# Inaugural Lecture

# HIV & cardiovascular risk in South Africans

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**HIV & cardiovascular risk in South Africans**

**Outline of lecture**



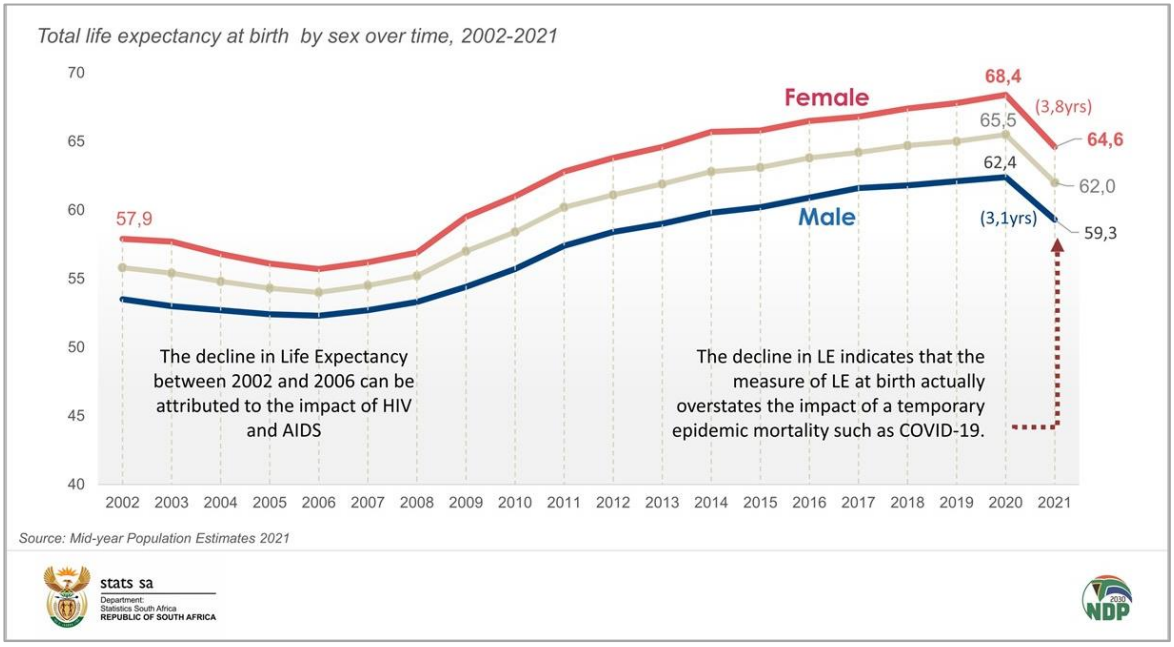
## 1. Introduction

The Covid-19 pandemic has changed our world and as we are confronted with the way the pandemic affected the health and life of people globally, we are easily overwhelmed. How are we ever going to overcome this enormous challenge? It reminds me of years back when we started the PURE (Prospective Urban and Rural Epidemiology) study. The prospect of a 10-20 year longitudinal study loomed before us and led to silence during that very first meeting, it was an elephant in the room. Then the late Prof Esté Vorster used the saying “hoe eet mens ‘n olifant – een happie op ‘n slag!”

That is research – one step at a time or one day at a time. It is like building a huge puzzle – you sort the pieces, pay attention to detail – the shapes and patterns and with time you gain knowledge and realise you overcame this huge elephant that loomed ahead.

As we currently see with the coronavirus, the human immunodeficiency virus (HIV) had a devastating effect on the health of those infected and the life expectancy dropped to 52 years in 2005, before antiretroviral treatment became available. Although the coronavirus pandemic did influence the life expectancy of South Africans, the current life expectancy is estimated at 64 years as seen in the figure from the Department of Statistics, South Africa (SA), indicating the projected

decline in life expectancy which is due to the rise in excessive deaths during the Covid-19 pandemic.[1]




Unlike in the case of the coronavirus, after years of intensive research, there is still no vaccine available for the human immunodeficiency virus. During a recent International AIDS Society conference, Prof Lynn Morris of the University of the Witwatersrand, said that if HIV vaccine research had the sort of funding devoted to COVID-19, it might have been a different story.[2] However, we should remember that the human immunodeficiency virus, is a far bigger challenge for a vaccine than a more typical virus such as the coronavirus. Whereas the natural immune reaction to the coronavirus (SARS-CoV-2) sooner or later clears the virus from most people's bodies, HIV is not cleared. As a retrovirus, HIV, instead of being eliminated, disappears by integrating into the body's own cells' nuclear DNA, where it is invisible to the immune system.[2] The human immunodeficiency virus is also far more variable and mutable than the corona virus, and can develop resistance both to drugs and to antibodies more easily.[2]

## 2. Background

### *2.1. HIV in South Africa*

The human immunodeficiency virus is a global health epidemic and in SA an estimated 8,2 million people are living with HIV in 2021, which is the highest number globally, with an HIV prevalence rate of approximately 19,5% in South Africans (15-49 years) [3]. There is no cure for HIV infection. However, of the 38 million people living with HIV worldwide, 25,4 million people are now on treatment. That means 12,6 million people are still waiting,[4] but with increasing access to effective

treatment and care, including for opportunistic infections, HIV infection has become a manageable chronic health condition, enabling people living with HIV to live a long and healthy life.[5]

### *2.2. HIV and cardiovascular disease*

As the life expectancy of people living with the human immunodeficiency virus (PLHIV) increased, the aetiology of mortality in sub-Saharan Africa (SSA) has shifted to a combination of communicable and non-communicable diseases, such as cardiovascular disease (CVD).[6] A proceedings report of the Academy of Science of South Africa (2020) indicated that about 69% of the South African population older than 40 years are battling multimorbidity, which is defined as two or more simultaneous existing chronic diseases.[7] Diseases such as hypertension,[8] metabolic syndrome (constellation of risk factors for CVD),[9] dyslipidaemia,[10] chronic low-grade inflammation as seen in HIV, diabetes mellitus[11] as well as factors related to an unhealthy lifestyle,[12] all contribute to multimorbidity. In a Western world context, in Washington DC, Levy et al., indicated the health burden of metabolic comorbidities or multimorbidity, and emphasized the evolving health care needs of people ageing with HIV.[13] In the paper the authors stressed that the epidemiology of metabolic comorbidities leading to CVD in HIV-infected populations, need to be an important public health priority.[13] In the figure from Levy et al., 2017, bar graphs indicate the prevalence of metabolic comorbidities among all HIV patients in the DC Cohort, by sex at birth, age group, and race/ethnicity.[13]

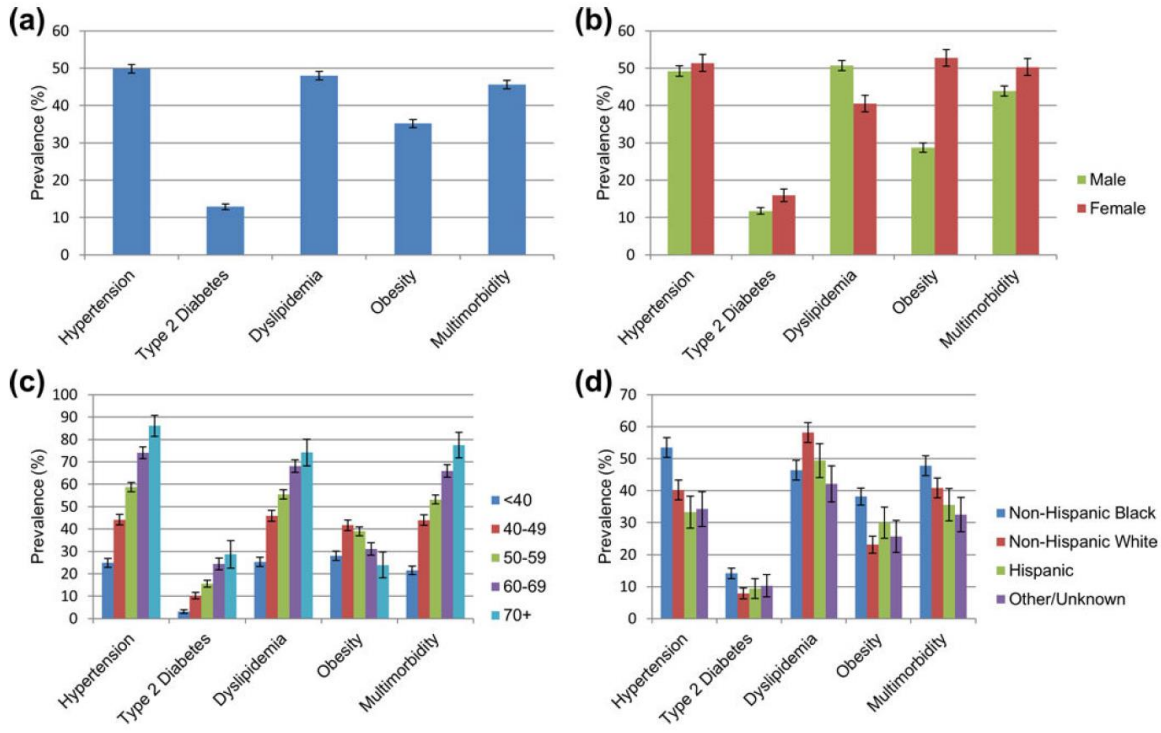



**Figure 1.**
Bar graphs of the prevalence of metabolic comorbidities (a) among all HIV patients in the DC Cohort and by (b) sex at birth, (c) age group, and (d) race/ethnicity, 1/1/2011-6/30/2015 (n=7018).

In light of the above, it is clear that the Covid-19 pandemic, the high prevalence of PLHIV and the greater CVD risk seen in SSA, place additional strain on the public health care system of South Africa, threatening the future well-being and health of people residing in this region.

Why are PLHIV more prone to develop co-morbidities and CVD?

In a review article, addressing HIV-related cardiovascular diseases, and focussing on cardiovascular physiology and pathophysiology, Dominick et al., highlighted that persistent immune activation is seen in PLWHIV despite effective antiretroviral therapy (ART) and maintained viral suppression.[14] This immune activation or low-grade inflammation seen in PLWHIV play a role in multimorbidity and the development of CVD.

Dominick et al., generated a hypothesis that includes the identification of the core pathways mediating CVD onset that may assist future research efforts aimed at elucidating underlying mechanisms.[14] The effects of lifestyle risk factors, ART, and low-grade immune activation show a proinflammatory state, leading to endothelial dysfunction and CVD in the figure, taken from the publication.[14] Vascular endothelial dysfunction plays a significant role in CVD onset, also in PLHIV.

Prolonged exposure to the virus, as well as viral protein toxicity, are associated with vascular injury and immune activation, which are often promoted by the treatment.[15]

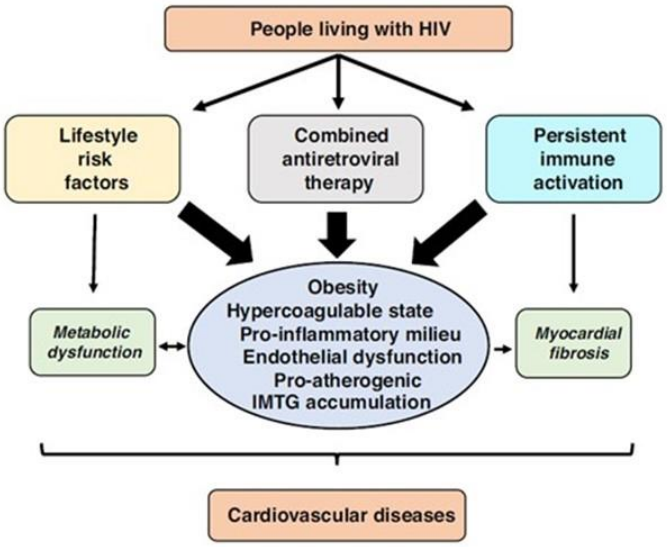



Fig. 2. Human immunodeficiency virus (HIV)-related cardiovascular disease onset, a unifying concept. IMTG, intramyocardial triglycerides.

Dominick et al.

*AJP-Heart Circ Physiol* • doi:10.1152/ajpheart.00549.2019 • www.ajpheart.org


According to Dominick et al., their review focused on the SSA context, but they also recognised that not much is known about the prevalence of CVD risk factors in PLHIV in SSA, despite the region being the epicentre of the global HIV epidemic.[14] Indeed, HIV-mediated cardiovascular disease still remains poorly understood, especially in a SSA context. Most of the research was done in the Western world where besides, ethnic, cultural and socio-economic differences, HIV-1 subtype B prevails, while HIV-1 subtype C is more prevalent in South Africa. The lack of knowledge with regards to cardiovascular risk and HIV in South Africa prompt us, at HART, to investigate the CVD risk in the HIV infected PURE participants and later lead to the North-West University (NWU) leg of EndoAfrica study[16].

### 3. HART research: HIV and cardiovascular risk

The Hypertension in Africa Research Team (HART) of the NWU added some pieces to the CVD risk in PLHIV in SA puzzle, thus to the body of knowledge, through the EndoAfrica and PURE studies. The EndoAfrica study, titled “Vascular endothelial dysfunction: The putative interface of emerging cardiovascular risk factors affecting populations living with and without HIV in Sub-Saharan Africa” commenced in the Western Cape in 2015. The North West leg of the EndoAfrica study, conducted by HART, consist of a baseline and an 18-month follow-up data collection phase which was completed December 2019. The NWU-EndoAfrica study included 278 HIV infected and 104 HIV free participants. The South African leg of the international PURE study, which was

performed in the North West province, tracked changes in lifestyles, CVD risk factors and chronic diseases over a period of 10 years (2005-2015). During the baseline data collection phase in 2005, 322 of the 2010 participants were identified as being HIV infected and we followed them for 10 years.

Thus, since starting my PhD, our research focussed on blood pressure measures and hypertension prevalence, metabolic syndrome and the lipid profile, chronic low-grade inflammation, as well as factors related to an unhealthy lifestyle, among PLHIV which might increase their cardiovascular risk.

### *3.1. HIV and blood pressure*

Hypertension is common in HIV-infected adults and this is attributed to a combination of traditional risk factors, HIV-specific factors, and ART. Knowledge of the mechanisms of hypertension in HIV-infected adults will be critical to public health efforts to prevent hypertension, cardiovascular disease, and premature mortality in HIV-infected adults.[17] The latter is especially important in SA where more than 1 in 3 adults live with high blood pressure (BP).[8] In a review article, focusing on SSA populations, reporting on BP estimates in treated HIV-infected patients against untreated and HIV-uninfected controls, we found that the majority of studies reported treated HIV-infected patients to present with lower BP and hypertension estimates compared to untreated patients and uninfected controls.[18] The figure below, taken from the publication by Phalane et al., 2020, indicate these findings. [18]

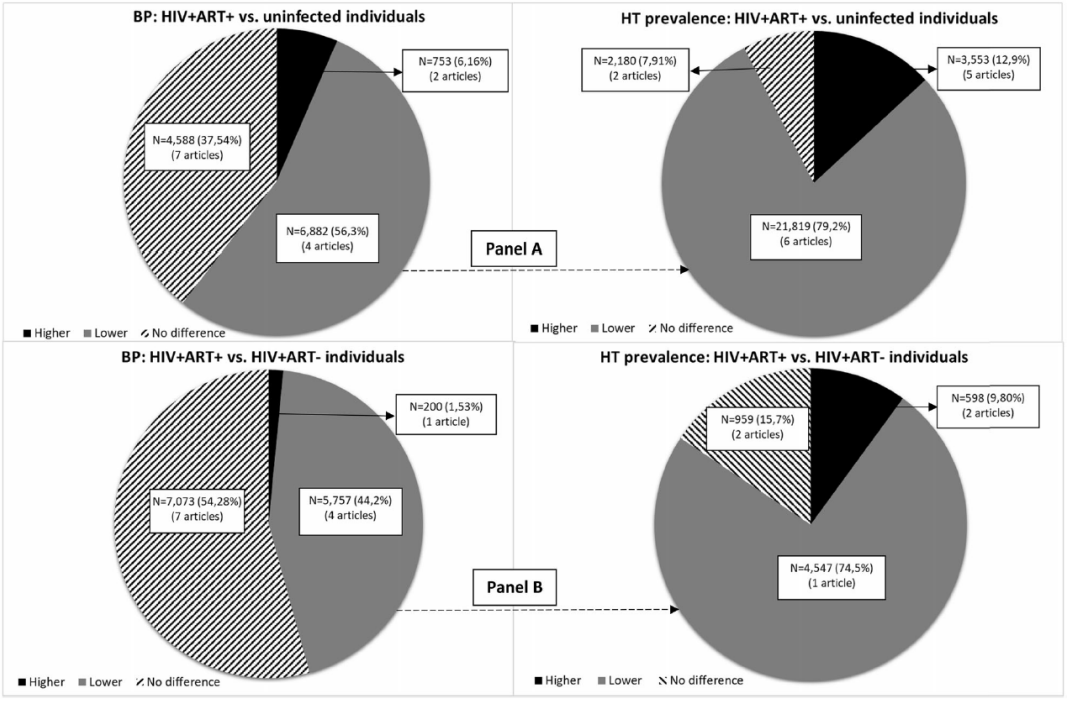



**Fig. 2 Do HIV-infected patients using ART have higher blood pressure (or prevalence of hypertension) than uninfected controls and untreated HIV-infected patients? a** Comparison of treated HIV-infected vs. uninfected patients; **b**: comparison of treated HIV infected vs. untreated HIV-infected patients. The number of eligible patients (and total number [*N*] of participants in these studies) indicating higher blood pressures/prevalence of hypertension (yes), lower blood pressure/prevalence of hypertension (no) or no difference in blood pressure/prevalence of hypertension between HIV-infected patients using ART compared uninfected controls and untreated HIV-infected individuals. N number; BP blood pressure; HT (%) hypertension prevalence; HIV human immunodeficiency virus; ART antiretroviral therapy; $HIV^-$ HIV-uninfected; $HIV^+ART^+$ HIV-infected individuals taking antiretroviral therapy, $HIV^+ART^-$, HIV-infected individuals not taking antiretroviral therapy.

We reported lower systolic blood pressure (SBP) levels in the untreated HIV infected PURE participants compared to their HIV free controls.[19,20,21] HART contributed to, and two HART researchers (Carla M Fourie and Aletta E Schutte) were co-authors of a systemic review and meta-analysis of cardiometabolic traits in SSA, reporting that HIV infection was associated with lower SBP, while treatment was associated with higher lipid levels.[22]

In our article reporting the 10-year follow-up of the cardiometabolic factors of the HIV infected PURE participants, we indicated that although South African's ART roll-out programme was implemented in April 2004,[23] the 320 HIV infected participants we followed, were not aware of their HIV positive status and were ART naïve. Five years later 46% of the participants were on ART and after 10 years 85% as indicated in figure from the publication of Phalane et al., 2019.[23]

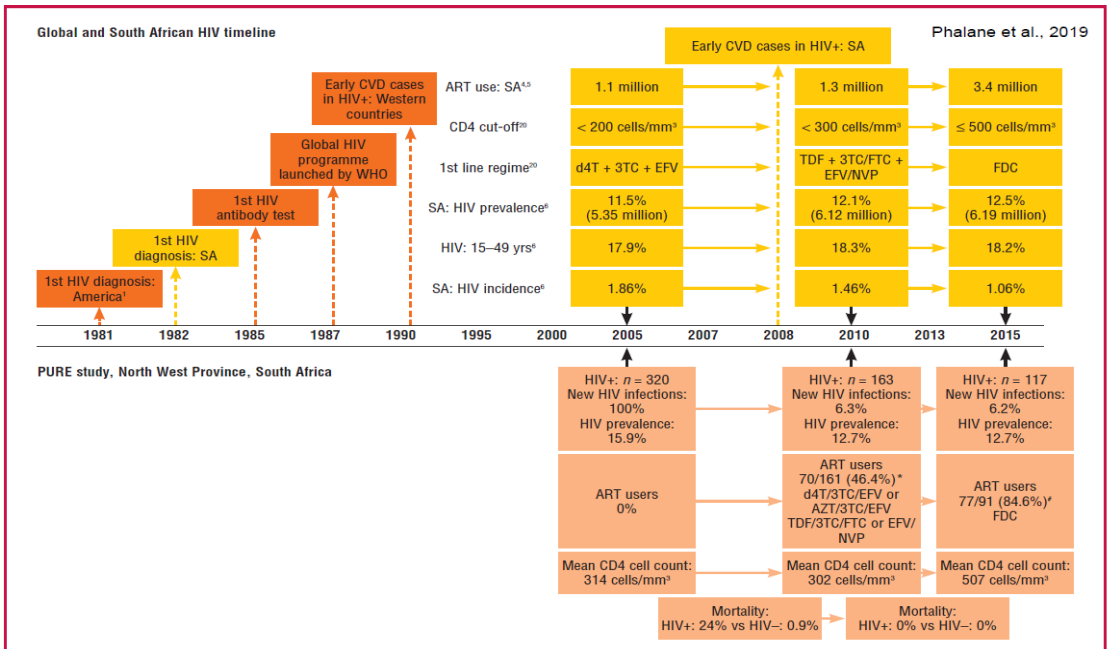



**Fig. 1.** Selected events in tracking HIV, ART and CVD. HIV, human immunodeficiency virus; ART, antiretroviral therapy; FDC, fixed-dose combination; d4T, stavudine; TDF, tenofovir; 3TC, emtricitabine; EFV, efivarenz; FTC, lamivudine; NVP, nevirapine; AZT, zidovudine; SA, South Africa; WHO, World Health Organisation; CVD, cardiovascular disease; PURE, Prospective Urban and Rural Epidemiology study; HIV+, HIV infected; HIV–, HIV uninfected; vs, versus. *Out of the 163 HIV-infected individuals who were followed in 2010, information on the use of ART was available for 151 participants. #Out of the 117 HIV-infected individuals who were followed in 2015, information on ART use was available for 91 participants.

We found that the ART had an effect on blood pressure and the systolic blood pressure (SBP) of the participants we followed was no longer lower than those HIV free.[21,23,24,25] The SBP of EndoAfrica study participants, where 80% were on treatment, did not differ between those infected and those HIV free.[16,26] Although the treatment seems to have influenced the SBP, it did not affect the prevalence of hypertension. The hypertension prevalence did either not differ[16,23] or were lower in those infected than those HIV free,[26,27] which is in agreement of the hypertension prevalence mainly seen in SSA.[18]

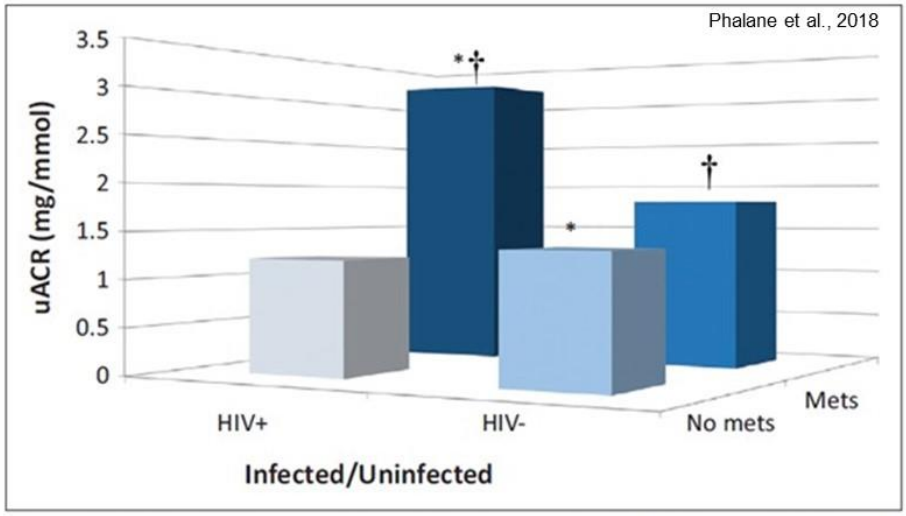
The pathophysiology mechanisms for hypertension in HIV-infected adults may include microbial translocation, chronic inflammation, immune suppression and reconstitution, viral tropism, lipodystrophy, and HIV-related renal disease.[17] We measured C-reactive protein (CRP) as indication of chronic inflammation and it is interesting to note that the CRP was higher in the HIV infected PURE participants during the 10 year follow-up study.[23] In this particular study the γ-glutamyltransferase (GGT) measures were 143% higher in those infected. The reason for this finding is unclear, but oxidative stress might have played a role. The EndoAfrica HIV infected participants did not present with higher CRP levels, however, their GGT levels were 231% higher than in those HIV free.[16] These findings warrant further investigation and may contribute to the clarification of the inflammatory mechanism of hypertension. Indeed, larger, multinational

prospective studies are needed to determine the mechanistic factors that precede and predict hypertension in HIV-infected adults.[17]

Direct ART effect may explain some, but not all, of the increased risk of hypertension in HIV-infected adults, however other risk factors were identified for hypertension such as lipodystrophy, immune reconstitution, dyslipidemia, microalbuminuria and renal dysfunction.[17] While following the HIV-infected PURE participants and their controls over 10 years, notable differences were seen in lipid measures and estimated glomerular filtration rate (eGFR) in the HIV-infected group.[23] Contrary to expectations, the eGFR of the HIV-infected participants showed an increase, suggesting an improvement of renal function with ART use.[23] However, continued increases in eGFR in the future, may reach the hyperfiltration range, which precedes the development of renal disease.

### *3.2. HIV and lipids*

We investigated the renal function in the HIV infected participants who also presented with the metabolic syndrome (MetS).[28] The MetS is diagnosed when someone has three or more of the risk factors that may lead to CVD namely, hypertension, abdominal obesity, hyperglycaemia (high blood sugar levels), high triglyceride (TG) and low high density lipoprotein (HDL) levels. We found that the HIV-infected participants with MetS had an almost twofold higher urinary albumin-creatinine ratio (uACR) compared to their uninfected counterparts as shown in the figure from the publication by Phalane et al., 2018.[28] A ratio of albumin to creatinine of less that 30 (mg/mmol) is normal, a ratio of 30-300 (mg/mmol) signifies microalbuminuria. Microalbuminuria reflects renal dysfunction and is also a marker of systemic endothelial damage,[29] which is linked to an elevated risk of kidney damage, cardiovascular disease and mortality.[30,31] Thus our findings may indicate the development of renal disease in the PLHIV already burdened by HIV and the MetS.




HIV, human immunodeficiency virus; HIV+, infected with human immunodeficiency virus; HIV-, human immunodeficiency virus uninfected; MetS, metabolic syndrome; uACR, urinary albumin-creatinine ratio (uACR). Bars with the same symbol differ significantly.

$\dagger p = 0.032$; $*p = 0.047$

**FIGURE 1:** Urinary albumin excretion for HIV-uninfected and HIV-infected individuals with and without metabolic syndrome after adjusting for age, sex and waist circumference.

Dominick et al., identified the core pathways mediating CVD onset, and the authors indicated, among others, metabolic dysfunction and TG accumulation.[14] As metabolic dysfunction and hypertriglyceridemia (high TG levels) are closely related to MetS, we determined dyslipidaemia and MetS in 300 HIV infected, ART naïve, and 300 age, sex, body mass index (BMI) and locality matched HIV free PURE participants.[19] We reported no difference in the prevalence of MetS between those infected and those HIV free. However, their lipid profile differed, and those infected had a higher TG/HDL ratio and higher TG levels as well as lower total cholesterol (TC), HDL and low density lipoprotein (LDL) levels than their matched controls.[19] It is generally accepted that HDL has anti-inflammatory/antioxidant effects.[31] The lower HDL levels in combination with higher TG levels, may lead to an increased delivery of cholesterol to the arterial wall. Here it is then taken up by macrophages, and atherogenic foam cells are formed that may lead to increased atherogenesis.[31] The HIV Nef protein, which is abundant during untreated HIV, initiate atherogenesis in the arterial wall, which play a role in endothelial dysfunction and atherosclerosis.[31]

The patterns of lipid changes varies during the course of HIV disease. In untreated disease, elevations in TG and low HDL levels predominate,[31] which is in agreement with our findings.[19] This is illustrated in the figure taken from the publication of Dillon et al., 2013.[22]

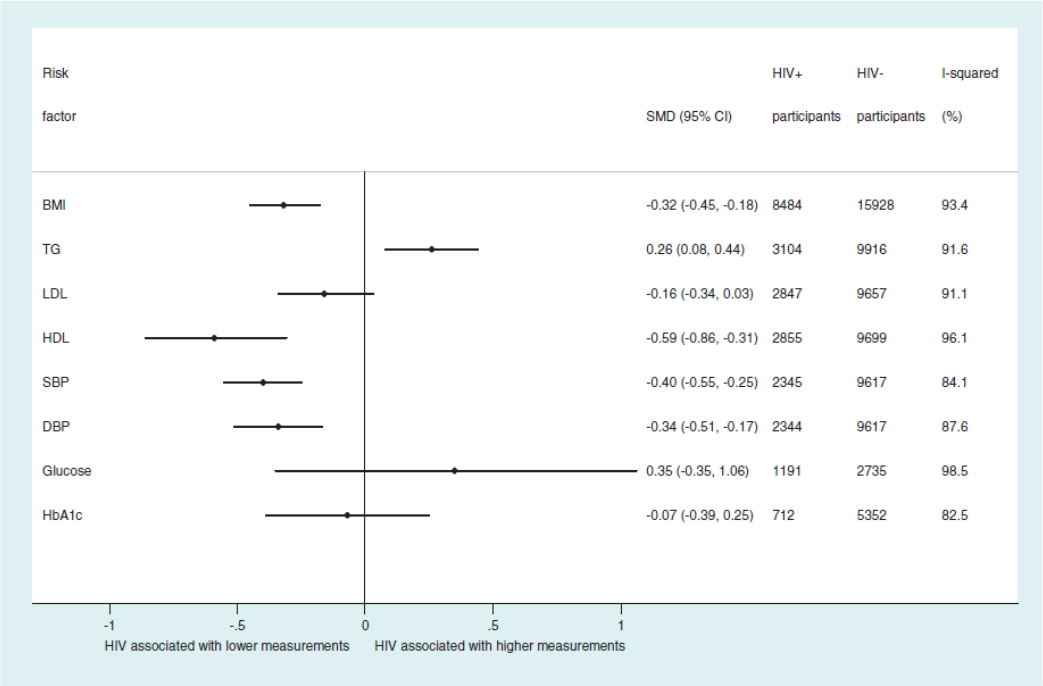



**Figure 3** Summary of overall estimates from random-effects meta-analyses of associations between HIV and individual cardiometabolic risk factors. SMD, standardized mean difference; CI, confidence interval; BMI, body mass index; TGs, triglycerides; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycated haemoglobin

As treatment for PLHIV became more readily available and more of the PURE participants were using ART, we observed changes in their lipid profile. This is also in agreement with the literature, as it is known that changes in the serum concentrations of TC, LDL and HDL occurs during treatment for HIV disease.[31] Following the PURE participants for 10 years, we found the LDL levels and TC:LDL ratio to be higher and the TG levels similar between those infected and their uninfected controls.[23] Among the EndoAfrica study participants, we found higher TC levels among those infected, and as seen among the followed PURE participants, no difference in TG levels.[16] Fat accumulation and viremia may influence increased circulating TG levels,[14] however, most of our participants were on treatment which controlled the viremia, and no abdominal fat accumulation were present in those living with HIV.[16,23] The latter may explain the similar TG levels we reported. Furthermore, the nucleoside reverse transcriptase inhibitors class antiretroviral treatment, tenofovir and lamivudine/emtricitabine is not associated with dyslipidemia.[31] All our HIV infected participants were on the first line ART regimen for South Africa, which included tenofovir and lamivudine/emtricitabine. We indeed observed changes in the lipid levels of those living with HIV, but the levels were not in the ranges of the reference values for dyslipidaemia and/or the levels associated with the development of CVD[32].

*3.3. HIV and inflammation*

Besides identifying metabolic dysfunction and pro-atherogenic TG accumulations as part of the core pathway mediating CVD, Dominick et al., also identified a pro-inflammatory milieu and endothelial dysfunction as underlying mechanisms driving the process leading to CVD.[14] The activation of the immune system and inflammation, which persists in PLHIV even after effectively using ART, is a hallmark of HIV infection.[31] The exact mechanism that drive immune activation in HIV infection is unclear. However, potential contributors among others are residual HIV replication, microbial translocation, and inflammatory lipids.[31] Mechanisms such as an altered cytokine profile and decreased lipid clearance (as we see with low HDL levels), may explain the dyslipidaemia and atherosclerosis seen in HIV infection.[31]

The glycoprotein, gp120 is critical for HIV entry, and is involved in upregulation of pro-inflammatory cytokine interleukin-6 (IL-6),[33] secreted by various cells including inflammatory cells and endothelial cells,[34] which cover the inner surface of blood vessels. Interleukin-6 form part of the altered cytokine profile seen in HIV infection and may promote lipid peroxidation and the production of reactive oxygen species.[31] Reactive oxygen species may further contribute to formation of oxidized, modified HDL[31] which my lead to atherosclerosis and endothelial dysfunction.[14,33] The acute-phase protein CRP is induced by IL-6,[33,34] and increased concentrations of CRP and IL-6 have been independently associated with CVD events in patients with HIV.[14,31] Researchers suggest that CRP play an active role in the pathophysiology of cardiovascular disease and elevated levels (>3mg/L) have been associated with atherosclerotic disease, congestive heart failure, atrial fibrillation, myocarditis, and aortic valve disease.[34]

We reported CRP levels of > 3mg/L in the treatment naïve PURE participants, and > 5mg/L in those with a nadir (<200 cells/mm$^3$) CD4 cell count.[19,20] In our conclusion, we suggested inflammatory injury of the endothelium after these elevated levels of both CRP and IL-6, together with high TG and low HDL levels, were seen in the HIV infected participants without ART.[20] Although, we did not find any indication of a pro-thrombotic state, there was an indication of accelerated vascular ageing and probable early atherosclerosis in the older HIV-infected participants.[20]

Later, after ART was induced and being used more by PLHIV in South Africa, the CRP levels did not differ among those with and without HIV in the EndoAfrica study (with 80% on treatment).[16] This, however, was not the case among the PURE participants after being followed for 10 years, and with 85% using treatment.[23] We found the CRP levels of the PURE HIV infected participants to be 1½ times higher than those HIV free.[23] The low-grade inflammation (indicated by the higher

CRP levels), and the lipid changes seen after 10 years, [23] may reflect the development of a pro-atherogenic profile, which may lead to atherosclerotic disease, and is associated with an increased risk of CVD.[34]

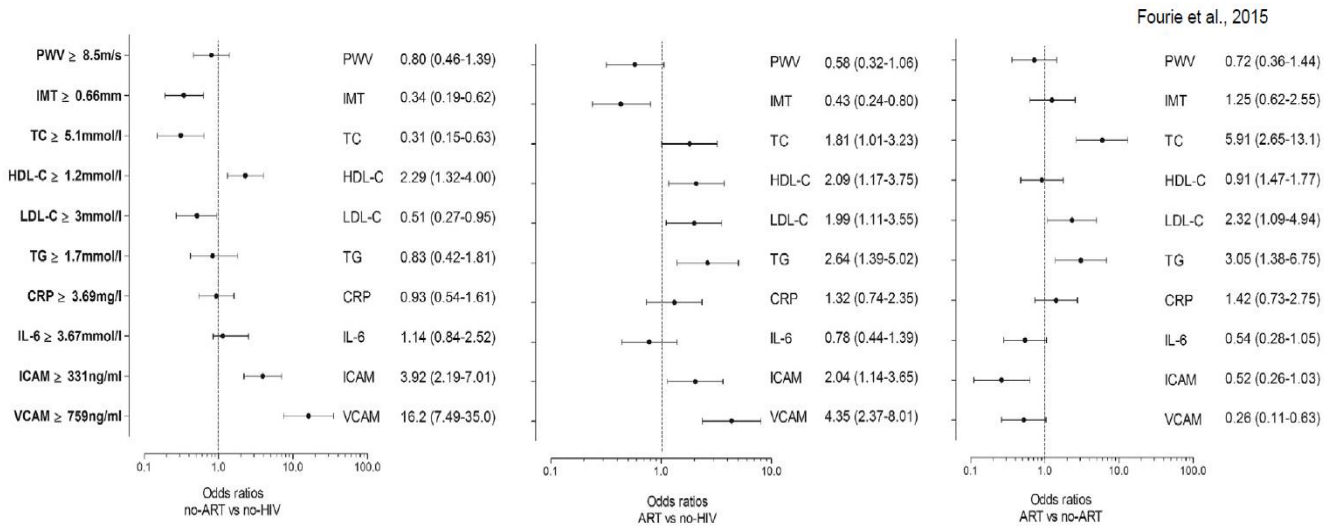
*3.4. HIV and endothelial function*

Indeed, research of the last twenty years have suggested that CRP may contribute to the inflammatory process of endothelial cells and development of endothelial dysfuntion.[35] The endothelium consists of a single cell layer of endothelial cells, lining the vascular system, and is known to play an important role in the modulation of vascular tone, dynamic permeability, thrombogenicity, inflammation, and angiogenesis.[36] Endothelial dysfunction is increasingly recognized as contributor to pathophysiology of many diseases,[36] and research has shown the importance of endothelial dysfunction for the development and progression of cardiovascular disease.[35]

Endothelial dysfunction triggered by HIV infection has been identified as a critical link between the infection, inflammation, immune activation and atherosclerosis.[33] The HIV protein, gp120, is actively secreted into the endothelial cell and may have significant direct effects on the endothelium.[33]

The protein injures the endothelium and end up in the endothelial cell, which may lead to endothelial dysfunction and stiffness of the vessel wall.[35] Endothelial dysfunction precedes morphological atherosclerotic changes, and may contribute to the development of lesions and atherosclerosis.[35] The latter may explain inflammatory injury of the endothelium and probable the early sub-clinical atherosclerosis we identified in the older (>50 years) HIV-infected, ART naïve participants.[20]

Furthermore, studies have demonstrated that CRP promotes endothelial activation by expression of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1).[34] When the endothelial cells are activated by pro-inflammatory stimuli, such as CRP, the expression of adhesion molecules is increased and are considered early markers of endothelial activation and systemic inflammation.[35] In a five year follow-up study of the PURE HIV infected participants, endothelial activation (increased levels of both ICAM-1 and VCAM-1) was clear in those living with HIV, and the endothelial activation was more prominent in the never-treated group than in their treated counterparts.[21] The endothelial activation was not accompanied by increased inflammation (as measured with CRP and IL-6), nor was it reflected by increased arterial stiffness or sub-clinical atherosclerosis as seen in the figure taken from the publication by Fourie et al, 2015. [21] We concluded that antiretroviral treatment seemed to reduce endothelial activation.




In endothelial activation, pro-inflammatory cytokines and adhesion molecules attract monocytes and T-cells which are stimulated to enter the endothelium.[37] In the endothelium, macrophages, which differentiate from monocytes, phagocytize oxidized low-density lipoproteins, leading to foam cell formation and eventually the formation of plaques and atherosclerosis.[37] Atherosclerosis is a chronic inflammatory disease which affects the structure (morphological changes) as well as the function of arteries.[38] The carotid artery wall consists of three layers, namely the innermost layer (tunica intima), the middle layer (tunica media), and the outermost layer (tunica adventitia or externa).[38] Atherosclerotic lesions are predominant in the intima[38] and the rupture of advanced plaques leads to stroke, myocardial infarction, and thrombosis.[37] Morphological changes such as atherosclerotic lesions and plague formation, affect both the structure and function of the endothelium, leading to endothelium dysfunction which may increase the CVD risk. A measurement that is useful to assess atherosclerosis or sub-clinical vascular disease, in a non-invasive approach, is the ultrasonic detection of carotid plaque and carotid intima media thickness (CIMT).

Assessment of endothelial function, on the other hand, consists of measures such as pulse wave velocity (PWV) and flow-mediated dilation (FMD). Pulse wave velocity, a measurement of arterial stiffness, has been used as an important marker of cardiovascular risk.[35] The carotid-femoral relation (cfPWV) is considered the gold standard for large artery stiffness,[35,36] as it is non-invasive and express PWV values directly related to the aorta.[35] The PWV values are lower in healthy young individuals, but increases with age, as the elastic properties of blood vessels decrease.[35] Arterial

stiffness is generally increased in persons with heart failure,[36] and associated with risk factors such as coronary artery disease, diabetes mellitus, arterial hypertension, diastolic function and age.[35] When used in clinical practice, PWV analysis may identify changes that might be related to vascular health and endothelial dysfunction, even before the occurrence of signs and symptoms.[35]

Flow mediated dilation is used to measure vascular endothelial function of the brachial artery and utilizes imaging, usually ultrasound, to measure the arterial dilation.[36] Brachial FMD is an indirect measure of endothelial dependent vasodilation mediated by nitric oxide (NO) release by the endothelium.[36] Impaired NO bioavailability (lower FMD percentage) suggest impaired release of endogenous vasodilators in response to ischemia,[36] and it is one of the early, reversible manifestations of endothelial dysfunction.[35]

HIV-induced endothelial dysfunction is a major contributing factor to CVD in PLHIV.[33] Research indicated significantly impaired endothelial function, as demonstrated by reduced flow-mediated dilation, in HIV-infected patients compared to those HIV free.[33] However, in a review article on HIV infection and arterial stiffness, Leite et al., reported conflicting results of increased aortic stiffness among PLHIV and their HIV free controls.[39] Nonetheless, the review indicated emerging evidence that HIV itself and immune activity affect vascular health and the large arteries.

Besides a higher PWV in never treated, older than 50 years HIV-infected participants compared to their age, sex, BMI and locality matched controls,[20] we did not find sub-clinical atherosclerosis, increased large artery stiffness, nor imparted vasodilation in any of our studies.[21,16,40,26] It is important to remember that the older than 50 years PURE participants, where the higher PWV were seen, were newly identified as being HIV infected and never used ART when the data was collected. Furthermore, we determined carotid-radialis pulse wave velocity[20] and not the “golden standard” carotid-femoral pulse wave velocity (cfPWV). Five years after the PURE participants were identified as being HIV infected, we reported endothelial activation among those living with HIV.[21] The endothelial activation was more pronounced in those ART naïve, however, the odds for the latter participants for higher PWV measurements did not differ from those using ART or HIV free.[21] Using the 10 year follow-up PURE data we again assessed the participants for sub-clinical atherosclerosis and increased large artery stiffness, this time using cfPWV.[40] In the latter study, where the mean age was 53 years, and with 67% of the participants being infected for more than five years and 82% on ART, the measures of arterial structure and function were similar between those infected and their age, sex and locality matched controls.[40] This is illustrated in the figure taken from the recent publication by Phalane et al., 2021.[40]

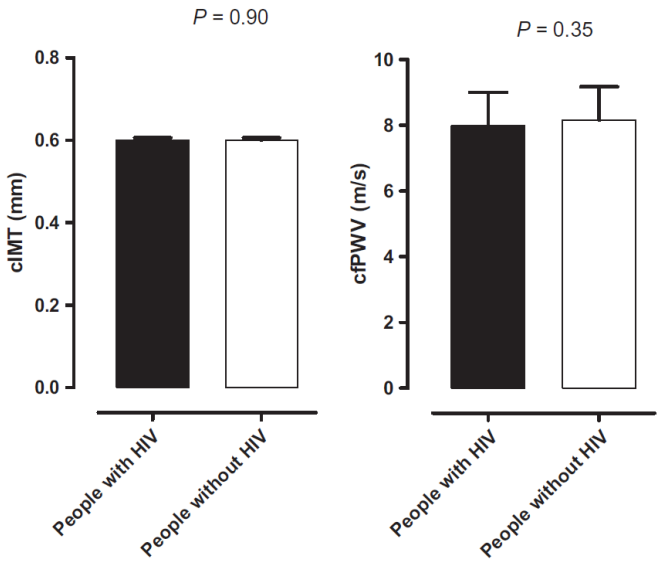



Fig. 2 Carotid intima-media thickness (cIMT) and carotid-femoral pulse wave velocity (cfPWV) in people with HIV ($N$ = 120) *vs.* age-, sex- and locality-matched controls without HIV ($N$ = 125). cIMT and cfPWV were adjusted for mean arterial pressure. *N*, number of participants.

We assessed the endothelial dependent vasodilation and cfPWV cross-sectionally in the EndoAfrica study.[16,26] We reported that despite the PLHIV being older, the analysis suggested that PLHIV, and using treatment, do not have poorer endothelial or vascular function compared to the HIV free participants.[16]

Our data by this time suggested a probable protective role of ART on the vasculature, but we only used data collected in the North West province of South Africa. We therefore investigated the possible influence that the multi-ethnic and multi-lingual society of South Africa, may have on vasodilation, by including two different regions namely the Western Cape and North West Province.[26] Although ethnic, cultural and socio-economic differences need further investigation, we found that the endothelial function of PLHIV were not worse than that of those HIV free.[26]

A limitation of our studies are the rather small study population. Therefore, in a recently submitted manuscript (August 2021), we included 572 participants (matched for age and sex) of the PURE and EndoAfrica studies.[27] In this combined analyses we investigated the age related differences in vascular function and structure between PLHIV and those HIV free.[27] Our results showed that HIV positive status did not associated with measures of vascular structure and function in any of the

age groups. Furthermore, PLWH did not have a worse cardiovascular profile and early vascular ageing was not evident in these people compared to those without HIV.

## 4. Summary

Thus, what does our part of the research puzzle on HIV and CVD risk in our cohorts tell us this far?

- HIV infection was associated with lower SBP, while treatment was associated with normalisation of the lipid levels. This was in agreement of the literature in SSA.[22]
- In untreated disease, elevations in TG and low HDL levels predominate,[31] which is in agreement with our findings.[19] We indeed observed changes in the lipid levels of PLHIV using ART, but no dyslipidaemia.
- Our article on the metabolic syndrome and renal function indicated that multimorbidity may affect renal function in PLHIV and should be investigated further. This is seen in the literature where microalbuminuria has been shown to be independently associated with hypertension in PLHIV. [17]
- Our research indicates that HIV itself and immune activity seem to affect vascular health. This is in agreement of that found by several studies.[17,33,39]
- From the literature and also from the PURE 10-year follow-up study, it is clear that ART has improved the morbidity and mortality associated with HIV. Although results on increased vascular ageing are controversial,[39,14,33] our results suggest a probable protective role of ART on the vasculature.
- Lastly, in the EndoAfrica study we observed interruption or defaulting of ART among the participants. Interrupting ART may increase inflammation and further increase the risk of cardiovascular disease,[37] and this needs to be investigated further.

## 5. Future directions

*5.1. From the literature*

- According to Fahme et al.[17]

  Pathophysiologic mechanisms for hypertension in PLHIV may include microbial translocation, chronic inflammation, immune suppression and reconstitution, as well as HIV-related renal disease. Large, multinational cohort studies are needed to strengthen our knowledge.

- According to Dominick et al.[14]

  Despite the progress made in terms of understanding the multifactorial nature of CVD in PLHIV, several gaps in our knowledge remain in the understanding of the underlying

mechanisms driving this process. Furthermore, the complex interplay between lifestyle risk factors, ART side effects and persistent immune activation needs further clarification.

- According to Chia et al.[36]
  Despite the wide array of techniques, our understanding of the human endothelium system is still not fully complete.
- According to Kearns et al.[37]
  In PLHIV, even those on ART, the interaction of the virus with immune cells and endothelial cells can trigger several molecular events, including increased oxidative stress, and inflammasome formation. The contribution of these pathways to immune cell activation and inflammation in the vasculature, still requires further investigation.
- According to Anand et al.[33]
-

  More detailed exploration into the mechanisms of HIV-induced endothelial dysfunction is needed to formulate targeted approaches to prevent and treat HIV-related vascular diseases. Also to provide vital information to guide clinicians on the most appropriate approach to prevent and treat CVD in this high-risk population.

*5.2. The way forward – HART*

From our research, it thus seems that the cardiovascular risk among the participants of our HIV infected cohorts is not as high as researchers fear, in fact our research suggests a probable protective role. Larger longitudinal studies need to be conducted which include the South African population at large in order to confirm our findings. Confirmation of our findings will be good news for the South African health care system, as it means that PLHIV do not add an extra burden on multimorbidity, by having an increased risk to develop CVD compared to the general population.

*5.2.1. Collaboration - HART*

- Continue our collaboration with the EndoAfrica study.
- We established collaboration with the group of Prof Per Björkman of Department of Translational Medicine, Lund University, and Department of Infectious Diseases, Skåne University Hospital, Sweden. For this collaboration we submitted a joint paper on low-level viremia during ART. Hopefully this collaboration will help us to add more pieces to the puzzle of cardiovascular risk in South Africa.
- We had discussion with Prof Mark Siedner from Harvard Medical School to visit the Africa Health Research Institute in KZN. If we succeed to establish collaboration with

the researchers of the institute in KZN, we might gather more insight in the cardiovascular risk in South Africa. As mentioned, our research focussed on the North West province and we only recently included PLHIV from the Western Cape. Including KZN would really help to gain knowledge on the cardiovascular risk of PLHIV in South Africa.

- Collaboration discussions with Prof Nigel Crowther from the University of Witwatersrand and the National Health Laboratory Service are also in the pipeline.

5.2.2 *Adding pieces to the puzzle - HART*

- Kearns et al., indicated increased oxidative stress and inflammasome formation in PLHIV, and in those on ART. [37] It is known that HIV-induced reactive oxidative stress likely contributes to endothelial dysfunction through direct effects on the endothelium, and/or indirectly through monocytes/macrophages contacting the vessel wall.[34] We found much higher levels of gamma–glutamyltransferase (GGT), which may indicate oxidative stress, among the participants living with HIV,[16,23] while the alcohol use levels did not differ. These interesting findings need further elucidation.
- The first line ART regimen in SA includes tenofovir, therefore, the possibility of renal disease, hyperfiltration as well as the renin levels among PLHIV in South Africa will add another piece to the puzzle.

5.2.3 *Community engagement - HART*

- Expand our involvement with the AIDS day activities at the clinic through our puppet show and talks to the PLHIV from the Potchefstroom community to more clinics and community activities.

## 6. Acknowledgements


- EndoAfrica study
  - The founders of the study and EndoAfrica consortium partners:
    Prof Hans Strijdom, Centre for Cardiometabolic Research in Africa, Division of Medical Physiology, Faculty of Medicine and Health Sciences, Stellenbosch University, Stellenbosch, South Africa. He is also the coordinator of the EndoAfrica project.
    Prof Nandu Goswami, Gravitational Physiology and Medicine Research Unit, Division of Physiology, Medical University of Graz, Graz, Austria.
    Patrick de Boever, Health Unit, Flemish Institute for Technological Research (VITO), Mol, Belgium; Centre for Environmental Sciences, Hasselt University, Diepenbeek, Belgium; Department of Biology, University of Antwerp, Wilrijk, Belgium
    Tim Nawrot, VITO, Hasselt University, Belgium.

- Department of Science and Innovation of the Republic of South Africa for the funding of the EndoAfrica NWU leg, especially Mr Toto Matshediso, deputy director strategic partnership, for his support and help in this regard.
- Dr Ingrid Webster and Dr Corli Westcott from the Stellenbosch University, for all their help support and especially the training sessions.
- This research would not have been possible without the contribution and dedication of the recruitment team, voluntary participants, research staff, postgraduate students and interns at the Hypertension Research and Training clinic and laboratory at the North-West University.

- The PURE study
  - The late Prof Annemarie Kruger who was the organiser, manager and principal investigator of the PURE study from the start till her death – she helped to make it all happen.
  - The researchers who were part of the study since 2005, Dr E Wentzel-Viljoen, who was the assistant manager, Prof Alta Schutte, who was the coordinator for Physiology, Prof Johan Potgieter – coordinator Psychology, Prof Herman Strydom – coordinator Social Work and Dr Colette Underhay – coordinator Human Movement Sciences. Also Ms Mada Watson who was responsible for the HIV testing and counselling at that time.
  - Since this is a longitudinal study ongoing since 2005, new researchers join as time went by and we acknowledge Prof Lanthé Kruger, the current principal investigator, Prof Minrie Greeff, Prof Salomé Kruger, Prof Marlien Pieters.
  - We are grateful towards the participants of this study, the PURE-SA research team, the field workers and supporting staff in the Africa Unit for Transdisciplinary Health Research (AUTHeR), North-West University, South Africa, without whom this research would not have been possible. This include Dr S Yusuf (PURE-International) and the PURE project staff at the PHRI, Hamilton Health Sciences and McMaster University, ON, Canada.
- The Hypertension in Africa Research Team (HART)
  - All my colleagues at HART, the researchers, the clinic management team, the support staff and especially also all the post-doctoral fellows and post-graduate students over the years.

**References**


1. Statistics South Africa. http://www.statssa.gov.za/wp-content/uploads/2021/07/Life-expectancy-by-sex-final.jpg (accessed 19 August 2021).
2. Morris L, *HIV vaccines and immunotherapy: Quo vadis?* Prime Session, 11th IAS Conference on HIV Science, 2021. https://www.aidsmap.com/news/jul-2021/hiv-vaccines-and-immunotherapies-would-be-further-along-if-they-had-resources-covid (accessed 20 August 2021).
3. Midyear population estimates 2021. http://www.statssa.gov.za/publications/P0302/P03022021.pdf (accessed 19 August 2021).
4. 2020 AIDS data book. https://www.unaids.org/en/resources/documents/2020/unaids-data (accessed 19 August 2021).
5. World Health Organization HIV/AIDS. https://www.who.int/news-room/fact-sheets/detail/hiv-aids (accessed 19 August 2021).
6. Gouda HN, Charlson F, Sorsdahl K et al. Burden of non-communicable diseases in sub-Saharan Africa, 1990–2017: results from the Global Burden of Disease Study 2017. *Lancet Global Health,* 2019; 7(10): e1375-e1387.
7. Improving the prevention and management of multimorbidity in sub-Saharan Africa (2020). Academy of Medical Sciences (UK), Academy of Science of South Africa (ASSAF) *Proceedings report*. https://research.assaf.org.za/handle/20.500.11911/139 (accessed 20 August 2021).
8. Heart Foundation Blood Pressure. https://www.heartfoundation.co.za/blood-pressure/ (accessed 20 August 2021).
9. Peer N, Lombard C, Steyn K, et al. High prevalence of metabolic syndrome in the Black population of Cape Town: The Cardiovascular Risk in Black South Africans (CRIBSA) study. *European Journal of Preventive Cardiology,* 2015; 22(8):1036–1042. DOI:10.1177/2047487314549744.
10. Ntusi N. Dyslipidaemia in South Africa. *South African Medical Journal,* 2018; 108(4):256-257. DOI:10.7196/SAMJ.2018.v108i4.13265.
11. Erzse A, Stacey N, Chola L, et al. The direct medical cost of type 2 diabetes mellitus in South Africa: a cost of illness study. *Global Health Action,* 2019; 12:1636611. DOI:10.1080/16549716.2019.1636611.
12. Dwane N, Wabiri N, Manda S. Small-area variation of cardiovascular diseases and select risk factors and their association to household and area poverty in South Africa: Capturing emerging trends in South Africa to better target local level interventions. *PLOS One*, 2020; 15(4): e0230564. DOI:10.1371/journal. pone.0230564.
13. Levy ME, Greenberg AE, Hart R, et al. A for the DC Cohort Executive Committee. High Burden of Metabolic Comorbidities in a Citywide Cohort of HIV Outpatients: Evolving Health

Care Needs of People Aging with HIV in Washington, DC. *HIV Medicine,* 2017; 18(10): 724–735. DOI:10.1111/hiv.12516.

14. Dominick L, Midgley N, Swart L-M, et al. Review Integrative Cardiovascular Physiology and Pathophysiology: HIV-related cardiovascular diseases: the search for a unifying hypothesis. *American Journal of Physiology-Heart* and *Circulatory Physiology*, 2019; 318:H731–H746. DOI:10.1152/ajpheart.00549.2019.
15. Monsuez JJ, Charniot JC, Escaut L, et al. HIV-associated vascular diseases: structural and functional changes, clinical implications. *International Journal Cardiology*, 2009; 133:293–306, 2009. DOI:10.1016/j.ijcard.2008.11.113.
16. Fourie CMT, Botha-le Roux S, Smith W, et al. Vascular function and cardiovascular risk in a HIV infected and HIV free cohort of African ancestry: Baseline Profile, Rationale and Methods of the Longitudinal EndoAfrica-NWU Study. *BMC Infectious Diseases,* 2020; 20:473-486. DOI:10.1186/s12879-020-05173-6.
17. Fahme SA, Bloomfield GS, Peck R. Hypertension in HIV-Infected Adults Novel Pathophysiologic Mechanisms. *Hypertension*, 2018; 72:44-55. DOI:10.1161/HYPERTENSIONAHA.118.10893
18. Phalane E, Fourie CMT, Mels CMC, et al. A comparative analysis of blood pressure in HIV-infected patients versus uninfected controls residing in sub-Saharan Africa: a narrative review. *Journal of Human Hypertension*, 2020. DOI:10.1038/s41371-020- 0385-6.
19. Fourie CM, van Rooyen JM, Kruger A, et al. Lipid abnormalities in a never -treated HIV-1 subtype C-infected African population. *Lipids,* 2010; 45(1):73-80. DOI:10.1007/s11745-009-3369-4.
20. Fourie CMT, Van Rooyen JM, Pieters M, et al. Is HIV-1 associated with endothelial dysfunction in a population of African ancestry in South Africa? *Cardiovacular Journal of Africa,* 2011; 22(3):134-140. DOI:CVJ-21.049.
21. Fourie CMT, Schutte AE, Smith W, et al. Endothelial activation and cardiometabolic profiles of treated and never-treated HIV infected Africans. *Atherosclerosis,* 2015; 240:154-160. DOI:10.1016/j.atherosclerosis.2015.03.015.
22. Dillon DG, Gurdasani D, Riha J, et al. Association of HIV and ART with cardiometabolic traits in sub-Saharan Africa: a Systematic review of HIV, ART and cardiometabolic traits in sub-Saharan Africa. *International Journal of Epidemiology,* 2013; 42(6):1754-71. DOI:10.1093/ije/dyt198.
23. Phalane E, Fourie CMT, Mels CMC, et al. A 10-year follow-up study of demographic and cardio-metabolic factors in HIV infected South Africans. *Cardiovascular Journal of Africa*, 2019; 30:1-5. DOI:10.5830/CVJA-2019-034.

24. Fourie CMT, Van Rooyen JM, Kruger A, et al. Soluble urokinase Plasminogen Activator Receptor (suPAR) is associated with metabolic changes in HIV-1 infected Africans: a prospective study. *Inflammation,* 2012; 35:221-229. DOI:10.1007/s10753-011-9308-6.
25. Botha S, Fourie CMT, Rooyen JM, et al. Cardiometabolic Changes in Treated Versus Never Treated HIV-Infected Black South Africans: The PURE Study. *Heart, Lung and Circulation,* 2014; 23: 119–126. DOI:10.1016/j.hlc.2013.07.019.
26. Swart C, Lammertyn L, Strijdom H, et al. Endothelial function in a South African cohort living with HIV: The EndoAfrica study. *Cardiovascular Journal of Africa,* 2021. DOI-10-5830-CVJA-2021-026.pdf
27. Louwrens A, Fourie CMT, Botha-Le Roux S, et al. Age-related differences in the vascular function and structure of South Africans living with HIV. *Journal of Epidemiology & Community Health,* submitted August 2021.
28. Phalane E, Fourie CMT, Schutte AE. The metabolic syndrome and renal function in an African cohort infected with Human Immunodeficiency Virus. *Southern African Journal of HIV Medicine*, 2018; 19(1):2078-6751. DOI:10.4102/sajhivmed.v19i1.813.
29. Efstratiadis G, Tziomalos K, Mikhailidis DP, et al. Atherogenesis in renal patients: A model of vascular disease? *Current Vascular Pharmacology*, 2008; 6(2): 93– 107. DOI:10.2174/157016108783955374.
30. Baekken M, Os I, Sandvik L, et al. Microalbuminuria associated with indicators of inflammatory activity in an HIV-positive population. *Nephrology Dialysis Transplantation,* 2008; 23(10):3130–3137. DOi:10.1093/ndt/gfn236.
31. Kelesidis T, Currier JS. Dyslipidemia and Cardiovascular Risk in Human Immunodeficiency Virus Infection. *Endocrinology and Metabolism Clinics of North America*, 2014; 43(3):665–684. DOI:10.1016/j.ecl.2014.06.003.
32. The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts). European Guidelines on cardiovascular disease prevention in clinical practice. *European Heart Journal,* 2016; 37:2315–2381. DOI:10.1093/eurheartj/ehw106.
33. Anand AR, Rachel G, Parthasarathy D. HIV Proteins and Endothelial Dysfunction: Implications in Cardiovascular Disease. *Frontiers in Cardiovascular Medicine,* 2018; 5:185. DOI: 10.3389/fcvm.2018.00185.
34. Sproston NR and Ashworth JJ. Role of C-Reactive Protein at Sites of Inflammation and Infection. Frontiers in Immunology, 2018; 9:754. DOI: 10.3389/fimmu.2018.00754.

35. Storch AS, de Mattos JD, Alves R, et al. Methods of Endothelial Function Assessment: Description and Applications. *International Journal of Cardiovascular Sciences*, 2017; 30(3):262-273. DOI: 10.5935/2359-4802.20170034.
36. Chia PY, Teo A, Yeo TW. Overview of the Assessment of Endothelial Function in Humans. *Frontiers in Medicine,* 2020; 7:542567. DOI: 10.3389/fmed.2020.542567.
37. Kearns A, Gordon J, Burdo TH, et al. HIV-1–Associated Atherosclerosis: Unravelling the Missing Link. *Journal of the American College of Cardiology,* 2017; 69(25): 3084–3098. DOI:10.1016/j.jacc.2017.05.012.
38. Milutinovic A, Suput D, Zorc-Pleskovic R. Pathogenesis of atherosclerosis in the tunica intima, media, and adventitia of coronary arteries: An updated review. *Bosnian Journal of Basic Medical Sciences,* 2020; 20(1):21-30. DOI:10.17305/bjbms.2019.4320.
39. Leite LHM, Cohen A, Boccara F. Review: HIV infection and aortic stiffness. *Archives of Cardiovascular Disease,* 2017; 110:495—502. Doi:10.1016/j.acvd.2017.03.001.
40. Phalane E, Fourie CMT, Schutte AE, et al. Arterial structure and function in Africans with HIV for > 5 years: longitudinal relationship with endothelial activation and cardiovascular risk markers. *HIV Medicine,* 2021. DOI:10.1111/hiv.13111.