The metabolic syndrome and renal function in an African cohort infected with Human Immunodeficiency Virus for at least 5 years

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It all starts here ™

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PREFACE

This dissertation consists of five chapters and forms part of the degree Magister Scientiae in Physiology. Chapter One includes a background and motivation. Chapter Two consists of a literature review regarding human immunodeficiency virus infection, antiretroviral therapy, the metabolic syndrome, renal function and aims, objectives and hypotheses. Chapter Three describes the detailed methodology of the study. Chapter Four consists of a research article written according to the instructions of the Journal of Acquired Immunodeficiency Syndrome (JAIDS). The final chapter (Chapter Five) summarises the main findings of the study, and includes a reflection on the hypotheses. References are provided at the end of each chapter according to the style of the JAIDS.

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CONTRIBUTION OF AUTHORS

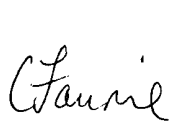
The following persons contributed to the compiling and completion of the dissertation:

Ms. E Phalane:
Responsible for the literature review, statistical analyses, interpreting the results and writing up all chapters of this dissertation including the research article.

Prof. CMT Fourie:
Provided professional supervision and guidance for the proposal and writing of the dissertation.

Prof. AE Schutte:
Provided intellectual guidance on statistical analyses and technical input, proposal and writing of the dissertation.

This is a statement from the authors confirming their individual contribution to the study and their permission that the manuscript may form part of this dissertation.



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SUMMARY

Motivation

The human immunodeficiency virus (HIV) is increasingly prevalent in South Africa, with approximately 6.12 million people living with HIV. As a result of the high infection rate, South Africa has the largest antiretroviral therapy (ART) roll-out programme, providing ART to approximately 3.1 million people. The introduction of ART has revolutionised the era of HIV in reducing the morbidity and mortality associated with HIV or acquired immunodeficiency syndrome (AIDS) opportunistic disease. However, after the introduction of ART, several studies reported metabolic derangements and renal disease with the use of ART.

The metabolic syndrome (MetS) is frequently reported in HIV-infected individuals and this population may be at higher risk due to the combination of HIV infection, ART and traditional risk factors.

Renal disease has also been highlighted in people living with HIV. HIV infection may directly infect the glomerular epithelial cells and podocytes, inducing renal injury. The ART is also potentially nephrotoxic and may augment the effect exerted by HIV and pre-existing kidney diseases.

The MetS and kidney disease have been reported in HIV-infected individuals, however, it has not been fully elucidated how kidney function is affected by HIV, ART and the MetS. Apart from HIV, the MetS and kidney disease have important public health implications as both are associated with increased risk of cardiovascular disease. As a result, these comorbidities may complicate the progression and management of HIV. Studies reporting on the combined effects of HIV, the MetS and renal function among the African population are scant.

Aim

In this study, we therefore determined the prevalence of the MetS and the association thereof with renal function in an African cohort infected with HIV for at least five years.

Methodology

We included 114 HIV-infected and 114 HIV-free participants matched for age, sex and locality. This is a sub-study of the Prospective Urban and Rural Epidemiological study (PURE) in South Africa, as approved by the Health Research Ethics Committee of the North-West University (approval number: NWU-00035-16-S1 and NWU-00016-10-A1). Of the 114 HIV-infected participants, 87 were infected for 10 years and 27 for 5 years. The HIV-infected participants on ART were using first-line regimen, namely a fixed-dose combination of tenofovir, efavirenz and emtricitabine. Anthropometric measurements such as height, weight and waist circumference (WC) were measured according to standardised procedures prescribed by the International Society for the Advancement of Kinathropometry, while body mass index (BMI) was also calculated. Duplicate brachial blood pressure (BP) measurements were performed in a sitting position, at an interval of five minutes, using the validated OMRON M6 (Omron Healthcare, Kyoto, Japan). We also performed duplicate central systolic blood pressures (cSBP) with the Sphygmocor XCEL device (Atcor Medical Pty. Ltd., Sydney, Australia), with the participant in the supine position.

Blood collection was done by a qualified nurse after an overnight fast. We performed biochemical analysis for serum glucose, total cholesterol, high-density lipoprotein cholesterol, low density lipoprotein cholesterol, γ-glutamyl transferase and C-reactive protein. Mid-stream spot urine was used to determine albumin and creatinine levels and we calculated creatinine clearance (CrCl), estimated glomerular filtration rate (eGFR), and calculated urinary albumin-creatinine ratio (uACR). HIV status was determined from whole blood, according to the South African Department of Health guidelines. We defined the MetS using the criteria of the International Diabetes Federation.

Results

The prevalence of the MetS was lower in the HIV-infected participants (77.3% of the HIV-infected were on ART) as compared to their uninfected counterparts (28% vs. 44%, $p=0.0013$). The HIV-infected group had lower BMI and WC (all $p<0.001$), as well as lower cSBP and branchial blood pressure (all $p\leq 0.021$). With regard to renal function, the CrCl was higher in the HIV-infected participants compared to their uninfected counterparts ($p<0.001$). There was no difference in eGFR and uACR in the

two groups (p=0.99 and p=0.72 respectively). When adjustment was done for WC, the cSBP (p<0.001) and brachial blood pressure remained significant (p=0.05), and CrCl, eGFR and uACR were similar (p>0.27).

With regard to the use of ART, the HIV-infected participants taking ART also presented with lower cSBP and brachial blood pressures (all p=0.01). CrCl was lower in the HIV-infected participants taking ART than the uninfected participants (p=0.002), whereas eGFR and uACR were similar between the two groups (all p>0.11).

When we compared HIV-infected and uninfected participants with the MetS, the blood pressures were similar (all p>0.46). Of those with the MetS, 46% and 17% of the HIV-infected and the uninfected participants respectively had microalbuminuria. The HIV-infected participants and those with the MetS had 43% higher uACR compared to the uninfected participants with the MetS (p=0.032). CrCl was lower in the HIV-infected group with the MetS than the uninfected group with the MetS (p=0.05), but eGFR was not different between these two groups (p=0.21).

General conclusion

HIV-infected participants with the MetS had a twofold higher uACR compared to their uninfected counterparts, despite similar age and sex distribution and a lower prevalence of the MetS. These findings suggest that a combination of the MetS and HIV may alter glomerular permeability. The presence of the MetS and renal dysfunction may therefore increase the risk of cardiovascular disease in the HIV-infected population.

Keywords

Human immunodeficiency virus, metabolic syndrome, renal function, kidney disease, tenofovir, South Africa.

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LIST OF ABBREVIATIONS

ABC:	Abacavir
AIDS:	Acquired Immunodeficiency Syndrome
Ang II:	Angiotensin II
Apo B:	Apolipoprotein B
ART:	Antiretroviral therapy
ATP III:	Adult Treatment Panel III
AZT:	Zidovudine
BMI:	Body mass index
BP:	Blood pressure
β:	Beta
CCR-5:	Chemokine co-receptor type-5
CCXR-4:	Chemokine receptor type-4
CI:	Confidence interval
CKD: EPI	Chronic Kidney Disease: Epidemiology Collaboration
cm:	Centimetres
CrCl:	Creatinine clearance
CRP:	C-reactive protein
cSBP:	central Systolic blood pressure
CVD:	Cardiovascular disease
DBP:	Diastolic blood pressure
DNA:	Deoxyribonucleic acid
d4T:	Stavudine
EFV:	Efavirenz
eGFR:	estimated glomerular filtration rate
ETB:	Extra pulmonary tuberculosis

FDC:	Fixed-dose combination
FTC:	Emtricitabine
HbA1c:	Glycated haemoglobin
HBV:	Hepatitis B virus
HDL-c:	High density lipoprotein cholesterol
HIV:	Human Immunodeficiency syndrome
IDF:	International Diabetes Federation
IFN:	Interferon alpha
IL-1:	Interleukin 1
IL-6:	Interleukin 6
IN:	Integrase
Kg:	Kilogram
Kg/m^2:	Kilograms per meter squared
LDL-c:	Low density lipoprotein cholesterol
LPV/r:	Lopinavir/ritonavir
MAP:	Mean arterial pressure
MCP-1:	Monocyte chemoattractant protein-1
MetS:	Metabolic syndrome
mm^3:	Millimetre cube
ml/min:	Millimetre per minute
mg/mmol:	Milligram per millimole
mmHg:	Millimetre Mercury
mmol/l:	Millimole per litre
mtDNA:	Mitochondrial deoxyribonucleic acid
N:	Number
Na^+:	Sodium

NAFLD:	Non-alcoholic fatty acid liver disease
NEF:	HIV-1 integrase factor
neg:	Negative
NNRTIs:	Non-nucleoside reverse transcriptase inhibitors
NRTIs:	Nucleoside reverse transcriptase inhibitors
NVP:	Nevirapine
OGGT:	Oral glucose tolerance test
p:	Probability value
PCOS:	Polycystic ovary syndrome
PIs:	Protease inhibitors
pos:	Positive
PP:	Pulse pressure
RNA:	Ribonucleic acid
RT:	Reverse transcriptase
PURE	Prospective Urban and Rural Epidemiological study
SA:	South Africa
SADoH:	South African Department of Health
SCr:	serum creatinine
SBP:	Systolic blood pressure
SD:	Standard deviation
SU:	Surface glycoprotein
TB:	Tuberculosis
TC:	Total cholesterol
Tenofovir:	Tenofovir disoproxil fumarate
TG:	Triglycerides
TGF-β:	Transforming growth factor-β

TM:	Transmembrane protein
TNF-α:	Tumour necrosis factor α
uACR:	Urinary albumin: creatinine ratio
U/l:	Units per litre
USA:	United States of America
µmol/l:	Micromole per litre
VLDL:	Very low density lipoprotein
vs:	Versus
WC:	Waist circumference
WHO:	World Health Organization
WHR:	Waist-to-hip ratio
3TC:	Lamivudine

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CHAPTER 1

Background and motivation

1.1. Background

The Human Immunodeficiency Virus (HIV) continues to be a global epidemic, with the latest figures indicating an estimated 38.8 million people infected worldwide. Sub-Saharan Africa contributes 75.4% of new HIV infections globally.[1] According to Statistics South Africa, the prevalence of HIV was estimated at 6.19 million in 2015,[2] making South Africa (SA) the country with the highest HIV infection and roll-out programme of antiretroviral therapy (ART) in the world.[3] Despite decreasing the morbidity and mortality associated with the Human Immunodeficiency Virus / Acquired Immune Deficiency Syndrome (HIV/AIDS) opportunistic diseases, several studies suggest that the use of ART increases the risk of developing hypertension,[4-6] abnormal fat distribution,[5,7-9] dyslipidaemia[10,11] and hyperglycemia,[4,12] which constitute the criteria for the metabolic syndrome (MetS).[13] With the increase in the prevalence of HIV infection and the use of ART, the adverse effects of ART on MetS are expected to increase.

The coexistence of the MetS and kidney disease has been highlighted in HIV-infected individuals.[14] It is suggested that kidney disease observed in HIV-infected patients not only reflects the effect of HIV infection and its treatment, but also the presence of hypertension and diabetes.[15] Both MetS and kidney disease are major risk factors for cardiovascular disease (CVD)[16,17] and the risk may be augmented in HIV-infected persons with the coexistence of these comorbidities. Therefore, due to the use of ART in HIV patients, the risk of developing CVD may also increase,[18] making MetS and kidney disease an important health concern. Moreover, CVD is a major cause of morbidity and mortality in HIV-infected patients.[19] Therefore, understanding the development of the MetS and its association with renal function in this already chronically ill HIV-infected population is essential.

The prevalence of the MetS in HIV-infected patients has been widely documented in the literature.[4,20-22] However, it remains controversial, with some reporting a higher[21] and others a lower prevalence.[23] This observation is also debatable when compared with the general population.[20,24] Findings in a study by Fourie et al.[25] conducted among black rural and urban South Africans, showed that the prevalence of MetS was 11.5% using the National Cholesterol Education Programme Adult Treatment Panel III criteria (ATP III), and 22.6% using the International Diabetes Federation criteria (IDF)

amongst HIV-infected, ART-naive participants. Tesfaye and others.[26] reported a higher prevalence of 15.6% vs. 12.3%, and 22.6% vs. 17.9% of HIV infection compared to the general population of Southern Ethiopia using ATP III and IDF criteria respectively. Mbunkah and collegues.[4] reported a prevalence of 8% among the general population of the South-West region in Cameroon, 15.6% for HIV-infected ART-naive and 24.4%, among HIV-infected individuals taking ART. Krishman et al.[22] conducted a prospective study on an HIV-infected cohort before and after ART initiation. They reported the prevalence of MetS at 47% for whites, 27% for blacks and 24% for Hispanics at ART initiation, and the prevalence increased after ART initiation for those without MetS at baseline in all ethnic groups. This group was also characterised by being of older age.[22]

In the general population, old age and female gender are significantly associated with the risk of developing the MetS.[26-28] Old age is an important risk factor for developing MetS in both HIV-infected and uninfected;[20] and age is an important indicator for metabolic diseases and CVD.[20] The HIV population is growing older[29] and seems to develop CVD risk factors earlier than the HIV-uninfected.[30]

The well documented lipid metabolic changes in HIV-infected, ART-naive individuals include elevated triglycerides (TG) and low high-density lipoprotein cholesterol (HDL-c).[20,25] The lipid changes with the use of ART include elevated TG,[31-33] low-density lipoprotein cholesterol (LDL-c),[34] and total cholesterol (TC),[35,36] and decreased HDL-C.[37] Protease inhibitors (PI) are frequently associated with lipid alterations, accounting for 70% to 80% of lipid alteration in individuals on ART.[10] The use of stavudine and lamivudine is associated with an increase in the TG, LDL-c and decrease in HDL-c.[38]

A study conducted by Mbunkah et al.[4] showed that hyperglycaemia is associated with MetS in the HIV-infected population. A cross-sectional study of an HIV-infected population reported 46.3% of the participants with higher glucose levels and that this was an important risk factor for developing the MetS.[12] Apart from the standard MetS criteria, it has also been found that serum C-reactive protein (CRP) levels are higher in the HIV-infected population, as expected,[25] indicating an elevated level of inflammation. When an HIV-infected population additionally presented with the MetS, the population had higher levels of CRP compared to their counterparts.[23] In a cross-sectional study by Guimareesa et al.[39], 53% of people with MetS treated for HIV had

higher levels of CRP, compared to 26% of people not treated with MetS, suggesting that ART may potentially increase CRP.

Although the prevalence of the MetS is common in HIV-infected taking ART and not taking ART, the former presents frequently with elevated body weight and waist circumference.[5] In the latter study, elevated waist circumference and body weight were common with the use of PIs and NNRTIs.[5] As with HIV-uninfected population, the HIV-infected individuals also have a higher prevalence of obesity especially in women than men.[7] However, another study reported that the HIV-infected participants using ART have lower BMI (15.9%) compared to the HIV-uninfected population (3%).[6]

High blood pressure has also been noted in HIV-infected patients and in addition, a cross-sectional study on an HIV population with and without treatment concluded that hypertension is an important risk factor for the development of MetS.[4] Schutte et al.[40] found that over a five-year period, HIV infections were associated negatively with an increase in blood pressure in Africans. The development of hypertension is often associated with the use of PIs in HIV treated patients.[5] On the other hand, a systemic review and meta-analysis of studies including 29 755 individuals in the adult black population living in Sub-Saharan Africa, reported that the HIV-infected, ART-naive population had lower systolic and diastolic blood pressure compared to the HIV-uninfected.[40] In the latter study, the use of ART was not associated with blood pressure.[40]

With respect to lifestyle factors and the MetS, it is well documented that unhealthy lifestyle behaviours might increase the incidence of the MetS in HIV-infected patients.[41,42] Samaras et al.[23] reported an association between current smoking and decreased physical activity as risk factors for developing the MetS in HIV-infected individuals. In a cross-sectional study done among a treated HIV-infected population, higher alcohol use and smoking were observed in those with the MetS; however, it was not statistically associated with MetS.[38]

In addition to the MetS, kidney disease is also becoming increasingly prevalent in the HIV-infected population. In particular, an independent relationship has been highlighted between HIV infection and renal impairment.[43] Kidney disease in HIV-infected patients is commonly characterised by higher urinary excretion of protein and

elevation of creatinine.[44-46] The pathology of kidney disease in HIV-infected individuals may include ART nephrotoxicity, HIV infection itself and metabolic factors such as hypertension and diabetes.[14,47,48] Although studies have reported improved immune function and renal improvement with the use of tenofovir,[49] this drug is also potentially nephrotoxic.[50] Recent studies in Sub-Saharan Africa, including HIV-infected individuals taking ART, highlighted that 25% have decreased estimated glomerular filtration rate (eGFR) and 72% have microalbuminuria.[51] Thus, these morbidities might have consequences on the choice of ART regimens and monitoring of renal diseases.

1.2. Motivation

The South African population is faced with a triple burden of HIV, cardiovascular and metabolic diseases, thereby reducing the quality of life in the population.[11] Previous studies have reported on the MetS in HIV-infected individuals, but the information is mostly reported among populations in America[21,23,52] and Europe[20,31,33] where HIV-1 subtype B is the predominant cause of HIV infections.[53,54] In SA, the HIV-1 subtype C is mostly predominant.[53] HIV-1 is considered to be more virulent and pathogenic than HIV-2. The HIV-1 subtype C is more diverse, spreads quickly, and can be transmitted by numerous routes.[54]

HIV infection is also associated with an increasing burden of kidney disease, which may be exaggerated with the HIV treatment.[55] Limited studies in the HIV-infected have reported on the increasing prevalence of both the MetS and kidney diseases.[14] MetS and kidney disease are associated with an elevated risk of cardiovascular disease[17,56] and present a serious public health implication, especially on the progression and management of HIV.[55] As HIV-infected individuals have a higher risk of developing renal disease, hypertension, dyslipidaemia and diabetes,[57,58] understanding the development of the MetS with renal dysfunction in this population is essential. The burden of the kidney disease is expected to increase with the increasing occurrence of the MetS, HIV and the use of ART. Although accumulating evidence suggests increased cardiometabolic risk among the HIV-infected population using ART, there are still contradictory findings regarding the prevalence of the MetS in the general population, the HIV-infected population, and the influence of ART – especially over the long term. Several studies have reflected on the existence of the MetS and kidney diseases in HIV-infected people,[4,47] however, studies emphasising the combination of

the MetS and HIV on renal function are scant. Therefore, to our knowledge, this study is the first to investigate the MetS and the association thereof with renal function in a unique South African population infected with HIV for at least five years.

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CHAPTER 2

Literature review

2. Introduction

The metabolic syndrome (MetS) is associated with increased risk of developing kidney disease and cardiovascular disease,[1,2] which consequently complicates the management of the human immunodeficiency virus (HIV).[3] HIV-associated nephropathy, which is commonly reported in HIV-infected individuals is also linked with the prompt progression of HIV to acquired immune deficiency syndrome and mortality.[4,5] Hence, it is vital to identify kidney dysfunction early in this population.

2.1 Human immunodeficiency virus and antiretroviral treatment

South Africa is a developing country facing a high burden of HIV infections.[6] In the face of having the highest HIV prevalence, it is also the country with the largest ART roll-out programme in the world.[7]

The HIV life cycle is a continuous process wherein the virus binds and fuses with the CD4 T-cell receptor to gain entry in to the CD4 T-lymphocytes.[8] HIV replicates itself inside the CD4 T-lymphocyte, and this lymphocyte responds by activating the immune response against the virus.[9] As this viral replication persists, the virus will affect and destroy the majority of the CD4 T-lymphocytes to such a point where the body's immune system can no longer adequately perform its functions.[8] At this stage the viral replication outstrips the immunological response and ability to produce CD4 T-lymphocytes.[8] The individual is then susceptible to opportunistic diseases and infection as the immune system weakens and CD4 cell count decreases, and this stage is known as AIDS.[9,10] The use of treatment at this stage may result in immune reconstitution.[9] The use of ART has therefore produced appreciable rewards in reducing the mortality associated with HIV/AIDS opportunistic diseases.[11]

However, ART is also associated with a higher prevalence of hypertension,[12,13] abnormal fat distribution,[14] dyslipidemia[15] and hyperglycemia[12,16] in the HIV-infected population. The main focus of the South African health system is treating and curing acute and emergency diseases, whereas non-communicable diseases do not receive enough consideration.[17] These co-morbidities associated with ART make the management of HIV infection in this population costly and multifaceted.[18,19] The majority of HIV-infected patients on ART in SA are simultaneously taking treatment for more than one non-communicable disease such as hypertension and diabetes in

addition to the ART.[20] In a developing country with limited resources, this high burden of HIV and its co-morbidities is placing a significant burden on the health system and finances for the management of the HIV infection and its co-morbidities.[19]

It is essential to understand the development of co-morbidities such as metabolic and cardiovascular diseases in the HIV-infected population, in order to employ effective preventative and treatment strategies.

2.1.1. HIV and basic virology

The human immunodeficiency virus was discovered in the 1980s[21] and is transmitted either through unprotected sex, mother to child transmissions, needle injection or contact with HIV-infected blood.[22,23] HIV belongs to a family of human retroviruses known as *Retroviridae, of the* genus *Lentivirus.*[24,25] It is divided into HIV-1 and HIV-2. These viruses vary in their genome structure but has a similar basic structure.[24,26] HIV-1 is the most common cause of HIV infections worldwide. Three varied genetic groups are classified for HIV-1, namely M (major group), O (an outlier group) and N (non-M/non-O group).[27-29] The M group is the predominant HIV causative worldwide.[29] HIV-1 is further divided into three subtypes A, B and C.[29,30] The subtype C is the most prevalent strain in Southern African countries, Ethiopia and India.[29] Subtype A is the second leading causative strain and is mostly reported in Africa and North America, with subtype B being the third leading cause of HIV-1 cases predominantly in America, Western Europe and Australia.[29,31] HIV-2 cases are mostly reported in western Africa and extend to other countries such as Europe and India.[29,31] HIV-1 is considered more virulent and pathogenic than HIV-2.[32] The HIV-1 subtype C is more diverse, and spreads quickly because it can be transmitted by various routes.[32] Its rapid spread can also be explained by the fact that the subtype C has numerous NF-kappa B sites as compared to the non-subtype C.[33,34]

2.1.2. HIV replication cycle

The HIV replication cycle is divided into seven steps, namely entry/fusion, reverse transcription, integration, transcription, translation, assembly, and release and maturation (see Figure 2.1).[35]

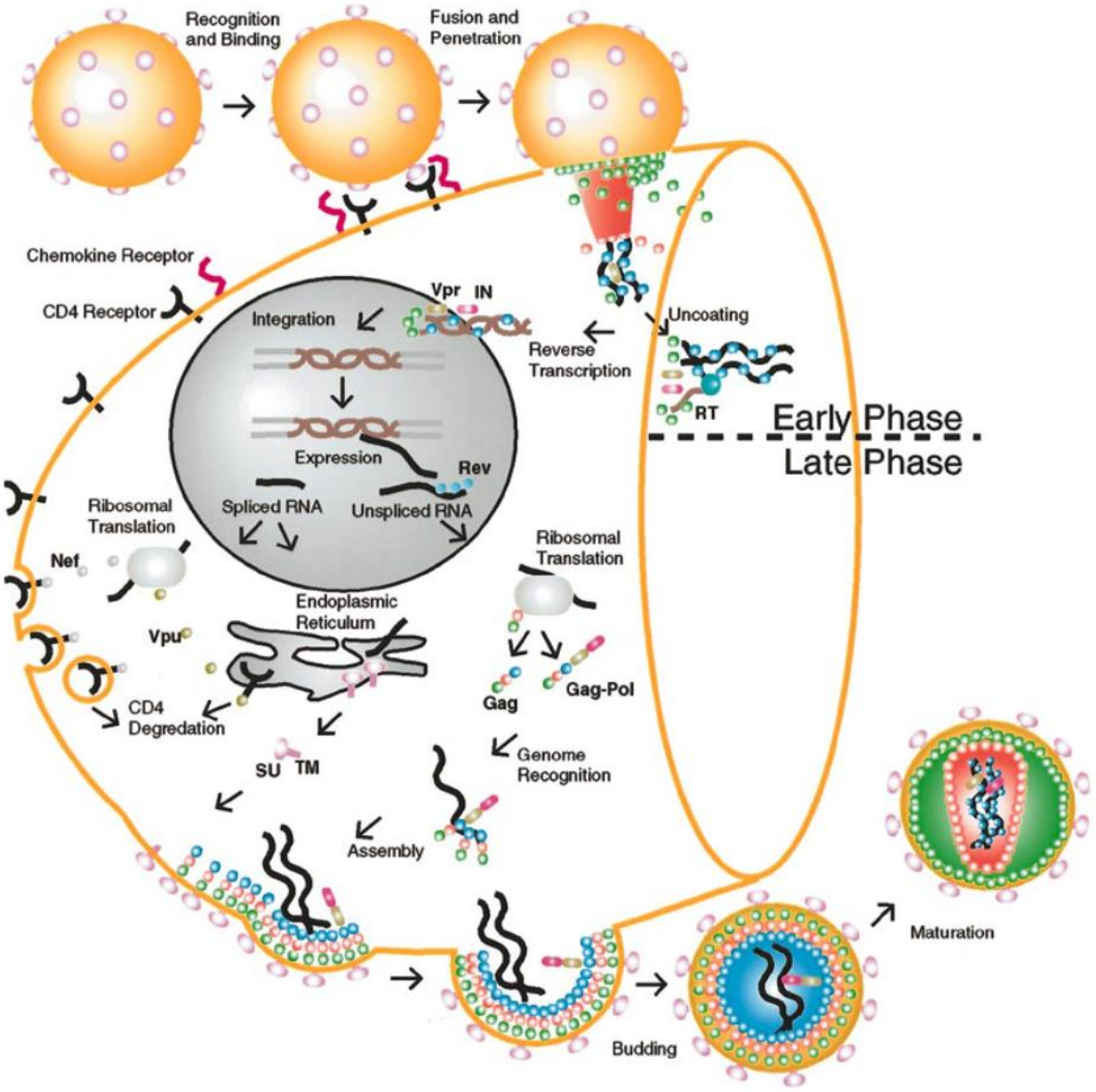

Figure 2.1: The HIV replication cycle (Turner and Summers,1999)[35].

IN, Integrase, RNA, Ribonucleic acid, NEF, HIV-1 integrase factor, TM, Transmembrane protein, RT, reverse transcriptase, SU, Surface glycoproteins.

The virus requires a host to survive and replicate.[30] HIV enters the body using an envelope protein on its outer membrane by binding to the CD4 T-helper cells.[35] The chemokine co-receptor type-5 (CCR-5) and CXC chemokine receptor type-4 (CXR-4) facilitate the completion of the binding of the viral particle to the CD4 T-helper cells. After the HIV has bound to the CD4 T-lymphocytes, CCR-5 and CXR-4, the viral particles are released inside this target cell.[36] The enzyme, reverse transcriptase copies the sequence of the ribonucleic acid (RNA) strands inside the virus, to form

viral deoxyribonucleic acid (DNA).[37,38] The viral DNA enters the nucleus of the host with assistance of the integrase enzyme. This integrated virus is referred to as a provirus. New viral RNA is formed by the provirus DNA by a process called transcription. The viral RNA relocates out of the infected cells' nucleus. The formation of viral proteins and enzymes is then initiated by the viral RNA coding.[39-41] The viral proteins, enzymes and RNA relocate to the outer cell's membrane to form an outgrowth. The outgrowth is released from the membrane and releases new virus particles, thereby spreading to other parts of the body. The infected cell will be destroyed, and the cycle will repeat itself continuously.[38] Consequently, this will lead to a higher viral load and a lower CD4 cell count in the body. The CD4 T-lymphocytes (Helper T cells) are white blood cells, which play an essential role in cell-mediated immunity.[42] Their functions include secretion of inflammatory markers such as interleukin-6 (IL-6)[43] and tumor necrosis factor-α (TNF-α), which respond to tissue damage or infection.[43] CD4 T-lymphocytes also play a role in T and B cell proliferation which is important in controlling immune responses.[42] The CD4 cell count expressed as cells/mm^3 is the measure of the immune status with regard to the progression of the HIV virus in the body.[9] Viral load is the measure of the HIV RNA viral load, and is expressed as copies/m.[2,9] Uncontrolled HIV replication results in the dysfunction of the immune response by infecting and destroying the CD4 T-lymphocytes, with the end result of thymus dysfunction.[44] The thymus is responsible for the generation and maturation of CD4 T-lymphocytes.[42] The chronic stimulation of the immune system is associated with an altered inflammatory status (increased C-reactive protein and IL-6) in the HIV-infected population.[42,45]

2.1.3. Prevalence of HIV

HIV infection continues to be a global epidemic, with an estimated 38.8 million people infected worldwide.[46] Sub-Saharan Africa adds 75.4% of the new HIV infections globally.[46] The death rate as a result of HIV/AIDS has reduced from 1.8 million in 2005 to 1.2 million in 2015 globally.[46] The prevalence of HIV in SA increased from an estimated 4.02 million in 2002 to 6.12 million by 2015.[6] This is mainly due to more people having access to treatment and thus longer lifespans.[47] The number of people living with HIV and taking ART has increased tremendously in both men and women. The global estimate of men and women living with HIV and taking ART in 2015 was 39% and 42.4% respectively.[46]

2.1.4. Treatment of HIV in South Africa

The introduction of ART in combating HIV has significantly improved the lives of HIV positive patients worldwide,[48] by decreasing morbidity and mortality caused by HIV/AIDS-related opportunistic diseases.[49] In SA, HIV treatment was introduced on 1 April 2004, after the government approved the comprehensive HIV/AIDS care, management and treatment for SA which was compiled by the Department of Health.[50-52] The South African ART roll-out programme in 2004 included less than two million HIV-infected people[51] and the number of people on ART has increased significantly to an estimated 3.26 million in the year 2015.[53] The number of HIV-infected patients taking ART is expected to increase with the on announcement given by the Minister of Health on the 10 May 2016 that all HIV-infected patients will initiate ART from 1 September 2016 irrespective of their CD4 cell count.[54] Given that SA already houses the largest ART roll-out programme globally, the financial constraint on the health system is expected to increase. The South African Minister of Finance, Pravin Gordhan, on February 2016 announced that the budget allocated for the Department of Health was R183.6 billion for the year 2015.[55]

Different ART regimens are used in developed and developing countries,[7] and it would be expected that the side effects of the therapy may differ between countries.[56] In SA, the choice of ART often depends on financial constraints and the availability of ART treatment to individuals.[57] The guidelines of ART have evolved from the past to the current. Some of the noted changes include a switch from the use of three separate pills to the new fixed-dose combination (FDC)[58] and an increase in the CD4 cell count that permits initiation of ART (see Table 2.1).[59,60]

The ART treatment used in SA is divided into a first, second and third-line regimen. These regimens are comprised mainly of non-nucleoside reverse transcriptase inhibitors (NNRTIs), nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors (PIs) as either three separate drugs or as fixed-dose combinations which are mostly preferred and recommended.[60] In 2012 the South African Minister of Health announced the switch from the use of three separate antiretroviral drugs to the recent, single and FDC tablet.[60] However the separate drugs are also still being used in SA.

Table 2.1: Change in the Antiretroviral Therapy Guidelines for people living with human immunodeficiency virus by the World Health Organization (WHO) and South African Department of Health (SADoH) over the years

Guidelines on when to start initiation of ART treatment per Organisation	
World Health Organization	**South African Department of Health**
2002[61]	
In resource-limited settings, ART should be initiated in HIV-infected adolescents and adults when they have: WHO stage IV of HIV disease (clinical AIDS), regardless of the CD4 count; WHO stages I, II or III of HIV disease, with a CD4 count below 200/mm^3; WHO stages II or III of HIV disease with TLC below 1200/mm^3.	At this stage it was still a battle to be won for the Health community as they were negotiating with Government to initiate ART in HIV-infected patients.
2004[62]	
Clinically advanced HIV disease: WHO Stage IV HIV disease, irrespective of the CD4 cell count; WHO Stage III disease with consideration of using CD4 cell counts < 350/mm^3 to assist decision-making. WHO Stage I or II HIV disease with CD4 cell counts < 200/mm^3	SA adopted the WHO guidelines.
2006[63]	
Adults: CD4 ≤ 200 cells/mm^3, WHO stage 2 or 3 & CD4 ≤ 200 cells/mm^3 or WHO stage 4 irrespective of CD4 cell count **Pregnant:** WHO stage 1 or 2, CD4 ≤ 200 cells/mm^3, WHO stage 3 & CD4 ≤ 350 cells/mm^3 or WHO stage 4 irrespective of CD4 cell count. **HIV/ TB co-infection:** In case of tuberculosis (TB) start at presence of active TB or CD4 ≤ 350 cells/mm^3	WHO recommendation were made also for resource limited countries such as South Africa
2009[64]	
Adults: CD4 ≤ 350 cells/mm^3 irrespective of clinical symptoms, WHO stage 1 and 2 after testing for CD4 cell count or WHO stage 3 &4 irrespective of CD4 cell count. **Pregnant:** CD4 ≤ 350 cells/mm^3 irrespective of clinical symptoms, WHO stage 1 & 2 after determining CD4 cell count or WHO stage 3 & 4. **HIV/ TB co-infection:** All patients with active TB, and start TB treatment followed by HIV treatment.	
2010[65,66]	
Adults: CD4 ≤ 350 cells/mm^3, WHO clinical stage 1 & 2 if CD4 ≤ 350 cells/mm^3 or stage 3 & 4 irrespective of CD4 cell count **Pregnant:** CD4 ≤ 350 cells/mm^3 irrespective of clinical symptoms or WHO stage 3 & 4 irrespective of CD4 cell count **HIV/ TB co-infection:** Presence of active TB irrespective of CD4 cell count or individuals who require HBV treatment irrespective of CD4 cell count	**Adults:** CD4 ≤ 200 cells/mm^3 irrespective of clinical stage, CD4 ≤ 350 cells/mm^3 or WHO stage IV irrespective of CD4 cell count **Pregnant:** All pregnant women irrespective of CD4 cell count or WHO stage **HIV/ TB co-infection:** All patients with active TB or multi-drug resistant/ extremely drug resistant irrespective of CD4 cell count.
2011[67]	
Adults: CD4 ≤ 350 cells/mm^3 **Pregnant:** CD4 ≤ 350 cells/mm^3	**Adults:** CD4 ≤ 200 cells/mm^3 **Pregnant:** CD4 ≤ 350 cells/mm^3

HIV/ TB co-infection: CD4 ≤ 350 cells/mm^3 start ART 2-8 weeks after anti-TB treatment	**HIV/ TB co-infection:** CD4 ≤ 350 cells/mm^3 start ART 2-8 weeks after anti-TB treatment
2013[11,68]	
Adults: CD4 ≤ 350 cells/mm^3 and WHO stage 3 & 4 or CD4 ≤ 500 cells/mm^3 regardless of WHO clinical stage **Pregnant:** All pregnant women **HIV/ TB co-infection:** individuals with active TB, HBV infection or severe chronic liver disease regardless of WHO clinical stage.	**Adults:** CD4 ≤ 350 cells/mm^3 irrespective of WHO clinical stage or WHO stage 3 & 4 irrespective of CD4 cell count **Pregnant:** All pregnant and breastfeeding women irrespective of CD4 cell count **HIV/ TB co-infection:** All types of TB (in patients with TB/HIV drug resistant or sensitive TB, excluding extra pulmonary TB) or patients with Cryptococcus meningitis or TB meningitis (defer ART for 4-6 weeks)
2015[59,60]	
Adults: ART be initiated in all adults living with HIV at any CD4 cell count. As a priority, ART should be initiated in all adults with severe or advanced HIV clinical disease (WHO clinical stage 3 or 4) and individuals with CD4 count ≤ 350 cells/mm^3. **Pregnant:** ART should be initiated in all pregnant and breastfeeding women living with HIV at any CD4 cell count and continued lifelong. **HIV/ TB co-infection:** ART should be initiated in all patients with TB irrespective of CD4 cell count and all patients with chronic liver disease irrespective of CD4 cell count	**Adults:** CD4 ≤ 500 cells/µl irrespective of clinical stage. Priorities should be those with: CD4 ≤ 350 cells/µl or Severe/advanced HIV disease (WHO clinical stage 3 or 4), regardless of CD4 count **Pregnant:** Start ART in all pregnant and breastfeeding on the same day irrespective of the CD4 cell count and WHO clinical stage. **HIV/ TB co-infection:** active TB disease (including drug-resistant & EPTB or Known hepatitis B viral (HBV) co-infection irrespective of CD4 cell count and WHO clinical stage.
2016 and the future	
On 10 May, 2015 the South African Minister of Heath, Aaron Motswaledi announced that all HIV-infected individuals will be initiated on ART irrespective of their CD4 cell by September 2016.	

WHO, World Health Organization; SADoH, South African Department of Health; HIV, Human immunodeficiency virus; TB, Tuberculosis; ART, Antiretroviral therapy; EPTB, Extra pulmonary tuberculosis; SA, South Africa.
WHO Stage 1, asymptomatic; Stage 2, mild symptoms; Stage 3, advanced symptoms; Stage 4, severe symptoms.

This switch to FDC is significant because it enhances the South African national ART roll-out programme as the use of one pill is associated with better adherence.[69,70] Purchasing one pill as opposed to the three separate have proven cost effective according to the Minister of Health.[58] The other advantages includes its efficacy,[71,72] acceptable dosing and uniform supply.[58] However, the use of the FDC is limited to specific patient groups in terms of the availability of stock and number of individuals on ART.[58] The special patients group to be given the FDC include the following by order of priority, first being all ART-naive patients newly starting ART, all the HIV positive pregnant women, recognised patients receiving stavudine, emtricitabine and efivarenz (d4T, FTC and EFV) and stable patients with tuberculosis (TB) co-infection and other co-morbidities.[58,73] With the recent announcement of the test-and-treat

programme more pressure will be placed on the supply and demand of the FDC because it is the preferred first line of defence as opposed to the three separate drugs.

This provision of the ART to all HIV-infected individuals would be beneficial in managing the spread and progression of HIV. However, the literature has already reported unfavourable effects of ART on metabolic disease and renal impairment.[15,74] It may be expected that the cardiovascular disease risk associated with such comorbidities will also increase in this population.

The Department of Health[60,68] recommends the first-line regimen as the first line of defence against the HIV replication; hence all HIV-infected patients are initiated with this regimen. The first-line regimen is comprised of two NRTIs and one NNRTI. The second-line regimen is given to patients that default and which become resistant to the virus. It includes two NRTIs with either one NNRTI or PI. The third-line regimen is initiated after the individual has failed to respond to both the first- and second-line regimens and mainly includes PIs (see Table 2.2).

Table 2.2: South African guidelines for the first- second- and third-line regimens (South African Department of Health, 2015)[60]

First-line regimen		
Population	**Drug**	**Comments**
Adolescents >15 years and weighing >40kg Adults All HIV/TB co-infection All HBV co-infection	TDF + 3TC (or FTC) + EFV provide as fixed-dose combination (FDC)	Replace EFV with NVP in patients: With significant psychiatric co-morbidity or intolerance to EFV Where the neuropsychiatric toxicity of EFV may impair daily functioning, e.g. night shift workers
Adults and adolescents on d4T	Change d4T to TDF (No patient must be on d4T)	Switch to TDF if virally suppressed and the patient has normal creatinine clearance, even if d4T well tolerated. If VL>1000 copies/mL, manage as treatment failure and consider switching to second-line.
Adolescents <15 years or weight <40kg	ABC + 3TC + EFV	If adolescent weight <40kg, align with paediatric regimen.
Contraindication	**Substitution drug**	**Comments**
Contraindication to EFV: Significant psychiatric co-morbidity Intolerance to EFV Impairment of daily function (shift workers)	TDF + FTC (or 3TC) + NVP or LPV/r	If CD4 <250 females and <400 males, give NVP 200mg daily for 2 weeks, then 200mg BD. CD4 ≥250 females and ≥400 males, use LPV/r 2 tablets 12 hourly.
TDF contraindication: Creatinine clearance of <50 mL/min	ABC+ 3TC + EFV (or NVP)	Renal disease or the use of other nephrotoxic drugs e.g. aminoglycosides MDR treatment
Second-line regimen		
First-line virological failure	**Drugs**	**Comments**
Failing on a TDF-based first-line regimen	AZT + 3TC + LPV/r AZT + TDF + 3TC + LPV/r (If HBV co-infected)	If non-adherent, address causes of nonadherence If the VL >1000 copies/mL at any point, intensify adherence and repeat VL in 2 months. If VL remains at >1000 copies/mL after 2 months, then switch to second-line regimen.
Failing on a d4T or AZT-based first-line regimen	TDF + 3TC (or FTC) + LPV/r	
Dyslipidaemia (total cholesterol >6 mmol/L) or diarrhoea associated with LPV/r	Switch LPV/r to ATV/r	
Anaemia and renal failure	Switch to ABC	
Third-line regimen		
Failing any second-line regimen Decision should be based on expert consultation and genotype resistance, and supervised care	Most likely regimens may contain: Raltegravir, Darunavir/Retravirine adjusted according to genotype interpretation and patient history	An expert panel will manage patients failing on second-line therapy. The drugs for third-line will be managed centrally. Should take into account prior exposure and predictable mutations.

HIV, Human immunodeficiency virus; TB, Tuberculosis; HBV, Hepatitis B virus; TDF, Tenofovir; FTC, Emtricitabine; 3TC, Lamivudine; d4T, Stavudine; AZT, Zidovudine; ABC, Abacavir; NVP, Nevirapine; EFV, Efavirenz; LPV/r, Lopinavir/ritonavir; FDC, Fixed-Dose combination; VL, Viral load; BD, twice daily.

2.2. Metabolic syndrome

2.2.1. Definition of the metabolic syndrome

The MetS is defined as a constellation of several risk factors of cardiovascular disease in one individual such as obesity, dyslipidemia, high blood pressure and hyperglycemia.[75-77] The Mets is a complex disorder and a multifactorial syndrome.[78,79] Obesity and insulin resistance are reported as the major driving forces of the MetS, due to the higher and increasing prevalence of these individual components of the MetS.[80-82] Other reported factors that play a role include ageing, lack of exercise, hormonal fluctuation and proinflammatory profiles.[78,79]

Different criteria exist for defining the MetS such as the International Diabetes Federation (IDF)[80], National Cholesterol Education Program Adult Treatment Panel III (NCEP: ATPIII)[83], World Health Organization (WHO)[83], American Association of Clinical Endocrinologist (AACE),[84] and European Group for Study of Insulin Resistance (EGIR)[85] and JIS (Joint Interim Statement).[86] Many epidemiological surveys on the MetS use the NCEP: ATP III in conjunction with IDF and WHO.[84,85,87] Nevertheless in 2006 a new world-wide definition was produced as part of a Consensus Statement from the IDF (see Table 2.3).[80]

The IDF critique is unique because it has gender and ethnic-specific values for both males and females. It has not included ethnic-specific waist circumference cut-offs for Sub-Saharan Africans, thereby suggesting use of the European cut-offs. This is important because the waist circumference differs significantly by ethnicity and sex.[80,88,89] In South Africa, several studies have suggested optimal cut-off values for men and women among black South Africans.[90-92] However, the IDF is considered the most reliable definition of the MetS; as it includes combination of both WHO and ATP definitions of the MetS.[93]

Table 2.3. The International Diabetes Federation Consensus Criteria for the Metabolic Syndrome[80]

Absolutely required	Central obesity (defined by waist circumference*): 94 cm for men, 80 cm for women with ethnic specific values **Europids**** M (≥ 94 cm) and W (≥ 80 cm) In the USA, the ATP III values (102 cm men; 88 cm women) are likely to continue to be used for clinical purposes **South Asians** M (≥ 90 cm) and W (≥ 80 cm) , Based on a Chinese, Malay and Asian-Indian population **Chinese** M (≥ 90 cm) and W (≥ 80 cm) **Japanese***** M (≥ 90 cm) and W (≥ 80 cm) **Ethnic South and Central Americans** Use South Asian recommendations until more specific data are available **Sub-Saharan Africans** Use European data until more specific data are available **Eastern Mediterranean and Middle East (Arab) populations** Use European data until more specific data are available
Criteria	Obesity, plus two of the four criteria below
Hyperglycaemia	(FPG) ≥ 100 mg/dL (5.6 mmol/L), or previously diagnosed type 2 diabetes If above 5.6 mmol/L or 100 mg/dL, OGTT is strongly recommended but is not necessary to define presence of the Syndrome.
Dyslipidaemia	≥ 150 mg/dL (1.7 mmol/L) or specific treatment for this lipid abnormality
Dyslipidaemia (second, separate criteria)	HDL-c: < 40 mg/dL (1.03 mmol/L) in men < 50 mg/dL (1.29 mmol/L) in women or specific treatment for this lipid abnormality
Hypertension	≥ 130 mmHg systolic or ≥ 85 mmHg diastolic or on treatment

* If BMI is >30kg/m², central obesity can be assumed and waist circumference does not need to be measured. ** In future epidemiological studies of populations of Europids origin, prevalence should be given using both European and North American cut-points to allow better comparisons. *** Originally different values were proposed for Japanese people but new data support the use of the values shown above. IDF, International Diabetes Federation; M, men; W, women; USA, United State of America; ATP III, Adult Treatment Panel III; FPG, fasting plasma glucose; OGTT, oral glucose tolerance test; HDL-c, high-density lipoprotein cholesterol.

Several opponents have, however, criticised the use of the MetS as a clinical tool for predicting future CVD[94] and diabetes.[94,95] Some of these critiques include that the choice and number of the individual components comprising this syndrome are not substantiated by enough scientific evidence.[96] It has also been suggested that the MetS is not an effective predictor of future CVD.[95,96] It may mislead medical practitioners and patients in terms of the treatment strategies which focus more on lifestyle modification.[96] Lifestyle modification has proven to be challenging without professional guidance and support.[87] The risk given by the MetS as a syndrome does not differ with the risk given by the individual components.[97-99] Adding to the above-mentioned, there is no actual consensus on the treatment guidelines for the MetS.

Treatment is mostly dependent on the risk factors which are presented by the individual.[96] Contrary to this, Grundy argued that the MetS comprises several risk factors that increase the risk of CVD and diabetes when clustered in one individual,[1,100] and that this syndrome does exist.[100] The components which define the MetS are considered to be scientifically in line with the syndrome, as obesity and IR seem to underlie the development of this syndrome.[101,102] Also noted was the recognition that the individual components of the MetS can also predict risk of CVD, such as IR and obesity.[100] It is suggested that a multiplicative risk would predict greater risk compared to a single risk factor.[100] The risk of developing CVD increases geometrically rather than linearly with the increase of the risk factors.[103] Several studies reported that the risk of developing CVD is doubled, and risk for type 2 diabetes mellitus is five times higher in individuals with the MetS than patients without the MetS,[87,104,105] showing its potential in predicting future CVD risk.

The MetS is therefore an easy tool to use for classifying people with clustering of the CVD risk factors, which might indicate elevated long-term risk for developing CVD.[100] Hence, the MetS is not suggested as a tool for short-term risk prediction that precedes the treatment of the risk factors with medication.[100] Taking into consideration all these above mentioned critique regarding the use of the MetS, for the purpose of this study the MetS will be defined by the clustering of several risk factors in one individual. Multiple risk factors carry greater risk as opposed to single risk; however the individual risk carried by the individual components will not be ignored.[100] The MetS is an effective tool which is easy to use and cost-effective for predicting future CVD.[104,105] This might be advantageous in a resource-limited country, such as SA. The MetS in the HIV- infected population may not be attributed to the traditional factors only, but also to the HIV and the use of ART.[56,106] Therefore this population could present with increased risk. The population is already burdened by HIV and ART,[107] and it will thus be important to determine the multiple risk contributed by risk factors in this population.

2.2.2. Prevalence of the metabolic syndrome in Africa and South Africa

It has been projected that a quarter of the global population has the MetS.[80] Ogbera et al.[108] reported a prevalence of 86% in a study which included 963 patients with type 2 diabetes mellitus. The most frequent MetS components in this African population was the following by order: elevated low density lipoprotein cholesterol (LDL-c) (80%), lower high density lipoprotein cholesterol (HDL-c) (65%), elevated total cholesterol (TC) (46%) and triglycerides (TG) (22%).[108] A cross-sectional and descriptive study done in Botswana including 256 participants reported a prevalence of 50% using ATP III. The study population was comprised of mainly older individuals, and women.[109] In addition, a prevalence of 13% and 18% for the MetS was reported using ATP III and IDF criteria respectively, with the study population comprising mostly of the elderly and women. The typical features of the MetS in black South Africans includes elevated HDL-c and fasting plasma glucose (FPG) in women and increased BP, FPG and TG in men.[90] In addition, Kruger and Nell reported that the MetS in black South Africans is comprised mainly of elevated WC in both women and men, whilst elevated glucose levels is only in women.[110] Motala et al.[90] reported a prevalence of the MetS with both IDF (27%) and ATP III (19%) and the MetS was independently associated with being a woman. Similarly, a cross-sectional study done in Ethiopia showed a prevalence of the MetS of 13% and 18% using ATP III and IDF respectively and the Mets was also frequently associated with overweight, women and use of alcohol and tobacco in men.[111] With respect to sex, women (32%) had a slightly higher prevalence of the MetS as compared men (31%) in a cross-sectional study done in Cape Town, SA.[86] Furthermore, in a cross-sectional study by Schutte and collegues[112] involving urban African women in SA the prevalence of the MetS was 25% using the IDF definition.[112] The study was done in urban setting, hence urban settings is usually associated with sedentary lifestyle and dense processed food. From these prevalence reports, it seems that age, the female sex and overweight are commonly associated with the MetS

2.2.3. Prevalence of the metabolic syndrome in people living with HIV

A cross-sectional study including 492 HIV-infected individuals from Cameroon reported a prevalence of 33% for the MetS using the IDF criteria.[113] In the latter study participants were mostly taking a NRTI based regimen.[113] Fourie et al.[114] indicated a prevalence of 15% of the MetS using IDF criteria among untreated HIV-infected from

rural and urban settings of SA.[114] The HIV-infected individuals presented with higher triglycerides: high density lipoprotein cholesterol ratio and C-reactive protein than the HIV-uninfected.[114] In a meta-analysis including 55 095 HIV- infected participants the prevalence of the MetS was 18% and 25% using IDF and ATP lll criterion, respectively.[56] The HIV-infected taking ART and with the MetS have a higher prevalence of MetS (21%) as compared to their infected counterparts not taking ART (9%) and d4T was the common ART used.[115] Another study in Italy reported a higher prevalence of the MetS in HIV-infected participants taking ART (19%) as compared to those not taking ART (14%) and HIV-free participants (5%).[116] Furthermore, Julius et al.[107] reported the MetS prevalence of 20% in HIV-infected individuals taking ART for more than one year. On the other hand, a cross-sectional study done in urban settings of the Eastern Cape Province, SA, reported similar prevalence of the MetS between the HIV-infected taking ART (23%) and not taking ART (23%).[106] The use of ART in this population was associated with high ratio of visceral to subcutaneous fat, increased levels of TG and LDL-c levels.[106] Given these reported studies, the HIV-infected individuals seem to have higher prevalence of the MetS as compared to the HIV-uninfected population. With regard to the HIV-infected population, the prevalence of MetS seems is higher in the HIV-infected population taking ART than ART-naive. This may suggest an association between ART and the MetS. Factors such as sex, ART and use of different criteria for the MetS may contribute to the differences in the reported prevalence of the MetS. The MetS in people living with HIV may be influenced by several factors such as traditional and cardiometabolic risk factors, HIV infection and the use of ART.[14,56,107] This population would seem to be at higher risk of developing MetS as compared to the general population.

2.3. Non-modifiable risk factors, metabolic syndrome and HIV

2.3.1. Age

Ageing is associated with deterioration in the immune function.[43] The immune dysfunction coupled with ageing leads to increased vulnerability to disease and stress in an individual, hence immunosenescence.[43] Immunosenescence is defined as an ongoing processes that results in the loss of functioning of the immune system with ageing. It involves biological changes such as a decline in the functioning of the hematopoietic stem cells,[117] cytoplasmic natural killer cells,[118] antigen-presenting cells,[117] formation and functioning of naive T-cell lymphocytes,[119] and decreased CD4

T-lymphocytes cells.[119] Increased vulnerability of the immune system decreases the ability of the system to fight infection and diseases.[120]

HIV hampers the immune system primarily through the destruction and depletion of CD4 T-lymphocytes that are fundamental in the immune response towards infectious agents.[121,122] A suppressed immune system is characterised by increased viral load and lower CD4 cell count; thereby increasing the chance of acquiring opportunistic diseases.[123] This immune suppression is further hampered by the effect of ageing on the immune system, making older HIV-infected individuals more vulnerable.[124] The HIV infection and ageing decrease the production and regulating ability of the CD4 T-lymphocytes.[125] It occurs through thymus dysfunction,[121] diminished hematopoietic stem cells[126] and production of naive CD4 T-lymphocytes.[125] The HIV-infected population have an accelerated immunosenescence as they age.[127] This event of ongoing suppression of the immune system affords chronic immune activation in this population,[128,129] resulting in endless viral replication and cell apoptosis.[124,130] The immune system can no longer adequately replenish the mature CD4 T-cells lost through cell death due to destruction of the thymus, liver and bone marrow.[131,132] The process of immunosenescence causes destruction in functional capabilities of the immune system and the ability to appropriately respond to metabolic stress.[127]

The HIV-infected population age more rapidly and develop age-related illnesses earlier as compared to the HIV-uninfected population.[127] Older HIV-infected individuals have delayed immunological reconstitution in response to antiretroviral therapy.[133] In a study done by Goetz et al.[134] older individuals presented with lower CD4 cell reconstitution as compared to the younger individuals. The elderly are deprived of the advantageous effect of the ART to increase the CD4 cell count due to loss of stimulation of CD4 T-lymphocytes regeneration and altered immune function.[132] Supporting evidence showed that the frequency of CD4 T-cell loss was higher in HIV-infected individuals aged 40 years and above as compared to 16-20 years younger individuals.[135] In a longitudinal study including HIV treated individuals the prevalence of the MetS increased with age, demonstrating a linear relationship.[136] In HIV-infected individuals ageing is associated with a higher odds ratio of developing the MetS.[137] In addition, a cross-sectional study involving 710 HIV-infected participants prevalence of the MetS was 5% in individuals aged < 30 years and 27% for individuals aged between 50-59 years.[16]

Ageing is significantly associated with risk of developing the MetS and the relationship is linear.[90] For instance, Bonora et al.[138] showed that 43% of the individuals with MetS were older than 60 years as compared to 27% of the individuals younger than 60 years.[138] And in a cross-sectional study done in Nigeria the prevalence of the MetS reached the highest point at age 44-55 years.[139] In univariate analysis the adjustment of age reduces the prevalence of the MetS whereas in unadjusted models the prevalence is higher.[90] The peak age of having the MetS was at 55-64 years in women and 65-74 years in men amongst urban South Africans.[86] Furthermore, the relationship between older age and the MetS reach its peak above the age 70 years.[86] In essence these two populations are at increased risk for the MetS as they grow older. Nevertheless, older individuals with HIV might be at increased risk compared to the HIV-uninfected as a result of immune suppression, chronic inflammation and accelerated ageing.

2.3.2. Sex

The differences in the prevalence of the MetS in men and women may be explained in part by the cut-off values for waist circumference and high-density lipoprotein cholesterol (HDL-c) for the definition of the MetS.[140] Women have a higher prevalence for obesity than men, which increases their risk of developing the MetS.[141] Abdominal obesity, which is higher in women, is associated with elevated circulating fatty acids and cytokines in the liver.[140] This might result in immature development of insulin resistance (IR), dyslipidemia and hypertension.[140] African men generally have a higher alcohol consumption,[142] which increases the risk of central obesity and elevated triglycerides.[143] Higher prevalence of smoking is also evident in the men.[108] The prevalence of the MetS varied between Philippine men (12%) and women (17%), using the IDF criteria.[144] Another study also reported a higher prevalence of the MetS for women (86%) as compared to the men (83%). In concert with the above studies, the prevalence of the MetS in Japanese women was 6 % and 4% for men.[145] However, in a cross-sectional study done in Europe, more men (31%) than women (26%) had the MetS.[146] The most common individual components observed in men with the MetS include elevated BP,[86,90,111,147] fasting plasma glucose,[90,148] and higher TG.[86,90,109] Women with the MetS present mostly with obesity,[111,147,148] decreased HDL-c[37,90,149] and dyslipideamia.[86]

There are sex differences in the MetS in the HIV-infected population, with some studies reporting a higher prevalence of the MetS in women than men[107] and, vice versa.[150] These differences may be attributed in part to the traditional risk factors of the MetS and HIV infection.[18] In a study including 4 010 HIV participants using ART, 12% of women had central obesity as compared to 7% in men.[151] The study also reported that women are less physically active as compared to men, with low physical activity being associated with risk of developing obesity.[142] Similarly, Mbunkah *et al*.[12] reported that more women than men have higher prevalence of the MetS: 18% vs. 4% respectively. The observation was also supported by EI-Sadar and collegues,[152] showing a higher prevalence of the MetS in women as compared to men: 12% vs. 10% respectively. Nonetheless, the male gender is associated with higher prevalence of hypertension and dyslipidemia in HIV-infected individuals.[18,151,153] Dyslipidemia, hypertension and smoking is also observed among HIV infected men.[151] In a study including HIV-infected individuals more men (71%) than women (29%) presented with the MetS.[16] Similarly, a higher prevalence of the MetS of 67% was shown in men, and 33% in women.[154] Clearly, findings have been controversial, and seem to be affected by the specific populations targeted by each study.

2.3.3. Locality

Location plays an important role in the development of non-communicable diseases such as obesity and high blood pressure.[109,155] SA is a developing country undergoing epidemiological and demographic transition.[156,157] Non-communicable disease continues to be on the rise in South Africa due to urbanisation, epidemiologic and demographic transition.[158] Rural areas are characterised by a lack of infrastructure and high agricultural activity, whereas the urban areas are characterised by over-population, industrial and infrastructure development.[159] The dietary intake in the rural areas of SA is comprised mainly of low fat and sugar content, and higher carbohydrates and fibre.[160] The urban diet consist of high fats with low carbohydrates and fibre.[161] Energy-dense food is rich in saturated fats, which are associated with higher levels of triglycerides and cholesterol.[17]

The diet of rural dwellers has a high salt content, which is associated with the risk of developing high blood pressure.[162,163] As a result of industrialisation and infrastructure, more urban dwellers are physically inactive compared to rural dwellers. Rural dwellers usually engage in an intense physical activities such as farming, household chores

and walking long distances to shops;[164] however, this activities are lessened due to urbanisation and modernisation.[165] Lack of physical activity is associated with the risk of high blood pressure[112] and obesity.[142] Physical activity is one of the recommended interventions in the modification of obesity and high blood pressure. The use of tobacco and alcohol is higher in the urban population but is increasing in the rural areas.[17] With ongoing urbanisation and epidemiological transition[157] in the rural areas, there is a change in dietary habits, physical activity, and alcohol and tobacco products usage, which increases the risk of developing non-communicable diseases.[165]

In a study of 1 259 rural and urban participants in the Free State Province of SA, the prevalence of the MetS was higher in the rural settings (52%) compared to urban settings (40%).[166] However, in a cross-sectional study of HIV-infected individuals residing in rural and urban settings, the prevalence of the MetS was higher in the urban participants compared to the rural participants: 36% vs. 16% respectively.[154] Sixty-six percent of the individuals were less physically active and had the MetS, compared to 30% of individuals with moderate physical activity.[154] A study conducted in urban Ethiopia showed that prevalence of the MetS was at 13%, and at 18% with ATP III and IDF respectively.[111] Again, in a study done in the urban setting of Cape Town, SA, the prevalence of the MetS was estimated at 31%.[86] In a cross-sectional study of 947 rural black South Africans, the MetS was 26%.[90] Though the prevalence of the MetS is higher in urban settings, it is increasing in rural settings.

2.4. Modifiable risk factors, the metabolic syndrome and HIV

2.4.1. Body composition

Obesity is a growing global epidemic and is associated with elevated risk of cardiovascular disease.[167,168] The factors that influence the development of obesity are multifactorial, and include both genetic and lifestyle factors.[169] Lifestyle factors such as lack of physical activity and unhealthy diet contribute significantly to the development of obesity.[170] Obesity and overweight is categorised by using a body mass index (BMI), that records <18 kg/m^2 as underweight, 18-24.5 kg/m^2 as normal body weight, 25-30 kg/m^2 as overweight, and ≥30 kg/m^2 as obese. These figures are set by the WHO Obesity Task Force.[171]

Of concern is that obesity is an important component of the MetS, as it underlies the development of this syndrome.[105] The on-going increase of the MetS may be

explained in part by the epidemic of obesity.[172,173] The prevalence of obesity worldwide is estimated at 12% (half a billion) according to the World Health Organization,[174] and according to the National Health and Examination survey of 2003-2006 in the United States, men and women who are overweight have a higher risk of developing the MetS.[175] The risk of developing the MetS is further elevated in obese adults.[175]

Visceral adipose tissue releases adipocytokines such as leptin, resistin, TNF-α, lL-6 and angiotensin ll (Ang II).[176] These adipocytokines may induce insulin resistance and are associated with increased prothrombotic and proinflammatory states.[176] Studies have reported that individuals with visceral adipose fat have a decreased level of adiponectin.[177] Adiponectin plays a protective part against the development of inflammation, hypertension and atherosclerotic diseases, which may be related to the MetS.[177,178] Central obesity is associated with an increased risk of CVD and type 2 diabetes mellitus.[179] For instance a, longitudinal study including black South Africans, indicated abdominal obesity to increase the five-year risk for developing metabolic and cardiovascular diseases.[180]

With respect to HIV infection, HIV is associated with underweight and fat wasting [181] and the use of ARTs such as PI and NNRTIs, is also associated with abnormal fat distribution.[182] In a prospective cross-sectional study, 41% of the HIV-infected population had abdominal obesity, which might be attributable to the use of ART.[113] ART induces abnormalities in fat deposition that often manifest as lipodystrophy, depending on which treatment regimen is used.[183] Patients with lipodystrophy have altered lipid metabolism and abnormalities in fat distribution. Lipodystrophy can present as lipoatrophy, lipohypertrophy, or mixture of both.[184,185] Lipoatrophy is described as fat loss usually occurring in the limbs and face, whereas lipohypertrophy is fat accumulation on the breast, neck and back (buffalo hump).[186] Taking into consideration the effect of the different ART classes, PIs are commonly responsible for the accumulation of visceral adipose fat,[187] and the NRTIs (stavudine (d4T) and zidovudine (AZT) usually favour the development of lipoatrophy.[188]

The NRTIs might induce lipoatrophy by deterring the DNA polymerase-γ which is responsible for the production of mitochondrial deoxyribonucleic acid (mtDNA) in the adipocytes.[189] This observation is supported by other reports of significant correlation between lipodystrophy and exhausted mtDNA in HIV-infected patients on ART.[190,191]

AZT and d4T induce impaired mitochondrial functioning and cell death in cultured adipose cells through the exhaustion of the mtDNA.[192] This cell death causes depletion of adipocyte cells in the lipoatrophic adipose fat tissue.[193] Furthermore, the use of PIs modifies the adipocyte differentiation and secretion of leptin and adiponectin.[194] HIV-associated lipodystrophy is associated with lower levels of mitochondrial ribonucleic acid for leptin and adiponectin.[195,196] Leptin plays a role in controlling weight gain by controlling satiety and hunger, whereas adiponectin decreases the fatty acid and triglycerides levels by magnifying oxidation of the fat in the tissue.[197] The use of NRTIs is associated with lipoatrophy in the HIV-infected individuals.[198]

Estrada et al.[199] demonstrated that 18% of individuals with lipodystrophy had the MetS, compared to 10% of individuals without lipodystrophy with the MetS. The HIV-infected individuals with lipodystrophy in that study population had decreased leptin levels compared to the HIV-uninfected (3 ng/ml vs. 9 ng/ml).[199] In addition, to a South African perspective HIV-infected individuals taking ART presents with lower total adiponectin and high molecular weight as compared to ART naïve and these parameters are inversely associated with BMI.[200] Another cross-sectional study done in South Africa reported a higher BMI among individuals using ART as compared to those not using ART, 24% vs. 26% respectively.[201] HIV-infected individuals not receiving ART are commonly underweight. However, with the use of ART, fat gain and fat loss in certain regions of the body becomes apparent.

2.4.2. Blood pressure

The presence of hypertension is associated with high morbidity and mortality rates due to renal failure, myocardial infarction and stroke.[202] The development of hypertension is multifactorial and influenced by genetic, environmental and lifestyle factors such as age, sex, locality, western diet, lack of physical activity, obesity, stress and socioeconomic status.[17,141,203] According to the World Health Organization, 48% of the global population has hypertension and approximately 7.5 million deaths are attributed to high blood pressure.[204] The prevalence of hypertension in Sub-Saharan Africa is estimated at 16%.[205] The South African National Health Survey in 2003 showed that 13% of men and 18% of women are hypertensive.[162] In a study with a 35 125 cohort from the WHO Study of Global Aging and Adult Health, the prevalence of hypertension in those older than 50 years was estimated at 78% for SA.[141] In this

study, hypertension was significantly associated with overweight, obesity, socioeconomic status, female gender and heavy alcohol intake.

HIV infection is associated with low blood pressure. This observation is sometimes reversed with the use of ART.[114,206] In a cross-sectional study of HIV-infected individuals not using ART, lower blood pressure levels were observed compared to matched HIV-uninfected individuals.[114] Similarly, a systematic review and meta-analysis of HIV-infected individuals showed that HIV infection is correlated with low systolic and diastolic blood pressure.[207] With respect to individuals taking ART, a cross-sectional study including 710 HIV-infected individuals taking ART reported that 26% of the individuals had blood pressure of ≥ 130/85 mmHg.[16] Additionally, a higher prevalence of hypertension was shown in patients on ART (17%) compared to the patients without ART (2%), and the risk of developing hypertension was associated with a longer duration on ART and obesity.[115] These patients were using d4T-based treatment, which has been implicated in increasing blood pressure.[115] Similarly, a higher prevalence of 43% for systolic blood pressure (≥130 mmHg) was reported for individuals taking ART as opposed to those not taking ART (36%).[106]

To the contrary, a similar prevalence of high blood pressure of 36% and 37% was reported respectively for individuals on ART and those not taking ART.[137] In an analysis of 20087 HIV-infected individuals, the use of ART was not significantly associated with blood pressure.[207] Findings by Agrawal et al.[208] showed that 21% of the participants using ART were hypertensive and 79% were normotensive individuals. Furthermore, there was no correlation between hypertension, the use of ART and the individual drugs.[208]

The effect of ART on blood pressure is often determined by the type of ART regimen.[12] The use of PIs is associated with systolic blood pressure of ≥ 130 mmHg.[13] The prevalence of hypertension in individuals using d4T was 16% compared to 14% for normotensive participants.[209] Those using AZT were at 54% compared to 50% for normotensives, and those using PI were 50% compared to 36.% for normotensive participants.[209] Prolonged use of Indanavir, but not Lopinavir/ritonavir (LPV/r), is correlated with risk of developing hypertension.[210] Furthermore, a longitudinal study including HIV-infected individuals on a first-line ART regimen showed a proportion increase in individuals with HT from 3.9% at baseline to 16% at 5-year follow-up.[211]

Borkum et al.[212] also reported a mean increase in office SBP of 111±14 mmHg at baseline to 116±14 mmHg at six months follow-up among HIV-infected individuals. However, in a cross-sectional study including HIV-infected patients, blood pressure was 122/75 mmHg in patients on NRTIs compared to 127/79 mmHg in patients not taking the treatment.[213] Overall, it seems that HIV-infected individuals taking ART have a higher prevalence of blood pressure compared to those not taking ART. The risk seems elevated with the use of PIs.

2.4.3. Lipid disorders

The lipid disturbances associated with HIV infection itself include elevated TG, decreased HDL-c and LDL-c.[114,214,215] The low levels of HDL-c in ART-naive individuals indicate chronic inflammation.[216] A possible effect of HIV on lipid metabolism is suggested by disturbances in the cytokine status and reduced lipid removal and elevated hepatic production of very low density-lipoprotein cholesterol (VLDL).[217] With the altered cytokine status, TNF-α, interferon alpha (IFN-α) and IL-6 stimulate lipid peroxidation, with the latter usually resulting in the production of free radicals, which can cause cell damage.[182] IFN-α is directly proportional to higher TG, total cholesterol (TC) and VLDL.[218] When the CD4 lymphocyte cell count is reduced in the blood, serum TG increases and HDL-c decreases.[215]

An alteration of the lipid metabolism is observed as early as three months after the initiation of ART in the HIV-infected population.[219] One should bear in mind that each class of ARTs exerts different effects on the lipid metabolism.[181] Patients taking ART show lower HDL-c even with immune reconstitution, suggesting that the treated individuals also constitute a certain level of chronic inflammation.[216] ART with a combination of tenofovir disoproxil fumarate (tenofovir) reduces the TG, LDL-c and TC levels more than a combination of d4T and lamivudine without tenofovir.[15,220] Individuals using NNRTIs such as nevirapine exhibit increased serum concentration of HDL-c,[221,222] TC and LDL-c.[183]

Higher occurrences of lipid alteration ranging from 70-80% have been reported with the use of PI treatment in HIV-infected individuals.[182] Long-term use of PIs is linked with elevated TG, LDL-c and lower HDL-c,[223,224] however, the effect on TG is reduced once optimal viral suppression is achieved.[224] The PIs promote higher TG levels by encouraging the hepatic TG production through upregulation of mRNA production in

the hepatic cells.[225] This upregulation of mRNA stimulates enzymes in the hepatic cells which are responsible for the biosynthetic pathway of TG, resulting in hepatic buildup of TG-rich lipoparticles.[225]

The physiological mechanism of HIV and ART on lipid metabolism in HIV-infected individuals is not well understood, but seems to be multifactorial,[181] and includes mitochondrial toxicity, inhabitation of lipogenesis and adipocyte differentiation.[181,218] In a cross-sectional study including 280 HIV-infected individuals taking ART, dyslipidemia was characterised by increased TC (27%), TG (43%), LDL-c (31%) and decreased HDL-c (58%).[226] This observation may be explained by the frequent use of NRTIs, PIs and the use of lipid lowering drugs.[226] A finding by Berhane et al.[14] showed that 48.2% and 12.1% of the HIV-infected individuals taking ART had dyslipidemia and lipodystrophy respectively. In the latter case, the most prevalent lipid abnormality was decreased HDL-c level at 33%, followed by TC: HDL-c ratio (26%), TG (18%), LDL-c (7%) and lastly, TC (7%).[14] Similar trend of lipid disorder is also reported in South African population living with HIV, with majority of individuals presenting with decreased HDL-c (49%), followed by hypercholesterolaemia (40%), hypertryglyceridaemia (26%).[227] The prevalence of increased TG is 54% in individuals using ART as compared to 42% for those not using ART.[137] With regard to HDL-c, 46% of the treated individuals had lower HDL-c levels compared to 40% of the untreated individuals.[137] However, other studies report lower prevalence of lipid abnormalities in those taking ART, for instance a study including participants taking NNRTIs reported a prevalence of dyslipidemia of 90% in those not taking ART and 85% in those taking ART.[228] Nonetheless, in the latter study it was only decreased HDL-c which was common in ART naïve as compared to those taking ART, and TC, TG and LDL-c were elevated in those taking ART as compared to those not taking ART.[228] To summarise, ART is associated with poor lipid profile in the HIV-infected population, especially with the use of PIs and NNRTIs. The effect of the individual drugs within the same class may have different outcomes on the lipid profile. The duration of the ART may also have a different impact on the lipid profile.

2.4.4. Hyperglycemia

There seems to be no marked difference with regard to fasting plasma glucose in the HIV-infected compared to the HIV-uninfected population.[114] The prevalence of hyperglycemia was similar in individuals receiving ART (12.7%) and not receiving ART

(12.9%),[137] and 13% of the HIV-infected individuals had high fasting plasma glucose versus 7% of the HIV-uninfected.[137] Similarly a cross-sectional study done in SA also reported similar prevalence of dysglycaemia among HIV-infected taking ART (22%) and not taking ART (26%).[229] The glucose levels may not always be influenced by HIV or the use of ART in the HIV-infected population. In a cross-sectional study 25% of the participants with high fasting plasma glucose had the MetS.[14] Hyperglycemia is a frequent metabolic component in HIV-infected individuals.[12] In the latter study, 53% of the individuals using the first-line regimen, 20% using the second-line regimen, and 15% of those not using ART developed hyperglycemia.[12] In the HIV-uninfected participants and the HIV-infected ART-naive participants the prevalence was similar, at 14% and 15% respectively.[12] In a cross-sectional study including 242 HIV-infected individuals taking ART, the prevalence of diabetes was 15%.[210] In the latter study, diabetes mellitus was associated with longer duration on ART in multivariate analysis.[210] Sinxadi et al.[227] reported a prevalence of 24% for impaired fasting glucose (IFG), 10 % for impaired glucose tolerance (IGT) and 2% for diabetes among HIV-infected individuals on fist-line regimen for median duration of 18 months. The use of efavirenz rather than nevirapine is associated with elevated risk of developing diabetes (hazard risk ratio of 1.27).[230]

2.4.5. Lifestyle factors

Lifestyle factors include factors such as diet, use of tobacco and alcohol, physical activity, psychological stress and socioeconomic status.[17] Lifestyle factors contribute to the differences observed in the different components of the MetS.[231,232] High energy dense food, lack of physical activity, smoking and the use of alcohol have a profound negative impact on the development of diseases such as obesity, hypertension, diabetes, and hypertriglyceridemia, which eventually result in MetS.[165,233]

Low physical activity is associated with the risk of developing high blood pressure[112] and obesity.[142] A lack of exercise is also associated with the development of the MetS, with high prevalence in individuals with low exercise (37%) as compared to their counterparts (27%).[138] Exercise has a positive impact on immune function through improving the functioning of the T-lymphocytes by reducing its depletion.[234] A study documented that exercise successfully minimises short and long-term chronic inflammation through conservation of the T-lymphocytes,[235] which are rapidly depleted in the HIV-infected population.[125] Positive outcomes of physical activity in the HIV-

infected population include improved T-lymphocytes functioning and the CD4 cell count.[234] Exercise is suggested by medical practitioners as a constructive way to manage weight loss and fitness, thereby reducing the risk of metabolic diseases associated with obesity.[236]

Ogbera et al.[108] reported no difference in smoking and drinking alcohol amongst participants with MetS (9% and 23%) and without the MetS (9% and 21% respectively).[108] HIV-infected individuals who smoke have a higher T-cell activation and immune activation than the HIV-infected individuals who do not smoke.[237] Findings from strategies for the management of antiretroviral therapy in a trial study reported that the HIV-infected individuals with higher levels of smoking and dyslipidemia showed elevated levels of CRP and IL-6.[238]

Healthy nutrition improves the functioning of the immune system,[239] whereas inadequate nutrition and metabolic stress are associated with wasting in the HIV-infected population, with evidence of long-term inflammation.[240] Long-term inflammation can also alter the absorption of food, leading to increased nutrient deficiency in the HIV-infected population.[240]

With regard to psychological stress, several studies have reported an important association with the MetS.[241,242] Stress can either be acute or chronic, and it may be induced by work, health, finances and environment related circumstances.[243] Findings from a systematic review suggested that long-term psychological stress is an important risk factor in developing the MetS.[243] Individuals with the MetS present with activation of the neuroendocrine stress axis, which suggests chronic stress as an underlying factor in its development.[241] Chronic stress is also linked to elevated release of neurohormones like corticosteroids and cortisol, which may prompt components of the MetS such as IR and obesity.[244,245]

The development of the MetS is also affected by socioeconomic status.[246] A study done in Nigeria reported that participants with higher socioeconomic status have elevated systolic blood pressure and fasting plasma glucose more than those with low and middle status.[247] A prevalence of thirty percent was shown among South African working as corporate executives.[248] In terms of sex, prevalence of the MetS is higher in married men who receive a high income, and lower in those not working.[246]

Educated and highly paid women have a lower prevalence of the MetS whereas unemployed women have a higher prevalence.[246]

2.5. Inflammation, the metabolic syndrome and HIV

Inflammation refers to the cascades of actions that take place when the body's immune system reacts to injury or infection by releasing and activating white blood cells and producing cytokines.[249] Inflammation can be either acute or chronic.[249,250] Acute inflammation occurs in response to injury or infections that are localised, whereas chronic inflammation occurs in response to ongoing immune response to chronic illness or infections, which lead to immune activation.[43,127] Several biomarkers such as CRP play a role in inducing inflammation.[249] CRP is an acute phase protein and a well-documented biological indicator of inflammation.[251] The concentration of CRP < 1 mg/L indicates low risk, 1-3 mg/L intermediate risk, and > 3 mg/L indicates high risk for CVD.[252] CRP has also been implicated in the development of type-2 diabetes mellitus (T2DM).[80] It has been suggested that CRP should be added as part of the criteria for MetS.[253,254]

In a case-control prospective study including 14,719 participants, the number of the MetS components was positively associated with CRP (for each 1, 2, 3, 4 and 5 component/s of the MetS, the levels of CRP were 0.68, 1.1, 1,9, 3.0 and 5.8 mg\L respectively).[255] Patel et al.[256] reported an increase of CRP levels from approximately 1 mg/L with the presence of one MetS component, to 3 mg/L with the presence of more than three of the MetS components. There is thus a linear relationship between the CRP levels and the MetS, or the number of the individual MetS components.[255-259]

A cross-sectional study reported higher CRP (3 mg/L) in HIV-infected individuals compared to (2 mg/L) HIV-uninfected individuals.[114] With regard to CRP and ART, a cross-sectional study involving 171 HIV-infected participants reported CRP levels of 1 mg/L in individuals on ART compared to 0.3 mg/L in individuals not receiving ART.[260] The use of NNRTIs and NRTIs was associated with higher CRP levels than the use of PIs.[260] In a prospective study of 60 HIV-infected participants using ART, CRP levels were similar in the individuals with and without MetS. Similarly, in a cross-sectional study including 788 HIV-infected participants, the CRP levels were 7.0 mg/L in individuals with the MetS, compared to 6.0 mg/L in those without the MetS.[258] In summary, CRP levels seem to be elevated in HIV-infected individuals compared to

their uninfected counterparts. The use of ART is still controversial, depending on the type and class of ART regimen used.

2.6. Renal function

Kidney disease have been commonly observed in the history of HIV infection[261,262] and is more likely to complicate the management and development of HIV.[3] In addition to the effect of HIV in renal function,[263] factors such as metabolic syndrome and traditional factors may further exacerbate renal function.[264,265] HIV-infected individuals with chronic kidney disease are predisposed to acute kidney disease and end-stage renal disease, which further add to the morbidity and mortality associated with other metabolic diseases such as hypertension and diabetes mellitus [266,267]

2.6.1. Measurements of renal function

Renal function is measured using various markers such as creatinine clearance (CrCl),[268] estimated glomerular filtration rate (eGFR),[268] and the urinary albumin-creatinine ratio (uACR).[269]

CrCl is the volume of blood plasma which is cleared of creatinine per unit time.[270] Creatinine is a metabolic by-product of skeletal muscle and is directly proportional to muscle mass.[271] Hence, serum creatinine levels are affected by muscle mass and other factors such as dietary intake, age, sex and ethnicity.[272,273] CrCl is a substitute measure for eGFR and has been substantiated for the African population.[274]

The flow rate of the filtered fluid through the kidneys is described by eGFR.[275] GFR cannot be estimated directly, and is measured using endogenous filtration markers such as creatinine.[271] The eGFR determined using the Chronic Kidney Disease Epidemiology collaboration (CKD-EPI) formula without the inclusion of ethnicity, has a lower percentage of overestimation compared to the CKD-EPI for ethnicity (15% vs. 34%).[276] A systematic review showed that the use of the CKD-EPI would lead to a smaller average bias in clinical practice as compared to Modification of Diet in Renal Disease.[277] Other methods such as the Cockcroft-Gault equations to calculate CrCl have been utilised in Africa[270] to determine renal function, but lately, the creatinine-based CKD-EPI has proven to approximate GFR better.[275] Studies have shown that creatinine-based CKD-EPI equations are adequate in both HIV-infected individuals[278,279] and uninfected individuals.[280] Decreased eGFR has been commonly

reported in Africans living with HIV infection;[281,282] however, it differs considerably as a result of the various methods utilised.[4,283]

uACR is a reliable and useful method to express microalbuminuria.[269] uACR is classified in three categories: albuminuria, microalbuminuria and macroalbuminuria.[284] Albuminuria is characterised by a normal to mild excretion of albumin of < 3 mg/mmol whereas microalbuminuria is between 3-30 mg/mmol, indicating moderate excretion of albumin.[271] Macroalbuminuria levels at > 30 mg/mmol show severe albumin excretion.[271]

In normal circumstance the kidneys almost do not excrete albumin.[285] However, when the kidneys are impaired, this protein might escape and be excreted in the urine.[285] Hence the excretion of albumin reflects kidney impairment through altered glomerular permeability,[265] and may also indicate early risk for kidney disease.[284] The presence of microalbuminuria may also present the presence of endothelial impairment[286] in conjunction with increased albumin permeability through the glomerulus. It is suggested that uACR independently predicts cardiovascular risk even at levels below the threshold for microalbuminuria, and in various clinical backgrounds.[287-289] In the general population, uACR is also associated with elevated cardiovascular disease risk in patients with and without hypertension and diabetes.[290-292]

2.6.2. Factors associated with renal dysfunction

Renal function is influenced by various factors such as age, sex, race, HIV and antiretroviral therapy.[262,293,294] Renal function declines with ageing, but substantial differences exist between people.[295] Previously, a decline in renal function with ageing was seen as part of natural ageing but it has lately become clear that it is associated with adverse outcomes in the elderly.[43] Age above 40 years predisposes a higher risk of renal dysfunction .[263] It is suggested that kidney function decreases with ageing at a rate of 0.4 ml/min (CrCl) per year. [296]

Chronic kidney disease commonly occurs among Africans[273] and is four times higher in Africa than in industrialised countries.[297] HIV-associated nephropathy is considered a potential cause of end-stage kidney diseases in African-Americans.[5] On the other hand, findings by Overton et al.[272] showed that the Caucasian race is associated with renal dysfunction in HIV-infected individuals.

Several studies have reported a higher risk of renal impairment in HIV-infected individuals than in their uninfected counterparts.[264,293] Szczech and others[263] reported an independent association between HIV infection and microalbuminuria. Low CD4 cell count and detectable viral load are associated with risk of renal impairment.[295] Low CD4 cell count is a predictor of eGFR <60 ml/min/1.73m^2. A CD4 cell count of <200 cells/µl is an independent risk factor of microalbuminuria.[3] In a retrospective study it was reported that CD4 cell count <50 cells/µL and older age are associated with an increase in serum creatinine in individuals on tenofovir.[298]

The introduction of tenofovir in HIV-infected individuals is associated with reduced eGFR and can occur as early as two months after initiation.[299] Nonetheless, the use of ART is associated with improved immune status, which is beneficial for renal function.[300,301] The improvement of renal function with the use of tenofovir occurs after 12 months.[299] PIs, especially ritonavir, is independently associated with the risk of developing microalbuminuria.[3] The combination of tenofovir and PIs causes a greater decline in eGFR.[302,303] The South African Department of Health recommends monitoring of CrCl in HIV-infected individuals taking tenofovir, especially if used in combination with PIs.[60] Monitoring is recommended to be done at baseline, every 3.6 months and thereafter annually, and discontinuation of tenofovir is encouraged if CrCl is < 50 ml/min.[60] In cases where tenofovir was discontinued, renal function was improved during follow-up, although not to the normal level.[304,305] Findings of a systematic review and meta-analysis showed that CrCl reduced by -3.9ml/min among individuals taking tenofovir as opposed to those not taking tenofovir.[306]

An observational study including 60 000 HIV-infected individuals in Zambia reported that participants with lower eGFR at initiation of tenofovir represented with further decrease in eGFR at follow-up.[283] Studies in developed countries showed a 25% reduction in kidney function after initiation of tenofovir among HIV-infected Americans.[307] Nonetheless, Reid and others[308] reported improvement in eGFR among individuals with mild to moderate kidney impairment after the use of ART. Findings from a South African cohort also indicated improvement in eGFR over the 12 months of initiating tenofovir.[299]

2.6.3. HIV, antiretroviral therapy and renal function

Kidney disease in HIV-infected patients involves different renal pathologies.[309] The pathology may directly be facilitated by HIV, coexisting factors such as hypertension and diabetes mellitus, and nephrotoxic drugs.[310] Kidney disease is associated with cardiovascular disease risk and complicates the progression of the HIV infection.[263] It is suggested that HIV directly exerts its effect by infecting the epithelial cells and podocytes, which are responsible for sustaining the basement membrane of the glomerulus.[267,311] Concurrently, HIV is independently associated with the risk of developing microalbuminuria, irrespective of the known traditional risk factors of kidney diseases.[263] A cross-sectional study including newly diagnosed HIV-infected patients, reported a prevalence 15% of microalbuminuria.[264] In that study microalbuminuria was associated with TG, low CD4 cell count, LDL-c and HDL-c. In addition, Szczech *et al*.[263] showed a prevalence of 11% of microalbuminuria in HIV-infected individuals, compared to 2% in the uninfected. Furthermore, a cross-sectional study done in Nigeria reported a higher prevalence of 21% for microalbuminuria in HIV-infected patients.[312] Findings by Msango and colleques[313] showed that 25% of participants had moderate decline in eGFR, and 39% had severe decline in eGFR.

Kidney disease risk in HIV-infected patients might occur dependent or independent of HIV.[314] In the former pattern, HIV infection alters renal function.[311] In such cases, the introduction of ART such as tenofovir improves the renal function by exerting its antiviral effects.[301] With HIV-independent kidney risk, individuals have had improved immune status as a result of chronic use of ART. Kidney disease risk is a result of factors such as ageing, pre-existing kidney disease and nephrotoxicity.[314] These individuals might have a more tenofovir-induced decline in renal function. Tenofovir is the commonly preferred ART for the treatment of HIV infection in SA because of its efficacy, tolerable side-effects and antiviral activity.[58] However, tenofovir is associated with various forms of renal impairment in developed and developing countries, particularly among the underweight elderly with progressed HIV infection, preexisting renal disease, and the simultaneous use of PI.[305,313,314]

Nevertheless, the effect of tenofovir on the kidneys is controversial: some authors report that tenofovir has nephrotoxic effects[271] whereas others report none/moderate nephrotoxic effects on the kidneys.[308] The prevalence of nephrotoxicity associated with tenofovir is estimated at 2.4%.[295] Randomised clinical trials have reported renal

safety with the use of tenofovir in relatively healthy HIV-infected patients.[315,316] However, case-reports and observational studies suggest that tenofovir is associated with nephrotoxicity.[315,317] The reported controversy may be due to clinical trials that have strict inclusion and exclusion criteria, whereas in routine clinical practice patients may have accompanying diseases, medication and history that may predispose them to tenofovir nephrotoxicity.[315,317]

The immune reconstitution and suppressed viral load with the use of tenofovir may explain the renal improvements in those using tenofovir.[301] Before the introduction of ART, the prevalence of microalbuminuria in HIV-infected individuals was approximately between 19% to 31%,[2,300] whereas in the post-ART era it is estimated between 8.7% to 11%.[293,318] Also, a retrospective study done in Johannesburg, South Africa, that included 890 HIV-infected individuals taking tenofovir, showed that 64.4% had normal kidney function, 30.4% had mild kidney dysfunction, and 5.2% had moderate kidney function.[295] Additionally, Kamkuemah et al.[299] also found lower prevalence of renal decline whereby 79% had normal eGFR, 19% had mild, and 2% had moderate reduction in eGFR. In contrast, a prospective study including 175 HIV-infected South Africans from the North-West Province reported that 61% of the individuals on tenofovir had severe acute kidney injury compared to 28% of those not taking tenofovir.[262] The individuals on a tenofovir-based regimen had higher median serum creatinine and lower renal recovery.

The mechanisms by which tenofovir damages the kidneys has not been fully elucidated, despite the understanding of tenofovir's renal elimination. The primary renal damage with the use of tenofovir includes proximal tubular dysfunction.[317] Proximal tubular cells are the main site of renal injury as a result of their complementary membrane transporters and mitochondria, which support tenofovir build-up.[319] Approximately 30% of tenofovir is uniformly excreted unchanged in the urine through active secretion by proximal tubular cells.[320] This active transport is accomplished/ facilitated by organic anion transporters, namely the hOAT1 and OAT3 in the basolateral membrane.[321] Consequently, tenofovir is secreted to the tubular lumen by the apical membrane transporters, MRP-4 and MRP-2.[322] Genetic polymorphism in these membrane transporters may mediate excessive build-up of tenofovir in the proximal tubule.[323] In addition, these transporters inherently favour the

build-up of tenofovir in the proximal tubular cells, making it a main target of tenofovir nephrotoxicity.

Elevated intracellular levels of tenofovir may deplete mitochondrial DNA[319] content by hindering mitochondrial DNA polymerase γ,[324] inducing changes in the make-up/ composition of the mitochondria in the proximal tubular epithelial cells.[325] Changes in mitochondrial structure lead to depletion in production of adenosine triphosphate, and the proximal tubular cells cannot adequately guarantee resorption of ions and small molecules like glucose, phosphate, uric acid and b2-microglobulin.[319] This inadequate resorption, permits secretion of the ions and molecules in the urine.

2.6.4. The metabolic syndrome and renal function

The MetS is commonly prevalent in progressed stages of chronic kidney diseases and this may imply that the MetS is an independent predictor for development and progression of kidney disease.[326] It seems that the MetS per se, or its individual components, have an effect on the kidney function by inducing effects such as hyperfiltration, focal segmentation and glomerulosclerosis (see Figure 2.2).[268]

It is postulated that the MetS and hypertension act by modifying the integrity of the endothelium, causing elevated filtration of albumin through the glomerular.[327] Microalbuminuria seen in hypertension might reflect elevated intraglomerular pressure and subsequent damage to the lining of the endothelial cells, leading to albumin leakage.[328]

With regard to obesity and renal function, there are suggestions that obesity is involved in the development of focal segmental glomerulosclerosis and glomerulomegaly.[329,330] Coexistence of both proteinuria and glomerulomegaly has been reported in obese individuals.[331] Various pathways for development of kidney diseases in obesity have been postulated, including such states as modification of the kidneys to increased body weight, which leads to elevated excretory load, insulin resistance, and retention of sodium and renal lipotoxicity.[332,333]

Several studies have implicated the role of dyslipidaemia in decreased kidney function and accelerated kidney disease.[334,335] TG-rich lipoproteins, including VLDL and intermediate-density lipoprotein may induce glomerulosclerosis[336] and mesangial cell proliferation.[337] With regard to HIV-infected individuals, a cross-sectional study

including newly diagnosed HIV-infected Nigerians reported a positive association between TG and microalbuminuria.[264]

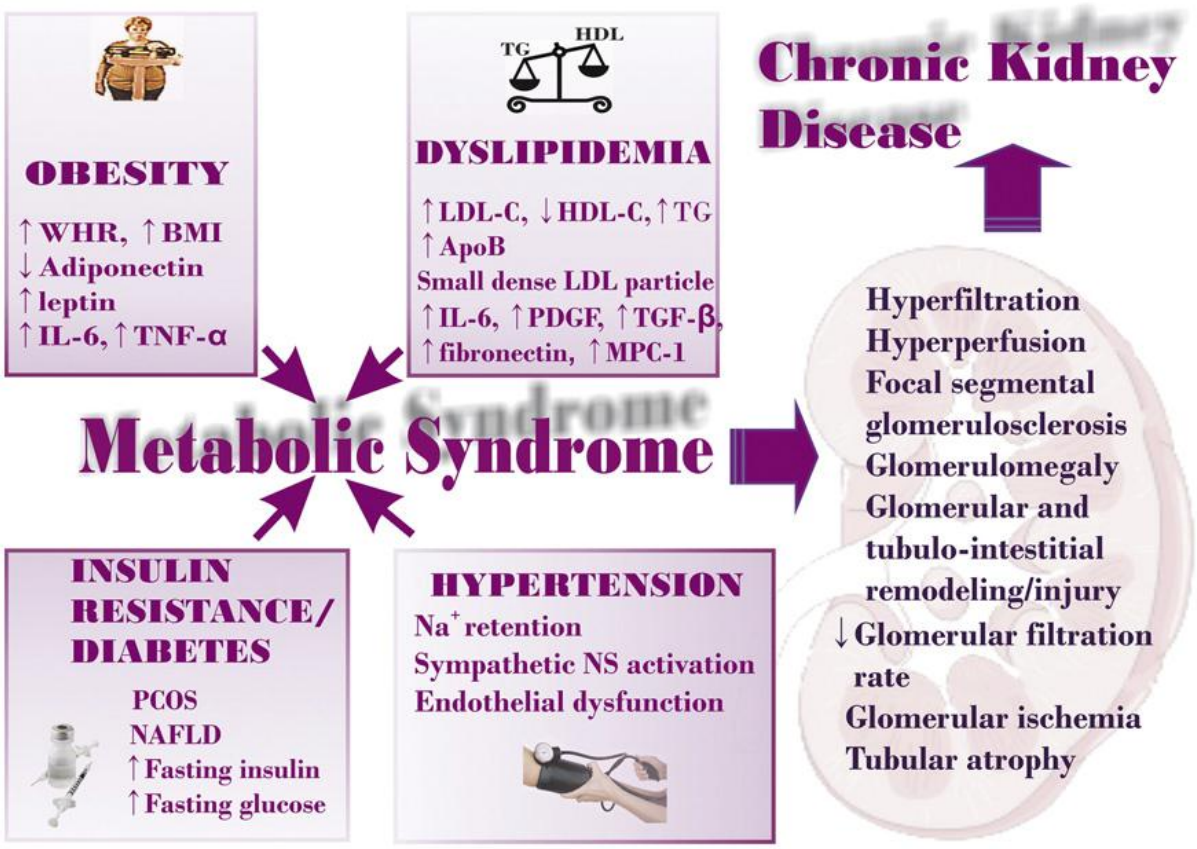

Figure 2.2: The metabolic syndrome and kidney disease (Gluba et al.[268]). WHR, waist-to-hip ratio; BMI, body mass index; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; LDL-c, low density lipoprotein-cholesterol, HDL-c; high density lipoprotein-cholesterol; TG, triglycerides; ApoB, apolipoprotein B; PDGF, platelet derived growth factor; TGF-β, transforming growth factor-β; MPC-1, monocyte chemoattractant protein-1; PCOS, polycystic ovary syndrome; NAFLD, non-alcoholic fatty liver disease; Na^+, sodium.

A positive association has been observed between MetS and eGFR, which may imply a state of hyperfiltration, often driven by hypetension.[338] High blood pressure is a known source of increased hydraulic pressure of the glomerular capillary and increased glomerular filtration.[339] Hyperfiltration is an indicator of future renal deterioration and kidney diseases.[340] It is often seen in proportion to the hyperfiltration in hypertensive and diabetic patients.[340] The MetS is linked to endothelial damage, which alters the permeability permeating protein excretion leading to microalbuminuria. These altered permeability's might be due to hemodynamic factors

or structural disturbances in the integrity of the endothelium or the intracellular matrix.[341]

A number of the MetS components are associated with both microalbuminuria and eGFR < 60 ml/min/1.73m.[342] In a study including American Indians, the risk of developing microalbuminuria was 2.3 times higher in those with three or more of the MetS components than those without the MetS.[343] These studies underline the role of the MetS in development of kidney diseases.

2.6.5. HIV, the metabolic syndrome and renal function

The risk of decline in kidney function is higher in HIV-infected individuals as a result of the direct effects of HIV and ART on the kidney.[262] HIV is chronically manageable with ART,[20] and the chronic effects of HIV and ART also increase the risk of metabolic disease. The MetS is associated with a higher occurrence of microalbuminuria in HIV-uninfected individuals,[268] and the MetS is rapidly prevalent among HIV-infected individuals.[12] Recent findings reported a prevalence of 37% and 9% for microalbuminuria (3-30 mg/mmol) among HIV-infected individuals respectively, with and without the MetS.[344] Microalbuminuria is an early marker of renal and kidney dysfunction, and is associated with higher cardiovascular morbidity and mortality.[261,287] In the latter study, the existence of the MetS augmented the risk of microalbuminuria.[344] Similarly, Hadigan et al.[3] reported microalbuminuria in 27% of HIV-infected participants with the MetS. These findings from the above studies suggest the possible role of the MetS in renal impairment in HIV infection.

2.7. Summary

The prevalence of the MetS reported in the literature remains controversial, with some reporting higher and others lower prevalence in HIV-infected individuals, and also when compared with the general population. The risk of developing the MetS in the HIV-infected population may be influenced by HIV infection, the use of ART, and traditional risk factors, or a mixture of all these factors together. Kidney diseases are commonly reported in HIV-infected individuals and often reflect the effect of comorbidities such as the MetS. Both the MetS and kidney diseases are seen in HIV-infected individuals, and the MetS is associated with an elevated risk of kidney impairment. It is important to investigate the MetS and its association with renal function in HIV-infected individuals taking ART, as it is likely that the combination of

HIV, the MetS and renal disease may occur in a substantial proportion of the SA population in the future.

2.8. Aim, objectives and hypotheses

The aim of this study is to determine the prevalence of the MetS and renal function in a South African cohort infected with HIV for at least five years.

The following objectives were formulated:

- To determine and compare the prevalence of the MetS in HIV-infected participants and in a HIV-free control group, matched according to age, sex, and locality;
- To determine whether renal function (uACR, eGFR and CrCl) is compromised in the HIV-infected participants with/without the MetS, when compared to their HIV-free counterparts.

The following hypotheses were formulated:

- The prevalence of the MetS will be higher in HIV-infected participants as compared to the HIV-free participants;
- HIV-infected participants will present with increased uACR, lower eGFR and CrCl;
- Measures of renal function will be adversely affected in the HIV-infected participants with the MetS as compared to the HIV-infected without the MetS, and the HIV-uninfected with and without the MetS.

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CHAPTER 3

Methodology

3. Methodology

3.1. Study design and population

The Prospective Urban and Rural Epidemiological (PURE) study is a multinational longitudinal study examining the changes in lifestyle and causes of chronic diseases, through periodic standardised data collection.[1] The study focuses on urban and rural areas in 17 different low- and middle-income countries, including South Africa. The main objectives of PURE are to: (i) investigate the association between societal effects and non-communicable diseases; and (ii) assess the link between societal elements and prevalence of non-communicable disease incidences and variations in the incidence of certain risk factors.[1]

In SA, the PURE study participants were randomly recruited door-to-door from two main sites: Potchefstroom (urban) and Ganyesa (rural) in the North West Province (see Figure 3.1.). Data was collected on three occasions, with baseline data collected in 2005 and follow-up data in 2010 and 2015. Participants were continuously followed over this period by fieldworkers, and information on events and mortality was captured.

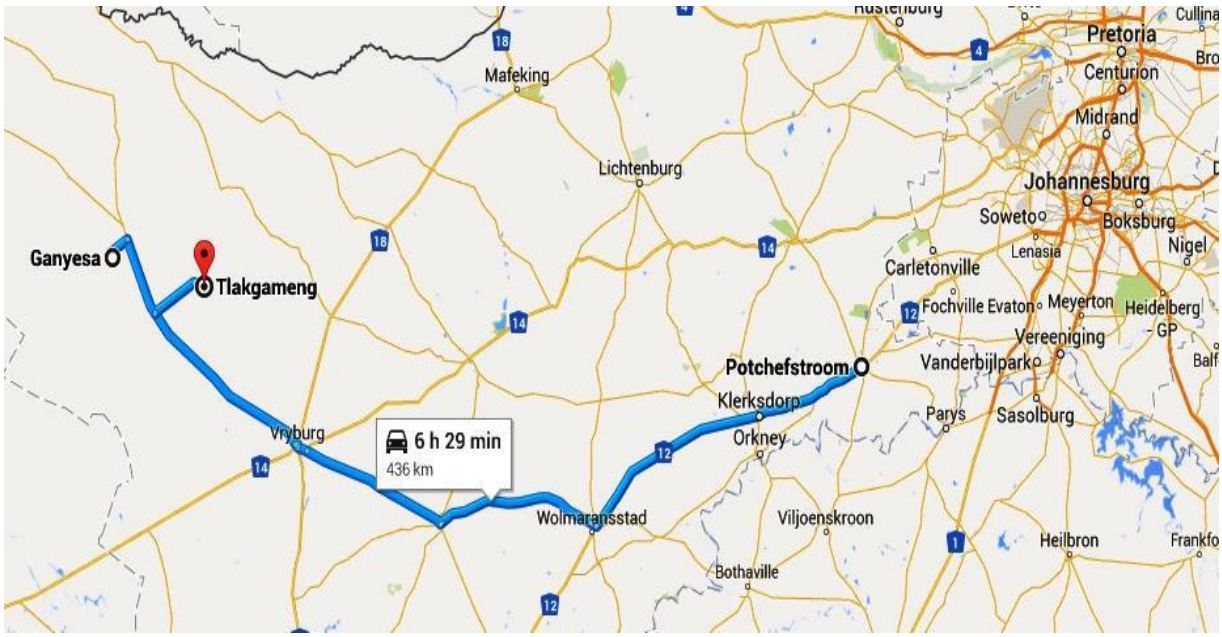

Figure 3.1: Areas used in the PURE study in the North West Province for the SA leg of the study.

The PURE study included black men and women older than 35 years, without reported chronic illness. The study also included participants infected with the human immunodeficiency virus (HIV) unaware of their status, and were all newly identified at

baseline (2005). During the five-year (2010) and 10-year (2015) follow-up study, all the HIV-free participants were tested again for HIV to identify the participants who were infected since the previous collection.

During the baseline data collection, a total of 2 010 apparently healthy black men and women were recruited. Of the 2 010 baseline participants, 746 were men and 1 264 were women, whereas 1 004 were from urban areas and 1 006 from rural areas. Of these 2 010 participants, 322 (16%) were identified as being HIV positive. In 2010, one thousand two-hundred ninety-two participants took part in the follow-up, and 214 were HIV-infected. Over the period, 233 died and 485 were lost to follow-up. During the 10-year follow-up done in 2015, 923 participants were followed, of which 161 (17%) were HIV positive. For this cross-sectional sub-study, 114 HIV-infected participants with complete research data set measurements were matched according to age, sex and locality, with 114 HIV-free participants as controls. Of these 114 HIV-infected participants, 27 (24%) and 87 (76%) of the participants were identified with HIV for five and 10 years, respectively.

3.2. Ethical aspects

This study is nested in PURE study and both studies were approved by the Health Research Ethics Committee (HREC) of the North-West University in South Africa (approval number: 00016-10-A1, PURE study and approval number: NWU-00035-16-S1, current study). Permission was also given by the Department of Health (North West Province) and from the Tribal Chief from each specified area. The study protocols conformed to the principles of the Declaration of Helsinki.

This sub-study made use of existing data from the 2015 data collection, and no further recruitment was required. During the 10-year PURE follow-up study, the fieldworkers performed house-to-house visits to every active participant, informing them about the planned upcoming follow-up measurements. Although the PURE study is a longitudinal study and research is on-going, out of respect for the participants, the fieldworkers obtained re-consent from all current active participants prior to the study. The research information was conveyed in the participant's home language by trained African field workers, fluent in both English and Tswana. Both at baseline and 10 years later, the participants were also given the opportunity to directly question the researcher, and a week to think about taking part in the study before they signed the

consent form. An independent person reconfirmed consent and emphasised that participation is completely voluntarily and that they may withdraw from the study at any point without being penalised in any way. The measurements took place in private rooms or enclosures with the researcher/s and participants. Unique numbers were used to identify the participants in all research procedures. Before taking part in the measurements, participants were asked to fast overnight for at least eight hours, not to smoke, exercise or climb stairs at least 30 minutes before measurements were taken. After data collection, all participants were given individual post-counselling with regard to their health status, and a referral to their local clinic/hospital if needed. The participants were thanked and given incentives to cover any costs incurred while voluntarily taking part in the study.

The participants also gave specific written consent for HIV testing. They were given pre-counselling by a qualified HIV counsellor before they were tested, and individual post-counselling after the procedure. The results were provided to the participants by the counsellor during these individual sessions, and the counsellor was trained to give professional emotional support where needed. The participants were referred to a clinic/hospital for follow-up if they tested positive.

3.3. Research measurements

A detailed layout of the experimental protocol and data collection procedures for data collection was previously described, and was consistent from baseline to follow-up.[1,2] The data, collected in the 2015 follow-up session, is relevant to this sub-study, and is discussed below. All measurements were clearly explained to the participants to ensure that they fully understand what was required of them. The measurements were done in line with standard operating procedures by trained personnel using validated apparatus, which is seen as the golden standard for specific measuring.

Figure 3.2: Data collection at Ganyesa, North West Province

3.3.1. Questionnaires

Questionnaires were conveyed in the home language of the participants with the help of trained field workers. The PURE SA adult questionnaire was used to collect data on socio-economic and demographic information, current health status, medical and family history, medication, tobacco and alcohol use. The Adapted BAECKE questionnaire was used to determine the physical activity index.[3]

3.3.2. Anthropometry

Anthropometric measurements were done according to standardised procedures as prescribed by the guidelines adopted at the National Institutes of Health, sponsored by the Airlie foundation[4] and the International Society for the Advancement of Kinanthropometry (ISAK).[5] Participants were measured with minimal clothing and barefoot by two researchers in a private enclosure. The values for each measurement were recorded in the PURE adult questionnaire for each participant. The height of the participants was measured to the nearest 0.1 cm with a stadiometer (Leicester height

measure, Seca, Birmingham, UK) and weight was measured on a portable electronic scale to the nearest 0.01 kg (Precision Health Scale, A & D Company, Japan). Body Mass Index (BMI) was determined using the values of weight (kg) and height (m^2), BMI (kg/m^2) = weight/height^2. Waist circumference (WC) was measured at the narrowest point between the lower rib border and the iliac crest, and was recorded to the nearest 0.1 cm with a steel tape (Lufkin, Cooper Tools, Apex NC, USA).

3.3.3. Blood pressure

The participants were allowed to rest for 10 minutes before the blood pressure measurement was taken. The measurement was taken twice at intervals of five minutes, using a validated OMRON M6 device (Omron Healthcare, Kyoto, Japan). An appropriate cuff size was used, and it was placed on the right arm over the brachial artery with the arm supported at heart level and in a relaxed position. This last measurement was used in analyses; pulse pressure and mean arterial pressure were calculated. Central systolic blood pressure (cSBP) was measured using the Sphygmocor XCEL device (Atcor Medical Pty. Ltd., Sydney, Australia), with the participant in a supine position.

3.3.4. Biological sample collection

Sterilised needles were used for each participant and blood was drawn by a qualified registered nurse, thus ensuring that all necessary precautions were taken. Venous blood samples were collected from the arm using a winged infusion set (See Figure 3.3). The serum and plasma were prepared according to standardised procedures, snap frozen and stored at -80 degrees Celsius (°C) until analysis. In the rural areas the samples were frozen at -20 °C for no longer than five days before being transferred to -80 °C freezers. The freezers have an alarm system which alerts personnel responsible for the laboratory through cell phone messages, if there are any changes in temperature. Midstream spot urine samples were collected in the morning. The urine samples were stored in a freezer at -80 °C until further analysis.

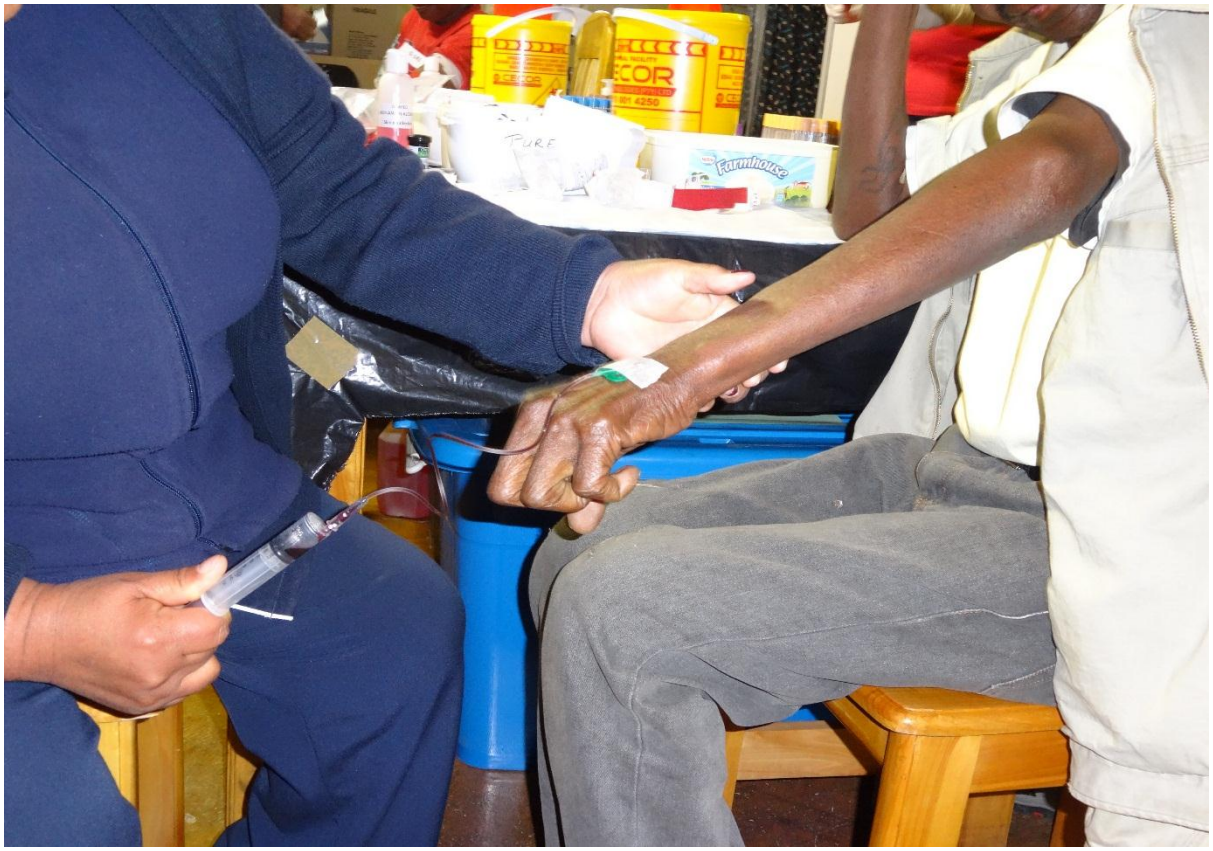

Figure 3.3: Collection of venous blood from the arm using a winged infusion set

3.3.5. Biochemical analyses

The serum samples were analysed using a Cobas Integra 400 Roche Clinical System (Roche Diagnostic, Indianapolis, IN) and glucose, total cholesterol, triglycerides and high-density lipoprotein cholesterol (HDL-c), low density lipoprotein-cholesterol (LDL-c), γ-glutamyl transferase and creatinine levels were determined. The C-reactive protein (CRP) was determined by means of a particle-enhanced turbidimetric assay. Glycated haemoglobin (HbA1c) was determined using the D-10 Haemoglobin testing system (Bio-Rad, #220-0101) by means of ion-exchange, high-performance liquid chromatography.

The Cobas Integra 400 plus (Roche,® Basel, Switzerland) was used to analyse the urinary creatinine and albumin levels by means of a kinetic colorimetric assay.

All the biochemical analyses for this sub-study were done in the research laboratory of the Hypertension in Africa Research Team (HART) at the North-West University.

3.3.6. HIV testing and counselling

The participants received counselling before and after HIV counselling done by a qualified counsellor in a private room. The HIV status of the participants was determined from whole blood according to the protocol of the South African Department of Health, using the first response rapid HIV card test (Premier Medical Corporation Limited, Daman, India). In the case of a positive result, the test was confirmed with Abon (Biopharm Corporation Limited Hanyzhou, China) rapid card, according to standard procedures (see Figure 3.4). During the follow-up in 2015 the participants that were HIV positive during 2005 and 2010 were not tested again.

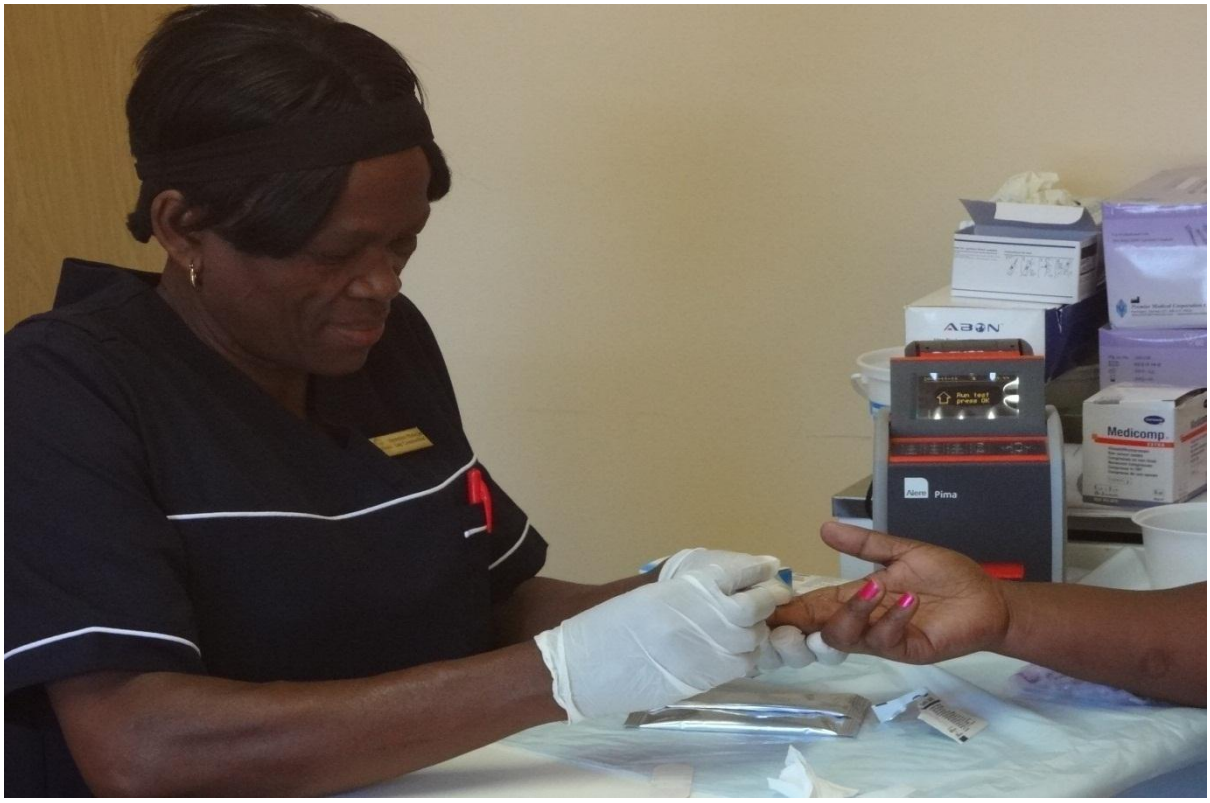

Figure 3.4: HIV testing by a counsellor using the rapid card test

Their CD4 count was measured the at the research site using finger-prick blood and a point-of-care device, PIMA^{TM} CD4 (Alere, Jena, Germany). Of the 114 HIV- infected participants, 77.3% were on the first-line antiretroviral regimen, as either separate or fixed-dose combinations of tenofovir, efavirenz and emtricitabine. The remaining participants did not use ART.

3.3.7. The metabolic syndrome definition

The metabolic syndrome (MetS) was defined using the criteria of the International Diabetes Federation (IDF): individuals with WC > 94 cm for men and > 80 cm for women and any of the other two: BP > 130/85 mmHg, TG > 1.7 mmol/l, HDL-c < 1.03 mmol/l for men and < 1.29 mmol/l for women and glucose > 5.6 mmol/l.[6]

3.3.8. Renal function

Renal function using creatinine clearance (CrCl), estimated glomerular filtration rate (eGFR) and urinary albumin-creatinine ratio (uACR). CrCl (ml/min) was determined with the Cockcroft-Gault formula as follows: [(140-age) * (weight in kg) * (1.23 if male OR 1.04 if female)] / (serum creatinine in µmol/l).[7] The guidelines of the South African Department of Health recommend discontinuation of tenofovir disoproxil fumarate (tenofovir) if CrCl <50 ml/min and this cut-off value was used in this study as the HIV-infected participants were taking tenofovir.[8] We calculated eGFR using the Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) equation without inclusion of black ethnicity,[9] and a cut-off value of eGFR < 90 ml/min/1.73m^2 was used.[10] eGFR is influenced by ethnicity, and inclusion of ethnicity overestimates the eGFR by 34% as compared to 16% without the inclusion of ethnicity.[9] This finding is also substantiated for the South African population.[9] uACR was calculated and a cut-off value of 3-30 mg/mmol was used, which defines microalbuminuria.[7]

3.4. Statistical analyses

Statistical analyses were done using Statistica version 13 (Stasoft Inc., Tulsa, OK) to analyse the data in this cross-sectional study. According to the sensitivity analysis done by the Statistician the study should be able to detect an effect size of 0.2 with power of 80% with the sample size of 200. The significance level is set at 0.05 for determining the association between HIV status and the prevalence of the MetS.

The normality of the data was tested and descriptive statistics were done for all normally distributed variables and presented as means and standard deviations. Log transformed variables were presented as geometric means and 5th and 95th percentiles. Independent t-tests were used to compare continuous variables (including age, weight, height, body mass index, blood pressure, lipid profile, glucose, C-reactive protein, renal function markers and lifestyle factors) in the HIV-infected and the matched HIV-free control group. The Chi-square test was used to compare categorical

variables (such as gender, location, medication use) between the groups. ANCOVAs were used to compare HIV-infected and uninfected groups while adjusting for WC.

ANCOVAs were used to compare uACR between the HIV-infected with/without MetS, and the uninfected with/without the MetS after adjusting for age, sex and WC. Comparisons between the two groups were done using the Bonferroni test. A bar graph was plotted using the adjusted least square means. Lastly, we performed multi-variate adjusted regression analyses with renal markers (CrCl, eGFR and uACR) as dependent variables in the three groups, namely the total group, and the HIV-uninfected and infected groups. This was done to determine the contributions of the MetS components towards renal function in the different groups. The independent variables that were entered into the model included age, sex, cSBP, WC, HDL-c, TG, HbA1c, CRP, MetS, HIV status, CD4 cell count and ART.

3.5. References

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Chapter 4

The metabolic syndrome and renal function in an African cohort infected with Human Immunodeficiency Virus for 5-10 years

4.1. Summary of author's instructions: *Journal of AIDS*

- A title page must be included in the manuscript file. Include on the title page: *a*) complete manuscript title; *b*) authors' full names, academic degrees, and affiliations (the affiliation should reflect the institution where the actual work was done and, if different, the present or permanent address should be indicated as a footnote to that author's name); *c*) name and address for correspondence, including fax number, telephone number, and e-mail address; *d*) address for reprints if different from that of corresponding author; *e*) meetings at which parts of the data were presented (including title of conference, city, and date); *f*) sources of support; and *g*) a running head of no more than 40 characters.
- The abstract should be structured and limited to 250 words depending on article type. It must be factual and comprehensive. Limit the use of abbreviations and acronyms, and avoid general statements (e. g. "the significance of the results is discussed"). List 3 to 6 key words or phrases.
- Organise the manuscript file into sections with appropriate section headings. The sequence should be as follows: title page, abstract/key word page, introduction, methods, results, discussions, acknowledgments, references, tables, figures and figure captions.
- The references should be numbered in the order in which they are cited in the text. JAMA reference style should be used. If there are more than three authors, list only the first three authors and then use et al. Refer to the List of Journals Indexed in Index Medicus for abbreviations of journal names.

The metabolic syndrome and renal function in an African cohort infected with Human Immunodeficiency Virus for 5-10 years

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Running head: Metabolic syndrome and renal function in HIV

4.2. Abstract

Objectives: The human immunodeficiency virus (HIV) is often accompanied by renal dysfunction. It is expected that the metabolic syndrome (MetS) may exacerbate renal impairment further. We therefore determined the prevalence of the MetS and the association thereof with renal function in a South African cohort infected with HIV for 5-10 years.

Methods: This study included 114 HIV-infected and 114 HIV-free individuals matched for age, sex and locality. We examined cardiovascular, anthropometric and metabolic measurements and determined that the MetS. Renal function was assessed using creatinine clearance (CrCl), estimated glomerular filtration rate and urinary albumin-creatinine ratio (uACR).

Results: The prevalence of the MetS was lower in the HIV-infected individuals as compared to the uninfected (28% vs. 44%, p=0.013). The HIV-infected group presented with a lower body mass index and waist circumference (WC) (all $p<0.001$), as well as blood pressures ($p\leq0.0021$). When comparing the HIV-infected with the MetS and to the HIV-free with the MetS, no differences in blood pressure were seen. With regard to renal function, the HIV-infected with the MetS had 43% higher uACR compared to the uninfected with the MetS, after adjusting for age, sex and WC (p=0.032).

Conclusion: uACR was almost two-fold higher in the HIV-infected Africans with the MetS, despite the low prevalence of the MetS, compared to their uninfected counterparts. The combination of HIV and the MetS seemed to increase the risk for renal impairment.

Key words: Human immunodeficiency virus, metabolic syndrome, microalbuminuria, renal function, South Africans

4.3. Introduction

The global burden of the human immunodeficiency virus (HIV) continues to rise with approximately 38.8 million people being infected worldwide. Sub-Saharan Africa contributes 75.4% of new infections globally.[1] In South Africa, the prevalence of HIV was estimated at 6.12 million for the year 2015.[2] The introduction of antiretroviral therapy (ART) to HIV-infected individuals has significantly improved mortality and morbidity,[3] and the HIV infection has now become a manageable chronic disease.[4] However, the beneficial effects of ART are often overshadowed by co-morbidities such as abnormal fat distribution,[5,6] hypertension,[7,8] and dyslipidaemia.[9,10]

These co-morbidities form part of the metabolic syndrome (MetS),[7,11] a multifaceted syndrome defined by a constellation of several cardiovascular risk factors.[12,13] The MetS is commonly reported among people living with HIV infection, and the prevalence is not affected by ART use (25% vs. 23% on ART and ART naïve respectively).[14] The MetS is also an independent risk factor for renal disease[15] and it is not clear if it is the MetS *per se* or its individual components that are the cause of the observed renal impairment.[15,16]

HIV infection is independently associated with microalbuminuria among black and white Americans.[17] Furthermore, Okpa et al.[18] reported the prevalence of microalbuminuria at 15% among newly diagnosed HIV-infected individuals in Nigeria. Microalbuminuria does not only reflect renal dysfunction, but is also a marker of systemic endothelial damage,[19] which is linked to an elevated risk of kidney damage, cardiovascular disease and mortality.[20] Kidney disease contributes significantly to the morbidity and mortality in HIV-infected individuals.[21]

Tenofovir disoproxil fumarate, part of the first-line antiretroviral therapy regimen in South Africa since April 2010,[22] is potentially nephrotoxic.[23] The prevalence of tenofovir-associated nephrotoxicity is estimated at 2.4% and the effect is considered to be mild and tolerable.[24] The prolonged use of tenofovir affects kidney function more than any other non-nucleoside reverse transcriptase inhibitors.[25-27]

Scant studies report on the prevalence of the MetS and the association thereof with renal function among the South African population living with HIV infection. Since renal dysfunction is related to both the use of ART (tenofovir) and the MetS, we hypothesise that the HIV-infected population using ART and suffering from the MetS

may be at particular risk for renal impairment. Therefore, the aim of this study is to determine the prevalence of the MetS and the association thereof with renal function in a South African cohort, infected with HIV for 5 -10 years.

4.4. Methods

Study design and population

The Prospective Urban and Rural Epidemiological (PURE) study is a multinational longitudinal study examining the changes in lifestyle and causes of chronic diseases, through periodic standardised data collection.[28] The PURE study focuses on urban and rural areas in 17 different low- and middle-income countries, including South Africa. In the North West Province of South Africa, the PURE study participants were randomly recruited door-to-door from two main sites: Potchefstroom (urban) and Ganyesa (rural). Data collection was done on three occasions, with baseline data collected in 2005 and follow-up data in 2010 and 2015.

The inclusion criteria of the PURE study specified black men and women older than 35 years. During baseline, the HIV-infected individuals were unaware of their status and were newly identified as being HIV-infected. For this cross-sectional study, we matched the data from the 10-year follow-up study (2015) for 114 HIV-infected individuals (77.3% taking ART) with 114 HIV-uninfected participants according to age, sex and locality (urban and rural areas). Of the 114 HIV-infected participants, 27 had been infected with HIV for five years (24%), 87 were infected for 10 years (76%,) and one participant had missing information. The participants (n=228) had complete datasets for all MetS components. The study population is outlined in Figure 4.1.

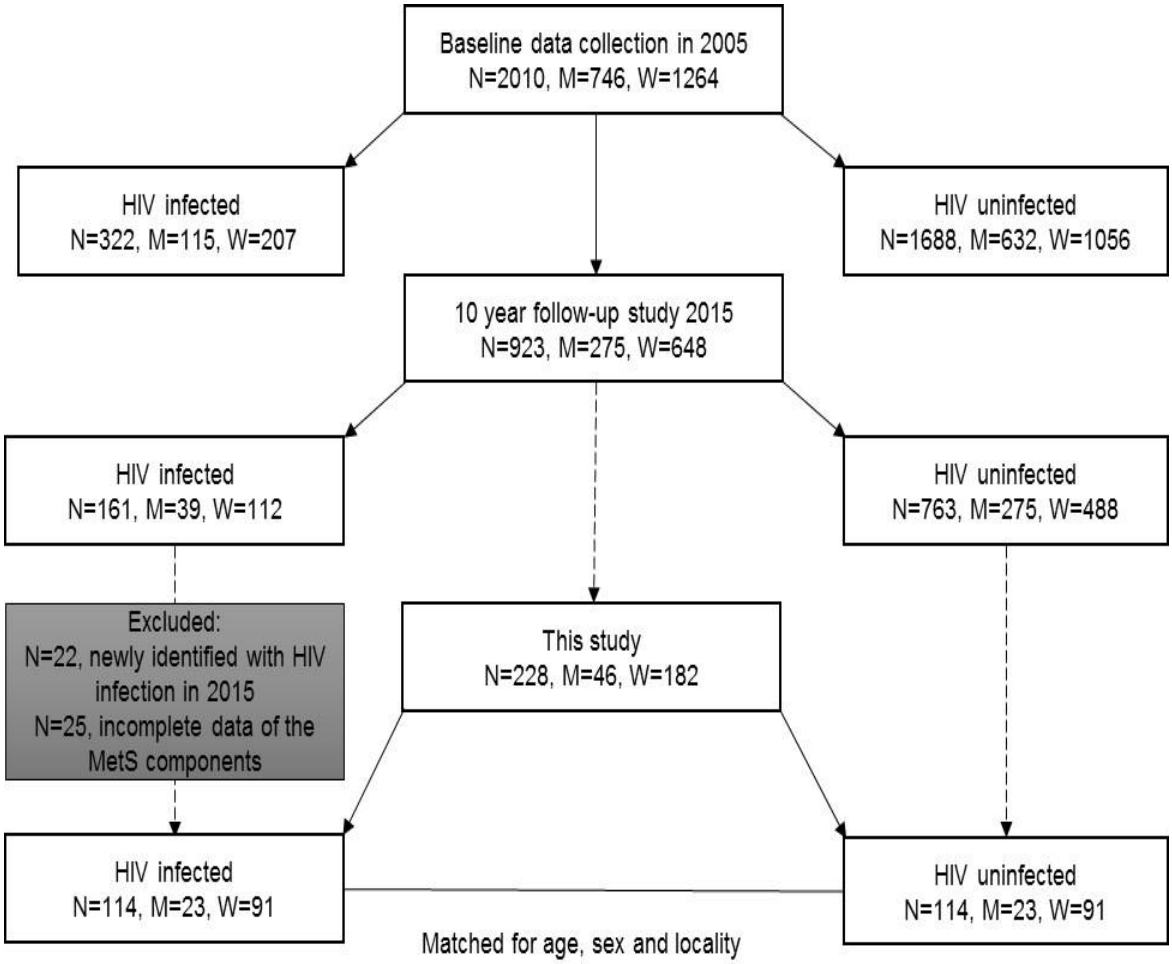

Figure 4.1: Outline of the study

N, number of participants; M, men; W, women; HIV, human immunodeficiency virus.

Ethical considerations

Both the PURE study and this sub-study were approved by the Health Research Ethics Committee (HREC) of the North-West University in South Africa (approval number: 00016-10-A1 and NWU-00035-16-S1). The study protocols conformed to the principles of the Declaration of Helsinki. The research information was conveyed in the participant's home language by trained African field workers fluent in both English and Tswana. The participants gave informed consent to take part in the study.

Questionnaires

Questionnaires were used to collect data on socio-economic and demographic information, current health status, medical and family history, medication, as well as tobacco and alcohol use.

Anthropometry

Anthropometric measurements were done according to standardised procedures, as prescribed by the guidelines of the International Society for the Advancement of Kinanthropometry (ISAK).[29] The height of the participants was measured to the nearest 0.1 cm with a stadiometer (Leicester height measure, Seca, Birmingham, UK), and weight was measured on a portable electronic scale to the nearest 0.1 kg (Precision Health Scale, A & D Company, Japan). Body mass index (BMI) was calculated. Waist circumference (WC) was measured at the narrowest point between the lower rib border and the iliac crest, and was recorded to the nearest 0.1 cm with a steel tape (Lufkin, Cooper Tools, Apex NC, USA).

Blood pressure measurements

The participants were allowed to rest for 10 minutes before blood pressure (BP) measurements were taken. Duplicate brachial blood pressure measurements were taken in the sitting position at five-minute intervals, using the validated OMRON M6 device (Omron Healthcare, Kyoto, Japan). An appropriate cuff size was used, and it was placed on the right arm over the brachial artery, with the arm supported at heart level and in a relaxed position. Pulse pressure and mean arterial pressure were calculated. Duplicate central systolic blood pressure (cSBP) was measured with the Sphygmocor XCEL device (Atcor Medical Pty. Ltd., Sydney, Australia), with the participant in the supine position.

Biological sample collection

Prior to measurements the participants were asked to fast overnight for a period of eight hours. Venous blood samples were collected using a winged infusion set. The serum and plasma were prepared according to standardised procedures and were stored at -80 degrees Celsius until analyses. A spot urine sample was collected and stored at -80 °C until analyses.

Biochemical analyses

The serum samples were analysed using the Cobas Integra 400 plus (Roche® Basel, Switzerland) and glucose, total cholesterol (TC), triglycerides (TG), low density lipoprotein-cholesterol (LDL-c), high density lipoprotein-cholesterol (HDL-c), γ-glutamyl transferase and creatinine levels were determined. Serum C-reactive protein (CRP) levels was determined by means of a particle-enhanced turbidimetric assay. Glycated haemoglobin (HbA1c) was determined using the D-10 Haemoglobin testing system (Bio-Rad, #220-0101) by means of ion-exchange high-performance liquid chromatography.

The urinary creatinine and albumin levels were analysed with the Cobas Integra 400 plus (Roche,® Basel, Switzerland) by means of a kinetic colorimetric assay.

HIV testing and counselling

Participants were counselled before and after HIV testing by a registered counsellor. The HIV status was determined from whole blood according to the protocols of the South African Department of Health, using the first response rapid HIV card test (Premier Medical Corporation Limited, Daman, India). In the case of a positive result, the test was confirmed with an Abon (Biopharm Corporation Limited Hanyzhou, China) rapid card test. For the CD4 count analyses, finger-prick blood was collected and the CD4 counts were determined at the research site using a point-of-care device, the PIMA^{TM} CD4 (Alere, Jena, Germany). Of the 114 HIV-infected participants, 77.3% were on the first-line ART regimen, namely the fixed-dose combination of tenofovir, efivarenz and emtricitabine.

The metabolic syndrome definition

We defined the MetS using the criteria of the International Diabetes Federation (IDF): individuals with WC > 94 cm for men and > 80 cm for women and any of the other two: BP > 130/85 mmHg, TG > 1.7 mmol/l, HDL-c < 1.03 mmol/l for men and < 1.29 mmol/l for women and glucose > 5.6 mmol/l.

Renal function

Creatinine clearance (ml/min) was calculated using the Cockcroft-Gault formula as follows: [(140-age) * (weight in kg) * (1.23 if male OR 1.04 if female)] / (serum creatinine in µmol/l).[23] For creatinine clearance (CrCl) a cut-off value of < 50ml/min was chosen, as the South African Department of Health recommends the use of

tenofovir to be discontinued below this cut-off point.[30] We calculated the estimated glomerular filtration rate (eGFR) (ml/min/1.73 m^2) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations.[23] Black ethnicity was not included in the formula.[31] We used the cut-off value of eGFR < 90 ml/min/1.72m^2.[32] Urinary albumin creatinine ratio (uACR) was calculated and a cut-off value of 3-30 mg/mmol, which defines microalbuminuria, was used.[23]

Statistical analyses

Statistical analyses were performed using Statistica version 13 (Stasoft Inc., Tulsa, OK). Descriptive statistics were done for all the normally distributed variables, and were presented as means and standard deviations. Variables which were not normally distributed were log transformed, and presented as geometric means and 5^{th} and 95^{th} percentiles. An independent t-test was used to compare the means of the groups, and the Chi-square test was used for proportions of the categorical variables. ANCOVAs were used to compare HIV-infected and uninfected groups while adjusting for WC. We also compared uACR between the HIV-infected with/without the MetS, and the HIV-uninfected with/without the MetS, after adjusting for age, sex and WC. Bonferroni tests were used in post hoc comparisons between the groups. The adjusted least square means were used to plot a bar graph comparing the four groups mentioned. Finally, we performed multi-variate adjusted regression analyses with renal markers (CrCl, eGFR and uACR) as dependent variables in the total group, and in the HIV- uninfected and HIV-infected group to determine the contributions of the MetS components towards renal function in the different groups. The independent variables that were entered into the model included age, sex, cSBP, WC, HDL-c, TG, HbA1c, CRP, MetS, HIV status, CD4 cell count and ART. The significance level was set at $p \leq 0.05$.

4.5. Results

Characteristics of the HIV-infected and matched HIV-free group are presented in Table 4.1. As a result of matching age, sex and locality, the groups were similar. The prevalence of the MetS was lower in the HIV-infected group than the HIV-free group (28% vs. 44%, p=0.013). In those on ART (N=85) the prevalence of the MetS was 24.2% and those not taking ART (N=23) the prevalence of the MetS was 30.4%.

Table 4.1: Characteristics of the HIV-uninfected and infected individuals

	HIV-uninfected N= 114	HIV-infected N= 114	*p*
Men, N (%)	23 (20.2)	23 (20.2)	-
Age, years	53.3 ± 5.5	53.4 ± 5.6	0.874
Urban N (%)	46 (40.4)	46 (40.4)	-
Anthropometry			
WC, cm	91.6 [70.5; 122.6]	81.7 [64.6; 109.1]	<0.001
BMI, kg/m^2	27.4 [18.0; 44.5]	22.8 [16.1; 34.5]	<0.001
Cardiovascular measurements			
SBP, mmHg	133 ± 21	126 ± 24	0.021
DBP, mmHg	88 ± 12	83 ± 14	0.003
PP, mmHg	45 ± 14	43 ± 15	0.309
MAP, mmHg	103 ± 14	97 ± 16	0.006
cSBP, mmHg	129 ± 18	120 ± 18	<0.001
Biochemical variables			
TC, mmol/l	4.53 ± 1.60	4.52 ± 1.01	0.904
LDL-c, mmol/l	2.79 ± 1.02	2.67 ± 0.89	0.362
TG, mmol/l	1.18 [0.53; 2.85]	1.10 [0.50; 2.40]	0.317
HDL-c, mmol/l	1.25 [0.69; 2.27]	1.32 [0.74; 2.53]	0.250
Glucose, mmol/l	5.34 [3.85; 9.03]	5.18 [4.35; 6.48]	0.529
HbA1c,%	5.92 [5.00; 8.80]	5.46 [4.90; 6.30]	<0.001
CRP, mg/l	1.11 [0.05; 16.1]	1.38 [0.04; 43.7]	0.072
γ-glutamyltransferase, U/l	23.2 [1.46; 256]	23.4 [0.70; 236]	0.963
HIV related parameters			
CD4 cell count, cell/mm^3	-	519 ± 263	-
≤ 500 cells/mm^3, N (%)	-	54/106 (50.9)	-
≤ 200 cells/mm^3, N (%)	-	9/106 (8.5)	-
Renal function			
SCr, µmol/l	55.9 ± 11.4	57.0 ± 12.8	0.499
CrCl, ml/min	116 [72.0; 208]	97.9 [56.9; 165]	<0.001
CrCl < 50 ml/min, N (%)	0/113	1/113	0.316
eGFR, ml/min/1.73 m^2	103 [83.2; 123]	103 [74.3; 120]	0.985
eGFR, < 90 ml/min/1.73 m^2, N (%)	14 (12.3)	14 (12.3)	1.000
uACR, mg/mmol	1.43 [0.43; 14.6]	1.89 [0.52; 14.7]	0.720
uACR, 3–30 mg/mmol, N (%)	18/102 (17.7)	27/100 (27.0)	0.110
Health behaviours			
Self-reported alcohol use, N (%)	34/113 (30.1)	35/111 (31.5)	0.815
Self-reported tobacco use, N (%)	41/113 (36.3)	43/111 (38.7)	0.704
Medication use			
Anti-hypertensive med, N (%)	35 (30.7)	14/109 (12.8)	0.001
Diuretics, N (%)	38 (33.3)	20/109 (18.4)	0.010
Statins, N (%)	6 (5.3)	1/109 (6.4)	0.063
Anti-inflammatory med, N (%)	8 (7.0)	7/109 (6.4)	0.859
Anti-diabetic med, N (%)	10 (8.8)	0/109 (0.0)	0.002
Anti-coagulant med, N (%)	9 (7.9)	3/109 (2.8)	0.089
Antiretroviral therapy (ART), N (%)	-	85/110 (77.3)	-
≥ 5 years on ART, N (%)	-	38/59 (64.4)	-
Metabolic syndrome, N (%)	50 (43.9)	32 (28.1)	0.013

Data are arithmetic mean ± SD or geometric mean (5th and 95th percentile intervals) for logarithmically transformed variables. SD, standard deviation; CI, confidence interval; HIV, human immunodeficiency virus; N, number of participants; WC, waist circumference; BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; PP, pulse pressure, MAP, mean arterial pressure; cSBP, central systolic blood pressure; TC, Total cholesterol; LDL-cholesterol, low density lipoprotein-cholesterol; HDL-cholesterol, high density lipoprotein- cholesterol; HbA1c%; glycated haemoglobin; CRP, C-reactive protein, SCr, serum creatinine; CrCl, creatinine clearance; eGFR, estimated glomerular filtration rate; uACR, urinary albumin-creatinine ratio; Med, medication; ART, antiretroviral therapy.

The HIV-infected group had lower WC (p<0.001) and BMI (p<0.001) compared to the HIV-uninfected. When comparing the cardiovascular measurements, the brachial systolic BP (p=0.021), diastolic BP (p=0.003), cSBP (p=0.006) and mean arterial pressure (p<0.001) were higher in the HIV-free group as compared to the HIV-infected. With regard to the renal function measurements, the HIV-infected had lower CrCl (p<0.001), but there was no difference in eGFR and uACR.

When WC was adjusted, diastolic BP, mean arterial pressure and cSBP remained higher in the HIV-uninfected participants (Supplementary Table S1), but the brachial systolic BP (p=0.057) and CrCl (p=0.304) no longer differed.

To determine the potential influence of ART we repeated the comparative analyses between the HIV-uninfected and the HIV-infected group using ART (N=85), (Supplementary Table S2). The results remained the same.

We further compared HIV-infected and uninfected groups with the MetS (Table 4.2). The blood pressures did not differ between the groups. However, the WC (p=0.023) and BMI (p=0.010) were lower in the infected group. The HIV-infected group had lower CrCl (p=0.050), and a greater proportion had microalbuminuria (46% vs. 17%, p=0.007) compared to the uninfected, supported by a tendency of higher uACR in the HIV-infected group (p=0.065).

We also compared uACR between the HIV-infected and uninfected, with and without the MetS (Figure 4.2), while adjusting for age, sex and WC. The mean uACR of the HIV- infected group with the MetS (3.16 mg/mmol) was almost double that of the HIV-uninfected with the MetS (1.81 mg/mmol) (p=0.032) despite similar ages and blood pressures. When comparing the HIV-infected without the MetS to those with the MetS, the uACR tended to be lower (1.21 mg/mmol; p=0.11). The uACR was also lower in the HIV-uninfected group without the MetS (1.34 mg/mmol), compared to the infected group with the MetS (p=0.047).

Table 4.2: Characteristics of the HIV-uninfected and HIV-infected with the metabolic syndrome

	HIV-uninfected with MetS N= 50	HIV-infected with MetS N= 32	*P*
Men, N (%)	5 (10.0)	4 (12.5)	0.723
Age, years	53.8 ± 6.5	53.5 ± 6.1	0.844
Urban N (%)	19 (38.0)	16 (50.0)	0.283
Anthropometry			
WC, cm	102 [85.5; 129]	94.6 [80.0; 120.3]	0.023
BMI, kg/m^2	32.6 [24.4; 50.0]	27.8 [19.0; 44.0]	0.010
Cardiovascular measurements			
SBP, mmHg	139 ± 12	137 ± 29	0.782
DBP, mmHg	91 ± 10	90 ± 14	0.789
PP, mmHg	48 ± 11	47 ± 19	0.839
MAP, mmHg	107 ± 09	106 ± 18	0.772
cSBP, mmHg	133 ± 15	129 ± 20	0.457
Biochemical variables			
TC, mmol/l	4.54 ± 1.07	4.66 ± 1.16	0.699
LDL-c, mmol/l	2.88 ± 1.11	2.69 ± 1.00	0.523
TG, mmol/l	1.47 [0.28; 2.72]	1.66 [0.67; 6.70]	0.432
HDL-c, mmol/l	1.02 [0.77; 1.61]	1.34 [0.55; 2.34]	0.316
Glucose, mmol/l	5.71 [3.84; 12.9]	5.56 [4.35; 7.96]	0.684
HbA1c,%	6.28 [5.20; 11.7]	5.71 [5.10; 6.60]	0.040
CRP, mg/l	1.04 [0.05; 15.6]	1.50 [0.04; 29.6]	0.555
γ-glutamyltransferase, U/l	22.7 [2.01; 224]	25.6 [0.70; 325]	0.797
HIV related parameters			
CD4 cell count, cell/mm^3	-	497 ± 239	-
≤ 500 cells/mm^3, N (%)	-	15/30 (50)	
≤ 200 cells/mm^3, N (%)	-	2/30 (6.7)	-
Renal function			
SCr, µmol/l	55.9 ± 12.2	57.0 ± 12.8	0.694
CrCl, ml/min	133 [86.5; 218]	113 [71.3; 192]	0.050
CrCl < 50 ml/min, N (%)	-	-	-
eGFR, ml/min/1.73 m^2	100 [87.2; 112]	104 [82.5; 129]	0.211
eGFR, < 90 ml/min/1.73 m^2, N (%)	7 (14.0)	3 (9.38)	0.532
uACR, mg/mmol	1.43 [0.49; 20.5]	2.80 [0.47; 25.8]	0.065
uACR, 3–30 mg/mmol, N (%)	8/46 (17.4)	13/28 (46.4)	0.007
Health behaviours			
Self-reported alcohol use, N (%)	9/49 (18.4)	10 (31.3)	0.181
Self-reported tobacco use, N (%)	14/49 (28.6)	12 (37.5)	0.400
Medication use			
Anti-hypertensive medication, N (%)	22 (44.0)	8 (25.0)	0.081
Diuretics, N (%)	24 (48.0)	9 (25.1)	0.073
Statins, N (%)	3 (6.0)	0 (0.0)	0.158
Anti-inflammatory medication, N (%)	4 (8.0)	2 (6.3)	0.766
Anti-diabetic medication, N (%)	9 (18)	0 (0.0)	0.011
Anti-coagulant medication, N (%)	6 (12.0)	2 (6.3)	0.392
Antiretroviral therapy (ART), N (%)	-	24 (75.0)	-
≥ 5 years on ART, N (%)	-	14/22 (14.0)	-

Data are arithmetic mean ± SD or geometric mean (5th and 95th percentile intervals) for logarithmically transformed variables. SD, standard deviation; CI, confidence interval; HIV, human immunodeficiency virus; N, number of participants; WC, waist circumference; BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; PP, pulse pressure, MAP, mean arterial pressure; cSBP, central systolic blood pressure; TC, Total cholesterol; LDL-cholesterol, low density lipoprotein-cholesterol; HDL-cholesterol, high density lipoprotein- cholesterol; HbA1c%, glycated haemoglobin; CRP, C-reactive protein, SCr, serum creatinine; CrCl, creatinine clearance; eGFR, estimated glomerular filtration rate; uACR, urinary albumin-creatinine ratio; Med, medication; ART, antiretroviral therapy.

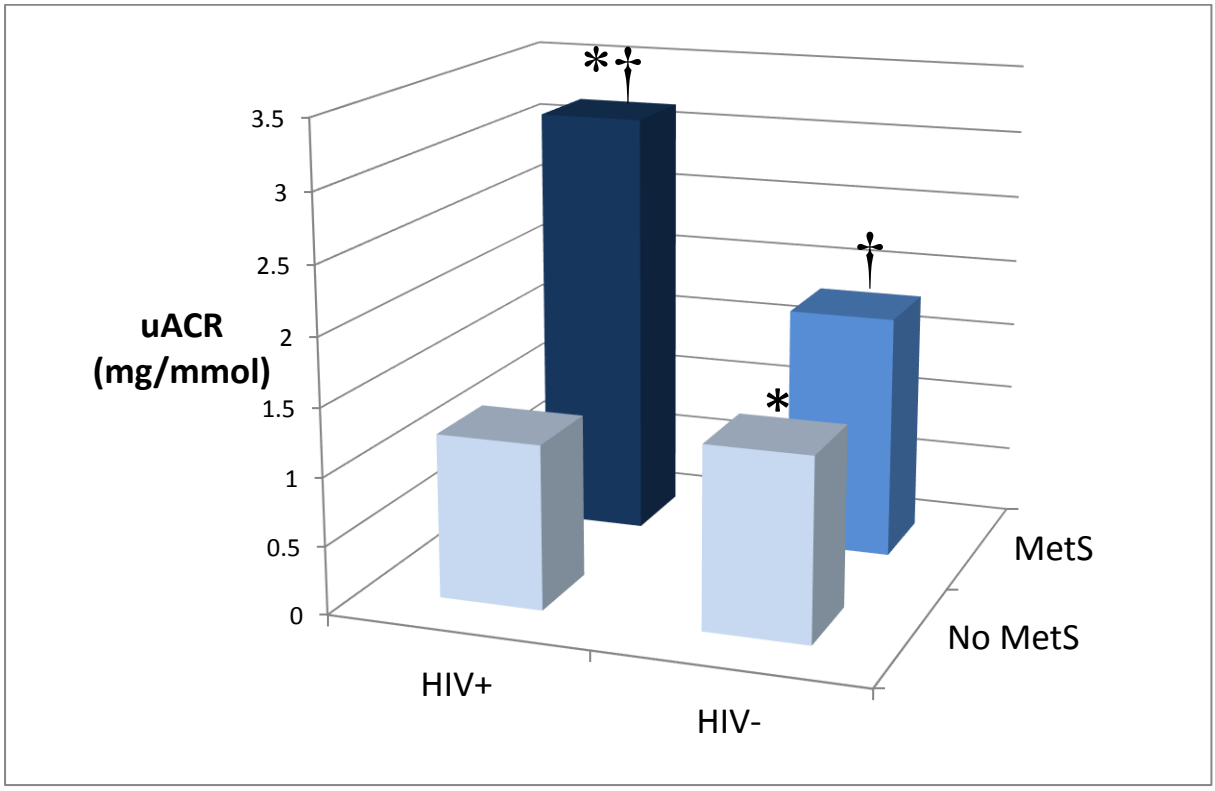

Figure 4.2: Urinary albumin excretion for HIV-uninfected and HIV-infected with/without the metabolic syndrome after adjusting for age, sex and waist circumference

HIV, Human immunodeficiency virus; HIV+, infected with human immunodeficiency virus; HIV-, human immunodeficiency virus unifected; MetS, metabolic syndrome; uACR, urinary albumin-creatinine ratio (uACR).Bars with the same symbol differ significantly ($p<0.05$).

We performed multiple regression analysis for renal markers (CrCl, eGFR and uACR) in the total group, HIV-uninfected, and HIV-infected groups. In the total group, the presence of the MetS was positively associated with eGFR ($p=0.008$).

In both the HIV-infected and uninfected groups, age, WC and TG were associated with CrCl, and age was negatively associated with eGFR. CD4 cell count was positively associated with CrCl ($p=0.009$) in the HIV-infected group. The use of ART did not associate with any markers of renal function. When viewing the results for uACR, women were associated with increased uACR in the total group ($p=0.001$), the HIV-uninfected ($p=0.001$), and were borderline in the HIV-infected group ($p=0.086$). uACR was also positively associated with cSBP in the total group ($p<0.001$), the HIV-uninfected group ($p<0.001$) and the infected group ($p=0.008$). uACR was negatively associated with WC in the total group ($p<0.001$), the uninfected ($p<0.001$) and infected

(p=0.049). In the HIV-infected, the presence of the MetS was positively associated with eGFR (p=0.039) and had a borderline association with uACR (p=0.059).

Table 4.3: Multiple regression analysis with markers of renal function as dependent variables

Total group (HIV-uninfected and HIV-infected, N= 228)

	CrCl, ml/min		eGFR, ml/min/1.72m^2		uACR, mg/mmol	
	R^2 = 0.533		R^2 = 0.227		R^2 = 0.202	
	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
Age, years	-0.28 (-0.37; -0.19)	**<0.001**	-0.44 (-0.56; -0.32)	**<0.001**	-0.01 (-0.13; 0.12)	0.970
Sex, women, men	-0.01 (-0.10; 0.09)	0.983	0.16 (0.04; 0.28)	**0.011**	-0.25 (-0.38; -0.12)	**<0.001**
cSBP, mmHg	0.06 (-0.04; 0.16)	0.265	0.01 (-0.12; 0.14)	0.873	0.36 (0.22; 0.5)0	**<0.001**
WC, cm	0.63 (0.51; 0.75)	**<0.001**	-0.15 (-0.31; 0.01)	**0.053**	-0.36 (-0.54; 0.19)	**<0.001**
HDL-C, mmol/l	0.01 (-0.10; 0.11)	0.883	0.05 (-0.08; 0.18)	0.465	-0.02 (-0.17; 0.12)	0.754
TG mmol/l	-0.21 (-0.31; -0.11)	**<0.001**	-0.11 (-0.24; 0.02)	0.086	0.11 (-0.03; 0.25)	0.120
HbA1c, %	-0.02 (-0.12; 0.07)	0.625	-0.12 (-0.24; 0.01)	0.079	-0.02 (-0.16; 0.12)	0.766
CRP, mg/l	0.02 (-0.07; 0.12)	0.638	-0.03 (-0.15; 0.09)	0.655	0.00 (-0.13; 0.13)	0.975
HIV status, neg/pos	-0.06 (-0.15; 0.05)	0.279	-0.06 (-0.18; 0.07)	0.385	0.12 (-0.02; 0.29)	0.087
MetS, no/yes	0.09 (-0.03; 0.23)	0.141	0.22 (0.06; 0.38)	**0.008**	0.11 (-0.06; 0.28)	0.217

HIV-uninfected, N= 114

	R^2 = 0.583		R^2 = 0.232		R^2 = 0.270	
	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
Age, years	-0.28 (-0.41; -0.16)	**<0.001**	-0.45 (-0.62; -0.28)	**<0.001**	-0.02 (-0.20; 0.15)	0.809
Sex, men, women	0.04 (-0.09; 0.18)	0.522	0.15 (-0.03; 0.34)	0.109	-0.38 (-0.57; -0.19)	**<0.001**
cSBP, mmHg	0.05 (-0.08; 0.18)	0.471	-0.07 (-0.25; 0.11)	0.440	0.39 (0.21; 0.57)	**<0.001**
WC, cm	0.71 (0.56; 0.86)	**<0.001**	-0.13 (-0.33; 0.08)	0.229	-0.42 (-0.63; -0.21)	**<0.001**
HDL-C, mmol/l	-0.05 (-0.20; 0.09)	0.487	-0.05 (-0.25; 0.15)	0.622	-0.05 (-0.25; 0.15)	0.629
TG mmol/l	-0.24 (-0.37; -0.11)	**<0.001**	-0.17 (-0.34; 0.01)	0.065	0.06 (-0.13; 0.23)	0.622
HbA1c, %	-0.07 (-0.20; 0.06)	0.319	-0.13 (-0.30; 0.05)	0.151	0.08 (-0.10; 0.26)	0.363
CRP, mg/l	-0.02 (-0.15; 0.11)	0.759	-0.02 (-0.19; 0.15)	0.804	0.12 (-0.06; 0.30)	0.193
MetS, no/yes	0.09 (-0.08; 0.26)	0.288	0.14 (-0.09; 0.36)	0.228	-0.02 (-0.25; 0.21)	0.891

HIV-infected, N= 114

	R^2 = 0.425		R^2 = 0.231		R^2 = 0.152	
	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
Age, years	-0.31 (-0.46; -0.10)	**<0.001**	-0.43 (-0.61; -0.26)	**<0.001**	0.03 (-0.16; 0.22)	0.775
Sex, men, women	-0.02 (-0.18; 0.13)	0.769	0.15 (-0.02; 0.33)	0.091	-0.17 (-0.36; 0.02)	0.086
cSBP, mmHg	0.10 (-0.06; 0.27)	0.221	0.12 (-0.07; 0.31)	0.235	0.29 (0.08; 0.50)	**0.008**
WC, cm	0.50 (0.30; 0.69)	**<0.001**	-0.19 (-0.41; 0.04)	0.106	-0.25 (-0.49; -0.11)	**0.049**
HDL-C, mmol/l	0.02 (-0.15; 0.19)	0.813	0.07 (-0.13; 0.27)	0.498	-0.04 (-0.26; 0.17)	0.709
TG, mmol/l	-0.21 (-0.39; -0.03)	**0.023**	-0.09 (-0.30; 0.11)	0.389	0.13 (-0.10; 0.35)	0.266
HbA1c, %	0.09 (-0.13; 0.22)	0.625	-0.08 (-0.23; 0.12)	0.457	-0.19 (-0.40; 0.03)	0.099
CRP, mg/l	0.07 (-0.09; 0.23)	0.370	0.01 (-0.18; 0.19)	0.951	-0.12 (-0.32; 0.08)	0.228
MetS, no/yes	0.09 (-0.12; 0.30)	0.409	0.26 (0.02; 0.50)	**0.039**	0.26(-0.01; 0.52)	0.059
CD4 count, mm^3	0.21 (0.06; 0.36)	**0.009**	0.17 (-0.01; 0.35)	0.062	-0.13 (-0.33; 0.06)	0.179
ART, no/ yes	0.07 (-0.09; 0.23)	0.219	0.13 (-0.06; 0.31)	0.190	0.07 (-0.13; 0.27)	0.506

β, partial regression coefficient; R^2, adjusted R^2; 95% CI, 95% confidence interval of β; HIV, human immunodeficiency syndrome; N, number of participants; eGFR, estimated glomerular filtration rate; uACR, urinary albumin creatinine ratio; CrCl, creatinine clearance; cSBP, central systolic blood pressure; WC, waist circumference; HDL-C, high density lipoprotein cholesterol; TG, triglycerides; HbA1c%, glycated haemoglobin; CRP, C-reactive protein; MetS, metabolic syndrome; pos, HIV positive; neg, HIV negative; ART, antiretroviral therapy. eGFR, uACR, CrCl, cSBP, WC, HDL-C, TG, CRP were logarithmically transformed. Independent variables included in the model include: age, sex, cSBP, WC, HDL-C, TG, HbA1C, CRP, CD4 cell count and ART. All independent variables were added at the same time. Bold values indicate p ≤ 0.05.

4.6. Discussion

We aimed to determine the prevalence of the MetS and to evaluate renal function in a South African cohort infected with HIV for at least 5 years. Our main finding was that Africans with HIV infection and the MetS had a 43% higher urinary albumin excretion compared to the HIV-uninfected with the MetS. The development of renal dysfunction in the HIV-infected group was supported by a markedly lower CrCl. However, it is also noteworthy that only 28% of the HIV-infected group had the MetS, compared to 44% of their matched, uninfected counterparts.

The latter is in agreement with a cross-sectional study by Jacobson *et al*.[33] who reported a lower prevalence of the MetS in the HIV-infected group as opposed to the uninfected group. Our finding of a prevalence of the MetS of 28% among the HIV-infected blacks is supported by Julius et al.[10] who reported a prevalence of 20% among black South Africans using ART. Hirigo et al.[34] reported a similar prevalence of 24% using the IDF critique among Ethiopian HIV-infected individuals on ART. In contrast, other studies have reported a higher prevalence of the MetS in HIV-infected individuals, compared to the uninfected.[7,14,35] In this study, the HIV-infected group had lower obesity and blood pressure measurements, with no differences in lipid and glucose levels, whereas in previous studies the higher prevalence of the MetS was driven by a higher prevalence of impaired metabolic components in HIV-infected groups. The IDF criterion requires central obesity as a prerequisite, and any other two metabolic components to meet the criteria of the MetS.[36] The lower obesity and blood pressures in our HIV cohort may explain the lower prevalence of the MetS in the HIV-infected participants.

In our study, the majority of HIV-infected participants (77%) were taking ART, which is associated with improved immune status[30] and either improvement or alteration of the MetS components.[7,37] However, different ART regimens may exert different effects on the metabolic components,[6,7] which may further explain the lower WC and blood pressure among those on ART in this study. An observational study including HIV-infected participants on ART (with tenofovir and efavirenz as part of the regimen), also reported no association between the use of ART and blood pressure among Indians,[38] while Kaplan *et al*.[39] reported a lower prevalence of overweight and hypertension among the HIV-infected, compared to uninfected individuals. However, when we compared the HIV-infected and uninfected participants with the MetS, no difference

was seen in the blood pressure measurements. This indicated the important role of BP in the development of the MetS, which may be independent of the HIV status.

Without considering the prevalence of the MetS, we found that 27% of the HIV-infected had microalbuminuria compared to only 18% of the uninfected individuals. Szczech *et al*.[17] reported a higher prevalence of urinary albumin excretion in a HIV-infected group as compared to uninfected counterparts, 11% vs. 2% respectively. Okpa *et al*.[18] also reported a prevalence of 15% of microalbuminuria among newly diagnosed HIV-infected Nigerians. However, the latter two studies did not assess the MetS.

We aimed to determine whether renal function is affected in those both with HIV and the MetS. Microalbuminuria was considerably higher in the HIV-infected with MetS (46%) compared to their uninfected counterparts (17%) despite similar ages and gender distribution. This supports the role of the MetS in early renal dysfunction in HIV-infected patients. Our finding of 46% microalbuminuria is higher than a recent report by Pirro et al.[40] indicating a prevalence of 17% of microalbuminuria in the HIV-infected with the MetS in Italy. However, a control group was not included in the latter study.

Urinary albumin excretion is a well-known marker of renal dysfunction and may precede systematic endothelial dysfunction,[20] with glomerular permeability to albumin increasing as endothelial dysfunction develops.[41] The MetS is frequently reported in HIV-infected individuals on ART, and is associated with both microalbuminuria and endothelial dysfunction.[40] Furthermore, HIV infection may directly infect the glomerular epithelial cells resulting in excretion of albumin.[42] Since tenofovir has nephrotoxic potential,[43] it may further augment the effect of the HIV infection on the kidneys. Thus, the combination of the MetS, HIV, and tenofovir may exacerbate the glomerular permeability, explaining the high albumin excretion in our participants burdened with both the MetS and HIV infection.

In the multivariate analyses, renal function was associated with cardiovascular risk factors rather than HIV-associated factors, and ART was not associated with any of the renal function markers. Some studies have reported improvements in renal function with the use of ART and suppressed viral load.[44,45] In our study, a CD4 cell count was beneficially associated with CrCl and eGFR, showing that improved immune systems may protect against renal dysfunction.[45] In addition, during the pre-

ART era, the prevalence of microalbuminuria (defined by uACR ≥ 3-30 mg/mmol) in HIV-infected individuals was estimated at between 19% to 31%,[21,46] whereas in the post-ART era it was estimated at between 8.7% to 11%.[47,48]

Urinary albumin excretion is an important marker of renal dysfunction and cardiovascular disease risk, even at subclinical levels. Utilisation of uACR may prove beneficial as it is suggested that the substantial renal impairment seen in individuals taking tenofovir is due to pre-existing renal dysfunction, which might be intensified with tenofovir.[49] It may help to identify HIV-infected individuals with MetS who are potentially at higher risk of renal dysfunction.

This study should be interpreted within the context of its strengths and potential limitations. A limitation of our study is the small sample size of those with the MetS. However, the HIV-infected individuals were infected for at least five years, and were matched according to age, sex and locality to a control group in order to limit confounders. There was incomplete data on the duration of the ART, but we were able to determine duration of at least five years, and the participants were on fixed-dose combinations. Tuberculosis testing was not done for this study, however, information on chronic medication was available. Since this was a cross-sectional study, the associations do not indicate cause and effect. This is a well-controlled study and to our knowledge, is the first study to investigate the combination of the MetS and renal function in an African cohort infected with HIV.

In conclusion, HIV-infected Africans with the MetS had an almost two-fold higher urinary albumin excretion compared to the HIV-free controls with the MetS. The combination of HIV and the MetS indicated an elevated risk for the development of renal disease and cardiovascular disease, and could increase the risk of cardiovascular morbidity and mortality in HIV-infected individuals.

4.7. Acknowledgements

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Supplementary Table S1: Characteristics of HIV-uninfected and infected after adjusting for waist circumference

	HIV-uninfected N= 114	**HIV-infected N= 114**	***p***
Cardiovascular measurements			
SBP, mmHg	133 ± 251	127 ± 251	0.057
DBP, mmHg	87 ± 141	83 ± 141	0.029
PP, mmHg	46 ± 157	43 ± 157	0.278
MAP, mmHg	102 ± 170	97 ± 170	0.032
cSBP, mmHg	128 ± 200	121 ± 200	0.004
Biochemical variables			
TC, mmol/l	4.49 ± 11.9	4.56 ± 11.9	0.645
LDL-c, mmol/l	2.72 ± 16.4	2.74 ± 16.4	0.911
TG, mmol/l	0.05 [1.02; 1.23]	0.06 [1.05; 1.27]	0.642
HDL-c, mmol/l	0.11 [1.21; 1.38]	0.10 [1.20; 1.36]	0.731
TG/HDL-C ratio	-0.06 [0.77; 0.98]	-0.04 [0.80; 1.03]	0.603
Glucose, mmol/l	0.72 [5.01; 5.47]	0.72 [5.00; 5.45]	0.937
HbA1c,%	0.77 [5.68; 6.00]	0.47 [5.38; 5.68]	0.007
CRP, mg/l	-0.001 [0.67; 1.49]]	0.186 [1.03; 2.30]	0.146
Renal markers			
CrCl, ml/min	2.04 [104; 114]	2.02 [100; 110]	0.304
eGFR, ml/min/1.73 m^2	2.01 [101; 106]	2.01 [100; 105]	0.573
uACR, mg/mmol	0.17 [1.21; 1.87]	0.25 [1.44; 2.24]	0.270

Data are adjusted means ± SD or -95% and +95% CI for logarithmically transformed variables. SD, standard deviation; CI, confidence interval; HIV, human immunodeficiency virus; N, number of participants; DBP, diastolic blood pressure; SBP, systolic blood pressure; PP, pulse pressure, MAP, mean arterial pressure; cSBP, central systolic blood pressure; TC, Total cholesterol; LDL-cholesterol, low density lipoprotein-cholesterol; HDL-cholesterol, high density lipoprotein- cholesterol; TG:HDL-C, Triglycerides: High density lipoprotein-cholesterol; CRP, C-reactive protein, CrCl, creatinine clearance; eGFR, estimated glomerular filtration rate; uACR, urinary albumin-creatinine ratio.

Supplementary Table S2: Characteristics of the HIV-uninfected and infected taking antiretroviral therapy

	HIV-uninfected N= 114	**HIV-infected taking ART N= 85**	***P***
Men, N (%)	23 (20.2)	17 (20.0)	0.975
Age, years	53.4 ± 5.5	53.0 ± 5.3	0.636
Urban N (%)	46 (40.4)	35 (41.2)	0.906
Anthropometry			
WC, cm	91.6 [70.5; 123]	82.6 [65.50; 109]	<0.001
BMI, kg/m^2	27.4 [18.0; 44.5]	23.0 [15.6; 35.9]	<0.001
Cardiovascular measurements			
SBP, mmHg	133 ± 21	125 ± 23	0.014
DBP, mmHg	88 ± 12	82 ± 13	0.001
PP, mmHg	45 ± 14	43 ± 15	0.308
MAP, mmHg	103 ± 14	97 ± 15	0.003
cSBP, mmHg	129 ± 18	118 ± 16	<0.001
Biochemical variables			
TC, mmol/l	4.54 ± 1.16	4.50 ± 0.92	0.794
LDL-c, mmol/l	2.79 ± 1.02	2.61 ± 0.87	0.201
TG, mmol/l	1.18 [0.53; 2.85]	1.14 [0.54; 2.35]	0.689
HDL-c, mmol/l	1.25 [0.69; 2.27]	1.34 [0.79; 2.53]	0.148
Glucose, mmol/l	5.34 [3.85; 9.03]	5.15 [4.26; 6.10]	0.491
HbA1c,%	5.92 [5.00; 8.80]	5.41 [4.90; 6.20]	<0.001
CRP, mg/l	1.11 [0.05; 16.1]	1.71 [0.04; 44.7]	0.160
γ-glutamyltransferase, U/l	23.2 [1.46; 256]	26.7 [1.24; 225]	0.538
HIV related parameters			
CD4 cell count, cell/mm^3	-	525 ± 264	-
≤ 500 cells/mm^3, N (%)	-	37/78 (47.4)	-
≤ 200 cells/mm^3, N (%)	-	7/78 (8.97)	-
Renal function			
Scr, µmol/l	55.9 ± 11.4	56.4 ± 12.6	0.787
CrCl, ml/min	116[72.0; 207]	100 [56.0; 167]	0.002
CrCl, < 50 ml/min, N (%)	0/113 (0.00)	1/84 (1.09)	0.245
eGFR, ml/min/1.73 m^2	103 [83.2; 123]	102 [79.2; 120]	0.504
eGFR, < 90 ml/min/1.73 m^2, N (%)	14 (12.3)	8 (9.41)	0.523
uACR, mg/mmol	1.43 [0.43; 14.6]	1.89 [0.48; 22.6]	0.109
uACR, 3–30 mg/mmol, N (%)	18/102 (17.7)	19/76 (25.0)	0.232
Health behaviours			
Self-reported alcohol use, N (%)	34/113 (30.1)	25/84 (29.8)	0.961
Self-reported tobacco use, N (%)	41/113 (36.3)	33/84 (39.3)	0.667
Medication use			
Anti-hypertensive medication, N (%)	35 (30.7)	11 (12.9)	0.003
Diuretics, N (%)	38 (33.3)	16 (18.8)	0.023
Statins, N (%)	6 (5.3)	1 (1.2)	0.122
Anti-inflammatory medication, N (%)	8 (7.0)	6 (7.1)	0.991
Anti-diabetic medication, N (%)	10 (8.8)	0 (0.0)	0.005
Anti-coagulant medication, N (%)	9 (7.9)	3 (3.5)	0.200
≥ 5 years on ART, N (%)	-	38/59 (64.4)	-
Metabolic syndrome, N (%)	50 (43.9)	24 (24.2)	0.024

Data are arithmetic mean ± SD or geometric mean (5th and 95th percentile intervals) for logarithmically transformed variables. SD, standard deviation, CI, confidence interval; HIV, human immunodeficiency virus; N, number of participants; WC, waist circumference; BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; PP, pulse pressure, MAP, mean arterial pressure; cSBP, central systolic blood pressure; TC, Total cholesterol; LDL-cholesterol, low density lipoprotein-cholesterol; HDL-cholesterol, high density lipoprotein- cholesterol; CRP, C-reactive protein, SCr, serum creatinine; CrCl, creatinine clearance; eGFR, estimated glomerular filtration rate; uACR, urinary albumin-creatinine ratio; Med, medication; ART, antiretroviral therapy

CHAPTER 5

Summary of the main findings, concluding remarks and recommendations

5.1 Introduction

This summative chapter includes an interpretation and discussion of the main findings of this study, compared to the literature. The original hypotheses, which were set in Chapter 2, are discussed according to the findings of this study, and conclusions are made. This is followed by recommendations for future research regarding renal impairment in individuals with the metabolic syndrome (MetS), who are also infected with the human immunodeficiency virus (HIV).

5.2 Interpretation of the main findings and a comparison with the relevant literature

Due to reports on an increasing prevalence of the MetS and renal dysfunction in HIV-infected individuals,[1,2] the aim was to determine the prevalence of the MetS and renal dysfunction in a South African cohort infected with HIV for at least five years (76% had been infected for longer than five years). This was accomplished by analysing data of 228 participants (114 HIV-infected and 114 HIV-uninfected) of the PURE (2015) study, matched for age, sex and locality. The study group was compared, based on their HIV status and the presence of the MetS, and reported a lower prevalence of the MetS in the HIV-infected group compared to the uninfected group. With respect to renal function, urinary albumin excretion was higher in the HIV-infected with the MetS than in their uninfected counterparts with the MetS. Furthermore, creatinine clearance (CrCl) was reduced in the HIV-infected group, compared to the uninfected individuals. Considering these findings, the original hypotheses are now addressed.

5.2.1 Hypothesis 1: The prevalence of the MetS will be higher in the HIV-infected individuals compared to the HIV-free individuals

We reported a lower prevalence of the MetS in the HIV-infected compared to their uninfected counterparts, hence the hypothesis is rejected. In the literature, the prevalence of the MetS seems to be controversial.[1,3] However, several authors suggest that HIV-infected individuals are at higher risk of the MetS as a result of either the HIV infection itself, antiretroviral therapy (ART), or their synergistic action coupled with traditional risk factors.[1,4,5] It was therefore suggested that our HIV-infected group would have a higher prevalence of the MetS, but the results indicated the opposite. Our findings are similar to those reported in a cross-sectional study done in the United States by Jacobson et al.[6] that reported a higher prevalence of the MetS in HIV-uninfected than infected individuals. HIV infection is associated with an increase in

triglycerides (TG), high density lipoprotein-cholesterol (HDL-c),[7,8] lower blood pressure[9] and underweight.[10] With the use of ART there seems to be a shift towards improved TG and an increase in blood pressure and body weight, which were borderline higher.[9,11]

The HIV-infected participants in this study presented with lower blood pressure, waist circumference (WC) and body mass index (BMI), despite the effect of ART on metabolic components, which is reported in the literature.[1] The fixed-dose combination used by the participants in this study did not seem to affect the MetS components. Frequently, metabolic derangement such as high blood pressure and body fat distribution are reported, especially with the use of lopinavir/ritonavir,[12] which forms part of the second-line regimen used in South Africa (SA).[13] In other studies in which the prevalence of the MetS is higher, it is driven by a higher prevalence of impaired lipid profiles, obesity, high blood pressure and hyperglycaemia.[1,5] In our study, the two groups presented with similar lipid profiles and glucose levels.

Central obesity is an important defining component of the MetS.[14] In HIV-infected individuals, obesity is less commonly reported and may be due to the slimming effects of HIV.[10,15] Hence, HIV-infected individuals only occasionally meet the cut-off value of the MetS criteria.[3,16] There are no precise cut-off values of WC for populations in Sub-Saharan Africa; the IDF criteria recommend the use of the European cut-off values.[14] Studies have shown that black and white populations have different body composition, and this may underestimate or overestimate the prevalence of central obesity, and ultimately of MetS.[17]

5.2.2 Hypothesis 2: HIV-infected individuals will present with increased uACR, lower eGFR and CrCl, compared to their uninfected counterparts

There was no difference in the urinary albumin creatinine ratio (uACR) and estimated glomerular filtration rates (eGFR) between the HIV-infected and uninfected participants. However, the CrCl was lower in the HIV-infected participants than in the uninfected participants. Therefore, the hypothesis is partially accepted.

In the literature, it is commonly reported that the HIV-infected are at increased risk of renal impairment, characterised by increased urinary albumin excretion,[18] decreased CrCl and eGFR,[19-21] but in this study there were no differences for these measures between the two groups except for CrCl, which is a useful measure to approximate the

kidney's filtration capacity. Mizushima et al.[22] reported that 18% of the HIV-infected on tenofovir weighing < 55 kg had reduced CrCl, compared to 8% of those that weighed > 55 kg. In the present study, the mean weight of the HIV-infected participants was 58 kg. Underweight individuals are potentially at a higher risk for greater drug exposure and consequently, more toxicity.[19,23] We reported lower BMI and WC in the HIV-infected group, which may explain the lower CrCl in the HIV-infected individuals. In addition, Nishijama et al.[24] reported that low WC and BMI are associated with a decrease in CrCl in HIV-infected individuals. When adjustment was made for WC in our study, the CrCl level did not differ between the two groups. The possible role of WC in altering the CrCl was further supported by the results in the multiple regression, wherein WC was positively associated with CrCl. Brennan *et al*.[25] suggested that renal impairment among the HIV-infected taking tenofovir might be linked to pre-existing kidney pathology, which may be augmented by tenofovir.

Reduced eGFR is frequently reported in HIV-infected individuals. A cross-sectional study including 1092 HIV-infected participants taking tenofovir in South Africa reported that 79% had normal eGFR (> 90 ml/min/1.73m^2), 19% had mildly reduced eGFR (60-89 ml/min/1.73m^2) and 2% had moderately reduced eGFR (30-59 ml/min/1.73m^2).[26] In the latter study the introduction of tenofovir for more than 12 months was associated with improvements in renal function.[26] However, Overton et al.[27] reported that 43% of HIV-infected patients had reduced eGFR < 90 ml/min/1.73m^2, and a decline in eGFR was associated with the use of tenofovir or stavudine and hypertension.

In contrast to our findings in the total HIV-infected vs uninfected groups, other studies have reported a higher urinary albumin-creatinine ratio in HIV-infected individuals.[28,29] In those studies, HIV-infected individuals with microalbuminuria presented with higher blood pressure and lower CD4 cell counts, whereas in our study the HIV-infected individuals presented with lower blood pressure and only 8.5% had a CD4 cell count of < 200 cell/mm^3.

5.2.3 Hypothesis 3: Measures of renal function will be adversely affected in HIV-infected participants with the MetS as compared to the HIV-infected without the MetS, and HIV-uninfected with and without the MetS

The combination of HIV and the MetS seems potent, as this group presented with significantly higher urinary albumin excretion than the HIV-uninfected with and without

the MetS. With regard to CrCl, the HIV-infected with the MetS had lower CrCl than the uninfected individuals with the MetS, although there were no differences with regard to eGFR. Therefore the hypothesis is partially accepted.

Microalbuminuria and HIV have been linked in previous studies,[18] with microalbuminuria as an early marker for the development of renal diseases[30] and alteration of the endothelium.[31] Szczech et al.[18] recently reported an independent association between HIV infection and microalbuminuria. Microalbuminuria is widely expressed as uACR[32] and elevates cardiovascular risk in various clinical settings,[33] even at lower concentrations than the definition of microalbuminuria.[34,35] Moreover, microalbuminuria often obscures the progression of the HIV infection,[36] due to its association with an increase in future cardiovascular disease, morbidity and mortality.[28]

HIV infection may directly exert its effects on the kidney by infecting the glomerular epithelial cells or podocytes, which may lead to a higher excretion of the albumin into the urine.[37] This effect of HIV on the kidney may further be exacerbated by the nephrotoxic effect of ART.[38] However, in this study, HIV status and ART were not associated with renal function markers in the multiple regression analyses. The majority of the study participants presented with a higher CD4 cell count, which is linked to improvement in renal function.[2,39,40]

Where the abovementioned studies focused on HIV and uACR independent of the MetS, we noted an increase in the number of individuals with microalbuminuria, from 27% in the HIV-infected to 46% HIV-infected with the MetS, whereas in the uninfected group there was no increase (17.7% to 17.4% respectively). These results indicate the combination of the MetS and HIV infection augmenting the risk of renal impairment. The literature has reported increased risk of developing microalbuminuria with the presence of the MetS in the HIV-infected that are taking ART.[41] The study further reported that the combination of both microalbuminuria and the MetS is associated with increased risk of endothelial dysfunction.[41]

In this study we observed different findings for uACR and eGFR, indicating that HIV-infected participants with the MetS possibly present with alteration in endothelial permeability rather than glomerular filtration.

5.3 Reflection on the main findings

This study has shown that the HIV-infected participants with the MetS have significantly higher urinary albumin excretion, which may be indicative of early risk for renal and endothelial dysfunction. Microalbuminuria may lead to or follow the initiation of endothelial dysfunction.[42] Several studies have reported on the prevalence of both microalbuminuria and endothelial dysfunction, and this may suggest the existence of a link between these conditions.[41] The MetS is linked to endothelial and epithelial injury, which result in leakage of protein, causing microalbuminuria.[43]

The interaction of HIV and MetS may synergistically or *per se* induce renal injury.[2,18,44] It is well recognised that the MetS and microalbuminuria are associated with increased risk of cardiovascular disease.[45,46] Both the MetS and renal disease are frequently reported in HIV-infected individuals. Several mechanisms could be involved in this observation; however, the mechanism has not yet been fully elucidated.

The HIV-infected participants with and without the MetS further presented with a decline in CrCl. However, there was no significant difference in the number of participants with CrCl below 50 ml/min in the HIV-infected and uninfected individuals. Figure 5.1 below presents a summary of the main findings of this study.

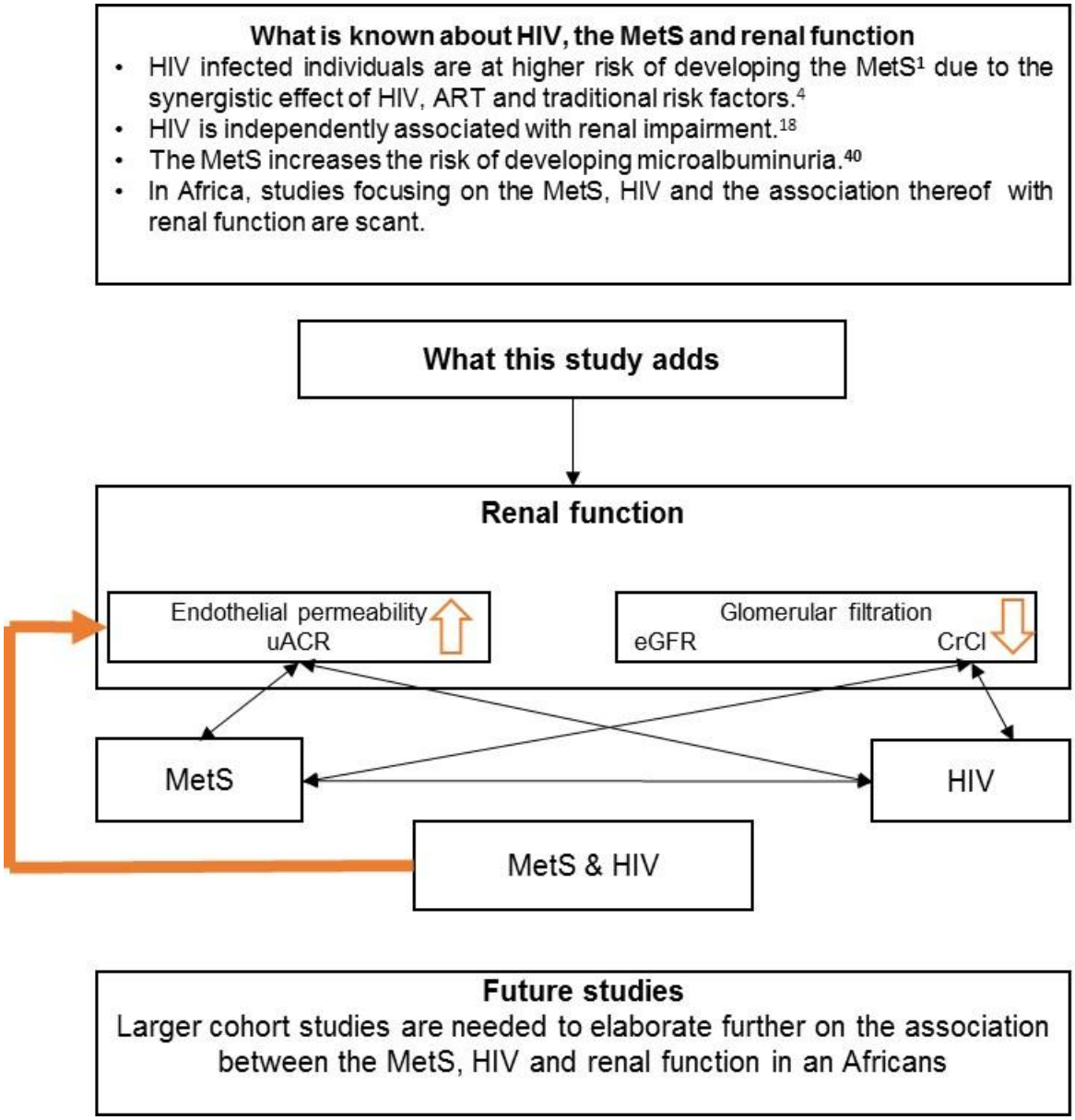

Figure 5.1: Summary of the main findings of this study

HIV, human immunodeficiency virus; MetS, metabolic syndrome; uACR, urinary albumin-creatinine ratio; eGFR, estimated glomerular filtration rate; CrCl, creatinine clearance.

Based on the findings of this study and the existing literature, we can reason that HIV-infected individuals with the MetS are at increased risk of kidney dysfunction, which is further associated with increased risk of cardiovascular disease. Furthermore, the MetS is an important risk factor of both kidney dysfunction and cardiovascular morbidity and mortality. It thus seems that HIV-infected individuals bear a greater burden of future cardiovascular disease risk as a result of the combination of HIV, the MetS and kidney disease. Future studies could elaborate further on the long-term

association between HIV, the MetS and urinary albumin excretion in HIV-infected populations.

5.4. Limitations, confounding factors and chance

It is important to consider factors that may have influenced the results of this study. These include the methodology, statistical analyses and interpretation of the results. The cross-sectional observational design of this study only specifies the current state of health and associations found, and therefore cannot imply causality.

The participants in this study resided in rural and urban areas of the North West Province and as a result, this sample population cannot be regarded as a representative sample of all HIV-infected South Africans. As participation was voluntary, results may be biased towards individuals interested in their health or healthy behaviours. This present study falls under the PURE study which aimed to identify causes of chronic diseases and lifestyle modification, hence it was not specifically designed to address the hypotheses formulated in this present study. However, it is well known that SA has the highest HIV infection in the world and having mentioned that, studies focusing on the HIV, the MetS and renal function are sparse in SA. The study was well designed, followed a strict protocol and was done under controlled conditions.

With regard to the results, the possibility of chance should be taken into account. Despite using univariate regression analyses, there is a possibility that some associations may be due to chance. In addition, multiple adjustments were made to known cofounders such as age, sex and locality.

5.5. Recommendations

- The PURE study is a longitudinal study with a follow-up of 10 years. It is suggested that the long-term association between the MetS and uACR be investigated in HIV-infected individuals.
- The effect of ART on the renal function and the MetS should also be considered in future studies. Complete information on the duration of ART should be done to shed more light on the effect of ART on the renal function.
- Future studies should include larger cohorts to enable broader statistical analyses.
- Proper medical diagnosis of the renal function could be done to confirm the results of this study.

- The renal function markers should be compared from baseline to follow-up, from the initiation of ART and during ART use.

5.6. Perspectives

This study highlights a higher risk of renal dysfunction with combination of HIV and the MetS, and the consequences for renal function. These findings are important for South Africa, which houses the largest number of people living with HIV and has an extensive ART roll-out programme.[47] The ART roll-out is expected to increase further in light of the test-and-treat programme that commenced in September 2016.

Increased uACR is an important risk factor for development of renal diseases and cardiovascular diseases risk. This could further increase the burden of the reported cardiovascular and renal diseases already observed as a consequence of traditional risk factors. Utilisation of uACR point-of-care devices may prove beneficial, especially in resource-limited countries. It therefore could be of help to identify HIV-infected individuals with the MetS who are potentially at higher risk of renal dysfunction.

5.7 References

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Appendix A: Ethics approval for the PURE study and this sub-study

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Ethics Committee
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ETHICS APPROVAL OF PROJECT

The North-West University Research Ethics Regulatory Committee (NWU-RERC) hereby approves your project as indicated below. This implies that the NWU-RERC grants its permission that provided the special conditions specified below are met and pending any other authorisation that may be necessary, the project may be initiated, using the ethics number below.

Project title: PROSPECTIVE URBAN AND RURAL EPIDEMIOLOGY STUDY (PURE STUDY)

Project Leader: Prof A Kruger

Ethics number: NWU-00016-10-A1
(Institution - Project Number - Year - Status)
Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Approval date: 2015-01-20 **Expiry date:** 2020-01-20

Special conditions of the approval (if any): None

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following:

- *The project leader (principle investigator) must report in the prescribed format to the NWU-RERC:*
  - *annually (or as otherwise requested) on the progress of the project,*
  - *without any delay in case of any adverse event (or any matter that interrupts sound ethical principles) during the course of the project.*
- *The approval applies strictly to the protocol as stipulated in the application form. Would any changes to the protocol be deemed necessary during the course of the project, the project leader must apply for approval of these changes at the NWU-RERC. Would there be deviated from the project protocol without the necessary approval of such changes, the ethics approval is immediately and automatically forfeited.*
- *The date of approval indicates the first date that the project may be started. Would the project have to continue after the expiry date, a new application must be made to the NWU-RERC and new approval received before or on the expiry date.*
- *In the interest of ethical responsibility the NWU-RERC retains the right to:*
  - *request access to any information or data at any time during the course or after completion of the project;*
  - *withdraw or postpone approval if:*
    - *any unethical principles or practices of the project are revealed or suspected,*
    - *it becomes apparent that any relevant information was withheld from the NWU-RERC or that information has been false or misrepresented,*
    - *the required annual report and reporting of adverse events was not done timely and accurately,*
    - *new institutional rules, national legislation or international conventions deem it necessary.*

The Ethics Committee would like to remain at your service as scientist and researcher, and wishes you well with your project. Please do not hesitate to contact the Ethics Committee for any further enquiries or requests for assistance.

Yours sincerely

Linda du Plessis

Digitally signed by Linda du Plessis
DN: cn=Linda du Plessis, o=NWU, Vaal Triangle Campus, ou=Vice-Rector: Academic, email=linda.duplessis@nwu.ac.za, c=ZA
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Prof Linda du Plessis
Chair NWU Research Ethics Regulatory Committee (RERC)

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Health Sciences Ethics Office for Research, Training and Support
Health Research Ethics Committee (HREC)

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11 October 2016

Prof CMT Fourie
Physiology

Dear Prof Fourie

APPROVAL OF YOUR APPLICATION BY THE HEALTH RESEARCH ETHICS COMMITTEE (HREC) OF THE FACULTY OF HEALTH SCIENCES

Ethics number: NWU-00035-16-S1

Kindly use the ethics reference number provided above in all correspondence or documents submitted to the Health Research Ethics Committee (HREC) secretariat.

Study title: The metabolic syndrome in an African cohort infected with Human Immunodeficiency Virus for at least 10 years

Study leader/supervisor: Prof CMT Fourie

Student: E Phalane

Application type: Single study

Risk level: Medium

You are kindly informed that your application was reviewed at the meeting held on 12/04/2016 of the HREC, Faculty of Health Sciences, and was approved on 11/10/2016.

The commencement date for this study is 11/10/2016 dependent on fulfilling the conditions indicated below. Continuation of the study is dependent on receipt of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation up to a maximum period of three years when extension will be facilitated during the monitoring process.

After ethical review:

Translation of the informed consent document to the languages applicable to the study participants should be submitted to the HREC, Faculty of Health Sciences (if applicable).

The HREC, Faculty of Health Sciences requires immediate reporting of any aspects that warrants a change of ethical approval. Any amendments, extensions or other modifications to the proposal or other associated documentation must be submitted to the HREC, Faculty of Health Sciences prior to implementing these changes. Any adverse/unexpected/unforeseen events or incidents must be reported on either an adverse event report form or incident report form at Ethics-HRECIncident-SAE@nwu.ac.za.

A monitoring report should be submitted within one year of approval of this study (or as otherwise stipulated) and before the year has expired, to ensure timely renewal of the study. A final report must be provided at completion of the study or the HREC, Faculty of Health Sciences must be notified if the study is temporarily suspended or terminated. The monitoring report template is obtainable from the Faculty of Health Sciences Ethics Office for Research, Training and Support at Ethics-Monitoring@nwu.ac.za. Annually a number of studies may be randomly selected for an external audit.

Please note that the HREC, Faculty of Health Sciences has the prerogative and authority to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.

Please note that for any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the HREC, Faculty of Health Sciences. Ethics approval is required BEFORE approval can be obtained from these authorities.

The HREC, Faculty of Health Sciences complies with the South African National Health Act 61 (2003), the Regulations on Research with Human Participants (2014), the Ethics in Health Research: Principles, Structures and Processes (2015), the Belmont Report and the Declaration of Helsinki (2013).

We wish you the best as you conduct your research. If you have any questions or need further assistance, please contact the Faculty of Health Sciences Ethics Office for Research, Training and Support at Ethics-HRECApply@nwu.ac.za.

Yours sincerely

Dr Wayne Towers

HREC Chairperson

Prof Minrie Greeff

Ethics Office Head

Current details: (13210572) C:\Users\13210572\Documents\HREC\HREC - Applications\2016 Applications\Applications 03 - 12 April 2016\NWU-00095-16-S1 (CMT Fourie-E Phalane)\NWU-00095-16-S1(CMT Fourie-E Phalane)-AL\NWU-00095-16-S1(CMT Fourie-E Phalane)-AL.docm
11 October 2016

File reference: 9.1.5.3

Appendix B: Confirmation of editing of the dissertation

CLAUDIA BOFFARD
Academic Documents Editor
14 Shannon, 6th Road, Hyde Park, Johannesburg

boffard@mweb.co.za 011 325 4950 076 523 0617

28 November 2016

Letter of Confirmation

This will confirm that I have language edited the dissertation:

The metabolic syndrome and renal function in an African cohort infected with the Human Immunodeficiency Virus for at least five years
byEdith Phalane.

All errors identified were corrected and marked with the 'track changes' function.
The document was edited in accordance with the latest conventions of English style and expression.

Claudia Boffard

Claudia Boffard

Appendix C: Turn it in originality report

Turnitin Originality Report

28149866:Phalane_Edith_dissertation_turn_it_in.pdf by EDITH PHALANE

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Appendix D: Solemn declaration