

The cardiovascular profile of HIV-infected South Africans of African descent: a 5-year prospective study

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Innovation through diversity



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AFFIRMATION BY AUTHORS

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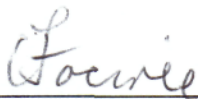
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This is a statement from the co-authors confirming their individual role in the study and giving their permission that the article may form part of this dissertation.



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SUMMARY

Motivation

In South Africa, cardiovascular disease and the high prevalence of Human Immunodeficiency Virus (HIV) infection significantly decreases the quality of life among the African population. Although treatment of HIV infection is known to increase quality of life and life expectancy, several negative effects, such as the development and worsening of the prevalence of cardiovascular disease, could emerge in the African population. Cardiovascular risk management among the HIV-infected population has become a dilemma in clinical practice, and research is limited to the Caucasian population in the Northern hemisphere, most likely infected with the HIV-1 subtype B virus. A thorough understanding of the cardiovascular risk, especially among the HIV-infected South African population, is therefore of crucial importance in order to be able to manage this epidemic, and the associated cardiovascular involvements thereof, thereby finding ways to increase the quality of life in this population.

Aim

The aim of this study was to evaluate the cardiometabolic profile of HIV-infected black South Africans over a period of 5 years, as well as to determine associations between antiretroviral treatment and cardiometabolic variables.

Methodology

This 5-year prospective study, which is embedded in the international Prospective Urban and Rural Epidemiology (PURE) study, included African participants from the North-West province, South Africa. During the baseline study in 2005, from a total of 2,010 participants (1,004 urban and 1,006 rural), 300 were newly identified as being HIV-infected. After 2005, treatment was initiated as recommended by the World Health Organisation at a CD4 cell count of ≤ 200 cells/mm³ for those participants seeking treatment. During the follow-up study in 2010, a total of 137 of the HIV-infected participants were successfully followed-up. From this group, 66 participants received treatment, while 71 were never treated. Seven participants were excluded from follow-up as they discontinued treatment for unknown reasons.

Anthropometric measurements included height, weight, hip- and waist circumference, followed by the calculation of body mass index (BMI). Regarding cardiovascular measurements, brachial systolic- and diastolic blood pressures, pulse pressure, pulse wave velocity, augmentation index and carotid intima-media thickness were determined. Biochemical variables included total cholesterol, low- and high-density lipoprotein cholesterol (LDL-C and HDL-C), triglycerides, glucose, glycated haemoglobin, C-reactive protein and HIV status. Mean values, mean change and percentage change were determined. *P*-values between treated and never treated HIV-infected groups were determined by using the standard analysis of variance (ANOVA) and analysis of covariance (ANCOVA) tests, as well as independent and dependent *t*-tests. Multiple regression analyses were used in order to determine independent associations between variables.

Results and Conclusion

The treated HIV-infected group presented with an increase ($p=0.030$) in pulse pressure (PP) over 5 years, which is substantiated by the much higher ($p=0.023$) percentage change in PP, compared to their never treated counterparts. This was still the case when adjustments were made for gender, age and follow-up body mass index. Only in the treated participants was an increase in systolic blood pressure percentage change ($p=0.029$) observed, while no differences were found in diastolic blood pressure between the groups. This is probably the reason for the greater percentage change in PP seen among the treated participants.

As was expected, the treated group in our study presented with a worse lipid profile than the never treated participants, which included higher total cholesterol ($p<0.001$), LDL-C ($p<0.001$) and triglyceride ($p=0.034$) levels in 2010. Albeit total cholesterol levels were still in a desirable range, LDL-C levels were above optimal, which might present with atherogenic properties. Oxidation of LDL-C, resulting in the formation of atherogenic oxidised LDL-C, could be further increased by higher PP levels.

Unlike the never treated participants, waist circumference of the treated participants tended to increase ($p=0.06$) over the 5 years, while their BMI levels remained unchanged. This confirms the known correlation between lipodystrophy and antiretroviral treatment. In the light of the above mentioned, the prevalence of vascular structural- and functional changes was expected. However, we found no differences in central systolic blood pressure ($p=0.23$), carotid-dorsalis-pedis pulse wave velocity ($p=0.56$), augmentation index ($p=0.28$), or carotid intima-media thickness ($p=0.80$) between the two groups at follow-up.

In conclusion, we observed a higher percentage change in PP, a more detrimental lipid profile, as well as abdominal fat accumulation amongst the treated HIV-infected participants. However, no differences in vascular structure (intima-media thickness) or function (central systolic blood pressure, carotid-radialis pulse wave velocity and augmentation index) were seen after 5 years in the treated group. Nonetheless, it could be speculated that the higher percentage change in PP and worse lipid levels could be an early indication of the development of arterial stiffness, probably due to antiretroviral treatment. Therefore, whether the treatment of the HIV-infected South Africans might lead to arterial stiffness, vascular aging or accelerated atherosclerosis, is yet to be seen.

Keywords: Human Immunodeficiency Virus, antiretroviral treatment, pulse pressure, dyslipidaemia, South Africa.

Die kardiovaskulêre profiel van MIV-geïnfekteerde Suid-Afrikane van Afrika oorsprong: 'n prospektiewe studie oor 5 jaar

OPSOMMING

Motivering

Kardiovaskulêre siekte en die hoë voorkoms van Menslike Immuniteitsgebrekvirus (MIV) infeksie veroorsaak 'n betekenisvolle afname in die lewenskwaliteit van die Suid-Afrikaanse populasie. Hoewel die behandeling van MIV infeksie daarvoor bekend is om lewenskwaliteit en lewensverwagting te verhoog, kan verskeie negatiewe effekte steeds na vore kom, soos byvoorbeeld die ontwikkeling en verhoogde voorkoms van kardiovaskulêre siekte in die Afrikane populasie. Die bestuur van kardiovaskulêre risiko in die MIV-geïnfekteerde populasie het 'n probleem in die kliniese praktyk geword en navorsing is beperk tot die Kaukasiese populasie in die Noordelike halfrond, wat waarskynlik geïnfekteer is met die MIV-1 sub tipe B virus. 'n Grondige kennis van die kardiovaskulêre risiko, veral onder die MIV-geïnfekteerde Suid-Afrikaanse populasie, is daarom van groot belang om sodoende die mens in staat te wees om hierdie epidemie en die kardiovaskulêre invloed daarvan te bestuur en dáárdeur wyses te kan vind om die lewenskwaliteit van hierdie populasie te verhoog.

Doel

Die doel van hierdie studie was om die kardiovaskulêre profiel van MIV-geïnfekteerde swart Suid-Afrikane te bestudeer oor 'n tydperk van 5 jaar, asook om die assosiasies tussen antiretrovirale behandeling en kardiometaboliese veranderlikes te bepaal.

Metodologie

Hierdie 5-jaar prospektiewe studie, wat deel uitmaak van die internasionale "*Prospective Urban and Rural Epidemiology* (PURE)" studie, het Afrikane deelnemers van die Noordwes provinsie, Suid-Afrika, ingesluit. Van die totale 2010 deelnemers (1004 stedelik en 1006 landelik), was 300 nuut geïdentifiseer as MIV-geïnfekteer tydens die basislyn studie in 2005. Na 2005 is behandeling, soos voorgeskryf deur die Wêreld Gesondheidsorganisasie, geïnisieer by 'n CD4 seltelling van ≤ 200 selle/mm³ vir daardie deelnemers wat behandeling verlang het. Tydens die opvolgstudie in 2010, is 'n totaal van 137 van die MIV-geïnfekteerde deelnemers suksesvol

opgevolg. Van hierdie groep het 66 deelnemers behandeling ontvang, terwyl 71 onbehandeld was. Sewe deelnemers is van die opgevolgde groep uitgesluit omdat hul behandeling gestaak het vir onbekende redes.

Antropometriese metings het liggaamslengte en -gewig, asook heup- en middel omtrek ingesluit, gevolg deur die berekening van liggaamsmassa-indeks. Met betrekking tot die kardiovaskulêre metings, is bragiale sistoliese- en diastoliese bloeddruk, polsdruk, polsgolfsnelheid, verhogingsindeks en karotis intima-media dikte bepaal. Biochemiese veranderlikes het totale cholesterol, lae- en hoë-digtheidlipoproteïen cholesterol (LDL-C en HDL-C), trigliseriedes, glukose, gliseerde hemoglobien, C-reaktiewe proteïen en MIV status ingesluit. Gemiddelde waardes, gemiddelde verandering en persentasie verandering is bepaal. *P*-waardes tussen behandelde en onbehandelde MIV-geïnfekteerde groepe is bepaal deur gebruik te maak van gestandaardiseerde analise van variansie- (ANOVA) en analise van kovariansie (ANKOVA) toetse, asook onafhanklike- en afhanklike *t*-toetse. Meervoudige regressie-analises is gebruik om onafhanklike assosiasies tussen veranderlikes te bepaal.

Resultate en Gevolgtrekking

Die behandelde MIV-geïnfekteerde groep het daar 'n toename ($p=0.030$) in polsdruk (PD) oor 5 jaar getoon, wat bevestig is deur die aansienlik hoër ($p=0.023$) persentasie verandering in PD in vergelyking met die onbehandelde deelnemers. Hierdie bevinding was onveranderd, selfs na aanpassings vir geslag, ouderdom en liggaamsmassa-indeks (gemeet tydens die opvolgstudie). 'n Toename in die persentasie verandering van sistoliese bloeddruk ($p=0.029$) is slegs in die behandelde deelnemers opgemerk, terwyl geen verskille in diastoliese bloeddruk tussen die groepe gevind is nie. Laasgenoemde kan waarskynlik die rede vir die groter persentasie verandering in PD, wat ons by die behandelde deelnemers opgemerk het, wees.

Soos wat verwag is, het die behandelde groep in ons studie 'n swakker lipied profiel as die onbehandelde deelnemers gehad, wat hoër totale cholesterol- ($p=0.001$), LDL-C- ($p<0.001$) en trigliseried ($p=0.034$) vlakke in 2010 ingesluit het. Hoewel totale cholesterolvlakke steeds binne die wenslike perke geval het, was LDL-C vlakke hoër as optimaal, wat 'n aanduiding van aterogeniese eienskappe mag wees. Die oksidasie van LDL-C, wat die formasie van aterogeniese geoksideerde LDL-C veroorsaak, kan verder verhoog word deur hoër PD vlakke.

In teenstelling met die onbehandelde deelnemers, het die middel omtrek van die behandelde deelnemers verhoog ($p=0.06$) oor die 5 jaar, terwyl hul liggaamsmassa-indeks onveranderd gebly het. Dit is dus bevestigend van die bekende korrelasie wat tussen lipodistrofie en

antiretrovirale behandeling bestaan. In die lig van die bogenoemde, is die voorkoms van vaskulêre strukturele- en funksionele veranderinge verwag. Ons het egter geen veranderinge in die sentrale sistoliese bloeddruk ($p=0.23$), karotis-dorsalis polsgolfsnelheid ($p=0.56$), verhogingsindeks ($p=0.28$), of karotis intima-media dikte ($p=0.80$) tussen die groepe gevind tydens die opvolgstudie nie.

Ten slotte het ons 'n hoër persentasie verandering in PD, 'n meer nadelige lipied profiel, asook abdominale vet akkumulاسie onder die behandelde MIV-geïnfekteerde deelnemers waargeneem. Na 5 jaar is daar egter geen verskille in vaskulêre struktuur (intima-media dikte) of -funksie (sentrale sistoliese bloeddruk, karotis-dorsalis polsgolfsnelheid en verhogingsindeks) waargeneem in die behandelde groep nie. Nietemin kan daar steeds gespekuleer word dat die hoër persentasie verandering in PD en die swakker lipiedvlakke 'n vroeë indikاسie van die ontwikkeling van arteriële styfheid kan wees, wat moontlik aan antiretrovirale behandeling toegeskryf kan word. Dus, of die behandeling van MIV-geïnfekteerde Suid-Afrikaners tot arteriële styfheid, vaskulêre veroudering of versnelde aterosklerose mag lei, moet steeds vasgestel word.

Sleutelwoorde: Menslike Immuniteitsgebrekvirus, antiretrovirale behandeling, polsdruk, dislipidemie, Suid-Afrika.

PREFACE

The *article format* was used for the completion of this dissertation. The chosen journal for this project is *HIV medicine*. This dissertation is written in English, while an Afrikaans summary of the article has been included at the beginning of the dissertation, as required by the institution. This is a format approved and recommended by the North-West University, consisting basically of a manuscript, which is ready for submission to a peer-reviewed journal. The manuscript is accompanied by an in-depth literature review and an interpretation of the results.

The layout of this dissertation is as follows:

- Chapter 1, the introductory chapter, consists of a background, motivation, aim, objective and hypotheses of the study.
- Chapter 2 consists of a complete literature study, divided mainly into three parts, including HIV, cardiovascular risk factors and HIV, as well as treatment of HIV in South Africa.
- Chapter 3 consists of the research article, which includes instructions for authors of the journal *HIV medicine*, an introduction, the materials and methods, results, discussion, conclusion and acknowledgements of the research study.
- Chapter 4 consists of concluding remarks, a critical discussion of the findings and recommendations.

A reference list is provided at the end of each chapter, according to the Vancouver referencing style, as prescribed by the journal *HIV medicine*.

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LIST OF ABBREVIATIONS

AIDS	---	Acquired Immunodeficiency Syndrome
AIx	---	Augmentation index
ANCOVA	---	Analysis of covariance
ANOVA	---	Analysis of variance
ART	---	Antiretroviral treatment
bDBP	---	Brachial diastolic blood pressure
BMI	---	Body mass index
bSBP	---	Brachial systolic blood pressure
cdPWV	---	Carotid-dorsalis pulse wave velocity
CG	---	Cockcroft-Gault
crPWV	---	Carotid-radialis pulse wave velocity
cSBP	---	Central systolic blood pressure
CVD	---	Cardiovascular disease
DNA	---	Deoxyribonucleic acid
ESC	---	European Society of Cardiology
ESH	---	European Society of Hypertension
GFR	---	Glomerular filtration rate
HbA1c	---	Glycated hemoglobin
HDL-C	---	High-density lipoprotein cholesterol
HIV	---	Human immunodeficiency virus
hsCRP	---	High-sensitivity C-reactive protein
IMT	---	Intima-media thickness
LDL-C	---	Low-density lipoprotein cholesterol
NCEP-ATPIII	---	The National Cholesterol Education Program-Adult Treatment Panel III
NNRTI	---	Non-nucleoside reverse transcriptase inhibitor
NRTI	---	Nucleoside reverse transcriptase inhibitor
PI	---	Protease inhibitor
PP	---	Pulse pressure
PURE	---	Prospective Urban and Rural Epidemiology
RNA	---	Ribonucleic acid
TC	---	Total cholesterol
TG	---	Triglyceride
UNAIDS	---	Joint United Programme on HIV/AIDS
WC	---	Waist circumference
WHO	---	World Health Organisation



CHAPTER 1

Introduction

1.1 BACKGROUND

Cardiovascular disease contributed to an estimated 17.1 million deaths globally in 2004, which represents 29% of all deaths globally [1]. Sub-Saharan Africa is by far the region in the world most affected by the human immunodeficiency virus (HIV) with 22.5 million people living with HIV, which accounts for 68% of the HIV infections globally [2]. In the HIV-infected population, especially those receiving antiretroviral treatment (ART), cardiovascular disease is known to be the cause of morbidity and mortality [3]. Thus, cardiovascular disease plays a role in the high mortality and morbidity rates among the HIV-infected [3] and, together with the high prevalence of HIV infection in South Africa [2], research in the field of cardiovascular risk in this population is of utmost importance.

Several cardiovascular risk factors that play a role in HIV infection have been identified (Fig. 1). Metabolic effects associated with cardiovascular risk include dyslipidaemia, which can be recognised by elevated total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) levels, as well as decreased high-density lipoprotein cholesterol (HDL-C) levels [4]. It has been reported that elevated TC levels form part of a predominant HIV associated lipid profile; also in the case of treatment [4]. In contrast, lower TC levels were previously found in our HIV-infected African population [5]. Although HIV infection and advanced HIV disease have been associated with a decrease in LDL-C [6,7], it is thought to be elevated in treated HIV-infected individuals [8]. HIV infection itself is commonly associated with lower HDL-C levels [6,9], also in the African population [5], which could result in the loss of protection against atherosclerosis [10]. Regarding ART, treatment does not seem to have any effect on HDL-C metabolism [11] and HDL-C levels remain low [12]. In HIV-infected individuals, hypertriglyceridemia also occurs [7,13], which is thought to be the result of insulin resistance [13]. In addition, ART (especially with protease inhibitors) is associated with metabolic side effects, which includes elevation in TG levels [14].

Another metabolic effect includes hyperglycaemia. Even though no differences in plasma glucose concentrations were reported in HIV-infected individuals, studies have found significant increases in insulin resistance among these individuals [15] and when treatment is applied, the risk of Type 2 diabetes mellitus is increased fourfold [16].

It was proposed that inflammation in HIV-infected individuals is accompanied by an increase in high sensitivity C-reactive protein (hsCRP) levels [17], which are associated with established cardiovascular risk factors [18]. Additionally, studies have shown higher CRP levels (of >3 mg/dl) in treated HIV-infected persons, compared to never treated HIV-infected persons,

especially with non-nucleoside reverse transcriptase inhibitor regimens. This suggests a higher risk for stroke and myocardial infarction in the treated HIV-infected persons [18,19].

With regard to anthropometric changes and cardiovascular risk, obesity has been associated with a decrease in extremity and abdominal subcutaneous fat decreases, while abdominal visceral fat is increased in HIV-infected persons [20]. The presence of lipodystrophy has been well documented in HIV-infected individuals receiving ART [21,22].

The use of tobacco products is known to be much more prevalent among the HIV-infected [23], and has been associated with an increased risk of infections, certain cancers, as well as a decrease in response to ART [23,24]. A higher HIV viral load and lower CD4 cell counts have been detected in the case of alcohol consumption in HIV-infected individuals, especially when treated [25]. No 'safe' level of alcohol consumption could be suggested for HIV-infected individuals and individuals receiving ART [26].

When cardiovascular effects are associated with HIV and/or treatment, several factors should be taken into account (Fig. 2). Firstly, although a decrease of 3 mmHg in systolic blood pressure has been reported in HIV-infected individuals [27], ART (especially with non-nucleoside reverse transcriptase inhibitors), has recently been associated with an increase in blood pressure [28], thereby increasing cardiovascular risk.

With regard to atherosclerosis and vascular aging, higher pulse wave velocity (PWV) values [29] and a higher intima-media thickness (IMT) were found in HIV-infected persons [30]. Initiation of treatment could cause an even further increase in arterial stiffness [31], while the use of ART, especially nucleoside reverse transcriptase inhibitors has been shown to have significant detrimental effects on IMT levels [30].

Finally, when investigating renal dysfunction, higher creatinine levels have been found to occur in HIV-infected persons [32] and in addition, a lower CD4 count relates to lower creatinine clearance [33]. It should however be noted that the use of ART (with the exception of tenofovir), is associated with an increase in renal function [34].

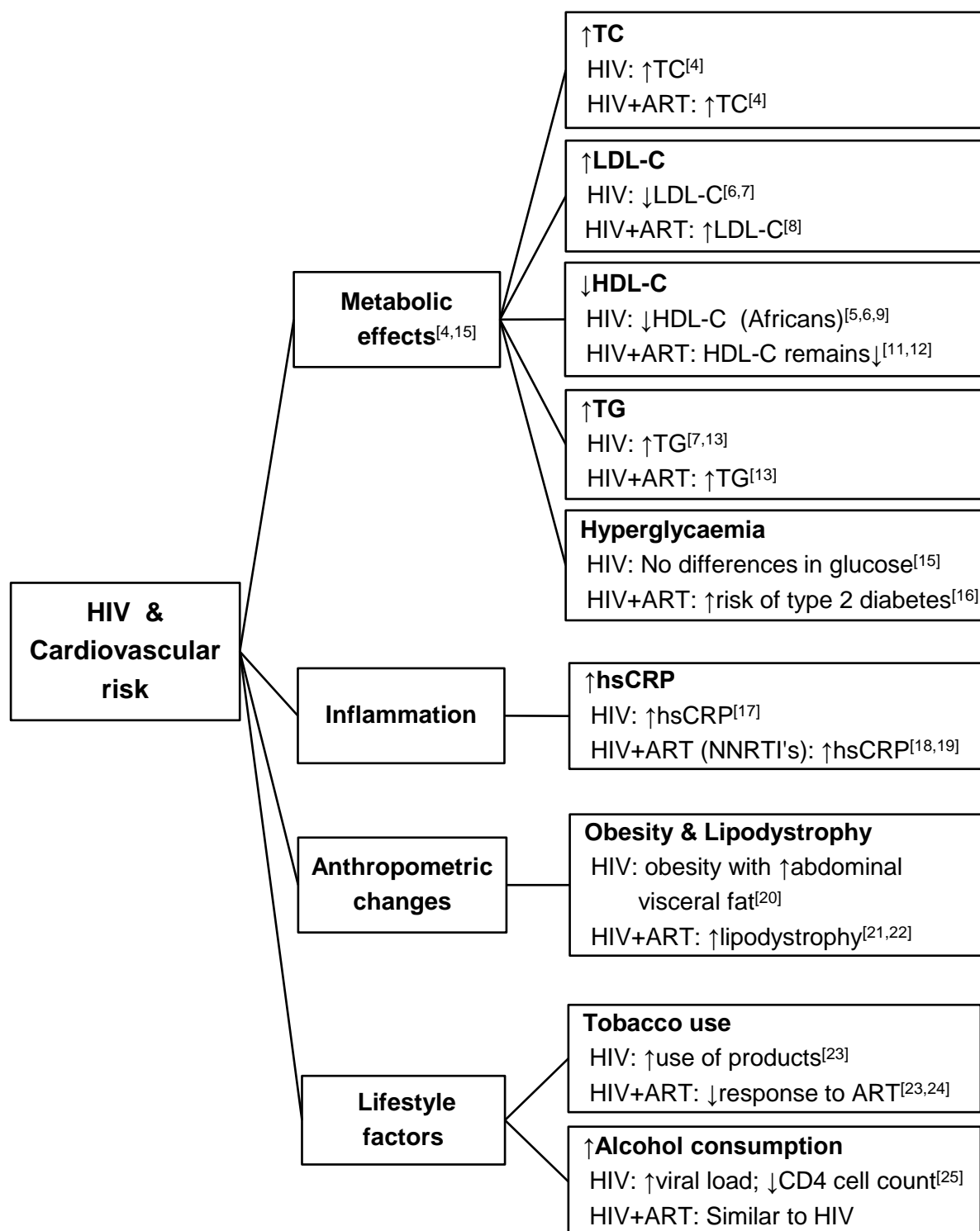


Fig. 1 Diagram of the associations between cardiovascular risk factors, HIV and ART. HIV, human immunodeficiency virus; ART, antiretroviral treatment; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; hsCRP, high-sensitivity C-reactive protein; NNRTIs, non-nucleoside reverse transcriptase inhibitors.

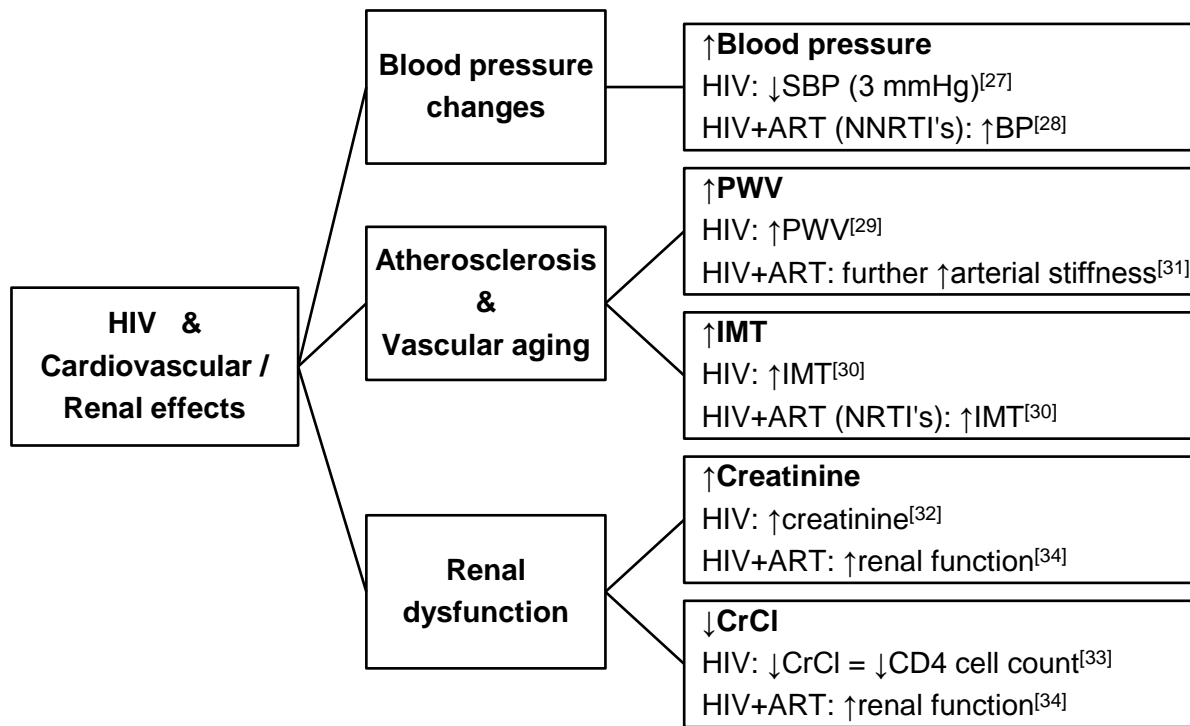


Fig.2 Diagram of the associations of HIV, cardiovascular- and renal effects. HIV, human immunodeficiency virus; ART, antiretroviral treatment; SBP, systolic blood pressure; NNRTIs, non-nucleoside reverse transcriptase inhibitors; PWV, pulse wave velocity; IMT, intima-media thickness; NRTIs, nucleoside reverse transcriptase inhibitors; CrCl, creatinine clearance.

1.2 MOTIVATION

Because of the very high prevalence of HIV-1 (subtype C) infection in South Africa, the management of this epidemic with antiretroviral treatment, resulting in the improvement of life quality, is of great significance. On the other hand, antiretroviral treatment is known to be associated with negative factors such as a worsened lipid profile and risk for the development of cardiovascular disease. The main motivation for this study, however, was to determine whether ART could be associated with changes in the cardiovascular and –metabolic profile of HIV-infected South Africans of African descent.

1.3 AIM

The aim of this study is to evaluate the cardiometabolic profile of HIV-infected South Africans, as well as to determine whether the use of antiretroviral treatment could lead to changes in the cardiometabolic profile of HIV-infected South Africans over 5 years.

1.4 OBJECTIVE

To determine whether antiretroviral treatment changes the cardiometabolic profile of HIV-infected South Africans (compared to never treated HIV-infected South Africans) over a period of five years.

1.5 HYPOTHESES

- After 5 years, treated HIV-infected South Africans will have a more detrimental cardiovascular profile than never treated HIV-infected South Africans.
- South Africans receiving treatment for HIV-infection will have a worse lipid profile and exhibit more fat accumulation after 5 years when compared to never treated South Africans.

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CHAPTER 2

Literature study

2.1 HUMAN IMMUNODEFICIENCY VIRUS

The significance of the human immunodeficiency virus (HIV) in sub-Saharan Africa was recently highlighted by the 2010 Joint United Programme on HIV/AIDS (UNAIDS) report on the global acquired immunodeficiency syndrome (AIDS) epidemic, as they reported sub-Saharan Africa to be the most HIV affected region in the world by far [1,2], with 22.5 million adults and children living with HIV, of which 1.8 million adults and children were newly infected with HIV in 2009 [2]. From the estimated 1.8 million AIDS-related deaths in adults and children globally, 1.3 million (72%) occurred in sub-Saharan Africa in 2009, of which 310,000 (24%) were found to occur in southern Africa [2]. Furthermore, in 2009, the prevalence of people living with HIV in South Africa, was estimated to be 11.3 million [2]. In sub-Saharan Africa, more women are HIV-infected than men, and about 40% of these infected adult women are living in South Africa [2].

HIV can be divided into HIV Type 1 (HIV-1) and HIV Type 2 (HIV-2) infection [3]. HIV-1 may be subdivided into groups M, N and O, whilst group M could further be divided into nine subtypes, including subtypes A-D, F-H, J and K [4,5]. From these types, subtype C is the most prevalent in southern and eastern Africa, while subtype B dominates in Australia, northern America, and Europe [6]. HIV is a lentivirus (member of the *Retroviridae* family) that eventually progresses to AIDS [3]. Retroviruses destroy the human immune system by producing an enzyme called reverse transcriptase. Active reverse transcriptase is needed for the viral ribonucleic acid (RNA) genome to be transformed into a proviral deoxyribonucleic acid (DNA) copy. The single-stranded RNA is also tightly bound to nucleocapsid proteins and other enzymes, which are needed for the development of the virion [3]. The HIV provirus integrates into the DNA of the host cell where it will be transcribed into viral messenger RNAs. Finally the messenger RNAs will be translated into HIV proteins, as well as into genomes for further virus generation [3,7].

HIV mostly infects helper T-cells, but is known to be able to infect any cell that expresses CD4 proteins, including cardiac myocytes [7]. Immune status, which is associated with clinical manifestations of HIV infection, is therefore indicated by the CD4 T-cell count (expressed as cells/mm³) [8]. Virological status, on the other hand, which is associated with imminent clinical status, can be indicated by the HIV RNA viral load (expressed as copies/mL) [8]. Differences in the balance between immunological and virological status give rise to the appearance of three different clinical stages of HIV infection, which is followed by the development of AIDS [8]. These stages include the acute HIV infection stage (where viral load reaches millions of copies/mL and the CD4 cell count becomes very low), the asymptomatic stage of infection (where viral load declines to 20,000-60,000 copies/mL while the CD4 cell count remains stable), followed by the generalised lymphadenopathy and AIDS-related complex stage (where viral load increases again to reach >120,000 copies/mL and the CD4 cell count declines to reach

<500 cells/mm³). When the latter continues, (and the CD4 cell count reaches <200 cells/mm³) AIDS develops, which is subjected to opportunistic infections [8]. It is interesting to note that a viral load of <50 copies/ml is considered to be below detection, in which case antiretroviral treatment (ART) is needed, and that HIV-infected women are prone to have lower viral loads than HIV-infected men [8].

2.2 CARDIOVASCULAR RISK FACTORS AND HIV

It has been proposed that the context of multiple risk factors should be considered in order to understand the effect of one single risk factor [9]. A risk factor can be defined as a factor, characteristic or measurable element which causes a person or a group of people to be prone to an increased rate of disease, which could present in an unwanted, unhealthy or unpleasant event [10,11]. Some examples of classic risk factors include dyslipidaemia, high blood pressure, hyperglycemia, obesity, smoking and diabetes [12,13].

Interestingly, in some cases, a component could be seen as both a risk marker and a risk factor. Such an example includes increased pulse wave velocity which is considered to be a risk factor in the case of future cardiovascular events, as well as to be a risk marker when it comes down to the clinical classification of cardiovascular disease [14]. Some examples of cardiovascular risk markers further include body mass index, waist circumference and waist-to-hip ratio, also collectively known as anthropometric indicators [15], as well as C-reactive protein which is known to be a sensitive systemic inflammatory marker and which could, among other things, cause plaque rupture and endothelial dysfunction [16,17].

On the other hand, the term 'mediator', also called an 'intermediate variable' [9], is suggested to follow a process or event which it mediates, and which precedes another process or event and thus the outcome [9,10]. Some examples of mediators (in this case of inflammation) are interleukin-6 and interleukin-1 β , as well as tumour necrosis factor- α [18].

2.2.1 Blood pressure, cardiovascular risk and HIV

According to the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC), blood pressure can be classified as optimal if systolic blood pressure (SBP) is <120 mmHg and diastolic blood pressure (DBP) is <80 mmHg, as normal (SBP 120-129 mmHg and DBP 80-84 mmHg), as high normal (SBP 130-139 mmHg and DBP 85-89 mmHg), and as hypertensive when SBP is \geq 140 mmHg and DBP is \geq 90 mmHg [19,20]. Hypertension, a

well-known cardiovascular risk factor, occurs more commonly as age increases, and an earlier development of hypertension in men than in women has previously been reported [21,22]. It is recommended that both SBP and DBP should be used in risk assessment [19], as both have been continuously associated with cardiovascular morbidity and mortality [23]. An increase in the incidence of cardiovascular disease has been found in participants whose pressure consisted of lower DBP levels, but interestingly, these findings were limited to those participants who also had higher SBP levels and therefore also elevated pulse pressure (PP) levels [24].

Elastin, one of many different structural proteins, is known to be the main elastic component in the large arteries. The elastic properties of these arteries could however be compromised, should degradation of the elastin and/or collagen proteins occur, resulting in an increase in arterial stiffness [25], which could have unfavourable hemodynamic effects [26]. Such effects include an elevation in PP that has been shown to cause the generation of reactive oxygen species, which again plays an inhibitory role in the endothelium-dependent vasodilator effect that acetylcholine has [26,27]. Increased PP could furthermore elevate the pulsatility and thereby damage the frail capillary veins (especially in the brain and the kidneys), increase wall stress, and accelerate arterial stiffening [26]. Thus PP elevation is in strong correlation with an increase in central arterial stiffness [28], and could be seen as a hemodynamic marker of large arterial stiffness [14]. According to the ESH-ESC guidelines for the management of arterial hypertension, cut-off values for normal-abnormal PP at different ages are yet to be determined [19], even though values of 50-55 mmHg have previously been suggested [29]. It should further be noted that central PP is considered to be a more accurate assessment than peripheral PP, because of the “amplification phenomena” between the aorta and peripheral arteries, which is being accounted for by central PP [19].

Elevations in inflammatory markers have been reported in large epidemiological studies in participants who fell in the pre-hypertensive (normal to high normal) range [30,31], in which case SBP is 120-139 mmHg and DBP is 80-89 mmHg [19]. Thus, blood pressure can be associated with chronic low grade inflammation [32] and is proposed to be a risk factor for the development of atherosclerosis [33].

It has been shown that hypertension in Africans occurs at a younger age, in which case it is more severe and accompanied by a more severe outcome of earlier damage to vital organs [34,35]. The hypertension rate among African populations is known to be one of the highest globally [36]. In the North-West Province (South Africa), uncontrolled hypertension occurs among the African adult population with a prevalence of 13.7-32.9%, depending on localisation [37]. In a study done by Tibazarwa *et al.* in Soweto, South Africa, 19% of the participants showed evidence of hypertension in both SBP and DBP forms, with no gender-related blood

pressure differences [38]. More studies have also found that 37% of Caucasian men and 28% of Caucasian women were hypertensive, compared to the much higher 50% and 43% of African men and women, respectively [39]. In addition, the literature shows that compared to Caucasian men, the blood pressure levels of African men are higher [40].

Together with an overall shorter life expectancy [41], a decrease of 3 mmHg in SBP has been reported in HIV-infected, untreated individuals from rural KwaZulu-Natal, South Africa [42]. Consistent with these findings, another study done on the African population has also found lower SBP levels, together with a lower prevalence of high blood pressure, in HIV-infected participants [43].

ART has been found to be associated with an increase in blood pressure, as was suggested in a study where on average, blood pressure was 3.2/2.7 mmHg higher in the treated HIV-infected group than in the never treated HIV-infected group [44]. In the same study, however, the type of ART was seen to play a role in the prevalence of higher blood pressure, as HIV-infected persons treated with non-nucleoside reverse transcriptase inhibitors had a higher blood pressure (of average 4.6/4.2 mmHg) than the never treated HIV-infected persons had [44].

2.2.2 Biochemical variables, cardiovascular risk and HIV

Dyslipidaemia

Dyslipidaemia has been considered an important risk factor with regard to cardiovascular disease (CVD) [45], and is associated with HIV infection itself [46]. Regarding ART, the characteristics of dyslipidaemia have been described as elevated triglyceride and total cholesterol levels, together with decreased high-density lipoprotein cholesterol levels, with or without elevated low-density lipoprotein cholesterol levels [46].

Total cholesterol

Elevated serum total cholesterol (TC) levels are known to play a role in coronary atherosclerosis and to correlate with coronary heart disease [47]. Indeed, a TC level of >209 mg/dl (>5.4 mmol/l) has been associated with a two-fold higher risk of hypertension [48]. Furthermore, TC is known to be predicted by body mass index [49]. With reference to general cut-off values, the National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATPIII) has characterised a TC level of <5.18 mmol/l (<200 mg/dl) as desirable, 5.18-6.19 mmol/l (200-239 mg/dl) as borderline high and ≥6.22 mmol/l (≥240 mg/dl) as high levels for the Caucasian population [50].

A predominant HIV associated lipid profile, also in the case of ART, has been reported to include elevated TC levels [46]. In contrast, lower TC levels were found in never treated HIV-infected Africans compared to their HIV-uninfected counterparts [43], while another study showed lower TC levels to be associated with more advanced HIV disease and increased mortality [51].

High-density lipoprotein cholesterol

High-density lipoprotein cholesterol (HDL-C) consists of apolipoproteins, lipids, lipid transfer proteins and enzymes [52]; it is released from macrophages into plasma and transported from the liver, back into hepatocytes, where it is converted to bile acids [46,53]. Lower HDL-C and higher triglyceride levels are known to be accompanied by smaller lipoprotein particles [54]. HDL-C plays an important role in the protection against atherosclerosis through its “reverse cholesterol transport” function, where it will return to the liver, bile, and faeces [53], as well as in functioning as an anti-inflammatory and anti-oxidant agent [55]. This explains why an increase of 1 mg/dl (0.026 mmol/l) in HDL-C is associated with a reduction of 2% in CVD [56], as well as why a decrease in HDL-C levels will also cause a decrease in cardiovascular protection and an increase in cardiovascular risk [55]. The protective levels of HDL-C for the development of coronary heart disease are stipulated to be ≥ 1.55 mmol/l [57]. The NCEP-ATPIII has also characterised a serum HDL-C level of ≥ 1.04 mmol/l (≥ 40 mg/dl) in men and ≥ 1.30 mmol/l (≥ 50 mg/dl) in women as normal, while < 1.04 mmol/l (< 40 mg/dl) could be seen as a low HDL-C level [50]. The latter classifies HDL-C as a categorical risk factor of coronary arterial disease [50,58]. Studies showed that Africans and African Americans seem to have higher HDL-C levels than Caucasians [49,59,60].

HIV infection itself is commonly associated with lower HDL-C levels [51,61]. This was confirmed by another study where low HDL-C levels (of < 1.28 mmol/l) were found to be one of the most prevalent lipid abnormalities among the HIV-infected African population [43]. A decrease in HDL-C levels in HIV patients, as part of the innate immune system of the body, has also been reported [62,63]. Even though HDL-C particles still have the ability to promote cholesterol efflux in HIV-infected persons [64], the loss of protection against atherosclerosis by these particles becomes evident [65]. The latter can be caused by the impairment of cholesterol delivery through liver scavenger receptors because of the higher triglyceride content that HDL-C has in HIV infection [66]. It has been suggested that inflammation in general, which stimulates endothelial lipase and phospholipase A₂, is another cause of the decrease in HDL-C levels [67]. Regarding ART, treatment does not seem to have any effect on HDL-C metabolism [64] and HDL-C levels remain low [68]. In contrast, treatment with non-nucleoside reverse transcriptase inhibitors has been shown to normalise HDL-C levels [69]. Noteworthy: persons who have

received treatment for 1-3 years were shown to be four times less likely to have decreased HDL-C levels than were people who have received treatment for 3-6 years [63].

Low-density lipoprotein cholesterol

Low-density lipoprotein cholesterol (LDL-C) particles are composed of protein and thousands of cholesterol molecules as well as other lipids, and are therefore not a single molecule [70]. LDL-C function as the major carrier of cholesterol to the cells [71]. Once LDL-C reaches levels higher than 2.59 mmol/l (100 mg/dl), it becomes atherogenic and could therefore be categorised as above optimal (2.59-3.34 mmol/l or 100-129 mg/dl), borderline high (3.36-4.11 mmol/l or 130-159 mg/dl), high (4.13-4.88 mmol/l or 160-189 mg/dl), and very high, in the case of levels higher than 4.91 mmol/l or 190 mg/dl [72]. Thus an LDL-C level of <2.59 mmol/l (<100 mg/dl) could be considered as a normal value for the whole adult United States population, as was suggested by the NCEP-ATPIII in 2002 [58].

With modification and accumulation of LDL-C particles in the arterial wall, the particles become atherogenic and initiates the process of atherogenesis [71]. A reduction in LDL-C levels by 50% has previously been associated with a reduction in myocardial infarction (by 54%) and in major vascular events (by 47%), as well as to significantly reduce cardiovascular risk [73]. The development of atherosclerotic lesions, together with associated inflammatory processes, could be related to an accumulation of oxidative LDL-C, a key factor in the processes of plaque destabilisation [74].

In a study done among diabetic African men and women, LDL-C was found to be lower than in their Caucasian counterparts [75]. South African women from the greater Johannesburg area have been shown to have low LDL-C levels [76], despite the LDL-C levels of ≥ 3 mmol/l in 42% African women and 29% African men in the rural South African population in Soweto [77]. Furthermore, no association was found between serum lipids and LDL-C particle size in South African women of African descent [78].

Although HIV infection, and advanced HIV disease, have been associated with a decrease in LDL-C [51,79], it is thought to be elevated in treated HIV-infected individuals [80] with levels that could reach 168% from baseline values at initiation of treatment [81].

Triglycerides

A reciprocal clearance of triglycerides (TG) and HDL-C from the circulation seems to be the cause of both high TG and low HDL-C levels in the system, which are associated with smaller lipoprotein particle sizes [54,82]. The concentration of TG (which circulates within lipoproteins) is yet another cardiovascular risk factor [83], as TG has been established by the NCEP-ATPIII to be a marker of non-LDL-C atherogenic lipoproteins [58]. Cut-off values for TG levels include normal- (<1.70 mmol/l or <150 mg/dl), borderline high- (1.70-2.25 mmol/l or 150-199 mg/dl), high- (2.26-5.64 mmol/l or 200-499 mg/dl), and very high (≥2.26 mmol/l or ≥500 mg/dl) ranges [58]. The latter has been reported to be associated with risk for pancreatitis, as well as with ischemic, but not hemorrhagic stroke risk [58,84,85]. Guidelines have proposed the addition of a new second target of therapy in cases where TG levels were 2.26-5.20 mmol/l (200-400 mg/dl) [58]. Furthermore, high TG levels are also distinctive of insulin resistance and diabetes, and are therefore in high correlation with cardiovascular disease [49,86,87]. In another study, an independent link existed between elevated plasma TG levels and an increase in heart disease in women and men of 37% and 14%, respectively [88]. However, factors such as obesity, hypertension, some lipids, smoking, and diabetes, have been shown to confound this correlation between TG levels and the risk for cardiovascular disease [47,88].

It is noteworthy that the mentioned thresholds for abnormal TG levels have been suggested to be inappropriate for the African population [87]. A previous study has shown TG levels to be lower in the African American population than in their Caucasian counterparts [49]. This was also confirmed in the African population [60].

In HIV-infected individuals, hypertriglyceridemia occurs [79,89], which is thought to be a result of insulin resistance [89]. More recent studies have also found TG levels to be higher in HIV-infected participants [43,90]. In addition, it is well known that ART with especially protease inhibitors is associated with metabolic side effects, which includes elevation in TG levels [91,92]. Indeed, after 72 weeks of protease inhibitor-treatment, TG levels were found to be above 200 mg/dl (2.26 mmol/l) in 84% of the HIV-infected patients [93].

Glucose

Insulin stimulates glucose uptake, decreases the utilisation of free fatty acids [94], and thus, as an energy source, plays a critical role in the transport of glucose to the liver and skeletal muscle [95]. Normal glucose levels, classified as 5.0-5.6 mmol/l, are known to be disrupted by suboptimal insulin concentrations, which in turn could lead to glucose abnormalities [96,97]. Such abnormalities include diabetes, impaired glucose tolerance or impaired fasting glucose, and the presence of any of the mentioned aspects could be used in the diagnosis of hyperglycaemia [98]. Another factor that could contribute to the development of hyperglycaemia

is a decrease in glucose uptake by skeletal muscle, primarily because of the disposing role (80-90%) of the skeletal muscle [99,100]. Fasting hyperglycaemic levels could be identified as ≥ 5.6 mmol/l (100 mg/dl) and, in the case of glucose levels of 5.6–6.9 mmol/l (100–125 mg/dl), an oral glucose test has been strongly recommended for diagnosis of the above-mentioned glucose abnormalities [98]. Hyperglycaemia and insulin resistance have further been associated with dysfunctional nitric oxide signalling [101], and, by resulting in glycosylated proteins, hyperglycaemia was reported to induce oxidative stress thereby becoming a stimulus for pro-inflammatory responses [102].

Insulin resistance (a case of decreased biological and physiological responses to insulin) [103], will result in a need for higher than normal insulin concentrations in order to maintain normal glucose levels [104]. Hypersecretion of insulin will therefore be the result of an attempt to compensate for the lack of glucose transport to lipid cells and skeletal muscle [103]. High insulin resistance has been shown to increase cardiovascular risk by 2.5 times [105], as well as playing a pathogenic role in arterial hypertension [106]. In a 13-year follow-up study, insulin resistant hypertensive individuals developed several cardiovascular diseases and -events over the years, which included angina pectoris, myocardial infarctions, peripheral vascular disease, carotid plaques or stenosis, as well as cardiovascular deaths [107].

Disturbed glucose metabolism has previously been related to lipodystrophy, which includes the combination of lipoatrophy and abdominal lipohypertrophy [108]. With an expansion of adipose tissue and an increase in free fatty acids formation, as in the case of obesity [109], production of glucose and other lipids from the liver will be increased [110], while induced insulin resistance and beta-oxidation will reduce glucose uptake and oxidation in the skeletal muscles [111]. This increase in circulating free fatty acids and glucose concentrations, will result in the development of hypertension by the stimulation of pancreatic insulin secretion, causing increases in sodium reabsorption, which in turn will result in increased sympathetic drive [110].

In HIV infection, glucose phosphorylation, as well as glucose transport in skeletal muscle, is impaired and thus, by playing a role in lipodystrophy, causes insulin resistance [7]. Furthermore, the net effect of a cytokine, called tumour necrosis factor- α , is to induce an insulin resistant state, which in turn will be the cause of insulin hypersecretion [112]. Compared to uninfected individuals, studies have found significant elevated serum insulin concentrations and increased insulin resistance in HIV-infected individuals, but notably, no differences in plasma glucose concentrations were reported in those individuals [112].

Impairment in glucose metabolism could be attributed to pro-inflammatory adipokine disturbances, and could play a role in the increased cardiovascular risk that was shown to be

associated with antiretroviral treatment [113]. The risk of Type 2 diabetes mellitus has also been shown to increase fourfold when treatment was applied [114], however, even in the absence of protease inhibitor-based treatment, insulin resistance has still been shown to occur in HIV-infected persons [112].

Glycated haemoglobin

The formation of glycated haemoglobin (HbA1c) occurs by the irreversible glycation of lysine and valine residues within haemoglobin [115] and the HbA1c value is therefore an expression of the bound fraction of hemoglobin to glucose [116]. HbA1c is regularly used in clinical practice as an indicator of the average glucose concentration, as well as to determine average glycemic control (even more accurately than fasting blood glucose) over the course of 3-4 months (± 120 days) [116-118]. The American Diabetes Association has endorsed a HbA1c cut-off value of $\geq 6.5\%$ as the criteria for diagnosing diabetes, while a value of $\geq 5.7\%$ was recommended as part of the criteria for testing for diabetes (in asymptomatic adults) [119].

It has been proposed that HbA1c is a useful marker of insulin sensitivity in adults with normal glucose tolerance [120], while elevated serum concentrations predict a future diagnosis of Type 2 diabetes and future cardiovascular disease and mortality [121]. Indeed, other studies have shown an increase in HbA1c levels to be associated with an increase in cardiovascular disease risk [122] and that a 20-30% increase in cardiovascular events or mortality occurs for each 1% increase in HbA1c levels [123]. In accordance with these studies, another study on asymptomatic, nondiabetic African Americans found HbA1c to be independently related to an increase in left ventricular mass, a decrease in aortic distensibility, and thus an increase in aortic stiffness and pulse wave velocity, even after adjustment for age [124].

It has been said that the prevalence of diabetes mellitus would increase in the HIV-infected because of the improvement in lifespan among this population [118]. HbA1c data, which is associated with future diagnosis of diabetes [121], were suggested to be reliable for HIV-infected groups [118]. It should however be noted that the lifespan of erythrocytes could be affected by some medications used by HIV-infected individuals, and that HbA1c levels could therefore be “interfered” with by such medications [118].

C-reactive protein

Several studies have identified C-reactive protein (CRP) as a novel risk factor for CVD, for development of the metabolic syndrome, to predict target organ damage, and to attribute to traditional cardiovascular risk factors (such as glucose, TC, LDL-C, TG and high blood pressure levels) [33,51,125-127]. Indeed, Miller *et al.* found increased CRP levels to be accompanied by at least one abnormal risk factor [128]. Several guidelines have classified a high CRP level as >3 mg/l, while a normal level of CRP is classified as <1 mg/l [128].

CRP, an acute-phase reactant, is secreted by hepatocytes and adipocytes when stimulated by interleukin-6 [13,129]. CRP is considered to be the most extensively investigated plasma inflammatory biomarker and is the only inflammatory biomarker that can be used to predict the first atherothrombotic event [127,130,131]. Inflammation is known to play a central role in development of atherosclerosis and progression of CVD [131,132].

Several studies have reported a strong association between elevated CRP levels and an increase in stiffness of large arteries, together with increases in pulse pressure and pulse wave velocity [28,133-135]. In addition, some mechanisms have been proposed in which CRP could have a proatherosclerotic effect [130]. CRP is thought to cause metabolic and haemodynamic changes (such as stimulation of adhesion molecule expression, monocyte recruitment, ion channel modification and increased oxidative LDL-C uptake), which in turn could cause vascular damage and an increase in arterial stiffness [28,130,134,136]. In addition, elevated CRP levels could cause endothelial dysfunction, followed by an inflammatory reaction, which in turn could inhibit endothelium-dependent vasodilatation [137-139]. However, studies have found that CRP could in fact benefit the availability of nitric oxide [140], a vasodilator, which led to the notion that, in the case of acute inflammation, CRP is unrelated to arterial dysfunction and should be seen as a marker, rather than a mediator [130,141].

Furthermore, the development of hypertension is one of many CVDs that can be predicted by elevated CRP levels [142,143]. CRP levels were shown to strongly correlate with incident weight gain (adipose tissue mass), and body mass index [13,128,129]. More studies have implicated CRP to be the cause of several cardiovascular events such as necrosis that may lead to acute myocardial infarction in animals, as well as ischemic stroke [144,145].

In HIV-infection, inflammation (which is associated with chronic immune activation and a pro-inflammatory state), also plays a role in the development of vascular abnormalities, which in turn is the cause of an increase in cardiovascular risk in this population. The above mentioned were proposed to be accompanied by an increase in CRP levels, which have been associated with mortality in HIV-infected women [146-149]. However, CRP levels were shown not to be

related to CD4 cell counts or HIV-viral load [126], even though it can be seen as a marker of HIV disease progression [150].

Additionally, studies have shown higher CRP levels (of >3 mg/dl) in treated HIV-infected persons, compared to never treated HIV-infected persons, which suggests a higher risk for stroke and myocardial infarction in the treated HIV-infected persons. This was especially the case in treatment with non-nucleoside reverse transcriptase inhibitor drugs, but not with protease inhibitor drugs and there is thus a lack of clarity in this area [16,126].

2.2.3 Arterial function, cardiovascular risk and HIV

Atherosclerosis & vascular aging

Atherosclerosis, which is known to cause several CVD outcomes [101], can be defined as an inflammatory disorder [130], initiated by the retention and accumulation, as well as the oxidation of lipoproteins (including LDL-C) in the arterial wall where endothelial dysfunction is present, and thus where the permeability of the endothelial is increased. Eventually mature atherosclerotic plaque will form and plaque rupture will occur, damaging the intima of the arteries [101,151-154]. Different risk factor profiles for the different vascular beds (including coronary-, carotid-, peripheral- and aortic vascular beds) have been reported, which proves atherosclerosis to be a heterogeneous disorder [155,156]. The number of cardiovascular risk factors increases proportionally with the extent of atherosclerotic lesions [157].

Cardiovascular performance is determined by arterial function [130], and it has been said that arterial distensibility is regulated by the endothelium [134]. Dysfunction of the endothelium in arteries can lead to an increase in arterial stiffness [134], which is known to be a surrogate marker for CVD, as well as a predictor of cardiovascular risk and outcome [158-160]. Arterial stiffness is considered as a characteristic of vascular aging [134], and it has previously been proposed that vascular age could be used as a risk stratification tool [130].

In addition, arterial stiffness was reported to be dynamically dependent on functional and structural properties of the vascular wall (including vascular tone) [134,161], which differs according to the location of these properties in the arterial tree [134]. The latter plays a role in pressure wave reflection, and together with arterial stiffness, it causes the differences that exist between central and peripheral blood pressure, where central SBP and PP are lower than brachial SBP and PP.

Markers of atherosclerosis

In the artery, a pressure wave is created from the left ventricle when blood is ejected during systole. This wave then circulates towards the periphery where it will be reflected back in the direction of the central aorta [14,158]. The velocity at which the pressure wave travels is referred to as the pulse wave velocity (PWV), and is proportional to the arterial wall stiffness [14,158]. In the case of a normal PWV (where normal compliance of the vascular wall occurs), the reflected wave will reach the heart during diastole and will not combine with the ejected wave [14,158]. However, in the case of high blood pressure and aging where arterial stiffness is increased, a higher PWV will occur, which will cause an earlier return of the reflected wave (during systole), and in which case the reflected wave will be combined with the ejected wave [14,158]. The latter are known to result in pressure wave amplification (as described by the augmentation index), which could cause further increases in PP, SBP, as well as a decrease in coronary perfusion [158,162].

As already mentioned, assessment of arterial stiffness and wave reflections could be made by measurement of arterial PWV [61,134]. The ESH-ESC has described the measurement of aortic PWV as the gold standard method to use for the assessment of aortic stiffness [19,160,160], and the use of specifically carotid to femoral PWV was proposed as the most validated technique [158]. Increased aortic PWV have been shown to be associated with higher risk for cardiovascular events and mortality, as well as all-cause mortality. Indeed, risk was shown to increase by >10% with each 1 m/s increase in aortic PWV [160]. ESH-ESC guidelines have suggested elevated PWV levels to be >12 m/s [20]. Additionally, left ventricular diastolic dysfunction has been found to occur in persons with a high aortic augmentation index [163].

Early structural changes in the vasculature have been shown to be a surrogate marker for structural changes in the blood vessels, and could be identified by measuring the arterial intima-media thickness (IMT) of the common femoral, common carotid and the brachial arteries with the use of B-mode ultrasonography [158,164]. The prevalence of atherosclerosis is known to be frequent in the carotid arteries [158], and IMT is considered one of the strongest risk factors for atherosclerotic disease [164], and at the same time is associated with cardiovascular- and all-cause mortality [51]. According to the ESH-ESC guidelines a carotid wall thickness (IMT) of >0.9 mm falls into the category of subclinical organ damage [19].

Studies have shown that in HIV-infected individuals, vascular age is increased [165,166] to up to 12 years higher than chronologic age [51], while peripheral (brachial) arterial endothelial function is impaired [167]. In addition, Vlachopoulos *et al.* recently found that in early stages of HIV infection, wave reflections were decreased and PWV were similar in treatment naive individuals [61], while another study reported higher PWV values in HIV-infected (treatment

naive) persons [168]. Early atherosclerosis is promoted by HIV infection [169], and the elevation of carotid IMT is associated with premature atherosclerosis in HIV-infected persons [170]. A higher IMT and more rapid progression thereof were reported in HIV-infected persons [169,171-173], especially in the bifurcation carotid segment [169]. Previous studies have shown that CD4 counts of ≤ 200 were related to IMT progression [171], while IMT was further associated with mortality in HIV infection [51].

2.2.4 Renal function, cardiovascular risk and HIV

Many molecules are metabolised by the kidneys [174], and even when chronic kidney disease is absent, kidney function is still known to decline by 10 mL/min/1.73m² per decade [175]. Several methods can be used to determine renal function. One such method includes the isotopic determination of the glomerular filtration rate (GFR) [176] by using the Modification of Diet in Renal Disease formula [177], or by using the Modification of Diet in Renal Disease equation, which contains serum creatinine levels, gender, age, and race [178]. Other creatinine-based equations include the Chronic Kidney Disease Epidemiology Collaboration equation [178] and the Cockcroft-Gault equation [179].

An increased risk of cardiovascular complications, together with a higher mortality and morbidity rate have previously been associated with a decrease in GFR [180,181]. In young adults, a GFR of 90-125 mL/min/1.73m² is considered to be normal [175]. In addition, Bax *et al.* have reported an estimated GFR of <60 mL/min/1.73m² to be concurrent with a higher risk of recurrent vascular events in patients with CVD [182]. A normal creatinine clearance can be seen as 117 mL/min [183], while mortality has been shown to increase by 1% with every 1 mL/min decrease in creatinine clearance [184]. Elevated creatinine levels were previously associated more with cardiovascular events and mortality [174].

Albuminuria and renal function have been reported to be markers of chronic kidney disease and can therefore be associated with each other [182], while microalbuminuria has previously been associated with an increase in cardiovascular events and mortality [185]. Uric acid, on the other hand, has been shown to stimulate the proliferation of vascular smooth muscle [186], decrease the production of endothelial nitric oxide [187], and thereby induce endothelial dysfunction [186,187].

A reduction in activity of the renin-angiotensin system, as well as improvement of renal function should be used as treatment goals in order to decrease cardiovascular events [188], as a decrease in renal function could result in the acceleration of the atherosclerotic process [189].

Indeed, renal insufficiency is associated with greater atherosclerotic burden [174], cardiovascular mortality [183] and higher prevalence of cardiovascular risk factors, including hypertension, lipid abnormalities, and diabetes [190]. In turn, advanced atherosclerosis negatively affects renal function, and together with renal dysfunction, plays a role in chronic inflammation [191]. It should be noted that chronic kidney disease proved to be a risk factor which was greater in the African than in the general population, for multiple cardiovascular end points [192].

It has been shown that 30% of HIV-infected persons suffer from kidney dysfunction, and that HIV disease progression, morbidity, and mortality can be increased by kidney disease [193,194]. HIV infection plays a role in several renal syndromes, including HIV immune complex glomerulonephritis, antiretroviral-associated nephrotoxicity, and HIV associated nephropathy [195,196]. In HIV-infected persons lower GFR, as well as higher serum creatinine levels and proteinuria have been found to occur [194]. The latter two factors have further been associated with faster progression of HIV to AIDS [197], which gives cause to the recommendation of early initiation of ART [198], even though the type of ART does not seem to have an influence on the GFR [198]. In addition, a lower CD4 count relates to lower creatinine clearance [194], while a worse GFR correlates with extended time of HIV infection [198]. In contrast with these findings, Fourie *et al.* found no differences in the estimated creatinine clearance or serum creatinine levels between HIV-infected and HIV-uninfected Africans [43].

2.2.5 Anthropometry, cardiovascular risk and HIV

Obesity

Obesity, recognised as a global epidemic [199], is considered a risk factor and represents excessive body fat content in relation to stature [200]. It is well known that visceral obesity is associated with the development of insulin resistance, which can be caused by the release of free fatty acids, cytokines, other pro-inflammatory markers, and specifically large amounts of tumour necrosis factor- α by adipose tissue [83,100]. A concerning link exists between obesity and CVD [201]. The latter could be attributed to the association of obesity with an increased activation of the renin-angiotensin and sympathetic nervous systems, which could lead to enhanced vasoconstriction, vascular resistance, cardiac output, and fluid retention, which could in turn be the cause of hypertension [101,202,203]. Interestingly, malnutrition and obesity have been found to be prevalent in the same households in developing countries [204], and it has been reported that African women and Hispanics have the highest obesity rates, while African people also have the highest hypertension rates [131].

Several methods by which obesity can be measured have been proposed, including the measurement of waist circumference (to reflect abdominal obesity), as well as the measurement of hip circumference (to reflect gynoid obesity) [97]. Furthermore, body mass index (BMI), calculated by the formula: weight (kg)/stature (m^2), has been presented as a reliable predictor of total fat, and can therefore be used in the measurement of overweight and obesity [201]. It has been proposed that the strength of association that occurs between the risk for CVD and obesity, is the same for BMI, waist-to-hip ratio and waist circumference (WC) [205], however, controversy regarding this subject still exists.

According to the recommendations for weight classification by the National Institutes of Health, BMI could be categorised into underweight $<18.5 \text{ kg/m}^2$, normal weight $18.5\text{--}24.9 \text{ kg/m}^2$, overweight $25.0\text{--}29.9 \text{ kg/m}^2$, obesity Class I (high) $30.0\text{--}34.9 \text{ kg/m}^2$, obesity Class II (very high) $35.0\text{--}39.9 \text{ kg/m}^2$ and obesity Class III (extremely high) $>40.0 \text{ kg/m}^2$ [206]. However, controversy exists over the cut-off values across different ethnic groups, as for certain ethnic populations, a BMI of 30 kg/m^2 has been considered to be too lenient. These controversies were also found to occur across time in the same populations [201]. It is noteworthy that, in addition to obese individuals, being underweight is also implicated in the shortening of lifespan, which can be described by the U-shaped relationship that exists between BMI and risk of mortality and morbidity [201]. Previous studies have reported the existence of strong correlations between BMI and serum lipids, which was reflected by higher TC levels, lower HDL-C levels, and thus higher TC/HDL-C ratios [49]. In addition, BMI was linked to coronary artery disease by its association with leptin, which is known to regulate basal metabolism and food intake [207,208].

On the other hand, WC (represented by the smallest measurement between the lowest rib and the lateral iliac crest) [209], is described as an independent risk for mortality in the general population [210]. The ESH and ESC, as well as the NIH, have reported increased waist circumference, measured in a standing position, of $> 120 \text{ cm}$ and $> 88 \text{ cm}$ in men and women, respectively, to be evidence of visceral (abdominal or central) obesity [19,211]. Increased adiposity and obesity, on the other hand, have been defined by a waist-hip ratio of >0.90 in men and >0.85 in women [212]. With regard to HIV infection, obesity was reported to be higher in HIV-infected women than in their HIV-uninfected counterparts, especially when not treated [213,214].

Lipodystrophy

Lipodystrophy, a morphologic phenomenon, can be recognised by peripheral fat depletion (loss of fat in the extremities, buttocks and face), as well as by intra-abdominal adiposity (accumulation of fat in the abdomen, neck and breasts) [95,215]. Intramyocellular TG accumulation, higher oxidative LDL-C and lower HDL-C levels, as well as decreased insulin

action and disturbed glucose metabolism have further been associated with lipodystrophy [62,108]. It is well known that lipodystrophy is associated with increased cardiovascular risk [215,216]. Likewise, studies have reported associations between lipodystrophy syndrome and hypertension, higher myocardial infarction incidence and coronary artery disease, as well as diabetes [104].

In HIV-infected individuals, lipodystrophy (associated with abnormal fat distribution, glucose intolerance, and hyperlipidaemia) [217] has been reported to present a risk for sub-clinical coronary atherosclerosis [216], heart disease, diabetes, and early hypercholesterolemia [104]. Although lipodystrophy was found to be prevalent in only a few never treated HIV-infected persons [218], the presence thereof has been well documented in HIV-infected individuals receiving ART [104,215]. Interestingly, lipodystrophy can also cause the development of stigmatisation and a decrease in self-esteem to occur, which could have a negative effect on adherence to ART, and therefore alternate a person's quality of life [219].

2.2.6 Lifestyle, cardiovascular risk and HIV

Tobacco use

Tobacco smoke is considered a modifiable health risk factor [220] that could, in many ways, have a negative influence on the cardiovascular system, the respiratory tract, as well as the immune system where it could play a role in the inhibition and release of anti-inflammatory and pro-inflammatory mediators [221]. Tobacco smoke contains more than 4,000 components, of which carbon monoxide plays a role in smoke-related cardiovascular alterations [222]. Smoking could influence all phases of atherosclerosis, including endothelial dysfunction, plaque progression, more fibrous lesions, and thrombotic clinical events [222,223]. Indeed, smoking was reported to play a primary role in the development of fibroatheroma from atheroma [224]. Lower HDL-C and higher plasma cholesterol levels have further been associated with smoking [225]. Rodriguez *et al.* found LDL-C to be negatively and independently predicted by smoking, but this was only the case where less than one pack per day was smoked, and not in the case of more than one pack per day [49].

The use of tobacco is known to be much more prevalent among the HIV-infected, which is considered to be the cause of the higher morbidity in this population [220]. Tobacco use, together with HIV infection, has been associated with an increased risk of infections and certain cancers (because of its influence on the immune system), as well as a decrease in response to ART [220,226]. Several cardiovascular risk factors are also associated with ART [169], and the

consideration of cessation from tobacco use has therefore been recommended by intervention studies [59].

Alcohol use

Lower cardiovascular morbidity and mortality have been associated with regular alcohol consumption by healthy individuals, because of the regulatory effect on lipids, as well as a decrease in coagulation and platelet aggregation factors [227-229]. This is also supported by a study where alcohol consumption correlated with elevations in HDL-C levels [49]. However, a J-shaped relationship has been proposed between ischemic cardiovascular events and alcohol consumption [230-232], therefore underlining the negative effects of alcohol abuse.

An increased transmission risk and possible disease progression [233], with higher HIV viral loads and lower CD4 cell counts, has been detected in HIV-infected alcohol consumers, especially when treated [234]. Although the effects of alcohol on lipid abnormalities in HIV-infected persons could not be detected, the addition of ART is thought to result in further derangement of the lipid profiles [235]. Given these findings, no 'safe' level of alcohol consumption could be suggested for HIV-infected individuals and individuals receiving ART [236].

Physical activity and diet

Physical activity can be defined as "any body movement produced by the skeletal muscles that results in energy expenditure" [237]. It has been suggested that development of the metabolic syndrome in adulthood can be prevented by participation in organised sport in youth [238], yet, physical activity is decreasing among children and is resulting in rapid increases in the prevalence of childhood obesity [239].

Exercise is known to have several beneficial effects such as to improve quality of life, decrease overall mortality, prevent ischemic heart disease, decrease arterial blood pressure, as well as to protect against atherosclerosis by facilitating cytokine production [237,240]. In addition to lowering blood pressure and lipoprotein levels, as well as increasing shear stress and decreasing vasoconstrictors [241], exercise has been proven to improve endothelial function by stimulating nitric oxide production and inhibiting nitric oxide inactivation, thereby enhancing nitric oxide bioavailability [241]. Another beneficial effect of exercise includes the reversing or controlling of lipodystrophy [242], together with increases in HDL-C levels and decreases in TG and LDL-C levels [237]. Supplementary, a mean decrease of 3 mmHg in systolic and diastolic arterial pressure has been associated with physical activity in normotensive individuals, while a decrease of as much as 6-7 mmHg has been found in hypertensive individuals [243]. Therefore

it is clear why physical inactivity can be seen as a risk factor for CVD [239,241] and coronary artery disease [237].

With regard to people living with HIV, exercise has been suggested to enhance muscle function and mass, as well as to have a positive effect on immune status by increasing the activity of CD4 and other immune cells in these people [244]. However, previous studies have shown that a decline in physical endurance could occur as a result of HIV infection [245], and that the physical ability of severe immunodeficient HIV-infected persons to exercise was very limited [246]. Noteworthy: exercise in HIV-infected persons was shown to reduce TG levels by 21% and TC levels by 11% when combined with a healthy diet [247], and care should therefore be taken to optimise nutritional status, in combination with exercise, in the management of HIV infection [248].

2.2.7 Family history, cardiovascular risk and HIV

According to the ESH and ESC, factors such as stroke, diabetes, hypertension, peripheral artery disease, premature coronary heart disease, renal disease, and dyslipidaemia should be included in order to obtain a complete family history [19]. A familial history of ischemic heart disease has been found to be a risk factor for coronary heart disease [249], while a family history of stroke has further been said to be a non-modifiable risk factor for stroke [250,251]. This is supported by a South African study conducted in Soweto, where 4% of the 9% of people with a family history of stroke, suffered from acute stroke [252].

With regard to the management of HIV infection, familial history of diabetes mellitus has to be brought into consideration [95]. Even though self-reported family history is known to be specific, for many conditions it can also be very non-specific of nature [253].

2.3 TREATMENT OF HIV IN SOUTH AFRICA

ART was first introduced in 1996 in developed countries [254] and it was only after 2004 that two fully subsidised, free of charge ART regimens were made available to the public health sector in sub-Saharan Africa [255]. The introduction of ART in HIV-infected individuals transforms this disease from an acute illness to a controllable chronic condition, and thereby decreases morbidity and mortality, as well as increases life expectancy [46,256]. The ART rollout program in South Africa, is thought to be associated with a lower mortality rate [2]. In South Africa, more than 970,000 individuals are currently enrolled in these programmes. In the

process a total of more than 700,000 life-years have been gained to date [2]. However, with this life expansion, several metabolic and clinical complications may develop such as dyslipidaemia, lipodystrophy, and Type 2 diabetes mellitus [46,257-259]. Lipodystrophy, which was observed in 20-80% of HIV treated patients [260], has been associated with a 16% increase in risk of myocardial infarction per year of ART exposure [261].

ART mainly consists of nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs) [126]. In South Africa, and in this particular study, the first-line treatment consists of two NRTIs (including stavudine and lamivudine) and one NNRTI (including efavirenz or nevirapine) [255], while PIs are being excluded because of the negative effects these drugs have [262,263]. First-line treatment is provided through the large-scale, free of charge rollout programs in South Africa [264], and ART is initiated when CD4 cell counts reaches a level of ≤ 200 cells/mm³ [255]. It should however be noted that, in 2010, the World Health Organization (WHO) has suggested earlier initiation of ART (at a CD4 cell count of ≤ 350 cells/mm³) [2], even though in South Africa, so far this guideline has only been implemented for pregnant women and for patients suffering from tuberculosis [265].

2.3.1 Nucleoside reverse transcriptase inhibitors

NRTIs are classified as competitive inhibitors of HIV reverse transcriptase, and when incorporated into the proviral DNA, are known for its DNA chain termination process, as well as prevention of viral DNA replication [266,267]. NRTIs have been used to treat HIV-infected patients for more than ten years and it has been shown that these antiretroviral drugs could have severe or fatal toxic effects, which may mainly be associated with mitochondrial dysfunction. Such effects include peripheral neuropathy, myopathy, pancreatitis, nephrotoxicity, lactic acidosis and hepatic steatosis [268]. NRTIs have also been associated with hypercholesterolemia, as well as an early increase in TG and TC levels [269-271].

The two NRTIs which form part of the South African rollout program, are Stavudine (also called d4T with the trade names Zerit and Zerit XR), and Lamivudine (also called 3TC with the trade name Epivir). A specific side effect, among other negative effects, that is theoretically associated with the mentioned drugs, includes the development of lipodystrophy syndrome [104]. As on the first of April 2010, it has been asserted that Stavudine, as the preferred first-line treatment, should be progressively replaced with alternative treatments such as Tenofovir, representing equally effective alternatives [272]. The reason stated for this change in treatment

was that Tenofovir has fewer side effects, compared to the associated increased risk of metabolic complications which occur with long-term use of Stavudine [273].

2.3.2 Non-nucleoside reverse transcriptase inhibitors

In contrast to NRTIs, NNRTIs are classified as non-competitive substrate inhibitors [274] and are known for their inhibitive binding to the HIV reverse transcriptase, thereby causing conformational changes to occur in the enzyme [275].

An example of such a NNRTI includes Efavirenz, formerly known as DMP-266, with trade names Sustiva and Stocrin [104,276], which blocks the reverse transcription process where viral RNA is transcribed to DNA [277]. Efavirenz has theoretically been shown to play a role in lipodystrophy syndrome development [104], which was confirmed by a previous study where NNRTI treated individuals were found to have higher HDL-C, LDL-C, and TC levels than treatment naive individuals [80]. Other negative effects of NNRTIs include hepatotoxicity, hypertriglyceridemia, rash, adverse central nervous system effects [278], as well as teratogenic effects, which will implicate the use of contraception in sexually active young women receiving this treatment [279].

2.3.3 Treatment limitations in South Africa

In November 2003, the government decided to provide free ART programmes to the South African population [264], and on the first of April 2004, the rollout ART programmes were announced in four of the nine South African provinces [280]. Even though rollout ART programs are available in rural South Africa for free [281] and are known to have a preventative effect on the spreading of the disease [279], sub-Saharan Africa still has the highest global number of HIV-infected patients [2].

Campbell *et al.* previously conceptualised three different categories (material-, networking- and symbolic context) each consisting of different factors that could play a role in the effectiveness of the communities' response to HIV disease challenges [282]. Such factors included poverty, unemployment, gender relations, and stigma [282]. Stigma has further been said to have an impact on the effectiveness of HIV/AIDS prevention, management, and care [283,284]. Other possible factors that could also limit the effectiveness of ART include lack of community support, limitations in human resource capacity and insufficient supplies [285].

South Africa has the largest ART treatment program globally [286], and even though ART prices have been declining and ART has become more commonly available across sub-Saharan Africa [287], limited or no access to healthcare services in the rural areas of sub-Saharan Africa continues to play a significant role in the overall HIV disease burden on this subcontinent's population [288]. Indeed, it has been reported that only 30-38% of people in sub-Saharan Africa who acquire ART, are being reached by the ART programmes [286,287] and that these programs retain only 60% of these patients after two years [289]. Several factors that could influence the overall ART success rate include diet, drug stock outs, socioeconomic status, and distance to the hospital [254].

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CHAPTER 3

Research article

INSTRUCTIONS FOR AUTHORS

Journal: HIV Medicine

Manuscript style

An Original Article has an abstract, main text up to 3,500 words, a maximum of 20–50 references. The Abstract, of up to 250 words, should be divided into sections entitled Objectives, Methods, Results and Conclusions. Longer articles may be accepted at the discretion of the Editor. Purely basic science papers are considered outside the scope of the journal. Original Articles should comprise the following sections:

Title page

This should contain a concise article title, a shortened version (no more than 50 characters including spaces) for the running head, initials and surnames of the authors, their affiliations, and the full postal address, fax and telephone number, and e-mail address of an author to whom correspondence can be addressed. A list of key words (maximum five) is required as part of the submission.

Main text

This should start on a new page, and include Introduction, Methods, Results and Discussion sections. The suggested points of insertion for illustrations should be indicated. Authors should avoid abbreviations (except those commonly understood), long sentences, and many juxtaposed numbers in sentences.

References

These should be in the Vancouver style, i.e. numbered throughout the text in consecutive order using Arabic numerals in parentheses. The references should be listed in numerical order at the end of the paper using the following styles.

Tables, Figures and Illustrations

Tables should be numbered consecutively with Arabic numerals with a fully informative caption as a heading. Column headings should be brief, with units of measurement in parentheses. Vertical lines should not be used to separate columns. Electronic tables should be provided in an editable format (.rtf or .doc). All illustrations (including photographs) are classified as figures and should be numbered consecutively.

Acknowledgements

These should be brief and must include references to sources of financial and logistical support.



The cardiometabolic profile of HIV-infected South Africans of African descent: a 5-year prospective study

Running head: Cardiometabolic profile of HIV-infected Africans

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Keywords: Human immunodeficiency virus, antiretroviral treatment, pulse pressure, dyslipidaemia, South Africa

Abstract

Objective

To determine whether antiretroviral treatment changes the cardiometabolic profile of HIV-infected South Africans over a period of five years.

Methods

In this 5-year prospective study, embedded in the Prospective Urban and Rural Epidemiology (PURE) study, we compared the cardiometabolic profile of 66 treated (after baseline) and 71 never treated human immunodeficiency virus (HIV) -infected participants from the North-West province, South Africa. By using standard techniques, these participants' cardiometabolic, biochemical and lifestyle variables were assessed in 2005 and 2010.

Results

The treated group showed a remarkably higher percentage change in pulse pressure ($p=0.013$), systolic blood pressure ($p=0.046$) and CD4 cell count ($p=0.019$) levels over 5 years. During follow-up (2010), higher levels of total cholesterol ($p<0.001$), low-density lipoprotein cholesterol ($p<0.001$) and triglycerides ($p=0.043$) were observed in treated participants, compared to the never treated HIV-infected participants. The waist circumference of only the treated group tended to increase ($p=0.06$) over the 5-year period, while their body mass index remained unchanged.

Conclusions

Africans receiving ART had a greater increase in pulse pressure and systolic blood pressure, a worse lipid profile and tended to show an increase in abdominal fat accumulation when compared to never treated participants. No vascular functional (central systolic blood pressure, carotid-dorsalis pulse wave velocity and augmentation index) or structural (intima-media thickness) differences were observed after 5 years. Whether antiretroviral treatment will lead to increased arterial stiffness, vascular aging or accelerated atherosclerosis in this HIV-infected African population, remains uncertain.

Introduction

South Africa is by far the country most affected by the human immunodeficiency virus (HIV) in the world, with 5.6 million people living with HIV in 2009 [1]. In South Africa, where HIV-1 subtype C is the most prevalent [2], more than 970,000 individuals are currently enrolled in the large-scale, free of charge antiretroviral treatment (ART) rollout programme [1]. Even so, South Africa achieved treatment coverage of less than 40% in 2009 [1].

Atherosclerosis is initiated by the retention and accumulation, as well as the oxidation of lipoproteins (including low-density lipoprotein cholesterol) in the arterial wall where endothelial dysfunction is present [3,4]. Should degradation of the elastic proteins (elastin) occur, elastic properties of the large arteries could be compromised, resulting in an increase in arterial stiffness [5]. This leads to unfavourable hemodynamic effects [6], such as an elevation in pulse pressure that has been shown to be both a consequence and a cause of atherosclerosis [7], as well as to be associated with an increase in central arterial stiffness [8]. In HIV infection, atherosclerosis is mostly associated with the treatment of the infection [9,10]. ART has also been found to be associated with an increase in blood pressure of up to 3.2/2.7 mmHg [11].

In HIV-infected individuals, lipodystrophy (associated with abnormal fat distribution, glucose intolerance, and hyperlipidaemia) [12] is a risk factor for sub-clinical coronary atherosclerosis [9]. ART is known to bring about metabolic side effects, especially Stavudine, Nevirapine and Efavirenz [13,14]. The characteristics of ART-associated dyslipidaemia include elevated total cholesterol levels and low-density lipoprotein cholesterol levels (that could reach 168% from baseline values) [15], elevated triglyceride levels [16], as well as decreased high-density lipoprotein cholesterol levels, which could cause a decrease in cardiometabolic protection [17].

Keeping the above-mentioned in mind, the aim of this study was to determine whether treatment is either directly or indirectly associated with changes in the cardiometabolic profile of HIV-infected black South Africans over a follow-up period of 5 years.

Materials and methods

Study design and participants

The international Prospective Urban and Rural Epidemiology (PURE) study is an epidemiological, longitudinal multi-national study assessing changes in lifestyles and causes of development of cardiovascular risk factors and chronic diseases. A minimum follow-up period of 10 years is planned, targeting urban and rural areas in low and middle-income countries, including South Africa [18]. For this part of the South African PURE study, participants were randomly recruited from the North-West Province, South Africa. The inclusion criteria consisted of volunteers of ages older than 35 years, who did not use any chronic medication and did not have any self-reported diseases. This sub-study is embedded in the above-mentioned PURE study. The baseline data was collected in 2005 and the follow-up data in 2010 with the aim of addressing questions, such as the changes in metabolic and cardiovascular variables of newly identified HIV-infected participants, before and after antiretroviral treatment was introduced. The methodology appropriate to this sub-study will be discussed.

During the baseline study in 2005 (Fig. 1), participants ($n=300$) were unaware of their HIV status and had never received ART. As shown in Figure 1, 300 participants were newly identified as being HIV-infected. After identification, the infected participants were referred to the nearest clinic or hospital for a follow-up on the diagnoses. In South Africa, (as recommended by the World Health Organization), first-line ART consists of two nucleoside reverse transcriptase inhibitors (NRTIs) for which Stavudine and Lamivudine are used, as well as one non-nucleoside reverse transcriptase inhibitor (NNRTI) for which Efavirenz or Nevirapine is used. Treatment was initiated at a CD4 cell count of ≤ 200 cells/mm³ [19] and was supplied free of charge as part of the rollout program. During the follow-up study in 2010, a total of 137 from the initial 300 participants were again evaluated. From these participants, 66 were receiving treatment, while 71 were never treated and 7 participants discontinued treatment (reasons unknown) and were excluded from the study. Thus, participants were lost to follow-up over the five years due either to death ($n=69$) or a lack of reparticipation ($n=87$). Accordingly, a successful follow-up rate of 71% was obtained (which included participants that were followed-up, those that were deceased and those that discontinued treatment).

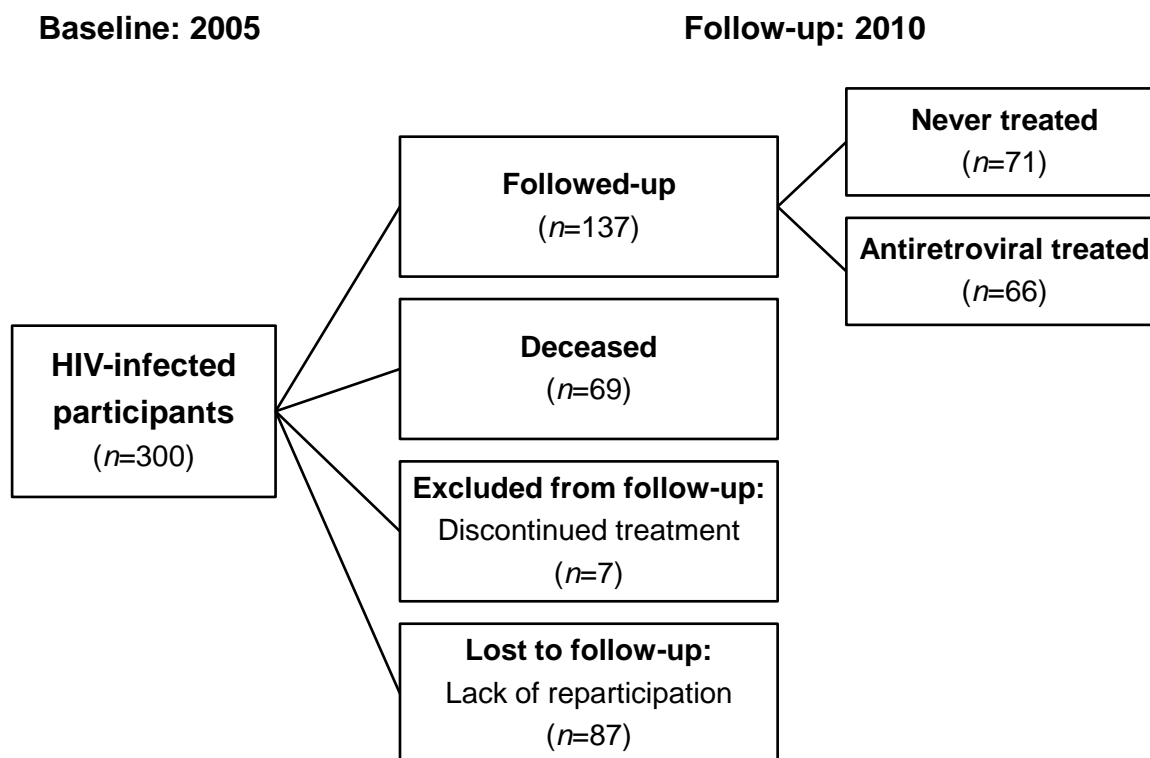


Fig. 1 Layout of the characteristics of HIV-infected participants in the baseline study.

Ethical considerations

The involved procedures were explained to each participant in his/her own language, followed by the signing of an informed consent form. The study protocol was approved by the Ethics Committee of the North-West University in Potchefstroom, South Africa, and complies with the Declaration of Helsinki (as revised in 2004).

Experimental protocol

Permission for the execution of this study was obtained from the provincial Department of Health, the local authorities, as well as the tribal Chief from the specific rural area. The experimental protocol for the data collection in 2010 was consistent with the protocol for the data collection in 2005, as was described in detail by Fourie *et al.* [20]. In short, lifestyle data (including self-reported current tobacco use, alcohol intake and medical history) were obtained by specially trained field workers in the participant's own language. During individual post-counselling, each participant was informed about his/her HIV status, blood pressure levels and fasting glucose levels, followed by the referral of the infected participants to the local clinic or hospital for further follow-up and CD4 cell count determination.

Anthropometric measurements

Standardised procedures were used to measure the height, weight, hip and waist circumference of each participant (at baseline and follow-up) with the Invicta Stadiometer (IP 1465; Precision Health Scale, A & D Company, Japan; and Holtain unstretchable metal tape) [21]. The body mass index (BMI) of each participant was determined by the following formula: BMI = weight (kg) / length (m²).

Cardiovascular measurements

Brachial systolic (bSBP) and diastolic blood pressure (bDBP) measurements were obtained with the validated OMRON HEM-757 device (Omron Healthcare, Kyoto, Japan) at baseline and follow-up. Measurements were taken with a 5-minute rest period in between, from which the last value was used. With each participant in a sitting position, with his/her right arm in a relaxed position and supported at heart level, measurements were performed on the right arm (brachial artery).

Central systolic blood pressure (cSBP) and augmentation index (AIx) measurements were obtained with the validated OMRON 9000AI device (Omron Healthcare, Kyoto, Japan), only at follow-up. Central PP (cPP) was calculated at follow-up by subtracting bDBP from central systolic blood pressure. Calculations were then made from the pulse wave by using the following formula: $AIx = P_2 / P_1$, where AIx denotes radial augmentation (expressed as a percentage of the pulse pressure), P_2 denotes the (second) systolic peak of the reflected wave and P_1 denotes the (first) systolic peak of the ejected wave.

Pulse wave velocity (PWV), a measurement of aortic stiffness [22], was measured on each participant's left side, while he/she was in a supine position. Noninvasively accessible superficial pulses and the Complior SP device (Artech-Medical, Pantin, France) were used to determine the measurement in a segment over the carotid radialis (crPWV), as well as the carotid dorsalis pedis (cdPWV) that was only measured in 2010.

A SonoSite Micromaxx ultrasound system (SonoSite, Inc., Bothel, WA, USA), with a 6-13 MHz linear array transducer was used to perform the carotid intima-media measurements. This was done only during follow-up in 2010. Images of the left and right common carotid artery were obtained from at least two optimal angles. The segments were then imaged and measured by prescribed protocols [23] and were digitised and imported into the Artery Measurement System's (Gothenburg, Sweden) automated software [24,25], where a dedicated analysis of the carotid intima-media thickness (IMT) was performed by one investigator. Analysis was done on a selected segment of maximum 10 mm with good image quality. The borders of the near and far wall of the intima-media, as well as the inner diameter of the vessel, are automatically

identified by the program. This was followed by the calculation of the mean IMT and vessel diameter throughout the 10 mm segment, by using around 100 discrete measurements. A visual inspection was also performed, and the automated analysis was manually corrected in the case of inappropriate results.

Blood, serum and plasma samples

Fasting blood samples were drawn from each participant's antebrachial vein with a sterile winged infusion set and syringes. Serum was then prepared according to the appropriate methods, followed by storage at -80°C in the laboratory until analysis. Serum collected in the rural areas, was first stored at -18°C for a maximum of five days, before it was transported to a laboratory facility and stored at -80°C.

Biochemical analysis

A determination of the total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), creatinine and high sensitivity C-reactive protein (hsCRP) levels were analysed using serum samples (Konelab20i™ auto-analyzer, Thermo Fisher Scientific Oy, Vantaa, Finland and Cobas Integra 400 Roche® Clinical System, Roche Diagnostics, Indianapolis, IN). The Friedewald formula [26] was used to calculate low-density lipoprotein cholesterol (LDL-C) in 2005, while the Cobas Integra 400 Roche® Clinical System (Roche Diagnostics, Indianapolis, IN) was used to determine LDL-C in 2010. Intra- and intercoefficients of variation for all assays were below 10%. The TC/HDL-C ratio and TG/HDL-C ratio were calculated. Creatinine clearance rate (CrCl) was estimated using the Cockcroft-Gault formula [27].

Glucose levels (fluoride) were also determined at baseline and follow-up (Vitros DT6011 Chemistry Analyzer; Ortho-Clinical Diagnostics, Rochester, New York, USA and Cobas Integra 400 Roche® Clinical System, Roche Diagnostics, Indianapolis, IN). In order to determine glycated haemoglobin (HbA1c) levels from EDTA plasma samples, the D-10 Haemoglobin testing system from Bio-Rad (#220-0101), which is based on ion-exchange high-performance liquid chromatography, was used.

Finally, HIV status was determined in 2005 by using the First Response (PMC Medical, India) rapid HIV card test with whole blood, and in 2010, HIV status was determined by using the SD BIOLINE HIV 1/2 3.0 (Standard Diagnostics, INC, Korea) test. In the case of a positive test, the test was repeated by using the Pareeshak (BHAT Bio-tech, India) card test in 2005; while in 2010 the First Response HIV card test 1-2.0 (Premier Medical Corporation Limited, India) was used. These tests cannot distinguish between subtypes. However, the subtype was most likely

subtype C as the epidemic prevalent in South Africa has been established as being HIV-1 subtype C [28] by means of serotyping as well as genotyping.

Statistical analysis

Statistical analysis was performed by using the Statistica version 10.0 (Statsoft Inc., Tulsa, OK). Independent *t*-tests were used to compare continuous variables, and for categorical variables, Chi-square tests were used. Data were expressed as arithmetic mean with 95% confidence intervals or % of *n*. Abnormally distributed data were *log*-transformed (as in the case of hsCRP and CD4 cell count) and these data were expressed as geometric mean with 5th and 95th percentile intervals.

The characteristics of participants in the baseline study were determined by means of independent *t*-tests and chi-square tests. Change within groups was calculated by subtracting baseline from follow-up data. The *p*-values for change within never treated and treated groups, respectively, were obtained with dependent *t*-tests. The percentage change between groups was calculated by using the formula: $2010-2005/2005 \times 100$. The *p*-values for change between never treated and treated groups were obtained with independent *t*-tests.

A forward stepwise multiple regression analysis was done with percentage change in pulse pressure (PP) as the dependent variable. The independent variables that were considered for the model included mean arterial pressure, TC, LDL-C, HDL-C, TG, TG:HDL-C ratio, hsCRP and HbA1c. Finally, we included ART (only in the case of the total HIV-infected group), baseline PP, as well as age, gender, body mass index, TC:HDL-C ratio, alcohol- and tobacco use (at follow-up), as independent variables, in this model.

In the comparison of variables indicating vascular structure and function differences (during follow-up) between never treated and treated groups, *p*-values were obtained by means of analysis of covariance (ANCOVA). In the case of cSBP, adjustments were made for gender, age and body mass index. Additional adjustments were made for mean arterial pressure (in the case of cdPWV and IMT), for heart rate (in the case of cdPWV and AIx) and for height (instead of body mass index) in the case of AIx.

Results

The differences between the participant groups (Fig. 1) at baseline are indicated in Table 1. From these results it is clear that those participants who were followed-up included less male participants, had lower bSBP and bPP levels than those who were lost to follow-up. Furthermore, followed-up participants were younger and also had lower TC:HDL-C and TG:HDL-C ratios, as well as lower hsCRP and HbA1c levels than those participants who died during the five years between baseline and follow-up. The latter group were older and had higher hsCRP and HbA1c levels than the participants who were lost to follow-up.

The characteristics of HIV-infected never treated and treated groups are compared, at both baseline (2005) and follow-up (2010), in Table 2. In total there were 137 HIV-infected participants, of whom 66 were treated and 71 participants were never treated after 2005. Less treated participants were living in urban regions during the study. At baseline only, the treated participants had a lower WC ($p=0.010$) and BMI ($p=0.009$). No differences were found in the cardiovascular variables between the groups in either 2005 or 2010. TC ($p<0.001$), LDL-C ($p<0.001$) and TG ($p=0.034$) levels were higher in the treated group compared to the never treated group at follow-up, while HDL-C levels were lower ($p=0.003$) at baseline. In the treated group, HbA1c levels were lower ($p=0.001$) at follow-up.

Table 1 Characteristics of HIV-infected participants ($n=300$) in the baseline study (2005)

Variable	Followed-up ($n=137$)	Excluded from follow-up ($n=7$)	Deceased ($n=69$)	Lost to follow-up ($n=87$)
Gender, male (%)	41 (29.9) ^a	4 (57.1)	28 (40.6)	43 (49.4) ^a
Age (years)	43.3 (42.1;44.4) ^b	47.6 (41.2;53.9)	47.0 (44.5;49.4) ^{bd}	42.6 (41.1;44.1) ^d
Anthropometric variables:				
WC (cm)	75.2 (73.4;77.0)	72.8 (64.8;80.7)	76.8 (74.2;79.3)	76.8 (74.4;79.1)
BMI (kg/m ²)	23.1 (22.1;24.1)	20.2 (16.7;23.8)	22.2 (20.8;23.5)	23.4 (22.2;24.5)
Cardiovascular variables:				
bSBP (mmHg)	122 (119;125) ^a	124 (105;143)	123 (117;129)	128 (123;134) ^a
bDBP (mmHg)	84 (81;86)	85 (73;97)	83 (79;87)	86 (82;89)
bPP (mmHg)	39 (37;41) ^a	39 (31;47)	40 (37;43)	43 (40;45) ^a
Biochemical variables:				
TC (mmol/l)	4.46 (4.25;4.66)	4.26 (3.10;5.43)	4.36 (4.04;4.67)	4.43 (4.14;4.72)
HDL-C (mmol/l)	1.27 (1.19;1.37)	0.91 (0.62;1.21)	1.17 (1.02;1.32)	1.26 (1.13;1.39)
LDL-C (mmol/l)	2.62 (2.47;2.77)	2.81 (1.67;3.95)	2.57 (2.31;2.82)	2.58 (2.33;2.82)
TG (mmol/l)	1.24 (1.11;1.37)	1.20 (0.86;1.54)	1.38 (1.20;1.57)	1.31 (1.14;1.48)
TC:HDL-C ratio	3.94 (3.68;4.20) ^{bc}	5.26 (2.96;7.57) ^c	5.07 (3.85;6.29) ^b	4.03 (3.71;4.36)
TG:HDL-C ratio	1.19 (1.02;1.36) ^b	1.56 (0.62;2.50)	1.90 (1.35;2.44) ^b	1.37 (1.10;1.63)
hsCRP (mg/l)	2.52 (-0.48;1.61) ^b	7.12 (0.36;1.69)	6.31 (-0.31;1.73) ^{bd}	2.84 (-0.58;1.58) ^d
HbA1c (%)	5.48 (5.41;5.55) ^b	5.80 (5.20;6.40)	5.63 (5.49;5.78) ^{bd}	5.45 (5.35;5.56) ^d
Glucose (mmol/l)	4.68 (4.53;4.83)	4.76 (4.07;5.45)	4.58 (4.42;4.74)	4.77 (4.62;4.92)
CrCl (ml/min)	86.5 (79.6;93.4)	84.0 (60.3;108)	82.9 (73.2;92.6)	185 (-7.04;378)
CD4 cell count (cells/mm ³)	311 (2.09;2.85)	391 (1.95;3.23)	260 (2.09;2.79)	321 (1.97;2.89)
Lifestyle variables:				
Alcohol use, n (%)	45 (32.9)	1 (14.3)	23 (33.3)	27 (31.0)
Tobacco use, n (%)	70 (51.1)	1 (14.3)	24 (34.8)	32 (36.8)

ART, antiretroviral treatment; WC, waist circumference; BMI, body mass index; bSBP, brachial systolic blood pressure; bDBP, brachial diastolic blood pressure; bPP, brachial pulse pressure; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; hsCRP, high-sensitivity C-reactive protein; HbA1c, glycated haemoglobin; CrCl, creatinine clearance. Data are expressed as arithmetic mean with 95% confidence intervals or % of n . hsCRP and CD4 cell count data are expressed as geometric mean (5th and 95th percentile intervals). P -values for comparison between groups were obtained with independent t -tests and for categorical variables with Chi-square tests. Same superscript letter are regarded as significant ($p \leq 0.05$) between individual groups.

Table 2 Characteristics of HIV-infected participants at baseline and follow-up

Variable	Baseline (2005)			Follow-up (2010)		
	Never treated (<i>n</i> = 71)	Treated after 2005 (<i>n</i> = 66)	<i>p</i> -value	Never treated (<i>n</i> = 71)	Treated (<i>n</i> = 66)	<i>p</i> -value
Gender, male (%)	24 (33.8)	17 (25.8)	0.30			
Locality, urban (%)	38 (53.5)	23 (34.9)	0.028			
Age (years)	42.9 (41.4;44.6)	43.7 (42.1;45.3)	0.49			
Anthropometric variables:						
WC (cm)	77.4 (74.7;80.1)	72.8 (70.6;75.0)	0.010	76.9 (74.5;79.4)	75.3 (72.8;77.8)	0.35
BMI (kg/m ²)	24.3 (22.7;25.9)	21.8 (20.7;22.8)	0.009	24.1 (22.6;25.7)	22.3 (21.0;23.5)	0.06
Cardiovascular variables:						
bSBP (mmHg)	125 (121;129)	120 (116;124)	0.12	122 (118;125)	124 (119;129)	0.43
bDBP (mmHg)	85 (82;88)	82 (79;86)	0.32	84 (81;86)	83 (80;86)	0.81
bPP (mmHg)	40 (38;43)	38 (35;40)	0.13	38 (35;40)	41 (38;44)	0.11
cPP (mmHg)				47 (43;51)	52 (49;56)	0.07
Biochemical variables:						
TC (mmol/l)	4.6 (4.3;4.9)	4.3 (4.1;4.6)	0.25	4.1 (3.8;4.3)	5.0 (4.7;5.4)	< 0.001
HDL-C (mmol/l)	1.4 (1.3;1.5)	1.1 (1.0;1.3)	0.003	1.2 (1.1;1.3)	1.3 (1.2;1.4)	0.18
LDL-C (mmol/l)	2.6 (2.4;2.8)	2.7 (2.5;2.9)	0.59	2.5 (2.3;2.7)	3.2 (2.9;3.5)	< 0.001
TG (mmol/l)	1.3 (1.1;1.5)	1.2 (1.1;1.3)	0.41	1.2 (1.0;1.4)	1.8 (1.3;2.2)	0.034
TC:HDL-C ratio	3.7 (3.3;4.1)	4.2 (3.9;4.6)	0.043	3.9 (3.5;4.3)	4.5 (3.9;5.0)	0.10
TG:HDL-C ratio	1.2 (0.9;1.5)	1.2 (1.0;1.4)	0.77	1.3 (0.9;1.8)	1.6 (1.1;2.1)	0.37
hsCRP (mg/l)	2.8 (-0.6;1.5)	2.2 (-0.5;1.7)	0.40	3.3 (-0.4;1.6)	4.1 (-0.4;1.7)	0.39
HbA1c (%)	5.5 (5.4;5.6)	5.4 (5.3;5.5)	0.17	6.0 (5.7;6.2)	5.5 (5.4;5.6)	0.001
Glucose (mmol/l)	4.7 (4.5;4.9)	4.7 (4.5;4.8)	0.65	4.9 (4.5;5.3)	4.9 (4.7;5.0)	0.96
CrCl (ml/min)	93.6 (83.2;104)	78.6 (69.9;87.3)	0.031	115 (105;124)	105 (96.1;113)	0.13
CD4 count (cells/mm ³)	518 (2.4;3.1)	234 (1.9;2.8)	< 0.001	285 (2.0;2.8)	344 (2.2;2.9)	0.07
Lifestyle variables:						
Alcohol use, <i>n</i> / <i>total</i> (%)	25/71 (35.2)	20/66 (30.3)	0.54	23/65 (35.4)	18/62 (29.0)	0.44
Tobacco use, <i>n</i> / <i>total</i> (%)	34/71 (47.9)	36/66 (54.6)	0.44	24/64 (37.5)	19/63 (30.2)	0.38

WC, waist circumference; BMI, body mass index; bSBP, brachial systolic blood pressure; bDBP, brachial diastolic blood pressure; bPP, brachial pulse pressure; cPP, central pulse pressure; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; hsCRP, high-sensitivity C-reactive protein; HbA1c, glycated haemoglobin; CrCl, creatinine clearance. Data are expressed as arithmetic mean with 95% confidence intervals or % of *n*. hsCRP and CD4 count data are expressed as geometric mean (5th and 95th percentile intervals). *P*-values for comparison between never treated and treated groups were obtained with independent *t*-tests and for categorical variables with Chi-square tests. *P*-values ≤0.05 are regarded as significant.

Table 3 shows the prevalence of hypertension and the use of anti-hypertensive and antiretroviral treatment of the HIV-infected never treated and treated groups compared.

Table 3 Prevalence in hypertension at follow-up and medication use in HIV-infected participants

Variable	Never treated (n = 71)	Treated (n = 66)	p-value
Hypertension, n (%)	29 (41)	20 (30)	0.28
Anti-hypertensive medication use, n (%)	11 (16)	9 (14)	0.76
Duration of antiretroviral treatment (years)			
4-5 years, n (%)	---	16 (24)	---
2-4 years, n (%)	---	29 (44)	---
<2 years, n (%)	---	21 (32)	---

Data are expressed as % of n. The *p*-values between groups were obtained with Chi-square tests. *P*-values ≤ 0.05 are regarded as significant.

In Table 4, change in the cardiometabolic profile within and percentage change between the never treated and treated HIV-infected participants (over a period of 5 years) is shown. Waist circumference within the treated group ($p=0.06$) and between the groups ($p=0.08$) tended to be higher with treatment. Although no changes were seen in bSBP within either of the groups, a difference ($p=0.029$) in percentage change was seen in bSBP between the groups where treated participants showed an increase and never treated participants a decrease over the 5-year period. Although PP tended to decrease ($p=0.06$) within the never treated group, these levels increased significantly ($p=0.030$) within the treated group, while the percentage change in PP was higher ($p=0.004$) in the treated group compared to the never treated group. HbA1c levels increased ($p<0.001$) within the never treated group. No differences were seen in the lipid ratios within or between the groups. The increase in percentage change in HbA1c was lower in the treated participants. CD4 cell count changed for the better ($p=0.012$) within the treated group over 5 years, and the percentage change in the latter group was also higher ($p=0.009$).

Table 4 Change and percentage change in the cardiometabolic profile of never treated and treated HIV-infected participants over 5 years (2005-2010)

Variable	Change within groups				Percentage change between groups		
	Never treated (n = 71)	P-value within never treated	Treated (n = 66)	P-value within treated	Never treated (n = 71)	Treated (n = 66)	P-value between groups
Anthropometric variables:							
WC (cm)	-0.4 (-2.0;1.1)	0.59	2.1 (-0.1;4.3)	0.06	-0.1 (-1.8;1.6)	4.2 (-0.4;8.8)	0.08
BMI (kg/m ²)	-0.2 (-1.0;0.7)	0.70	0.4 (-0.4;1.2)	0.28	0.3 (-2.7;3.3)	2.4 (-0.9;5.7)	0.34
Cardiovascular variables:							
bSBP (mmHg)	-3.1 (-7.0;0.7)	0.11	3.9 (-1.8;9.6)	0.18	-1.6 (-4.6;1.4)	4.5 (-0.3;9.3)	0.029
bDBP (mmHg)	-0.9 (-3.8;2.1)	0.57	0.5 (-3.2;4.2)	0.79	0.4 (-3.2;4.0)	2.1 (-2.4;6.5)	0.57
PP (mmHg)	-2.3 (-4.6;0.1)	0.06	3.5 (0.3;6.6)	0.030	-2.4 (-8.5;3.7)	13.3 (4.3;22.3)	0.004
Biochemical variables:							
TC:HDL-C ratio	0.2 (-0.1;0.5)	0.11	0.2 (-0.3;0.7)	0.34	10.8 (3.2;18.4)	7.8 (-2.8;18.4)	0.65
TG:HDL-C ratio	0.2 (-0.1;0.5)	0.26	0.4 (-0.0;0.9)	0.08	40.8 (9.3;72.4)	35.8 (7.1;64.5)	0.82
HbA1c (%)	0.4 (0.2;0.6)	< 0.001	0.1 (-0.0;0.2)	0.19	7.6 (3.7;11.6)	2.0 (-0.5;4.4)	0.016
CD4 count (cells/mm ³)	-0.1 (-0.3;0.1)	0.003	1.6 (-0.3;0.7)	0.012	-4.9 (-10.2;2.0)	9.2 (-11.4;34.5)	0.009

WC, waist circumference; BMI, body mass index; bSBP, brachial systolic blood pressure; bDBP, brachial diastolic blood pressure; PP, pulse pressure; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; HbA1c, glycated haemoglobin. Data are expressed as arithmetic mean with 95% confidence intervals. CD4 count data are expressed as geometric mean (5th and 95th percentile intervals). The *p*-values for change within groups were obtained with dependent *t*-tests and percentage change between groups was obtained with independent *t*-tests. *P*-values ≤0.05 are regarded as significant.

Figure 2 illustrates the difference in percentage change (over a period of 5 years) in PP, after adjustment for gender, age and BMI at follow-up, between the never treated and treated HIV-infected groups. Treated HIV-infected participants showed a significantly higher ($p=0.009$) percentage change in PP over 5 years than the never treated group.

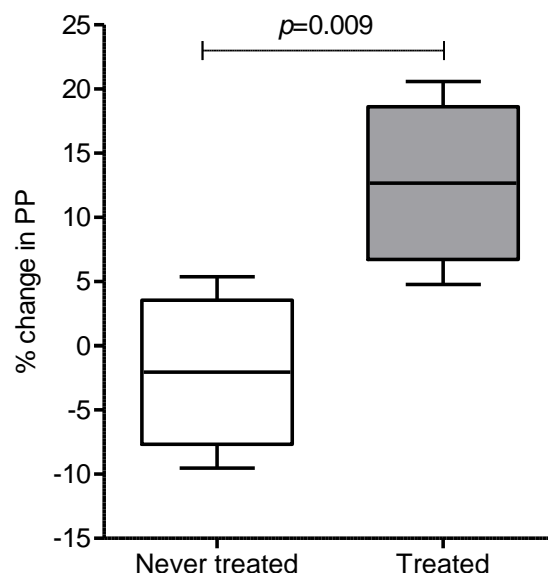


Fig. 2 Difference in percentage change in pulse pressure (PP) between never treated and treated HIV-infected participants over 5 years. P -values between groups were obtained with ANCOVA. Adjustments were made for gender, age and body mass index. P -value ≤ 0.05 are regarded as significant.

The central box encloses the standard error of mean, the horizontal line inside the box represents the mean; vertical lines (whiskers) represents 95% confidence intervals.

Table 5 indicates the differences in markers of arterial structure and function between never treated and treated groups at follow-up. From this table it is clear that no differences were found between the two groups.

Table 5 Difference in vascular structure and function between never treated and treated HIV-infected participants at follow-up

Variable	Never treated ($n = 71$)	Treated ($n = 66$)	p -value
cSBP (mmHg)	131 (126;135)	135 (130;140)	0.23
cdPWV (m/s)	8.41 (8.09;8.73)	8.29 (7.94;8.63)	0.61
Alx (%)	89.2 (86.2;92.2)	91.6 (88.5;94.7)	0.28
IMT (mm)	0.62 (0.59;0.64)	0.62 (0.59;0.65)	0.90

cSBP, central systolic blood pressure; cdPWV, carotid dorsalis pulse wave velocity; Alx, augmentation index; IMT, carotid intima-media thickness. Data are expressed as arithmetic mean with 95% confidence. P -values between groups were obtained with ANCOVA. Adjustments were made for gender, age and body mass index for all variables and additionally for mean arterial pressure (in the case of cdPWV and IMT), heart rate (in the case of cdPWV and Alx), as well as height (instead of body mass index) in the case of Alx. P -values ≤ 0.05 are regarded as significant.

A multiple regression analysis was performed within the total participant group (treated and never treated) with percentage change in PP as dependent variable (Table 6). The use of ART was significantly associated with an increase in the 5-year percentage change in PP ($p=0.020$).

Table 6 Forward stepwise regression analysis with 5-year percentage change in pulse pressure in the total HIV-infected group ($n=127$)

Variable	Percentage change in pulse pressure	
	$R^2 = 0.24$	p -value
Antiretroviral treatment (no/yes)	$\beta = 0.19$	0.020
Age (years)	$\beta = 0.20$	0.018
Baseline PP (mmHg)	$\beta = -0.47$	< 0.001

Independent variables included in the model: antiretroviral treatment, baseline pulse pressure (PP), as well as age, gender, body mass index, total cholesterol/high-density lipoprotein cholesterol ratio, alcohol use and tobacco use at follow-up. P -value ≤ 0.05 are regarded as significant.

Discussion

The most prominent finding of this 5-year prospective study is the larger percentage change in PP found in the treated HIV-infected group, compared to their never treated counterparts. This was accompanied by a worse lipid profile, as well as a tended increase in abdominal fat accumulation.

A significant percentage increase in PP occurred only within the treated group over the 5-year period, resulting in the much higher percentage change (13%) in PP in the treated participants. ART is known to play a role in the development of atherosclerosis by inducing early vascular injury and thereby initiating atherogenesis [29]. In this study ART was significantly and independently associated with an increase in central arterial stiffness, as represented by the increase in PP. The latter was further underlined by the presence of an increase in SBP, as well as the absence of a decrease in DBP that were found in the treated group.

PP is described to be both a consequence and a cause of atherosclerosis [7] as well as to be a risk factor for cardiovascular disease and -mortality [30]. Arterial wall properties and the episodic nature of cardiac contraction are both known to be determinants of PP [7]. An increase in arterial stiffness could thus be the cause of an elevation in PP levels [7]. On the other hand, an elevation in PP could accelerate arterial stiffening and increase arterial wall stress [6]. Elevated PP causes mechanical fatigue and vascular endothelial damage, leading to the development of atherosclerosis and resulting in an increase in arterial stiffness [7]. Alternatively, an increase in PP levels could double the stretch stimulus in the arterial wall, resulting in an increase of angiotensin II production, which is known to have vasoconstrictive properties [31]. The result of such an increase in arterial stiffness is an elevation in central wave reflection, again leading to an increase in PP levels. Arterial stiffness is also known to be associated with vascular aging [31] and studies have established HIV-infection to be associated with an increase in vascular age [32,33] of up to 12 years older than chronological age [34]. Furthermore, ART is known to play a role in the development of atherosclerosis by inducing early vascular injury [29].

In light of the above, we expected to observe a detrimental vascular profile in the treated group. However, no difference in known markers of vascular function (cSBP, cdPWV and A1x) or structure (IMT) was observed during follow-up between those who used treatment and those who were never treated. Nonetheless, ART was associated with percentage change in PP in the total HIV-infected group, underlining the inevitable effect of ART on changes in the vascular function in the treated participants. Thus it seems that the treatment leads to early changes in the vascular wall, which might in turn lead to future vascular aging and/or accelerated

atherosclerosis and future cardiovascular events. It is speculated that more significant changes will be seen in subsequent follow-up studies in these participants.

Although no differences were seen in blood pressure at either baseline or follow-up between our groups, the treated participants showed a higher percentage change in bSBP over 5 years than their never treated counterparts. In a previous study a lower blood pressure in the HIV-infected, compared to the uninfected participants was found in the North-West province [20], which was in agreement with a study in KwaZulu-Natal, South Africa, where HIV-infected, never treated individuals had lower SBP levels (of 3.5 mmHg) than HIV-uninfected participants [35]. Treatment (also with NNRTIs) was, however, associated with an increase in blood pressure of 3.2/2.7 mmHg [11], which seems to be in accordance with the findings of this study.

Even though no differences in lipid ratios were seen between the groups, TC, TG and LDL-C levels were significantly higher in the treated group in 2010, which was not the case in 2005 when participants only started receiving treatment. The worse lipid profile of our treated group was in concert with several other studies, which reported ART regimens, including Nevirapine and Efavirenz, to be associated with a normalisation in HDL-C levels, together with an increase in TC, TG and LDL-C levels [13,36,37]. The latter is in accordance with our findings. HDL-C levels were however lower than the prescribed protective levels of 1.55 mmol/l (60 mg/dl) against the development of coronary heart disease [38] and TG levels of the treated group reached borderline high levels [39]. These findings could contribute to the development of atherosclerosis [40] and therefore increase the risk for cardiovascular disease.

Furthermore, LDL-C levels were above optimal (thus >2.59 mmol/l or 100 mg/dl) [40] at baseline and at follow-up. The oxidation of LDL-C in the vascular wall provides it with pro-inflammatory and atherogenic properties [41]. Thus, LDL-C plays a distinct role in the development of atherosclerosis [41]. It should be mentioned that controversy still exists over the cut-off values for lipid variables across different ethnic groups [42].

Previous studies have reported the presence of lipodystrophy in HIV-infected individuals receiving ART, which included some of the regimens that were used in this study (e.g. Stavudine, Lamivudine and Efavirenz) [43,44]. Likewise, we found that the waist circumference of the treated participants increased borderline significantly ($p=0.06$) over 5 years, while this was not seen in the never treated group. In both groups, however, BMI levels remained unchanged. This increase in waist circumference is an indication of abdominal fat accumulation and is associated with higher cardiovascular risk [45].

The strength of this study is that data on the cardiometabolic profile of the treated HIV-infected African population, are extremely limited. In fact, to the best of the authors' knowledge, this is

the first study to report associations of central arterial stiffness and ART in HIV-infected black South Africans. This study further showed a 71% successful follow-up rate. Except for the fact that the followed-up group consisted of less male participants and had lower SBP and PP levels than the lost to follow-up group, no further differences were seen between these groups at baseline, highlighting the comparability of our followed-up participants to those who were lost to follow-up.

Regarding limitations, the participants in this study were treated for a maximum of 5 years, and it might be that the effects of ART on the cardiometabolic profile of these participants could become clearer, should they be exposed to treatment or longer. For this study we used the brachial PP as it was the only PP measurement that was measured during both baseline and follow-up. However, the calculated central PP was borderline higher ($p=0.07$, Table 2) in the treated group compared to the never treated group, supporting our brachial PP results. Finally, the use of carotid-femoral PWV is proposed to be the most validated technique to measure arterial stiffness [46], but in this study, carotid-radialis PWV was measured, as well as carotid-dorsalis PWV only at follow-up, which could explain the lack of differences seen in arterial stiffness between our groups. Even though all apparatus for the measurement of lipid values are calibrated as prescribed, apparatus used for these measurements in 2005 differed from those used in 2010. Therefore we decided to only use lipid ratios (and not TC, LDL-C, HDL-C and TG values) in the analysis of change in the lipid profile over five years, both within each group and between the groups.

In conclusion, an increase in large arterial stiffness (as indicated by change in PP) was significantly and independently associated with the use of ART in Africans infected with HIV-1. Although no functional (PWV) or structural (IMT) arterial differences were seen after 5 years between treated and never treated groups, the greater percentage change in PP might be an early indication of the gradual development of arterial stiffness in those receiving treatment. Lastly, the cardiometabolic results we observed are in agreement with previous research and do not seem to be influenced by either ethnicity or HIV-1 subtype differences.

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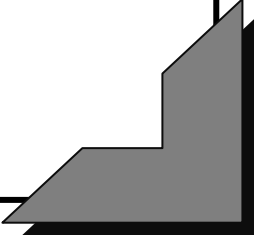
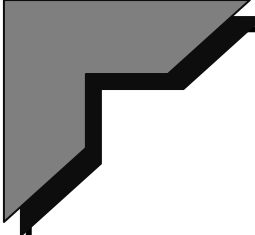
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CHAPTER 4

Concluding remarks and findings

4.1 Introduction

In this chapter, the main findings are summarised, compared to the relevant literature, discussed and concluded in order to reveal the effects of antiretroviral treatment (ART) on the cardiovascular profile of HIV-1 (most likely subtype C) infected South Africans. Recommendations to future researchers will also be included.

4.2 Summary of main findings

The main focus of this study was to determine the associations between ART use and the cardiometabolic profile by comparing 66 treated to 71 never treated participants. We observed an increase in pulse pressure (PP) levels over a period of 5 years, only in the treated group. This was accompanied by a significantly higher percentage change in PP and brachial systolic blood pressure among the treated, compared to the never treated participants. The first hypothesis (as presented in Chapter 1, Page 6) is therefore accepted, namely that ART seems to worsen the cardiovascular profile of HIV-infected South Africans, compared to those that were never treated.

Even though no differences in lipid ratios were seen between the groups, TC, TG and LDL-C levels were significantly higher in the treated group in 2010, which was not the case in 2005 when participants only started receiving treatment. The treated group further tended to show an increase in waist circumference over 5 years, accompanied by a tendency to have a higher percentage change in waist circumference than the never treated group. However, body mass index levels remained unchanged. Abdominal fat accumulation thus seemed to occur within the treated participants, which could lead to an increase in cardiovascular risk and further deterioration of the cardiovascular system over time. The second hypothesis were therefore accepted.

4.3 Comparison to relevant literature

Research regarding any association between ART and cardiometabolic variables, especially among the African population of South Africa, is extremely limited, which makes this a very unique study. Our study is similar to a previous study in a Caucasian population, which found blood pressure levels to increase by up to 3.2/2.7 mmHg in the presence of ART [1]. In South African, a high prevalence of hypertension occurs [2]. Still, the mean systolic and diastolic blood

pressure levels of both the groups in our study were in the normotensive range, which could be due to HIV-infection itself [3] and/or the type of ART regimen used [1].

Furthermore, and in concert with our findings, higher total cholesterol, LDL-C and triglyceride levels are seen with ART use [4-6]. Regarding the suggested lipid cut-off values, LDL-C levels in our treated group were above optimal (thus >2.59 mmol/l or 100 mg/dl) [7], while HDL-C levels were lower than the prescribed protective levels of 1.55 mmol/l (60 mg/dl) against the development of coronary heart disease [8]. It should be mentioned that controversy still exists over the cut-off values for anthropometric lipid variables across different ethnic groups [9]. Unexpectedly, we found glycated haemoglobin (HbA1c) levels to be lower in the treated HIV-infected group. It should however be noted that the lifespan of erythrocytes, and therefore HbA1c levels, could be affected by some ART regimens [10], thereby questioning the reliability of HbA1c data in treated HIV-infected groups.

Finally, in our study, we came across a tended increase in abdominal fat accumulation within the treated group. The latter is in accordance with previous studies which have reported the presence of lipodystrophy in treated HIV-infected persons [11,12], which could have adverse cardiovascular consequences [13]. It should be noted that the findings from our study are in agreement with previous research and do not seem to be influenced by our unique ethnic group or HIV-1 subtype differences.

4.4 Discussion of main findings

ART is known to have both beneficial and detrimental effects. In this study we therefore focused on determining if ART exerts any effects on the cardiometabolic profile of HIV-infected participants. An increase in PP levels, as was found among the treated participants in our study, is associated with accelerated arterial stiffening and increased arterial wall stress [14], as well as early vascular injury [15], early vascular aging [16] and the possible development of atherosclerosis [15] in other studies. Further, higher PP levels have been proposed to be responsible for an increase in oxidised LDL-C levels [17]. Oxidised LDL-C, in turn, could contribute to vascular inflammation [18] and aortic stiffening [19], while a low HDL-C level could cause a lowering in protection against atherosclerosis [20]. In our study, even though we did not measure oxidised LDL-C, we did come across an increase in PP levels during the 5-year period, as well as higher TC, LDL-C and TG levels in the treated group in 2010.

Triglyceride levels, a marker of non-LDL-C atherogenic lipoproteins [21], further reached borderline high levels which, because of its atherogenic characteristics, might have contributed to the central arterial stiffness that we found among the treated HIV-infected participants.

On the other hand, the CD4 cell count in the treated group proved to be borderline higher and to have increased considerably more over the 5-year period than in the never treated group. Even so, these participants still had CD4 cell counts lower than the most recent prescribed level (of ≤ 350 cells/mm³) at which treatment should be initiated. Higher oxidised LDL-C levels could probably further play a role in the higher PP levels [17] that we found among the treated participants, as oxidised LDL-C is yet another factor that could play an atherogenic role [18], leading to an increase in arterial stiffness. However, this scenario remains speculative as we did not measure oxidised LDL-C levels.

In the light of the above-mentioned, we expected to see a change in vascular structure or function among the treated HIV-infected participants, but to our surprise, this was not the case. Thus, how the increase in PP that were found in the presence of ART in our study will influence the above, remain unclear. The black South African population is historically not prone to develop coronary artery disease [22], which might have influenced our findings.

Additionally, as was described by the multiple regression analysis, no lipid variables contributed in the treated group. ART did however show a strong correlation with percentage change in PP, indicating that ART does have some kind of influence on the PP levels in the HIV-infected participants. It should be kept in mind that the majority (44%) of the treated participants received treatment for a period of 2-4 years. It might be that the effects of ART on the cardiometabolic profile of these participants could become clearer, should they be exposed to treatment or longer.

As was mentioned, both groups had mean systolic and diastolic blood pressure levels within the normotensive range; however, we found 32% of the treated and 41% of the never treated group to be hypertensive. In addition, 14% of the treated and 16% of the never treated participants reported the use of anti-hypertensive medication. Nonetheless, no significant differences in either hypertensive status or anti-hypertensive medication use were found between the two groups. These findings could possibly be explained by the lowering blood pressure effect of HIV infection.

Finally it should be mentioned that the deceased participant group (as was showed in Table 1) consisted of the highest triglyceride/high-density lipoprotein cholesterol and high-sensitivity C-

reactive protein levels at baseline, which might implicate the detrimental role of HIV infection among this population.

4.5 Conclusion

Treatment with antiretroviral regimens is associated with an increase in PP and an increase in systolic blood pressure levels over 5 years. Additionally, during follow-up, higher total cholesterol, LDL-C and triglyceride levels were seen in HIV-infected South Africans. This, however, is not accompanied by structural (IMT) or functional (PWV) changes in the vasculature and no atherosclerosis or end organ damage was observed. Nonetheless, the higher PP levels could be an indication of early vascular changes and/or the development of atherosclerosis, or a higher risk for future cardiometabolic diseases, but future research is needed in order to establish this hypothesis.

4.6 Chance and Confounding

It is of importance to critically reflect on some of the factors that may have confounded the results of this study.

After 2005, participants started to receive treatment over the period of 5 years as their CD4 cell count declined below 200 cells/mm³. Although the duration of treatment is shown in Table 3, we were not able to determine the exact duration (months) of each participant's treatment. Furthermore, after baseline only a limited number of participants visited the local clinic for the determination of their CD4 cell counts (after they were informed of a positive HIV status); hence we have a small amount of CD4 cell count data available at baseline. Even though this study had an appropriate follow-up rate of 71%, it should be kept in mind that a large number of these participants were deceased.

Some methodological issues could have confounded the results. Although the gold standard measurement for PWV includes the measurement of the carotid-femoralis PWV, we only measured carotid-radialis and carotid-dorsalis PWV. Central SBP, carotid-dorsalis PWV, augmentation index and carotid IMT were only determined during the follow-up study. In addition, no inflammatory or endothelial dysfunction markers were measured, except for CRP, which did not show any significant results. Finally we did not test for any opportunistic infections (this was only reported on questionnaires) and did not elaborate on the causes of mortality of those deceased. These non-measured factors might thus have weakened the study, however,

this study is of a prospective nature and scheduled follow-up studies will be able to incorporate these factors. Statistical results were investigated from a physiological perspective and statistical significance does not necessarily indicate physiological significance.

4.7 Recommendations

It is recommended for future studies that:

- Studies should be conducted where all treated participants receive treatment for a longer period than 5 years. Even though 44% of the participants received ART for 2-4 years, only 24% received treatment for 4-5 years, while a total of 32% received ART for a period of less than 2 years.
- More variables, such as inflammatory markers, adhesion molecules and oxidised LDL-C, could be analysed in order to better establish the association between inflammation, ART, vascular changes and blood pressure levels.
- Gold standard pulse wave velocity measurements, such as carotid-femoralis pulse wave velocity, should be measured at both baseline and follow-up, as only carotid-radialis and carotid-dorsalis pulse wave velocity data were available for this study.

4.8 Final remarks

Even though the incidence of HIV is significantly declining in South Africa [23], this country is well known for the largest HIV epidemic globally, which is estimated to be 5.6 million people living with HIV [23]. This epidemic, as well as the treatment requirement thereof, is accompanied by a high prevalence of cardiovascular disease [24]. The latter could increase the already high burden of hypertension and cardiovascular events on the South African population.

On 1 April, 2004, free-of-charge treatment rollout programmes were initiated in four of the nine provinces for the first time [25]. In the meantime, ART access in poor countries increased [25] and in 2009, 37% of sub-Saharan Africans who were eligible for treatment, received ART [23].

Apart from the increase in cardiometabolic risk associated with ART among South Africans (as was clearly shown in our study), the higher demand than capacity for ART further places an enormous burden on the economy of South Africa, as government funds are required to improve health services and treatment programmes. Treatment could assist in the decrease of HIV viral load and disease progression, HIV transmission rate and mortality [26,27], but has adverse cardiovascular effects. Thus, prevention of HIV infection still remains the best solution.

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Twenty years from now you will be
more disappointed by the things
that you didn't do than by the ones
you did do.

So throw off the bowlines.

Sail away from the safe harbour.

Catch the trade winds in your sails.

Explore. Dream. Discover.

-Mark Twain-

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