Table 4. 12: Predictors of Metabolic CVD risk factors among ART naïve HIV infected people.

Predictor variable		Metabolic CVD risk factors							
		HTN	**Diabetes**	**Low HDL-C**	**High TC**	**High TG**	**High TC/ HDL-C**	**High Apo B/ Apo A**	**TG/HDL-C**
Age(years):	≤50	1 [ref]	1 [ref]	1 [ref]	___	___	___	___	___
	>50	**4.05**#	**1.17***	0.61					
Gender:	females	____	____	____	____	1 [ref]	____	____	___
	males					1.89			
Alcohol use:	No	___	___	1 [ref]	____	____	1 [ref]	____	____
	Yes			0.41			**0.11***		
Tobacco use:	No	1 [ref]	1 [ref]	1 [ref]	____	___	____	___	____
	Yes	3.91	**7.95***	0.41					
VL (copies/ml):	≤50	___	____	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]
	>50			**7.37***	0.16	1.50	**2.51***	**2.45***	**1.58**#
Abdominal Obesity:	No	1 [ref]	____	___	1 [ref]	1 [ref]	___	1 [ref]	1 [ref]
	Yes	**10.95***			**7.67***	**3.44**#		2.45	2.76
CD 4 count:	>500	1 [ref]	____	____	1 [ref]	___	____	1 [ref]	___
(cells/mm^3)	301-500	0.95			0.18			0.60	
	≤300	**0.16***			**0.04***			0.23	
Chi-square		15.54	33.78	20.02	13.89	4.39	14.01	10.72	6.32
p-value		0.01	0.001	0.0001	0.01	0.22	0.001	0.03	0.04
Nagelkerke		34.7%	57.8%	30.2%	39.2%	8.7%	27.5%	27.4%	11.3%
Hosmer & Lemeshow		0.70	0.86	0.67	0.86	0.98	0.20	0.39	0.15
-2Log –likelihood		55.31	36.61	86.64	35.24	74.31	56.64	53.39	90.88
Classification –accuracy		76.9%	91.0%	72.2%	83.3%	77.9%	83.5%	70.2%	67.1%

Values are reported as Adjusted Odds ratio; *p-value is significant at <0.05, #p value=0.05 is marginally significant. VL-viral load, HDL-C high density lipoprotein cholesterol, LDL-Clow density lipoprotein cholesterol, TC total cholesterol, TG triglycerides, Apo B apolipoprotein B, Apo A apolipoprotein A, HTN hypertension, ARV antiretroviral.

People more than 50 years of age were 4.05 times (p value 0.05) more likely to be hypertensive and 1.17 times (p value 0.0001) more likely to be diabetic than people of less than 50 years of age.

People who used alcohol were less likely to have a high TC/HDL-C ratio (p value 0.046) than people who did not use alcohol.

Tobacco users were 7.95 times (p value 0.03) more likely to have diabetes mellitus than non-tobacco users.

People with a viral load of more than 50 copies/ml were more likely to have a low HDL-C concentration (p value 0.001), a high TC/HDL-C ratio (p value 0.01), a high ApoB/ApoA ratio (p value 0.01) and high TG/HDL-C ratio (p value 0.05) compared to people with a viral load of less or equal to 50 copies/ml.

The likelihood of having hypertension was 10.95 times (p value 0.001) more in people with than in people without abdominal obesity. In addition people with abdominal obesity were 7.67 times (p value 0.04) more likely to have a high TC concentration and 3.44 times (p value 0.05) more likely to have a high TG concentration compared to people without abdominal obesity.

People with a CD4 count of less or equal to 300cell/mm^3 were less likely to be hypertensive (p value 0.048) and hypercholesterolaemic (p value 0.03) compared to people with a CD4 count of more than 300 cells/mm^3.

Predictors of Lipoprotein subclasses in ART naïve HIV infected people are presented in table 4.14.

Table 4. 13: Univariate linear regression to examine association of covariates with lipoprotein subclasses among ART naive HIV infected people.

Predictor variable	Lipoprotein subclasses			
	Large HDL-p	**Small HDL-p**	**Large LDL-p**	**Small LDL-p**
Age:	-0.08 (0.47)	0.07 (0.55)	0.12 (0.28)	-0.12 (0.27)
Gender:	-0.16 (**0.14**)*	0.16 (**0.14**)*	0.07 (0.55)	-0.07 (0.53)
Alcohol:	-0.18 (**0.10**)*	0.18 (**0.10**)*	0.07 (0.53)	-0.07 (0.52)
Tobacco use	-0.05 (0.66)	0.05 (0.66)	0.01 (0.93)	-0.01 (0.90)
VL:	-0.06 (0.59)	0.06 (0.59)	0.11 (0.31)	-0.12 (0.31)
CD4 count (cells/mm^3):	0.20 (**0.16**)*	-0.20 (**0.16**)*	-0.10 (0.51)	0.01 (0.95)
BMI (kg/m^2):	0.12 (0.33)	-0.12 (0.33)	0.14 (**0.20**)*	-0.14 (**0.19**)*
WC	0.15 (**0.18**)*	-0.15 (**0.18**)*	0.20 (**0.07**)*	-0.19 (**0.08**)*
HDL-C:	0.18 (**0.10**)*	-0.18 (**0.10**)*	-0.03 (0.77)	0.03 (0.76)
LDL-C:	-0.03 (0.80)	0.03 (0.80)	0.02 (0.84)	-0.03 (0.82)
TC:	-0.01 (0.92)	0.01 (0.92)	0.04 (0.70)	-0.04 (0.69)
TG:	-0.18 (**0.10**)*	0.18 (**0.10**)*	0.12 (0.27)	-0.12 (0.27)
TG/HDL-C	-0.18 (**0.10**)*	0.18 (**0.10**)*	0.16 (**0.16**)*	-0.16 (**0.15**)
TC/HDL-C	-0.24 (**0.03**)*	0.24 (**0.03**)*	0.06 (0.58)	-0.07 (0.56)
ApoB/ ApoA	-0.20 (**0.07**)*	0.20 (**0.07**)*	0.13 (**0.10**)*	-0.13 (**0.10**)*
Hypertension:	-0.06 (0.57)	0.06 (0.57)	0.07 (0.52)	-0.07 (0.50)
Diabetes:	-0.03 (0.79)	0.03 (0.79)	0.04 (0.74)	-0.04 (0.74)
Met S:	-0.12 (0.29)	0.12 (0.29)	0.29 (0.27)	-0.29 (0.26)
Physical activity	-0.10 (0.35)	0.10 (0.35)	-0.08 (0.45)	0.08 (0.45)
Fruit and vegetable intake	0.004 (0.97)	-0.004 (0.97)	0.10 (0.37)	-0.10 (0.36)

Values are reported as Beta coefficient (p-value), * p value ≤ 0.25 are candidates for multivariate modelling. HDL-C high density lipoprotein cholesterol, LDL-C low density lipoprotein cholesterol, TC total cholesterol, BMI body mass index, VL-viral load, TG triglycerides, ApoB apolipoprotein B, ApoA apolipoprotein A, Met S metabolic syndrome, HDL-p High density lipoprotein-particles, LDL-p Low density lipoprotein-particles, ART antiretroviral therapy.

Table 4. 14: Predictors of Lipoprotein subclasses in ART naive HIV infected people.

Predictor variable	Large HDL-particles		Small HDL-particles		Large LDL-particles		Small LDL-particles	
	1st Model	Last Model	1st Model	Last Model	1st Model	Last Model	1st Model	Last Model
Gender:	-0.09 (0.59)	____	0.08 (0.64)	____				
Alcohol:	-0.14 (0.37)	____	0.16 (0.31)	____				
CD4 count (cells/mm^3):	0.25 (0.13)	0.28 (**0.056**)#	0.27 (0.09)	-0.28 (**0.056**)#				
Abdominal obesity	0.19 (0.26)	0.29 (**0.047**) *	-0.20 (0.24)	-0.29(**0.047**) *	0.24 (0.046)	0.26 (**0.028**) *	-0.23 (0.049)	-0.26 (**0.03**) *
HDL-C:	-0.11 (0.59)	____	0.12 (0.54)	____				
TG/HDL-C	0.23 (0.18)	____	-0.25 (0.14)	____	0.16 (0.18)	____	-0.16 (0.18)	____
TC/HDL-C	-0.005 (0.98)	____	0.03 (0.86)	____				
ApoB/ ApoA	-0.008 (0.97)	____	-0.006 (0.98)	____	0.04 (0.74)	____	-0.04 (0.72)	____
F-test	1.34	4.05	1.34	4.05	2.35	5.02	2.36	4.93
p-value	0.26	0.03	0.26	0.03	0.08	0.03	0.08	0.03
R^2	23.4%	16.5%	23.4%	16.5%	9.4%	6.7%	9.4%	6.6%
Adjusted R^2	6.0%	12.4%	6.0%	12.4%	5.4%	5.4%	5.4%	5.2%

Values are reported as Beta coefficient (p-value). *****p-value is significant at <0.05, #p value is marginally significant. HDL-C high density lipoprotein cholesterol, LDL-C low density lipoprotein cholesterol, TC total cholesterol, TG triglycerides, Apo B apolipoprotein B, Apo A apolipoprotein A, ART antiretroviral therapy

CD4 count was positively associated with the large HDL-particles (p value 0.056) and negatively with the small HDL-particles.

Abdominal obesity was positively associated with large HDL-particles (p value 0.047) and negatively with small HDL-particles. In addition abdominal obesity was positively associated the large LDL-particles (p value 0.023) and negatively with the small LDL-particles (p=0.03) (Table 4.14).

4.3.7 Framingham 10-year cardiovascular disease (CVD) risk estimation in ART naïve HIV infected participants**.**

The distribution of ART naïve HIV infected participants by Framingham risk category is presented in Figure 4.3.

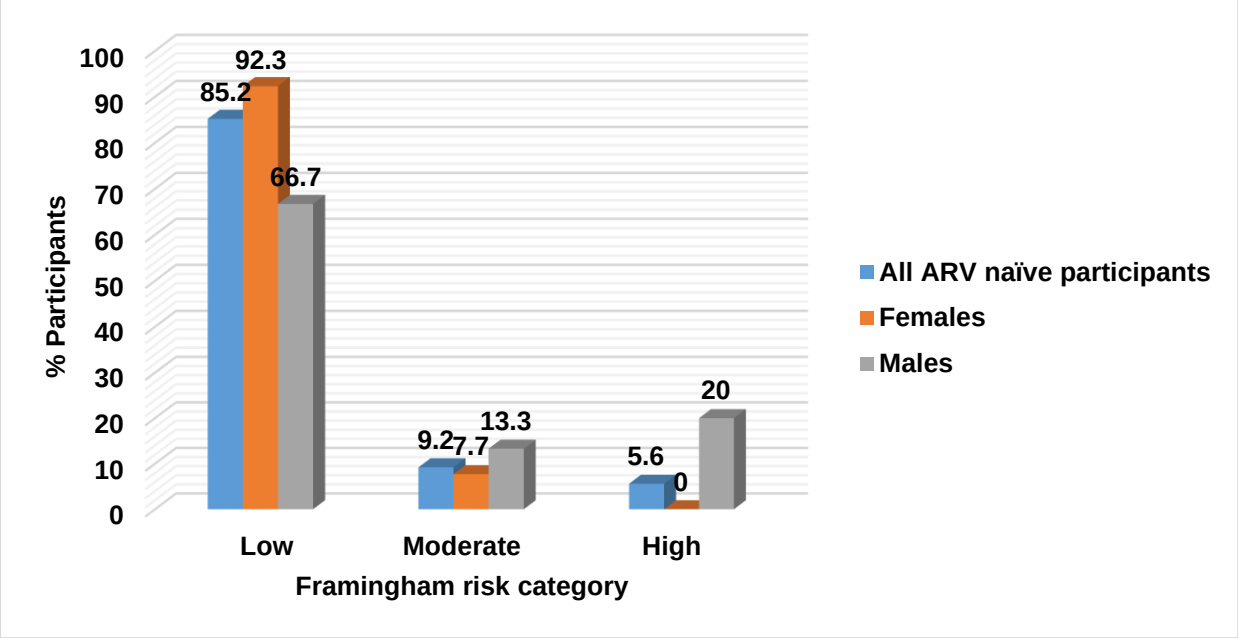

Figure 4. 3: Distribution of ART naive HIV infected participants by Framingham risk category

The majority of ART naïve HIV infected participants (85.2%) had a low 10- year CVD risk. The prevalence of participants with moderate and high 10-year CVD risk was 9.2% and 5.6% respectively. The prevalence of individuals at low, moderate and high 10-year CVD risk was 92.3%, 7.7%, 0% among ART naïve HIV infected females and 66.7%, 13.3% and 20% among ART naïve HIV infected males, respectively. According to the Framingham risk score ART naïve HIV males have a higher risk of developing

cardiovascular disease in the next 10 years than ART naïve HIV infected females (Figure 4.3).

4.4 PHASE 2 RESULTS

4.4.1 Socio-demographic data.

The socio-demographic characteristics of people on ART is presented in Table 4.15.

Table 4. 15: Socio-demographic data of ART HIV infected participants by gender

Variables	**ART treated HIV infected participants N=214**	**ART treated HIV infected females N=171**	**ART treated HIV infected males N= 43**	**p-value**
Age (years)	44.8 ± 11.8	43.5 ± 11.6	49.9 ± 11.1*****	**0.001**
Marital status n (%)				
Not Married	164(76.6)	137 (80.1)	27 (62.8) *	**0.03**
Married	50(23.4)	34 (19.9)	16 (37.2) *	
Educational level n (%)				
Primary	114(53.3)	86 (50.3)	28 (65.1)	0.12
Secondary	98(45.8)	83 (48.5)	15 (34.9)	
University	2(0.9)	2 (1.2)	0 (0)	
Work status n (%)				
Unemployed	149 (69.6)	129 (75.4)	20 (46.5)	**0.001**
Retired	17 (7.9)	9 (5.3)	8 (18.6)	
Employed	48(22.4)	33 (19.3)	15 (34.9)	
Tobacco use n (%)				
No	169(79.0)	143 (83.6)	26 (60.5)	**0.001**
Yes	45(21.0)	28 (16.4)	17 (39.5)	
Alcohol use n (%)				
No	167 (78.0)	142 (83.0)	25 (58.1)	**0.001**
Yes	47(22.0)	29 (17.0)	18 (41.9)	
Fruit and vegetable intake n (%)	N=157	N=127	N=30	
≥5 servings/day	7 (4.5)	6 (4.7)	1 (3.3)	1.00
<5 servings/day	150 (95.5)	121 (95.3)	29 (96.7)	
Physical activity	N=157	N=127	N=30	
>600 MET-min	119 (75.8)	90 (70.9)	29 (96.7)	**0.002**
<600 MET-min	38 (24.2)	37 (29.1)	1 (3.3)	
ARV regimen	**N=200**	**N=160**	**N=40**	
NVP (NNRTI)-based HAART	25 (12.5)	22 (13.8)	3 (7.5)	
EFV (NNRTI)-based HAART	172 (86)	136 (85.0)	36 (90.0)	
Alluvia (PI)-based HAART	3 (1.5)	2 (1.3)	1 (2.5)	

Data is presented as number (percentage), HIV human immunodeficiency virus, ARV antiretroviral, NVP nevirapine, EFV efavirenz, NNRTI non-nucleoside reverse transcriptase inhibitor, PI protease inhibitor, HAART highly active antiretroviral therapy, MET-min metabolic equivalent of task-minute.

Of the 214 people on ART, 171 (79.9%) were females and 43 (20.1%) were males. The mean age of people on ART was 44.8 ± 11.8 years and males were significantly older than females (49.9 ± 11.1 vs 43.5 ± 11.6, p=0.00). The percentage of unmarried participants was 164 (76.6%) with more females than males unmarried (80.1% vs 62.8%, p<0.05). About 53.3% of participants had primary level and 45.8% had secondary level of education. Unemployment among people on ART was 69.6 %, and higher in females (75.4%) than in males (46.5%). A significantly higher proportion of males than females used tobacco (39.5% vs 16.4%, p<0.05) and consumed alcohol (41.9% vs 17.0%, p<0.05) respectively. Males were more physically active than females (96.7% vs 70.9%, p=0.002), while fruit and vegetable intake was similar between the two groups (Table 4.15).

In Figure 4.4 the distribution of ART regimen use is presented.

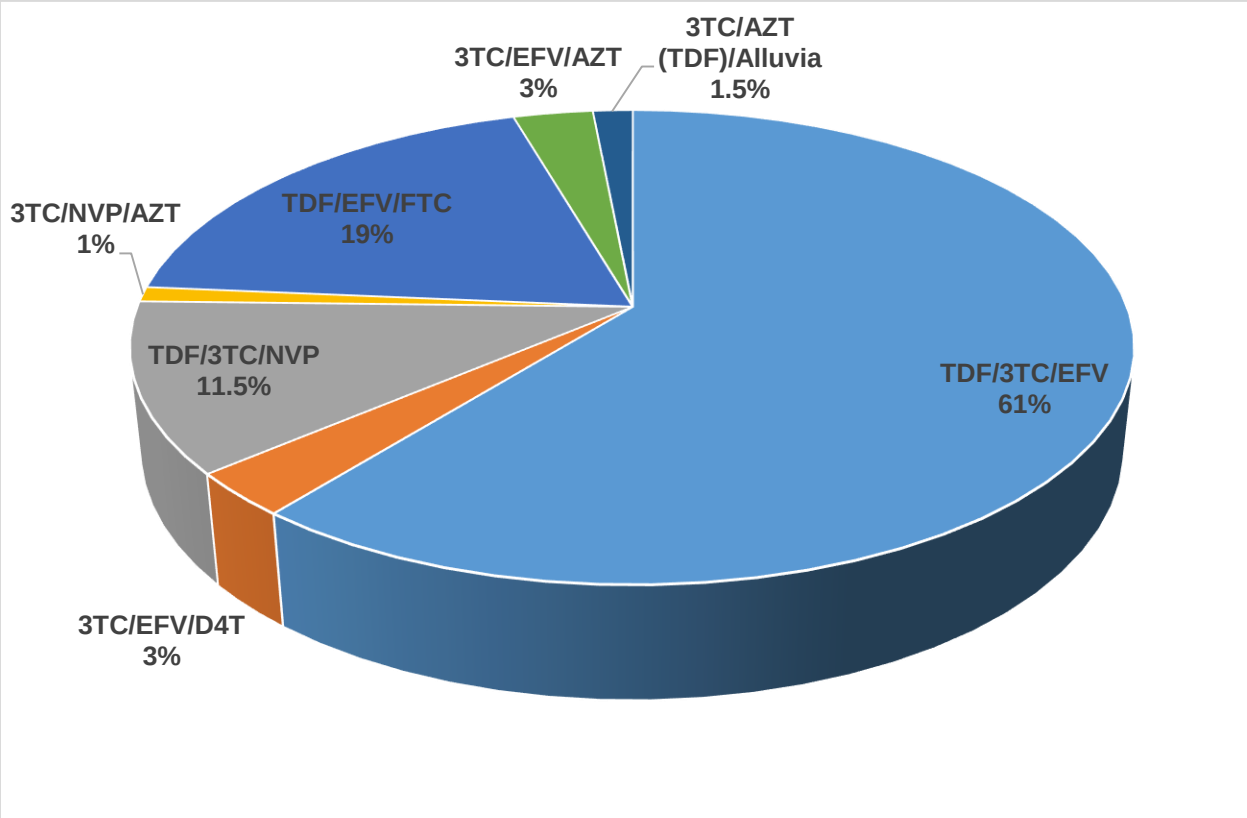

Figure 4. 4: Distribution of ARV regimen used by participants
NVP nevirapine, 3TC lamivudine, AZT zidovudine, TDF tenofovir, EFV efavirenz, FTC emtricitabine, D4T Stavudine, Alluvia- lopinavir/ritonavir.

Information on ART used and duration of treatment was only available in 200 of the participants, 160 females and 40 males because files for some participants could not be located. The most commonly used treatment was EFV-based ART in both males (90%) and females (85%) (Table 4.16). The majority of participants were treated with combinations of tenofovir (TDF)/lamivudine (3TC)/ efavirenz (EFV) (61%) followed by emtricitabine (FTC)/ EFV/TDF (19%) and nevirapine (NVP)/ 3TC/TDF (11.5%) (Figure 4.4). Nevirapine and Emtricitabine were available as 200mg, lamivudine, tenofovir and Stavudine were available as 300mg and efavirenz was given as 600mg.

The duration of ART for 200 participants is presented in Figure 4.5

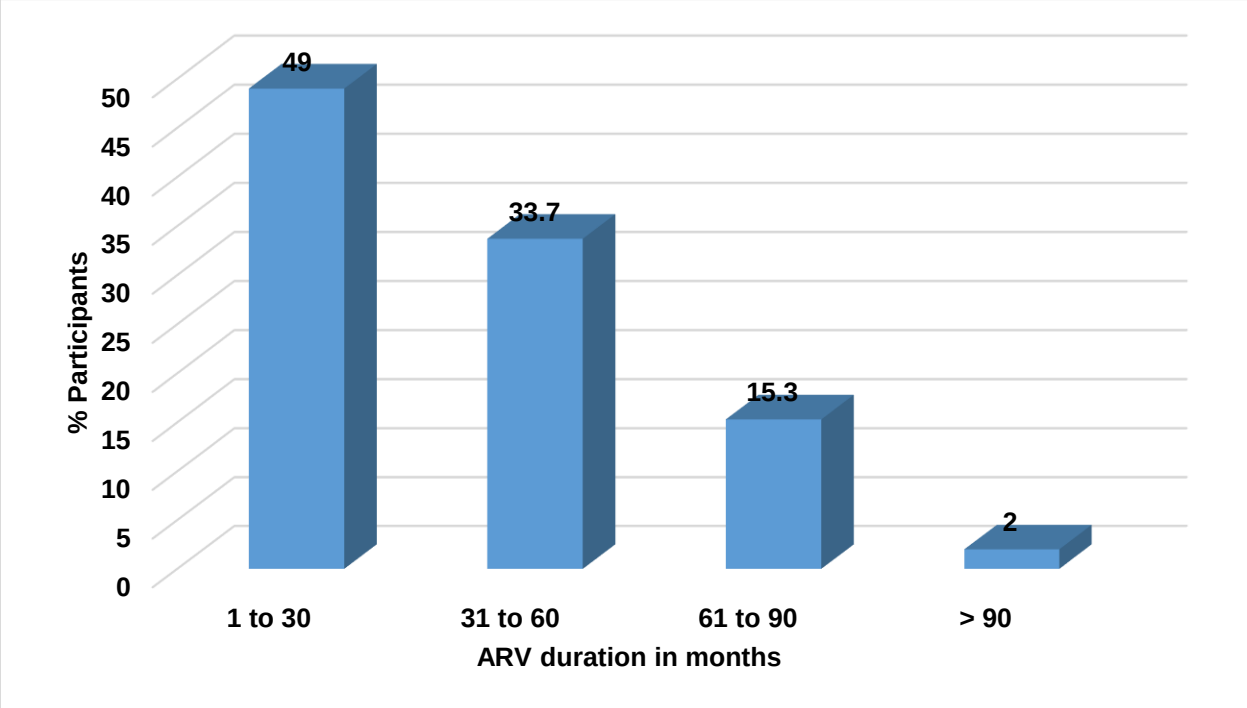

Figure 4. 5: Distribution of people on ART according to duration of treatment

Duration of ART among participants ranged from 1 month to 121 months with a mean duration of 36.1±24.4 months. About 49% of participants had taken ART between 1 to 30 months, while 33.7% had received treatment between 31 to 60 months. The proportions of people on longer duration of treatment were 15.3% (61 to 90 months) and 2% (more than 90 months) (Figure 4.5).

4.4.2 Anthropometric measurements and blood pressure

Anthropometric measurements and blood pressure of participants on ART are presented in Table 4.16.

Table 4. 16: Anthropometric measurements and blood pressure of ART HIV infected participants

	ART treated HIV infected participants N=214	**ART treated HIV infected females N=171**	**ART treated HIV infected males N=43**	**P-value**
SBP (mmHg)	117.3 ± 16.9	116.5 ± 16.1	120.5 ± 19.7	0.23
DBP (mmHg)	77.4 ± 10.2	77.3 ± 10.3	77.9 ± 9.9	0.74
Weight (kg)	61.7 ± 14.3	61.7 ± 14.9	61.5 ± 11.7	0.94
Height (cm)	1.61 ± 0.09	1.61 ± 0.09	1.59 ± 0.07	**0.001**
BMI(kg/m^2)	24.0 ± 5.0	24.6 ± 5.0	21.4 ± 3.9	**0.001**
WC (cm)	84.84±11.81	85.4 ± 12.1	82.6 ± 10.2	0.12
HC (cm)	96.7 ± 10.9	97.9 ± 11.3	91.9 ± 7.4	**0.001**
WHR	0.88 ± 0.09	0.87 ± 0.09	0.89 ± 0.07	0.06

Data is expressed as mean ± standard deviation. P-value is significant at less than 0.05. HIV human immunodeficiency virus, ART antiretroviral therapy, WC waist circumference, HC hip circumference, WHR waist hip ration, BMI body mass index.

Mean blood pressure in males and females on ART was similar. Mean body mass index was higher in females than in males (p=0.001), as was the hip circumference (p=0.001), while there was no significant difference in waist to hip ratio (Table 4.16).

4.4.3 Biochemical parameters

Comparison of biochemical parameters between male and female participants on ART is presented in Table 4.17.

Table 4. 17: Biochemical parameters in ART HIV infected participants by gender

	ART treated HIV infected N=214	**ART treated HIV infected females N=171**	**ART treated HIV infected males N=43**	**p-value**
TC (mmol/l)	4.60 ± 1.11	4.66 ± 1.08	4.34 ± 1.22	0.14
HDL-C (mmol/l)	1.36 ± 0.40	1.39 ± 0.36	1.20 ± 0.51	**0.03**
LDL-C (mmol/l)	2.66 ± 0.99	2.72 ± 0.94	2.38 ± 1.18	0.09
TG (mmol/l)	1.03 [0.80-1.48]	1.00 [0.77-1.39]	1.43 [0.93-1.95]	**0.001**
TC/HDL-C	3.61 ± 1.12	3.52 ± 1.06	4.00 ± 1.30	**0.03**
TG/HDL-C	0.78 [0.54-1.23]	0.73 [0.52-1.11]	1.13 [0.76-2.34]	**0.0001**
Apo A (mg/dl)	123.9 ± 27.9	125.3 ± 27.7	118.0 ± 28.0	0.15
Apo B (mg/dl)	82.5 ± 26.3	83.3 ± 25.5	78.7 ± 29.2	0.36
Apo B/ Apo A	0.70 ± 0.28	0.70 ± 0.28	0.70 ± 0.29	1.00
Lp (a) (mg/dl)	84.9[39.4-155.0]	86.5[40.1-157.8]	84.0[29.8-130.0]	0.40
hsCRP (mg/l)	4.38 [2.00-8.37]	4.89 [2.04-8.75]	3.86 [1.32-6.65]	0.32
Glucose (mmol/l)	4.93 ± 0.63	4.94 ± 0.60	4.88 ± 0.76	0.64
Large LDL-p (%)	82.4 ± 6.2	82.5 ± 6.5	81.7 ± 4.2	0.35
Small LDL-p (%)	17.2 ± 4.2	17.0 ± 4.1	18.3 ± 4.3	0.09
Large HDL-p (%)	62.9 ± 11.1	63.6 ± 10.8	59.8 ± 11.9	0.08
Small HDL-p (%)	37.1 ± 11.2	36.4 ± 10.9	40.1 ± 11.9	0.07
CD4 count (cells/mm^3)	461.9 ± 235.3	485.5 ±234.1	364.3 ± 216.6	**0.001**
Viral load (copies/ ml)	≤ 50*	≤ 50	≤ 50	0.14
Mean duration of ART in months (N=200)	36.1 ± 24.4	37.0 ± 24.3	32.5 ± 24.6	0.31

* 85% of ART HIV infected participants had a viral load of less or equal to 50. Data expressed as mean ± standard deviation, median [interquartile range]. N total number of participants, HIV human immunodeficiency virus, ARV antiretroviral, SBP systolic blood pressure, DBP diastolic blood pressure, TC total cholesterol, HDL-C high density lipoprotein cholesterol, LDL-C low density lipoprotein cholesterol, TG triglycerides, Apo A apolipoprotein A, Apo B apolipoprotein B, Lp (a) lipoprotein (a), hsCRP high sensitivity C-reactive protein, LDL-p low density lipoprotein particle, HDL-p high density lipoprotein particle.

Lower mean values of HDL-C concentration (p=0.03) and TC/HDL ratio (p=0.03) and higher median value of TG (p=0.001) and TG/HDL-C ratio (p=0.0001), were observed in males than in females. There was no significant difference in mean values of TC, LDL-C, Apo A, ApoB, ApoB/ApoA ratio, Lp (a), glucose and median values of hsCRP between males and females. LDL and HDL particle proportions were not significantly different between males and females. However, the proportions of large particles were higher than the small particles in both males and females. The mean CD4 count was lower in males than in females (p=0.001), while the median viral load was similar between the two groups. Of the 214 participants on ART, 178 (85%) had undetectable viral load (≤ 50 copies/ml). Participants had taken ART for a mean duration of 36.1 ± 24.4 months (Table 4.17).

4.4.4 Cardiovascular risk factors.

The prevalence of cardiovascular risk factors in males and females on ART is presented in Table 4.18.

Table 4. 18: Prevalence (%) of CVD risk factors in ART HIV infected participants by gender

	ART treated HIV infected participants N=214 n (%)	**ART treated HIV infected females N=171 n (%)**	**ART treated HIV infected males N=43 n (%)**	**P-value**
Tobacco use	45 (21.1)	28 (16.5)	17 (39.5)	**0.001**
Alcohol	47 (22.1)	29 (17.1)	18 (41.9)	**0.001**
Obesity	27 (12.7)	25 (14.6)	2 (4.8)	0.12
Abdominal obesity	51 (23.9)	44 (25.9)	7 (16.3)	0.23
Hypertension	56 (26.2)	40 (23.4)	16 (37.2)	0.08
Diabetes	10 (4.7)	6 (3.5)	4 (9.3)	0.12
Low HDL-C	91 (43.8)	71 (42.3)	20 (50.0)	0.38
Hypercholesterolaemia	69 (33.2)	58 (34.5)	11 (27.5)	0.46
Hypertriglyceridaemia	35 (16.8)	21 (12.5)	14 (35.0)	**0.001**
High TC/HDL (≥5)	28 (13.5)	17 (10.1)	11 (27.5)	**0.01**
High TG/HDL-C (≥1.49)	38 (18.3)	23 (13.7)	15 (37.5)	**0.001**
ApoB/ApoA (≥ 0.68)	93 (45.4)	74 (44.8)	19 (47.5)	0.86
Metabolic Syndrome	20 (9.6)	15(8.9)	5 (12.5)	0.56

ART antiretroviral therapy, HIV human immunodeficiency virus, CVD cardiovascular disease, ApoB apolipoprotein B, apo A apolipoprotein A, TC total cholesterol, HDL-C high density lipoprotein cholesterol. Diabetes – glucose ≥ 7mmol/l and/or history of diabetes, hypertension- systolic blood pressure ≥140 and/or diastolic blood pressure ≥90 or history of high blood pressure, abdominal obesity- waist circumference - > 80cm for females and > 94 cm for males, obesity- body mass index ≥ 30 kg/m^2 , low HDL- ≤1.3 for females and ≤1.1 for males, hypercholesterolemia- TC ≥5mmol/l, hypertriglyceridemia- TG≥ 1.7mmol/l, high TC/HDL-C >5, high ApoB/ApoA >0.68, high TG/HDL ratio ≥1.49, Met Syndrome- metabolic syndrome

Tobacco use was reported by 21.1% of participants, and its use was higher among males than among females (39.5% vs 16.5%, p=0.001). Similarly, 22.1% of participants on ART consumed alcohol, with a higher proportion of males than females consuming alcohol (41.9% vs 17.1%, p=0.001). The prevalence of hypertriglyceridaemia (35.0% vs 12.5%, p=0.001), high TC/HDL ratio (27.5% vs 10.1%, p=0.01) and high TG/HDL-C ratio (37.5% vs 13.7%) was higher in males than in females. The prevalence of metabolic syndrome was similar between males and females (Table 4.18). None of the participants indicated a family history of CVD.

4.4.5 Partial correlation of viral load and cd4 count with CVD risk factors after controlling for age, gender and duration of ARV treatment.

Table 4. 19: Partial correlation of viral load and CD4 with cardiovascular disease risk factors count after controlling for age, gender and duration of treatment, in people on ART.

		Alcohol use	**Tobacco use**	**SBP**	**DBP**	**WC**	**BMI**	**Glucose**	**hsCRP**	**TC**	**HDL-C**	**TG**	**ApoB/ Apo A**	**TC/ HDL-C**	**TG/ HDL-C**
CD4 count	r	0.06	-0.01	0.00	0.05	-0.02	-0.04	-0.04	-0.04	0.07	0.13	0.05	0.03	-0.05	0.07
	p	0.46	0.91	1.00	0.49	0.76	0.65	0.59	0.60	0.35	0.10	0.48	0.65	0.55	0.35
Viral load	r	0.13	-0.05	-0.03	-0.08	-0.13	-0.13	-0.04	-0.01	-0.11	-0.21*	0.10	0.15	0.15	0.14*
	p	0.09	0.49	0.69	0.28	0.08	0.09	0.58	0.89	0.16	**0.01**	0.20	**0.05**	**0.06**	**0.04**

* Significant correlation, r- correlation coefficient, p- level of significance, SBP systolic blood pressure, DBP diastolic blood pressure, WC waist circumference, BMI body mass index, hsCRP high sensitivity CRP, TC total cholesterol, HDL high density lipoprotein cholesterol, TG triglycerides, ApoB apolipoprotein B, Apo A apolipoprotein A.

The partial correlation of viral load and CD4 count with CVD risk factors after controlling for age, gender and duration of ART is presented in Table 4.19.

After controlling for age, gender and duration of ART, viral load was correlated negatively with HDL-C (r=- 0.21, p=0.01) and positively with the TG/HDL-C ratio. A positive correlation of marginal significance was observed for Apo B/ Apo A (r=0.15, p=0.05) and TC/HDL-C (r=0.15, p=0.06) with viral load. No other correlation were observed (Table 4.19).

4.4.6 Regression analysis to determine predictors of CVD risk factor in ART HIV infected people

The univariate regression analysis was used to determine which factors to include in multivariate regression analysis. Univariate regression analysis is presented in Tables 4.20, 4.21, 4.23 and 4.25.

Predictors of tobacco use, alcohol consumption and physical inactivity among people on ART is presented in Table 4.22.

Table 4. 20: Univariate logistic regression to examine association of covariates with behavioural CVD risk factors among ART HIV infected people

Variables		Behavioural CVD risk factors		
		Tobacco use	**Alcohol consumption**	**Physical inactivity**
Age:	≤50	1 [ref]	1 [ref]	1 [ref]
	>50	**2.82***	1.23	0.73
Gender:	females	1 [ref]	1 [ref]	1 [ref]
	males	**3.34***	**3.53***	**0.08***
Educational status:	primary	1 [ref]	1 [ref]	1 [ref]
	Secondary and above	**0.56***	**0.51***	1.05
Marital status:	Unmarried	1[ref]	1 [ref]	1 [ref]
	Married	**2.5***	1.34	0.91
Work status:	Unemployed	1 [ref]	1 [ref]	1 [ref]
	Employed	0.83	1.07	0.63

Values are reported as Unadjusted Odds ratio. *p value ≤ 0.25 are candidates for multivariate modelling. ART antiretroviral therapy

Table 4. 21: Univariate linear regression to examine association of covariates with behavioural CVD risk factors among HIV infected people on ART

Variables		**Behavioural CVD risk factor :**
		fruit and vegetable intake
Age		0.02 (0.83)
Gender		-0.09 (0.29)
Education status		-0.09 (0.27)
Marital status		-0.11 (**0.18**) *
Work status		-0.003 (0.97)

Beta (p-value), *p value ≤ 0.25 are candidates for multivariate modelling. CVD cardiovascular disease, ART antiretroviral therapy.

Table 4. 22: Predictors of behavioural CVD risk factors among people on ART

Variables		Behavioural CVD risk factors			
		Tobacco use	**Alcohol consumption**	**Physical inactivity**	**Fruit and vegetable intake**
Age:	≤50	1 [ref]			
	>50	2.08	_____	_____	
Gender:	females	1 [ref]	1 [ref]	1 [ref]	
	males	**2.45***	**3.31***	**0.08***	
Educational status:	primary	1 [ref]	1[ref]		
	Secondary and above	1.33	0.57	_____	
Marital status:	Unmarried	1[ref]			
	Married	**3.13***	_____	_____	-0.11 (0.18)^
Interaction: Education status by marital status		**0.17#**	_____	_____	_____
Chi-square; p-value		21.98; 0.001	13.81; 0.001	11.76; 0.001	R^2 – 1.2%
Nagelkerke		15.2%	9.6%	10.8%	F test, p-value -1.83; 0.18
Hosmer and Lemeshow		0.63	0.99	-	
-2 Log likelihood		198.16	211.51	162.02	
Classification accuracy		79.4%	78%	75.8%	

Values are reported as Adjusted Odds ratio. *p-value is significant at <0.05, #p value =0.05, is marginally significant. ART antiretroviral therapy, CVD cardiovascular disease, ^multivariate linear regression was used and values are reported as Beta coefficient (p-value).

Males were more physically active than females (p value 0.02) and were more likely to use tobacco (p value 0.03) and alcohol (p value 0.001) than females.

Married people who had secondary education and above were less likely to use tobacco (p value 0.05) than married people with primary education (Table 4.22).
None of the examined socio-demographic factors were associated with a low intake of fruits and vegetables (Table 4.22).

Predictors of metabolic CVD risk factors in people on ART are presented in Table 4.24.

Table 4. 23: Univariate logistic regression to examine association of covariates with Metabolic CVD risk factors among HIV infected people on ART.

Predictor variable	Metabolic CVD risk factors								
	HTN	**Diabetes**	**Low HDL-C**	**High TC**	**High TG**	**High TC/HDL-C**	**High ApoB/Apo A**	**High TG/ HDL-C**	**Met S**
Age (years): ≤50	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1[ref]	1 [ref]
>50	**3.40**[1]	**6.45**[1]	**1.80**[2]	1.16	**2.55**[1]	**4.25**[1]	**1.50**[2]	**2.61**[1]	**3.68**[1]
Gender: females	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1[ref]	1 [ref]
males	**1.94**[2]	**2.80**[2]	1.37	0.72	3.77	**3.37**[1]	1.11	**3.40**[1]	0.92
Alcohol use: No	1 [ref]	1[ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1[ref]	1 [ref]
Yes	**0.61**[2]	0.90	0.82	**1.65**[2]	1.09	0.99	1.07	0.69	0.62
Tobacco use: No	1[ref]	1[ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1[ref]	1 [ref]
Yes	0.76	0.40	**0.53**[2]	1.20	0.74	0.79	0.79	0.85	**0.50**[2]
Log VL: <1.71	1 [ref]	1[ref]	1 [ref]	1 [ref]	1 [ref]	1[ref]	1 [ref]	1[ref]	1 [ref]
>1.71	**0.3**[1]	0.53	**3.65**[1]	**0.62**[2]	1.53	1.05	**3.38**[1]	**2.29**[1]	1.11
ARVduration: >60	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1[ref]	1 [ref]
(months) 30-60	**1.8**[2]	0.73	0.94	0.79	**0.48**[2]	**0.28**[1]	1.19	**0.40**[2]	0.92
<30	1.06	0.73	1.30	**0.42**[1]	**0.48**[2]	**0.31**[1]	1.57	**0.62**[2]	**0.41**[2]
Abdominal Obesity: No	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1[ref]	
Yes	**2.01**[1]	1.38	**2.17**[1]	**2.06**[1]	**2.52**[1]	1.59	**1.49**[2]	**2.48**[1]	____
CD 4 count: >500	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1[ref]	1 [ref]
(cells/mm^3) 301-500	1.37	0.50	0.79	1.29	0.96	0.89	1.07	0.95	0.74
≤300	1.22	0.60	0.79	1.12	0.83	0.85	1.33	1.26	**0.48**[2]
PA: >600 MET-min/week	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1[ref]	1 [ref]
<600MET-min/week	**0.59**[2]	0.00	**1.73**[2]	**2.10**[1]	1.22	0.68	1.15	1.29	**2.40**[1]
F/V intake	1.01	1.23	**0.81**[2]	1.03	**0.64**[2]	0.84	1.01	**0.66**[1]	0.99
ARV drug: EFV-HAART	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]	1 [ref]
NVP-HAART	0.54	0.00	0.83	0.67	0.90	0.81	0.68	1.10	0.60

Values are reported as Unadjusted Odds ratio (95% CI); [1]p-value <0.05 and [2]p-value is from 0.05 to 0.25 are candidates for multivariate modelling. PA- physical activity, F/V fruit and vegetable, VL viral load

Table 4. 24: Predictors of Metabolic CVD risk factors among HIV infected people on ART

Predictor variable	Metabolic CVD risk factors								
	HTN	**Diabetes**	**Low HDL-C**	**High TC**	**High TG**	**High TC/ HDL-C**	**High Apo B/ Apo A**	**High TG / HDL-C**	**Met S**
Age (years): ≤50	1 [ref]	1 [ref]	1 [ref]	_____	1 [ref]	1 [ref]	1 [ref]	1[ref]	1 [ref]
>50	**4.67[1]**	**5.66[1]**	**2.27[1]**		**2.91[1]**	**3.29[1]**	1.70	2.8	**1.10[1]**
Gender: females	_____	1 [ref]	____	____	___	1 [ref]	___	1[ref]	___
males		1.83				**2.94[1]**		1.90	
Alcohol use: No	1 [ref]	_____	___	1 [ref]	____	___	____	___	____
Yes	0.57			1.69					
Tobacco use: No	_____	_____	1 [ref]	_____	____	____	____	____	1 [ref]
Yes			0.49						1.61
VL (copies/ml): <50	1 [ref]	_____	1 [ref]	____	_____	_____	1[ref]	1[ref]	___
>50	**0.08[1]**		**3.82[1]**				**3.83[1]**	2.71	
ARVduration: >60 months	1 [ref]	_____	_____	_____	1 [ref]	1 [ref]	_____	1[ref]	1 [ref]
30-60months	2.32				0.48	**0.29[1]**		0.49	0.48
<30 months	0.32				0.45	**0.31[1]**		0.29	0.34
Abdominal Obesity: No	1 [ref]	____	1 [ref]	1 [ref]	1 [ref]	____	1 [ref]	1[ref]	
Yes	2.48		1.10	**2.10[1]**	2.08		1.71	**2.98[1]**	_____
PA : >600 MET-min/week	1 [ref]	____	1 [ref]	____	____	____	___	___	1 [ref]
<600 MET-min/week	0.27		0.54						0.22
CD 4 count: >500	_____	_____	___	____	___	____	___	____	1 [ref]
301-500									0.31
≤300									0.12
Low F/Veg intake	_____	_____	0.92	_____	**0.56***	_____	_____	0.67	_____
Abd. obesity by PA	____	___	7.18	____	____	___	___	____	____
Gender by ARV months: >60	____	____	____	____	____	____	____	1[ref]	____
30-60								0.46	
<30								6.07	
Chi-square; p-value	28.46;0.01	8.45; 0.02	23.68; 0.001	6.81; 0.03	13.91;0.02	21.39;0.01	15.39; 0.02	28.78; 0.01	27.71; 0.01
Nagelkerke	27.0%	12.3%	19.4%	4.5%	16%	19.1%	9.7%	28.7%	41.4%
Hosmer & Lemeshow	0.28	0.89	0.71	0.34	0.85	0.98	0.97	0.29	0.94
-2Log –likelihood	130.14	72.25	183.23	256.71	110.44	133.94	265.82	118.25	52.90
Classification accuracy	81.7%	95.3%	67.8%	69.6%	84.1%	87.4%	61.8%	82.6%	91.0%

Values are reported as Adjusted Odds ratio. [1]p-value <0.05 is significant. *****p=0.05, marginally significant. PA-physical activity, VL-viral load, PA-physical activity, F/V-fruit and vegetable intake, HDL-C high density lipoprotein cholesterol, LDL-C low density lipoprotein cholesterol, TC total cholesterol, TG triglycerides, Apo B apolipoprotein B, Apo A apolipoprotein A, Met S metabolic syndrome, HTN hypertension, ART antiretroviral therapy, MET-min metabolic equivalent of task-minute.

People more than 50 years of age were more likely to be hypertensive (p value <0.05) and diabetic (p value <0.05) compared to people less 50 years of age. In addition an age of more than 50years increased the likelihood of having metabolic syndrome (p value< 0.05), a high concentration of TG (p value <0.05), a high TC/HDL-C ratio (p value <0.05) and a low concentration of HDL-C (p value <0.05) compared to an age of less than 50 years.

Males were 2.94 times (p value <0.05) more likely to have a high TC/HDL-C ratio compared to females.

People with a viral load of more than 50 copies/ml (> log 1.71) were less likely to be hypertensive but were more likely to have a low HDL-C concentration and a high ratio of ApoB/ApoA than people with a viral load of less than 50 copies/ml.

People on ART for less than 60 months were less likely to have a high TC/HDL-C ratio than people on ART for more than 60 months.

The likelihood of having a high TC concentration was 2.10 times (p value <0.05) and a high TG/HDL-C ratio was 2.98 times (p value <0.05) more in people with than in people without abdominal obesity.

A low intake of fruit and vegetable was associated with a high concentration of TG (p value <0.05) (Table 4.24).

Predictors of Lipoprotein subclasses in ART HIV infected people are presented in Table 4.26

Table 4. 25: Univariate linear regression to determine association of covariates with Lipoprotein subclasses in HIV infected people on ART

Predictor variable	Lipoprotein Subclasses			
	Large HDL-p	**Small HDL-p**	**Large LDL-p**	**Small LDL-p**
Age:	-0.05 (0.44)	0.05 (0.53)	0.02 (0.76)	-0.10 (**0.14**)*
Gender:	-0.13 (**0.06**)*	0.13 (**0.06**)*	-0.05 (0.47)	0.12 (**0.08**)*
Alcohol:	-0.10 (**0.16**) *	0.10 (**0.15**)*	0.03 (0.68)	0.01 (0.91)
Tobacco use:	0.040 (0.60)	-0.04 (0.62)	0.05 (0.45)	-0.04 (0.62)
VL:	-0.06 (0.38)	0.06 (0.37)	0.03 (0.67)	-0.01 (0.89)
CD4 count (cells/mm^3):	0.16 (**0.03**) *	-0.16 (**0.03**)*	-0.06 (0.39)	0.11 (**0.11**)*
ARV duration (months):	0.12 (**0.10**) *	-0.13 (**0.08**)*	-0.07 (0.33)	-0.01 (0.87)
ARV drug:	0.002 (0.98)	0.002 (0.98)	0.004 (0.96)	0.04 (0.64)
BMI (kg/m^2):	-0.06 (0.39)	0.06 (0.36)	-0.01 (0.85)	0.04 (0.62)
WC	-0.15 (**0.03**)*	0.16 (**0.03**)*	0.004 (0.95)	0.03 (0.65)
HDL-C:	0.05 (0.45)	-0.05 (0.47)	0.01 (0.94)	-0.06 (0.42)
LDL-C:	0.04(0.62)	-0.04 (0.59)	0.15(**0.03**)*	-0.20 (**0.004**)*
TC:	-0.02 (0.77)	0.017 (0.81)	0.12 (**0.08**)*	-0.18 (**0.01**)*
TG:	-0.28 (**0.0001**)*	0.27 (**0.0001**)*	-0.02(0.83)	0.04(0.60)
TG/HDL-C	-0.22 (**0.002**)*	0.21 (**0.002**)*	-0.02 (0.81)	0.06 (0.38)
TC/HDL-C	-0.07 (0.32)	0.07 (0.33)	0.09 (**0.21**)*	-0.07 (0.34)
ApoB/ ApoA	-0.02 (0.76)	0.02 (0.79)	0.19 (**0.01**)*	-0.25 (**0.001**)*
Hypertension:	-0.10 (**0.17**)*	0.08 (0.26)	-0.07 (0.32)	-0.05 (0.44)
Diabetes:	-0.13 (**0.06**)*	0.13 (**0.06**)*	-0.02 (0.73)	0.06 (0.42)
Met S:	-0.13 (**0.08**)*	0.13 (**0.07**)*	-0.05 (0.52)	0.10 (**0.16**)*
Fruit and vegetable intake	0.03 (0.69)	-0.04 (0.64)	0.02 (0.83)	-0.03 (0.76)
Physical activity	0.07 (0.47)	-0.08 (0.95)	0.01 (0.95)	-0.08 (0.33)

Values are reported as Beta coefficient (p-value). *p value ≤ 0.25 are candidates for multivariate modelling. HDL-C high density lipoprotein cholesterol, LDL-C low density lipoprotein cholesterol, TC total cholesterol, BMI body mass index, VL-viral load, TG triglycerides, ApoB apolipoprotein B, ApoA apolipoprotein A, Met S metabolic syndrome, HDL-p High density lipoprotein-particles, LDL-p Low density lipoprotein-particle.

Table 4. 26: Predictors of Lipoprotein subclasses in HIV infected people on ART

Predictor variable	Large HDL-p		Small HDL-p		Large LDL-p		Small LDL-p	
	1st Model	Last Model	1st Model	Last Model	1st Model	Last Model	1st Model	Last Model
Age:							-0.11 (0.15)	____
Gender:	-0.04 (0.61)	____	0.04 (0.61)	____			0.13 (0.07)	____
Alcohol:	-0.12 (0.12)	-0.12 (0.09)	0.12 (0.11)	0.12 (0.09)				
CD4 count(cells/mm^3):	0.14 (0.09)	0.18 (**0.02**)*	-0.15 (0.07)	-0.19 (**0.01**)*			0.07 (0.30)	____
ARV duration (months):	0.09 (0.28)	____	-0.08 (0.31)	____				
Abdominal obesity	-0.19 (0.01)	-0.16 (**0.03**)*	0.23 (0.01)	0.20 (**0.01**)*				____
LDL-C:					0.10 (0.58)	____	-0.05 (0.80	____
TC:					-0.003 (0.98)	____	-0.15(0.39)	____
TG/HDL-C	-0.18 (0.048)	-0.20 (**0.01**)*	0.17 (0.06)	0.17 (**0.02**)*				
TC/HDL-C					-0.08 (0.41)	____		
ApoB/ ApoA					0.19 (0.04)	0.19 (**0.01**) *	-0.21 (0.02)	-0.27 (**0.0001**) *
Hypertension:	0.03 (0.69)	____						
Diabetes:	-0.08 (0.30)	____	0.08 (0.30)	____				
Met S:	0.02 (0.81)	____	-0.06 (0.54)	____			0.15 (0.04)	0.14 (**0.04**) *
F-test	2.94	6.06	3.55	6.48	2.22	7.3	3.45	7.99
p-value	0.003	0.0001	0.001	0.0001	0.07	0.01	0.002	0.0001
R^2	13.6%	12.3%	14.4%	13.0%	4.3%	3.5%	11.4%	7.6 %
Adjusted R^2	9.0%	10.3%	10.3%	11.0%	2.3%	3.0%	8.1%	6.7 %

Values are reported as Beta coefficient (p-value). *p-value is significant at <0.05. HDL-C high density lipoprotein cholesterol, LDL-C low density lipoprotein cholesterol, TC total cholesterol, TG triglycerides, Apo B apolipoprotein B, Apo A apolipoprotein A, ART antiretroviral therapy.

CD4 count was positively associated with the large HDL-particles (p value 0.02) and negatively with the small HDL-particles (p value 0.01).
Abdominal obesity was negatively associated with the large HDL-particles (0.03) and positively with the small HDL-particles (p value 0.01).
The ratio of TG/HDL-C was negatively associated with the large HDL-particles (p value 0.01) and positively with the small HDL-particles (p value 0.02).
The ratio of ApoB/ApoA was positively associated with the large LDL-particles (0.01) and negatively with the small LDL-particles (p value 0.0001). Metabolic syndrome had a positive association with the small particles of LDL (p value 0.04) (Table 4.26).

4.4.7 Framingham 10-year cardiovascular disease (CVD) risk in ART HIV infected participants.

The distribution of people on ART according to Framingham risk category is presented in Figure 4.6.

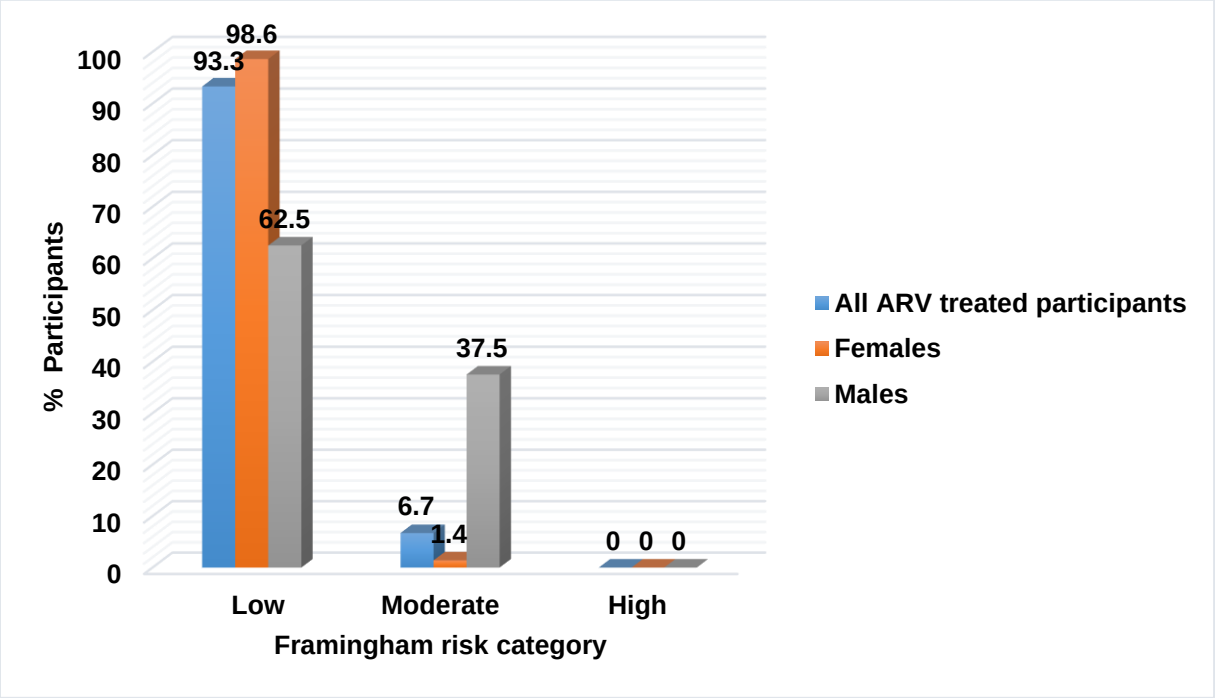

Figure 4. 6: Distribution of people on ART according to Framingham risk category

The majority of participants had a low risk score with a higher percentage of females (98.6%) than males (62.5%). A higher percentage of males (37.5%) had a moderate 10-year risk than females (1.4%). None of the participants on ART had a high 10-year CVD risk according to Framingham risk assessment tool (Figure 4.6).

4.4.8 DAD 5-year cardiovascular disease (CVD) risk in ART HIV infected participants.

The distribution of people on ART according to the DAD risk category and the comparison between Framingham and the DAD risk estimations are presentated in Table 4.27.

Of the 164 participants, 68.9 % had a low risk, 27.4% had a moderate risk and 3.7% had a combined high and very high risk for developing a CVD event in next 5-years. However, considering all (214) participants, 66.8% had a low risk, 29% had a moderate risk, 1.9% had a high risk and 2.3% had a very high risk for developing CVD in next 5-years.

Comparison of the Framingham risk scores with the DAD risk scores in 164 participants who met the Framingham criteria gave a level of agreement of 73.8% (Kappa=0.23; 95% CI: 0.10-0.35; p value 0.001) (Table 4.27).

Table 4.27 Distribution of participants according to DAD and a comparison between CVD risk estimation using DAD and Framingham equations

		FRAMINGHAM (N=164)		
	Cardiovascular disease risk	Low risk	Moderate risk	High risk
DAD (N=164)	Low risk	113	0	0
	Moderate risk	37	8	0
	High risk and very high risk	3	3	0
Agreement		73.8 %		
Kappa (p-value)		0.23 (0.001)		
95% CI for Kappa		0.10 – 0.35		

	DAD risk estimations on all participants			
	Low risk (<1%)	Moderate risk (1-5%)	High risk (5-10%)	Very high risk (>10%)
All participants N=214	143 (66.8 %)	62 (29 %)	4 (1.9%)	5 (2.3%)

CHAPTER 5

DISCUSSION

Introduction

Cardiovascular disease (CVD) is considered the second most common cause of mortality after cancer in HIV infected people (Giannarelli et al., 2011) and investigation of the risk factors for CVD is warranted in order to strengthen management strategies. In the present study conducted in a rural area, Limpopo Province, cardiovascular risk factors among ART naïve HIV infected people, ART HIV infected people and among HIV negative people were assessed.

Cardiovascular risk factors in ART naïve HIV infected and uninfected people.

Published results have indicated that the prevalence of CVD risk factors is higher in ART naïve HIV infected individuals compared to uninfected individuals (Islam et al., 2012, Freiberg et al., 2013). In the present study, a higher prevalence of low HDL-C concentration was observed in the ART naïve HIV infected group compared to the uninfected group which is in agreement with other studies (Rose et al., 2008, Riddler et al., 2008, Fourie et al., 2010, Dillon et al., 2013). The lower concentration of HDL-C among ART naïve HIV infected people is thought to be mediated via an inflammatory process in which high concentration of serum amyloid A produced in HIV infection tend to substitute the apoA in HDL-C leading to its increased catabolism (Kontush and Chapman 2006, Marsche et al., 2013). An association of high viral load with low HDL-C concentration previously reported by Rose et al. (2008) and Riddler et al. (2008), was also observed in the present study. As a low HDL-C concentration is considered second to age in contributing to CVD risk (Cotter et al., 2011), the low HDL-C concentration in

the present study may therefore contribute to CVD risk among ART naïve HIV infected people. Furthermore the proportion of the atherogenic small LDL-particles was higher in HIV infected than uninfected people conferring increased CVD risk, while no difference was observed in the proportion of HDL-particles. The regression analysis in the present study showed that a decrease in waist circumference among ART naïve HIV infected people was associated with an increase in the proportions of atherogenic small LDL and HDL-particles, while a decrease in CD4 count was associated with an increased proportion of small HDL-particles. These associations provide evidence that if HIV infection is not controlled, there will be increased proportions of the atherogenic small LDL and HDL particles and reduced proportions of large LDL and HDL particles, a pattern that promotes growth of an atheroma (Otvos et al., 2006) and possible risk of CVD events. None of the serum lipids or lipid ratios in ART naïve HIV infected people in the present study were significant predictors of LDL and HDL particles, indicating that the serum lipids may not explain the distribution of the subclasses (Otvos et al., 2006). However, the absence of an association between the serum lipid or lipid ratios with small LDL and HDL particles among ART naïve HIV infected people in the present study require further research.

Even though tobacco use, obesity and abdominal obesity in the present study were similar in ART naïve HIV infected and uninfected people, as was reported in other studies (Fourie et al., 2010, Carey et al., 2013), there were some gender differences. Irrespective of HIV status, the use of tobacco was more common in men than in women, while obesity and abdominal obesity were more common in women than in men. These findings may be indicative of some societal influences. In most black African communities, tobacco use by men is culturally accepted (Egbe et al., 2014). Several other studies have reported higher smoking rates in males than in females (Desalu et al., 2009, Batista et al., 2013, Gilani and Leon 2013). The association of male gender and tobacco use observed in the present study and also by Kruse et al., 2014 renders it a priority group for tobacco cessation. The higher prevalence of obesity and abdominal obesity in women than in men in the present study was also reported by Grantham and Henneberg. (2014) and may be driven by cultural values that favours women with large body size as a sign of fertility, wealth and prosperity (Kanter and Caballero 2012). HIV

infection has been associated with lower BMI (Dillon et al., 2013) but the present study showed no change in BMI with infection, a possible indication of an early to mid-stage HIV infection which was confirmed by a CD4 count of nearly 400cells/mm^3. According to the Framingham risk score in the present study, six in every hundred ART naïve HIV infected people may suffer a CVD event in the next ten years, and all the people with this high risk were males. This risk may be associated with the high use of tobacco among males. Similar to the present study findings, ART naïve HIV individuals with a high 10 year Framingham risk score in a study conducted in Norway, were all men (Bergersen et al., 2004).

Several CVD risk factors were similar in ART naïve HIV infected and uninfected people, and showed no gender difference. Irrespective of HIV status and gender, the intake of fruit and vegetable was low. In agreement to this finding, a study conducted in a South African rural and semi-rural area, similar to Dikgale HDSS, found that the intake of fruit and vegetables in that community was low (Peltzer and Promtussananon 2004). Overcoming this problem particularly in rural areas may therefore remain a challenge, as evidence suggests an association of perceived expense, and low income with low fruit and vegetable intake (Peltzer and Promtussananon 2004, Oguntibeju et al., 2013). A low level of fruit and vegetable intake may lead to deficiencies in vitamins, predisposing people to a wide range of diseases including CVD, cancers and cataracts (Peltzer et al., 2012, Oguntibeju et al., 2013) and may accelerate HIV disease progression (Nkengfack et al., 2013). Irrespective of HIV status, physical activity was low among both HIV positive and negative people in the present study. However, a previous study conducted among women in this community using an objective method showed that the women were physically active (Cook et al., 2010). Literature suggests that subjective methods tend to produce higher physical inactivity than objective methods (Cook et al., 2007), possibly due to recall bias. But participants in the present study were old, a possible explanation of the high prevalence physical inactivity. The benefits of physical activity are well documented (Veljkovic et al., 2010, Liu et al., 2012, Dufour et al., 2013) but these benefits need to be communicated to the community. According to regression analysis in this study, older age increased the likelihood of being physically inactive, while the male gender was associated with being physically

active. Alcohol use was similar for ART naïve HIV infected and uninfected people in the present study, which was also reported by Fourie et al. (2010). However, among HIV uninfected people, males used more alcohol than females, while no gender difference was observed among HIV infected people. Of note is the higher alcohol use in HIV infected than uninfected women, which may be due to the fact that majority of HIV infected women were unmarried (Shisana et al., 2014). Consistent with present study findings, Figueroa-Cosme et al. (2010) reported higher alcohol use in HIV infected than uninfected women. These findings probably indicate a close relationship of alcohol use and risk of HIV infection in women. An association of low levels of education and the use of alcohol was observed among ART naïve HIV infected people in Dikgale HDSS.
In the present study, the mean TG concentration and the prevalence of hypertriglyceridaemia did not differ by HIV status or gender. Some studies have demonstrated disparate TG results (Oduola et al., 2009, Fourie et al., 2010, Kumar et al., 2011). Variations in TG results observed in various studies were attributed to the difference in the stage of HIV infection, with TG concentration escalating as HIV infection progresses (Grunfeld et al., 1992, Armstrong et al., 2011). According to the regression analysis, in the present study abdominal obesity among ART naïve HIV infected people was a predictor of high concentration of TG in this study.

Lipid ratios (TC/HDL-C, TG/HDL-C, ApoB /ApoA) are regarded as stronger predictors of CVD than serum lipids (Lewington et al., 2007). However in the present study the ratios were similar in HIV infected and in uninfected people, with no gender difference. In agreement to the present study findings Carey et al. (2013) and Daniyam and Iroezindu (2013) found no difference in lipid ratios between HIV infected and uninfected people. Evidence from the present study suggests that an increase in viral load will lead to increased lipid ratios. Thus the similarities observed between HIV infected and uninfected people in the present study coupled with a CD4 count of nearly 400cells/ mm^3 may indicate that participants had an early to mid-stage HIV infection. The concentration of hsCRP which is a measure of inflammation was not different between HIV infected and uninfected people and between genders. The present study finding is supported by Baker et al. (2010) who found a similar concentration of hsCRP in ART naïve HIV infected and in uninfected people. But Neuhaus et al. (2010) reported a higher

concentration of hsCRP in ART naïve HIV infected than in uninfected people, disagreeing with present study finding. According to Lau et al. (2006) and Kuller et al. (2008) the concentration of hsCRP is dependent on the stage of the HIV infection, with high concentration present in advanced stage of infection. As participants in the various studies may be at different stages of infection, the results may therefore be contradictory. HIV infected males in the present study had a higher concentration of hsCRP than uninfected males, suggestive of a possible increase in the risk for CVD (Pearson et al., 2003). This difference may be explained by the high viral load in HIV infected males, since an association of high hsCRP concentration with high viral load was previously reported (Lau et al., 2006, Dumitrescu et al., 2013).

The high prevalence of hypertension which was similar in HIV infected and uninfected rural people in this study, presents a major public health problem. In another study, hypertension was more common in HIV uninfected than infected people, but these participants were not age and gender matched (Carey et al., 2013). The present study found that as HIV infection progressed as marked by decreased CD4 count, the risk of developing hypertension was reduced. Published results on this association are however conflicting. An inverse association of HIV infection and BP, which is in agreement with present study finding, was reported in a meta-analysis of sub-Saharan studies (Dillon et al., 2013), while other studies found no association between HIV infection and BP (Bergersen et al., 2003, Lategan et al., 2014). ART naïve HIV infected and uninfected people in the Dikgale HDSS community had an equal burden of diabetes mellitus, findings also observed in studies from Australia (Samaras et al., 2009) and India (Carey et al., 2013). The prevalence of diabetes mellitus was similar for males and females in the present study, while findings from the DAD study suggested an increased risk in the male gender. Evidence from the present study, suggests no risk for diabetes mellitus from HIV infection, but shows increased risk from age and tobacco use, confirming previous reports (Brar et al., 2007, Capeau et al., 2012). Metabolic syndrome (MetS) presents a greater risk of developing CVD than its individual components (Calvo and Martinez 2014). In the present study, ART naive HIV infected and uninfected people had a similar burden of MetS which showed no gender difference. In agreement with the present study finding, a study from North West Province of South Africa involving

both rural and urban dwellers found a similar prevalence of MetS in ART naïve HIV infected and uninfected people. But a higher burden of MetS in ART naïve HIV infected than in uninfected people was reported from Cameroon (Ngatchou et al., 2013) and Italy (Bonfanti et al., 2007). The inconsistencies surrounding the prevalence of MetS between HIV infected and uninfected people may be due to differences in HIV variables in study populations. It therefore remains unclear whether the prevalence of MetS is higher in ART naïve HIV infected than uninfected people.

HIV infected participants on ART

The majority (96%) of HIV infected participants on ART in the present study were on the 1st line non-nucleoside reverse transcriptase inhibitors. The use of NNRTI was associated with a low atherogenic lipid profile and could defer occurance of premature CVD in HIV infected people (Minnaar and van der Merwe 2008, Swanson et al., 2009, Murphy et al., 2010). In the present study, three of the participants (1.5%) were on the ATV-based ART option, while 5 participants (2.5%) were receiving d4T-based ART that has been discouraged due to its toxicity (WHO 2013). The mean ART duration in this study was 36 months (range: 1-121 months). Most of the participants (85%) had undetectable viral load and a mean CD4 count of 462 ± 235 cell/mm^3.

Cardiovascular risk factors in HIV infected people on ART.

In this population of HIV infected people on ART, the prevalence of behavioural CVD risk factors was high. At least one fifth of participants used tobacco and the majority of these were males. The people on ART are in constant consultations with health care providers and therefore the prevalence was expected to be lower than that reported in the general South African population (Stassen, 2013). Thus despite counselling tobacco use in HIV infected people on ART in this study has remained high. The strongest predictor of tobacco use among people on ART was male gender, while people with higher education and who were married were less likely to use tobacco.

The use of alcohol in the present study was high among males on ART which is similar to a study from North West Province, South Africa (Van Rooyen et al., 2014). This high prevalence of alcohol use may suggest a potential for new HIV infections and re-infections, due to risky sexual behaviour (Kalichman et al., 2007, Rehm et al., 2012, Schneider et al., 2014). The use of alcohol in people on ART as in the general population, is however still surrounded with controversy. Carter et al. (2011) argued that alcoholic beverages such as red wine have health benefits and Carrieri et al. (2012) reported that moderate alcohol use may reduce the risk of coronary disease events. However, according to Bryant et al. (2006) there is no safe quantity of alcohol intake in HIV infected people. Thus whether the consumption of alcohol is beneficial or harmful to HIV infected people on ART remains unclear. Male gender was a predictor of alcohol use in this study as was also reported by Soboka et al. (2014).

In the present study, most people on ART were physically active (75.8%) similar to a Malawian study (Muronya et al., 2011). Lower physical activity than in the present study was reported among Rwandese on ART (Frantz et al., 2013), probably owing to a different instrument for data collection. Informal discussions with people on ART revealed that they were more likely to know more on the benefits of physical activity as they received this information from nurses. Thus the majority engaged in activities such as walking, jogging, skipping and household chores such as fetching firewood and water. Males were more physically active than women, a finding suggesting that more women of all ages on ART need to be encouraged to take up physical activities.

Fruit and vegetable intake among people on ART was low as was reported among adult Malawians (Muronya et al., 2011) and South Asians (Simkhada et al., 2014) on ART. Even though none of the available variables could explain the low intake of fruits and vegetables, most participants in the present study cited non availability of fruits and vegetables in some villages coupled with unaffordability as reasons for low intake of fruits and vegetables. The low intake of fruits and vegetables observed in the present study remain a major challenge as it increases the risk of nutritional deficiencies, CVD and interferes with HIV disease management (Koebnick et al., 2005, Takahashi et al., 2010).

Among the metabolic risk factors, hypertension was observed in nearly a quarter of HIV infected participants on ART, consistent with a study in Senegal (Diouf et al., 2012) and Ethiopia (Berhane et al., 2012). However other studies with younger study populations than the present study reported a lower prevalence (Julius et al., 2011, Edward et al., 2013, Muhammad et al., 2013, Carey et al., 2013). The prevalence of hypertension was not different between males and females in the present study. Of the 42 HIV infected participants on ART with a history of hypertension, approximately 60% had their blood pressure controlled, while 40% had raised blood pressure. Factors contributing to the uncontrolled blood pressure are varied including poor adherence to treatment, poor lifestyle and high salt intake (Faselis et al., 2011). A further investigation on the causes of uncontrolled blood pressure in this community in order to design an intervention program is needed. While HIV infected people on ART tend to experience an increase in BP (Palacios et al., 2006) the relationship of ART and BP is conflicting. Some studies reported no association (Thiebaut et al., 2005, de Arrunda Junior et al., 2010, Ogunmola et al., 2014), while others reported an association between ART and BP (Seaberg et al., 2005, Wilson et al., 2009). According to the present study, regression analysis did not show a relationship between ART duration and hypertension, but demonstrated age as a significant preditor of hypertension, adding to the growing evidence that the traditional risk factors for BP are operating in people on ART (Thiebaut et al., 2005, de Arrunda Junior et al., 2010).

In the present study, diabetes mellitus in both HIV infected males and females on ART was low as was reported in other studies (Manuthu et al., 2008, Dave et al., 2011, Julius et al., 2011, Muronya et al., 2011). A high burden of diabetes mellitus among Indians on ART as reported by Carey et al. (2013), may be due to their use of stavudine based ART, shown to have serious effects on glucose metabolism (Kalra et al., 2011). The use of PIs by majority of Senegalese (Diouf et al., 2012) may explain the high prevalence of diabetes mellitus. It is possible that the use of newer, better ART combinations with little effects on glucose metabolism (Noor et al., 2004) may explain the low prevalence of diabetes mellitus in the present study. Moreover the participants in the present study were young reducing their risk of developing diabetes mellitus, as age was found to predict diabetes mellitus in this study, as in a study by (Dave et al.,

2011). However, cumulative exposure of ART was shown to increase the incidence of diabetes mellitus in the D.A.D study from United States (De Wit et al., 2008).

Obesity was found in more than a tenth of HIV infected participants on ART in this study which is similar to a study from Nigeria (Muhammad et al., 2013). However a lower prevalence of obesity was reported from Ethiopia (Abebe et al., 2014), while a study from India (Carey et al., 2013) reported a higher prevalence of obesity in people on ART. Abdominal obesity (visceral lipohypertrophy) experienced with ART was observed in nearly a quarter of participants in the present study. Other African cohort studies reported a lower prevalence (Muhammad et al., 2013, Edward et al., 2013), while a higher prevalence was reported from India (Carey et al., 2013). According to Crum-Ciaflone et al. (2008), the variations in obesity and abdominal obesity may partly be explained by differences in ART duration and the different cut-off values for the BMI and waist circumference used in various studies. The prevalence of obesity and abdominal obesity was similar in males and females on ART, contrasting the pattern in the general African populations that shows a higher prevalence of obesity and abdominal obesity in females than in males (Kanter and Caballero 2012). A possible explanation may be based on the young age of female participants, in addition to the advice on weight reduction received by both males and females from nurses.

The prevalence of metabolic syndrome was low and similar in males and in females on ART. Documented prevalence of metabolic syndrome is varied, possibly resulting from its heterogeneous nature and variations in prevalence of components (Jerico et al., 2005, Ayodele et al., 2012, Muhammad et al., 2013, Malangu et al., 2014).

Antiretroviral therapy increases triglyceride (TG) concentration to beyond pre-infection levels, with early PI inducing substantial TG increases (Hejazi et al., 2013). In the present study, the prevalence of hypertriglyceridaemia was low, maybe due to the NNRTI based ART used. The use of stavudine base ART in Ethiopians (Berhane et al., 2012) and Indians (Carey et al., 2013), may explain the more than twice the prevalence in these populations. The high concentration of TG in the present study, was more common in males than in females, as males were significantly older than females.

Abdominal obesity and older age in the present study increased the risk of having high TG concentration, in agreement with Hejazi et al. (2013). On the other hand, the risk of having a high TG concentration was reduced by increased intake of fruit and vegetables as has been reported in other studies (Koebnick et al., 2005, Takahashi et al., 2010).

According to Estrada and Portilla (2011) serum HDL-C concentration hardly return to normal levels in HIV infected people on ART, with NNRTIs giving better improvement than PIs. Low HDL-C concentrations in people on ART are likely due to the exchange of TG for cholesterol ester carried in HDL by cholesterol ester transfer protein (CETP) forming a TG-rich HDL which is rapidly metabolized (Rader 2006). Thus the prevalence of a low HDL-C concentration is high among people on ART (Julius et al., 2011, Edward et al., 2013, Muhammad et al., 2013). There was no significant difference in prevalence of low HDL-C concentration between females and males in the present study. Older age and high viral load were independent predictors of a low HDL-C concentration in the present study. These results showed the importance of suppressing the viral load to minimize the presence of low HDL-C concentration as people on ART age.

High TC concentrations caused by ART were common in participants on ART in this study which was also reported in other studies (Muronya et al., 2011, Julius et al., 2011, Edward et al., 2013, Muhammad et al., 2013). There was no difference in the prevalence of hypercholesterolaemia between males and females in this study. This similarity may be linked to the similarity in prevalence of abdominal obesity among males and females, since abdominal obesity in the present study is a significant predictor of hypercholesterolaemia. None of the participants in the present study was using lipid lowering drugs, possibly accounting for the high prevalence of abnormal lipid levels. Lipid ratios are regarded as better predictors of CVD than individual lipids (Lewington et al., 2007, Duprez et al., 2011). In the present study, a high TC/HDL-C ratio was more common in males than in females. The regression analysis identified male gender, older age and longer ART duration as independent predictors of the TC/HDL-C ratio in this study. These findings have important implications as these variables will intersect at some point increasing the risk of high TC/HDL-C ratio and development of CVD. A similar prevalence of the high ApoB/ApoA ratio was found in males and in females. The

association of ApoB/ApoA ratio with high viral load in the present study indicates the importance of suppressing the virus load. More males than females had a high prevalence of high TG/HDL-C ratio, probably due to the older age in males than females. According to regression analysis, abdominal obesity a common feature in HIV infected people on ART was associated with a high TG/HDL-C ratio.

The high median CRP concentration in this study as in other studies (Boger et al., 2009, Borato et al., 2012) indicated possible ongoing inflammation in participants receiving ART. The levels were however, similar in males and females.

Large LDL and HDL particles are considered less atherogenic than the small particles (Otvos et al., 2006). In the present study, the majority of people were on EFV + NRTI regimen and had a lipoprotein subclass profile characterized by a predominance of a high proportion of large LDL and HDL particles, as determined by the polyacrylamide gradient gel electrophoresis (PAGGE). The lipoprotein subclass proportions were not significantly different between males and females. In agreement to the present findings, studies from developed countries that used the nuclear magnetic resonance spectroscopy (NMR) to determine particle concentrations, found an association of EFV + NRTI with a less atherogenic particle profile consisting of high concentrations of the large LDL and HDL particles (Stein et al., 2008, Riddler et al., 2008). In the present study, an increase in CD4 count was associated with an increase in proportion of large HDL-particles and decreased proportion of small HDL-particles. The presence of abdominal obesity and a high TG/HDL-C ratio was associated with a decrease in the proportion of large HDL-particles and an increase in the proportion of small HDL-particles. The association of TG/HDL-C ratio and HDL-particles that predicted CVD events in HIV infected people on ART (Duprez et al., 2009, Baker et al., 2010) may be used to estimate the HDL-particles adding more information to CVD risk estimation since subclasses are not routinely analysed. Perhaps surprisingly, was an association of increased ApoB/ApoA ratio with increased proportion of large LDL-particles observed in the present study. In a study conducted in America, ART was shown to increase the concentration of large LDL-particles but not small LDL-particles (Baker et al., 2011). Therefore the increase in ApoB/ApoA ratio might be a reflection on the increase in

proportion of the large LDL-particles, since ApoB is largely present in LDL-particle. Metabolic syndrome in the present study was associated with an increase in proportion of small LDL-particles. In contrast, Bittar et al. (2012) found a significant association of small LDL particles with high TG and low HDLC concentrations in an HIV infected cohort treated with protease inhibitors.

5-year DAD and 10-year Framingham risk estimation in HIV infected people on ART.

A low prevalence of high 10-year CVD risk (Framingham risk score ≥ 20%) among HIV infected participants on ART in this study may be that our cohort was relatively young and predominantly composed of females. In addition studies have shown a high prevalence of high 10-year CVD risk with the use of PIs but not with the use of NNRTIs (Kaplan et al., 2007, Reinsch et al., 2012) that were predominantly used by our cohort. According to the Framingham risk score, no one of the HIV infected people on ART in the present study had a high risk of developing a CVD in the next ten years. Although our study population was young, using the DAD risk equation, 31.1% of participants had a 5-year moderate to high CVD risk.

Our study found the level of agreement between the Framingham and DAD risk estimation equations to be 73.8 percent, which was similar to an agreement level of 77.4% reported by Nery et al. (2013). Despite this level of agreement observed in our study, the Framingham equation underestimated the risk for CVD in 43 of the 164 participants, when compared to the DAD risk score. These results suggest that the use of the Framingham equation in people infected with HIV receiving ART may lead to the exclusion of some individuals to benefit from more aggressive CVD prevention. Similar to our findings, literature suggests that the Framingham equation underestimates the risk of CVD in Africans [Klug et al., 2015].

Summary on CVD risk factors in ART naïve HIV infected participants and in HIV infected participants on ART in the present study.

The present study provides evidence that HIV infected participants naïve to ART and those on ART residing in rural Dikgale HDSS are not spared from the burden of CVD risk factors. According to the present study, ART naïve HIV infected participants had a worse CVD risk profile than those on ART. This finding is in contrast to other studies that reported higher CVD risk factors among ART than among ART naïve HIV infected people (Manfredi 2009, Edward et al., 2013, Carey et al., et al. 2013, Kagaruki et al., 2014). The inconsistency between the present study findings and the above mentioned studies may probably lie with the difference in the ART combinations used. The majority of participants in the study by Manfredi et al. (2009) and Kagaruki et al. (2014) were on PI-based ART while most participants in the study by Carey et al. (2013) were on stavudine-based ART, drugs associated with CVD risk factors. In the study by Edward et al. (2013), more than three quarters of participants on NNRTI-based ART, were much older than those naïve to ART, presumably contributing to higher risk factors in those on ART. The present study findings are likely a result of the use of NNRTI based ART coupled with the younger age of those on treatment compared to treatment naïve participants.

According to Cotter et al. (2011) a low HDL-C concentration is second only to age in contributing to overall CVD risk suggesting that the risk of not being on ART in terms of morbidity and mortality maybe greater than the metabolic disturbances attributable to ART. Evidence from the present study shows that the prevalence of a low HDL-C concentration was lower in those on ART than in ART naïve HIV infected people. In addition, ART naïve HIV infected people had twice the prevalence of obesity, hypertension, diabetes mellitus and metabolic syndrome than people on ART. The proportions of the atheroprotective large LDL and HDL particles were higher in those on ART than in ART naïve HIV infected people. Furthermore the majority of people on ART were physically active, while the majority of ART naïve HIV infected were physically inactive. However, the prevalence of hypercholesterolaemia was twice as

high in people on ART than in ART naïve HIV infected people. The fact that people on ART were aware of their status and showed an understanding of a healthy lifestyle as briefed during their monthly consultations at clinics, could possibly explain the low prevalence of some risk factors observed in the present study. Overall, according to 10-year Framingham risk score estimation, HIV infected people on ART compared to those naïve to ART in the present study had a better 10 –year CVD risk score.

Strengths and limitations of the present study

This study is one of the first to reflect on the wide spread roll-out of the ART in a rural South African population. The study included the analysis of the non-routinely analysed lipoprotein subclasses in addition to a wide range of CVD risk factors as well as the determination of the 5-year and 10-year CVD risk, adding on to the prediction of CVD risk in the rural population of Dikgale HDSS. Dietary intake (fruit and vegetable) and physical activity in HIV infected people on ART who are constantly receiving counselling on lifestyle changes as part of HIV care have not been reported from Limpopo Province and therefore our study provides information that can be used to evaluate the impact of lifestyle counselling on HIV infected people on ART in Dikgale HDSS. Standardized techniques were used which included the WHO STEPS questionnaire and the repeated measurements of BP and HIV, as well as the use of controls in analysing the biochemical parameters and CD4 count.

There are some limitations within the present study that have to be taken into account when interpreting our results.

- Our study was cross-sectional in design, therefore we cannot conclude that the associations between covariates and CVD risk factors are causal, but it provides information that serves as basis for future longitudinal studies.
- Information on tobacco use, alcohol use, physical activity and fruit and vegetable intake was obtained using the WHO STEP questionnaire. This is considered to be a reliable instrument, however recall bias may have influenced the results.

- We included two (2) HIV negative controls per one (1) HIV infected case, in order to increase the power of the study (Niven et al., 2012).
- Non-random sampling was used to recruit HIV infected participants on ART. However, recruitment was conducted for a whole month cycle, giving all patients collecting their medication equal opportunity to participant in the study. We did not include controls during recruitment, as our goal was to assess the CVD risk in HIV infected on ART. While our sample may therefore not be representative of the whole population of HIV infected South Africans receiving ART, we are not aware of any other study in our HIV infected population that has attempted to assess the dietary intake and physical activity, lipoprotein subclasses and to estimate the 10-year CVD risk using the Framingham risk equation. The present study therefore provides valuable and useful information for comparison with other published studies from both developing and developed countries.
- The Framingham risk equation was not developed for estimation of CVD risk in HIV infected population, however the NCEP ATP III guidelines for CVD risk stratification of HIV infected people use the Framingham risk equation for predicting an individual's likelihood of suffering CVD in future (Dube et al., 2003) In addition, the Framingham risk prediction has perfomed reasonably well in HIV infected patients (Schambelan et al., 2008). Moreover, a good level of agreement (77.4%) between Framingham risk equation and the DAD (Data collection on Adverse Effects on Anti-HIV Drugs Cohort) risk equation, developed from results of HIV infected people was reported (Nery et al., 2013) and some concordance between the Framingham risk equation, WHO/ISH Risk prediction chart for Africa and the SCORE equations were reported (Edward et al., 2013).
- Since there is no standardized procedure for performing the PAGGE, we adopted the Invitrogen protocol for running 4-16 % precast gels for separation of markers with diameter range of 3nm to 30nm. We determined our lipoprotein subclass proportions using the PAGGE method, while studies on lipoprotein subclasses in HIV infected people conducted in developed countries used the nuclear magnetic resonance spectroscopy (NMR) method. Quantification of lipoprotein subclasses by PAGGE and NMR is based on differences in lipoprotein particle

size, thus IDL, LDL, and Lipoprotein (a) (Lp (a)) particles whose diameter sizes overlap cannot be distinguished by these methods (Freedman et al., 1998). However, investigations by Otvos et al. 1999 showed that Lp (a) even at high concentrations would not significantly influence the estimation of LDL particle subclasses. Furthermore, these two methods had a correlation of 0.86 (Blake et al., 2002) and 70% agreement (Ensign et al., 2006).

CHAPTER 6

CONCLUSION

The present study obtained high prevalence of low fruit and vegetable intake in both ART naïve HIV infected people and in people on ART. The association of low fruit and vegetable intake with hypertriglyceridaemia observed among people on ART may be indicative of an indirect role of low fruit and vegetable intake in increasing the risk of CVD. While the prevalence of physical inactivity was high among ART naïve HIV infected people, the majority of people on ART were physically active.

The ART naïve HIV infected rural population in Dikgale HDSS had significantly lower levels of lipids than HIV uninfected rural population. The rural population on ART had a lipid profile that was less atherogenic than people naïve to ART. Higher proportions of large particles than the small particles of LDL and HDL were observed in people on ART. A similar pattern was obtained in ART naïve HIV infected people, however the ratio of large to small particles for HDL as well as for LDL was higher in people on ART when compared to ratios in ART naïve HIV infected participants.

Except for the prevalence of hypercholesterolaemia, the prevalence for most CVD risk factors was lower in HIV infected people on ART than in HIV infected people naïve to ART.

While 5.6% of ART naïve HIV infected people had a high risk of developing CVD in the next 10-years, none of the people on ART had such a high 10- year CVD risk, according to the Framingham equation. However, the high proportion of people on ART with a moderate to high 5-year CVD risk, observed with the DAD risk equation, clearly represents a considerable health burden in the future that can possibly be reduced by increasing educational programs on CVD prevention for people on ART.

CHAPTER 7

RECOMMENDATIONS

In the present study, a high number of ART naïve HIV infected people over the age of 50 years was observed which is similar to what has been reported from other rural areas of South Africa (Wallrauch et al., 2010). Therefore greater efforts should be directed at getting the older age group to screen for HIV.

Routine testing of lipoprotein subclasses in resource limited countries is not practiced as methods are laborious, while the NMR equipment is expensive. According to present study findings, the TG/HDL-C ratio can be used to give an indication of the proportions of large and small HDL-particles that are predictors of CVD events in HIV infection. More studies of lipoprotein subclasses particularly in black populations on ART, using NMR are needed to confirm the use of AIP.

While ART is known to increase TG concentration, a high intake of fruit and vegetable associated with reduced TG concentration in the present stdy, should be encouraged in people on ART as it may provide a natural means to counteract the effect of ART on TG concentration.

Long term monitoring of CVD risk factors in people on ART residing in Dikgale HDSS, at 5-year intervals is needed to confirm current findings.

According to Clarke and Mouse (2009), the cytochrome P450 gene (CYP 3) involved in ART drug metabolism is expressed differently in Caucasians and in people of African descent. Based on this evidence there is a need to develop and evaluate a race/ethnicity-specific CVD risk estimation tool for HIV infected Africans on ART.

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APPENDICES

Appendix I: Ethics clearance certificate (i)

09-09-11:15:12

UNIVERSITY OF LIMPOPO
Medunsa Campus

MEDUNSA RESEARCH & ETHICS COMMITTEE

CLEARANCE CERTIFICATE

P O Medunsa
Medunsa
0204
SOUTH AFRICA

Tel: 012 - 521 4000
Fax: 012 - 560 0066

MEETING: 06/2011

PROJECT NUMBER: MREC/HS/137/2011: PG

PROJECT :

Title: Effects of Human Immunodeficiency Virus and its treatment on lipid levels and lipoprotein subclass patterns in a rural population of Limpopo Province, South Africa

Researcher: Mrs F Mashinya
Supervisor: Prof M Alberts
Co-supervisor: Dr JP Van Geertruyden
Department: Medical Science
School: Health Sciences
Degree: PhD Medical Science

DECISION OF THE COMMITTEE:

MREC approved the project.

DATE: 18 August 2011

PROF GA OGUNBANJO
CHAIRPERSON MREC

Note:
i) Should any departure be contemplated from the research procedure as approved, the researcher(s) must re-submit the protocol to the committee.
ii) The budget for the research will be considered separately from the protocol.
PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.

Appendix II: Ethics clearance certificate (ii) for ammendments

UNIVERSITY OF LIMPOPO
Medunsa Campus

MEDUNSA RESEARCH & ETHICS COMMITTEE

CLEARANCE CERTIFICATE

MEETING:	**06/2011** **04/2013**
PROJECT NUMBER:	**MREC/HS/137/2011: PG**
PROJECT :	
Title:	Effects of Human Immunodeficiency Virus and its treatment on lipid levels and lipoprotein subclass patterns in a rural population of Limpopo Province, South Africa
Researcher:	Mrs F Mashinya
Supervisor:	Prof M Alberts
Co-supervisor:	Dr JP Van Geertruyden
Department:	Medical Science
School:	Health Sciences
Degree:	PhD Medical Science

DECISION OF THE COMMITTEE:

MREC approved the project.	**DATE:**	18 August 2011
MREC approved the amendments to the project:	**DATE:**	11 April 2013

PROF GA OGUNBANJO
CHAIRPERSON MREC

The Medunsa Research Ethics Committee (MREC) for Health Research is registered with the US Department of Health and Human Services as an International Organisation **(IORG0004319)**, as an Institutional Review Board **(IRB00005122)**, and functions under a Federal Wide Assurance **(FWA00009419)**
Expiry date: **11 October 2016**

Note:
i) Should any departure be contemplated from the research procedure as approved, the researcher(s) must re-submit the protocol to the committee.
ii) The budget for the research will be considered separately from the protocol.
PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.

Finding Solutions for Africa

Appendix III: Provincial Department of Health approval letter

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF HEALTH

Enquiries: Selamolela Donald Ref:4/2/2

MRS Mashinya F
University of Limpopo
Medunsa cumpas

Greetings,

Re: Effects of Human Immunodeficiency Virus and its treatment on lipid levels and lipoprotein subclass patterns in rural population of Limpopo Province, South Africa

1. The above matter refers.
2. Permission to conduct the above mentioned study is hereby granted.
3. Kindly be informed that:-
    - Further arrangement should be made with the targeted institutions.
    - In the course of your study there should be no action that disrupts the services.
    - After completion of the study, a copy should be submitted to the Department to serve as a resource.
    - The researcher should be prepared to assist in the interpretation and implementation of the study recommendation where possible.

Your cooperation will be highly appreciated.

Head of Department

14/06/2013
Date

18 College Street, Polokwane, 0700, Private Bag x9302, POLOKWANE, 0700
Tel: (015) 293 6000, Fax: (015) 293 6211/20 Website: http/www.limpopo.gov.za

Appendix IV: Department of Health-Capricorn District approval letter

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA
DEPARTMENT OF HEALTH – CAPRICORN DISTRICT

REF: S/10

ENQ: TLEANE MM

DATE: 2013/09/09

POLOKWANE EAST

ATTENTION: ASSISTANT MANAGERS

Kindly be informed that following student from the University of Limpopo Felistas Mashinya student number: 201118041has been granted permission to conduct research on the effect of HIV and its treatment on lipid levels and lipoprotein subclasses.

The required period for the research is estimated at six months starting from October 2013 ˙ May 2014 as the requirement from the university.

The district request your clinic to give her the necessary support.

Your cooperation and in this regard will be highly appreciated.

2013.09.09.

DISTRICT EXECUTIVE MANAGER

Appendix V: Consent forms

UNIVERSITY OF LIMPOPO (Medunsa Campus) ENGLISH CONSENT FORM

Statement concerning participation in a Research Project*.

Name of Project

Effects of Human Immunodeficiency Virus and Its treatment on Lipid levels and Lipoprotein subclass patterns in a rural population of Limpopo Province, South Africa.
I have heard the aims and objectives of* the proposed study and was provided the opportunity to ask questions and given adequate time to rethink the issue. The aim and objectives of the study are sufficiently clear to me. I have not been pressurized to participate in any way.
I understand that participation in this Project* is completely voluntary and that I may withdraw from it at any time and without supplying reasons. This will have no influence on the regular treatment that holds for my condition neither will it influence the care that I receive from my regular doctor.

I know that this Project* has been approved by the Medunsa Research Ethics Committee (MREC), University of Limpopo (Medunsa Campus. I am fully aware that the results of this results of this Project* will be used for scientific purposes and may be published. I agree to this, provided my privacy is guaranteed.

I hereby give consent to participate in this Project*.

.....

.....

Name of patient/volunteer Signature of patient or guardian.

....

.....

Place. Date. Witness

Statement by the Researcher

I provided verbal and/or written* information regarding this Project*
I agree to answer any future questions concerning the Project* as best as I am able.
I will adhere to the approved protocol.

....

Name of Researcher Signature Date Place

UNIVERSITY OF LIMPOPO (Medunsa Campus) SEPEDI CONSENT FORM

Setatamente mabapi le go tšea karolo ka go Protšeke *.

Leina la Protšeke *
Tšhušumetšo ya kokwanatlhoko le kalafo ya yona godimo ga kelo ya makhura le mehuta ya magoro a disepediši tša makhura mo mading a batho bao ba dulago nagalegaeng ya Limpopo, Africa-Borwa.
Ke badile/ke kwele ka ga tshedimošo mabapi le *maikemišetšo le morero wa* dinyakišišo tšeo di šišintšwego gomme ke ile ka fiwa monyetla wa go botšiša dipotšišo gomme ka fiwa nako yeo e lekanego gore ke naganišiše ka ga taba ye. Ke tloga ke kwešiša maikemišetšo le morero wa dinyakišišo tše gabotse. Ga se ka gapeletšwa go kgatha tema ka tsela efe goba efe.

Ke a kwešiša gore go kgatha tema Protšekeng * ke ga boithaopo gomme nka tlogela go kgatha tema nakong efe goba efe ntle le gore ke fe mabaka. Se se ka se be le khuetšo efe goba efe go kalafo yaka ya ka mehla ya maemo a ka gape e ka se huetše le ge e ka ba tlhokomelo yeo ke e humanago go ngaka yaka ya ka mehla.
Ke a tseba gore Protšeke tše* di dumeletšwe ke Medunsa Research Ethics Committee (MREC), Yunibesithi ya Limpopo (Khamphase ya Medunsa). Ke tseba gabotse gore dipoelo tša Protšeke tše * di tla dirišetšwa merero ya saense gomme di ka phatlalatšwa. Ke dumelelana le se, ge fela bosephiri bja ka bo ka tiišetšwa.

Mo ke fa tumelelo ya go kgatha tema Protšekeng *.
.....

.....
Leina la molwetši/ moithaopi Mosaeno wa molwetši goba mohlokomedi.
....
.....
Lefelo. Letšatšikgwedi.
Tlhatse

Setatamente ka Monyakišiši

Ke fana ka tshedimošo ka molomo le/goba yeo e ngwadilwego * mabapi le Protšeke ye .*
Ke dumela go araba dipotšišo dife goba dife tša ka moso mabapi le Protšeke ka bokgoni ka moo nka kgonago ka gona.
Ke tla latela melao yeo e dumeletšwego.
....
Leina la Monyakišiši Mosaeno Letšatšikgwedi Lefelo

Appendix VI: Participant information leaflet

Invitation to take part in a Health Project **Memo ya go tšea karolo go projeke ya tša maphelo.**
You are invited to take part in a health project titled "**Prevention, control and intergrated management of chronic diseases in a rural community, Phase 2: Establishment of the prevalence of diseases and their risk factors in Dikgale health and demographic surveillance system (HDSS) site, South Africa**". Le memiwa go tšea karolo go karolo ya bobedi ya projeke ya tša maphelo yeo e bitšwago "**Prevention, control and intergrated management of chronic diseases in a rural community, Phase 2: Establishment of the prevalence of diseases and their risk factors in Dikgale health and demographic surveillance system (HDSS) site, South Africa**".

About the Project. **Tshedimošo ka ga projeke**
This project is conducted by the Department of Medical Science, University of Limpopo, Turfloop Campus in collaboration with the VLIR-University of Antwerp, Belgium. Projeke ye e dirwa ke ba lefapha la dithuto tša mahlale a maphelo Unibesithing ya Limpopo, Turfloop, ka tšhomišano le VLIR-Unibesiti ya Antwerp, Belgium.
The aim of the project is first to determine the prevalence of Chronic Diseases and their risk factors in the Dikgale community. The chronic diseases that will be studied include: Obesity Tobacco use Alcohol consumption Low intake of fruits and vegetables High levels of sugar in blood High levels of fat in blood High blood pressure Lung diseases HIV/AIDS

Maikemišetšo a projeke ye ke go nyakišiša ka go ata ga malwetši a go tšea nako ye telele le dika goba dihlolwa tša ona mo baduding ba Ga-Dikgale.
Malwetši ao le dika goba dihlolwa tša ona ke tše di akaretšago:

- Go nona kudu
- Go fola
- Go nwa bjala
- Swikiri e ntši madding
- Makhura a mantši mading
- Madi a magolo
- Malwetši a mafahla
- Bolwetši bja HIV/AIDS

Ka morago ga fao, go tlo hlomiwa lenaneo la go sedimoša batho ka malwetši le dika tše , le go kaonafatsa ditirelo tsa maphelo dikliniking, maikemišetšo a lona ele go go alafa le go fokotša go ata ga malwetši le dika goba dihlola tšeo.

To attain this aim anthropometric measurements (height, weight etc) and blood tests will be required to be performed from randomly selected people residing in Dikgale.

Gore maikemišetšo a a atlege, go tlo tšewa ditekatekano tša boima bja mmele le diteko tša madi go batho bao bakgethilwego go tšwa metsaneng ya ga-Dikgale.

How was I selected as to be a participant?

Naa ke kgewthilwe bjang go ba motšeakarolo?

Dikgale HDSS database was developed using census information gathered by the field workers who visited your home. Each resident of Dikgale was then assigned a specific identification number.
From this database people are chosen randomly and you were one of those who were chosen.
Please note your participation is voluntary.

Go ilwe ga dirwa datapeisi ya badudi ba Ga-Dikgale go šomišwa dinyakišiso tša paloya batho (census) tšeo di dirilwego ke bašomišane le rena ba "fieldwork" bao ba ilego ba etela malapa a lena. Modudi o mongwe le o mongwe wa Dikgale o ile a fiwa nomoro ye kgethegilego ya boitsebišo. Dinomoro tša batšeakarolo di ile tša kgethwa ka tokologo gomme o bile yo mongwe wa bao ba kgethilwego.
Tsebang gore go tšea karolo ga lena ga se ka kgapeletšo.

How will you participate in this project?

Naa o tlo tšea karolo bjang mo projekeng ye?

Information about participants will be gathered in three steps
Step 1: Interview questions
Step 2: measurements of height, weight, waist and blood pressure
Step 3: Blood test for sugar, fats, TB and HIV/AIDS

Go tlo šomišwa mekgwa ye meraro ya go nyaka šedi ka ga batšeakarolo.
Mokgwa wa pele: Karabo ya dipotšišo poledišanaong
Mokgwa wa bobedi: Go ela botelelele, boima bja mmele le botelele bja nnoka le go ela madi a magolo
Mokgwa wa boraro: Go ela swikiri le makhura ka mading. Go lebelela bolwetši bja TB le HIV/AIDS ka mading.

STEP 1

KGATO YA PELE

You will be asked questions about your:

- Age
- Education
- Tobacco and alcohol use
- History of diabetes and raised blood pressure

Le tlo botšišwa dipotšišo tša go amana le lena tšeo di balago:

- Mengwaga ya lena
- Thuto yeo o e fihletšego
- Tshomišo ya motsoko le dinotagi
- Histori ka ga bolwetši bja swikiri le ba madi a magolo

STEP 2.

KGATO YA BOBEDI

Health workers will take simple measurements of your:

- Height
- Weight
- Waist circumference
- Blood Pressure

Bašomedi ba tša maphelo ba tlo kgopela go le tšea dikelo tše di latelago tša mmele wag ago:

- Botelele bja mmele
- Boima bja mmele
- Kalo ya nnoka
- Kelo ya madi

How will the anthropometric measurements be taken?

Naa boima bja mmele bo tlo tšeiwa bjang?:

(a) For weight:

(a) Boima bja mmele
You will be asked to take off your shoes and wear light clothes and stand on the weighing balance and your weight will be recorded Le tlo kgopelwa gore le hlobole dieta ebile le apare dikobo tše bofefo goba tše sese gomme le namele godimo ga sekala, boima bja lena bo tla ngwadiwa.
(b) For Height: **(b) Botelele:**
You will be asked to take off your shoes and climb on the stadiometer and your height will be recorded Le tlo kgopelwa gore le hlobole dieta ebile gomme le namele godimo ga "stadiometer", botelele bja lena bo tla ngwadiwa.
(c) For waist and hip measurements: **(c) Tekatekano ya dinoka**
You will be asked to wear light clothes. A measuring tape will be wrapped around your waist and hip and your waist and hip circumference will be recorded Le tlo kgopelwa gore le apare dikobo tše bofefo goba tše sese gomme "measuring tape"e tlo tatetšwa dinokeng tša lena gomme tekatekano ya dinoka tša lena e tla ngwadiwa.
(d) For Blood Pressure **(d) Thlatlhobo ya Madi a magolo**
You will be asked to rest for 5 minutes there after the cuff belt will be placed on your hand and blood pressure measured. Le tlo kgopelwa go dula fase tekano ya metsotso ye mehlano gomme lepanta la go dira diteko tsa madi a magolo le tlo bofsa letsogong.
STEP 3 **KGATO YA BORARO**
Donating Blood samples. **Go fana ka madi.**

You will be asked to donate blood samples (20ml) for several blood test. You will be asked to
fast for overnight (at least 12 hours) before blood is collected and will be provided breakfast
food parcel.
People from NEW START programme will ask you to take an HIV test. They will not be
given your name but only your database identification number.

Le tlo kgopelwa go fana ka madi a go lekana "20ml", ao a tlogo šomišwa go dira di teko tša
malwetši a go tšea nako.
Le tlo kgopelwa gore le se je bošego kamoka goba nako ya go feta di-iri tše lesome pedi pele
madi a tšewa.
Le tlo fiwa sephutelwana sa dijo ka morago ga go tšewa ga madi.
Ba lenaneo la NEW START batlo kgopela go dira diteko tša HIV gomme ba ka se tsebe leina la
motšeakarolo ka ge batlo fiwa nomoro ya motšea karolo fela.

What will happen if the project results, shows that I need further medical attention?

Na go tlo diragala eng, ge projeke ye e humana ke na le bolwetši bja go nyaka kalafo ka morago ga diteko tše?

If any participant is found to have results that require further medical attention the participant will be refered to the Hospital or primary health clinic.

Ge gona le motšeakarolo o dipoelo tša gagwe di nyakago thušo ya tša maphelo, motšeakarolo oo o tlo fiwa lengwalo la go ya sepetlele goba kliniking go hwetša thušo ya kalafo

How will the project ensure that my results are confidential?

Naa projeke e tlo kgonthišiša bjang gore dipoelo tša ka ga di be phatlalatša?

All people involved in this project will sign a confidentiality form. The project will not use your name but will use you identification number retrieved from the database.

Bašomedi ba projeke ye ba sainile kwano ya go se phatlalatše dipoelo tša motšeakarolo mo setšhabeng. Gape go ka se šomišwe leina la gago ka ge go tlo šomišwa nomoro ya boitsebišo yeo o filwego yona ke data peise.

Blood tests to be conducted / Diteko tša madi tšeo di tlogo dirwa.

Serum lipids (Cholesterol, LDL cholesterol, HDL cholesterol and Triglycerides) / Makhura ka mading
Glucose and Insulin / Swekere ka mading
C-reactive proteins
HIV test **(Pre- and post counseling will be provided)** / kokwana ya HIV
Coagulation factors / Diteko tša go gatla ga madi
If necessary, the following tests will be performed/ Ge go wokega, re ka dira diteko tše di latelago: viral load, CD4 count, liver function tests, lipoprotein particle subclasses, HIV resistant mutants.
Anonymity
All blood test results will be treated with strict confidentiality. Dipoelo tša diteko tša madi di tla swarwa ka tlhokomelo ye kgolo gore di se tsebagale setshabeng.

Appendix VII: WHO Step quetionnairre

ument illance Chronic Disease

<SOUTH AFRICA, LIMPOPO PROVINCE / DIKGALE HEALTH AND DEMOGRAPHIC SURVEILLANCE SITE>

Survey Information / TshedimoŠo ka ga dinyakiŠiŠo

Location and Date / Lefelo le Letšatši		Response / Dikarabo	Code
1	Cluster/Centre/Village ID / Nomoro ya Motse (go ya ka DHDSS)	⌊_⌊_⌊_⌊_⌋	I1
2	Cluster/Centre/Village name / Leina la Motse		I2
3	Interviewer ID or Initials / Nomoro ya Mmotšišiši	⌊_⌊_⌊_⌊_⌋	I3
4	Date of completion of the instrument / Letšatši la go tlatsa form ye	⌊_⌊_⌋ ⌊_⌊_⌋ ⌊_⌊_⌊_⌊_⌋ dd mm year Letšatši Kgwedi Ngwaga	I4

- ---------- ✂

Nomoro ya Motšeakarolo ⌊_⌊_⌊_⌋ ⌊_⌊_⌊_⌋ ⌊_⌊_⌊_⌋			
Consent, Interview Language and Name / Tumelelo ya go tšea karolo, Leleme la poledišano, Le Leina		**Response / Karabo**	**Code**
5	Consent has been read and obtained / Kwano ya go tšea karolo e badilwe ebile e hweditšwe	Ee 1 Aowa 2 **Ge eba Aowa, GONA FETŠA POLEDIŠANO**	I5

6	Interview Language *[Insert Language]* / Leleme la Poledišano	English / Sekgowa 1 *Pedi / Sepedi* 2	I6
7	Time of interview (24 hour clock) / Nako ya Poledišano (go ya ka di-iri tše masomepedinne tša letšatši)	⌊_⌋⌊_⌋ : ⌊_⌋⌊_⌋ hrs mins Di-Iri Metsotso	I7
8	Family Surname / Sefane		I8
9	First Name / Leina la Mathomo		I9
Additional Information that may be helpful / Tlaleletšo yeo e ka thušago			
10	Contact phone number where possible / Nomoro ya mogala ge ele gona		I10

Step 1 Demographic Information
Legato la pele TshedimoŠo ya tŠa bodudi

Nomoro ya Motšeakarolo
⌊_⌋⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋⌊_⌋

TŠE BOHLOKWA: Tshedimošo ya tša bodulo			
Potšišo		**Karabo**	**Code**
11	Sex (*Record Male / Female as observed*) / Bong (*Monna/ Mosadi*)	Male / Monna 1 Female / Mosadi 2	C1
12	What is your date of birth? / Letšatši la Matswalo? *Don't Know 77 77 7777 /* *Ga ke tsebe 77 77 7777*	⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋⌊_⌋ *If known, Go to C4 / Ge* *a tseba, E ya go C4* dd mm year Letšatši Kgwedi Ngwaga	C2
13	How old are you? O na le mengwaga e me kae?	Years / Mengwaga ⌊_⌋⌊_⌋	C3

14	In total, how many years have you spent at school or in full-time study (excluding pre-school)? / Ka moka, o tsere mengwaga ye mekae o le sekolong goba o ithuta se sengwe (Nako ya Pre-school ga e balwe)?	Years /Mengwaga ⌊_⌋⌊_⌋	C4

Step 1 Behavioural Measurements / Legato la pele Kelo ya maitshwaro

⌊_⌋⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋ Nomoro ya Motšeakarolo

CORE: Tobacco Use / DIPOTŠISO TŠE BOHLOKWA: Tšhumišo ya Motsoko

Now I am going to ask you some questions about various health behaviours. This includes things like smoking, drinking alcohol, eating fruits and vegetables and physical activity. Let's start with tobacco. /
Bjale ke tlo go botšiša dipotšišo mabapi le tsa maphelo. Go akaretša go kgoga, go nwa bjala, go ja dienywa le merogo le go thobolla mmele. A re thome ka ka motsoko.

	Question / Potšišo	Response / Karabo		Code
15	Do you currently smoke any **tobacco products**, such as cigarettes, cigars or pipes? *(USE SHOWCARD)* / Naa gona bjale o kgoga e mengwe ya metšoko ya go swana le sekerete, sikara goba peipe? *(ŠOMIŠA KARATA YA GO HLALOŠA)*	Yes / Ee No / Aowa	1 2 *If No, go to T6* / *Ge eba aowa gona fetela go T6*	T1
16	Do you currently smoke tobacco products **daily**? / Naa le kgoga mehuta ye tšatši le tšatši?	Yes /Ee No / Aowa	1 2 *If No, go to T6* / *Ge eba aowa gona fetela go T6*	T2
17	How old were you when you **first started** smoking daily? If not known probe by asking questions such as when you	Age (years) / Mengwaga	⌊_⌋⌊_⌋ *If Known, go to T5a* /	T3

	started was your first child already bornand how old is that child presently?/	Don't know / Ga ke tsebe 77	*Ge a tseba eya go T5a*	
18	Do you remember how long ago it was? *(RECORD ONLY 1, NOT ALL 3) /* Naa o gopola gore ke lebaka le le kaakang? *(TLATŠA GA TEE FELA E SEGO*	In Years / Mengwaga	⌊_⌋⌊_⌋ *If Known, go to*	T4a
		OR in Months / GOBA Dikgwedi	⌊_⌋⌊_⌋ *If Known, go to*	T4b
		OR in Weeks / GOBA Dibeke	⌊_⌋⌊_⌋	T4c
18	On average, **how many** of the following do you smoke each day? *(RECORD FOR EACH TYPE, USE SHOWCARD) /* Ka palogare, naa o kgoga e me kae metšoko ye e latelago? *(TLATŠA MEHUTA KAMOKA, MMONTŠHE KARATA)* *Don't Know / Ga ke tsebe 77*	Manufactured cigarettes / Sekerete	⌊_⌋⌊_⌋	T5a
		Hand-rolled cigarettes / Sekerete sa go bofiwa goba go tatwa	⌊_⌋⌊_⌋	T5b
		Pipes full of tobacco / Peipe ye e tletšego motšoko	⌊_⌋⌊_⌋	T5c
		Cigars, cheroots, cigarillos / Sikara go ya ka mehutahuta	⌊_⌋⌊_⌋	T5d
		Other / E mengwe	*Ge eba ye mengwe e ya* ⌊_⌋⌊_⌋ *go T5other goba T9*	T5e
		Other (please specify) / E mengwe(Hlaloša):	⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋ *Go to T9 / E ya go T9*	T5other

EXPANDED: Tobacco Use / DIPOTŠIŠO TŠA TLALELETŠO: Tšhomišo ya Motsoko				
Question / Potšišo		**Response Karabo**		**Code**
Please note that questions 27 – 29 and 33 are for people who are not currently smoking. (That is those who have never smoked or those who had smoked but stopped smoking). **Lemoga gore dipotšišo 27 -29 le 33, ke tša bao ba sa folego gona bjale. (Bao ba sakago ba fola goba bao ba bego ba fola feela ba emiša go fola).**				
19	In the past, did you **ever** smoke **daily**? / Lebakeng la go feta naa o ile wa	Yes / Ee	1	T6
		No / Aowa	2 *If No, go to T9* Ge eba	
20	How old were you when you **stopped** smoking **daily**? /	Age (years) / Megwaga		T7
		Don't Know / Ga ke tsebe 77	⌊_⌋⌊_⌋ *If Known, go to T9 /*	

	Naa o be ona le mengwaga e me kae ge o tlogela go kgoga tšatši ka tšatši?		*Ge ba tseba fetela go T9*	
22	How **long ago** did you stop smoking daily? *(RECORD ONLY 1, NOT ALL 3)* Naa ekaba lebaka le le kae o tlogetše go kgoga tšatši ka tšatši? *(TLATŠA KARABO E TEE FELA E SEGO KAMOKA)* *Don't Know / Ga ke tsebe 77*	Years ago / Mengwaga ya go feta	⌊_⌋⌊_⌋ *If Known, go to T9 /* *Ge ba tseba fetela go T9*	T8a
		OR Months ago / GOBA Dikgwedi tša go feta	⌊_⌋⌊_⌋ *If Known, go to T9 /* *Ge ba tseba fetela go T9*	T8b
		OR Weeks ago / GOBA Dibeke tša go feta	⌊_⌋⌊_⌋	T8c
23	Do you **currently use** any **smokeless tobacco** such as *[snuff, chewing tobacco, betel]*? *(USE SHOWCARD)*	Yes / Ee No / Aowa	1 2 *If No, go to T12/* Ge eba aowa	T9
24	Do you **currently use smokeless tobacco** products **daily?** / Naa gona bjale o šomiša motšoko	Yes / Ee No / Aowa	1 2 *If No, go to T12 /*	T10
25	On average, how many **times a day** do you use …. *(RECORD FOR EACH TYPE, USE SHOWCARD)* Ka palogare/ average, naa ka letšatsi o o šomiša ga kae? …. *(TLATŠA MEHUTA KAMOKA, ŠOMIŠA KARATA GO HLALOŠA)* *Don't Know / Ga ke tsebe 77*	Snuff, by mouth / Sneife, Ka molomong	⌊_⌋⌊_⌋	T11a
		Snuff, by nose / Sneife ka nkong	⌊_⌋⌊_⌋	T11b
		Chewing tobacco / Motšoko wa go jewa	⌊_⌋⌊_⌋	T11c
		Other / O mongwe	⌊_⌋⌊_⌋	T11e
		Other (specify) / O mongwe (Hlaloša)	*If Other, go to T11other,* ⌊_⌋⌊_⌋ *else go to T13* *Ge eba o mongwe fetela goT11other,* goba go *T13*	T11other

26	In the past, did you **ever use** smokeless tobacco such as snuff, chewing tobacco **daily. /** Mo lebakeng le le fetilego, naa **o ile wa šomiša** motšoko wa go hloka muši bjalo ka sneife goba motšoko wa go jewa?	Yes / Ee	1	T12
		No / Aowa	2	
27	During the past 7 days, on how many days did someone **in your home** smoke when you were present/ / Mo matšatšing a 7 a go feta, naa go na le matšatši a makae mo go ilego gwa ba le yo mongwe **ka lapeng** yo a ilego a fola o le gona?	Number of days / Matšatši	__	T13
		Don't Know / Ga ke tsebe	77	
28	During the past 7 days, on how many days did someone smoke in closed areas **in your workplace** when you were present? / Mo matšatšing a 7 a go feta, naa go na le matšatši a makae mo go ilego gwa ba le yo mongwe ka **mošomong wa gago** yo a ilego a fola o le gona?	Number of days / Matšatši	__	T14
		Don't Know or do't work in closed areas / Ga ke tsebe goba ga ke šome mafelong a tšwaletšego	77	

CORE: Alcohol Consumption / DIPOTŠIŠO TŠE BOHLOKWA: Tšhomišo ya Bjala				
The next questions ask about the consumption of alcohol./ Dipotšišo tša go latela di amana le tšhomišo ya bjala.				
Question / Potšišo		**Response / Karabo**		**Code**
29	Have you **ever** consumed an alcoholic drink such as beer, wine, spirits, fermented cider or *traditionally fermented beer*? *(USE SHOWCARD OR SHOW EXAMPLES) /*	Yes / Ee	1	A1a
		No / Aowa	2 *If No, go to D1* *Ge eba aowa fetela go D1*	
30	Have you consumed an alcoholic drink within the **past 12 months**? / Naa o ile wa nwa bjala mo kgweding tše lesome pedi (12) tša go feta?	Yes / Ee	1	A1b
		No / Aowa	2 *Ge eba aowa fetela go D1*	
31		Daily / Tšatši ka tšatši	1	A2

	During the past 12 months, **how frequently** have you had at least one alcoholic drink? *(READ RESPONSES, USE SHOWCARD) /* Mo dikgweding tše lesome pedi (12) tša go feta, naa o ile wa nwa bjala makga a ma kae? *(BALA DIKARABO, O ŠOMIŠE KARATA)*	5-6 days per week / Matšatši a 5-6 ka beke 1-4 days per week / Matšatši a 1-4 ka beke 1-3 days per month / Matšatši a 1-3 Ka kgwedi Less than once a month / Ka tlase ga ga-tee ka kgwedi	2 3 4 5		
32	Have you consumed an alcoholic drink within the **past 30 days**? / Naa o ile wa nwa bjala mo	Yes / Ee No / Aowa	1 2	*If No, go to D1/ Ge eba aowa*	A3
33	During the past 30 days, on how many **occasions** did you have at least one alcoholic drink? / Mo matšatšing a masome tharo (30) a go feta, naa o ile wa nwa bjala makga a ma kae?	Number / Nomoro Don't know / Ga ke tsebe 77	└─┴─┘		A4
34	During the past 30 days, when you drank alcohol, **on average**, how many **standard alcoholic drinks** did you have during one drinking occasion? *(USE SHOWCARD) /* Mo matšatšing a masome tharo (30) a go feta moo o ilego wa nwa bjala, naa ka palogare o ile wa nwa bjala bjo bo kaakang lekgeng le tee? *(ŠOMIŠA KARATA GO HLALOŠA)*	Number / Nomoro Don't know / Ga ke tsebe 77	└─┴─┘		A5
35	During the past 30 days, what was the **largest number** of standard alcoholic drinks you had on a single occasion, counting all types of alcoholic drinks together? / Mo matšatšing a masome tharo (30) a go feta, naa nomoro e kgolo ya bjala bjo o bo nwelego mo lebakeng le tee ge o akaretša dino kamoka ke eng?	Nomoro Don't know / Ga ke tsebe 77	└─┴─┘		A6

36	During the past 30 days, how many times did you have for **men**: **five or more** for **women**: **four or more** standard alcoholic drinks in a single drinking occasion? / Mo matšatšing a masome tharo (30) a go feta, ke ga kae mo o ilego wa nwa dino tša go feta tše:- Hlano goba go feta (ge mmotšišwa e le monna) Nne goba go feta (ge mmotšišwa e le mosadi Lebakeng le tee?	Number of times / Ga Don't know / Ga ke tsebe 77 ⌊__⌋⌊__⌋	A7

EXPANDED: Alcohol Consumption / DIPOTŠISO TŠA TLALELETŠO: Tšhomišo ya bjala			
37	During the past 30 days, when you consumed an alcoholic drink, how often was it with meals? Please do not count snacks. / Mo matšatšing a masome tharo (30) a go feta, mao ebego o nwa	Usually with 1 Sometimes with meals / Le diio 2 Rarely with meals / Ga se gantši 3 Never with meals / Ntle le dijo 4	A8
38	During each of the **past 7 days**, how many standard alcoholic drinks did you have each day? *(USE SHOWCARD)* / Mo matšatšing a šupa(7) ago feta, naa o nwele bjala bjo bo kaakang tšatši le lengwe le le lengwe? *(ŠOMIŠA KARATA GO HLALOŠA)* *Don't Know / Ga ke tsebe 77*	Monday / Mosupologo ⌊__⌋⌊__⌋	A9a
		Tuesday / Labobedi ⌊__⌋⌊__⌋	A9b
		Wednesday / Laboraro ⌊__⌋⌊__⌋	A9c
		Thursday / Labone ⌊__⌋⌊__⌋	A9d
		Friday / Labohlano ⌊__⌋⌊__⌋	A9e
		Saturday / Mokibelo ⌊__⌋⌊__⌋	A9f
		Sunday / Lamorena ⌊__⌋⌊__⌋	A9g

CORE: Diet / DIPOTŠIŠO TŠA BOHLOKWA: Tša Dijo

The next questions ask about the fruits and vegetables that you usually eat. I have a nutrition card here that shows you some examples of local fruits and vegetables. Each picture represents the size of a serving. As you answer these questions please think of a typical week in the last year / Dipotšišo tše di latelago ke tša mabapi le dienywa le merogo yeo o ejago. Ke nale karata ya dijo yeo e laetšago dienywa le merogo e tlwaelegilego ya gae. Seswantšho se sengwe le se sengwe se laetša kalo ya dijo. Ge o araba dipotšišo ka kgopelo nagana ka beke ye e tlwaelegilego mo ngwageng wa go feta.

Question / Potšišo		Response / Karabo	Code
39	In a typical week, on how many days do you **eat fruit**? *(USE SHOWCARD) /* Mo bekeng yeo e tlwaelegilego, na o **ja dienywa** matšatši a [illegible]	Number of days / Palo ya matšatši ⌊__⌋ *If Zero days, go to D3* Don't Know / Ga ke tsebe 77	D1
40	How many **servings** of fruit do you eat on **one** of those days? (*USE SHOWCARD) /* [illegible]	Number of servings / Palo ya go ja ⌊__⌋ Don't Know / Ga ke tsebe 77	D2
41	In a typical week, on how many days do you **eat vegetables**? *(USE SHOWCARD) /*	Number of days / Palo ya matšatši ⌊__⌋ *If Zero days, go to D5* Don't Know / Ga ke tsebe 77 *Ge di sa*	D3
42	How many **servings** of vegetables do you eat on one of those days? *(USE SHOWCARD) /* [illegible]	Palo ya go ja Don't Know / Ga ke tsebe 77 ⌊__⌋	D4

EXPANDED: Diet / DIPOTŠIŠO TŠA TLALELETŠO: Tša Dijo			
43	What type of **oil or fat is most often** used for meal preparation in your household? *(USE SHOWCARD) (SELECT ONLY ONE)* Naa o šomiša **makhura** a mohuta mang ge o apea? *(ŠOMIŠA KARATA GO HLALOŠA) (KGETA YE TEE FEELA)*	Vegetable oil / Makhura a merogo 1 Lard or suet 2 Butter or ghee / Botoro 3 Margarine / Margarine 4 5 *If Other, go to D5 other* / Other / A mangwe *Ge eba se sengwe fetela go D5other* None in particular 6 None used / Ga ke šomiše Makhura 7 Don't know / Ga ke tsebe 77	D5
		Other / Se sengwe ⌊_____⌋	D5other

44	On average, how many meals per week do you eat that were not prepared at a home? By meal, I mean breakfast, lunch and dinner. /	Number / Palo Don't know / Ga ke tsebe 77 ⌊__⌋⌊__⌋	D6

CORE: Physical Activity / DIPOTŠITŠO TŠE BOHLOKWA: Thobollo ya mmele			
Next I am going to ask you about the time you spend doing different types of physical activity in a typical week. Please answer these questions even if you do not consider yourself to be a physically active person. Think first about the time you spend doing work. Think of work as the things that you have to do such as paid or unpaid work, study/training, household chores, harvesting food/crops, fishing or hunting for food, seeking employment. *[Insert other examples if needed].* In answering the following questions 'vigorous-intensity activities' are activities that require hard physical effort and cause large increases in breathing or heart rate, 'moderate-intensity activities' are activities that require moderate physical effort and cause small increases in breathing or heart rate. / Bjale ke ile go go botšiša ka nako yeo o e tseyago o thobolla mmele mo bekeng. Ka kgopelo araba dipotšišo le ge o ša ipone o le motho wa go fela a ithobolla mmele. Nagana pele ka nako yeo o etšeago o soma. Nagana ka mošomo ele dilo tšeo o di dirago go swana le mošomo wa go lefšwa goba wa go se lefšwe, go ithuta, mešomo ya ka gae, go lema, go thea dihlapi goba go nyakana le mošomo.[Tsentšha mehlala ye mengwe ge go hlokega]. Ge o fetola dipotšišo tše latelago;tseba gore ge re bolela ka mošomo o boima re ra gore mošomo woo o dirago gore o hemele godimo le pelo e kibela godimo, mola mošomo o boleta e le woo o dirago gore o se hemele godimo kudu le pelo e se kibela godimo kudu.		
Question / Potšišo	**Response / Karabo**		**Code**
Work / Go šoma			
Please note that in this case **work means all paid and unpaid working activities**. This sections should be filled by even those who are unemployed			
45	Does your work involve vigorous-intensity activity that causes large increases in breathing or heart rate like *[carrying or lifting heavy loads, digging or construction work]* for at least 10 minutes continuously? *[INSERT EXAMPLES] (USE SHOWCARD) /* Naa mošomo wag ago o nyaka	Yes / Ee No / Aowa	1 2 *Ge eba aowa fetela go P 4*	P1
46	In a typical week, on how many days do you do vigorous-intensity activities as part of your work? /	Number of days / Palo ya matšatši ⌊__⌋		P2

47	How much time do you spend doing vigorous-intensity activities at work on a typical day? / Naa o tšea nako e kaakang ge o šoma boima mo letšatšng le le tee?	Hours : minutes / Di-iri : metsotso	⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins Di-iri metsotso	P3 (a-b)
48	Does your work involve moderate-intensity activity, that causes small increases in breathing or heart rate such as brisk walking *[or carrying light loads]* for at least 10 minutes continuously? *[INSERT EXAMPLES] (USE SHOWCARD)* / Naa mošomo wa gago o nyaka	Yes / Ee No / Aowa	1 2 *If No, go to P 7 / Ge eba aowa fetela go P 7*	P4
49	In a typical week, on how many days do you do moderate-intensity activities as part of your work? / Mo bekeng ya e tlwaelegilego ke	Number of days / Palo ya matšatši	⌊__⌋	P5
50	How much time do you spend doing moderate-intensity activities at work on a typical day? / Naa o tšea nako e kaakang ge o šoma mošomo o bofefo, mo letšatšing le le tee?	Hours : minutes / Di-iri : metsotso	⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins Di-iri metsotso	P6 (a-b)
Travel to and from places / Go ya le go boa mafelong a go fapana				
The next questions exclude the physical activities at work that you have already mentioned. Now I would like to ask you about the usual way you travel to and from places. For example to work, for shopping, to market, to place of worship. *[Insert other examples if needed]* / Dipotšišo tše di latelago di akaretša mošomo o boima wo re šetšego re boletše ka ona. Bjale ke rata go go botšiša mabapi le go ya le go boa mafelong a go fapana. Mohlala: Go sepela go ya mošomong, mabenkeleng mmarakeng goba mafelong a go rapela.[TŠENTŠHA MEHLALA E MENGWE GE GO HLOKEGA].				
51	Do you walk or use a bicycle *(pedal cycle)* for at least 10 minutes continuously to get to and from places? / Naa o a sepela goba o šomisa paesekela (ya materapo) go lekana nako ya metsotso e lesome goba go feta ge o eya goba o bowa mafelong?	Yes / Ee No / Aowa	1 2 *Ge eba aowa fetela go P 10*	P7

52	In a typical week, on how many days do you walk or bicycle for at least 10 minutes continuously to get to and from places? / Mo bekeng naa o tšea matšatši a	Number of days / Palo ya matšatši ⌊__⌋	P8
53	How much time do you spend walking or bicycling for travel on a typical day? / Naa o tšea nako e kaakang mo letšatšing go sepela goba o šomiša paesekela?	Hours : minutes / Di-iri : metsotso ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins Di-iri Metsotso	P9 (a-b)

CORE: Physical Activity, Continued / DIPOTŠITŠO TŠE BOHLOKWA: Thobollo ya mmele,				
Question / Potšišo		**Response / Karabo**		**Code**
Recreational activities / Maitapološo				
The next questions exclude the work and transport activities that you have already mentioned. Now I would like to ask you about sports, fitness and recreational activities (leisure), *[Insert relevant terms]*. / Dipotšišo tše di latelago a di akaretše mošomo le mokgwa wa dinamelwa tšeo o šetšego o di boletše. Bjale ke rata go go botšiša mabapi le tša dipapadi, tša boitekanelo le tša boitapološo (boiketlo) [tsentšha tšeo di swanetšego)				
54	Do you do any vigorous-intensity sports, fitness or recreational *(leisure)* activities that cause large increases in breathing or heart rate like *[running or football]* for at least 10 minutes continuously? *[INSERT EXAMPLES] (USE SHOWCARD)* /	Yes / Ee No / Aowa	1 2 *If No, go to P 13* / *Ge e le aowa eya go P 13*	P10
55	In a typical week, on how many days do you do vigorous-intensity sports, fitness or recreational *(leisure)* activities? / Mo bekeng a naa o raloka papadi ye e thata, goba o tsenela tša boitekanelo, goba tša boitapološo (boiketlo) ga kaakang?	Number of days / Matšatši	⌊__⌋	P11
56	How much time do you spend doing vigorous-intensity sports, fitness or recreational activities on a typical day? / A naa o tšea nako e kaakang o raloka papadi yeo ye boima, goba o tsenetše tša boitekanelo goba tša boitapološo mo letšatšing?	Hours : minutes / Di-iri: metsoso	⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins di-iri mets	P12 (a-b)
57		Yes / Ee	1	P13

	Do you do any moderate-intensity sports, fitness or recreational *(leisure)* activities that cause a small increase in breathing or heart rate such as brisk walking, *[cycling, swimming, volleyball]* for at least 10 minutes continuously? *[INSERT EXAMPLES] (USE SHOWCARD) ?* A naa o tsenela le tše dingwe tša	No / Aowa	2 *If No, go to P16 /* *Ge ele aowa eya go P16*	
58	In a typical week, on how many days do you do moderate-intensity sports, fitness or recreational *(leisure)* activities? / Mo bekeng a naa o tsenela tše tša dipapadi, tsa botekanelo goba tša boitapološo (boiketlo) tšeo di sego boima kudu ga kae?	Number of days / Matšatši	⌊__⌋	P14
59	How much time do you spend doing moderate-intensity sports, fitness or recreational *(leisure)* activities on a typical day? / A naa o tšea nako e kaakang o tsenetše tšeo tša dipapadi, tša boitekanelo goba tša boitapološo (boiketlo) tšeo di sego boima kudu ka letšatši ?	Hours : minutes / Diiri:metsotso	⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins di-iiri metsotso	P15 (a-b)

EXPANDED: Physical Activity / DIPOTŠIŠO TSA TLALELETŠO: Thobollo ya Mmele
Sedentary behaviour / Mokgwa wa go dula felo go tee
The following question is about sitting or reclining at work, at home, getting to and from places, or with friends including time spent sitting at a desk, sitting with friends, traveling in car, bus, train, reading, playing cards or watching television, but do not include time spent sleeping. *[INSERT EXAMPLES] (USE SHOWCARD) /* Potšišo e e latelago e mabapi le go dula fase goba go ithekga ka sengwe mosomong, ka gae, go ya le go bowa mafelong a itseng, goba le bagwera go akaretšwa nako eo o e tšerego o dutše setulong, o dutše le bagwera, o sepela ka sefatanaga, pese, setimela, o bala, o raloka dikarata, goba o bogetše thelebišene, e fela ga e akaretše nako yeo o e tsšrego o robetše [TSENTŠHA MEHLALA] [BONTŠHA KA KARATA]

60	How much time do you usually spend sitting or reclining on a typical day? / A naa o fela o tšea nako e kaakang ka letšatši o dutše fase goba o ithekgile ?	Hours : minutes / Di-iri:metsotso	⌊_⌊_⌋ : ⌊_⌊_⌋ hrs mins di-iri mets	P16 (a-b)

CORE: History of Raised Blood Pressure / DIPOTŠITŠO TŠE BOHLOKWA: Tša madi a magolo				
Question / Potšišo		**Response / Karabo**		**Code**
61	Have you ever had your blood pressure measured by a doctor or other health worker? / A naa o ile wa bofša lepanta la	Yes / Ee No / Aowa	1 2	H1
62	Have you ever been told by a doctor or other health worker that you have raised blood pressure or hypertension? / A naa o kile wa botšwa ke ngaka	Yes / Ee No / Aowa	1 2 *If No, go to H6 / Ge ele aowa, eya go H6*	H2a
63	Have you been told in the past 12 months? / A naa o boditšwe mo dikgweding tše lesome-pedi tša go feta?	Yes / Ee No / Aowa	1 2	H2b

EXPANDED: History of Raised Blood Pressure / DIPOTŠIŠO TŠA TLALELETŠO: Tša madi a magolo				
64	Are you currently receiving any of the following treatments/advice for high blood pressure prescribed by a doctor or other health worker? / A naa ga bjale o nwa dihlare tše/o fiwa maele ka tša madi a magolo tšeo o di/ao o a fiwago ke ngaka goba mošomedi o mongwe wa tša maphelo?			
	Drugs (medication) that you have taken in the past two weeks / Diokobatši (dihlare) tšeo o di nwelego mo dibekeng tše pedi tša gofeta	Yes / Ee No / Aowa	1 2	H3a
	Advice to reduce salt intake / Keletšo ya go fokotša letswai dijong	Yes / Ee No / Aowa	1 2	H3b
	Advice or treatment to lose weight / Keletšo goba kalafo ya go fokotša boima bja mmele	Yes / Ee No / Aowa	1 2	H3c
		Yes / Ee	1	H3d

	Advice or treatment to stop smoking / Keletšo goba kalafo ya go tlogela go kgoga motšoko	No / Aowa	2	
	Advice to start or do more exercise / Keletšo ya go thoma go itšhidulla kudu	Yes / Ee No / Aowa	1 2	H3e
65	Have you ever seen a traditional healer for raised blood pressure or hypertension? / A naa o ile wa bonwa ke ngaka ya setšo mabapi le madi a magolo?	Yes / Ee No / Aowa	1 2	H4
66	Are you currently taking any herbal or traditional remedy for your raised blood pressure? /	Yes / Ee No / Aowa	1 2	H5

CORE: History of Diabetes / DIPOTŠITŠO TŠE BOHLOKWA: Tša mabapi le bolwetši bja swikiri					
Question / Potšišo		**Response / Karabo**			**Code**
67	Have you ever had your blood sugar measured by a doctor or other health worker? / A naa o kile wa lekolwa mabapi le	Yes / Ee No / Aowa	1 2		H6
68	Have you ever been told by a doctor or other health worker that you have raised blood sugar or diabetes? /	Yes / Ee No / Aowa	1 2	*If No, go to M1 / Ge eba aowa*	H7a
69	Have you been told in the past 12 months? / A naa o boditšwe mo dikgweding tše lesomepedi tša go feta ?	Yes / Ee No / Aowa	1 2		H7b

EXPANDED: History of Diabetes / DIPOTŠIŠO TSA TLALELETŠO: Tša bolwetsi bja swikiri				
70	Are you currently receiving any of the following treatments/advice for diabetes prescribed by a doctor or other health worker? / A naa ga bjale o hwetša kalafo/keletšo ya mabapi le bolwetši bja swikiri tšeo o di fiwago ke ngaka goba mošomedi o mongwe wa tša maphelo?			
	Insulin / Tšhwaana ya taolo ya swikiri mo	Yes / Ee No / Aowa	1 2	H8a
	Drugs (medication) that you have taken in the past two weeks / Diokobatši (dihlare) tšeo o di nwelego mo dibekeng tše pedi tša go feta	Yes / Ee No / Aowa	1 2	H8b
	Special prescribed diet / Dijo tšeo o di kgethetšwego ke ngaka ?	Yes / Ee No / Aowa	1 2	H8c
	Advice or treatment to lose weight / Keletšo goba kalafo ya go fokotša boima bja mmele	Yes / Ee No / Aowa	1 2	H8d
	Advice or treatment to stop smoking / Keletšo goba kalafo ya go tlogela go kgoga motšoko	Yes / Ee No / Aowa	1 2	H8e
	Advice to start or do more exercise /	Yes / Yes No / No	1 2	H8f

	Keletšo goba kalafo ya go itšhidulla kudu			
71	Have you ever seen a traditional healer for diabetes or raised blood sugar? / A naa o ile wa bonwa ke ngaka ya setšo mabapi le bolwetši bja swikiri goba swikiri e ntši mo mading ?	Yes / Ee No / Aowa	1 2	H9
72	Are you currently taking any herbal or traditional remedy for your diabetes? / A naa ga bjale o nwa mešunkwane goba dihlare tša setšo go alafa bolwetši bjoo bja swikiri ?	Yes / Ee No / Aowa	1 2	H10

Step 2 Physical Measurements

Nomoro ya Motšeakarolo

|_|_|_|_| |_|_|_|_| |_|_|_|_|

CORE: Height and Weight				
Question		**Response**		**Code**
73	Interviewer ID		|_|_|_|	M1
74	Device IDs for height and weight	Height Weight	|_|_| |_|_|	M2a M2b
75	Height	in Centimetres (cm)	|_|_|_|.|_|	M3
76	Weight *If too large for scale 666.6*	in Kilograms (kg)	|_|_|_|.|_|	M4
77	**For women:** Are you pregnant?	Yes No	1 *If Yes, go to M 8* 2	M5
CORE: Waist				
78	Device ID for waist		|_|_|	M6
79	Waist circumference	in Centimetres (cm)	|_|_|_|.|_|	M7
CORE: Blood Pressure				
80	Interviewer ID		|_|_|_|	M8
81	Device ID for blood pressure		|_|_|	M9

82	Cuff size used	Small Medium Large	1 2 3	M10
83	Reading 1	Systolic (mmHg)	⌊_⌋⌊_⌋⌊_⌋	M11a
		Diastolic (mmHg)	⌊_⌋⌊_⌋⌊_⌋	M11b
84	Reading 2	Systolic (mmHg)	⌊_⌋⌊_⌋⌊_⌋	M12a
		Diastolic (mmHg)	⌊_⌋⌊_⌋⌊_⌋	M12b
85	Reading 3	Systolic (mmHg)	⌊_⌋⌊_⌋⌊_⌋	M13a
		Diastolic (mmHg)	⌊_⌋⌊_⌋⌊_⌋	M13b
86	During the past two weeks, have you been treated for raised blood pressure with drugs (medication)	Yes No	1 2	M14

EXPANDED: Hip Circumference and Heart Rate				
87	Hip circumference	in Centimeters (cm)	⌊_⌋⌊_⌋⌊_⌋.⌊_⌋	M15
88	Heart Rate			
	Reading 1	Beats per minute	⌊_⌋⌊_⌋⌊_⌋	M16a
	Reading 2	Beats per minute	⌊_⌋⌊_⌋⌊_⌋	M16b
	Reading 3	Beats per minute	⌊_⌋⌊_⌋⌊_⌋	M16c

Step 3 Biochemical Measurements

Nomoro ya Motšeakarolo

⌊_⌋⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋

CORE: Blood Glucose				
Question		**Response**		**Code**
89	During the past 12 hours have you had anything to eat or drink, other than water?	Yes No	1 2	B1
90	Technician ID		⌊_⌋⌊_⌋⌊_⌋	B2
91	Device ID		⌊_⌋⌊_⌋	B3
92	Time of day blood specimen taken (24 hour clock)	Hours : minutes	⌊_⌋⌊_⌋ : ⌊_⌋⌊_⌋ hrs mins	B4
93	Fasting blood glucose	mmol/l	⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋	B5

	Choose accordingly: mmol/l or	mg/dl	⌊_⌋⌊_⌋⌊_⌋.⌊_⌋	
94	Today, have you taken insulin or other drugs (medication) that have been prescribed by a doctor or other health worker for raised blood glucose?	Yes No	1 2	B6
CORE: Blood Lipids				
95	Device ID		⌊_⌋⌊_⌋	B7
96	Total cholesterol *Choose accordingly: mmol/l or mg/dl*	mmol/l mg/dl	⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋.⌊_⌋	B8
97	During the past two weeks, have you been treated for raised cholesterol with drugs (medication) prescribed by a doctor or other health worker?	Yes No	1 2	B9

EXPANDED: Triglycerides and HDL Cholesterol				
98	Triglycerides *Choose accordingly: mmol/l or mg/dl*	mmol/l mg/dl	⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋.⌊_⌋	B10
99	HDL Cholesterol *Choose accordingly: mmol/l or mg/dl*	mmol/l mg/dl	⌊_⌋.⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋.⌊_⌋	B11

Appendix VIII: Tuberculosis and HIV questionnairre

CORE: History of TB				
Question / Potšišo		**Response / Karabo**		**Code**
	Have you had cough with sputum for more than 21 days O kile wa kgohlora sehuba se setala gofeta matšatšia	Yes / Ee	1	
		No / Aowa	2	
	Coughing with blood? O kile wa kgohlora madi	Yes / Ee	1	
		No / Aowa	2	
	Low grade fever during the night? O kile wa ba le mmele wa go fiša gannyane bošego	Yes / Ee	1	
		No / Aowa	2	
	Have you felt a general weakness, loss of weight or loss of appetite? O kile wa kwa o felelwa ke maatla, mmele wa gago o fela goba o se na takatso ya dijo	Yes / Ee	1	
		No / Aowa	2	
	Have you ever been checked for TB? O kile wa hlolwa ge eba ga o na bolwetši bja mafahla	Yes / Ee	1	
		No / Aowa	2	

EXPANDED: History of TB				
	Are you currently receiving any of the following treatments/advice for TB prescribed by a doctor or other health worker? / A naa o amogela e nngwe ya dihlare / keletšo tše di latelago tša bolwetši bja mafahla ka taela ya ngaka goba mošomedi wa tša maphele Streptomycin(STM): ⌊⌋ Rifampicin(RMP): ⌊⌋ Pyrazinamide(PZA): ⌊⌋ Isoniazid(INH): ⌊⌋ Ethambutol(EMB): ⌊⌋ Any Others:			
		Yes / Ee	1	

	Drugs (medication) that you have taken in the past two weeks / Dihlare (kalafi) tšeo o di šomisitšego mo dibekeng tše pedi tša go feta	No / Aowa 2	
	Where do you get your treatment for TB? Na o hwtša kae kalafi ya gago ya bolwetši bja mafahla		
	Have you been treated for TB in the past? Na o ile wa alafiwa bolwetši bja mafahla mo nakong ye e fitilego	Yes / Ee 1 No / Aowa 2	
	Where were you treated for TB? Na o ile wa alafiwa kae bolwetši bja mafahla		
	Have you ever been treated for by any herbal or traditional remedy for TB in the past? Na o ile wa alafša bolwetši bja mafahla ka sehlare / moriana sa/wa setšo mo nakong ye e fitilego	Yes / Ee 1 No / Aowa 2 No / Aowa 2	
	Are you currently taking any herbal or traditional remedy for TB? / Na o nwa sehlare goba moriana wa setšo sebakeng sa bolwetši bja	Yes / Ee 1 No / Aowa 2	

CORE: History of HIV		
Question / Potšišo	**Response / Karabo**	**Code**

	Have you ever been tested for HIV? Na o kile wa dira diteko tša	Yes / Ee No / Aowa	1 2	
	Have you ever been tested for HIV in the last 12 months? Na o kile wa dira diteko tša kokwanathloko mo dikgweding tse	Yes / Ee No / Aowa	1 2	
	Do you know your HIV status? Na o tšeba leemo la gago lakokwanathloko	Yes / Ee No / Aowa	1 2	
	Have you ever receive a blood donation? Na o kile wa tšwelwa madi	Yes / Ee No / Aowa	1 2	
	Did you get advice in regard to avoid HIV transmission? Na o fiwa keletso mabapi le go thibela phetelo ya kokwanathloko	Yes / Ee No / Aowa	1 2	

EXPANDED: History of HIV

Are you currently receiving any of the following treatments/advice for HIV prescribed by a doctor or other health worker and for how long? /

Na ga bjale o fiwa kalafo / ketelšo e nngwe ya tšeo di latelago mabapi le kokwanathloko ke ngaka goba mošomedi e mongwe wa tša maphele

Viramune(NVP): ⌊__⌋ Trizivir: ⌊__⌋ Zerit (d4T): ⌊__⌋ AZT: ⌊__⌋ Combivir: ⌊__⌋ Atripla: ⌊__⌋ Any Others:

Drugs (medication) that you have taken in the past two weeks / Dihlare (kalafi) tšeo o di šomisitšego mo dibekeng tše pedi tša go feta	Yes / Ee No / Aowa	1 2	

	Where do you receive treatment for HIV? Na o hwetša kae kalafo ya kokwanathloko			
	Are you currently taking any herbal or traditional remedy for HIV? / Na o nwa sehlare goba moriana wa setšo sebakeng sa kokwanathloko	Yes / Ee No / Aowa	1 2	

Appendix IX: Laboratory procedures

a) Total Cholesterol estimation in serum

The estimation of cholesterol in serum was based on an enzymatic colorimetric method. Cholesterol ester is hydrolysed by the introduced water molecule and the reaction is catalysed by the enzyme cholesterol esterase, yielding free cholesterol and fatty acids. Free cholesterol is oxidised by oxygen and the reaction is catalysed by the enzyme cholesterol oxidase yielding cholest-4-en-3-one and hydrogen peroxide. Two molecules of hydrogen peroxide react with phenol and 4- aminoantipyrine catalysed by the enzyme peroxidase yielding red quinoneimine and four water molecules. Colour intensity is directly proportional to the cholesterol concentration in the original sample. The colour absorbance is measured using a spectrophotometer at 510nm wavelength.

Cholesterol levels were determined using the ILab 300 Plus Chemistry System analyser (Instrumentation Laboratory Company, Italy) and cholesterol reagents suuplied by Beckman coulter. Initially the lyophilised cholesterol reagent consisting of cholesterol esterase, oxidase, peroxidase and 4-aminoantipyrine was reconstituted by adding exactly 23ml of deionised water. The reagent was stable for 15 days after preparation at 2-8°C. The controls were reconstituted by adding 5ml of deionised water to the vial according to the manufacturer's instruction. The ILab 300 Plus Chemistry System was calibrated using Referril G calibrator. Four microliters (µl) of clear serum was mixed with 320 µl of cholesterol reagent inside the analyser and incubated for 8.5 minutes. After incubation the absorbance of each reaction vial was read at 510nm wavelength. Normal and abnormal control samples were run pararell to the test samples. The machine used calibration ratio to calculate the cholesterol concentration. The results were reported as mmol/L. The reference range for serum total cholesterol is 3.7-5.0 mmol/L. Any result above 5.0 mmol/L was considered high and abnormal.

b) High density lipoprotein cholesterol estimation in serum

HDL cholesterol estimation in serum was based on an enzymatic colorimetric method. Anti-human β-lipoprotein antibody binds to lipoprotein (LDL, VLDL, Chylomicrons) other than HDL when mixed with serum sample. The antigen-antibody complexes formed block

enzyme (cholesterol esterase and cholesterol oxidase) reaction with all lipoproteins except HDL-C. Cholesterol esterase and cholesterol oxidase therefore reacts with HDL-C only. Hydrogen peroxide produced by enzyme reactions with HDL-C yields a blue coloured complex upon oxidative condensation of the chromogen. The absorbance of blue complex is read at 620nm wavelength. The concentration of the blue coloured complex is proportional to the concentration of HDL-C.

HDL-C levels were determined using ILab 300Plus Chemistry system analyser (Instrumentation Laboratory Company, Italy). The reagents R1 (Good's buffer ph 7.0, 4-aminoantipyrine, peroxidase, ascorbate oxide, anti-human β-lipoprotein antibody, preservatives) and R2 (Good's buffer pH 7.0, cholesterol esterase, cholesterol oxidase, N-ethyl- N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxy-4-fluoroaniline) were supplied in a ready to use state by Beckman and Coulter. The reagent was stable for 15 days at 2-8Oc upon opening. The controls were made up by reconstituting the lyophilized serum with 5ml of deionised water and were gently swirled for 30 minutes to mix. The ILab 300 Plus Chemistry System was calibrated using Referril G calibrator. Inside the analyser, three µl of clear serum was mixed with 300µl of R1 and incubated for 180 seconds. One hundred µl of R2 were added to reaction mix and incubated for 242 seconds. Absorbance was read using ILab Plus Chemistry System at 620nm wavelength. Normal and abnormal quality control samples were run pararell to test samples. The machine used calibration ratio to calculate the HDL-C concentration. The results were reported in mmol/L. The reference range for HDL-C in males is >1.1 mmol/L and for females is >1.3 mmol/L. Results below the lower limits were considered low and abnormal.

c) Triglyceride estimation in serum

Triglycerides estimation in serum was based on an enzymatic colorimetric method. The test is based on the hydrolysis of triglyceride by the enzyme lipoprotein lipase to free glycerol and three fatty acids. Glycerol is phosphorylated by adenosine triphosphate and this reaction is catalysed by glycerol kinase to yield glycerol-3-phosphate. Glycerol-3-phosphate is oxidised by oxygen in the presence of glycerol phosphate oxidase to yield

dihydroxyacetone phosphate and hydrogen peroxide. Hydrogen peroxide oxidises 4-chlorophenol and 4-aminoantipyrine yielding a red quinoneimine. The reaction is catalysed by the enzyme peroxidase. The colour absorbance is read at 510nm wavelength using spectrophotometer inside analyser. The concentration of the red coloured complex is proportional to the concentration of triglycerides in original sample.

Triglyceride levels were determined using ILab 300 Plus Chemistry System analyser (Instrumentation Laboratory Company, Italy) following manufacturer instructions. The reagent consisting of lipoprotein lipase, glycerol kinase, glycerol phosphate oxidase, peroxidase, 4-chlorophynol and 4-aminoantipyrine were supplied in a ready to use state by Beckman and Coulter. The reagent was stable for 15days at 2-8^{o}C upon opening. The controls were made up by reconstituting the lyophylised serum with 5ml of deionised water and were gently swirled for 30 minutes to mix. The ILab 300 Plus Chemistry System was calibrated using Referril G calibrator. Inside the analyser, three µl of clear serum was mixed with 300µl of triglyceride reagent and incubated for 350 seconds. Absorbance was read at 510nm wavelength. Normal and abnormal quality control samples were run pararell to test samples. Machine used calibration ratio to calculate triglyceride concentration. The results were reported as mmol/L. The reference range for triglycerides is less than 1.7 mmol/L. Any result equal or above 1.7mmol/L was considered high and abnormal.

d) LDL-C estimation in serum

The LDL-C was calculated from total cholesterol, high density lipoprotein cholesterol and triglyceride levels according to the Friedewald formula (Friedewald et al., 1972), when TG was less than 400mg/ml. LDL-C = TC- [HDL-C+TG/2.2].

Non HDL-C estimation in serum

Non HDL-C was determined by subtracting the concentration of cholesterol in HDL from that in total plasma.

e) Glucose estimation in plasma

Glucose estimation in plasma was based on Trinder's enzymatic colorimetric method. The test is based on oxidation of glucose by oxygen in the presence of glucose oxidase to yield hydrogen peroxide and gluconic acid. Hydrogen peroxide reacts with phenol and 4-aminoantipyrine with the reaction catalysed by peroxidase to yield red quinoneimine. The colour absorbance is read at 510nm wavelength. The intensity of the red coloured complex is proportional to the concentration of glucose in original sample.

Glucose levels were determined using ILab 300 Plus Chemistry System analyser (Instrumentation Laboratory Company, Italy) following manufacturer instructions. The lyophilised glucose oxidase reagent (glucose oxidase, peroxidase, phenol, 4 aminoantipyrine) (Beckman coulter) was reconstituted by adding 12ml of deionised water to reagent bottle and mixed well by inversion. The reagent was stable for 15 days at 2-8^{o}C upon opening. The controls were made up by reconstituting the lyophilised serum with 5ml of deionised water and swirling gently for 30 minutes to mix. The ILab 300 Plus Chemistry System was calibrated using Referril G calibrator. Inside the analyser, four µl of plasma was mixed with 300µl of glucose oxidase reagent and incubated for 180 seconds. Absorbance was read at 510nm wavelength. Normal and abnormal quality control samples were run pararell to test samples. Machine used calibration ratio to calculate glucose concentration. The results were reported as mmol/L. The reference range fasting glucose is 3.9-5.8mmol/L. A fasting blood glucose level equal to or greater than 7.0mmo/L was considered high, abnormal and signified diabetes mellitus.

f) High Sensitivity C - reactive protein estimation in serum

High sensitivity C-reactive protein estimation in serum was based on a rate turbidimetric assay.

The test is based on agglutination of CRP when mixed with latex reagent. An anti-hs CRP antibody coated particles binds to hs-CRP in the patients sample resulting in the formation of insoluble aggregates causing turbidity. The rate of aggregate formation is directly proportional to the concentration of hs-CRP in the sample.

The hs-CRP levels were determined using the IMMAGE Immunochemistry System, (Beckman Coulter, USA) following manufacturer instructions. The hs-CRP antibody coated particles, buffer 4, and diluent were supplied ready to be used by Beckman coulter. They were stable for 30 days at 2-8ºC with evaporation caps in place. The IMMAGE Immunochemistry System analyser was calibrated using the calibrator 5 Plus. The machine mixed 4.5µl of sample with 209µl of reagent consisting of 42µl of antibody coated particle, 125µl of buffer and 42µl diluent. The IMMAGE analyser measured the rate of aggregate formation and automatically calculated the concentration of hs-CRP in original sample. Normal and abnormal quality control samples were run pararell to test samples. The reference range for hs-CRP is less than 3.

g) Apolipoprotein AI estimation in serum

Apo AI estimation in serum was based on a nephelometric assay. The test is based on the reaction of Apo AI (sample) and Apo AI antibody to form Apo AI-Apo AI antibody aggregates. The measurement of the rate of increase in light scattered from antigen-antibody complexes present in the solution is proportional to the concentration of Apo AI in the original sample.

Apo AI levels were determined using the IMMAGE Immunochemistry System (Beckman Coulter, USA) following manufacturer instructions. The Apo AI reagent, diluent 2 and buffer 1 (Beckman coulter) were stable for 30 days at 2-8ºC with evaporation caps in place. The IMMAGE analyser was calibrated using the Apolipoprotein calibrator (APO CAL). The machine mixed 0.58µl of sample with 341.42µl of reagent consisting of 21µl of antibody, 300µl of buffer 1 and 20.42µl of diluent 2. The machine measured the rate of increase in light scattered from Apo AI-Apo AI antibody complexes present in the solution. The Virgil lipid control level 1-4 was run pararell to test samples. The machine used the calibration ratio to calculate the concentration of Apo AI in original sample. The reference range for APO AI in males and females are 90-170mg/dl and 107-214mg/dl respectively.

h) Apolipoprotein B estimation in serum

Apo B estimation in serum was based on a nephelometric assay. The test is based on the reaction of Apo B (sample) and Apo B antibody to form Apo B-Apo B antibody aggregates. The measurement of the rate of increase in light scattered from antigen-antibody complexes present in the solution is proportional to the concentration of Apo B in the original sample.

Apo B levels were determined using the IMMAGE Immunochemistry System (Beckman Coulter, USA) following manufacturer instructions. The Apo B reagent, diluent 2 and buffer 1 were supplied ready to be used by Beckman coulter and were stable for 30 days at 2-8^{o}C with evaporation caps in place. The IMMAGE analyser was calibrated using the Apolipoprotein calibrator (APO CAL). The machine mixed 0.58µl of sample with 341.42µl of reagent consisting of 21µl of antibody, 300µl of buffer 1 and 20.42µl of diluent 2. The machine measured the rate of increase in light scattered from Apo B-Apo B antibody complexes present in the solution. The Virgil lipid control level 1-4 was run pararell to test samples. The machine used the calibration ratio to calculate the concentration of Apo B in original sample. The reference range for Apo B in males and females are 56-162mg/dl and 51-171mg/dl respectively.

i) HIV Testing

HIV testing was performed on plasma using Determine HIV 1 / 2 Antigen/ Antibody Combo kit (Inverness Medical, Japan) and Double check kit (Inverness Medical, Japan) following manufacturer's instructions).

Determine HIV1/2 Antigen/Antibody Combo

Determine HIV1/2 Antigen/Antibody Combo is an immunochromatographic test for the qualitative detection of p 24 antigen and antibodies to HIV 1 and HIV 2 in plasma. The test is based on mixing the test sample with a biotinylated anti-p 24 antibody and selenium colloid-antigen conjugate embedded on test strip. This mixture continues to migrate through the solid phase (test strip) to the immobilized avidin, recombinant antigens and synthetic peptides at the patient window sites.

If antibodies to HIV 1 and/or HIV 2 are present in the specimen the antibodies bind to the antigen-selenium colloid and to the immobilized recombinant antigens and synthetic peptides forming a red bar at the patient HIV antibody window site. If antibodies to HIV 1 and /or HIV 2 are absent the antigen-selenium colloid flows past the patient window, and no red bar is formed at the patient HIV antibody window site.
If p 24 antigen is present in the specimen, the antigen binds to the biotinylated anti- p 24 from the sample pad and the selenium colloid anti p24 antibody and it binds to an immobilized avidin forming a red bar at the patient HIV antigen window site. If p 24 antigen is not present both the biotinylated anti-p 24 and selenium colloid anti-p 24 antibody flow past the patient window and no red bar is formed at the patient HIV antigen window site. A procedural control bar is incorporated in the assay device.

The test was performed at room temperature and all samples and reagents were allowed to reach room temperatures before being used. Using a micropipette with disposable tips, fifty µl of plasma was applied to the sample pad, on the test strip. The strip was incubated for 25minutes. The strips were visually read, as either positive if a red bar appeared on patient antigen, antibody window and control window or negative if a red bar appeared only on the control window. Absence of a control bar irrespective of the result on patient window invalidated the test and it was repeated. The kits were used before their expiration date.

Double Check gold kit

Double check gold is an immunoassay for the qualitative detection of antibodies to human immunodeficiency virus types 1 and 2 (HIV 1 and HIV 2) in human plasma. The test is based on the immobilization of recombinant proteins representing the immunodominant regions of the envelop and gag proteins of HIV 1 and the gp36 molecule of HIV 2 at the test region of the nitrocellulose strip. An antibody binding reagent is embedded at the control region of the strip. HIV 1 and HIV 2 proteins, linked to colloidal gold are impregnated on the gold pad, placed between the sample pad and the nitrocellulose strip. After addition of plasma to sample port and subsequent addition of wash reagent, the specimen flows into the cassette and onto test strip. If antibody specific to HIV 1and / or

HIV 2 proteins are present in the sample, they will react with colloidal gold conjugated particles. A positive reaction is indicated by the presence of two coloured bands in the test region (marked T) and in the control region (marked C). A negative test is indicated by only one band in the control region.

The test was performed at room temperature and all samples and reagents were allowed to reach room temperatures before being used. Using a micropipette with disposable tips, 25 µl of plasma was applied to the sample port, on the test cassette. Two drops of wash reagent were immediately added to the sample port. The cassette was incubated for 15 minutes and results were visually read. A positive result had two bands on the test region and the control region, while a negative result had one band on the control region. The absence of a control band irrespective of the result on patient window invalidated the test and the test was repeated. The kits were used before their expiration date.

j) CD4 Count Determination

CD4 count test was perfomed following manufacturer's instructions. The test is based on the determination of absolute counts of T-helper cells in whole blood using the PIMA Analyser (Alere Limited, United Kingdom) and disposable PIMA CD4 test cartridge (Alere Limited, United Kingdom). After the insertion of the PIMA CD4 test cartridge into analyser, peristaltic movement transports the sample into the incubation compartment where the sample interacts with specific antibodies labelled with two different fluorescent dyes emitting light at two different wavelengths (dye1 and dye 2). One antibody is an anti-human CD3 monoclonal antibody conjugated to dye 1. The second antibody is an anti-human CD4 monoclonal antibody conjugated to dye2. After incubation, the stained sample is transferred into the detection channel of the cartridge. The PIMA analyser is equipped with miniaturized multi-colour fluorescence imaging optics. Fluorescence signals are detected by an on-board camera and analysed using proprietary software algorithms and on board an embedded computer. The T-helper cells carry both CD3 and CD4 surface antigens and therefore emit light at wavelengths specific for both antibody-dye conjugate. This allows the specific differentiation of T-helper cells from other blood

cell types carrying only one of the two surface antigens. Results are displayed by the PIMA Analyser as cells/µl.

The test was initiated by adding whole blood sample to the PIMA CD4 cartridge. The cartridge was held in an upright position in order to view the control window and ensure that sufficient sample was loaded. Enough sample was loaded when the capillary visible in the control window was filled with blood. The clip of the sample collector (on cartridge) was squeezed between thumb and index finger and the sample collector was removed from the cartridge in one continuous upward motion. The sample collector was disposed in biohazard waste container. The orange plastic cap was completely closed. The run test command was selected on the PIMA analyser and it automatically opened the window for cartridge insertion. The cartridge was inserted following the direction indicated by the arrow on cartridge. After the analysis the PIMA analyser prompted the removal of PIMA CD4 test cartridge. The results were calculated automatically by PIMA analyser and displayed on screen. Cartridges containing beads (PIMA beads) representing normal CD4 count (670-1244c/µl mean: 957c/µl) and low CD4 count (132-252 c/µl mean: 192c/µl) were supplied by Inverness Medical, Japan and were run with each batch of samples as controls. The PIMA beads were stable up to 6 months upon opening.

k) Viral load determination

Viral load testing, using the branched deoxyribonucleic acid (DNA) technique (Siemens, South Africa) was performed by Toga Molecular Biology and Pathology medical laboratory that is South African National Accreditation System (SANAS) accredited for ISO 17025.

Appendix X: Lipoprotein and its subclasses in HIV infected individuals: A Review of the Literature.

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Lipoprotein and its subclasses in HIV-infected individuals: A Review

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Abstract

Studies on dyslipidaemia in human immunodeficiency virus (HIV) infected people have reported on lipoproteins and lipoprotein subclass profiles. Lipoprotein subclasses are regarded as more accurate measures of cardiovascular disease (CVD) risk than levels of lipoproteins. In this review, the primary objective was to compare and contrast the distribution patterns of lipoprotein and lipoprotein subclasses in highly active antiretroviral therapy (HAART) naïve people and those on HAART based on available literature. PubMed, Science Direct and Google were searched using a combination of various keywords, and relevant English language articles were selected. HIV infection is associated with a decrease of high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), total cholesterol (TC), with increased triglycerides (TG) accompanied by noticeable decreases in total HDL-particles (HDL-p), small dense HDL-p, total LDL-particles (LDL-p) and small dense LDL-p. Acquired immunodeficiency syndrome (AIDS) is associated with increase in small dense LDL-p and decreased HDL-p. HAART, especially protease inhibitor (PI)-based, is associated with increase in lipoprotein levels and levels of total LDL-p and small dense LDL-p, while the non-nucleoside reverse transcriptase inhibitors (NNRTI)-based HAART is associated with smaller increase in lipoprotein levels and significant increase in HDL-p. In addition to a predominance of small dense LDL-p, patients on HAART, especially PI-based have low HDL-p levels. This subclass pattern increases the risk of CVD in HIV-infected people. The use of NNRTI-based HAART or newer PI drugs such as atazanavir, associated with a less atherogenic subclass profile could defer premature CVD in HIV-infected people. As most studies were conducted in Western countries and in people whose ethnicity is different from the ethnicity of people from sub-Saharan Africa, there is a need to analyse lipoprotein subclasses in HIV- infected people, especially in sub-Saharan Africa where HIV infection is most prevalent.

Keywords: Lipoprotein, lipoprotein subclasses, dyslipidaemia, HIV, HAART.

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Introduction

Due to a longer survival time attributable to HAART, cardiovascular disease is becoming a significant cause of death among people infected with HIV (Duprez, Kuller, Tracy, Otvos, Cooper, Hoy, Neuhaus, Paton, Friis-Moller, Lampe, Liappis & Neaton, 2009). Alterations in lipid metabolism by both HIV infection and HAART cause dyslipidaemia which may lead to the development of atherosclerosis. The persistent inflammation present in HIV-infected people may also accelerate the development of atheroma (Malvestutto & Aberg, 2010). HIV associated dyslipidaemia is characterised by increased levels of TG and decreased levels of total TC, LDL-C and HDL-C (Rose et al., 2006). Decreases in total HDL-p, small dense HDL-p, total LDL-p and small dense LDL-p are evident in HIV infection (Stein et al., 2008; Baker et al., 2010). Some HAART regimens, especially PI-based regimens have been associated with severe dyslipidaemia characterised by high levels of TC, LDL-C, TG and low levels of HDL-C (Riddler et al., 2006) coupled with increased levels of total LDL-p, small dense LDL-p and low levels of HDL-p (Swanson et al., 2009).

However, decreases of TC, TG, non-HDL-C in dyslipidaemic HIV-infected people switched to atazanavir/ritonavir-based regimen from lopinavir/ritonavir-based regimen were reported (Murphy, Berzins, Zala, Fichtenbaum, Dube, Guaraldi, Torriani, Belsey, Mitchell & Stein, 2010). Some NNRTI-based HAART result in smaller increase in TC, LDL-C, and TG with noticeable increase in HDL-C and HDL-p (Minnaar & van der Merwe, 2008; Swanson et al., 2009). Standard lipid measures have long served as important predictors of atherosclerosis and CVD risk. However, evidence indicates that lipoprotein particle size and subclass patterns may provide more accurate assessment of CVD risk than standard lipid measurements (Rosenson, 2004). In an effort to accurately predict the CVD risk in HIV-infected subjects, studies on lipoprotein subclass distribution have been carried out (Stein et al., 2008; Duprez et al., 2009; Swanson et al., 2009; Baker et al., 2010; Saumoy et al., 2011). Here, we review the literature on lipoprotein and lipoprotein subclass distribution in HIV-infected people both HAART naïve and those undergoing HAART and the current antiretroviral (ARV) switching strategies for management of dyslipidaemia.

Methodology

Search strategy and selection criteria

Online databases of PubMed, Google and Science Direct were searched using. keywords such as "lipids", "lipoproteins", lipoprotein subclasses", "HIV", "HAART", "dyslipidaemia", "LDL-p functions", "HDL-p functions", "AIDS",

"NNRTIs", "protease inhibitors", "Nevirapine and Efavirenz", "Polyacrylamide gradient gel electrophoresis", "nuclear magnetic resonance". Various combinations of these words were used. The 'related citation' tab in PubMed and the 'related articles' tab in Science Direct were also used. Studies meeting the following criteria were included:

Target population: Studies whose population comprised HIV-infected people either naïve to HAART treatment or on HAART or both. *Outcome*: Studies whose outcome was lipoprotein or lipoprotein subclass distribution or both.

Specimen analysed: Both serum and plasma were considered. Serum or plasma has been used in determination of lipoprotein and lipoprotein subclasses (Mallol, Rodriguez, Brezmes, Masana & Correig, 2013). In the SMART study, baseline lipids were measured from serum and the one month follow-up lipid levels of same participants were determined from plasma. The lipid changes obtained from the two different samples were reported (Lampe et al., 2010).

Technique used to analyse samples: Both nuclear magnetic resonance (NMR) spectroscopy and polyacrylamide gradient gel electrophoresis (PAGGE) were considered. Most studies utilised the nuclear magnetic resonance (NMR) spectroscopy to determine the lipoprotein subclasses, except one study (Feingold et al., 2003) that utilised polyacrylamide gradient gel electrophoresis (PAGGE). The two techniques PAGGE and NMR were evaluated and their correlation was reported as 0.86 (Blake, Otvos, Rifai & Ridker, 2002), while an agreement of 70% was reported elsewhere (Ensign, Hill & Heward, 2006).

Study designs: Case controls, cohorts, randomised clinical trials and cross-sectional studies. The lipoprotein and lipoprotein subclasses from studies with similar designs and study populations were compared irrespective of the laboratory technique or sample type used in the studies.

Results

The online search strategy identified 296 articles. Articles not meeting target population (47), articles not meeting outcome (197) and articles not written in English language (16) were excluded. Thirteen articles (13) were added after manual search from retrieved articles. A total of 49 relevant articles were chosen and full text manuscripts were read (Figure 1). Tables 1-3 summarize the characteristics and results of studies compared in this review.

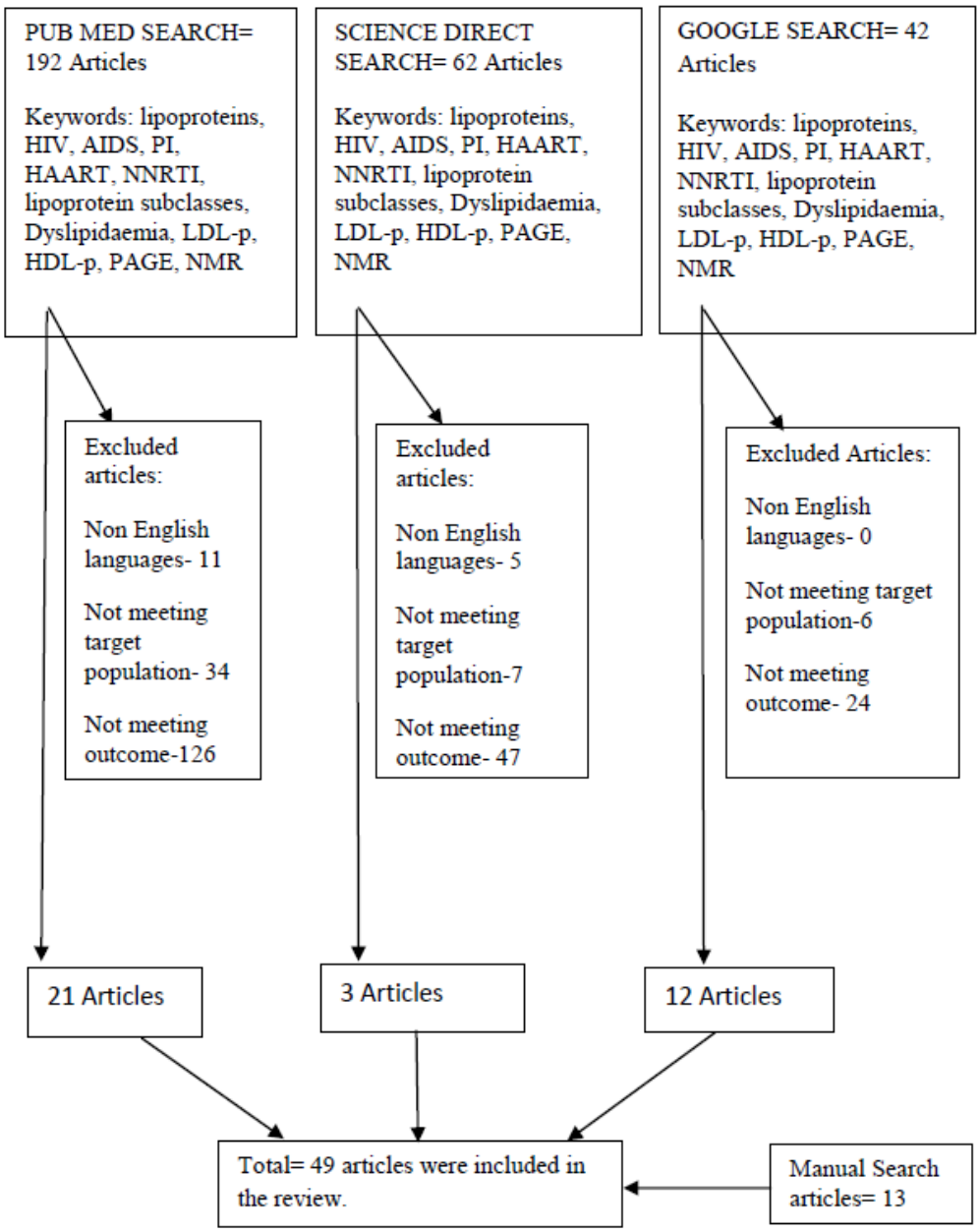

Figure 1: Search design

Table 1: Lipoprotein distributions in reviewed studies

Author (Year)	Study design / Location	Lab method	Sample type	Study population (number)		Lipoprotein levels TC(mmol/l)	HDL-C (mmol/l)	LDL-C(mmol/l)	TG(mmol/l)
Feingold (1993)	Case control/ America	SBM	Plasma	Males	HIV -ve	5.09 (0.078)	1.14 (0.023)	3.36 (0.072)	1.13 (0.045)
					HIV +ve, aids, ARV naive	3.96(0.222) *	0.78(0.031) *	2.12(0.191) *	2.47(0.229) *
Fourie (2010)	Case control/ South Africa	SBM	Serum	Males & females	HIV -ve	5.03 ± 1.33	1.70 ± 0.71	2.80 ± 1.14	1.15 ± 0.75
					HIV +ve, HAART naive	4.42 ± 1.25*	1.23 ± 0.58*	2.80 ± 1.14*	1.15 ± 0.75*
Baker (2010)	Case control/ America	SBM	Serum	Males & females	HIV –ve (29)	4.95(4.16-5.72)	1.22(1.09-1.45)	2.80(2.41-3.76)	1.27(0.87-1.62)
					HIV +ve, HAART naive (32)	4.35(3.83-4.82)	0.89 (0.781.15) *	2.80(2.10-2.95)	1.42(1.02-2.01)
Yone (2011)	Case control/ Cameroon	SBM	Serum	Males & females	HIV+ve, ART naive (138)	4.45±1.89	1.27±0.65	2.59±1.76	1.51(1.25-1.92)
					HIV+ve, 2NRTI (3TC, d4T/AZT)+NNRTI (NVP/EFV) (138)	5.07±2.35[#]	1.27±0.67	3.44±2.30[#]	1.51(1.11-1.96)
Badiou (2003)	Cohort, Case control/ France	SBM	Serum	Males & females	HIV –ve (14)	5.19±0.16	1.61±0.08	3.18±0.13	0.88±0.13
					HIV+ve (141T/ 10 UT) , M0 (24)	4.49±0.27	0.97±0.07	2.60±0.23	2.01±0.21
					HIV+ve lopinavir/ritonavir, M1 (24),	5.53±0.25[$]	1.14±0.05	3.08±0.23	4.20±0.64[b]
Rose (2008)	Case control/ Australia	SBM	Plasma	Males	HIV-ve (33)	4.9±0.8	1.4±0.4	2.9±0.9	1.3±0.8
					HIV +ve, treatment naïve (11)	3.8±1.4*	0.8±0.3*	1.9±0.8*	1.6±0.6*
					HIV +ve, currently untreated (14)	4.7±1.0[#]	0.8±0.2*	2.8±1.0[#]	1.7±0.6*
					HIV +ve, untreated combined (25)	4.1±1.1*	0.8±0.2*	2.4±1.0	1.7±0.6*
					HIV +ve, on PI treatment (28)	4.5±1.2[#*]	0.8±0.2*	2.3±0.6[#]	2.3±0.9*
Tien (2010)	Case control/ America	SBM	Plasma	females	HIV –ve (361)	-	1.32(1.14-1.63)	2.46(1.92-3.13)	0.99(0.69-1.36)
					HIV +ve, HAART naive (128)	-	1.06(0.85-1.35)	2.56(2.10-3.21)	1.06(0.79-1.45)
					HIV +ve, on HAART(588)	-	1.22(0.98-1.50)	2.59(2.00-3.21)	1.33(0.93-1.88)
Riddler 2008	Case control/ America	SBM	Plasma	Males	HIV –ve(609)	5.12±0.94	1.30±0.33	3.11±0.87	1.65±1.53
					HIV +ve, HAART naive(77)	4.48±1.04	1.17±0.42	2.61±0.91	1.55±0.93
					HIV +ve, on HAART(396)	5.15±1.37[+]	1.16±0.34[+]	2.87±1.03[+]	2.76±3.20[+]
Oduola	Cohort, Case	SBM	Plasma	Males &	HIV –ve (10)	4.39±0.49	1.23±0.17	2.74±0.64	1.19±0.49

(2009)	control/ Nigeria			females	HIV –ve, M15 (10)	4.46±0.40	1.25±0.21	2.52±0.35	1.46±0.13
					HIV+ve, NRTI+EFV, M24 (changes)	0.85(0.26-1.47) [b]	-	-	0.21 [b]
Podzamc zer (2011)	Randomized Clinical trial/ Spain	SBM	Plasma	Males & females	HIV +ve, HAART naïve assigned NVP+TDF/FTC	4.03	1.00	2.37	1.48
					HIV+ve,NVP+TDF/FTC,M12(changes obtained)	0.63[++]	0.25[++]	0.39[++]	0.00[++]
					HIV+ve,HAART naïve assigned ATV/r+TDF/FTC	3.99	1.01	2.30	1.50
					HIV+ve,ATV/r+TDF/FTC,M12(change obtained	0.51	0.10	0.27	0.31
Hazra (2012)	Cross sectional, Case control/ Latin America & Jamaica	SBM	Plasma	Males & females - children <2years	HIV exposed uninfected (HEU) (681)	3.78±0.95	-	-	1.39±0.82
					HIV +ve (All combined) (83)	3.79±0.78	-	-	1.82±1.02*
					HIV +ve treatment naive (34)	3.14[+]	-	-	1.57[+]
					HIV +ve, PI containing ART (33)	4.36[+]	-	-	2.38[+]
					HIV +ve, Non-PI containing ART (16)	3.94[+]	-	-	1.21[+]
Brewins ki (2011)	Cross sectional/ Latin America & Jamaica	SBM	Plasma	Males & females children ≥2years	HIV+ve,PI containing ART (PI + NRTI) (311)	4.40(3.70-5.07) [+]	-	-	1.32(0.99-1.93) [+]
					HIV+ve,NNRTI containing ART (NNRTI + NRTI) (112)	3.88(3.21-4.40) [+]	-	-	0.84(0.62-1.17) [+]
					HIV+ve, PI + NNRTI containing ART(PI + NNRTI + NRTI) (54)	4.64(3.88-5.25) [+]	-	-	1.29(1.03-1.86) [+]
					HIV +ve,HAART naïve assigned NRTI +EFV (171)	[illegible]	[illegible]	[illegible]	[illegible]

SBM standard biochemical method, HAART highly active antiretroviral therapy, ART antiretroviral therapy, NNRTI non-nucleotide reverse transcriptase inhibitor, NRTI nucleotide reverse transcriptase inhibitor, 3TC lamivudine, d4T stavudine, AZT zidovudine, ATV/r atazanavir/ritonavir, NVP nevirapine, EFV efavirenz, IT interupted treatment, UT uninterupted treatment, M month, DC drug conservative, VS viral suppression, L/R lopinavir/ritonavir, HEU HIV exposed uninfected, PI protease inhibitor, *p<0.05 vs HIV ve, #p<0.05 vs HAART naïve, b p <0.05 vs baseline levels, ^p<0.05 vs untreated combined, +p<0.05 comparing 3 groups, a p<0.05 vs DC at M1, ++p<0.05 compared to changes in ATV/r.

Table 2: Lipoprotein Subclass distributions in Reviewed studies

Author (Year)	Study design/ Location	Lab method	Sample type	Study population (number)		Lipoprotein subclass levels					
						T- LDL-P (nmol/l)	L-LDL-p (nmol/l)	S-LDL-p (nmol/l)	T-HDL-p (µmol/l)	L-HDL-p (µmol/l)	S-HDL-p (µmol/l)
Feingold (1993)	Case control/ America	PAGGE	Plasma	Males	HIV -ve	-	-	-	-	-	-
					HIV +ve, aids, ARV naive	-	-	predominant	-	-	-
Riddler (2008)	Case control/ America	NMR	Plasma	Males	HIV –ve (609)	1379 (1113-1464)	352(213-490)	985 (642-1294)	34.3 (30.6-37.9)	-	-
	MAC study				HIV +ve, HAART naive (77)	1181 (969-1459)*	333(203-466)	886 (584-1174) *	30.5* (26.4-34.7)	-	-
					HIV +ve, on HAART (396)	1468 (1137-1816) *	264(130-469) *	1096 (757-1497) *	32.3* (28.1-37.5)	-	-
Baker (2010)	Case control/ America	NMR	Serum	Males & females	HIV –ve (29)	-	-	-	30.4 (25.5-33.1)	6.40 (4.20-8.80)	21.5 (17.1-24.4)
					HIV +ve, HAART naive (32)	-	-	-	23.9* (20.2-27.8)	3.80* (2.50-6.78)	18.4* (13.9-21.0)
Stein (2008) (A5152s)	Randomised clinical trial/ America	NMR	Plasma	Males & females	HIV +ve, HAART naive assigned NRTI +L/R (31	1128 (869-1486)	-	855 (587-1102)	21.8 (18.6-26.1)	3.2 (2.2-4.8)	-
					HIV +ve,NRTI + L/R,M6 (changes obtained)(31)	135 (-115-312) [b]	-	127 (-162-357)	5.1 [b] (1.6-9.7)	0.1 (-1.1-2.6)	-
					HIV +ve, HAART naive assigned EFV + L/R (28)	1158 (861-1252)	-	759 (575-960)	22.7 (19.1-26.9)	3.7 (2.1-5.6)	-
					HIV +ve, EFV+L/R, M6(changes obtained)(28)	414 (120-740) [b]	-	371 [b] (-9-720)	8.3 [b] (5.9-10.8)	1.3 [b] (-0.8-3.0)	-
					HIV+ve,HAART naïve assigned NRTI +EFV(23)	1089 (941-1257)	-	855 (584-1068)	23.3 (21.2-27.6)	2.5 (1.9-4.9)	-
					HIV+ve,NRTI+EFVz,M6(changes obtained)(23)	64 (-65-167)	-	101 (-162-207)	5.3 [b] (2.4-9.3)	1.1 (-0.5-2.5)	-
Tien (2010)	Case control/ America	NMR	Plasma	females	HIV –ve (361)		-				
					50th percentile	1010	-	574	30	-	19
					75th percentile	1341		824	35	-	24
					HIV +ve,HAARTnaïve(128)		-				
					50th percentile	1038		547	26	-	16
					75th percentile	1320		859	29	-	20

					HIV +ve, on HAART(588)						
					50th percentile	1119	-	607	29	-	18
					75th percentile	1465	-	1032	33	-	22
Lampe (2010)	Randomised IT SMART America	NMR	Serum & Plasma	Males & females	HIV+ve, (DC) , BL (log,particles) (154)	7.09±0.38	5.38±1.17	6.52±1.02	3.37±0.26	1.44±0.91	2.84±0.68
					HIV+ve, (DC), M1(log,particles) (154) (changes obtained)	-0.05±0.27	-0.07±1.22	0.02±0.76	-0.12±0.23	0.03±0.65	-0.10±0.61
					HIV+ve, (VS), BL (log,particles) (175)	7.15±0.39	5.29±1.2	6.55±1.38	3.38±0.27	1.42±1.06	2.86±0.56
					HIV+ve, (VS), M1 (log,particles) (175) Changes obtained	-0.03±0.25	0.04±1.24	0.06±0.86	-0.01±0.21 [a]	0.05±0.70	-0.04±0.42
Baker (2011)	Randomised IT SMART America	NMR	Serum & Plasma	Males & females	HIV+ve, (DC) , BL (126)	1106	-	-	25.9	4.9	17.7
					HIV+ve,(DC),M2 (changes obtained) (126)	-	-	-	0.0(-2.5 to 2.8)	0.1(-1.0 to1.1	-0.6(-3.5to2.8
					HIV+ve, (VS), BL (128)	1084	-	-	25.9	4.4	17.0
					HIV+ve, (VS), M2 (Changes obtained) (128)	-	-	-	1.8(-1.5 to 5.2) [a]	1.0(0.5to2.6 [a]	0.4(-3.0to3.6

T-LDL-p Total low density lipoprotein particle, L-LDL-p large low density lipoprotein-particle, S-LDL-p small low density lipoprotein-particle, T-HDL-p total high density lipoprotein-particle, L-HDL-p large high density lipoprotein-particle, S-HDL-p small high density lipoprotein-particle, M month, BL baseline, PAGGE polyacrylamide gradient gel electrophoresis, NMR nuclear magnetic resonance, , *p<0.05 vs HIV-ve, b p <0.05 vs baseline levels, a p<0.05 vs DC at M2.

Table 3: Effects of Switching ARVs on Lipoproteins and Lipoprotein subclasses

Author (Year)	Study design/ Location	Lab method	Sample type	Study population (number)	Switch from	Switch to	Outcomes	Notes
Valantin (2010)	Randomised Trial/(France)	SBM	Plasma	2 NRTI (not TDF or FTC) + PI or NNRTI Males & females (91)	2NRTI backbone (not TDF and FTC)	TDF + FTC Backbone 12 weeks	↓TC, ↓LDL-C, ↓TG, ↓HDL-C	Significant differences in all lipid parameters between groups. Slight and significant decrease of HDL-C in TDF +FTC group.
Saumoy (2011)	Randomised trial/Spain	SBM	Serum	2 NRTI(not ABC or TDF) + PI or NNRTI Males & females (62)	2 NRTI backbone	TDF +FTC → 48weeks *or* ABC + 3TC → 48weeks	↓TC,↓LDL-C, ↓apo B, ↓TG,↓HDL-C ↔apo A, ↓TC:HDL-C, ↑apo A: apo B ↑TC, ↑LDL-C, ↑HDL-C, ↑TG, apo B, ↑apo A, ↑apoA1:apo B, ↑TC:HDL-C	TC,LDL-C, apoB had significant declines and HDL-C, apo A1, TG had no significant declines Only apo A1 had significant increases.
Anunworanich (2008)	Randomised trial/ Thailand	SBM	Serum	ddI + d4T+ SQV/r Males & females (35)	d4T + ddI	TDF+3TC 24&48weeks	↓TC, ↓LDL-C, ↓TG, ↓HDL-C	Significant decrease in TG, TC, HDL-C by end of study.
Fisher (2009)	Randomised trial/(UK)	SBM	Serum	AZT + 3TC + EFV Males & females (234)	AZT + 3TC	TDF + FTC 48weeks	↓TC, ↓LDL-C, ↓TG, ↓HDL-C	Greater declines in Tenofovir group at week 24. Significant difference in TC and TG between FTC/TDF and AZT/3TC.
Moyle (2006)	Randomised trial/(UK)	SBM	Serum	tNRTI containing HAART Males & females (105)	d4T / AZT	TDF48weeks→ or ABC48weeks→	↓TC, ↓LDL-C, ↓TG, ↓HDL-C ↑TC, ↑LDL-C, ↑HDL-C, ↑TG	Significant differences in TC and LDL-C between TDF and ABC groups.
Murphy (2010)	Randomised trial/America	SBM & NMR	Plasma	LPV/r ; ABC-based ~6years Males & females (50)	LPV/R ; ABC	ATV/r for 24weeks	↓TC, ↓LDL-C, ↓TG, ↓TC:HDL-C, ↓VLDL-p, ↓LDL-p, ↓small LDL-p	significant decrease in TC,TG,VLDL-p, LDL-p, small LDL-p in switched group
Sension (2009)	Randomised trial/America, Australia,Europe	SBM	Plasma	PI based ≥ 3months Males & females (246)	PI	ATV 24weeks Immediate vs delayed	↓TG, ↓apo B, ↓LDL-C, ↓non-HDL-C, ↓TC	Significant greater reductions in TC,TG, apo B,non-HDL-C occurred in immediate group than delayed Tenofovir not used, unboosted ATV used.

Soriano (2008)	Randomised trial/ Spain	SBM	Plasma	LPV/r based HAART ≥3months Males & females (189)	LPV/r	ATV or ATV/r vs no switch 48weeks	↓TC, ↓TG	Significant decrease in atazanavir than no switch group. Greater decrease in ATV compared to ATV/r group.
Parienti (2007)	Randomised trial/France	SBM	Serum	EFV + NRTI (d4T, ABC, TDF) ≥ 41 months. Males & females(37)	EFV	NVP vs no switch 52weeks	↓LDL-C	Significant decrease in LDL-C in NVP arm but not EFV arm. ABC & TDF based regimen associated with LDL-C.
Calza (2005)	Randomised trial/ Italy	SBM	Plasma	PI + 2NRTI ≥ 12months Males & females (130)	PI	NVP or EFV vs lipid lowering agents	↓TC, ↓LDL-C, ↓TG, ↑HDL-C	Significant reductions in TG, TC, LDL-C with NVP than EFV. NVP had greater HDL-C increase than EFV. Lipid lowering agents had significant favourable lipid changes than ARV switching.

HAART highly active antiretroviral therapy, PI protease inhibitor, NRTI nucleoside reverse transcriptase inhibitor, NNRTI non-nucleoside reverse transcriptase inhibitor, ATV/r atazanavir/ritonavir, LPV/r lopinavir/ritonavir, NVP nevirapine, EFV efavirenz, d4T Stavudine, ddI didanosine, SQV/r Saquinavir/ritonavir, TDF tenofovir, FTC emtricitabine, AZT zidovudine, 3TC lamivudine, ABC abacavir , TC total cholesterol, LDL-C low density lipoprotein cholesterol, HDL-C high density lipoprotein cholesterol, TG triglycerides, apo A apolipoprotein A, apo B apolipoprotein B, SBM standard biochemical method, NMR nuclear magnetic resonance.

Changes in Lipoproteins in HAART naive HIV- infected people

Case control studies conducted in America (Feingold et al., 1993; Riddler et al., 2008) and Australia (Rose et al., 2008) involving adult male participants reported significant decline in TC, LDL-C, HDL-C ($p<0.05$) in HAART naïve HIV- infected compared to seronegative participants. Higher TG levels ($p<0.05$) were obtained (Feingold et al., 1993; Rose et al., 2008) in HIV-infected HAART naïve compared to seronegative males while non-significant difference in TG levels were obtained elsewhere (Riddler et al., 2008) between HIV-infected HAART naïve and seronegative males. The discord in levels of TG levels in studies Rose et al. (2008) and Riddler et al. (2008) could be a result of differences in stage of HIV infection, since TG levels increase as the HIV infection progresses to AIDS and CD4 count decreases (Feingold et al., 1993).

Two case control studies involving combined adult males and females, conducted in South Africa and America reported significant decline ($p<0.05$) in TC, LDL-C, HDL-C (Fourie, Van Rooyen, Kruger & Schutte, 2010) and non-significant difference ($p>0.05$) in TC, LDL-C coupled with a significant difference in HDL-C levels ($p<0.01$) (Baker et al., 2010), between HAART naïve HIV-infected and seronegative participants. Significantly high TG levels ($p<0.05$) were obtained by Fourie et al. (2010) while non-significant TG levels ($p>0.05$) were obtained by Baker et al. (2010) between HAART naïve HIV-infected and seronegative participants.

In another case control study from America involving female participants (Women's Interagency HIV Study), a higher LDL-C level 2.56mmo/l (non-significant) was reported for 128 HIV-infected HAART naïve participants compared to 2.46mmol/l for uninfected participants. The higher LDL-C levels in treatment naïve HIV-infected compared to uninfected females could have been confounded by the fact that 96% of the HIV-infected females had taken treatment at some stage, and hence the observed difference could be a result of the trailing effect of treatment. The TG levels were higher, while HDL-C levels were lower in the HAART naïve HIV-infected than seronegative females; unfortunately, level of significance was not indicated (Tien et al., 2010). The discordances in TG levels may be related to HIV disease progression as CD4 count seems inversely related to TG levels (Armstrong et al., 2011).

In a cohort study conducted in Nigeria, involving combined males and females, HDL-C and TC levels were significantly lower ($p<0.05$) in HAART naïve HIV-infected than seronegative people while TG and LDL-C levels were not significantly different ($p>0.05$) between the two groups, for the greater part of the study period (Oduola et al., 2009). The above studies from both Western and African countries have shown that lipoproteins are decreased in treatment naïve

HIV-infected people, however variations in lipoprotein levels are a result of differences in disease stage of participants from different studies. Additionally lipoproteins are decreased in both treatment naïve HIV-infected men and women.

Changes in Lipoproteins among patients on HAART

Traditionally, HAART combines two nucleoside reverse transcriptase inhibitors (NRTIs) with either NNRTI or PI (Minnaar & van der Merwe, 2008). The degree of dyslipidaemia and specific lipid changes differs among the different classes of antiretroviral drugs and even among the individual drugs within each class (Feeney & Mallon, 2011).

Two case control studies, one from Australia (Rose et al., 2008) with male participants on PI-based regimen and not taking lipid lowering drugs and another from America (Riddler et al., 2008) with male participants, 48% on PI-based, 44% on NNRTI-based (without PI) and 8% on triple NRTI regimen, all using lipid lowering drugs, found no significant difference ($p>0.05$) in TC and LDL-C levels, but significantly higher TG ($p<0.05$) and lower HDL-C levels ($p<0.05$) in treated groups than the HIV negative groups. The non-significant difference in TC and LDL-C levels (Riddler et al., 2008) was attributed to the lipid lowering drugs.

In Women's Interagency study, participants on HAART had higher TG and LDL-C and lower HDL-C levels compared to HIV negative women. The drugs and levels of significance were however not reported (Tien et al., 2010).

A case control study conducted in Cameroon, involving 138 HIV infected male and female participants on nevirapine (NVP) or efavirenz (EFV) combined with stavudine/lamivudine (d4T/3TC) or zidovudine/lamivudine (AZT/3TC) and 138 treatment naïve HIV infected male and female participants, found significantly higher TC and LDL-C levels ($p<0.02$) and no significant difference in HDL-C and TG levels between treatment naïve and those on treatment. There was no significant difference in prevalence of lipid abnormalities in patients on regimen that included d4T compared to AZT. Similar prevalence of lipid abnormalities was also found between NVP and EFV regimens (Yone et al., 2011). This study provides evidence that HDL-C is not significantly improved by HAART treatment and TG levels increase in the presence or absence of HAART treatment.

In a nested case control study conducted in America, involving males and females with (248) and without (480) CVD, previously randomised to intermittent treatment in the SMART study, no differences in TC, LDL-C, TG

were observed between CVD case and no CVD cases. However, lower HDL-C ($p=0.03$) was observed in CVD cases compared to controls (Duprez et al., 2009). This study shows low HDL-C as an important risk factor for the occurance of CVD.

One cohort case control study from France comprising male and female participants were grouped into three as HIV negative(14), interupted treatment (IT) (14) and uninterupted treatment (UT) (10) and followed up for 3 months. Two groups, IT and UT were combined due to small numbers and the 24 cases were treated with lopinavir/ritonavir (LPV/r)-containing regimen. At baseline, there was no significant difference in lipids between IT and UT groups. Significantly higher TG levels ($p<0.001$) and lower HDL-C ($p<0.001$) were observed in IT and UT compared to controls. The TC and TG levels from combined group increased ($p<0.05$) in 1 month. Between 1 and 3 months no statistical difference was observed except for increases in LDL-C ($p<0.05$), with a trend to further increase TC levels ($p=0.057$). There was no difference between IT and UT groups at 1 month and 3 months. At 3 months TC levels in IT group was similar to control whilst TC in UT became higher ($p<0.05$) than control. HDL-C levels remained lower in IT ($p<0.01$) and UT ($p<0.05$) than controls (Badiou et al., 2003). This study adds on the evidence that TG levels remains high while HDL-C remains low in absence of treatment or when treatment is interupted.

A Nigerian cohort study of 36 participants, (10 HIV negative, 10 HIV positive ARV naïve and 16 HIV positive on ARV) found no significant difference ($p>0.05$) in TC, HDL-C, LDL-C and TG throughout 15 months between treated group and HIV negative group, except during the 3rd month when TC and LDL-C were significantly lower in treated group (Oduola et al., 2009). These findings are contrary to the findings from another cohort study (Badiou et al., 2003) where significantly higher TC, LDL-C and TG were observed in people on ARV treatment compared to HIV negative people. Unfortunately the ARVs used in the study Oduola et al. (2009) were not specified.

Among three randomised clinical trials, that reported on lipoproteins in HAART treated males and females, two were conducted in America (Stein et al., 2008; Haubrich, Riddler, Di Rienzo, Kamarow, Powderly, Klingman, Garren, Butcher, Rooney, Haas, Mellows & Havlir, 2009) and one (Atazanavir/Ritonavir on a background of Tenofovir and Emtricitabine versus Nevirapine: ARTEN Study) conducted in Spain (Podzamczer, Andrade-Villanueva, Clotet, Tayler, Rockstroh, Reiss, Domingo, Gellermann, de Rossi, Cairns & Soriano, 2011). The HAART naïve HIV positive participants from A5142 study by Haubrich et al. (2009) were randomised equally to receive LPV/r plus EFV, two NRTI plus either LPV/r or EFV, and were followed up for 24 months, while treatment

naïve participants from A5152 study by Stein et al. (2008) were randomised to similar regimen but followed for 6 months. Baseline lipids TC, HDL-C, LDL-C, TG from A5152 study were similar in each of the 3 arms (p_{kw} >0.50), however all except TG levels were lower than normal ranges. After 6 months significant differences in TC and direct LDL-C levels (p<0.001), HDL-C level (p=0.053) and TG levels (0=0.051) were observed between arms. Highest increases were observed in the EFV + LPV/r regimen. Similar trends were reported after 24 months from A5142 study. The median increases in TC, HDL-C, and TG levels were significantly higher (p<0.05) in the EFV+LPV/r arm compared to NRTI+LPV/r arm or NRTI + EFV arm, however there was no significant difference in TC, HDL-C between NRTI+LPV/r arm and NRTI+ EFV arm, but TG levels were significantly higher in NRTI+LPV/r arm (Haubrich et al., 2009). The ARTEN study, followed treatment naive HIV positive participants for up to 12 months, after randomly assigning participants to NVP + TDF/FTC or ATV/r+ TDF/FTC. After 24 months, higher increases in TC (p=0.038), HDL-C (p<0.0001) and LDL-C (p=0.011) from baseline were observed NVP-group compared to ATZ/r group, while higher increases in TG levels (p=0.0001) from baseline were observed in ATZ/r group than NVP-group. The study outcome suggested a more favourable lipid profile with NVP than with ATZ/r when combined with TDF/FTC (Podzamcer et al., 2011). The findings of these three clinical trials show that NNRTI (NVP or EFV) on NRTI backbone is associated with a less atherogenic lipid profile.

In a randomised interruption trial (SMART study) involving 332 HIV infected male and female participants, of whom 174 participants were assigned to viral suppression (VS) group, treated with either PI-based (39.5%), NNRTI-based (43.4%), both classes (6.9%) and neither of these classes (10.2%) for 1 month and 156 participants assigned to drug conservative (DC) interrupted group which was repeatedly taken off treatment when CD4 count was >350 cells/mm^3 and retreated when CD4 count was < 250cells/mm^3, found significant declines in TC (p<0.001), LDL-C (p=0.011), HDL-C (p<0.001) and TG (p<0.001) in DC compared with VS group after 1 month. The lipid changes in DC group did not differ according to baseline ART class; however, changes in TC and LDL-C levels in DC versus VS groups were much greater in subjects taking lipid lowering drugs at baseline compared to those not taking these drugs (Lampe et al., 2010). In this study, the interruption of HAART resulted in replication of virus and decrease in CD4 count with resultant significant decrease in lipoproteins. The combined effect of HIV infection and lipid lowering drugs in DC group could have contributed to the much greater decreases in TC and LDL-C in those taking lipid lowering drugs at baseline compared to those not taking these drugs in the DC group.

In a cross-sectional study from Latin America, involving 477 HIV infected children (boys and girls ≥2years) being treated with PI+NRTI (311), NNRTI+NRTI (112) and PI+NNRTI+NRTI (54) regimens, the TC levels were different among the 3 regimens ($p<0.0001$) with the highest levels obtained in PI+NNRTI regimen, the TG levels were different among the 3 regimens ($p<0.0001$) with the highest levels obtained in PI+NRTI regimen. Children on PI+NRTI and PI+NNRTI+NRTI regimens were at increased risk for high cholesterol compared to children on NNRTI+NRTI regimen, adjusted odds ratios 2.7(95% CI: 1.3-5.6) and 2.7% (95% CI: 1.1-7.0) respectively, while children on PI-containing regimen were 3.5 times more likely to have high TG levels than children on NNRTI-containing regimen, adjusted odds ratio 3.5 (95% CI: 1.9-6.4) (Brewinski et al., 2011). Hazra et al. (2012) in another Latin America cross-sectional, case control study of 83 HIV-infected children (boys and girls <2years) on PI-containing ART (33), non PI-containing ART (16), treatment naive (34) and HIV-1-uninfected (681), reported no difference in mean TC levels between HIV-infected and HIV uninfected children ($p=0.89$), but TG levels were higher in HIV-infected than uninfected children ($p=0.0003$). TC levels were different by antiretroviral regimen ($p<0.0001$), with treatment naïve HIV-infected children having significantly lower mean TC level compared to the other groups. TG levels were different by ARV regimen ($p<0.0001$) with those receiving a PI-containing ART having significantly higher mean TG levels than non-PI-containing ART, treatment naïve HIV-infected and HIV uninfected groups. These lipid changes observed in children <2years receiving ARVs are similar to changes that have been reported in children ≥2years receiving similar ARV by Brewinski et al. (2011) (Hazra et al., 2012). Studies conducted in children also showed NNRTI+NRTI regimen as one that gives favourable lipid profiles.

Hyperlipidaemia management strategy involving switching of ARV class or switching of ARV drugs within the same class have shown improvement in lipoprotein levels. The effect of tenofovir (TDF) containing regimen indicates a lipid lowering action of this NRTI which is different from other ARV drugs in the same class. In randomised trials conducted in France (Valantin et al., 2010) and Spain (Saumoy et al., 2011) involving adult males and females who were switched from 2NRTI (not TDF or emtricitabine (FTC) or abacavir (ABC)) + PI /NNRTI to TDF+FTC+PI /NNRTI regimen in the France study and TDF+ FTC+PI /NNRTI or ABC+lamivudine (3TC)+PI/NNRTI regimen in the Spain study for at least 12 weeks, there were declines in levels of TC ($p<0.001$), LDL-C ($p=0.031$), TG ($p=0.034$) and HDL-C ($p=0.009$) compared to the control group (unswitched) in the France study, while in the Spain study a switch to TDF+FTC+PI/NNRTI had declined in levels of TC ($p=0.002$) and LDL-C ($p=0.040$) and no changes in HDL-C and TG levels compared to baseline levels. In contrast, switching to ABC + 3TC+PI/NNRTI regimen in the Spain study

resulted in no significant changes in levels of TC, LDL-C, HDL-C and TG compared to baseline levels. There were differences between the changes from baseline of TDF+FTC group and ABC + 3TC group in the Spain study in TC (p=0.003), HDL-C (p=0.031), TG (p=0.036) and LDL-C (p=0.061) (Saumoy, Ordonez-Llanos, Martinez, Barragan, Ribera, Bonet, Knobel, Negredo, Lonca, Curran, Gatell & Podzamczer, 2011). The use of the NRTIs abacavir and didanosine was found to be an independent risk factor for myocardial infarctions in the Data collection on adverse events of Anti-HIV Drugs (DAD) study (Sabin et al., 2008).

A randomised trial conducted in the United Kingdom (UK) involving adult males and females, switched from AZT+3TC+EFV to TDF+FTC+EFV regimen, and retaining a control group on AZT+3TC+EFV regimen, reported differences of the changes in TC (p=0.008) and TG (0.0008) between the two groups over 24 weeks; however, lipid changes up to week 48 were not significant (Fisher et al., 2009). A similar study in the UK involving males and females, stopped participants from taking AZT/d4T-containing HAART and switched them to either TDF or ABC containing HAART over 48 weeks, and observed changes in TC (p=0.003) and LDL-C (p=0.04) that significantly favoured TDF relative to ABC (Moyle et al., 2006). Ananworanich et al. (2008) in a randomised trial involving adult males and females, switched from d4T/didanosine (ddl) to TDF/3TC while receiving saquinavir/ritonavir (SQV/r) obtained differences in TC (p<0.0001), HDL-C (p<0.0001), LDL-C (p=0.0004) and TG (p=0.014) over 24 weeks, and remained significantly different from controls for up to 48 weeks except for LDL-C (p=0.13). In the NRTI class, switching to TDF is associated with favourable improvement in lipoproteins.

Randomised trials involving adult males and females, conducted in United States of America (USA) showed that switching from PI-regimen, especially LPV/r to atazanavir (ATV) for up to 24 weeks resulted in significant decline in TC, non HDL-C and TG levels (p<0.0001) and no significant difference in HDL-C levels compared to controls groups (unswitched group) (Sension et al., 2009; Murphy et al., 2010) while a randomised trial in Spain involving males and females switched from LPV/r to ATV or atazanavir/ritonavir (ATV/r) regimen observed significant decline in TC, TG levels (p<0.001) and no significant difference in HDL-C and LDL-C levels compared to controls(unswitched group). Greater decreases were obtained with ATV compared to ATV/r group (Soriano et al., 2008). These trials showed that the newer PI drugs are associated with less lipoprotein abnormalities.

In a randomised trial conducted in Italy the effect of two lipid lowering strategies (ARV switching and lipid lowering agents) on lipid levels were compared. In one group, males and females were treated with lipid lowering

agents, pravastatin (36) or bezafibrate (31) whilst remaining on PI-regimen for at least 48 weeks, while the other group was switched from a PI-regimen to NNRTI-regimen, EFV (34) or NVP (29) for at least 48 weeks. Significant reductions in levels of TG ($p<0.05$), TC ($p<0.05$), LDL-C ($p<0.05$) and increases in HDL-C levels with NVP than EFV regimen were observed. However, lipid lowering agents had significant declines in TG ($p<0.01$), TC ($p<0.01$) and LDL-C ($p<0.01$) than ARV switching. The percentage of participants who reached normal TG and TC levels was higher in pravastatin and bezafibrate treated group than in NVP and EFV treated group ($p<0.01$) (Calza et al., 2009). This study provided evidence that the lipid lowering drugs remain superior in lowering the lipoprotein levels. However, caution should be taken when choosing the lipid lowering drugs to avoid drug-drug interactions with ARV.

In a 52 week randomised trial, in which 18 males and females were switched from EFV+ (d4T/ABC/TDF) to NVP + (d4T/ABC/TDF) regimen while 19 males and females continued taking EFV+ (d4T/ABC/TDF), significant decrease in LDL-C levels ($p<0.05$) from baseline were reported in the NVP group but not in the EFV group, while TC, HDL-C and TG levels between the two groups were not significantly different (Parienti, Massari, Rey, Poubeau & Verdon, 2007). There is incremental evidence showing ARV switching as a beneficial strategy to reduce lipid levels to a lesser atherogenic lipid profile and defer premature atherosclerosis. However, some of the drugs providing favourable lipid levels may not be affordable for people in resource limited setting (RLS) where minimum costs of HAART are $113 per person-year for TDF/3TC+EFV, $119 per person-year for TDF/3TC+NVP and $122 per person-year for TDF/3TC+NVP (Médicins Sans Frontiéres, 2012).

Changes in Lipoprotein subclasses in HAART naive and HAART treated HIV infected people

Studies on lipoprotein subclass distribution in HIV infected people (both naïve to HAART and HAART treated) have largely been conducted in Western countries. The distribution of lipoprotein subclasses in males with AIDS from pre-HAART era show an increased prevalence of the LDL phenotype B which is characterised by increased TG and predominance of small dense LDL-p (Feingold et al., 1993). In the Multicenter AIDS Cohort Study (MACS), treatment naïve HIV-1-infected males had significantly lower small LDL-p and total HDL-p with non-significantly lower levels of large LDL-p compared with seronegative men (Riddler et al., 2008). In the Women's Interagency Study, the distribution of lipoprotein subclasses in HIV infected females varied at different percentiles. Throughout the 25^{th}, 50^{th}, and 75^{th} percentiles the total HDL-p and small HDL-p tended to be lower in the HIV infected HAART naïve compared to

the HIV-1 seronegative participants. The total LDL-p and small LDL-p concentration was similar in HIV HAART naïve when compared to seronegative participants throughout the 25^{th}, 50^{th}, 75^{th} and 90^{th} percentiles (Tien et al., 2010). In a mixed gender population, Baker et al. (2010) obtained lower total HDL-p ($p<0.01$); primarily the large HDL-p ($p<0.01$) and small HDL-p ($p=0.01$) in HIV infected than uninfected group. The findings from Riddler et al. (2008), Baker et al. (2010) and Tien et al. (2010) show that total, large and small HDL-particles decrease in treatment naïve HIV infected people. However LDL-particle findings from these studies were not consistent. The non-significant difference in LDL-p (Tien et al., 2010) between treatment naïve HIV infected and seronegative could be a reflection of the trailing effect of ARV, as 96% of participants had taken treatment at some stage.

The lipid level adjustments with HAART may not necessarily reflect on a favourable underlying subclass distribution. Most HAART regimens alter the lipoprotein subclass distribution and the determination of these subclass changes will provide accurate information regarding the risk for atherosclerosis posed by various ARV combinations. Lipoprotein subclass pattern of HIV infected men on HAART, in the MACS study was consistent with the atherogenic lipoprotein profile (ALP) with significantly higher median particle concentration of small dense LDL-P and total Very Low Density Lipoprotein-particle (VLDL-p) and lower numbers of large LDL-p and total HDL-p compared to HIV-1 seronegative. This ALP pattern was more pronounced in HAART treated men with a good clinical status (plasma HIV-1 ribonucleic acid (RNA) <50 copies/ml and CD4 count >350cells/mm^3), while men on HAART with poor clinical status had the lowest levels of all lipoprotein particles, small and large LDL-p and HDL-p a pattern similar to that of HAART naïve group in same study. PI (ritonavir or non ritonavir)-containing regimens were associated with significantly higher total VLDL-p, lower large LDL-p and non-significant increases in small dense LDL-p when compared to NNRTI-based regimen. NRTI regimen had lower large LDL-p and tended to have higher small dense LDL-p and lower total HDL-p compared to NNRTI. NNRTI based regimen were found to have more modest effect on particles. Both ritonavir and non ritonavir-containing regimen were associated with significantly higher total VLDL-p and lower large LDL-p and a trend to higher small dense LDL-p and lower HDL-p when compared to HIV seronegative men (Riddler et al., 2008). This study provides evidence that despite an increased CD4 count (good clinical status) an adverse pattern comprising of decreased HDL-p and increased small dense LDL-p is present in HIV infected people on HAART especially PI-based. This pattern may predispose to premature CVD.

In the Women Interagency study, the total HDL-p and small HDL-p tended to be lower in HAART treated group than HIV seronegative group throughout the

25th, 50th, and 75th percentiles, while total and small LDL-p tended to be higher in HAART treated than seronegative group throughout 50th, 75th, 90th, and 95th percentiles. Although HAART is associated with greater small dense LDL-p, the association was attenuated after the adjustment of TG and HDL-C, while HIV infection remained associated with lower small dense HDL-p after adjustment of TG and LDL-C levels. The study suggested that small dense LDL-p concentration confers little additional information regarding CVD risk beyond that of standard lipid in HIV infected women (Tien et al., 2010). Although though the lipoprotein patterns obtained in female population may not be generalised to male population, they are consistent with findings of the MACS study.

The A5152s study whose treatment naïve HIV-1-infected participants were on HAART for 24 weeks, had increased total VLDL ($p<0.001$), large VLDL-p ($p<0.001$), total LDL-p ($p<0.001$), small LDL-p ($p<0.001$), total HDL-p ($p<0.01$), and large HDL-p ($p<0.01$) from baseline after 24 weeks. Significant increases in total VLDL-p and large VLDL-p were observed in NRTI+lopinavir/ritonavir and EFV+lopinavir/r ($p<0.01$) but not NRTI+EFV group. The increase in total VLDL-p was greater in the EFV+lopinavir/r group than NRTI+EFV group ($p=0.008$) and tended to be greater than in the NRTI+ lopinavir/ritonavir group ($p=0.085$). Significant increases in total LDL-p were observed in the NRTI+lopinavir/ritonavir group ($p<0.05$), but were not different to NRTI+EFV ($p=0.316$). EFV+lopinavir/ritonavir group had large increases in total LDL-p ($p<0.01$), which were greater than those in the NRTI+EFV ($p=0.01$) and in the NRTI+ lopinavir/ritonavir ($p<0.013$). EFV+ lopinavir/ritonavir also had significant increases in small LDL-p ($p<0.01$) and these increases were greater than in the NRTI+EFV ($p<0.014$) and tended to be greater than in the NRTI+lopinavir/ritonavir ($p<0.071$). Changes in small LDL-p between NRTI+lopinavir/ritonavir and NRTI+EFV groups were not statistically significant. Total HDL-p increases in EFV+lopinavir/ritonavir group was statistically significant than in NRTI+EFV group ($p=0.036$) and tended to be higher than NRTI+lopinavir/ritonavir ($p=0.056$). Differences in large HDL-p between arms were not significant (Stein et al., 2008). The study findings show NRTI+NNRTI as a regimen associated with less atherogenic particle profile.

The SMART interruption studies involved two groups of participants the virally suppressed (VS) group in which treatment was continued and the drug conservative group (DC) in which treatment was discontinued when CD4 count was >350 cells/µl and retreated when CD4 count was <250 cells/µl. The SMART interruption study, in which participants were followed up for 1 month, reported significant declines in total, large, medium VLDL-p ($p<0.01$), and total and medium HDL-p ($p<0.01$) in the DC compared to VS group. There were no significant differences between DC and VS in total LDL-p, large LDL-p, small LDL-p, small HDL-p, and large HDL-p (Lampe et al., 2010). In a separate

SMART interruption study conducted for 2 months and 6 months, the HDL-p and apolipoprotein A1 levels increased among VS after starting ART. There was significant difference in the changes in total HDL-p ($p=0.001$) at 2 months, ($p<0.001$) at 6 months and large HDL-p ($p=0.03$) at 2 months, ($p =<0.001$) at 6 months between the DC and VS groups. Changes in small HDL-p were not significantly different between DC and VS at 2 months and 6 months. The LDL-p and apolipoprotein B levels did not differ significantly between VS and DC groups, and this was attributed to a high proportion of participants in SMART study who used an NNRTI-based regimen which is slow in raising these particles (Baker et al., 2011).

Switching from lopinavir/ritonavir to atazanavir/ritonavir in the Switch to Atazanavir and Brachial Artery Reactivity (SABAR) study, resulted in significant decreases in VLDL-p ($p=0.005$), LDL-p ($p<0.001$) and small LDL-p ($p<0.01$) when compared to baseline values. There were no significant changes in the control group throughout the 24 weeks in VLDL-p ($p=0.769$), LDL-p ($p<0.407$) and small LDL-p ($p=0.478$) when compared to baseline values. Between the atazanavir/ritonavir and control group (remained on lopinavir/ritonavir) differences in lipoprotein subclass changes were not significant (Murphy et al., 2010).

The distribution of lipoprotein subclasses in HIV infected people who suffered CVD were determined and compared to the distribution of HIV infected without CVD in the SMART study. There were no differences in total LDL-p, small LDL-p and total VLDL-p between CVD cases and no CVD cases. However, differences were observed in total HDL-p ($p<0.0001$), large HDL-p ($p<0.006$) and small HDL-p ($p<0.01$) between CVD cases compared to the controls. The reduced HDL-p in CVD cases could have contributed to the reduced cholesterol transportation back to the liver, hence more cholesterol retention in the vessels increasing the probability of plaque formation. This evidence suggests low HDL-p, as a more important risk factor for CVD in HIV infected people than traditional LDL-p in particular small dense LDL-p (Duprez et al., 2009; Baker et al., 2010).

Discussion

The early findings from pre-ARV era on lipid distribution pattern (Feingold et al., 1993) and subsequent findings reported in post ARV era (Riddler et al., 2008, Rose et al., 2008, Fourie et al., 2010) indicated dyslipidaemia characterised by decreases in TC, LDL-C and HDL-C, with an increase in TG levels in treatment naïve HIV infected people as compared to the seronegative people. As uncontrolled HIV infection progresses to AIDS, the TC, LDL-C and HDL-C levels continue to decline while TG continues to increase. High levels of

TG increase myocardial infarction (MI) risk in people with HIV infection and are regarded as independent risk factor for MI even after adjustment for other CVD risk factors (Worm et al., 2011). Studies have shown that lipoprotein subclasses, small LDL-p, large LDL-p, small HDL-p and large HDL-p levels decrease in treatment naïve HIV infected compared to uninfected people (Stein et al., 2008, Baker et al., 2010).

Alterations in lipoproteins in treated HIV infected people varies with the ARV combinations used. Unlike the early PI drugs (Lopinavir, Indinavir), newer PI drugs (Atazanavir, Duranavir) have less dyslipidaemic effects (Sension et al., 2009, Murphy et al., 2010). NNRTI drugs have even lesser dyslipidaemic effects compared to the PI drugs and are therefore considered safer than the PI drugs. However, HAART in general has been associated with increase in LDL-p, particularly small dense LDL-p and increases in HDL-p, though not to pre-infection levels (Stein et al., 2008). Put together ARV dyslipidaemia, particularly PI induced dyslipidaemia, may resemble the Atherogenic Lipoprotein Phenotype B, which is associated with increased risk of atherosclerosis and CVD. The down regulation and up regulation of lipoproteins is a result of a combination of mechanisms (Figure 2). The HIV virus via its accessory protein Nef, impairs the Adenosine Triphosphate (ATP)-binding cassette transporter subfamily A, member 1 (ABC A1) system resulting in decreased cholesterol efflux from macrophages and decreased HDL-C (Mujawar et al., 2006). Inflammation process triggers release of cytokines tumor necrosis factor (TNF) α and interleukin-6 (IL-6). TNF-α attenuates the ability of insulin to suppress lipolysis from fat cells and thus interferes with free fatty acid metabolism promoting hypertriglyceridemia (Oh & Hegele, 2007). IL-6 decreases lipoprotein lipase activity and increases phospholipase A2 and endothelial lipase activity resulting in the accumulation of TG-rich lipoproteins and an increase in catabolism of HDL (Rader, 2006). Protease inhibitor containing regimens are associated with abnormal accumulation of intramyocellular fat, leading to insulin resistance, which increases plasma apolipoprotein-B containing and TG-rich lipoprotein.

Furthermore, PI regimen impairs hydrolysis of triglyceride-rich lipoproteins by plasma and tissue lipases, resulting in high levels of TG (Purnell, Zambon, Knopp, Pizzuti, Achari, Leonard, Locke & Brunzell, 2000; Torriani, Thomas, Barlow, Librizzi, Dolan & Grinspoon, 2006). Structural homology between LDL-receptor related protein type 1 (LRP 1) and HIV protease may promote binding of PI to LRP 1. LRP 1 normally binds to lipoprotein lipase on capillary endothelium, which hydrolyses free fatty acids (FFA) from triglycerides promoting their accumulation in adipocytes. Thus PI binding to LRP1 would interfere with LRP1-lipoprotein lipase complex formation, reducing adipose storage capacity and increasing plasma triglyceride-rich lipoproteins (Carr,

Samaras, Chisholm & Cooper, 1998). Evidence from Calza et al. (2009) showed that the lipid lowering agents (pravastatin and bezafibrate) are more effective in improving the dyslipidaemia induced by ARV rather than ARV switching. Thus pravastatin with minimal interactions with ARV drugs could be a preferred statin in HIV infected people.

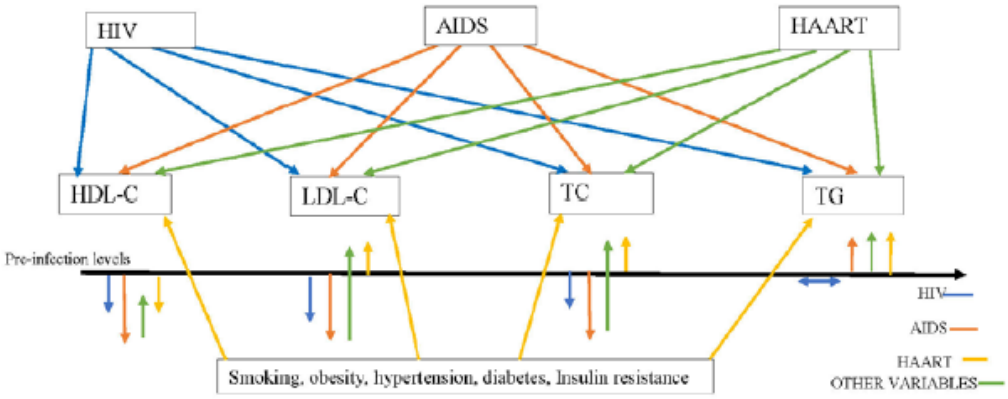

Figure 2: Schematic diagram representing the down regulation and up regulation of lipoproteins by HIV, AIDS, HAART intervention and other variables that may be present during HIV infection and its treatment. HIV Nef protein impairs ABC A1 system resulting in decreased HDL-C (A). Chronic inflammation (A, B,C) triggers release of TNF-α which attenuates insulin ability to suppress lipolysis resulting in hypertriglyceridemia and release of IL-6 which decreases lipoprotein lipase activity and increases phospholipase A2 and endothelial lipase activity resulting in the accumulation of TG-rich lipoproteins and an increase in catabolism of HDL. PI regimen impairs hydrolysis of triglyceride-rich lipoproteins by plasma and tissue lipases, resulting in high levels of TG (C). The other variables cause dyslipidaemia through the mediation of adiponectin (D).

The contribution of other metabolic conditions such as diabetes, insulin resistance and metabolic syndrome that normally cause similar lipoprotein abnormalities to HAART should be born in mind when managing dyslipidaemia in HIV infected people. In addition to lipid lowering drugs, dietary intake and physical activity remain a priority.

Conclusions and implications for future research

HIV infection decreases lipoprotein in treatment naïve HIV-infected people while the HAART increases lipoprotein levels to pre-infection levels and beyond (except HDL-C) depending on class of ARV. It is the view of the authors that the combined effect of HIV infection and HAART lowers HDL-p and raises LDL-p and this substantially increases the risk of CVD in HIV-infected people. Most studies on lipoprotein subclass distribution in persons with HIV infection were done in Western countries. While these studies could have been conducted in some areas of low socio-economic status similar to developing countries, differences in ethnic groups does exist. Additionally the effect of HAART on lipoprotein subclasses could vary in different African ethnic groups, where there is 50% polymorphism in the Apo C3 gene responsible for metabolism and clearance of triglyceride-rich lipoprotein particles and CYP 3 gene for metabolism of drugs (Clarke & Mousa, 2009). Differences in lipoprotein subclass profiles between the general African and Caucasian populations have been documented (Miljkovic-Gacic, Bunker, Ferrell, Kammerer, Evans, Patrick & Kuller, 2006). Therefore, there is a need to analyse lipoprotein subclasses in HIV-infected people (whether on HAART or not), especially in resource poor settings and in persons of different ethnic origin (Clarke & Mousa, 2009).

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Appendix XI: Cardiovascular risk factors in a treatment naïve Human immunodeficiency virus infected rural population in Dikgale, South Africa.

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ORIGINAL RESEARCH

Cardiovascular risk factors in a treatment-naïve, human immunodeficiency virus-infected rural population in Dikgale, South Africa

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Objective: The objective was to determine serum lipid, lipoprotein levels and cardiovascular risk factors in treatment-naïve human immunodeficiency virus (HIV)-infected rural African people in Limpopo province.
Design: This was a case control study.
Setting and subjects: The setting was Dikgale Health and Demographic Surveillance System Centre, Limpopo province. Treatment naïve HIV-infected and HIV-negative people participated in the study.
Outcome measures: Demographic, lifestyle and chronic disease data were collected using the World Health Organization stepwise approach to surveillance (STEPS) questionnaire. Biochemical parameters were tested using standard biochemical methods. HIV testing and CD4 counts were performed using the Alere Determine™ HIV 1/2 Ag/Ab kit and The Alere Pima™ Analyser. Insulin resistance, low-density lipoprotein cholesterol (LDL cholesterol), and non-high-density lipoprotein cholesterol (non-HDL cholesterol) levels were calculated.
Results: The mean age of participants (years) was 49.7 ± 16.6 More HIV-infected than HIV-uninfected women consumed alcohol (25.4% vs. 11.9%, *p*-value < 0.05), and the prevalence of abdominal obesity was higher in HIV-uninfected than in HIV-infected women (74.6% vs. 54.8%, *p*-value < 0.05). Levels of total cholesterol (TC), HDL cholesterol, non-HDL cholesterol, LDL cholesterol and apolipoprotein A1 (ApoA1) were significantly lower in the HIV-infected than in the HIV-uninfected group. The prevalence of low HDL cholesterol was higher in HIV-infected than in HIV-uninfected people (62.4% vs. 41.6%, *p*-value < 0.01). HIV infection increased the likelihood of low HDL cholesterol by 2.7 times (*p*-value 0.001). Male gender and alcohol use decreased the likelihood of low HDL cholesterol by 61% (*p*-value 0.002) and 48% (*p*-value 0.048), respectively. HIV infection was associated with low HDL cholesterol, ApoA1, LDL cholesterol and TC. Low CD4 count was associated with low body mass index, LDL cholesterol and high diastolic blood pressure.
Conclusion: The prevalence of cardiovascular risk factors was equally high in HIV-infected and in HIV-uninfected rural people, except for low HDL and alcohol consumption, which were significantly higher in HIV-infected people, while abdominal obesity was significantly higher in HIV-uninfected people. There is a need to raise awareness of cardiovascular risk factors in rural people in Limpopo province.

Keywords: abdominal obesity, alcohol, diabetes, hypertension, lipids

Introduction

Human immunodeficiency virus (HIV) is one of the greatest worldwide public health challenges. An estimated 22.5 million people lived with HIV in sub-Saharan Africa in 2007, which comprised approximately 68% of global infection.[1] Cardiovascular disease (CVD) is becoming a significant cause of morbidity and mortality in HIV-infected patients.[2]

HIV is a risk factor for CVD.[3] It induces chronic inflammation that leads to several CVD-associated risk factors, such as type 2 diabetes, insulin resistance (IR), hypertension and dyslipidaemia,[4-6] mediated by decreased adiponectin levels.[6] Dyslipidaemia in HIV infection is characterised by decreased levels of total cholesterol (TC), low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol.[7-10] Higher triglycerides (TG) levels were reported in advanced disease (acquired immune deficiency sydnrome).[11,12] Additionally, high levels of tobacco use, alcohol consumption and lower body mass index (BMI) are associated with CVD in HIV-infected people.[13-15]

Studies on the lipid profiles of treatment-naïve HIV-infected people in South Africa show contradictory results on TC and TG levels, while HDL cholesterol and LDL cholesterol levels were reported by only one study.[9,16] Little is known of CVD risk factors in HIV-infected people in the rural province of Limpopo. The purpose of our study was to determine whether or not CVD risk factors were more elevated in HIV-infected than in HIV-uninfected rural people. We also determined associations between CVD risk factors and HIV and CD4 count. We searched for major predictors of low HDL cholesterol, an independent risk factor for CVD.

Method

Study design and setting

The study was a cross-sectional nested case control substudy of the main project, *The Prevention, Control and Management of Chronic Disease project in a rural population, South Africa.* The study was conducted in Dikgale Health and Demographic Surveillance System (HDSS) Centre, situated 50 km north east of Polokwane, the capital city of Limpopo province. There are approximately 35 000 inhabitants in the Dikgale HDSS Centre, most of whom speak Northern Sotho.

Study participants

Eight hundred and fifteen randomly selected people participated in the main project. Participants who received pre-counselling were tested for HIV. Participants who tested

HIV-positive and who were not on antiretroviral treatment according to the questionnaire formed the cases (89). The controls (178), matched for gender and age (± 2 years), were randomly selected from those who tested negative for HIV.

Pregnant women were excluded from the study. Participants received information on the study prior to participation through door-to-door visits by trained fieldworkers and signed consent forms. Despite providing consent, some participants indicated that they did not want to know their results. Consenting participants were advised of participation dates, the local venue and times a week in advance. Participants received pre-counselling from trained counsellors on the scheduled date. The HIV results were revealed in post-counselling to participants who wanted to know their status. HIV-positive participants, and those with other abnormal biochemical abnormalities parameters, were referred to the local clinic or hospital for further analysis and management. Trained field workers revisited participants and administered the questionnaire.

Ethical considerations

Permission for the study was sought from tribal chiefs. Ethical approval was obtained from the Ethics Committee of the University of Limpopo, Medunsa Campus. Written informed consent was obtained from participants and guardians of minors (<18 years) prior to the study.

Data collection

The World Health Oganization (WHO) stepwise approach to surveillance (STEPS) questionnaire[17] was used to obtain information on the medical condition of the subjects. Tuberculosis and HIV information was obtained using an additional questionnaire. The data collection was conducted from August 2011 to April 2012.

Anthropometric measurements

Weight was measured using Omron® BF 400 (Omron Healthcare, Kyoto, Japan). Subjects were asked to take off their shoes and heavy coats. Weight was measured to the nearest 0.1 kg. Height was measured with a stadiometer. Barefoot subjects were asked to stand in an upright position. Height was measured to the nearest 0.1 cm. BMI was calculated by dividing weight (kg) by height (m^2). A BMI of 18.50–24.99 kg/m^2 was considered to be normal, 25–29.99 kg/m^2 overweight and ≥ 30 kg/m^2 obese.[18]

Waist circumference (WC) and hip circumference (HC) were measured using a measuring tape. WC was measured around the area between the last rib and the hip bone, while HC was measured around the widest part in the gluteal area. Both parameters were measured to the nearest 0.1 cm, and were used to calculate the waist to hip ratio.

Blood pressure measurements

Blood pressure (BP) was measured using the Omron® M5-1 (Omron Healthcare, Kyoto, Japan). The subjects were asked to sit and relax for five minutes before the first measurement was taken, and were restricted from talking during the measurement procedure. Three measurements were taken, with a few minutes' break inbetween. The mean of the last two values was calculated for systolic blood pressure (SBP) and diastolic blood pressure (DBP). High BP was defined as an SBP above 140 mmHg and/or a DBP above 90 mmHg,[19] and/or a self-reported history of antihypertensive drug use.

Blood collection

Fasting venous blood samples were drawn by registered nurses. Whole blood was used to measure CD4 count on the day of collection. Serum from clotted blood and plasma from whole blood were separated through centrifugation at 2 000 rpm for 15 minutes. Glucose and HIV status were analysed soon after centrifugation using plasma. The remaining samples were stored at –80°C until analysis.

Biochemical analysis

Triglycerides (TGs), TC, HDL cholesterol, glucose and creatinine levels were determined on ILab® 300 Plus Chemistry System (Instrumentation Laboratory Company, Milan, Italy). Insulin levels were measured on Beckman® Access Immunoassay System (Beckman Coulter, USA). Apolipoprotein B (ApoB), Apolipoprotein A1 (ApoA1) and high-sensitivity C- reactive protein (hsCRP) levels were measured on the IMMAGE® Immunochemistry System (Beckman Coulter, USA). Determine™ HIV-1/2 Ag/Ab Combo was initially used for HIV testing. Positive samples and 10% of negative samples were re-run using DoubleCheckGold™ Ultra HIV 1/2 kit. Both kits are Elisa®-based and supplied by Inverness Medical, Tokyo, Japan. CD4 count was measured using the Pima® Analyser (Inverness Medical, Tokyo, Japan). LDL cholesterol and IR were calculated using Friedewald[20] and homeostatic model assessment-insulin resistance[21] formulas, respectively. Non-HDL cholesterol was determined by subtracting the concentration of cholesterol in the HDL from that in the total plasma.

Statistical analysis

Statistical analysis was performed with Statistical Package for Social Science® version 20. Variables were tested for normality using frequency histograms and line graphs. Data that were not normally distributed were logarithmically transformed for analysis. To make a comparison of group differences in respect of socio-demographic characteristics and lipid profiles, the independent Student's *t*-test was used for continuous variables and the chi-square test for categorical variables. Normally and not normally distributed data were presented as mean ± standard deviation and median interquartile range, respectively. Bivariate correlation was used to establish associations between HIV, CD4 count and CVD-associated risk factors. Bivariate logistic regression was used to ascertain the individual influence of CVD-associated risk factors on HDL cholesterol levels. Multivariate stepwise forward and backward regression modelling was used to determine significant predictors of low HDL cholesterol levels.

Results

The mean age (years) of the participants was 49.7 ± 16.6. HIV-infected participants and HIV-uninfected participants had similar measurements in weight, height, BMI, WC, HC, hsCRP, insulin, DBP and SBP. Equally high prevalence rates of tobacco use (18% vs. 15.2%), IR (23.9% vs. 21.9%), hypertension (42.7% vs. 45.5%) and diabetes mellitus (13.5% vs. 13.5%) were observed between the HIV-positive and HIV-negative participants, respectively. The prevalence rate of low HDL cholesterol was significantly higher in HIV-infected than in HIV-uninfected individuals (62.4% vs. 41.6%, p-value < 0.01). A significantly higher percentage of HIV-positive women than HIV-negative women consumed alcohol (25.4% vs. 11.9%, p-value < 0.05), and abdominal obesity was present in a significantly higher percentage of HIV-negative women (74.6 % vs. 54.8%, p-value < 0.05), while their BMI remained below the threshold of 30 (Table 1).

Table 1: Characteristics of human immunodeficiency virus-infected and human immunodeficiency virus-uninfected rural African people

Characteristics	All participants		Males		Females	
	HIV-negative *n* = 178	HIV-positive *n* = 89	HIV-negative *n* = 52	HIV-positive *n* = 26	HIV-negative *n* = 126	HIV-positive *n* = 63
Age	49.7 ± 16.6	49.7 ± 16.8	54.4 ± 18.3	55 ± 18.8	47.7 ± 15.5	47.6 ± 15.6
Weight (kg)	68.3 ± 15.7	69.2 ± 17.9	66.4 ± 15.3	65.9 ± 11	69.1 ± 15.8	70.6 ± 19.9
Height (m)	1.60 ± 0.09	1.62 ± 0.09	1.66 ± 0.09	1.68 ± 0.09	1.58 ± 0.08	1.60 ± 0.08
Body mass index	26.2 ± 6.5	25.9 ± 7.2	23.7 ± 5.4	23.1 ± 5.2	27.2 ± 6.7	27 ± 7.7
WC (cm)	88.1 ± 14.4	85.5 ± 16.5	83.5 ± 13.5	80.9 ± 10.7	90.0 ± 14.3	87.3 ± 18.1
HC (cm)	102.3 ± 14.4	100.8 ± 13.5	93.8 ± 11.4	97.3 ± 7.4	105.8 ± 14.1	102.1 ± 15.2
WC to HC	0.87 ± 0.12	0.84 ± 0.09*	0.90 ± 0.15	0.83 ± 0.08*	0.85 ± 0.11	0.84 ± 0.09
DBP (mmHg)	81.8 ± 12.3	81.1 ± 15.8	83.1 ± 11.7	80.0 ± 13.2	81.3 ± 12.5	81.6 ± 16.8
SBP (mmHg)	129.5 ± 21.6	128.2 ± 24.8	136.0 ± 22.1	129.9 ± 26.4	126.8 ± 20.9	127.5 ± 24.3
Alcohol, *n* (%)	35 (19.7)	26 (29.2)	20 (38.5)	10 (38.5)	15 (11.9)	16 (25.4)*
Tobacco use, *n* (%)	27 (15.2)	16 (18)	17 (32.7)	8 (30.8)	10 (7.9)	8 (12.7)
IR,* *n* (%)	39 (21.9)	21 (23.9)	13 (25)	8(30.8)	26 (20.6)	13 (21)
Hypertension,** *n* (%)	81 (45.5)	38 (42.7)	29 (55.8)	10 (38.5)	52 (41.3)	28 (44.4)
Diabetes,*** *n* (%)	24 (13.5)	12 (13.5)	6 (11.5)	5 (19.2)	18 (14.3)	7 (11.1)
Abdominal obesity,**** *n* (%)	105 (59)	36 (41.4)*	11 (21.2)	2 (8)	94 (74.6)	34 (54.8)*
Low HDL cholesterol,***** *n* (%)	74 (41.6)	53 (62.4)**	10 (19.2)	14 (53.8)**	64 (50.8)	39 (66.1)

DBP: diastolic blood pressure, HC: hip circumference, HDL: high-density lipoprotein, IR: insulin resistance, SBP: systolic blood pressure, WC: waist circumference
Values are mean ± standard deviation, median interquartile range
* *p*-value < 0.05, ** *p*-value < 0.01
* Insulin resistance ≥ 2.5
** Hypertension or systolic blood pressure > 140, diastolic blood pressure > 90, and a history of high blood pressure
*** Diabetes glucose > 7 mmol/l and a history of diabetes
**** Abdominal obesity [waist circumference > 94 cm (males) and waist circumference > 80 cm (females)]
***** Low high-density lipoprotein cholesterol [high-density lipoprotein cholesterol ≤ 1.3 mmol/l (females), and high-density lipoprotein cholesterol ≤ 1.1 mmol/l (males)]

Total cholesterol, HDL cholesterol, non-HDL cholesterol and LDL cholesterol levels were significantly lower (*p*-value < 0.05) in the HIV-infected than in the control group, while no statistical difference was observed between TG levels (Table 2).

The pattern persisted in males, while LDL cholesterol and non-HDL cholesterol levels were not significantly different between HIV-positive and HIV-negative females. ApoB levels were not significantly different in HIV-positive than in HIV-positive people. ApoA1 levels were significantly lower in the HIV-positive group than in the control group. Glucose levels were significantly lower in HIV-infected than in the control group (*p*-value 0.04). Glucose levels remained significantly lower (*p*-value 0.01) in females, while no significant difference was observed in males.

HIV infection significantly correlated negatively with HDL cholesterol (r = -0.23, *p*-value < 0.00), ApoA1 (r = -0.24, *p*-value 0.00), LDL cholesterol (r = -0.18, *p*-value 0.00) and TC (r = -0.23, *p*-value 0.00), while CD4 count [only available for 52 participants (mean value 397 cells/µl)] significantly correlated positively with BMI (r = 0.37, *p*-value 0.00) and LDL cholesterol (r = 0.30, *p*-value 0.03), but negatively with DBP (r = -0.43, *p*-value 0.00) (Table 3).

Table 2: Biochemical characteristics of human immunodeficiency virus-infected and human immunodeficiency virus-uninfected rural African people

Characteristics	All participants		Males		Females	
	HIV-negative *n* = 178	HIV-positive *n* = 89	HIV-negative *n* = 52	HIV-positive *n* = 26	HIV-negative *n* = 126	HIV-positive *n* = 63
hsCRP (mg/l)	2.32 (0.88-6.09)	1.87 (0.68-5.64)	1.86 (0.87-3.17)	3.79 (0.93-10.03)	2.81 (0.88-6.99)	1.58 (0.61-5.23)
Glucose (mmol/l)	5.79 ± 2.78	5.22 ± 1.63*	5.29 ± 0.93	5.51 ± 2.13	6.00 ± 3.23	5.11 ± 1.38*
Insulin (uIU/ml)	6.28 (3.27-9.42)	4.91 (2.56-9.33)	5.80 (2.74-9.63)	4.85 (2.37-15.20)	6.37 (3.43-9.43)	4.91 (3.12-9.10)
TG (mmol/l)	1.18 (0.83-1.70)	1.12 (0.78-1.67)	1.29 (0.87-1.74)	1.03 (0.74-2.15)	1.14 (0.79-1.70)	1.15 (0.78-1.52)
TC (mmol/l)	4.74 ± 1.15	4.16 ± 1.27*	4.74 ± 1.00	3.92 ± 1.02*	4.75 ± 1.21	4.27 ± 1.35*
LDL cholesterol (mmol/l)	2.76 ± 0.95	2.37 ± 1.10*	2.69 ± 0.85	2.15 ± 1.03*	2.79 ± 0.99	2.47 ± 1.12
HDL cholesterol (mmol/l)	1.37 ± 0.35	1.17 ± 0.44*	1.39 ± 0.33	1.07 ± 0.36*	1.36 ± 0.36	1.21 ± 0.46*
Non-HDL cholesterol (mmol/l)	3.38 ± 1.05	2.99 ± 1.10*	3.36 ± 0.94	2.85 ± 0.98*	3.39 ± 1.10	3.05 ± 1.15
ApoB (mg/dl)	89.0 ± 27.9	83.9 ± 24.3	85.8 ± 26.3	78.8 ± 26.7	90.3 ± 28.5	86.3 ± 23.0
ApoA1 (mg/dl)	150.5 ± 32	133.4 ± 33.3*	153.4 ± 33.3	121.8 ± 25.8*	149.3 ± 31.4	138.5 ± 35.3*

ApoA1: apolipoprotein A1, ApoB: apolipoprotein B, HDL: high-density lipoprotein, hsCRP: high-sensitivity C-reactive protein, LDL: low-density lipoprotein, TC: total cholesterol, TG: triglycerides
Values are presented as mean ± standard deviation, median interquartile range
* *p*-value < 0.05
Cut-offs: Low-density lipoprotein cholesterol < 3 mmol/l, total cholesterol < 5 mmol/l, and triglycerides < 1.7mmol/l

Table 3: Bivariate correlations of cardiovascular disease-associated factors with human immunodeficiency virus and CD4 count

	HDL cholesterol	ApoA1	LDL cholesterol	TC	BMI	DBP
HIV	−0.239*	−0.240*	−0.179*	−0.225*	−0.02	−0.02
CD4 count	−0.04	−0.21	0.303**	0.25	0.370 *	−0.43*

ApoA1: Apolipoprotein A1, BMI: body mass index, DBP: diastolic blood pressure, HDL: high-density lipoprotein, HIV: human immunodeficiency virus, LDL: low-density lipoprotein, TC: total cholesterol
Values are correlation coefficients
* p-value < 0.01, ** p-value < 0.05

The current study focused on HDL cholesterol levels as the latter is regarded as a significant independent risk factor for CVD.[2,22-24] Bivariate analysis showed that an HIV-positive person is 2.3 times more likely to have low HDL cholesterol than an HIV-negative person (Table 4). HIV infection increased the likelihood of low HDL cholesterol by 2.7 times in a multivariate analysis. Overall, HIV-positive status, gender and alcohol consumption were significant predictors of low HDL cholesterol in this study.

Discussion

The mean age (years) of the participants was 49.7 ± 16.6. There was no significant difference in mean anthropometric values, BP measurements and insulin levels between HIV-positive and HIV-negative people. These similarities may indicate that our study population was in the early to mid stage of HIV infection, which was confirmed by the mean CD4 count of nearly 400 cells/µl. Similarities in BMI, WC and HC between treatment-naïve HIV-infected and HIV-uninfected people have also been observed elsewhere.[25]

The prevalence rates of diabetes mellitus, hypertension and IR were not different between HIV-positive and HIV-negative males and females. However, overall, the prevalence rates were high, putting both HIV-positive and HIV-negative people at risk of CVD occurrence.

Similar prevalence rates of diabetes mellitus between treatment-naïve HIV-infected and HIV-uninfected women were reported in the Menopause study,[25] and are consistent with those found in the current study. Higher prevalence rates of diabetes mellitus were reported in HIV-uninfected than in HIV-infected men (12% vs. 7%, p-value < 0.0001) by Womack et al.[26] Several hypothesis have been made about the relationship between HIV and diabetes, including the chronic inflammation state, leading to decreased adiponectin levels and IR, genetic susceptibility, obesity prevalence, sedentariness and the use of opiates.[4,25,27] A significant difference in the hypertension prevalence rate between HIV-infected and HIV-uninfected people was not found in this study. Similar results were reported by Baekken, Os, Sandvik and Oektedalen.[28] Other investigators found a significantly higher prevalence rate of hypertension in HIV-uninfected women relative to that in HIV-infected women (31.4% vs. 20.1%, p-value < 0.001), while no significant difference was observed in men.[29] These inconsistencies could relate to differences in HIV duration and different populations.[5]

Abdominal obesity was more predominant in HIV-negative than in HIV-positive females (59% vs. 41.1%, p-value < 0.05). Although this study did not include a dietary analysis, earlier reports found that the Dikgale HDSS community consumed high carbohydrates and low-fat, low-dietary fibre which may favour the development of abdominal obesity.[30] Similarly, a study conducted in a South African rural area showed obesity to be significantly more common in HIV-uninfected than in HIV-infected adults (24.5% vs. 20%, p-value < 0.001), and this difference was largely contributed by women (34% HIV-uninfected vs. 23.8% HIV-infected), while obesity rates were not significantly different in HIV-infected and HIV-uninfected men.[29]

A significantly higher prevalence rate of HIV-infected than HIV-uninfected females consumed alcohol (25.4% vs. 11.9%, p-value < 0.05), while similar rates were observed in HIV-infected and HIV-uninfected males. Dikgale HDSS is a poor rural community, and poverty is a driver of commercial sex work for most women, exposing them to the sale of alcohol and the risk of HIV infection. Significantly higher alcohol use by HIV-positive than HIV-negative women has been reported elsewhere.[31] The current study observed similar tobacco usage in HIV-positive and HIV-negative males and females. Higher tobacco use in HIV-positive than in HIV-negative people was reported elsewhere.[32]

Table 4: Predictors of low high-density lipoprotein cholesterol levels in participants in the Dikgale Health and Demographic Surveillance System Centre (n = 267)

Predictors	Bivariate logistic regression		Multivariate logistic regression	
	OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
HIV-positive	2.33 (1.37–3.96)	0.002	2.69 (1.52–4.75)	0.001
Male	0.35 (0.20–0.62)	< 0.001	0.39 (0.22–0.71)	0.002
Alcohol	0.49 (0.27–0.90)	0.02	0.52 (0.27–0.99)	0.047
WC obesity	1.63 (1–2.66)	0.05		
IR	1.21 (0.67–2.16)	0.53		
hsCRP	2.07 (0.97–4.40)	0.06		
Hypertension	0.91 (0.56–1.48)	0.71		
Smoking	0.48 (0.24–0.96)	0.04		
Diabetes	0.78 (0.38–1.59)	0.49		

CI: confidence interval, HIV: human immunodeficiency virus, hsCRP: C-reactive protein, IR: insulin resistance, OR: odds ratio, WC: waist circumference
Values are unadjusted odds ratio (95% confidence interval) from bivariate logistic regression, and adjusted odds ratio (95% confidence interval) from multivariate stepwise forward and backward logistic regression modelling

The lipid profile in HIV-infected people (89) was altered, compared to that in HIV-uninfected people (Table II). Our study showed significant decreases in TC, LDL cholesterol and HDL cholesterol in HIV-positive people compared to that in HIV-negative people. This lipoprotein pattern is associated with the potential risk of premature CVD developing.[33] The dyslipidaemia caused by the effects of the virus itself results from the inflammatory cytokine response to HIV infection.[34,35]

Several studies have reported similar decreases in TC, HDL cholesterol and LDL cholesterol,[36-39] while higher LDL cholesterol levels have been reported elsewhere.[40-42] Interestingly, a study[16] conducted on urban South African women reported no significant difference in TC between treatment-naïve HIV-positive and HIV-negative women, while HDL cholesterol and LDL cholesterol were not reported. The current study observed no significant difference in TG levels between HIV-positive and HIV-negative people for the whole group, and for males and females separately. Some studies have demonstrated disparate TG results.[9,38,42] Variations in TG levels were attributed to the difference in degree of immunosuppression, with TG levels escalating with an increase in immunosuppression.[11,43]

Consistent with the current study, Baker et al[44] reported no significant association between HDL cholesterol and CD4 count. By contrast, two earlier studies by El-Sadr et al[45] and Rose et al[46] reported significant associations between HDL cholesterol and CD4 count.

Even though the contribution of HIV to lipid abnormalities may be difficult to distinguish from that of classical cardiovascular risk factors, in the current study, after controlling for the effect of each risk factor, the multivariate regression model indicated HIV infection, male gender and alcohol use as significant predictors of low HDL cholesterol. Having 2.8 times more likelihood of low HDL cholesterol in HIV-infected people therefore increases the risk of CVD in this HIV-infected rural population. Similarly, Oka et al[12] reported an association between HIV disease and lipid metabolism in a Japanese male population.

The strength of this study was that standardised techniques were used, which included the WHO STEPS questionnaire and the repeated measurement of BP and HIV, as well as the use of controls in analysing the biochemical parameters.

Limitations of the study included the relatively small number of study subjects. Moreover, the sample largely comprised people living at home, thus it was skewed towards an older sedentary group. Migrant workers and people with employment were away from home when the study was conducted. This could have biased the results. A similar study is needed on the migrant population. Information on smoking and alcohol use was obtained using the WHO STEPS questionnaire, considered to be a reliable instrument.

Conclusion

The treatment-naïve, HIV-infected rural population in the Dikgale HDSS had significantly lower levels of TC, LDL cholesterol, HDL cholesterol and ApoA1, but not TG, than an HIV-uninfected rural population. The study also revealed a high prevalence of CVD risk factors, such as hypertension, diabetes, IR, tobacco use and alcohol consumption in treatment-naïve, HIV-infected people, which confirms an earlier report on the general population.[47] To our knowledge, this study is the first to have reported on CVD risk factors in treatment-naïve, HIV-infected people in the Limpopo province. HIV outreach programmes should focus more on the management of noncommunicable diseases in rural areas where elderly people reside. Further studies on subclasses of lipoproteins may provide more in-depth knowledge of the risk of CVD in this rural population.

Conflict of interest — The authors declare no conflict of interest.

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Appendix XII: Tobacco use among ARV treated HIV infected rural South Africans: Prevalence and its determinants

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Tobacco use among ARV treated HIV infected rural South Africans: Prevalence and its determinants

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Abstract

Tobacco use remains one of the major cardiovascular risk factors and its use in antiretroviral (ARV) treated human immunodeficiency virus (HIV) infected people may lead to activation of immune cells and rendering them more susceptible to HIV. We determined the prevalence of and factors associated with tobacco use in an antiretroviral treated HIV infected rural African people. The study was a cross-sectional, conducted in three ARV clinics in rural Dikgale Health and Demographic Surveillance System (HDSS), South Africa. Socio-demographic, tobacco and alcohol use data were collected using World Health Organisation stepwise approach to surveillance (STEPS) questionnaire. The Chi-square test was used to compare categorical variables between tobacco users and non tobacco users. The multiple logistic regression analysis was used to determine the predictors of tobacco use status. Of 214 ARV treated HIV infected participants, 171 (79.9%) were females and 43 (20.1%) were males. The mean age of participants was 44.8 ± 11.8 years. About 45 (21%) of participants were tobacco users. A higher proportion of males than females (39.5% versus 16.4%, p=0.02) used tobacco. Older age >50years (p=0.01), marital status (p=0.03) and alcohol consumption (p=0.001) were significant independent predictors of tobacco use. Tobacco use among ARV treated HIV infected rural people was common. Older age, alcohol consumption and marital status were the risk factors for tobacco use. There is need to scale up the awareness on how tobacco use, apart from being a risk factor for CVD, interferes with viral suppression despite treatment with antiretroviral drugs.

Keywords: Tobacco use, cardiovascular disease, antiretroviral, human immunodeficiency virus.

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Introduction

Tobacco is detrimental to health, and its use is one of the public health challenges. Tobacco use among ARV treated HIV infected people may reverse the improvement in immune status that is achieved from ARV treatment, through

the activation of immune cells and rendering them susceptible to HIV (Valiathan, Miguez, Patel, Arheart & Asthana, 2014). The use of tobacco also increases the risk of chronic obstructive diseases, lung cancer, decreased bone mineral density and pulmonary hypertension (Petrosillo & Cicalini, 2013; Shirley, Kaner & Glesby, 2013). Furthermore, tobacco use is also regarded as a risk factor for cardiovascular diseases (CVD) (CDC), that are becoming a common cause of mobidity and mortality in HIV infected people (Malvestutto & Aberg, 2010; Pallella & Phair, 2011; Podzamczer, 2013). While HIV infection itself and its treatment have been associated with increased risk for CVD (Podzamczer, 2013), the use of tobacco in HIV infected people may compound the risk of CVD occurance.

High prevalence of smoking in ARV treated HIV infected people was reported from developed countries by Tesoriero, Gieryic, Carrascal and Lavigne (2010) (59%) and Rubinstein, Harris, Rudy and Kapogiannis, et al. (2014) (38.7%). Studies from some African countries reported varied results: Desalu et al. (2009) (22.1%); Edward et al. (2013) (1.9%) and Muhammad, Sani and Okeahialam (2013) (10%). In South Africa, the study conducted by Waweru et al. (2013) in Johannesburg, reported prevalence of 15%. While South Africa bears the largest numbers of HIV infection in the world, information on prevalence of tobacco use in ARV treated HIV infected person, particularly from rural areas is scarce. The present study aimed at determining the prevalence and factors associated with tobacco use in an ARV treated rural African people in Limpopo Province.

Methodology

Study design

The study was cross-sectional sub-study of the main project on '*Cardiovascular risk factors in human immunodeficiency virus-infected rural people, Dikgale, South Africa'*. The study was conducted in Dikgale Health and Demographic Surveillance System (HDSS) centre active Primary Health Care clinics namely Seobi-Dikgale, Sebayeng and Dikgale.

Study participants

Two hundred and fourteen ARV treated HIV infected people, 15 years and older participated in the study. ARV treated HIV infected people were recruited to participate in the study by health professional nurses who received prior information regarding the study. Recruitment was conducted when people came to collect their ARV treatment and was performed throughout a full month cycle per clinic to allow an equal opportunity for HIV infected people to participate in the project. Those willing to participate were advised on the participation dates and time (7:00am) at their clinic, a week in advance. On the scheduled date, participants and guardians of minors (<18years) received information regarding

the study from the researcher and were requested to complete consent forms. Consenting participants went through completion of questionnaire.

Ethical approval was obtained from the Ethics Committee of University of Limpopo, Medunsa campus. Permission to conduct the study in the Dikgale HDSS clinics was obtained from the Department of Health-Provincial office and Primary Health Care Capricorn District office.

Data collection

The data collection was conducted from September 2013 to March 2014. The WHO stepwise approach to surveillance (STEPS) questionnaire (WHO) was used to obtain information on socio-demographic, tobacco use and alcohol consumption from the participants.

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences version 22. Frequency histogram and line graph was used to check normality of continuous variable (age). The independent Student t-test was used to compare continuous variables and the Chi-square test was used to compare categorical variables between tobacco and non-tobacco users.

Bivariate logistic regression was used to determine individual influence of socio-demographic factors on tobacco use. Factors significant at p-value ≤ 0.25 in bivariate regression were considered as candidates for multivariate modelling. The multivariate logistic regression modelling was used to determine association of each socio-demographic factor with tobacco use after controlling for other covariates in the model.

Results

Of the 214 ARV treated HIV infected participants, 171 (79.9%) were females and 43 (20.1%) males. The mean age of participants was 44.8 ± 11.8 years and males were older than females (49.9 ± 11.1 years versus 43.5 ± 11.6 years, p=0.001). The prevalence of tobacco use in the study was 21%, with more males than females using tobacco (39.5% versus 16.4%, p=0.002).

A higher proportion of married people used tobacco compared to the proportion of unmarried people who used tobacco (37.8% versus 19.5%, p=0.016). Alcohol consumption was more prevalent among tobacco users than non-tobacco users (60.0% versus 11.8%, p=0.001) (Table 1).

The present study examined the association of age, gender, marital status, educational level, work status and alcohol with tobacco use. In univariate analysis, age > 50 years (p=0.003), male gender (p=0.001), being married (p=0.01) and alcohol consumption (p=0.001) were positively associated with

tobacco use. No significant association was observed for tobacco use with educational level and work status (Table 2).

Controlling for covariates in a multivariate logistic regression model, age > 50 years (p=0.01), alcohol consumption (p=0.001) and marital status (p=0.03) remained positively associated with tobacco use (Table 3).

Table 1: Socio-demographic status in ARV treated HIV infected tobacco and non-tobacco users

Variables	Non-tobacco users N=169	Tobacco users N=45	P=value
N (males / females)	169 (26 / 143)	45 (17 / 28)	
Age < 50 years n (%)	129 (76.3)	24 (53.3)	0.01
Unmarried	136 (80.5)	28 (62.2)	0.02
Primary n (%)	85 (50.3)	29 (64.4)	0.08
Unemployed	130 (76.9)	36 (80.0)	0.80
Alcohol consumers	20 (11.8)	27 (60.0)	0.001

P-value < 0.05 is significant. Data is presented as number (percentage), HIV human immunodeficiency virus, ARV antiretroviral.

Table 2: Bivariate Association of socio-demographic characteristics with tobacco use among ARV treated HIV infected people.

Characteristics	Unadjusted OR	95% CI	p-value
Age: <50	1 [ref]		
> 50	2.82	1.42 – 5.60	0.003
Gender: Females	1 [ref]		
Males	3.34	1.60 – 6.95	0.001
Marital status: Unmarried	1 [ref]		
Married	2.50	1.23 – 5.10	0.01
Education: Primary level	1 [ref]		
Secondary level	0.56	0.28 – 1.10	0.09
Work status: Unemployed	1 [ref]		
Employed	0.83	0.37 – 1.88	0.66
Alcohol: Non consumers	1 [ref]		
Consumers	11.1	5.24 – 23.83	0.001

OR odds ratio, CI confidence interval

Discussion

The present study obtained a self-reported prevalence rate of tobacco use of 21% among ARV treated HIV infected people and was comparable to the self-reported smoking prevalence rate of 15% among HIV infected people in urban South Africa (Waweru et al., 2013). The prevalence of smoking reported from Nigeria by Edward et al. (2013) (1.9%), Muhammad et al. (2013) (10%) and Desalu et al. (2009) (22.1%) was varied.

Table 3: Multiple logistic regression coefficients and adjusted odds ratio (95% CIs) for socio-demographic factors associated with tobacco use in ARV treated HIV infected.

Characteristics	Adjusted OR	95% CI	p-value
Age: 30- 50	1 [ref]		
> 50	3.25	1.27 – 8.34	0.01
Gender: Females	1 [ref]		
Males	1.47	0.59-3.62	0.40
Marital status: Unmarried	1 [ref]		
Married	3.39	1.15-10.04	0.03
Education: Primary level	1 [ref]		
Secondary level	1.89	0.68-5.24	0.22
Alcohol: Non consumers	1 [ref]		
Consumers	11.90	5.05-28.02	0.001
Education by marital status	0.24	0.03-1.74	0.16

OR odds ratio, CI confidence interval, Model- 84.1 % classification accuracy

The low prevalence rates reported by Edward et al. (2013) and Muhammad et al. (2013) are in contrast to the present finding. Firstly, this difference may be partly due to the fact that our study reported on tobacco use (both smoke and smokeless tobacco) while the two studies by Edward et al. (2013) and Muhammad et al. (2013), reported only on cigarette smoking, yielding low prevalence. Secondly, the study population in the aforementioned studies was predominantly females who are less likely to smoke cigarettes. On the other hand, Desalu et al. (2009) had a higher male than female participants, probably being the reason for yielding a higher prevalence of smoking than the other two studies conducted in the same country. Higher prevalence of smoking than that of tobacco use in present study was reported from Brazil (28.9%) (Batista, de Albuquerque, Ximenes and Miranda-Filho et al., 2013) and from USA (59%) (Tesoriero et al., 2010) and (38.7%) Rubinstein et al. (2014) among HIV infected people. Lifestyle differences between the developed and African countries could partly explain the differences in smoking prevalence. Another explanation could be the high male participation in the studies from developed countries while in most African studies female participation is high, with males known to smoke more than females (Desalu et al., 2009; Batista et al., 2013; Gilani & Leon, 2013).

Increasing age was previously found to be associated with smoking (Gilani & Leon, 2013; Lo, Oeltmann, Odhiambo & Beynon, et al., 2013). In the present study, people older than 50 years had a 3.25 times (p-value 0.01) likelihood of using tobacco than people with an age of less or equal to 50 years. While an association of tobacco use and being unmarried was previously reported (Lawrence, Rose, Fagan & Moolchan, et al., 2010), the present study finding, that married people are 3.39 times more likely to use tobacco than unmarried people requires further elaboration. A significantly higher proportion of married than unmarried females (29.4% vs 13.1%, p=0.036) used tobacco, while there

was no significant difference in proportion of married than unmarried man who used tobacco. It is important to note that in the present study most of the female tobacco users were using snuff (smokeless) rather than cigarettes and the great number of married female tobacco users influenced our results. The interaction of education and marital status showed that married people who were educated had a lower probability of using tobacco as compared to the married people who were not educated, however level of significance was not reached. Alcohol consumers were 11.9 times more likely to use tobacco than non-alcohol consumers. This finding underscores the need to simultaneously counsel ARV treated HIV infected people on dangers of alcohol consumption during tobacco use counselling sessions. The association of alcohol with tobacco use was also observed by other investigators (Neblett, Hutton, Lau & McCaul, et al., 2011; Lo et al., 2013; Louwagie & Ayo-Yusuf, 2013). The alcohol and tobacco use habits are detrimental to health. Tobacco use increases the risk of CVD, in addition to chronic obstructive diseases, lung cancer, decreased bone mineral density and pulmonary hypertension (Petrosillo & Cicalini, 2013; Shirley et al., 2013). Furthermore, some investigators recently demonstrated that smoking was associated with immune cell activation in ARV treated HIV infected people rendering them more susceptible to further infection with HIV (Valiathan et al., 2014). While alcohol may not present direct risk for cardiovascular disease, alcohol consumption by people receiving ARV treatment should be discouraged as it may interfere with ARV adherence leading to HIV disease progression (Neuman et al., 2012), and possible risk of CVD mediated by the HIV infection itself (Podzamczer, 2013). The present study found no association of tobacco use with education level and work status, although in a previous report, unemployment and low education level were shown to be associated with tobacco use (Lawrence et al., 2010). Tobacco use is probably viewed as a means to relieve the stress due to unemployment.

Our study found tobacco use among ARV treated HIV infected rural people to be common, at a time when tobacco cessation is a priority. Quitting tobacco use is a challenge (Van Zyl-Smit, Allwood, Stickells & Abdool-Gaffar, et al., 2013), even when the resources which include medication and counselling are available. In developing countries particularly in rural areas, the problem may be compounded by poverty and not able to afford the medication that assist in ceasing tobacco use. Counselling is the common method in use. However, its effectiveness remains unclear. When completed, a clinical randomized trial comparing intensive counselling plus nicotine replacement therapy and intensive counselling only currently underway at University of Witwatersrand in South Africa will provide information on the effectiveness of counselling as a tobacco use cessation strategy (Clinical trials.gov). Increasing tax on tobacco, has resulted in decrease in tobacco use in the general population in South Africa from 23% in 2003 to 16% 2012 (Stassen, 2013). However, these gains may be

under threat from the ever growing tobacco illegal market (Stassen, 2013), where the price of tobacco is cheaper.

Our study had limitations. The cross sectional design of the study limits inference, but it gives a relationship hence the need for longitudinal study. Furthermore, tobacco use was self-reported and may have been underreported particularly in females as it may be socially unacceptable.

Conclusion

The present study showed that tobacco use among ARV treated HIV infected rural people was common. Older age, alcohol consumption and marital status were associated with tobacco use among ARV treated HIV infected people. There is needed to scale up the awareness on how tobacco use, apart from being a risk factor for CVD, interferes with viral suppression despite treatment with antiretroviral drugs. Follow up studies assessing knowledge on risk of tobacco use and attitude towards tobacco use cessation among ARV treated people attending PHC clinics in Dikgale, South Africa are warranted.

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Appendix XIII: Assessment of cardiovascular risk factors in people with HIV infection treated with ART in rural South Africa: a cross sectional study

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Assessment of cardiovascular risk factors in people with HIV infection treated with ART in rural South Africa: a cross sectional study

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Abstract

Background: The risk of cardiovascular diseases (CVD) in human immunodeficiency virus (HIV) infected people on antiretroviral therapy (ART) from some rural parts of Africa is not well known. We assessed CVD risk factors, the estimated 5-year Data collection on adverse effects of anti-HIV drugs (DA.) risk score and the 10-year Framingham risk score in persons with HIV infection on ART in a rural area in South Africa.

Methods: A cross-sectional study in which the data on demographic, lifestyle, and chronic disease were collected using the World Health Organization Stepwise approach to surveillance questionnaire. Biochemical parameters were tested using standard biochemical methods. CD4 counts were performed using PIMA analyser and viral load was tested using the branched deoxyribonucleic acid technique. Student *t* test and Chi square test were used on continuous and categorical variables respectively. Bivariate and multivariate logistic regression were used to analyze predictors of CVD risk factors. Estimates of 5 and 10-year CVD risk were calculated using online tools. The Cohen's kappa coefficient was used to assess the agreement between CVD risk equations.

Results: The mean age of participants was 44.8 ± 11.8 years; 79.9 % were females. Most of the participants (85 %) had an undetectable viral load and a mean CD4 count of 462 ± 235 cell/mm^3. The most common CVD risk factors were low high density lipoprotein cholesterol (HDL-C) (43.8 %), hypercholesterolaemia (33.2 %) and a high Apolipoprotein (Apo) B/ApoA ratio (45.4 %).Using the Framingham equation, 6.7 % of participants had a moderate to high 10-year CVD risk while the DAD risk equation showed that 31.1 % of participants had a moderate to high 5-year CVD risk. Most participants had a low CVD risk by both risk equations. The level of agreement between the two risk equations was 73.8 % (k = 0.23; 95 % CI 0.10–0.35; p value 0.001).

Conclusion: CVD risk factors were common among this rural population on ART. The high proportion of participants with a moderate to high CVD risk, observed with the DAD risk equation, clearly represents a considerable health burden that can possibly be reduced by increasing educational programs on CVD prevention for people on ART. There is however a need to develop and evaluate a race/ethnicity-specific CVD risk estimation tool for HIV infected Africans.

Keywords: Cardiovascular disease risk, Human immunodeficiency virus, Antiretroviral therapy

Background

Human immunodeficiency virus (HIV) or antiretroviral therapy (ART) through direct or indirect mechanisms may induce diabetes mellitus, dyslipidaemia, hypertension, lipodystrophy and endothelial dysfunction [1]. Furthermore, some studies have shown that traditional risk factors for cardiovascular diseases (CVD) such as low physical activity, increased body mass index (BMI), smoking, are frequently present in HIV infected people [2, 3].

A limited number of studies have assessed CVD risk factors in HIV infected people on ART from low-income countries [4–6]. Furthermore, the number of CVD risk

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factors, type and duration of ART reported in these studies varied. Most studies on CVD risk factors in persons with HIV infection were done in Western countries [7–11]. While some of these studies were conducted in areas with people of a low-socio-economic class similar to developing countries, differences in ethnicity, environmental, genetic factors, [12–14] and populations' uptake of smoking cessation campaign [9], may have influenced prevalence rates of CVD risk factors.

Studies on CVD risk factors among HIV infected people are however scarce in South Africa [15, 16]. A recent attempt to describe the CVD risk in rural people on ART from Mpumalanga in South Africa was made [17], but the use of ART was self-reported and according to the authors, the prevalence of CVD risk factors in that rural population on ART may be underestimated since there remains considerable stigma associated with HIV. In South Africa, the intersection of an epidemiological transition [18], a high number of people living with HIV [19] and a widespread adult treatment coverage that was close to 80 % by mid-2011 [20], presents a high risk of CVD among people infected with HIV and needs to be investigated. While CVDs are preventable, little is known regarding CVD risk factors in HIV infected rural South Africans on ART [17]. We determined the prevalence of CVD risk factors, the estimated 5-year DAD risk score and the 10-year Framingham risk score in persons with HIV infection on ART in a rural area in South Africa.

Methods

Study design

A cross-sectional study was conducted in the primary health care clinics, Seobi-Dikgale, Sebayeng and Dikgale. At least 74 people with HIV infection are treated at Seobi-Dikgale clinic, while 373 people and 377 people with HIV infection are treated at Sebayeng and Dikgale clinics respectively. The three clinics are situated within the Dikgale Health and Demographic Surveillance System (HDSS) site. Dikgale HDSS site is situated about 70 km to the Northeast of Polokwane, the capital city of Limpopo Province. The site consists of 15 villages. Dwellings in Dikgale HDSS are a mix of shacks, conventional brick houses and traditional mud huts in the Limpopo province of South Africa.

Study participants

During one month all patients, at least 15 years old who came to collect their ART were asked to participate in the study. Those willing to participate, were advised on the participation dates and time (7:00am) at their clinic and were advised to provide a fasting sample. Participants who could not come on the scheduled date were visited in their homes on an arranged date and participated. Participants were only included after written informed consent was obtained through the completion of a consent form approved by the Medical University of South Africa (MEDUNSA) Ethics committee. In the case of minors, written informed consent was obtained from their legal guardians. Pregnant women were excluded.

Ethical considerations

Ethical approval was obtained from the Ethics Committee of University of Limpopo, Medunsa campus. Permission to conduct the study in the Dikgale HDSS site clinics was obtained from the Department of Health-Provincial office and Primary Health Care Capricorn District office.

Data collection

The study was performed from September 2013 to March 2014. The World Health Organization stepwise approach to surveillance (WHO STEPS) questionnaire [21] was used to obtain information on dietary intake, physical activity, socio-demographic, tobacco use, alcohol consumption and medical history.

Anthropometric and blood pressure measurements

Anthropometric and blood pressure measurements were taken following procedures as previously described [22]. In brief, weight was measured to the nearest 0.1 kg, using Omron BF 400 (Omron Healthcare, Japan). Height was measure to the nearest 0.1 cm, using a stadiometer. BMI was calculated as weight (kg)/height (m^2). A BMI was considered as normal (18.50–24.99), overweight (25.00–29.99) and obese ($\geq$30.00) [23]. Waist circumference and hip circumference were measured to the nearest 0.1 cm, using a measuring tape. Both parameters were used to calculate the waist to hip ratio. The Omron M5-1 (Omron Healthcare, Kyoto, Japan) was used to measure blood pressure. High blood pressure was defined as a systolic blood pressure (SBP) above 140 mmHg and/or a diastolic blood pressure (DBP) above 90 mmHg [24] and/or self-reported history of antihypertensive medication. Metabolic syndrome was defined as any three of the following five risk factors; abdominal obesity (waist circumference $\rightarrow$ 88 cm for females and >102 cm for males), high TG concentration ($\geq$1.7 mmol/l), low HDL-C concentration ($\leq$1.3 for females and $\leq$1.1 for males), high Blood pressure and raised fasting plasma glucose concentration (>7 mmol/l) [25].

Blood collection

Fasting venous blood samples were drawn by registered nurses. Whole blood was used to measure CD4 counts on the day of collection. Serum from clotted blood and plasma from whole blood were separated through centrifugation at 2000 rpm for 15 min. Glucose was analysed

soon after centrifugation using plasma from sodium fluoride tubes. The remaining samples were stored at −80 °C until analysis.

Biochemical analysis

Triglycerides (TGs), total cholesterol (TC), high density lipoprotein (HDL) cholesterol and glucose levels were determined on ILab 300 Plus Chemistry System (Instrumentation Laboratory Company, Milan, Italy). Apolipoprotein B (ApoB), Apolipoprotein A1 (ApoA1) were measured on the IMMAGE Immunochemistry System (Beckman Coulter, USA). CD4 count was measured using the Pima Analyser (Inverness Medical, Tokyo, Japan). Viral load testing, using the branched deoxyribonucleic acid (DNA) technique (Siemens, South Africa) was performed by Toga Molecular Biology and Pathology medical laboratory that is South African National Accreditation System (SANAS) accredited for ISO 17025.

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Science version 22. Frequency histogram and line graph was used to check normality of continuous variable. The independent Student t-test was used to compare continuous variables and the Chi square test was used to compare categorical variables between males and females. Factors significant at p-value less or equal to 0.25 in bivariate regression were considered as candidates for multivariate modelling. Multivariate logistic regression was used to determine the significant predictors of CVD risk factors. The level of significance for statistical analysis was set at less than 0.05. Framingham 10-year CVD risk estimation was calculated for each participant above 20 years, with no diabetes and no history of CVD, by entering the following variables: age, gender, TC, HDL-C, SBP, smoking status and current treatment for high blood pressure, as required by the Framingham risk model tool [26]. Participants were regarded as low risk, moderate risk, or high risk when the risk score for developing CVD in 10 years was <10, 10–20 or >20 % respectively [27]. Variables included in the 5-year DAD risk estimation tool were age, sex, SBP, TC, HDL-C, diabetes mellitus, smoking status, family history of CVD, current use of abacavir, indinavir, or lopinavir and duration on indinavir and lopinavir [28]. The risk of developing coronary heart disease in the next 5-years was regarded as low (<1 %), moderate (1–5 %), high (5–10 %), or very high (>10 %) [29]. The level of agreement between DAD and Framingham risk equations was determined using Cohen's Kappa coefficient with 95 % CI. For comparison with Framingham, participants with high and very high scores according to the DAD equation were combined and considered as high risk group. Kappa coefficients was interpreted as poor agreement (<0), slight agreement (0.0–0.20), fair agreement (0.21–0.40), moderate agreement (0.41–0.60), substantial agreement (0.61–0.80) and perfect agreement (0.81–1.00) [30].

Results

Characteristics of participants

Two hundred and fourteen HIV infected people on ART, participated in the study. Of which 171 (79.9 %) were females and 43 (20.1 %) were males. The mean age of ART participants was 44.8 ± 11.8 years and males were significantly older than females (49.9 ± 11.1 vs 43.5 ± 11.6, $p = 0.001$). The percentage of unmarried participants was 164 (76.6 %) with more females than males (80.1 vs 62.8 %, $p < 0.05$). About 45.8 % had secondary level of education. Unemployment was 69.6 %, and higher in females (75.4 %) than in males (46.5 %).

The majority of participants were on efavirenz (86 %) and nevirapine (12.5 %) based ART. Only three of the participants (1.5 %) were on a lopinavir/ritonavir based ART regimen. The mean duration of ART in this study was 36 months (range 1–121 months). Most of the participants (85 %) had an undetectable viral load and a mean CD4 count of 462 ± 235 cell/mm^3.

Prevalence of behavioural and metabolic CVD risk factors

Tobacco and alcohol use were higher among males than females respectively (39.5 vs 16.5 %, $p = 0.001$) and (41.9 vs 17.0 %, $p = 0.001$), while more females than males were physically inactive (29.1 vs 3.3 %, $p = 0.002$). Hypertriglyceridaemia (35.0 vs 12.5 %, $p = 0.001$), high TG/HDL-C ratio (37.5 vs 13.7 %, $p = 0.002$) and high TC/HDL ratio (27.5 vs 10.1 %, $p = 0.01$) was present more often in males than in females (Table 1). None of participants indicated a family history of cardiovascular disease.

Predictors of metabolic CVD risk factors

People more than 50 years of age were more likely to be hypertensive ($p < 0.05$) and diabetic ($p < 0.05$) compared to people less 50 years of age. In addition an age of more than 50 years increased the likelihood of having metabolic syndrome ($p < 0.05$), a high concentration of TG ($p < 0.05$), a high TC/HDL-C ratio ($p < 0.05$) and a low concentration of HDL-C ($p < 0.05$) compared to an age of less than 50 years. Males were 2.94 times ($p < 0.05$) more likely to have a high TC/HDL-C ratio compared to females. People with a viral load of more than 50 copies/ml (>log 1.71) were less likely to be hypertensive but were more likely to have a low HDL-C concentration and a high ratio of ApoB/ApoA than people with a viral load of less than 50 copies/ml. People on ART for less than 60 months were less likely to have a high TC/HDL-C ratio than people on ART for more than 60 months. The

Table 1 Demographics, HIV information and CVD risk factors in ART treated HIV infected participants by gender

	All participants N = 214, n (%)	Females N = 171, n (%)	Males N = 43, n (%)	P-value
Demographics				
Age (years)	44.8 ± 11.8	43.5 ± 11.6	49.9 ± 11.1	*0.001*
Unmarried	164 (76.6)	137 (80.1)	27 (62.8)	*0.03*
Secondary education	98 (45.8)	83 (48.5)	15 (34.9)	0.12
Unemployed	149 (69.6)	129 (75.4)	20 (46.5)	0.001
HIV information				
CD4 count (cells/mm^3)	461.9 ± 235.3	485.5 ± 234.1	364.3 ± 216.6	*0.001*
Viral load (copies/ml)	≤50	≤50	≤50	0.14
Mean duration of ART in months (N = 200)	36.1 ± 24.4	37.0 ± 24.3	32.5 ± 24.6	0.31
NVP (NNRTI)-based ART*	25 (12.5)	22 (13.8)	3 (7.5)	
EFV (NNRTI)-based ART	172 (86)	136 (85.0)	36 (90.0)	
Alluvia (PI)-based ART	3 (1.5)	2 (1.3)	1 (2.5)	
CVD risk factors				
Tobacco use	45 (21.1)	28 (16.5)	17 (39.5)	*0.001*
	47 (22.0)	29 (17 %)	18 (41.9 %)	*0.001*
Alcohol use Physical activity <600 MET-min/wk)	38 (24.2)	37 (29.1 %)	1 (3.3 %)	*0.002*
Fruit and vegetables intake (<5 servings/day)	150 (95.5)	121 (95.3)	29 (96.7)	1.00
Obesity	27 (12.7)	25 (14.6)	2 (4.8)	0.12
Abdominal obesity	51 (23.9)	44 (25.9)	7 (16.3)	0.23
Hypertension	56 (26.2)	40 (23.4)	16 (37.2)	0.08
Diabetes	10 (4.7)	6 (3.5)	4 (9.3)	0.12
Low HDL	91 (43.8)	71 (42.3)	20 (50.0)	0.38
Hypercholesterolaemia	69 (33.2)	58 (34.5)	11 (27.5)	0.46
Hypertriglyceridaemia	35 (16.8)	21 (12.5)	14 (35.0)	*0.001*
TC/HDL (≥5)	28 (13.5)	17 (10.1)	11 (27.5)	*0.01*
High TG/HDL-C (≥1.49)	38 (18.3)	23 (13.7)	15 (37.5)	*0.001*
ApoB/ApoA (≥0.68)	93 (45.4)	74 (44.8)	19 (47.5)	0.86
Metabolic syndrome	20 (9.6)	15 (8.9)	5 (12.5)	0.56

Diabetes—glucose >7 mmol/l and/or history of diabetes, hypertension—systolic blood pressure >140 and/or diastolic blood pressure >90 or history of high blood pressure, abdominal obesity—waist circumference >88 cm for females and >102 cm for males, obesity—body mass index ≥30 kg/m^2, low HDL <1.3 for females and 1.1 for males, hypercholesterolaemia—TC ≥ 5 mmol/l, hypertriglyceridaemia—TG ≥ 1.7 mmol/l, high TC/HDL-C >5, TG/HDL-C ≥ 1.49, high ApoB/ApoA >0.68

* ART information was available for 200 participants

ART antiretroviral therapy, *HIV* human immunodeficiency virus, *NVP* nevirapine, *EFV* efavirenz, *CVD* cardiovascular disease, *ApoB* apolipoprotein B, *Apo A* apolipoprotein A, *TC* total cholesterol, *HDL-C* high density lipoprotein cholesterol. *MET-min/wk* metabolic equivalent of task-minutes/week

likelihood of having a high TC concentration was 2.10 times ($p < 0.05$) and a high TG/HDL-C ratio was 2.98 times ($p < 0.05$) more in people with than in people without abdominal obesity. A low intake of fruit and vegetable was associated with a high concentration of TG ($p < 0.05$) (Table 2).

Framingham risk scores

None of the 164 participants according to the Framingham estimation had a high risk of developing a CVD event in the next 10 years. However, about 6.7 % had a moderate risk and majority had a low risk (93.3 %).

DAD risk scores

Of the 164 participants, 68.9 % had a low risk, 27.4 % had a moderate risk and 3.7 % had a combined high and very high risk for developing a CVD event in next 5-years. However, considering all (214) participants, 66.8 % had a low risk, 29 % had a moderate risk, 1.9 % had a high risk and 2.3 % had a very high risk for developing CVD in next 5-years.

Comparison of the Framingham risk scores with the DAD risk scores in 164 participants who met the Framingham criteria gave a level of agreement of 73.8 % (Kappa = 0.23; 95 % CI 0.10–0.35; p value 0.001) (Table 3).

Table 2 Predictors of Metabolic CVD risk factors among ARV treated HIV infected people

Predictor variable	Metabolic CVD risk factors								
	HTN	Diabetes	Low HDL-C	High TC	High TG	High TC / HDL-C	High Apo B /Apo A	High TG/HDL-C	Met S
Age (years)									
≤50	1 [reference]	1 [reference]	1 [reference]	–	1 [reference]	1 [reference]	1 [reference]	1[reference]	1 [reference]
>50	*4.67*[†] (1.94–11.2)	*5.66*[†] (1.36–23.5)	*2.27*[†](1.04–4.95)		*2.91*[†](1.12–7.55)	*3.29*[†] (1.35–8.01)	1.70 (0.90–3.21)	2.8 (0.84–5.68)	*1.10*[†] (1.02–1.18)
Gender									
Females	–	1 [reference]	–	–	–	1 [reference]	–	1 [reference]	–
Males		1.83 (0.46–7.20)				*2.94*[†] (1.13–7.65)		1.90 (0.21–17.6)	
Alcohol use									
No	1 [reference]	–	–	1 [reference]	–	–	–	–	–
Yes	0.57 (0.18–1.77)			1.69 (0.85–3.36)					
Tobacco use									
No	–	–	1 [reference]	–	–	–	–	–	1 [reference]
Yes			0.49 (0.19–1.23)						1.61 (0.30–8.70)
Log VL									
<1.71	1 [reference]	–	1 [reference]	–	–	–	1 [reference]	1 [reference]	–
>1.71	*0.08*[†] (0.01–0.63)		*3.82*[†] (1.51–9.63)				*3.83*[†] (1.73–8.46)	2.71 (0.84–8.75)	
ARV-duration									
>60 months	1 [reference]	–	–	–	1 [reference]	1 [reference]	–	1 [reference]	1 [reference]
30–60	2.32 (0.65–8.34)				0.48 (0.14–1.68)	*0.29*[†] (0.09–0.90)		0.49 (0.13–1.87)	0.48 (0.09–2.75)
<30 months	0.32 (0.66-8.19)				0.45 (0.14–1.46)	*0.31*[†] (0.11–0.89)		0.29 (0.07–1.23)	0.34 (0.05–2.60)
Abdominal obesity									
No	1 [reference]	–	1 [reference]	1 [reference]	1 [reference]	–	1 [reference]	1 [reference]	–
Yes	2.48 (0.69–8.89)		1.10 (0.26–4.66)	*2.10*[†] (1.09–4.05)	2.08 (0.75–5.82)		1.71 (0.88–3.35)	*2.98*[†] (1.05–8.47)	
PA (MET-min/wk)									
>600	1 [reference]	–	1 [reference]	–	–	–	–	–	–
<600	0.27 (0.07–1.09)		0.54 (0.11–2.71)						
CD4 count									
>500	–	–	–	–	–	–	–	–	1 [reference]
301–500									0.31 (0.05–1.96)
≤300									0.12 (0.01–1.29)
F/veg intake	–	–	0.92 (0.18–4.79)	–	*0.56*[*] (0.31–1.01)	–	–	0.67 (0.41–1.10)	–
Abd. obesity by PA	–	–	7.18 (0.74–69.7)	–	–	–	–	–	–

Table 2 continued

Predictor variable	Metabolic CVD risk factors								
	HTN	Diabetes	Low HDL-C	High TC	High TG	High TC/ HDL-C	High Apo B /Apo A	High TG/HDL-C	Met S
Gender by ARV months									
>60								1 [reference]	
30–60	–	–	–	–	–	–	–	0.46 (0.02–10.9)	–
<30								6.07 (0.45–28.8)	
Classification accuracy	81.7 %	95.3 %	67.8 %	69.6 %	84.1 %	87.4 %	61.8 %	82.6 %	91.0 %

Values are reported as adjusted odds ratio (confidence interval)

PA physical activity, *VL* viral load, *F/V* fruit and vegetable intake, *HDL-C* high density lipoprotein cholesterol, *LDL-C* low density lipoprotein cholesterol, *TC* total cholesterol, *TG* triglycerides, *Apo B* apolipoprotein B, *Apo A* apolipoprotein A, *Met S* metabolic syndrome, *HTN* hypertension, *ARV* antiretroviral, *MET-min/wk* metabolic equivalent of task-minute/week, *Abd* abdominal

[1] p-value < 0.05 is significant

* p = 0.05, marginally significant

Table 3 Comparison between CVD risk estimation using DAD and Framingham equations

Cardiovascular disease risk	FRAMINGHAM (N = 164)			
	Low risk (<10 %)	Moderate risk (10–20 %)	High risk (>20 %)	
DAD (N = 164)				
Low risk (<10 %)	113	0	0	
Moderate risk (1–5 %)	37	8	0	
High risk and very high risk (>5 %)	3	3	0	
Agreement	73.8 %			
Kappa (p-value)	0.23 (0.001)			
95 % CI for Kappa	0.10–0.35			
	DAD risk estimations on all participants			
	Low risk (<1 %)	Moderate risk (1–5 %)	High risk (5.1–10 %)	Very high risk (>10 %)
All participants (N = 214)	143 (66.8 %)	62 (29 %)	4 (1.9 %)	5 (2.3 %)

N number, *DAD* data collection on adverse effects of anti-HIV drugs, *CVD* cardiovascular disease, *CI* confidence interval

Discussion

Although our study population was young, using the DAD risk equation 31.1 % of participants had a 5-year moderate to high CVD risk. Several factors may play a role in causing this high risk. In our study, most people were physically active (75.8 %). However, the use of a different instrument for data collection may explain variations observed in physical activity between our study and others [5, 31]. Most participants in our study cited non availability of fruits and vegetables coupled with unaffordability as reasons for low intake. This low intake remains a major challenge as it increases the risk of nutritional deficiencies [32, 33] and CVD incidence and mortality [34]. Consistent with our findings, a low intake of fruit and vegetable was reported among the general population of Dikgale HDSS [35].

A high rate of unemployment was observed in this study, as was reported in the general population in the same community [35]. This high unemployment rate coupled with stigma related to HIV infection, may predispose the HIV infected people to high levels of stress. Given that chronic stress predicts the occurance of CVD [36], interventions aimed at creation of jobs among HIV infected people may therefore play an important role in indirectly reducing the risk for CVD, by alleviating stress associated with unemployment.

Among metabolic risk factors, hypertension was observed in nearly a quarter of participants, consistent with a study in Senegal [37]. However other studies that included younger populations reported lower prevalence rates [6, 38–40]. The prevalence of hypertension was similar between males and females. Of the 42 HIV infected participants with a history of hypertension and were on medication, approximately 60 % had their blood pressure controlled, while 40 % had a raised blood pressure despite being on medication. Factors contributing to the uncontrolled blood pressure include poor adherence to treatment, high salt intake and large alcohol consumption (>3 drinks per day) [41]. Older age was a predictor of hypertension, and people with a low viral load were more likely to be hypertensive. Diabetes mellitus (DM) in males and females was low as reported among people on ART in other African studies [5, 38, 42, 43]. Unlike our study findings, a high prevalence of diabetes mellitus was reported among HIV negative and ART naïve HIV positive people in Dikgle HDSS [22], probably due to an older age than that of our cohort. The overall prevalence of diabetes mellitus among South Africans aged 30 years and above is estimated at 9 % equaling a 9.3 % prevalence for United States of America [44]. South Africa is undergoing epidemiological transition and prevalence of diabetes mellitus is thus expected to rise in future [45]. The prevalence of obesity (10 %) and abdominal obesity (22 %) in our study differed from prevalences reported from Africa [6, 46] and Asia [40, 47]. According to Crum-Ciaflone et al. (2008) [48], the variations in obesity and abdominal obesity may partly be explained by differences in ART duration and the different cut-off values for the BMI and waist circumference used in various studies. Females in our study were more likely to be obese than males, although the difference was not significant due to the small number of participants [49]. The prevalence of metabolic syndrome was low and similar in males and females. Reported prevalences of metabolic syndrome vary, possibly resulting from its heterogeneous nature and variations in prevalence of its components [39, 50–52].

The prevalence of hypertriglyceridaemia was low, maybe due to the NNRTI based regimen used. The use

of a stavudine containing regimen in certain other studies [40, 53, 54] may explain why a prevalence more than twice as high was observed in those studies. In our study high concentrations of TG, were more common in males than in females, but gender could not predict a high TG concentration in multivariate analysis. Abdominal obesity and older age were significant predictors of hypertriglyceridaemia as previously reported [55]. High TG levels in our study were associated with low intake of fruit and vegetables as has been reported in other studies [32, 33]. Although ART increases lipid levels, HDL-C may not return to normal levels thus high prevalence of low HDL-C has been observed in this and other studies [6, 38, 39]. Older age and high viral load were independent predictors of a low HDL-C concentration. These results show the importance of suppressing the viral load to minimize the risk for developing low HDL-C concentration when people on ART become older. Hypercholesterolaemia was present in a third of the participants which is similar to results from other studies [5, 6, 38, 39]. Visceral lipohypertrophy increased the likelihood of having a high total cholesterol concentration. None of our participants was using lipid lowering drugs, possibly accounting for the high proportions of dyslipidaemia. Lipid ratios are regarded as better predictors of CVD than individual lipids [56, 57]. A high ApoB/ApoA ratio was present in nearly 50 % of participants a possible reflection of high prevalence of low HDL-C. Its association with high viral load in our study predicts the benefits of effective suppression of virus load. Nearly 15 % of participants mostly males had a high TC/HDL-C ratio. Older age, longer ART duration and male gender, were predictors of a high TC/HDL-C ratio. These findings have important implications as these variables may increase the risk of developing CVD. A high TG/HDL-C ratio present in nearly a fifth of the participants mostly males was associated with abdominal obesity.

An earlier study conducted among ART naïve HIV infected and HIV negative people in Dikgale Health and Demographic Surveillance System site reported a high prevalence of CVD risk factors which was similar between the two groups [22], but a worse CVD risk profile compared to that of people on ART in the present study. Except for hypercholesterolaemia, the prevalence for most CVD risk factors in the present study is lower than that of ART naïve HIV infected and HIV negative in that study. A high prevalence of CVD risk factors among the general population in this rural area has previously been reported [58]. The fact that people on ART have monthly consultations at clinics and are informed of healthy lifestyles needed to manage co-morbidities could possibly explain the lower prevalence of CVD risk factors compared to ART naïve and HIV negative from same locality. Furthermore majority of participants (96 %) in the present study were receiving NNRTI based regimen associated with lesser CVD risk compared to PI based regimen [27].

Our study found a 73.8 % level of agreement between the Framingham and DAD risk estimation equations, which was similar to an agreement level of 77.4 % reported by Nery et al. (2013) [59]. Despite this level of agreement observed in our study, the Framingham equation underestimated the risk for CVD in 43 of the 164 participants, when compared to the DAD equation. These results suggest that the use of the Framingham equation in people infected with HIV receiving ART may lead to the exclusion of some individuals to benefit from more aggressive CVD prevention. The low rate of CVD risk as measured by the Framingham equation in our study may be that our cohort was relatively young and predominantly composed of females. Similar to our findings, literature suggests that the Framingham equation underestimates the risk of CVD in South Africans [60]. However, contrary to our findings, the Framingham equation overestimated the 10-year CVD risk among HIV infected Thais [9] and Brazilians [59] when compared to DAD equation.

Our study is one of the first that assessed a wide range of CVD risk factors as well as determined the 10-year CVD risk for persons with HIV infection on ART in a rural population in South Africa. Limitations of our study include its cross-sectional design, therefore we cannot conclude that the associations between covariates and CVD risk factors are causal. Information on tobacco use, alcohol use, physical activity and fruit and vegetable intake was obtained using the WHO STEP questionnaire. This is considered to be a reliable instrument, however recall bias may have influenced the results. Non-random sampling was used to recruit participants. However, recruitment was conducted for a whole month cycle, giving all patients collecting their medication equal opportunity to participant. Follow up on participants who failed to turn up for the agreed scheduled date helped to reduce selection bias. While our sample may not be representative of the whole population of HIV infected South Africans receiving ART our study provides valuable and useful information for comparison with other published studies from both developing and developed countries. We also acknowledge the small sample size of our study.

Conclusion

The CVD risk factors were common among this rural population on ART. While 6.7 % of participants had a moderate risk, none had a high risk of developing a CVD event in the next 10 years, according to Framingham risk score

equation. However, the high proportion of people with a moderate to very high risk of developing CVD in the next 5 years according to DAD risk score equation clearly represents a considerable health burden that can possibly be rectified by increasing educational programs on CVD prevention for people on ART. Whilst the DAD equation was not developed for Africans, its use instead of the currently used Framingham risk table in people on ART in South Africa, to identify people with a high CVD risk may help to reduce the burden of CVD on the health system. There is a need however to develop and evaluate a race/ethnicity-specific CVD risk estimation tool for HIV infected Africans.

Authors' contributions

MA, JPV contributed substantially to the conception and design of study. FM collected data, performed analysis, interpretation of data and drafted the manuscript. MA, JPV and RC were involved with the interpretation and presentation of data, and the critical review of the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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