

Interaction Between Immune Activation, Sleep Disturbances and Circadian Timing on Cardiometabolic Risk in HIV Positive Patients

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Supervisors: Karine Scheuermaier and Nigel Crowther

DECLARATION



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SENATE PLAGIARISM POLICY: APPENDIX ONE

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DEDICATION

This thesis has been five years in the making, with many pitfalls and challenges in the process. We have had complete shutdowns of research sites, community protests (on more than one occasion, at more than one research site), project changes and a global pandemic to contend with. Thank you to the BFRG, my supervisors, and the whole School of Physiology for their experience and support throughout this process.

I got married, had two beautiful children, nearly gave up a few times – especially at the beginning – but my supervisors pulled through, reminded me of why I started in the first place. At one particular point, I was gently reminded by my supervisors of my sense of pride, and of wanting to accomplish something strong, something greater – helping people living with HIV. Something that we are reminded can hit very close to home now and again.

In submission of this body of work, the weight of this achievement does not sit light on my shoulders. I'm not as young as I was when starting out on this project, and I look back and applaud that girl's determination to get this started, and now it is finished. I was reminded that the halls of universities were not originally made for women, much less young black women. When my great-grandmothers, and grandmothers had children, I doubt they ever dreamed that there would have been a day that their descendent, a girl child nonetheless, could grace the halls of The University of the Witwatersrand and pursue any kind of higher education, let alone a doctorate. I thank each generation of women for educating their daughters because my mother, a teacher, educated me. And I hope to be as inspiring to my own daughter, my sisters, my cousins, my nieces, all of them. There truly are no limits when someone believes in you.

I would like to extend my deepest gratitude to my friends my family with your ongoing and continued support. When I first registered for this degree, I was unmarried, planning a wedding to my now husband, and now here I am married for five years and with two wonderful children.

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My darling Alykhan, without whom none of this would be possible – your sheer excellence inspired me to dream. To want more for myself, and to rise to your level of education, even though it is not a competition – we really are in this together. Thank you for your ages of support, for your continued encouragement, and for your commitment to ensuring that our home, and our children, are well taken care of while I pursued this qualification. You truly are everything that I prayed for and everything that I didn't know I needed.

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I would like to extend my deepest gratitude to all our collaborators across the fields to everybody at the Chris Hani Baragwanath Adult HIV clinic in Soweto, Gauteng, at the management team at the Ndlovu research facility in Elandsdoorn, Limpopo, to the fieldworkers, nurses, community liaisons at the Wits/MRC Agincourt unit in Agincourt, Mpumalanga, and most importantly to all the participants who gave up their time and allowed us into their homes, who filled out questionnaires while in waiting rooms all to further the knowledge base in this country.

A lot can be said about the efforts that we as Africans have made to ensure that PLWH have access to the best possible care at all times one thing that has been afforded to me over the past decade at the school of Physiology is death they have been leaps and bounds in terms of patient care in terms of disease management and also access to healthcare sooner on in disease diagnosis. We now know that earlier time to ART is associated with better disease outcomes and that fixed those combination therapy improves adherence and thus increases longevity in PLWH. With the major drives by the government to ensure patient education by the production of many different kinds of posters, billboards, and advertisements by outreach programmes and by community education days, PLWH are now more equipped and more aware of how to best deal with the diagnosis and management of the disease. When I started my honours in 2012 the WHO guidelines were waiting for people to be clinically classified as having AIDS prior to starting antiretroviral therapy and it took major drives from the government and the WHO to ensure that people had access to this life saving medication earlier on in

their disease and now we're at a point with what the chest test to treat campaign more people have more access to better drugs than they ever have before.

We know that HIV does not discriminate based on race, creed, or gender. We know that this virus has been particularly hard to eradicate over the past four decades since its discovery, but the dedication of people in South Africa and across the world to try and improve lives of PLWH is exemplary. To see myself classified as one of those people makes me extremely proud of myself and also, I feel extremely privileged to be a part of such a global community. Working in various research sites over the past five years and seeing everybody's commitment to the cause of improving the lives of everyday South Africans is something that greatly inspires me. I commend the researchers, fieldworkers, data teams, nurses and doctors all the work that they continue to do.

As I sit and reflect on the sheer volume and magnitude of the work that has been done to put together this thesis I am rightly and acutely aware of the fact that designing and setting up projects analysing samples collecting data incomprehensively compiling it into readable format was and is a monumental task but also one that I have been privileged to undertake. For me to achieve the success that I have a lot of other people have had to carry the load. I would like to thank my husband for being my number one cheerleader so wonderful kids who still had a parent when mom was busy writing up her thesis.

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lifestyle since the beginning of time, one understands now more than ever the “standing on the shoulders of greatness” quote. Thank you both for never failing to listen to me, even when you didn’t understand what I was saying. Thank you for believing in me, and for being all-round brilliant people → Not too bad for a girl from the hood ;). For my siblings, (in order of appearance: Tyra, Dylan, Ethan, and Jordyn) the bar is high, and I’m looking forward to seeing you raise it even further.

To the reader, it is nice to think of biological science degrees as happening in a box. We sit behind computer screens with giant processors to run statistics, we analyse blood samples in sterile rooms for months and weeks after we’ve had initial contact with participants, and then take even more time to write and publish scientific articles that gets our knowledge out into the world. With studying PLWH, one is reminded that we as a country still have a lot to do. There is still a lot of disinformation, and this was highlighted time and again in my life. In my family, we have buried aunts and uncles; we’ve mourned with their children over the lack of knowledge and desire to seek out care and treatment for this disease. We can only hope and pray that our combined efforts impact the lives of those who truly need it the most.

Thank you for your time and consideration.

Kirsten

P.S. Daddy, please can you clap at my graduation now? We had a deal. Thanks.

RESEARCH OUTPUTS ARISING FROM THIS STUDY

First author:

- Redman, K.N., O'Brien, K.E., Ruiz, F.S., Rae, D.E., Gómez-Olivé, F.X., von Schantz, M. and Scheuermaier, K., 2023. Delayed circadian rhythms in older Africans living with human immunodeficiency virus (HIV). *Journal of Pineal Research*, 74(1), p.e12838. DOI: [10.1111/jpi.12838](https://doi.org/10.1111/jpi.12838)
- Redman, K.N., Karstaedt, A.S. and Scheuermaier, K., 2018. Increased CD4 counts, pain and depression are correlates of lower sleep quality in treated HIV positive patients with low baseline CD4 counts. *Brain, behavior, and immunity*, 69, pp.548-555. DOI: [10.1016/j.bbi.2018.02.002](https://doi.org/10.1016/j.bbi.2018.02.002)

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- Physiological Society of South Africa, July 2021: Circadian phase in older rural South Africans living with and without HIV: a preliminary report.
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- School of Public Health Biennial Research Day, July 2021: Insomnia and HIV predict higher levels of inflammation in people living in rural South Africa. Kirsten Redman, Nigel Crowther, Hugo Tempelman, Karine Scheuermaier
- Brain Function Research Group Research Day, 2018: Sleep and cardiometabolic disease in a rural cohort of HIV-positive patients receiving anti-retroviral therapy. Kirsten Redman, Dr. Karine Scheuermaier, Prof. Nigel Crowther.
- Airwaves Congress *Breathe Well, Sleep Well, Live Well* June 2014: Sleep disruption is associated with pain and increased CD4 counts in HIV positive patient. Kirsten Redman.
- SLEEP, July 2013: Sleep disruption is associated with pain and increased CD4 counts in HIV positive patients. Kirsten Redman, Alan Karstaedt , Karine Scheuermaier.
- Federation of African Immunological Science (FAIS), December 2012: (Title unknown). Kirsten Redman, Alan Karstaedt , Karine Scheuermaier.

ABSTRACT

Introduction

South Africa still has the highest burden of Human Immunodeficiency Virus (HIV) in the world. Over the past 15 years, antiretroviral (ARV) treatments for HIV have improved, ensuring that people living with HIV (PLWH) have life expectancies like people living without HIV (PLWOH). Studies have shown that PLWH are at higher risk of death from cardiovascular disease than PLWOH. Poor sleep is a common issue for PLWH – treated and untreated. Poor sleep is a contributor to weight gain and higher BMI, contributing to the development of cardiometabolic disease. Poor sleep increases inflammation, contributing to the development of cardiovascular disease. Most studies investigating sleep in PLWH have been done in the northern hemisphere, which may not adequately represent South Africa. We test the hypotheses that: 1. PLWH in South Africa have poor sleep quality using validated tools, 2. Identify if the presence of HIV affects the homeostatic or circadian components of sleep, and 3. Identify if inflammation is present in PLWH with poor sleep.

Methods:

To assess the presence of poor sleep quality in participants at the Nthabiseng HIV Clinic at the Chris Hani Baragwanath Academic Hospital, Soweto (urban), we used the Pittsburgh Sleep Quality Index. To assess the impact of being HIV positive on sleep architecture and timing (circadian) in a study based in Agincourt (rural), Mpumalanga, we used actigraphy and dim light melatonin onset. To assess the presence of poor sleep in participants in Elandsdoorn (rural), Limpopo, we used one question from the Patient Health Questionnaire-9. We also investigated the relationship between the presence of poor sleep in PLWH and C-reactive protein.

Results:

We found poor sleep (Elandsdoorn, 1,040 PLWOH and 887 PLWH (691 treated), poor sleep quality (Soweto, N=139) and circadian misalignment (Agincourt, N=36) in PLWH. We found a positive correlation between poor sleep and inflammation in PLWH, and evidence that PLWH with poor sleep and elevated CRP had an increased risk for developing cardiometabolic disease.

Discussion and conclusion:

Poor sleep is present in PLWH in South Africa, is associated with increased inflammation and could also be a contributor to increased cardiometabolic disease risk. Poor sleep in PLWH in South Africa needs to be investigated as a potential mediator of inflammation and cardiometabolic disease.

Keywords: HIV, sleep, circadian misalignment, inflammation, cardiometabolic disease

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To the teams at the Ndlovu Care Centre, and the Ndlovu Research Facility. Thank you for allowing us to continue with research, amidst all the pushback from government. Thank you for doing the good work that you do and for uplifting the community. Thank you to the study participants for your consistency and for staying in the respective studies to allow more researchers like me the opportunity to gain insights and learn from the data that you are providing.

For more information on these initiatives, please see:

Ndlovu Research Centre: <https://ndlovuresearch.org/>

Ndlovu Care Group: <https://ndlovucaregroup.co.za/>

MRC Wits-Agincourt Unit: <https://www.agincourt.co.za/>

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ABBREVIATIONS

ABC	Abacavir
3TC	Lamivudine
AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
ARV	Antiretroviral
ATV	Atazanavir
AZT	Zidovudine
BDI	Beck's Depression Inventory
CCR5	Chemokine receptor 5
CD4	Cluster of differentiation - 4
CHBAH	Chris Hani Baragwanath Academic Hospital
cIMT	Carotid intima media thickness
CMDR	Cardiometabolic disease risk
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
CRP	C-Reactive Protein
CVD	Cardiovascular disease
D4T	Stavudine
DLMO	Dim Light Melatonin Onset
DRV	Darunavir
DTG	Dolutegravir
EFV	Efavirenz
FTC	Emtricitabine
HIV	Human Immunodeficiency Virus
HPA	Hypothalamic-Pituitary Axis
ICAM-1	Intercellular Adhesion Molecule-1
IFN	interferon
IL	interleukin
INSTI	Integrase Strand Inhibitor
IPAQ	International Physical Activity Questionnaire
LPV/r	Lopinavir boosted with ritonavir
Nef	Negative factor
NREM	Non-Rapid Eye Movement
NVP	Nevirapine
PHQ	Patient Health Questionnaire
PLWH	People Living With HIV
PLWOH	People Living WithOut HIV
PMTCT	Prevention of Mother-to=Child Transmission
PSQI	Pittsburgh Sleep Quality Index
RAL	Raltegravir

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REM	Rapid Eye Movement
RPV	Rilviripine
SCN	Suprachiasmatic Nucleus
Tat	Transactivator of Transcription
TDF	Tenofovir Disoproxil Fumerate
TGF	Transforming Growth Factor
TNF	Tumour necrosis factor
VCAM-1	Vascular Cell Adhesion Molecule-1

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CHAPTER 1: INTRODUCTION

Human Immunodeficiency Virus (HIV) infection is now under control with antiretroviral therapy (ART) having become effective, easy to use (one dose combination, instead of 20 pills a day as in the early 2000s), and widely available and affordable. In most countries, HIV prevalence is below 1% (UNAIDS, 2024). However, there remain regions in the world where HIV prevalence is high and, worryingly, keeps increasing (Maluleke, 2022). One such region of the world is South Africa, a country of 64 million people, which yet holds a staggering 25% of the world's HIV population despite widely available ART.

With treatment, people living with HIV (PLWH) now have life expectancies close to that of people without HIV. Thus, the focus of care of PLWH has shifted from acute care of co-infections arising from HIV infection to long-term chronic care, including treatment adherence and non-communicable diseases (cardiometabolic, mental health, cognitive). Aside from other lifestyle behaviours (increased sedentary behaviour, diets rich in sugars and fat, lower physical activity), one of the factors associated with the rise in non-communicable diseases has been poor/insufficient sleep (disordered sleep). Cumulative evidence throughout the last 20 years has shown that disordered sleep is associated with adverse physiological consequences such as a higher risk of developing hypertension, overweight/ obesity, insulin resistance and immune dysregulation. It is also associated with worse mental health and cognitive function.

A recent review has shown that PLWH have higher risks of having disordered sleep and that the prevalence of poor sleep quality is ~55–60% in PLWH (O'Brien *et al.*, 2021; Wu *et al.*, 2015).

In the literature review, which leads to the aims and objectives of this dissertation, we will be focussing on understanding the context of HIV in South Africa, showing evidence of the rise in cardiometabolic diseases in PLWH, and of

disordered sleep in PLWH and we will examine sleep regulation and the importance of sleep in cardiometabolic disorders. I will then discuss ways that sleep is impacted by factors outside of an individual's control, namely infection and the immune system's response to infection. We will specifically focus on the role that HIV infection itself may modify sleep, and how the treatment of HIV impacts sleep. We will then explore cardiovascular diseases in persons living with HIV and discuss the understudied role that sleep may play in the development of cardiovascular disease. Finally, this leads to the aims of this thesis, and the hope that the findings herein will invoke as many questions as it attempts to answer.

1.1 Understanding HIV in South Africa

1.1.1 Prevalence

Sub-Saharan Africa still holds the title of highest global prevalence of HIV, of which South Africa represents a quarter of all cases. In 2008, the prevalence of HIV was 11.0% of the total population (Lehohla, 2008), and in 2021, this number increased to 13.9% of the population (Maluleke, 2022).

This thesis represents three projects in three different geographical locations in South Africa. In Limpopo, reflected in Chapter 4, the overall HIV prevalence is 8.9%, but in the 25–49 age group, the prevalence increases to 17.0% and 22.3% in men and women, respectively. In rural areas, the HIV prevalence was 7.4%, which is relatively high according to the report (SA News, 2024a)

Gauteng, represented in Chapter 2, has an overall HIV prevalence of 11.7% as of 2022. In the 25–49 age group, the prevalence increases to 14.1% and 24.9% in men and women, respectively. In the older group, 50–54 years old, prevalence increases to 32.2%. In rural areas, the HIV prevalence was 12.7%, which is higher than the average reported for the area (SA News, 2024c).

Mpumalanga, represented in Chapter 3, has the country's highest overall HIV prevalence of 17.4% as of 2022. In the 25–49 age group, the prevalence increases to 19.9% and 31.9% in men and women, respectively. HIV prevalence was higher in rural areas and farms (21.1%) and in rural informal areas (18.4%) and lower in urban areas (15.5%) of the province (SA News, 2024b). In Agincourt, where the study is based, the prevalence increases to 23%, with equal prevalence of HIV across men and women (23.0% and 22.9%, respectively; Gómez-Olivé *et al.*, 2018).

As much as South Africa has a large burden of HIV, we are doing well in our treatment of the infection, which is detailed in the section below.

1.1.2 Antiretroviral therapy and drug classes in South Africa

The management of HIV infection relies on antiretroviral therapy (ART), which involves the use of multiple pharmacological agents to suppress viral replication, restore immune function, and reduce HIV-related morbidity and mortality. These are some of the main goals of the various outreach and educational campaigns that have been run and are currently being run in South Africa – and will be discussed later on in this section.

The five major classes of antiretroviral drugs include nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase strand transfer inhibitors (INSTIs), and entry inhibitors.

NRTIs form the core of most ART regimens and function by mimicking and creating non-functional copies of natural nucleosides, thereby terminating viral DNA synthesis during reverse transcription. In South Africa, the commonly used NRTIs include tenofovir disoproxil fumarate (TDF), lamivudine (3TC), emtricitabine (FTC), abacavir (ABC), and zidovudine (AZT). Historically, stavudine (d4T) was widely prescribed but has since been phased out due to its

association with severe adverse effects, including peripheral neuropathy (Wadley *et al.*, 2011) and metabolic complications (Brinkman *et al.*, 1998; Gallant *et al.*, 2012; van Oosterhout *et al.*, 2012).

NNRTIs, such as efavirenz (EFV), nevirapine (NVP), and rilpivirine (RPV), inhibit reverse transcriptase by binding to a non-active site on the enzyme, inducing conformational changes that prevent the synthesis of viral DNA (Arts and Hazuda, 2012). Protease inhibitors (PIs), such as lopinavir boosted with ritonavir (LPV/r), atazanavir (ATV), and darunavir (DRV), target the HIV protease enzyme, which is essential for the cleavage of viral polyproteins into functional components required for the assembly of mature virions (Park and Morrow, 1993). INSTIs such as dolutegravir (DTG), which has recently (2019) been added to the South African landscape, and raltegravir (RAL) (2011) have become central to first-line therapy due to their potency and favourable safety profiles, inhibit the integrase enzyme responsible for incorporating viral DNA into the host genome (Meintjes *et al.*, 2015; Bangalee, Hangy, and Bangalee, 2022). DTG is preferred over RAG as DTG has been proven to have a higher barrier to resistance (Avihingsanon *et al.*, 2022).

Lastly, entry inhibitors prevent HIV from entering host cells by targeting various steps in the viral entry process. These include CCR5 antagonists such as maraviroc, fusion inhibitors like enfuvirtide, and newer agents such as fostemsavir and ibalizumab, which interfere with viral attachment and post-attachment mechanisms. Together, these drug classes form the basis of highly active antiretroviral therapy (HAART), which has transformed HIV from a fatal disease into a manageable chronic condition. The selection of specific agents within these classes is guided by factors such as drug resistance profiles, comorbidities, tolerability, and national treatment guidelines.

ART regimens typically consist of a combination of drugs from at least two different classes (Feng *et al.*, 2009), targeting distinct stages of the HIV replication cycle. As seen in Table 1, initially, there was only zidovudine available

for adults living with HIV with a CD4 count <200 cells/ μ L, and nevirapine (NVP) for the prevention of mother-to-child transmission. The use of different combinations of drugs is the gold standard for ART in South Africa, as seen in current NDoH guidelines for the treatment and management of HIV/AIDS (NDoH, 2019).

1.1.3 The evolution of treatment guidelines in South Africa

In this section, we will discuss how the guidelines for the treatment of HIV changed in South Africa: from the requirements for being initiated onto ART in 2012, up until the current guidelines. To understand where we are, we should first discuss where we come from. The changes to guidelines for ART initiation in South Africa were in line with the guidelines set out by the WHO and are highlighted in Table 1 below.

In 2012, when data collection for Chapter 2 of this thesis was underway, the guidelines for initiation onto ART for PLWH was that the CD4+ T-cell counts needed to be less than 200 cells/ μ L – this meant that people had to be clinically defined as having AIDS prior to receiving ART (NDoH, 2010). The proposed therapy, at this point, was largely stavudine (d4T) based. Stavudine, a non-nucleoside reverse transcriptase inhibitor (NNRTI), is known to have a high level of toxicity associated with its use, including pain (Wadley *et al.*, 2011; van Oosterhout *et al.*, 2012), lipodystrophy, and dyslipidaemia (van Oosterhout *et al.*, 2012) and thus higher cardiometabolic risk.

As per global evidence of better efficacy, or lower side effect profiles, better tolerability, lower dosages, guidelines are updated to replace certain therapies with newer molecules/combination therapies. This is evident in the 2010 WHO recommendations that motivated the switch from stavudine (d4T) to tenofovir (TDF) - based therapies for people already on d4T to reduce the side effects caused by d4T (Llibre *et al.*, 2006; *et al.*, 2008). In 2013, guidelines were updated again in that there was a shift from initiating people onto d4T to instead initiating them onto TDF therapies (NDoH, 2013), and in 2014, it was mandated that

nobody should be on d4T (Pillay, 2014). Currently, PLWH in South Africa should not be on d4T.

Barriers to access was also the eligibility criteria, detailed in Table 1, specifically the CD4+ T-cell counts. Prior to 2013, CD4+ T-cell counts thresholds for initiation onto ARVs from <200 cells/μL (clinically defined as AIDS). These were updated in 2013, whereby CD4+ T-cell count thresholds for initiation onto ARVs from <200 cells/μL were increased to CD4+ T-cell counts of <350 cells/μl, increasing access to ARVs. This was increased again in 2015 to <500 cells/μl (NDoH, 2015), and in 2019, there was no longer a threshold required for the initiation onto ARVs, ensuring that everyone who needed ARVs could have access to them (NDoH, 2019).

This means that people were able to conserve their immune function while still receiving ART, allowing them to maintain higher levels of CD4-positive T-cell counts for longer and reduce the risk of chronic immune activation. From 2015, there was a movement towards single-pill combinations in the form of fixed-dose combination (FDC) therapies away from free-pill equivalent therapies. This is true for both the FDC containing tenofovir (TDF), emtricitabine (FTC) and efavirenz (EFV) as well as for TLD (TDF, lamivudine (3TC) and dolutegravir (DTG). The single-pill combination at the time of the first study was a combination of efavirenz, emtricitabine, and tenofovir (Davies, 2013) and was rolled out in South Africa in 2014 (Pillay, 2014). The single-pill combination therapies improved adherence (Kruger-Swanepoel *et al.*, 2022), which was one of the key goals of the national strategic plan regarding HIV set out by the South African National Department of Health to ensure that PLWH in South Africa have normal or near-normal life expectancy (NDoH, 2023).

Table 1: Changes in South African treatment guidelines for the initiation, fast track and ineligible access to antiretroviral therapy from 2010 to 2024(current).

Year	Eligibility	Guidelines highlighted in the document	Document	1st line drugs
2004	Eligible	CD4 count <200 cells/μl	Operational Plan for Comprehensive HIV and AIDS Care, Management and Treatment for South Africa	New cases: AZT PMTCT: NVP
2010	Eligible to start Lifelong ART	CD4 count <200 cells/μl irrespective of clinical stage CD4 count <350 cells/μl in patients with TB/HIV, pregnant women WHO stage IV, irrespective of CD4 count MDR/XDR TB irrespective of CD4	The South African Antiretroviral Treatment Guidelines 2010	New patients: TDF + 3TC/FTC + EFV/NVP; On d4T: d4T + 3TC + EFV
	Fastrack (<14 days)	Pregnant women eligible for lifelong ART Patients with very low CD4 (<100) Stage 4, CD4 count not yet available OR MDR/XDR TB		
	Ineligible	Transfer to a wellness programme for regular follow-up and repeat CD4 testing 6-monthly. Advice on how to avoid HIV transmission to sexual partners and children. Initiate INH prophylaxis if asymptomatic for TB. Contraceptives and annual Pap smear		
2013	Eligible to start Lifelong ART	CD4 count <350 cells/μl irrespective of WHO clinical stage Irrespective of CD4 count All types of TB (In patients with TB drug resistant or sensitive, including extrapulmonary TB) WHO stage 3 or 4, irrespective of CD4 count	The South African Antiretroviral Treatment Guidelines 2013	TDF + FTC (or 3TC) +EFV (FDC preferred)
	Fastrack (<7 days)	HIV positive women who are pregnant or breastfeeding Patients with low CD4 <200 cells/μl Patients with Stage 4, irrespective of CD4 count Patients with TB/HIV co-morbidity with CD4 count <50 (Patients with cryptococcal meningitis or TB meningitis (defer ART for 4-6 weeks)		Contraindications to TDF AZT+ 3TC +EFV or (NVP)

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Year	Eligibility	Guidelines highlighted in the document	Document	1st line drugs
	Patients with CD4 count >350cells/μl	Transfer to a wellness programme for regular follow-up and repeat CD4 testing 6-monthly. Advise on how to avoid HIV transmission to sexual partners and children. Initiate INH prophylaxis if asymptomatic for TB. Provide counselling on nutrition and contraception, and do an annual pap smear.		Currently on d4T-based regimen TDF + FTC (or 3TC) + EFV
2014	All patients already on ART	1. All HIV positive patients eligible for initiating ART, including all pregnant women (regardless of CD4 count). 2. HIV positive pregnant women and breastfeeding mothers currently stable on singles 3TC, TDF and EFV. 3. Virologically suppressed patients on a d4T-based regimen with normal renal function. 4, 5, 6, 7 Stable patients receiving singles TDF, 3TC and EFV, with or without co-morbidities, as clinically appropriate.	NDoH Circular: Fixed Dose Combination February 2014. Urgent FDC rollout	FDC: TDF, FTC, EFV
	Other considerations	Adolescents >15 years and weighing >40 kg Adults: All TB co-infection and HBV co-infection Adults and adolescents on d4T: Change D4T to TDF (No patient must be on d4T)	National Consolidated Guidelines for the Prevention of Mother-to-Child Transmission of HIV (PMTCT) and the Management of HIV in Children, Adolescents and Adults	TDF + 3TC (or FTC) + EFV provide as fixed-dose combination (FDC)
Associated campaign to improve detection and treatment of PLWH: 90-90-90 campaign (see paragraph below for details).				
2015	Eligible to start	CD4 count ≤500 cells/μl irrespective of clinical stage (Prioritise those with CD4 ≤350 cells/μl) OR Severe or advanced HIV disease (WHO clinical stage 3 or 4), regardless of CD4 count OR irrespective of CD4 count or clinical stage: Active TB disease (including drug-resistant and EPTB) Pregnant and breastfeeding women who are HIV-positive Known hepatitis B viral (HBV) co-infection Prioritise those with CD4 ≤350 cells/μl or advanced HIV disease	National Consolidated Guidelines for the Prevention of Mother-to-Child Transmission of HIV (PMTCT) and the Management of HIV in Children, Adolescents and Adults	TDF + 3TC (or FTC) + EFV provide as fixed-dose combination (FDC); change d4T to TDF; for people <15 years old OR <40 kgs: ABC + 3TC + EFV
	Immediate initiation	All HIV-positive pregnant or breastfeeding women, as long as no active TB or contraindication to FDC (TDF/FTC/EFV) - in		

IMMUNE ACTIVATION, SLEEP AND CIRCADIAN TIMING IN HIV PATIENTS

Year	Eligibility	Guidelines highlighted in the document	Document	1st line drugs
		which case start AZT 300mg bd immediately and switch to FDC/alternative triple regimen at an appropriate time.		
	Fastrack (<7 days)	Patients with CD4 ≤ 200 cells/ μ l » HIV stage 4, even if CD4 is not yet available		
2016	Immediate initiation	All HIV-positive pregnant or breastfeeding women with no active TB. All HIV positive children, adolescents and adults, regardless of CD4 count, prioritizing those with CD4 ≤ 350 .	NDoH Circular: Implementation of the Universal Test and Treat Strategy for HIV-Positive Patients and Differentiated Care for Stable Patients	FDC (TDF/FTC/EFV)
	Contra-indications for DTG	Women who are trying to fall pregnant should not be prescribed dolutegravir. • Pregnancy should be excluded in women of childbearing age who are being initiated on dolutegravir-based HIV treatment. • Women of childbearing age who are taking dolutegravir should consistently use a highly effective method of contraception.		
		Associated campaign to improve detection and treatment of PLWH: <i>Universal test-to-treat campaign (see paragraph below for details).</i>		
2018	Eligible to start	All people living with HIV (PLWH), regardless of age, CD4 cell count and clinical stage.	Recommendations About the Use of the HIV Medicine Dolutegravir in Pregnancy in Response to the Potential Risk of Birth Defects	FDC (TDF/FTC/EFV)
2019 - present	Fast-track initiation	Pregnant women, infants and children under 5 years, and clients with advanced HIV disease should be prioritised for rapid initiation.	2019 ART Clinical Guidelines for the Management of HIV in Adults, Pregnancy, Adolescents, Children, Infants and Neonates	TLD → TDF, 3TC, DTG. Shift from EFV to DTG
	Defer	TB symptoms, TB diagnosis, other coinfections including cryptococcal meningitis, DS-TB, or bacterial pneumonia. Signs of active liver disease. Associated campaign to improve detection and treatment of PLWH: <i>Undetectable = Untransmittable (see paragraph below for details).</i>		

3TC – Lamivudine, FTC – emtricitabine, d4T – stavudine, DTG – Dolutegravir, TDF – tenofovir, EFV – efavirenz, PMTCT – Prevention of Mother to Child Transmission, TLD – Tenofovir, Lamivudine, Dolutegravir, NDoH – National Department of Health, EPTB – extrapulmonary TB, bd – bidaily, WHO – World Health Organisation, MDR/XDR – multidrug-resistant/extensively drug-resistant TB, ART – antiretroviral therapy.

The UNAIDS 90-90-90 campaign

The UNAIDS 90-90-90 campaign was initiated in 2014 with the target that by 2020, 90% of PLWH will be aware of their HIV-positive status, of those who are diagnosed with HIV, 90% need to be receive sustained ART, of those who are receiving ART, 90% should achieve viral suppression (UNAIDS, 2024). South Africa adopted this campaign in alignment with global standards in December 2014 with the goal of improving the lives of South African PWLH.

As of 2020, South Africa had 84,8% of PLWH aware of their status, 70,1% of those who were aware of their status were on treatment, and 87.4% of those on treatment were virally suppressed (Marinda *et al.*, 2020). In 2022, the ART coverage increased to 80.9%, with an estimated 5,700,000 PLWH on ART.

This campaign ensures that PLWH in South Africa are firstly aware of their status and ensures that they have access to life-saving medication before they experience the incidences of opportunistic infections and other diseases associated with HIV infection that are not treated.

Universal test-to-treat campaign

In alignment with the 90-90-90 campaign, in 2016, the NDoH rolled out the universal test-to-treat (UTT) campaign, where people testing positive for HIV for the first time are to be initiated onto ART, irrespective of the CD4+ T-cell count (NDoH, 2016). South Africa was one of the first countries to roll out UTT, which started on 1 September 2016. Starting ART soon after a positive HIV diagnosis means that people can preserve higher CD4+ T-cell counts if the infection is still in relatively early stages, i.e. not having progressed to AIDS or AIDS-defining illnesses.

This is particularly beneficial for people who get access to treatment early, as this preserves their immunity. Only in cases of active TB infection are people who test positive for HIV deferred from treatment for 2 weeks to treat the TB infection before initiating ART, as seen in the guidelines reflected in Table 1.

Undetectable = Untransmittable

As shown in the previous paragraphs, there is overall increased awareness of HIV and its management with ART in South Africa. The most recent campaign is a public health initiative to bring awareness to scientific evidence that shows that if a person living with HIV achieves an undetectable viral load, they cannot transmit HIV (Cohen *et al.*, 2016; Rodger *et al.*, 2016). Bringing awareness that having undetectable viral loads means that PLWH cannot transmit HIV would encourage more people to get tested for HIV and encourage them to be compliant with the ART regimen (NDoH, 2024). In 2020, an integrase strand transfer inhibitor (INSTI) Dolutegravir was introduced to replace efavirenz (Pillay, 2020). Dolutegravir was more effective at viral suppression, had a better tolerability profile, and improved patient adherence compared to efavirenz, leading to less discontinuation of therapy (Kanters *et al.*, 2020).

Prior to the introduction of DTG into the ARV programme, there were concerns about the effect the drug had on pregnancy, and thus, caution was suggested in women who are pregnant or planning on becoming pregnant (SAHPRA, 2018). Guidelines have been updated after evidence from Brazil showed that there was no risk of neural tube defects while on DTG during the first few weeks of pregnancy (Pereira *et al.*, 2021). Currently, Dolutegravir is available as first-line therapy as a fixed-dose combination with tenofovir and lamivudine, the single-pill combination (TLD). TLD use is currently encouraged by clinicians when initiating PLWH onto ART, along with an awareness campaign by the NDoH to improve the usage of TLD, as depicted in Figure 1.

TENOFOVIR, LAMIVUDINE & DOLUTEGRAVIR (TLD)

A new HIV Treatment. One pill, once a day

🩺 🧑‍⚕️ 💊

TLD is recommended by the World Health Organization for first-line treatment of HIV. It can also be used in second-line treatment of HIV.

WHO QUALIFIES FOR TLD?

- All people living with HIV
- TB patients only after finishing their TB treatment.
- There are other ART options to consider while on treatment for TB.

THE BENEFITS OF TLD ARE:

- To improve the lives of people living with HIV.
- To provide rapid viral suppression.
- High genetic barrier to resistance.
- Fewer side effects.
- Smaller tablet – easy to take.

TLD doesn't replace condoms – use a condom consistently and correctly

FOR MORE INFORMATION

- Visit your nearest healthcare provider/clinic
- Ask your healthcare provider
- www.health.gov.za
- www.bwisehealth.com
- National AIDS Helpline: 0800 012 322

Pregnant women and those planning for pregnancy should discuss the benefits and potential risks of TLD with their healthcare provider to make an informed choice.

TEE is recommended for women who want to get pregnant, and any other TEE patient who is happy with their treatment

TLD is manufactured and supplied by several pharmaceutical companies and comes in different colours, shapes and sizes – all contain the same ingredients. No one pill is better than the other.

Adcock Ingram	Aspen	Aurobindo	Cipla	Hetero	Macleods	Mylan	Sonke

health
Department of Health
REPUBLIC OF SOUTH AFRICA

Cheka Impilo

2019 NDP

www.health.gov.za

@HealthZA

Figure 1. TLD poster by the NDoH to increase awareness of the single-pill combination of Tenofovir, Lamivudine and Dolutegravir (NDoH, 2020).

As outlined in Figure 1 above, some of the goals of TLD are to provide rapid viral suppression and improve the lives of PLWH in South Africa – outlined in and in alignment with the campaigns that have been ongoing globally and locally.

Testing, treating, and goals past 2020

The combined impact of increased testing and increased coverage of ART is to ensure that more PLWH live as long as possible, allowing them to contribute to the economy and become active members of society. The life expectancy of PLWH on sustained ART is now increased to a similar life expectancy as people living without HIV (PLWOH) (Wandeler, Johnson and Egger, 2016; Trickey *et al.*, 2023), demonstrating the effectiveness of adhering to ART treatment. Though South Africa is only at 81.4% ART coverage, falling short of the 90-90-90 goals set out for 2020, South Africa's new goal is the 95-95-95 campaign. The goal of this campaign is that by 2030, 95% of PLWH know their HIV-positive status, of those PLWH, 95% need to be taking sustained ART, and of those on ART, 95% should achieve viral suppression.

1.2 Increased life expectancy and the emergence of non-communicable diseases in PLWH

PLWH on treatment are thus living longer lives (Wandeler, Johnson and Egger, 2016; Trickey *et al.*, 2023) and are more likely now to experience diseases of lifestyle and aging (non-communicable diseases, NCDs) as HIV is being managed as a chronic condition (Moyo-Chilufya *et al.*, 2023). Some of the NCDs that are on the increase for PLWH include hypertension, diabetes, hypercholesterolaemia and obesity (Patel *et al.*, 2019).

This is also true for South African PLWH (Gómez-Olivé *et al.*, 2018), as South Africa has an overweight/obesity rate of over 50% (20 million people out of an adult population of approximately 40 million; Lehohla, 2017). In a recent report by the South African Department of Health, 31% of South African men, and 68% of South African women were classified as being overweight or obese (Discovery Vitality, 2023; National Department of Health (NDoH), 2023), making South the second largest country for obesity prevalence in the world (after Mexico but before the USA). Obesity predisposes

to a plethora of other NCDs and independently contributes to the development of cardiovascular disease (CVD; Battineni *et al.*, 2021). Being HIV-positive is associated with an increased risk of developing cardiovascular disease (Vos *et al.*, 2016), including increased risk of cardiovascular events such as myocardial infarction (Triant *et al.*, 2007; Freiberg *et al.*, 2013). These increased risks are not negated by the use of ART, which can itself – directly and indirectly – also have cardiometabolic consequences.

1.3 Cardiometabolic consequences of HIV in PLWH on ART

The use of ART can lead to changes in body fat distribution, which can further lead to lipid disorders such as dyslipidaemia (Bacchetti *et al.*, 2006), but ART can also affect lipids and insulin sensitivity (Pinto and da Silva, 2018).

1.3.1 Dyslipidaemia in PLWH on ART

People with dyslipidaemia—characterised by elevated total cholesterol, triglycerides, or LDL-cholesterol, and/or reduced HDL-cholesterol—are at increased risk for developing cardiovascular disease (CVD) (Stein, Ferrari and Scolari, 2019; Du and Qin, 2023). Among people living with HIV (PLWH), viraemia has been associated with a dyslipidaemic profile marked by elevated triglycerides and reduced HDL-cholesterol (Kelesidis and Currier, 2014), suggesting an increased CVD risk, although the underlying mechanisms remain poorly understood.

Before initiating antiretroviral therapy (ART), PLWH typically present with low levels of total cholesterol, LDL-cholesterol, and HDL-cholesterol. However, ART initiation has been shown to significantly increase total and LDL-cholesterol levels, with little to no improvement in HDL-cholesterol (Riddler *et al.*, 2003), indicating that ART itself may contribute to dyslipidaemia. A meta-analysis by Dillon *et al.* (2013) found that in sub-Saharan African populations, HIV infection is associated with higher triglycerides and lower HDL cholesterol and HbA1c levels compared to HIV-negative individuals (Dillon *et al.* 2013). ART exposure, on the other hand, was linked to higher HDL and LDL cholesterol, lower triglycerides, and lower HbA1c levels compared to untreated PLWH.

Despite these lipid profile changes, PLWH often exhibit higher CVD risk than PLWOH, even when traditional risk markers (BMI, waist circumference, hypertension) are not as high. This suggests that additional, non-traditional factors may be contributing to CVD risk in PLWH. One such factor is sleep and circadian disruption, which has been shown to influence cardiovascular health in the general population (Buxton, 2010; Grandner et al., 2012).

In the South African context, a study of truck drivers (9.7% HIV positive) found that circadian misalignment among HIV-positive individuals and obstructive sleep apnoea across the cohort were associated with increased CVD risk (Roche et al., 2021). These findings highlight the role of sleep-related disturbances, such as circadian misalignment and sleep apnoea, in the development of CVD, particularly in PLWH.

Moreover, sleep disturbances in PLWH have been directly linked to increased risk of cardiovascular events (Polanka et al., 2019). This growing body of evidence suggests that poor sleep quality and disrupted circadian rhythms may amplify cardiovascular risk in PLWH, potentially through mechanisms involving inflammation, autonomic dysfunction, and metabolic dysregulation. These findings underscore the need to consider sleep health as a critical component in the management of cardiovascular risk among PLWH.

1.3.1.1. Non-traditional lifestyle factors that contribute to cardiovascular disease

People living with HIV (PLWH) face an elevated risk of developing cardiovascular disease (CVD), even when traditional risk factors such as hypertension or dyslipidaemia are present at lower levels compared to people living without HIV (PLWOH) (Freiberg et al., 2013; Shah et al., 2018; Eyawo et al., 2019). In addition to conventional risk factors, non-traditional lifestyle and psychosocial factors may also contribute to this increased risk.

Psychological stress and mood disorders, including depression and anxiety, have been shown to play a significant role in cardiovascular health (Furman et al., 2019; Derry et al., 2022). For example, a study in Korea found that PLWH experiencing both

depression and anxiety were more than twice as likely to have moderate or high CVD risk compared to those without these conditions (Park *et al.*, 2020).

Stress is particularly prevalent among PLWH and has been strongly linked to systemic inflammation (Fumaz *et al.*, 2012; Siyahhan Julnes *et al.*, 2016), which in turn contributes to arterial stiffness (Chow *et al.*, 2016)—a known precursor to endothelial dysfunction and cardiovascular disease. Depression has also been associated with immunological changes in PLWH, including reduced CD4 counts and increased viral loads, both of which are linked to heightened inflammation (Rivera-Rivera *et al.*, 2016).

Moreover, elevated depression scores have been directly correlated with increased levels of C-reactive protein (CRP), a marker of inflammation (Poudel-Tandukar *et al.*, 2014). A recent systematic review concluded that depression may act as an independent risk factor for CVD in PLWH (Polanka *et al.*, 2023). Taken together, these findings underscore the complex interplay between psychological health, inflammation, and cardiovascular risk in PLWH (Barnes, Lacson & Bahrami, 2017; Ou-Yang *et al.*, 2023).

1.3.2 PLWH and endothelial dysfunction

One of the potential causes of increased CVD risk in PLWH is due to endothelial dysfunction as a result of the action of HIV. Directly, HIV proteins involved in viral pathogenesis can impair endothelial function. Indirectly, these proteins may drive systemic inflammation and promote the generation of reactive oxygen species and adhesion molecules, further compromising vascular health. Treatment of HIV may contribute as well, and even in PLWH with undetectable viral loads, the secretion of these viral proteins may still be secreted at basal levels and can continue to contribute to endothelial dysfunction.

1.3.2.1. Pathways that could impact endothelial dysfunction during HIV infection: HIV Proteins

The negative regulatory factor (Nef) protein is strongly implicated in HIV pathogenesis, where it promotes viral replication, T-cell apoptosis, and persistent immune activation, all of which drive systemic inflammation. The transactivator of transcription (Tat) protein further amplifies this inflammatory milieu by enhancing cytokine production and disrupting normal cellular signalling, with downstream effects on sleep regulation (Clark *et al.*, 2005; Wang *et al.*, 2014) and neuroinflammation (Saro *et al.*, 2021; Yarandi, Duggan and Sariyer, 2021; Muvenda, Williams and Williams, 2024). Both Nef and Tat remain detectable even in people living with HIV (PLWH) on suppressive ART, sustaining a state of chronic low-grade inflammation (Mediouni *et al.*, 2012).

These proteins are known also to directly impact the function of the vascular endothelium, contributing to the development of endothelial dysfunction (Anand, Rachel and Parthasarathy, 2019). In endothelial cells, Nef can cause cellular apoptosis, generation of radical oxygen species, and release of monocyte chemoattractant protein-1 (MCP-1) (Wang *et al.*, 2014). This damage to endothelial cells caused by Nef can still persist even in PLWH with undetectable viral loads (Chevlanambi, 2019). The Tat protein similarly damages endothelial cells directly, and increases permeability of the blood brain barrier (Sun *et al.*, 2023).

An effect of this persistent immune activation is the upregulation of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) (Liu *et al.*, 2017; Guo *et al.*, 2024). These molecules are expressed on activated endothelial cells, monocytes, and macrophages, and facilitate leukocyte adhesion and transmigration across the vascular endothelium (Lamers *et al.*, 2012; Jiang *et al.*, 2023). Their increased expression in HIV infection contributes not only to vascular inflammation, but also to endothelial dysfunction, thereby linking chronic HIV-driven inflammation to heightened cardiovascular disease (CVD) risk.

Persistent Tat- and Nef-mediated immune activation drives secretion of proinflammatory cytokines such as interleukin-6 (IL-6), largely through monocyte and macrophage activation (Haij *et al.*, 2015). IL-6 acts as both a proinflammatory and atherogenic

cytokine, stimulating hepatic production of C-reactive protein (CRP) (Jiang *et al.*, 2023). Elevated IL-6 and CRP levels have been consistently observed in people living with HIV, even in those well controlled on ART, and are strongly associated with increased risk of non-AIDS comorbidities, particularly CVD and neurocognitive impairment (Kuller *et al.*, 2008; Duprez *et al.*, 2012).

Upregulation of ICAM-1 and VCAM-1 levels complements this cytokine-driven response, as endothelial activation creates an adhesive interface for immune cells, increasing vascular inflammation and sustaining high levels of IL-6 and CRP. This, as mentioned, can contribute to endothelial dysfunction and increase the risk for PLWH developing cardiovascular disease and having cardiovascular events, including myocardial infarction (Shah *et al.*, 2018).

The direct effects of HIV proteins on endothelial cells as well as the persistent inflammation that they drive, contribute to the development of endothelial dysfunction, and can add to the development of cardiovascular disease. Understanding the underlying factors that contribute to CVD in PLWH is essential for reducing CVD risk and mortality. One of the factors that contributes to cardiometabolic disorders and CVD is sleep. Poor sleep is associated with increased BMI, higher levels of inflammation, and overall increased risk of CVD.

1.3.2.2. Treatment of HIV and impact on endothelial dysfunction

Treatment of HIV with protease inhibitors gives rise to insulin resistance in 25-60% of patients (Behrens *et al.*, 2002), contributing to the development of diabetes. ART has also been shown to increase endothelial dysfunction in PLWH (Wang *et al.*, 2007), which contributes to the development of cardiovascular disease. Inflammation is one of the key drivers of endothelial dysfunction, and one of the easiest markers of inflammation is C-reactive protein (CRP). Not only is CRP associated with endothelial dysfunction, arterial stiffness, and elevated blood pressure (Hage, 2014), but higher levels of CRP are found in people with diabetes and dyslipidaemia. All these factors indicate that CRP would be a good biomarker for assessing CVD risk in PLWH.

1.3.3 Evidence of PLWH and death relating to CVD

PLWH have a higher risk of death as a result of CVD. In the context of ischaemic heart disease, PLWH have a 40 - 50% higher chance of death by myocardial infarction than PLWOH (Freiberg *et al.*, 2013; Shah *et al.*, 2018; Eyawo *et al.*, 2019) and some reports even mention a double risk for CVD events (Shah *et al.*, 2018). Even when adjusting for traditional risk factors for CVD, PLWH have a higher risk of mortality than PLWOH.

1.3.4 The mental health of PLWH on chronic ART

Along with problems with physical health on chronic treatment for HIV, PLWH are experiencing increased levels of mental health complaints, including depression and anxiety (Hoare *et al.*, 2021). Before the advent of ART, there was a high incidence of HIV-associated neurocognitive decline (HAND) that eventually led to death. However, taking ART prevents that decline and mortality, prolonging life for PLWH.

PLWH have more mental health complaints than PLWOH, with depression being the most common complaint. In South Africa, a study conducted in 2017 showed that the most common mental health complaints in people seeking HIV testing were alcohol use disorder (19,5%), followed by major depressive disorder (14,2%), generalised anxiety disorder (5%) and post-traumatic stress disorder (4.9%; Kagee *et al.*, 2017).

In 2024, in a study of ~18600 participants, PLWH had higher levels of psychological distress (27.4%) than PLWOH (20.1%). In PLWH, rating their health as either fair/poor was associated with 1.22 times higher odds of reporting psychological distress (Vondo *et al.*, 2024). Depression is known to impact quality of life in the general population, and for PLWH, depression is associated with lower adherence to ART (Yen *et al.*, 2022; Kyung *et al.*, 2023).

In the context of depression and mental health, sleep complaints, including poor sleep quality, insomnia, and short sleep duration, are all associated with increased risk of developing depression. And, for people with depression, sleep complaints are present in over 70% of confirmed cases.

Before we delve into poor sleep, its consequences, and what it means for PLWH with poor sleep, we first need to understand normal (healthy) sleep.

1.4 Healthy sleep, sleep regulation, sleep and circadian influences on hormonal diurnal rhythms, which impact cardiometabolic risk

1.4.1 Healthy sleep, homeostatic and circadian regulation of sleep

A healthy sleep duration in adulthood is considered to be between 7 and 9 hours per night. Sleep is under a two-process model of control, i.e. homeostatic (S) (see Figure 2) and circadian (C). Homeostatic control of sleep relies on the sleep-wake cycle and the build-up of sleep pressure (homeostatic sleep drive) throughout the biological day and dissipating the sleep pressure by going to sleep, and then waking up in the morning refreshed (Rechtschaffen and Kales, 1968).

In those 7–9 hours of sleep, there is a cycle between lighter sleep (non-rapid eye movement sleep (NREM stages 1 and 2), deep sleep (NREM stage 3) and rapid-eye-movement (REM) sleep (Rechtschaffen and Kales, 1968). The time taken to fall asleep is typically less than 20 minutes in healthy adults.

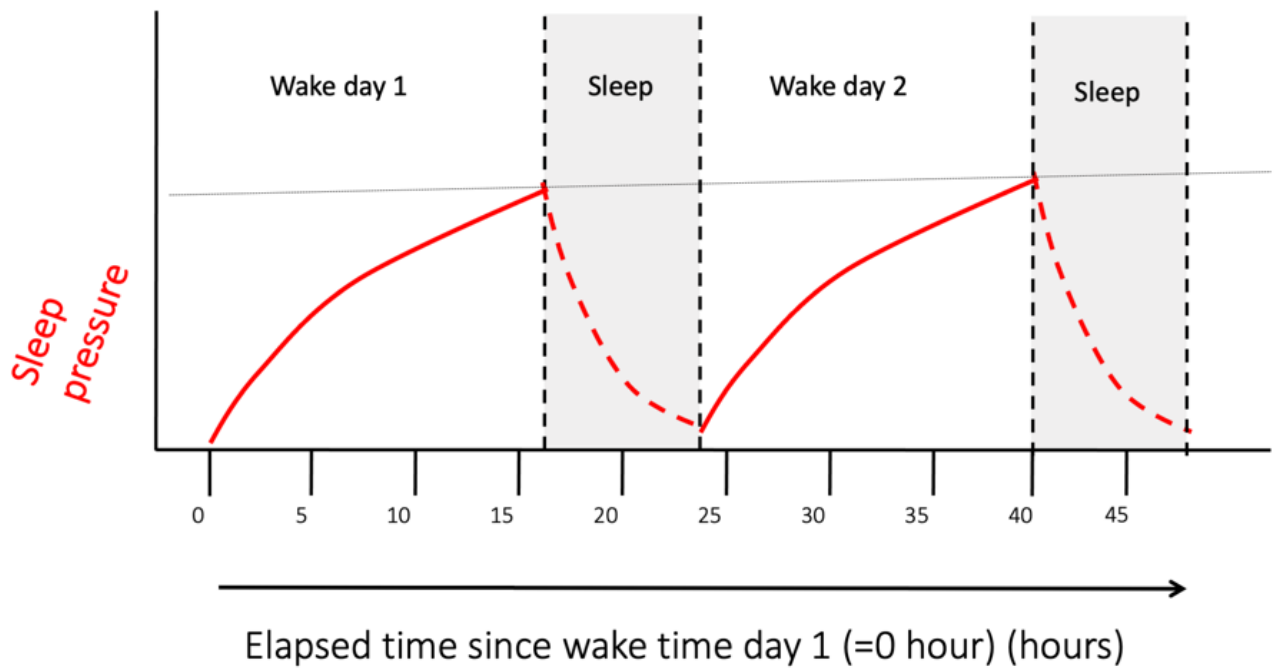


Figure 2: Graph showing the build-up of sleep pressure throughout the day and dissipation during the sleep period. Under perfect conditions, there is alignment between the homeostatic process of sleep and the circadian process of sleep whereby, as homeostatic sleep pressure builds up, the circadian drive through the suprachiasmatic nucleus of the hypothalamus (SCN) increases the signal for wakefulness, allowing for a consolidated bout of wake (16 h); and whereby the SCN starts promoting sleep ~1 hour before habitual bedtime by decreasing its firing to the wake nuclei, allowing for a consolidated bout of sleep (8 h).

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Under normal conditions, a person cycles through the day alert and awake, and then during the biological night, goes to sleep to dissipate sleep pressure. The homeostatic control of sleep is a sleep-dependent process. It involves sleep and wake nuclei in the hypothalamus as well as the build-up of adenosine in the synaptic cleft. Adenosine, a byproduct of ATP breakdown in the body, is considered a key regulator in the control of the sleep-wake system (Landolt, 2008). The build-up of adenosine in the synaptic cleft translates to the build-up of sleep pressure during time spent awake. The sleep pressure build-up throughout the day results in an individual experiencing tiredness towards the end of the day.

Adenosine levels decrease slightly when drinking coffee, as caffeine acts as a ligand that binds adenosine, making coffee an antagonist to tiredness and, therefore, sleep. Once caffeine is consumed, sleep pressure builds up again from the new baseline that has been set once some adenosine is bound to caffeine (Reichert, Deboer and Landolt, 2022). When an individual goes to sleep, the sleep pressure built up over the time spent awake dissipates throughout the sleep period, usually during the biological night. Throughout the sleep period, sleep pressure dissipates until there is no sleep pressure left, and they wake up in the morning feeling, hopefully, refreshed. Then the day starts again – as seen in Figure 2.

Sleep is categorised into different stages as per the American Academy of Sleep Medicine (AASM) guidelines (Berry *et al.*, 2017). Deep sleep (also known as NREM 3) occurs in the first half of the night, with lighter sleep occurring in the second half of the night, during rapid eye movement (REM) sleep, occurring more in the second part of the sleep episode. This is depicted in Figure 3 below, showing the different stages of sleep.

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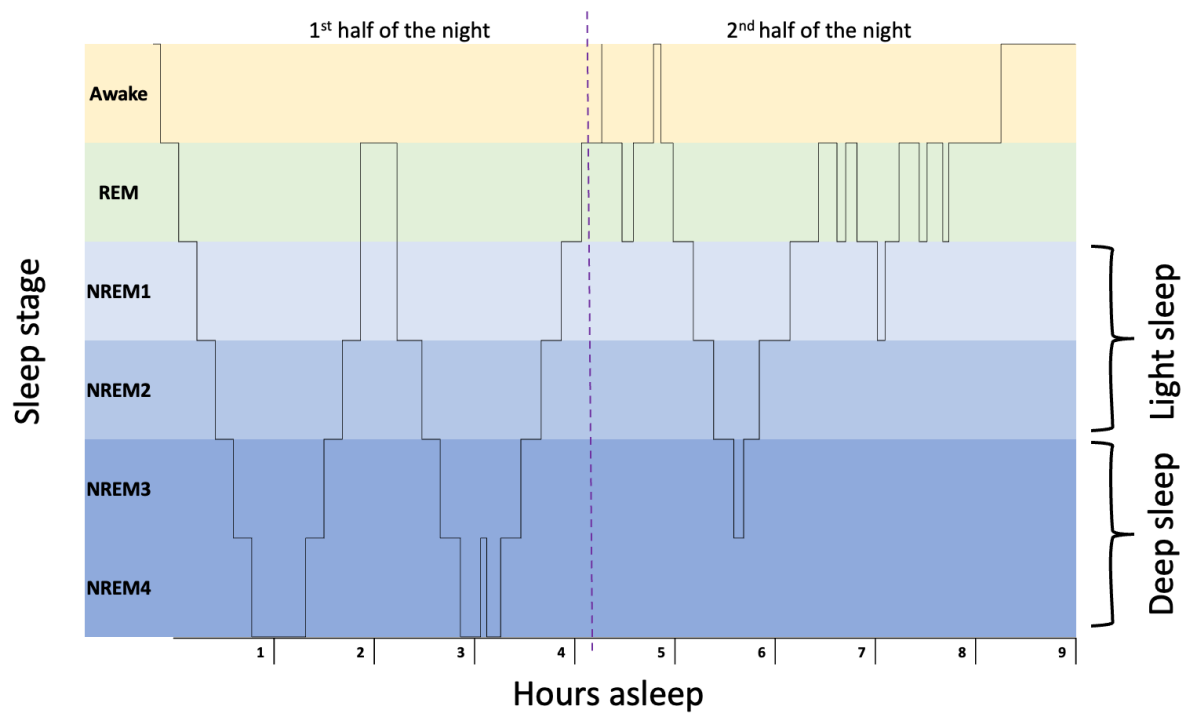


Figure 3: Cycles of sleep throughout the night. During the first half of the night (left of the dotted line), there is more slow wave sleep, and the second half (right of the dotted line) of the night has more light sleep and REM sleep.

Brain activity during REM sleep is close to that of wake, but without the bodily function and alertness of actual wakefulness (Chow *et al.*, 2016). An individual cycles through these stages (NREM 1, 2, 3 - REM) of sleep every night, with each sleep cycle lasting approximately 90 minutes (Figure 3). High amounts of sleep pressure, sleeping at an appropriate time, or underlying health conditions can impact the quality and the quantity of sleep (Bryant, Trinder and Curtis, 2004; Ibarra-Coronado *et al.*, 2015; Besedovsky, Lange and Haack, 2019).

Quantity of sleep is important, but so is the quality of sleep. If a person has poor sleep (due to disruptions, awakenings, or not reaching the phases of restorative deep NREM sleep), this can spill over into waking life and cause many deficits such as increased daytime sleepiness (Bryant, Trinder and Curtis, 2004; Ibarra-Coronado *et al.*, 2015; Besedovsky, Lange and Haack, 2019), reduced cognitive function (Wang *et al.*, 2022), reduced motor skills (Appleman *et al.*, 2016), increased reaction times (Van Dongen *et al.*, 2023) Mahajan and Velaga, 2020), and increased hunger (Spiegel *et al.*, 2004). The effects of poor sleep quality and insufficient sleep duration on hunger, weight gain and obesity will be discussed later in this chapter.

The second process controlling sleep is the sleep-independent process, known as the “biological clock” or “circadian rhythm”. The circadian control of sleep relies largely on light entrainment and is independent of the homeostatic control of sleep (Borbély, 1982). This process relies on the suprachiasmatic nucleus (SCN) within the hypothalamus, which is entrained by light. Light activates N-methyl-D-aspartate (NMDA) receptors within the SCN, which causes changes in the firing of the neuron cluster of the SCN. This firing has a near 24-hour rhythm, which allows entrainment to the Earth’s 24-hour light-dark cycle of the SCN’s outputs, e.g. behavioural cycles (sleep-wake, eating), physiological variables (blood pressure, heart rate, core body temperature, and hormonal release such as cortisol, melatonin, vasopressin, and aldosterone) and receptor expression (insulin receptors, for example). This sensitivity to light of the SCN follows a phase response curve (Khalsa *et al.*, 2003) with light in the late biological night/early biological day resulting in phase advances (earlier timing of the SCN), while light in the late biological day/early biological night results in phase delays (later timing of the SCN and hence of its outputs). It has different sensitivity to different wavelengths

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of light, with its highest sensitivity being to blue light (450 to 495 nm; Brainard *et al.*, 2001; Lockley, Brainard and Czeisler, 2003)

1.4.2 *Diurnal rhythms of hormones associated with cardiometabolic risk: sleep and circadian influences*

The three main outputs and thus good markers of circadian rhythm are core body temperature, melatonin and cortisol (Benloucif *et al.* 2008). Core body temperature peaks in the evening, and the lowest temperature usually occurs an hour before habitual wake time (Aschoff, 1967). As seen in the Czeisler studies, humans operate on a roughly 24-hour cycle, with melatonin being a fundamental marker of circadian phase in humans (Czeisler *et al.* 1981). These studies revealed just how powerful the circadian system is in ensuring that certain processes occur at the same time during the 24-hour period, and just how important natural light is for entraining this pathway. Hence the name: the light entrainment pathway – depicted in Figure 4 below. Hormones that are included in this section are summarised in Table 2.

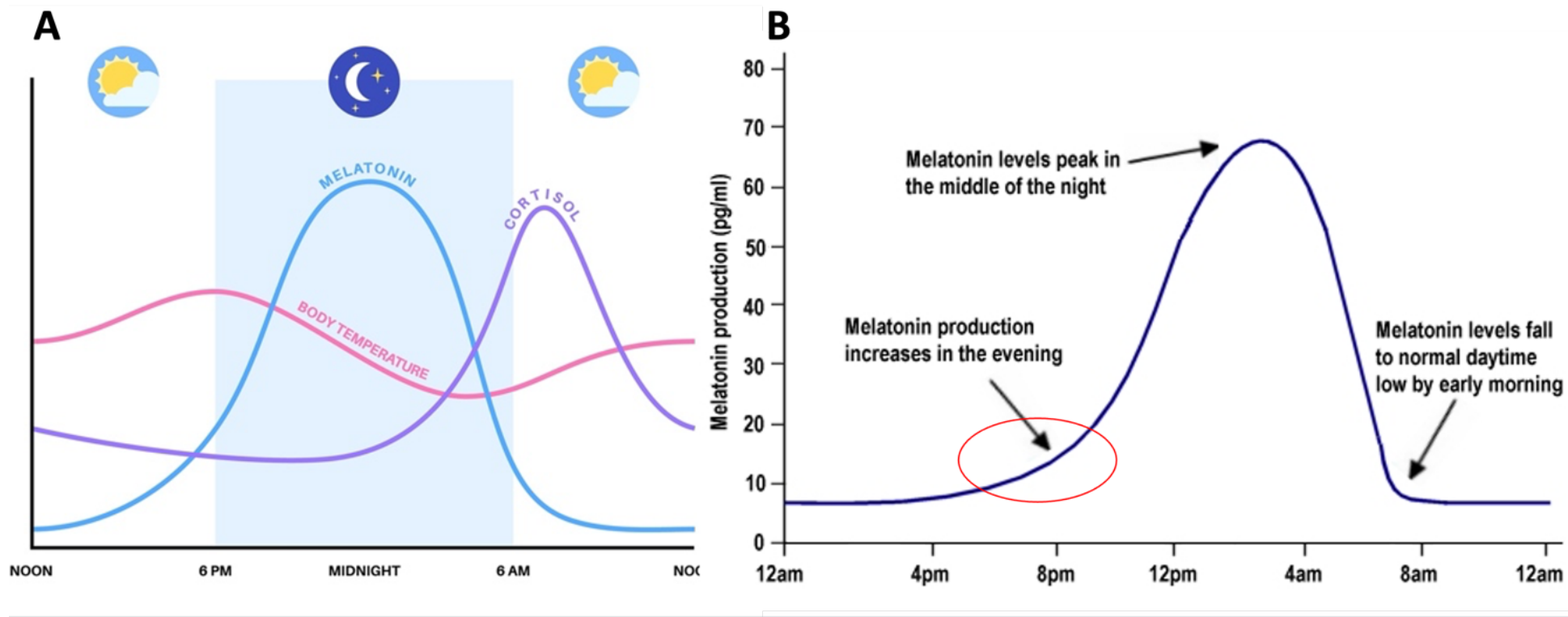


Figure 4: A) Melatonin and cortisol cycles throughout the day. During the biological night, melatonin peaks, with melatonin onset occurring in the evening (image from Shutterstock). B) Melatonin onset (circled in red) at 8 pm occurs early in the evening, 2 hours before bedtime.

Melatonin and cortisol

The hormones involved in the control of the sleep-wake cycle are melatonin and cortisol (Sharma *et al.*, 1989). Melatonin increases during the biological night, peaking in the hours before habitual sleep time (Burgess *et al.*, 2003). Cortisol levels increase during the biological day, peaking around 8 am, and tapering off towards the evening (Chan and Debono 2010). Both these hormones' levels shift away from normal when an individual works in shifts (Brum *et al.*, 2022), and this can contribute to an increased risk of developing disease. Overall, melatonin and cortisol actively influence sleep and circadian rhythms. Thus, melatonin signals the body for sleep by increasing at night, while cortisol promotes wakefulness by peaking in the early morning. Modifications in circadian rhythms may result in altered fluctuations in either of these hormones.

Appetite and satiety

Leptin and ghrelin are key hormones in the regulation of appetite and satiety throughout the day. Leptin, often called the "satiety hormone", follows a diurnal rhythm, with its levels rising through the biological day and peaking around 01:00 in the biological night. After peaking, leptin levels gradually decrease throughout the biological night, hitting a low point around 11:00 in the morning (van Aggel-Leijssen *et al.*, 1999). Leptin is produced in adipose tissue and is found in higher concentrations in persons who are obese (Obradovic *et al.*, 2021; Perakakis, Farr and Mantzoros, 2021). Ghrelin, known as the "appetite-stimulating hormone", is influenced by the sleep-wake cycle and energy intake (Cummings *et al.*, 2002; Markwald *et al.*, 2013; Qian *et al.*, 2019) and displays a circadian rhythm. Furthermore, leptin and ghrelin align with feeding and fasting periods tied to circadian patterns.

Insulin and glucose homeostasis

Similarly, insulin, which plays a significant role in glucose metabolism, increases in response to meal timings and follows a circadian rhythm (Buxton *et al.*, 2012; Morris *et al.*, 2015). Insulin levels are typically lowest during the biological night, between 3:00 and 5:00 am, corresponding to reduced insulin secretion and sensitivity. This decrease in insulin secretion aligns with the body's overall reduced metabolic activity during the night, indicating a circadian pattern in glucose metabolism (Boden *et al.*, 1996).

Other hormones: adrenaline and noradrenaline

Both adrenaline and nor-adrenaline have been shown to affect sleep. Adrenaline, an adrenocorticosteroid released by the adrenal gland in response to stress, negatively impacts sleep (Linsell *et al.*, 1985; Dumbell, Matveeva and Oster, 2016). i.e., when a person is stressed, the hypothalamic pituitary axis (HPA) is activated, and adrenaline is released. Levels of adrenaline are low during sleep, but increase slightly upon awakening. Adrenaline follows a circadian rhythm, and levels are affected by the functioning of the suprachiasmatic nucleus. Noradrenaline, a catecholamine hormone, is also synthesised by adrenal cells of the adrenal gland. Levels of noradrenaline are more affected by sleep-wake regulation than by a circadian clock.

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Table 2: The effects of hormones known to impact sleep, how they affect sleep, and whether they follow a circadian rhythm (Process C) or are impacted by homeostatic (Process S) sleep.

Hormone	Function	Role in Sleep	Circadian or homeostatic sleep	Reference
Melatonin	Sleep regulation	Facilitates sleep onset and plays a role in sleep maintenance throughout the night. Levels are negatively impacted by blue light exposure. Regulates circadian rhythm. Peaks at night, lowest during the day – see Figures 3A and B. Regulates circadian rhythms by syncing body clock via suprachiasmatic nucleus.	Marker of circadian phase	(Zisapel, 2018)
Cortisol	Stress hormone	Impacts alertness and wakefulness; elevated cortisol may disrupt sleep quality. Peaks early morning - soon after waking. Declines throughout the day, with a secondary small rise in late afternoon. See figure 3A.	Circadian	(Azmi <i>et al.</i> , 2021)
Leptin	Satiety	Levels increase during the first half of the night, then reduce in the second half of the night; align with sleep duration. Low levels of leptin aligned with increased hunger (i.e. in the morning upon waking up).	Under circadian control	(Hsuchou <i>et al.</i> , 2013; Mosavat <i>et al.</i> , 2021)
Ghrelin	Appetite-stimulating hormone	Levels of ghrelin are low during the biological night and increase during the biological day. Inversely related to leptin and sleep patterns.	Diurnal and affected by sleep disturbances	(Kulkarni, Singh and Zigman, 2024)
Insulin	Glucose homeostasis	Sleep deprivation and circadian misalignment (i.e. shift work) can reduce insulin sensitivity, impacting glucose control, predisposing individuals to diabetes and other metabolic disorders. Insulin sensitivity peaks during the day; disrupted sleep and circadian misalignment can impair glucose regulation and increase diabetes risk. Insulin levels nadir at 6 am and reach a peak in the afternoon. <i>Meaning: glucose tolerance and insulin sensitivity are higher in the morning than at night.</i>	Circadian	(Boden <i>et al.</i> , 1996; Kurose, Yabe and Inagaki, 2011; Buxton <i>et al.</i> , 2012; Morris <i>et al.</i> , 2015)

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Hormone	Function	Role in Sleep	Circadian or homeostatic sleep	Reference
Adrenaline	Other stress hormones	Increase can disrupt sleep; chronic stress may impair sleep quality. Levels of adrenaline are low during sleep, with levels rising in response to stress or early in the morning. Modulated by SCN effect on HPA axis activity.	Follows a circadian rhythm, reduced during sleep	(Linsell <i>et al.</i> , 1985; Dumbell, Matveeva and Oster, 2016)
Noradrenaline	Stress hormone	Levels are affected by the circadian rhythm, but are more affected by sleep-wake regulation.	Homeostatic	(Linsell <i>et al.</i> , 1985; Dumbell, Matveeva and Oster, 2016)

SCN = suprachiasmatic nucleus, HPA = hypothalamic-pituitary axis.

1.5 Cardiometabolic consequences of poor sleep and/or circadian misalignment

Long-term sleep deprivation leads to weight gain, which conversely leads to an increased risk of having disordered sleep (Beccuti and Pannain, 2011). Chronic sleep deprivation causes a disruption in the secretion and sensitivity to ghrelin, the hormone that controls appetite/hunger. Studies have shown that during periods of sleep deprivation, there is an increase in ghrelin secretion, increasing hunger levels – usually increasing cravings for foods that have a high carbohydrate content (Nedeltcheva *et al.*, 2009; Dashti *et al.*, 2015); as well as reducing leptin sensitivity – so an individual is not satiated even with their increase in caloric intake (Mosavat *et al.*, 2021).

Poor sleep and circadian misalignment have direct effects on cardiovascular disease risk. When there is a mismatch between the timing of sleep and the endogenous timing of sleep, this is called circadian misalignment. Such phenomena can be seen in shift work and forced desynchrony studies (Dijk *et al.*, 1999) and have been shown to have negative impacts on the immune system, sleep quality, and metabolism (Scheer *et al.*; Morris *et al.*, 2017).

Circadian misalignment is associated with higher plasma concentrations of inflammatory cytokines (IL-6, TNF- α) and plasma C-reactive protein (CRP) levels (Leproult, Holmbäck and Van Cauter, 2014; Morris *et al.*, 2016, 2017). These factors independently contribute to the pathogenesis of inflammation, contributing to cardiovascular disease. Disrupted sleep and circadian misalignment are associated with endothelial dysfunction (Morris *et al.*, 2016), which, as mentioned earlier, is one of the main drivers of cardiovascular disease (Cai and Harrison, 2000; Widmer and Lerman, 2014).

Chronic sleep disruption also affects platelet formation and platelet aggregation (Scheer *et al.*, 2011). Endogenous platelet activation was found to be under circadian control, with the highest expression of glycoprotein (Gp) IIb/IIIa molecules on platelets at ~8-9 am, suggesting that this may explain the higher risk for arterial thromboses in the morning (Scheer *et al.*, 2011).

Thus, modifications in sleep and circadian rhythms are associated with several physiological pathways, which are involved in cardiometabolic diseases through traditional cardiometabolic risk factors. In addition, sleep has a bidirectional relationship with the immune system, whereby poor sleep quality is associated with higher immune activation (Frey *et al.*, 2007; Besedovsky *et al.*, 2019), while higher immune activation may further disrupt sleep (Mullington *et al.*, 2000; Meier-Ewert *et al.*, 2004; Sharpley *et al.*, 2016). It has been hypothesised that poor sleep quality may enhance cardiovascular risk in people with chronic immune activation through non-traditional mechanisms, i.e. by further enhancing immune activation, which is associated with endothelial dysfunction and higher insulin resistance. In this next section, we will investigate the bidirectional relationship between sleep and the immune system, first by examining the central nervous system to immune system pathways, then by focusing on the bidirectional relationship between sleep and immunity.

1.6 Sleep and the immune system

As mentioned, there is a bidirectional relationship between the brain and the immune system that enables the brain to control inflammation, while inflammation, in turn, can affect neural activities in the brain, including sleep patterns. The brain, directly and indirectly, modulates inflammation, primarily through neural and humoral pathways.

1.7 Neural pathway influencing inflammation/immune response

1.7.1 The immune inhibitory effects of the parasympathetic nervous system

Neural pathways include activation of the parasympathetic nervous system via vagus nerve stimulation. Stimulation of the vagus nerve causes the release of acetylcholine (Li *et al.*, 2021), which suppresses the release of proinflammatory cytokines from vagus nerve-innervated tissue macrophages. Specifically, acetylcholine binds to the alpha 7 nicotinic acetylcholine receptor ($\alpha 7$ nAChR) on macrophages and other immune cells (Nakamura *et al.*, 2023), suppressing the production of pro-inflammatory cytokines (TNF- α , IL-1 β , and IL-6; Tracey, 2007).

During inflammation, the presence of IL-1 β and TNF- α stimulates the brainstem, triggering an anti-inflammatory reflex to reduce cytokine production, reducing inflammation (negative feedback). The vagus nerve, acetylcholine and cytokines form part of the cholinergic anti-inflammatory pathway (Tracey, 2007).

1.7.2 Immune stimulatory effects of the sympathetic nervous system

Sympathetic nervous system innervation also causes the release of noradrenaline, which modulates immune cell activity to produce proinflammatory cytokines such as IL-1 β (Goehler *et al.*, 1997) and TNF- α (Zielinski *et al.*, 2013). HIV is also known to impact the release of these pro-inflammatory cytokines (Buonaguro *et al.*, 1992), further adding to the inflammatory milieu.

1.7.3 HIV and suppression effects on the parasympathetic nervous system

HIV has been shown to negatively impact the cholinergic anti-inflammatory pathway (Ríos *et al.*, 2021). PLWH show reduced vagal tone, which is typically measured by heart rate variability (Mittal *et al.*, 2004). Vagal dysfunction is associated with altered cytokine networks in PLWH (Robinson-Papp *et al.*, 2018). The reduction in vagal tone is one of the possible mechanisms by which infection with HIV leads to chronic inflammation.

1.8 Humoral pathway: proinflammatory cytokines influence brainstem functions (including sleep-wake pathways)

The humoral pathway involves the blood-brain barrier, through which certain cytokines can pass. In the area postrema in the medulla, which is a circumventricular organ with permeable capillaries and sensory neurons, cytokines such as IL-1 β , IL-6, and TNF- α can directly interact with brain structures (Banks, Kastin and Broadwell, 1995; Schulz and Engelhardt, 2005; Yang, Huh and Choi, 2023).

Receptors for the cytokines mentioned above are found in structures within the brain: the surface of neurons, astrocytes and microglia cells, which are the primary neuro-immune controlling inflammatory processes in the brain. TNF- α receptors are widespread across the brain, including in the suprachiasmatic nucleus (SCN; Sadki *et al.*, 2007; Beynon and Coogan, 2010), and IL-1 β receptors are mostly present in the hypothalamus, hippocampus and cortex (Hammond *et al.*, 1999; DiSabato *et al.*,

2021). Modifications to the actions of these areas of the brain, due to increasing levels of these proinflammatory cytokines, impact sleep (O'Brien *et al.*, 2021).

Cytokine binding can activate intracellular pathways like the NF- κ B pathway, which regulates gene expression and the release of secondary mediators such as prostaglandins (Liu *et al.*, 2017). Some of the consequences of the activation of the NF- κ B pathway in the CNS include fatigue, fever, and loss of appetite – as a result of the activation of inflammatory cascades (Liu *et al.*, 2017; Anilkumar and Wright-Jin, 2024). Long-term triggering of these pathways in the case of chronic inflammation can affect memory, learning, and synaptic plasticity (Kaltschmidt *et al.*, 2006).

In the short term, cytokine-brain interactions provide a short-term response to infection. Acute inflammation allows for the resolution of illness and a return to health and homeostasis (Hannoodie and Nasuruddin, 2024). In the long term, however, chronic inflammation can lead to modifications in neurotransmitter systems, such as serotonin and dopamine pathways (Felger and Miller, 2012). This may also contribute to psychiatric issues like depression and anxiety (Raison, Capuron and Miller, 2006; Del Guerra *et al.*, 2013).

Thus, a feature of HIV – chronic inflammation – may directly affect the central nervous system, particularly the sleep-wake pathways. Conversely, the central nervous system has an impact on immune activation (inhibitory through the PNS and enhancing inflammation for the SNS), with HIV suppressing the inhibitory inflammatory pathway of the parasympathetic nervous system.

The next paragraphs present specific evidence of how sleep and circadian rhythms impact the immune response and how the immune response affects sleep.

1.9 Sleep and the immune system: evidence of a bidirectional relationship

Sufficient and good quality sleep is essential for the optimal function of the immune system, and when sleep is disrupted, there is increased immune activation or hypersensitivity of the immune system, and some of the immune cells are set into a state of incoordination (Bollinger *et al.*, 2009). Proper sleep is essential to maintain

adequate immune responses (Besedovsky, Lange and Haack, 2019), with alterations in sleep being associated with elevated levels of proinflammatory plasma cytokines.

1.9.1 Sleep, circadian rhythms and the immune response

Diurnal rhythms of immune markers indicate a sleep and/or circadian effect on the immune system

Interleukin-6 (IL-6), a proinflammatory cytokine, is secreted in a biphasic circadian pattern with two nadirs at about 08:00 in the morning and 21:00 in the evening and two peaks at approximately 19:00 in the evening and 05:00 in the morning. Similarly, regulatory T-cells increase during the biological night (during sleep), and are at nadir during the biological day (wake time; Bollinger *et al.*, 2009). When activated, regulatory T-cells downregulate the immune response and can thus dampen immune responses to infection. Regulatory T-cell function is lower during total sleep deprivation (Bollinger *et al.*, 2009).

There is evidence that disruptions to sleep, either with partial or total sleep deprivation, can lead to immune activation. When investigating the impact of sleep loss on C-reactive protein – a marker of inflammation – and the effect that sleep deprivation has on CRP, sleep deprivation was shown to increase CRP as well as increase blood pressure in a separate experiment (Meier-Ewert *et al.*, 2004). A study investigating inflammation in people with non-apnoea sleep disorders (ICD-9-CM codes: 9CM 307.4 – specific disorders of sleep of non-organic origin 780.5x: sleep disturbances) compared to people not diagnosed with non-apnoea sleep disorders. The study found that patients with non-apnoea sleep disorders had a 47% increased risk for developing autoimmune disorders (adjusted HR = 1.47, 95% confidence interval [CI] = 1.41-1.53). With specific autoimmune disorders like systemic lupus erythematosus and rheumatoid arthritis having an 81% and 45% increased risk, respectively (adjusted HR = 1.81 [1.50-2.18] (systemic lupus erythematosus), 1.45 [1.36-1.54] (rheumatoid arthritis); Hsiao *et al.*, 2015). Overall, we see that getting enough, good quality sleep is essential for the proper functioning of the immune system – both in the short and long term.

The impact of acute sleep disruption on the immune system

We have contextualised the impact of the immune system on sleep, and the other side of the bidirectional relationship is the impact of sleep, or rather, the lack thereof, on the immune system. I will first discuss acute sleep disruption, i.e. one night of sleep deprivation or short-term sleep restriction and then look at chronic sleep disruption.

Studies have shown that even one night of total sleep deprivation triggers an acute stress response, increasing cortisol levels and downregulating the immune system (Bryant *et al.*, 2004). Acute sleep disruption is associated with elevated levels of pro-inflammatory cytokines such as IL-1 β , IL-6 and TNF- α (which we are, at this point, rather familiar with), within hours of sleep loss. There are also changes in immune cell function - namely, a lack of function of antigen-presenting cells, such as dendritic cells, and reduced T-cell activation and proliferation (Garbarino *et al.*, 2021). The combined effects could open a person up to an increased risk of developing an infection.

Most of these deleterious effects are reversed by sufficient, good quality recovery sleep. This begs the question: What happens when one is unable to get enough sleep or sleep properly often enough?

The impact of chronic sleep disruption on the immune system

Chronic sleep disruption refers to more long-term sleep disruption, as opposed to acute sleep disruption, which focuses more on one or two nights of disrupted sleep. Conditions that fall under the banner of chronic sleep disruption would be seen in shift workers, or chronic sleep restriction, sleep apnoea, and insomnia (sleep maintenance insomnia or sleep onset insomnia). Chronic sleep disruption has been associated with increased, low-grade inflammation, producing higher levels of IL-6, C-reactive protein (CRP) and TNF- α (Irwin, Olmstead and Carroll, 2016). As much as some proteins are increased, there is reduced control of the immune system, disruption of the function of regulatory T-cells, preventing the control of the immune system and driving the low levels of inflammation. Furthermore, cytokines associated with adaptive immunity are reduced (IFN- γ).

Chronic sleep disruption is also associated with reduced natural killer cell function, reducing immunological surveillance – the long-term consequence of which would be an increased risk of cancer, as tumour cells may not be programmed for death as quickly (Garbarino *et al.*, 2021).

The immune response affects sleep

Infection by pathogens triggers an immune response. Recruitment of immune cells to the site of infection is facilitated by the release of specific cytokines (including interleukins, tumour necrosis factors (TNF- α and β), IL-1 β , IL-6, IL-8, IL-10, IL-12, IFN- γ , IFN- α (Yamauchi *et al.*, 2006; Irwin & Opp, 2017). Infection leads to central nervous system responses such as fever and typical behaviours associated with sickness, such as sleepiness, fatigue, mood changes like depression and anxiety, reduced appetite and increased pain sensitivity (Dantzer and Kelley, 2007).

Infection also leads to the activation of the nuclear factor kappa B (NF- κ B) pathway, which leads to the production of proinflammatory cytokines (TNF- α , IL-1 β and IL-6) (Liu *et al.*, 2017). A study published in 2015 investigating the relationship between sleep disturbance and systemic inflammation over a 5-year follow-up revealed that sleep disturbances directly influence the production of pro-inflammatory mediators such as cytokines and CRP (Cho *et al.*, 2015). These cytokines also have a physiological impact on the host, inducing a somnogenic (sleep-inducing) effect, as seen when the immune system is fighting an infection or dealing with an inflammatory challenge.

The table below uses the elements highlighted in the work by Michael Opp in 2005 as well as other authors, summarising different acute effects of cytokines on sleep in cellular, animal, and human models (Opp, 2005).

Table 3: Summary of different cytokines and their effects on sleep.

Cytokine	Effect on Sleep	Mechanisms/Observations	References
IL-1 β	Associated with an increase in NREM sleep	Increases Ca ²⁺ influx in hypothalamic neurons, altering GABAergic neurotransmission Decreases glutamatergic synaptic potentials in the hippocampus, leading to inhibition of wakefulness circuits. Reduces discharge rates of wake-related neurons in the preoptic area, promoting NREM sleep. Slow wave activity increases during periods of NREM sleep enhancement that follow administration. Synergistic effect along with IL-6, increases CRP production from the liver. Maximum CRP expression from hepatocytes exposed to IL-6 and IL-1 β .	(Irwin and Allen, 2009) (Brown <i>et al.</i> , 2012) (Jewett and Krueger, 2012) (Calabró, Willerson and Yeh, 2003)
TNF- α	Associated with an increase in NREM sleep, suppresses REM sleep	Application of TNF- α to the somatosensory cortex increases slow wave activity (<5 Hz) during NREM sleep. Localized TNF- α increases slow wave activity without affecting the amount of NREM sleep. Slow wave activity increases during periods of NREM sleep enhancement that follow administration. TNF- α works similarly to IL-1 β , indicating a role in sleep regulation; antagonizing TNF- α systems also reduces spontaneous NREM sleep. Stimulates the release of CRP from hepatocytes in a dose dependant manner.	(Rockstrom <i>et al.</i> , 2018) (Krueger <i>et al.</i> , 1998, 2011) (Rockstrom <i>et al.</i> , 2018) (Jewett and Krueger, 2012) (Calabró, Willerson and Yeh, 2003)
IL-6	Associated with mixed effects on NREM and REM sleep	Increases in NREM sleep with administration, but biphasic: initial increase followed by suppression (animal model). Increases slow wave sleep (SWS) and reduces REM sleep in humans. Sleep deprivation increases IL-6 in human plasma. Il-6 knockout (KO) mice show subtle differences in sleep patterns but retain normal rhythms, suggesting possible redundancy in cytokine networks. IL-6 KO mice show a diminished response to immune challenges such as LPS injections, with reduced NREM sleep but similar REM sleep suppression as control mice (animal model).	(Morrow and Opp, 2005) (Irwin and Opp, 2016) (Redwine <i>et al.</i> , 2000) (Morrow and Opp, 2005)

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Cytokine	Effect on Sleep	Mechanisms/Observations	References
		Binds to hepatocytes to induce the production of CRP via activating the JAK-STAT pathway, activating the transcription of STAT3 – producing CRP.	(Ngwa <i>et al.</i> , 2022)
Other Cytokines	Modulate sleep-wake behaviour	IL-4, IL-8, IL-13, IL-18, interferons (IFNs), and others are implicated in altering sleep patterns. IL-2, IFN γ , IL-18, NREM sleep-promoting properties in animal models. IL-8: higher levels associated with chronic insomnia in patients with atherosclerotic cerebral small vessel disease. IL-4, IL-13: associated with reducing NREM sleep in animal models. Most cytokines, when injected into laboratory animals, increase NREM sleep. Exceptions are IL-4, IL-10, and IL-13, which reduce NREM sleep, possibly due to inhibition of IL-1 β and TNF- α synthesis.	(Besedovsky, Lange and Haack, 2019) Besedovsky, Lange and Haack, 2019) (Wang <i>et al.</i> , 2020) (Walker and Borniger, 2019) (Irwin and Opp, 2016) (Rockstrom <i>et al.</i> , 2018; Garbarino <i>et al.</i> , 2021)

IL = interleukin, NREM = non-rapid eye movement, REM = rapid eye movement, LPS= lipopolysaccharide, IFN = interferon, KO - knockout

Having discussed the bidirectional relationship between sleep and the immune system, the following section of this chapter will explore how HIV may have direct pathological effects on the central nervous system, which may contribute independently to sleep changes during HIV.

1.10 HIV, inflammation and the brain

HIV has been shown to infect cell types in the brain (Borrajó *et al.*, 2021), including primarily perivascular macrophages, glial cells (Rojas-Celis *et al.*, 2019), and astrocytes. These cells can be considered as the primary neuro-immune cells controlling inflammatory processes within the central nervous system. These cell types make up a small portion of neuronal tissue, but there is growing evidence that they play a vital role in the viral reservoir set up by HIV early on in infection (Valdebenito *et al.*, 2021). This indicates the role that the infection of astrocytes plays in the viral reservoir (Valdebenito *et al.*, 2021). Astrocytes are key players in the regulation of the sleep-wake cycle and play a role in the promotion of sleep by releasing adenosine (with cumulated levels of adenosine in the brain being associated with higher sleep pressure, as reviewed in the paragraph about homeostatic sleep regulation).

In PLWH, infection of the astrocytes and microglial cells may lead to some of the changes in sleep architecture mentioned in this review. For example, the suprachiasmatic nucleus of the hypothalamus, the mammalian circadian pacemaker, is a network of astrocytes which could be infected with HIV. This, in turn, could lead to modifications in the molecular pathways leading to circadian entrainment, which could modify the sleep-wake regulation. Another way HIV could disrupt sleep-wake regulation may be through cytokines secreted by those activated cells (glial cells and macrophages), such as IL-1 β , IL-6, and TNF- α (Merrill and Chen, 1991). These cytokines have been shown to cause alterations in sleep in non-HIV-infected populations (Shoham *et al.*, 1987; Späth-Schwalbe *et al.*, 1998; Yoshida and Caruso, 2002).

We have thus considered in the sections above the relationship between sleep and immunity and how HIV may also directly affect the central nervous system, possibly

affecting the sleep-wake pathways. In the next section, we will show evidence of sleep alteration in PLWH.

1.11 Changes in sleep experienced by PLWH

In PLWH, there is evidence that the homeostatic and circadian control of sleep are modified. When HIV was first discovered in the 1980s, patients who presented with the infection had progressed so far into the disease state that there were several health issues, such as a high incidence of cancer, immunodeficiencies, and AIDS-related neurocognitive decline before finally, death. As more understanding grew about the disease, investigators began to assess the effects that HIV could have on factors such as sleep.

1.11.1 Changes to sleep architecture and diurnal rhythms of immune cells in PLWH

Early studies (pre-ART) revealed that PLWH, had a reduced total sleep time and increased sleep onset latency (time taken to fall asleep; Wiegand *et al.*, 1991) compared to age- and sex-matched controls. In a normal sleep period, sleep cycles through the three stages throughout the night, with more deep sleep occurring in the first half of the night, and more REM sleep happening in the second half of the night (see Figure 3). In untreated PLWH, HIV infection caused changes in sleep architecture that included shifts of slow-wave sleep to the second half of the night (White, 1992, 1995) and a reduction in REM sleep latency (time taken to get to REM sleep after sleep onset; Wiegand *et al.*, 1991; White *et al.*, 1995). REM sleep is under circadian control, suggesting that events during HIV infection may have an impact on circadian rhythms.

HIV preferentially infects CD4⁺ T-lymphocytes, causing cell death and a reduction in host immunity (Douek *et al.*, 2002). During the progression of primary HIV infection, the virus triggers immune cell activation and the release of cytokines (IL-1 β , IL-6, IL-10, TNF- α) and chemokines. Known sleep-inducing cytokines such as IL-1 β , TNF- α and IFN- α were found to be increased in persons living with HIV (Norris *et al.*, 2006). These cytokines were associated with increased daytime sleepiness and higher levels of fatigue (Krueger *et al.*, 1998; Krueger, 2008).

Patients infected with HIV show clinically significant changes in diurnal rhythms of immune cells in the early stages of infection, and throughout the progression of the disease (Bourin *et al.*, 1993). In people without HIV, natural killer (NK) cells, granulocytes, macrophages,

CD8⁺ T-cells, and gamma-delta T-cells increase during the biological day, and T-cells, B-cells, and CD4⁺ T-cells increase during the biological night (Suzuki *et al.*, 1997). As HIV infection progresses, it causes a gradual eradication of the diurnal rhythms of B-lymphocytes (CD3⁻, CD16⁺) and T-lymphocytes (CD2⁺, CD3⁺). However, HIV had no effect on the circadian rhythm of NK cell (CD3⁻, CD57⁺) levels, similar to uninfected individuals (Krueger *et al.*, 1998; Krueger, Rector and Churchill, 2007). This shows that the impacts of HIV are not solely to decrease the numbers of CD4⁺ T-cells, affecting immunity, but given that B-cells and T-cells exhibit circadian rhythms (Downton, Early and Gibbs, 2020), modifications to their expression could also impact how and when the immune system functions throughout the day.

Evidence suggests that there is an interplay between the genes that control elements of the circadian clock and HIV. Through deletion of ROR (Retinoic Acid Receptor-related Orphan Receptor) and *Bmal1* genes in cell lines, the circadian rhythm was impacted, as was the replication of HIV (Borrmann *et al.*, 2023).

1.12 Sleep in PLWH and receiving ART

In PLWH on ART, other sleep problems came to light. Some specific changes in sleep noted in PLWH while taking ART were the higher levels of sleep disruption and higher levels of insomnia present compared to matched controls (Crum-Cianflone *et al.* 2012; Low *et al.* 2012; Allavena *et al.*, 2016). Eighteen HIV-seropositive insomniacs were matched to HIV-seronegative insomniacs, and the PLWH still reported shorter sleep times than PLWOH (Low *et al.* 2012).

To evaluate the effects of HIV treatment on sleep, specific drugs were investigated, notably the non-nucleoside reverse transcriptase inhibitor (NNRTI) efavirenz, which was shown to reduce time spent in REM sleep. Patients also reported that they experienced changes in dreams while taking efavirenz (Gallego *et al.* 2004). This study found that patients on efavirenz had increased sleep onset latency, reduced sleep efficiency, and reduced REM sleep. There was also a positive correlation between serum efavirenz levels and sleep changes.

Efavirenz, an NNRTI, is a significant predictor of insomnia and sleep disruption in persons living with HIV. Persons being treated with efavirenz have reported higher levels of

insomnia (Núñez *et al.*, 2001), and in another study, sleep disturbance was reported by 35% of subjects using efavirenz at four weeks. This study found, when comparing efavirenz to protease inhibitor therapy, that sleep disruption was more common in the efavirenz group (Fumaz *et al.* 2002). However, after 6 months of treatment, the insomnia in the efavirenz group had decreased significantly (Fumaz *et al.* 2002). Many people who are initiated onto treatment with efavirenz report sleep changes, including insomnia, hypersomnia, and alterations to their dreams, but these symptoms usually reduce after 1 month of treatment (Clifford *et al.*, 2005; Simen *et al.*, 2015).

A study conducted in London showed that persons who were initiated onto ART with efavirenz had a reduction in the amount of time spent in stage 2 sleep (thought to be associated with daytime alertness) and an increase in the amount of time individuals spent in stage 4 (deep sleep) and rapid eye movement (REM) sleep. Both REM and deep sleep are associated with dreaming. These differences persisted to week twelve. Sleeping patterns at weeks two and twelve were significantly different to those seen prior to the initiation of ART. Patients noted that they felt less refreshed in the morning, but with no impact on daytime function. They were also more likely to remember their dreams from the night before (Moyle *et al.*, 2006).

Later studies have shown that efavirenz either has no effect (Crum-Cianflone *et al.*, 2012), or a protective effect against poor sleep (Allavena *et al.*, 2016), whereby being on either efavirenz or nevirapine reduced the odds of reporting sleep disruption in a multivariate analysis. Those studies were run in the USA and Europe, respectively. In a South Africa, a recent study from Khayelitsha showed that PLWH had worse sleep quality while they were taking efavirenz when compared to their sleep quality after being switched to Dolutegravir (Nightingale *et al.*, 2024). Overall, the effects on sleep observed with efavirenz therapy are still being reported, and the drug is currently (from 2020) being phased out in favour of dolutegravir (DTG) – an integrase inhibitor with fewer – but not absent – side effects (Fantauzzi and Mezzaroma, 2014; Taha, Das and Das, 2015). Thus, the ADVANCE study conducted in Johannesburg proved the safety and efficacy of DTG in a South African cohort, and since 2020 this drug has been used as first line ART rather than patients being initiated onto efavirenz (Venter *et al.*, 2019).

A meta-analysis conducted by Wu *et al.* in 2015 reported that the prevalence of sleep disturbances in PLWH was 58% (Wu *et al.*, 2015). Another meta-analysis was conducted on sleep studies in PLWH in sub-Saharan Africa, elucidating that the prevalence of sleep disturbances among PLWH varied greatly between countries (21–71%), but pooled data revealed a prevalence of 49.32% (95% CI 41.32–56.8%; Melese *et al.*, 2024). Factors associated with sleep disturbances in PLWH have remained the same across the various reviews of the literature, with depression (OR 2.78; 95% CI 1.21–6.40) seemingly at the forefront of the conversation. In addition, CD4+T-cell count <200 cells/ μ L (OR 3.15; 95% CI 2.41–4.15) was also associated with sleep disruption (Melese *et al.*, 2024). Both papers highlighted that sleep disturbances may be present when PLWH initiate onto ART, but neither paper was able to detect an overall impact on sleep by ART in either of the meta-analyses, perhaps because the participants in most of the studies had been treated for longer than the sleep effects may have lasted.

Infection with HIV is independently associated with increased risk of cardiovascular disease, and one of the proposed mechanisms is through the modulation of sleep and sleep pathways (Friis-Møller *et al.*, 2003; Triant *et al.*, 2007; Triant, 2013). We have discussed how disrupted sleep increases inflammatory pathways, but infection with HIV also leads to inflammation throughout all stages of infection (Vos *et al.*, 2016), even while on ART (Wada *et al.*, 2015; Kwame *et al.*, 2018). Pro-inflammatory states are associated with increased cardiometabolic disease, cardiac consequences, and reduced life expectancy (Alfaddagh *et al.*, 2020; Tejada *et al.*, 2022). Even in the advent of ART, PLWH are still prone to inflammation, and ART is associated with increased CVD risk (Stein and Hsue, 2012), which will be discussed in the next section.

1.13 Gaps in the literature

We have identified that sleep is a problem for PLWH, but little is known about sleep in PLWH in African populations. Is there sleep disruption present, and if so, is there a possible mechanism that could account for it? Many of the studies put forward in this review have highlighted the importance of sleep but fail to give insight as to the pathways or reasons that could explain the high level of sleep disturbance experienced by PLWH.

Furthermore, PLWH on treatment are living longer lives and are more likely now to experience some of the lifestyle diseases that are currently occurring in South Africa with

the overweight/obesity rate of over 50% (20 million people out of an adult population of approximately 40 million; Lehohla, 2017). In a recent report by the South African Department of Health, 31% of men and 68% of women were classified as being overweight or obese (Discovery Vitality, 2023; National Department of Health (NDoH), 2023). However, there is little data on the prevalence of sleep disturbances in PLWH in South Africa and whether this contributes to greater levels of inflammation and cardiometabolic disease.

1.14 Aims of this thesis

- 1) Describe sleep in South African PLWH
- 2) Investigate correlates of poor sleep quality/lower sleep duration in South African PLWH
- 3) Investigate the relationship between sleep quality and cardiometabolic disease risk in South African PLWH.

1.15 Objectives of this thesis

- 1) To describe sleep quality and its correlates, using validated questionnaires in a cross-sectional study of 139 urban PLWH in South Africa.
- 2) To investigate whether the sleep-wake cycle and circadian rhythms are modified in South African PLWH living in a rural township of the province of Mpumalanga, South Africa, using objective measures (actigraphy and the timing of the circadian pacemaker through the measurement of dim light melatonin onset).
- 3) To investigate the relationship between sleep complaints, inflammation and cardiometabolic risk in a cohort of 1915 people with and without HIV living in Limpopo, South Africa.

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CHAPTER 2: INCREASED CD4 COUNTS, PAIN, AND DEPRESSION ARE CORRELATES OF LOWER SLEEP QUALITY IN TREATED HIV POSITIVE PATIENTS WITH LOW BASELINE CD4 COUNTS

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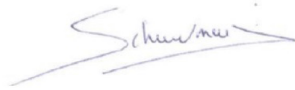
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Increased CD4 counts, pain and depression are correlates of lower sleep quality in treated HIV positive patients with low baseline CD4 counts

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ABSTRACT

Poor sleep quality leads to increased immune activation and immune activation leads to worse sleep quality. South African HIV positive patients typically have delayed start of treatment, which has been associated with CD4+ effector T cells being more spontaneously activated in chronically treated patients. This cross-sectional study investigated whether subjective sleep quality was associated with CD4+ T lymphocyte reconstitution in treated South African HIV+ patients.

One hundred and thirty-nine treated HIV+ patients (109 F, age average (SD) = 43 (9)) were recruited from Chris Hani Baragwanath Academic Hospital in Soweto, Johannesburg, South Africa. Participants completed questionnaires evaluating their subjective sleep quality (Pittsburgh Sleep Quality Index), daytime sleepiness (Epworth sleepiness scale), pain, and depression severity (Beck Depression Inventory). Univariate and multivariate analyses were run to determine the correlates of sleep quality in this population.

Patients had been on antiretroviral treatment for about 4 years and had increased their CD4 counts from a median at baseline of 82 to 467 cells/ μ L. They had overall poor sleep quality (average (SD) PSQI = 7.7 ($\pm$ 5), 61% reporting PSQI > 5, a marker of lower sleep quality), 41% had clinical depression (average (SD) BDI = 17 ($\pm$ 12)) and 55% reported pain. In two separate multivariate analyses, both the overall CD4 count increase from baseline ($p = 0.0006$) and higher current CD4 counts ($p = 0.0007$) were associated with worse sleep quality, when adjusting for depression severity ($p < 0.001$), daytime sleepiness ($p = 0.01$) and the presence of pain ($p < 0.01$).

In this cohort of treated South African HIV positive patients, poor sleep quality was associated with higher current CD4 counts, when adjusting for depression severity, daytime sleepiness and pain. Further studies should investigate the temporal relationship between HIV-related poor sleep quality and underlying immune activation.

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1. Introduction

1.1. Sleep and immunity

There is a well-described relationship between sleep and the immune system whereby sleep disturbances have been shown to enhance inflammatory states (Bollinger et al., 2009; Dinges et al., 1999; Irwin et al., 2006; Meier-Ewert et al., 2004; Mullington et al., 2009; Shearer et al., 2001). Conversely inflammatory states also lead to increased sleep disruption (Mullington et al., 2000;

Pollmächer et al., 1993). Accordingly, treatment with the pro-inflammatory cytokine IFN alpha has been shown to increase wake after sleep onset, with more spontaneous arousals and to decrease sleep efficiency (Raison et al., 2010). In contrast, treatment with a blocker of the pro-inflammatory cytokine TNF alpha (infliximab) of patients with severe depression and increased CRP levels was shown to decrease wake after sleep onset and improve sleep quality (Weinberger et al., 2015). These findings therefore strongly suggest that immune activation disrupts sleep.

1.2. HIV, sleep, and immunity

1.2.1. In untreated HIV positive patients

Before starting treatment, infection by the retrovirus human immunodeficiency virus (HIV) is associated with chronic activation

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of the immune system (Espindola et al., 2015). Early studies have shown that fatigue and lower sleep quality precede HIV diagnosis and are early symptoms of the infection (Darko et al., 1992, 1995, 1998; Norman et al., 1992, 1990, 1988). In untreated HIV positive patients, the slow wave sleep percentage decreased in the first part of the sleep episode compared to controls, and patients had increased wake after sleep onset (Ferini-Strambi et al., 1995; Norman et al., 1992). HIV-related sleep disturbances have been shown to be associated with the increased immune activation triggered by HIV infection both in rats (Opp et al., 1996) and in humans (Darko et al., 1992, 1995).

1.2.2. In treated HIV positive patients

However, even after starting antiretroviral therapy (ART), HIV positive patients continue reporting lower sleep quality. A 2005 review of 29 studies on HIV and sleep reported that an estimated 40 to 60% of HIV patients (treated or untreated) complain of poor sleep quality (Reid and Dwyer, 2005). A more recent meta-analysis including 27 studies of 9246 HIV positive participants found an overall prevalence of self-reported poor sleep quality of 58% (Wu et al., 2015). A study of 290 American HIV positive patients using actigraphy showed that 45% slept <6 h per night, 34% reported difficulty falling asleep, and 56% had fragmented sleep; with only 30% being good sleepers (Crum-Cianflone et al., 2012). Correlates of poor sleep quality in treated HIV positive patients highlighted by a large body of studies included low CD4 counts (Oshinaike et al., 2014; Seay et al., 2013), depression (Allavena et al., 2016; Barroso et al., 2015; Wibbeler et al., 2012), lower socioeconomic status (Allavena et al., 2016; Crum-Cianflone et al., 2012), the presence of peripheral neuropathy and/or pain (Crum-Cianflone et al., 2012; Vosvick et al., 2004), and treatment by the non nucleoside reverse transcriptase inhibitor (NNRTI) efavirenz (Gallego et al., 2004; Nunez et al., 2001; Oshinaike et al., 2014). Recent data show that immune activation may remain a factor in HIV-related lower sleep quality, whereby HIV-related lower sleep quality was shown to be associated with polymorphisms in cytokines including IL1, IL 6 and TNF alpha (Gay et al., 2015).

1.3. HIV in South Africa

To date, populations studied with respect to HIV and sleep have been mainly Caucasian, male and with first world access to care. In contrast, South Africa's HIV positive population, with its staggering prevalence of 12.2% (7 million) of South Africa's total population, is 60% female and 97% of African ancestry (Statistics South Africa, 2016). In addition, due to delayed initial presentation and past treatment initiation guidelines, the majority of HIV positive patients started on ART only when reaching a CD4 count below 200/ μ L. This delayed start of ART has been associated with higher spontaneous immune activation once CD4 counts have reconstituted (Okulicz et al., 2015). Taken together, these factors differentiate the South African treated HIV positive population in which, as far as we are aware, no study has been done on sleep quality.

We therefore undertook a cross-sectional study of treated HIV positive patients from a South African urban community to determine the prevalence and correlates of sleep quality, in particular focusing on the possible association with a CD4-related immune activation.

2. Methods

2.1. Population and study design

In this cross sectional study, 139 HIV positive patients with baseline CD4 count information were enrolled (152 patients

recruited) from the Adult HIV Clinic at the Chris Hani Baragwanath Academic Hospital in Soweto, Johannesburg. This tertiary hospital serves an urban community of African ancestry. A nursing sister made an announcement inviting patients who were over the age of 18 years to participate in the study, which was advertised as a study of how well people slept. Potential participants went with the investigator into a separate consulting room. Most patients were fluent in English. HIV counselors acted as translators for 3 of them. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Human Research Ethics Committee of the University of the Witwatersrand (M120411). Informed consent to participate in the study was obtained from each participant prior to enrolment. Recruitment took place over 4 months from May to August 2012.

2.2. Questionnaires

2.2.1. Sleep quality

The Pittsburgh Sleep Quality Index (Buysse et al., 1989) was used to assess sleep quality over the past 30 days. It comprises seven components each taking a value from 0 to 3. Therefore, the PSQI Global score ranges from 0 to 21. A global PSQI score ≤ 5 indicates normal sleep quality while a score >5 indicates poor sleep quality. We used the PSQI global score as the main outcome variable for the study.

2.2.2. Daytime sleepiness

The Epworth Sleepiness Scale (ESS) (Johns, 1991) was used to assess levels of daytime sleepiness. This questionnaire uses 8 questions pertaining to the likelihood in the past 30 days of dozing during the day in different types of circumstances (either being passive or active): each question can take any discrete value from 0 (never) to 3 (high chance). Therefore ESS total score ranges from 0 to 24. An ESS total score >10 indicates the presence of excessive daytime sleepiness and suggests an underlying sleep disorder.

2.2.3. Depression

Beck's Depression Inventory (BDI) (Beck et al., 1961) was used to assess the presence of depressive symptoms and their severity in our cohort. It comprises 21 questions assessing the degree of a depressive symptom on a discrete scale from 0 to 3, with 0 indicating absence of the depressive symptom to 3 indicating the most severe presentation of that symptom. Therefore BDI score ranges from 0 to 63. BDI scores >10 indicate depression, scores >17 indicate moderate to severe depression.

2.2.4. Questionnaires' internal consistency and validity testing for a population using English as a second language

Although most participants at the clinic were fluent in English, English was their second language. To ensure the questionnaires (PSQI, ESS, BDI) we used were reliable in this context, we ran a Cronbach's alpha to test for the internal consistency of each questionnaire and we also ran a factor analysis to assess their construct validity in this cohort.

2.2.5. General information questionnaire

Demographic data, including age, sex, employment status and significant comorbidities, past or present, were collected both from the patient and by checking the clinic record.

The presence of pain in the month prior to enrollment into the study and presence of pain on the study day were noted. If they reported pain on these two questions, we asked them to rate their pain on a 10-point Likert scale going from 1 to 10, with 1 showing the anchor 'no pain' and 10 showing the anchor 'worst pain ever felt' and also to color on a body diagram the site(s) of their pain.

2.3. CD4+ T cell count and HIV viral load

Venous blood was collected from each study participant at the clinic in the morning to determine current CD4 counts and viral loads (i.e. on the study day). From the patient record, we obtained baseline CD4 counts and viral loads prior to ART.

2.3.1. CD4+ T cell count determination

CD4+ T cell counts were determined using a routine haematology analyzer to first assess absolute white cell counts and perform flow cytometry using the PanLeucogating method for CD4+ T cell enumeration. (Glencross et al., 2002).

2.3.2. Viral load determination

Quantification of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma (viral load) was determined using the COBAS® AmpliPrep Instrument for automated specimen processing and the AmpliPrep/COBAS® TaqMan® HIV-1 Test, version 2.0 (v2.0). The test quantifies HIV-1 RNA over the range of 20–10,000,000 copies/mL.

2.3.3. Antiretroviral treatment information

The clinic's data management system was accessed for treatment information.

2.4. Analysis plan and statistical analyses

Subjective sleep quality measured by the Global PSQI score was used as the outcome variable.

2.4.1. Univariate analyses

We first ran univariate analyses on specific variables of interest, such as age, sex and employment status.

We then tested whether there was an association between Global PSQI score and HIV-associated markers and the presence of comorbidities. HIV markers tested were CD4+ T cell count and viral load at baseline and on the study day, time since diagnosis and time on treatment.

Our cohort started ART at a time when guidelines recommended ART only when CD4 counts dropped below 200/ μ L, corresponding usually to treatment late in the disease (>12 months after seroconversion). Okulicz et al. (2015) showed that delayed ART is associated with higher levels of CD4 effectors expressing markers of activation. Since we expected most of our participants to have started ART late in the disease, we hypothesized that the higher a patient's CD4 count reconstituted, the higher that patient's likely underlying immune activation at the time of the study. We also explored the hypothesis that the rate of CD4 reconstitution may be instrumental in leading to higher current underlying immune activation. To explore those two hypotheses, we therefore also calculated overall CD4 increase from baseline (CD4 current – CD4 baseline and CD4 increase over time ((CD4 current – CD4 baseline)/(time since diagnosis)) and tested the association of those two constructed variables with sleep quality.

For comorbidities, we tested each HIV-associated comorbidity, then we combined all HIV-related comorbidities as one variable, and finally the presence of any HIV- or non HIV-related comorbidity was tested.

Third, we tested whether there was a relationship between sleep quality as measured by the Global PSQI score and 1- depression as assessed by the BDI score, 2- subjective daytime sleepiness as assessed by the ESS score and 3- the presence and rating of pain in past month and on the study day.

Finally, we tested whether certain antiretrovirals were associated with changes in sleep quality as assessed by the PSQI, specifically:

1. stavudine, which causes peripheral neuropathies and therefore pain,
2. tenofovir which replaced stavudine in 2012,
3. efavirenz which can lead to sleep disturbances,
4. lopinavir/ritonavir, the protease inhibitor used in second line which has been shown to be associated with increased cytokine production which could influence sleep (Espindola et al., 2015).

Each of the antiretrovirals was studied as a separate variable reflecting whether the patients either took the antiretroviral or did not.

2.4.2. Multivariate analyses

Variable selection for multivariate analyses is described in detail in the [Supplementary material \(See Supplementary material 1 – variable selection for the multivariate analysis: methods and results; and Supplementary Table 1\)](#).

All continuous data were tested for normality. Viral loads were skewed and were log transformed prior to analysis. All analyses were carried out in SAS 9.1.3®.

2.4.3. Missing data

We did not have any missing data for the following variables: sex, employment, age, PSQI, ESS, BDI, current CD4 counts, baseline CD4 counts, CD4 change since baseline, ARV treatment.

For other variables, we had few missing data (between 1 and 14 observations) and therefore did not attempt to replace the missing data by imputations. Rather, we ran the univariate analyses with the slightly lower sample sizes when data were missing.

For the multivariate analysis, three patients had missing pain information and therefore the final models were run on all participants minus the three with the missing data.

3. Results

3.1. Demographics

[Table 1](#) shows the demographic, HIV-specific and sleep-specific characteristics of our sample. The study participants were mainly female (79%), in their early forties and mostly unemployed. They had been living with documented HIV on average for almost seven years (range: 23 days to 18 years, with 90% having had an HIV diagnosis for more than a year) and treated for just over four years (range 1 day to 12 years, with 90% having been on treatment for >6 months). Current CD4 counts were significantly associated with time since HIV diagnosis (Pearson's $r = 0.28$, $p = 0.008$) and time on treatment (Pearson's $r = 0.44$, $p < 0.0001$). At baseline, 81% had AIDS (CD4 count <200/ μ L) and 59% had <100 CD4/ μ L, frequencies which decreased to 19% and 6% at the time of our study, respectively. Most of the patients received tenofovir (41%), stavudine (25%) or zidovudine (22%) together with lamivudine (81%) and efavirenz (76%). Only 16% received lopinavir/ritonavir. Forty-nine patients had coexisting conditions, 29 HIV-related and 20 not HIV-related ([Table 1](#)). None had active immune reconstitution inflammatory syndrome documented at the time of study participation.

3.2. Questionnaires

3.2.1. Validation

Full information about the internal consistency (Cronbach's alpha) and construct validity (factor analysis) for the PSQI, ESS and BDI is shown in the [Supplementary material](#). In summary, the PSQI had a Cronbach's alpha of 0.78, the ESS of 0.76 and the

Table 1

Population characteristics and predictors of sleep disruption (outcome variable: Global PSQI score) in univariate and multivariate analyses.

	N = 139	%	Univariate			Multivariate 1 (Adj r ² = 0.27)			Multivariate 2 (Adj r ² = 0.26)			
			Beta	SE	p	Beta	SE	p	Beta	SE	p	
Population characteristics												
Age (years), mean (SD)	42.7 (9.2)		0.04	0.05	0.36							
Sex = female	109	79	1.92	1.04	0.07							
Employed	36	26	−1.58	0.96	0.10							
HIV characteristics												
Baseline CD4 count (/μL), med. [IQR]	82 [33–165]		−0.003	0.003	0.24							
Baseline CD4 count < 100/μL	82	59	1.14	0.85	0.18							
Current CD4 count (/μL), med. [IQR]	467 [233–604]		0.004	0.0014	0.005				0.006	0.002	0.0007	
CD4 increase (/μL)/year, med. [IQR]	46 [18–101]		−0.0006	0.004	0.89							
(Current CD4 − baseline CD4)/μL, med. [IQR]	297 [103–487]		0.005	0.0013	0.0007	0.004	0.001	0.0006				
Baseline viral load (log/μL), mean (SD)	4.5 (1.37)		0.25	0.32	0.45							
Current viral load (log/μL), mean (SD)	2.0 (0.98)		0.004	0.44	0.99							
Time since diagnosis (years), mean (SD)	6.8 (4.3)		0	0	0.95							
Time on treatment (years), mean (SD)	4.2 (2.9)		0.0003	0.0004	0.36							
Reported HIV-related comorbid conditions, past or present												
Cancer ¹	10	7.2										
Of which, current	9	6.5	−4.66	5.77	0.44							
Tuberculosis	23	16.5										
Of which, current	18	13	−2.24	2.8	0.39							
Pneumocystis carinii	4	3										
Of which, current	0	0										
Cryptococcal meningitis	5	3.6										
Of which, current	2	1.4										
Reported current HIV comorbidities	29	20.8	−0.62	1.07	0.56							
Overall comorbidities ² , including HIV comorbidities	49	35.3	0.94	0.89	0.29							
		N=139	%	Univariate			Multivariate 1 (Adj r ² = 0.27)			Multivariate 2 (Adj r ² = 0.26)		
				Beta	SE	p	Beta	SE	p	Beta	SE	p
Sleep, depression and pain												
PSQI global score, mean (SD) (0–21)		7.7 (5.0)										
PSQI> 5		84	61									
ESS global score, mean (SD) (0–24)		7.9 (5.9)		0.23	0.07	0.001	0.16	0.06	0.011	0.19	0.06	0.0038
ESS>10		43	31	2.03	0.9	0.03						
BDI score, mean (SD)		17.3 (12.5)		0.13	0.03	0.0001	0.10	0.03	0.0007	0.10	0.03	0.0008
BDI score >17 (clinical depression)		57	41	2.12	0.85	0.014						
Presence of pain in the past month		96	73	2.65	0.95	0.006						
Rating of pain in the past month (1–10)		6.5 (2.8)		0.34	0.18	0.007						
Presence of pain on the study day		74	55	2.82	0.82	0.0009	2.07	0.74	0.0058	4.77	1.37	0.0007
Rating of pain on the study day (1–10)		5.4 (2.7)		0.01	0.21	0.96						
Interaction between pain on the study day and current CD4 count												0.02
ARV treatment												
NRTI												
Zidovudine (AZT)		31	22.3									
Didanosine (ddI)		12	8.6									
Tenofovir (TDF)		57	41.0	−1.11	0.86	0.19						
Abacavir (ABC)		8	5.8									
Stavudine (d4T)		35	25.2	0.50	0.98	0.61						
Lamivudine (3TC)		112	80.6									
NNRTI												
Efavirenz (EFV)		106	76.3	−0.14	1.00	0.89						
Nevirapine (NVP)		1	0.7									
PI												
Lopinavir/ritonavir (LPV/r)		23	16.5	1.20	1.14	0.29						

Med.: Median, IQR: interquartile range, SD: standard deviation, PSQI: Pittsburgh Sleep Quality Index; ESS: Epworth Sleepiness Scale; BDI: Beck Depression Inventory; MEQ: Horne Ostberg Morning Evening type Questionnaire; ARV: anti-retroviral; NRTI: nucleoside reverse transcriptase inhibitors; NNRTI: non-nucleoside reverse transcriptase inhibitors; PI: protease inhibitors.

In univariate and multivariate models, PSQI global score was the outcome variable. Predictors of PSQI global score included in the multivariate models were selected through forward selection, backward elimination and Mallows's C(p) as described in [Supplementary Table 1](#). Multivariate model 1 included as a predictor 'CD4 increase from baseline' whereas Multivariate model 2 included as a predictor 'current CD4 count'.

¹ HIV related cancers included Kaposi sarcoma, lymphoma, thymoma, and anal carcinoma.

² Non HIV-related comorbidities included haemophilia, endometriosis, asthma, breast cancer and thyroid cancer.

BDI of 0.9. Factor analysis showed good construct validity for all questionnaires.

3.2.2. Results

Sixty-one percent of the participants had a PSQI > 5, corresponding to poor sleep quality, with an overall average global

PSQI score of 7.7 ([Table 1](#)). Thirty-one percent of patients reported higher than normal daytime sleepiness with an ESS score > 10 and an overall ESS score of 7.9. Clinical depression (as assessed by a BDI score > 17) was found in 41% of our patients; only 32% had no symptom of depression (BDI score < 10).

Pain in the past month was reported by 73% of the participants, and 55% reported currently experiencing pain, with overall moderate levels of pain (between 5 and 7 on the 1–10 visual scale).

3.3. Univariate analyses of the correlates of sleep quality

3.3.1. Demographic variables

We first tested the effects of sex, age and employment on subjective sleep quality using the global PSQI score. There was a trend towards an effect of sex with women reporting worse sleep quality than men (average $\pm$ SD PSQI women = 8.1 ± 4.8 vs. average $\pm$ SD PSQI men = 6.2 ± 5.3 , $p = 0.068$). There was no significant association between age or employment status and subjective sleep quality (Table 1).

3.3.2. HIV characteristics

We found no association between CD4 counts at baseline and current subjective sleep quality (Table 1). Higher current CD4 counts were associated significantly with lower sleep quality (Pearson's r value = 0.24, $p = 0.004$) (Table 1). This association with lower sleep quality held with higher CD4 change from baseline. The rate of change of CD4 count per year was not significantly associated with sleep quality.

There was also no significant relationship between subjective sleep quality and log viral loads at baseline or current log viral loads, nor with time since diagnosis or time on treatment.

Finally, we found no association between single or combined HIV comorbidities, nor between any comorbidity and sleep quality (Table 1).

3.3.3. Sleep, depression and pain

Higher daytime sleepiness (ESS) and depression (BDI) were significantly associated with lower sleep quality (Table 1).

Reporting the absence of pain in the past month, the PSQI was 6 ± 4.9 increasing to 8.6 ± 4.8 when pain was present ($p = 0.006$). Experiencing no current pain the PSQI was 6.3 ± 4.8 compared to 9.1 ± 4.8 ($p = 0.001$) for those reporting pain (Table 1). Accordingly, 85% of patients reporting pain on the study day also reported that pain disrupted their sleep (question 5i of the PSQI) vs. 35% of those who did not report pain on the study day (Chi Square = 28.8; $p < 0.0001$). The two most frequent sites of pain reported on the study day were lower back pain (in 37 (50%) patients with pain) and lower limb pain (in 18 (24%) patients with pain).

Higher ratings of pain in the past month were significantly associated with decreased sleep quality while this was not the case with the pain ratings done on the study day (Table 1).

3.3.4. Antiretroviral treatment

There was no association between any specific antiretroviral drug and sleep quality (Table 1).

3.4. Multivariate analysis of the correlates of sleep quality

3.4.1. Multivariate model 1

Results of the careful step-by-step variable selection for the multivariate analysis are presented in the [Supplementary material](#).

Our final 4-variable multivariate model obtained after this careful variable selection incorporated CD4 change from baseline, BDI, ESS, and 'presence of pain on the study day'. We also tested for an interaction between CD4 change from baseline and 'presence of pain on the study day' but this was not significant and therefore we left it out of the final model. When adding back individually one by one age, sex and employment (which had not been selected in the variable selection process), those variables remained non-significant and therefore the final model only included those

significant covariates: CD4 change since baseline, ESS, BDI and pain on the study day. This model is shown as 'Multivariate 1' in Table 1.

From this 'Multivariate 1' model, we found that CD4 change since baseline was a significant correlate of lower sleep quality whereby each additional increase by 100 CD4/ μ L from baseline would induce a 0.4 increase in PSQI score (adjusted $p = 0.0006$). Higher daytime sleepiness was also associated with lower sleep quality (1-point increase ESS associated with 0.16 increase in PSQI, adjusted $p = 0.011$). Higher depression severity was also associated with lower sleep quality (10-point increase BDI associated with 1 point increase in PSQI, adjusted $p = 0.0007$). Finally, the presence of pain on the study day was associated with lower sleep quality (people reporting pain had on average a 2-point higher PSQI score compared to those without pain, adjusted $p = 0.0058$).

3.4.2. Multivariate model 2

In terms of clinical usefulness of the model, a baseline CD4 count is frequently unavailable, particularly in the South African context. When testing CD4 change from baseline and current CD4 counts together as correlates of PSQI in the kitchen sink model and in a simple 2-variable model, we found collinearity between these two variables. Since current CD4 count was also a correlate of PSQI in the univariate analysis ($p = 0.005$) and is available at the time of a patient's visit, we decided to also test a model where we replaced 'CD4 change from baseline' with 'current CD4 count' but kept ESS, BDI and pain on the study day, as reported in Table 1 'Multivariate 2' model.

From this model, we found that current CD4 count was a significant correlate of sleep quality whereby if the current CD4 count was higher by 100 cells/ μ L, it was associated with a 0.6 point higher PSQI score (adjusted $p = 0.0007$).

Higher daytime sleepiness was also associated with lower sleep quality (each additional point in ESS was associated with an additional 0.189 point in PSQI score, adjusted $p = 0.0038$). Higher depression levels were associated with lower sleep quality (each additional 10 points in BDI score were associated with an additional point in PSQI, adjusted $p = 0.0008$). Presence of pain on the study day was associated with lower sleep quality (people reporting pain had on average a PSQI score of 4.77 points greater compared to those without pain, adjusted $p = 0.0007$). We also found a significant interaction between current CD4 count and 'pain on the study day'. Using an additional 100 cells/ μ L of the current CD4 count, if pain was present on the study day, the effect of current CD4 counts on sleep was negligible (an additional 0.087 points in PSQI score) whereas for patients who did not have pain, this led to a significantly lower sleep quality (an additional 0.6 points in PSQI score) ($p = 0.0238$).

4. Discussion

This cross-sectional study conducted in 2012 found overall poor sleep quality in 139 treated and controlled South African persons of African ancestry living with HIV, with lower sleep quality being associated with greater depression severity, daytime sleepiness and the presence of pain. For the first time, we also report in a treated HIV positive population, an association between higher current CD4 counts (and higher CD4 change from baseline) and lower sleep quality.

4.1. Sleep quality and daytime sleepiness

We found that patients reported a lower than normal level of sleep quality and higher daytime sleepiness, with an average PSQI score at 7.7 and ESS at 7.9. These findings are similar to what has been found in other HIV and sleep studies, using objective

measurements such as polysomnography or actigraphy (Lee et al., 2012; Low et al., 2012; Norman et al., 1992; Wiegand et al., 1991) or subjective sleep measurements such as the PSQI or ESS (Allavena et al., 2016; Lee et al., 2012, 2001; Low et al., 2012; Nokes and Kendrew, 2001; Salahuddin et al., 2009; Seay et al., 2013; Wibbeler et al., 2012). Poor sleep quality has been shown to impact daytime function (Aouizerat et al., 2013; Saberi et al., 2013), cognition (Byun et al., 2016) and overall quality of life (Wibbeler et al., 2012) in people living with HIV. In a country where 12% of the general population is affected by HIV, this information is relevant for the long term chronic care of the patients and to devise appropriately targeted interventions to alleviate this poor sleep quality.

4.2. Association between sleep quality and depression

Poor sleep quality was associated with higher depression severity, which was relatively high in our cohort, with 41% of patients having BDI scores above 17 (moderate - severe). This is similar to a study in HIV positive women (Junqueira et al., 2008) and in both male-predominant and female-predominant studies (Crum-Cianflone et al., 2012; Oshinaike et al., 2014; Wibbeler et al., 2012) where higher depression levels were also associated with worse sleep quality.

4.3. Association between poor sleep quality and pain

Lower sleep quality was also found in patients who reported pain. Fifty-five percent of patients reported pain on the study day (73% in the past month), with moderate ratings of pain comparable to another South African metropolitan cohort (Mphahlele et al., 2012). Pain is a common complaint of persons living with HIV and those who are on antiretroviral therapy (Barroso et al., 2015; Keltner et al., 2012; Mphahlele et al., 2015, 2012; Namisango et al., 2012; Yoshida and Caruso, 2002). Consistent with our findings, other studies have reported that pain was a correlate of lower sleep quality in treated HIV positive patients (Crum-Cianflone et al., 2012; Vosvick et al., 2004).

4.4. Association between poor sleep quality and higher current CD4 counts/ higher CD4 change from baseline

Even when adjusting for these known correlates of poor sleep quality in HIV positive patients (depression and pain), the relationship between higher current CD4 counts/higher CD4 change from baseline and poor sleep quality persisted. This was intriguing as other studies have either described no relationship between CD4 counts and sleep (Cohen et al., 1996; Nokes and Kendrew, 2001; Perkins et al., 1995) or an improvement of sleep quality when CD4 counts increase (Oshinaike et al., 2014; Seay et al., 2013), the latter seeming the most likely scenario as it would be expected that as patients decrease their viral load and improve their CD4 counts, their overall health would improve, together with sleep. In an early study of untreated patients with HIV however, higher sleep duration was associated with lower CD4 counts (Darko et al., 1992) while another on treated HIV positive patients found an association between increased fatigue and increased CD4 counts (Henderson et al., 2005). The relationship we found between higher CD4 counts and poor sleep quality may reveal an underlying immune mechanism involved in the pathophysiology of the HIV-related poorer sleep quality. This mechanism may not be as prominent in other studies where the ethnic and gender composition as well as the disease and treatment context differ from South Africa's, in particular with the historical delayed access to treatment until low CD4 counts are reached.

4.4.1. Elucidating the relationship between increased CD4 counts and poor sleep quality: Is it due to the unique ethnic/ sex composition of our population?

Our population was characteristic of South African HIV positive populations as it was entirely composed of people of African ancestry and was mainly female. The high number of women in the sample is consistent with the population who attend HIV clinics in South Africa (Grimsrud et al., 2016; Mphahlele et al., 2015, 2012). This high proportion of women is different from most studies on HIV and sleep where the HIV positive populations were mainly male and of Caucasian origins (Allavena et al., 2016; Lee et al., 2012, 2001; Low et al., 2012; Nokes and Kendrew, 2001; Salahuddin et al., 2009; Wibbeler et al., 2012). However some studies with a closer ethnic and sex composition to ours also did not find this relationship between poor sleep quality and higher current CD4 counts: an earlier study reporting on women in the San Francisco Bay area who were 59% African American and 14% Hispanic (Lee et al., 1999) found that lower CD4 counts were associated with higher daytime sleepiness and fatigue. A 2014 study in Nigeria where 70.7% of the participants were women (Oshinaike et al., 2014) reported that sleep quality was better with higher CD4 counts. Another study which included mainly women of African American or Caribbean origins described that better sleep quality (as measured only by one component (of 7) of the PSQI, and not by the PSQI global score) was associated with higher CD4 counts (Seay et al., 2013).

The relationship we found between poor sleep quality and higher current CD4 counts reflected also the relationship between poor sleep quality and higher change from baseline in CD4 counts. The studies mentioned above either did not report CD4 counts at baseline or reported CD4 counts at baseline that were higher than ours.

4.4.2. Elucidating the relationship between increased CD4 counts poor sleep quality: Linked to the delayed access to treatment of the South African HIV positive population?

The median baseline CD4 count, i.e. at treatment initiation, (82 CD4 T cells/ μ L) in our cohort was much lower than other studies on HIV (usually above 200 CD4 T cells/ μ L). Most of our patients had started treatment around 2008, about 3 years after being diagnosed with HIV. This level is similar to the median CD4 count at ART initiation of 119 cells/ μ L reported in another large cohort that same year in South Africa (Grimsrud et al., 2016) but is lower than the usual baseline CD4 count reported in most HIV studies. We are aware of one paper on sleep and HIV that reported baseline CD4 counts (207 cells/ μ L) (Allavena et al., 2016). Longitudinal HIV studies in countries where other HIV and sleep studies were run report nadir/baseline CD4 counts of 250 or more (Chevalier et al., 2016; Edelman et al., 2016; Silveira et al., 2016).

This relationship between poor sleep quality and higher current CD4 count (or higher CD4 change from baseline) may reflect an underlying immune activation, which may disturb sleep. A recent study by Gay et al. (2015) showed an association between poor sleep quality in treated HIV positive patients and cytokine levels/ cytokine polymorphisms, which also suggests that poor sleep quality in treated HIV positive patients may be caused by underlying immune activation. Chronic immune activation has been indeed evidenced in treated and well controlled HIV positive patients: a study by Okulicz et al. (2015) showed that later timing of treatment relative to seroconversion (above 12 months) was associated with a higher percentage of effector CD4 T lymphocytes expressing the HLA DR activation marker (Okulicz et al., 2015). This marker is typically present on CD4 effector T cells in patients who develop immune reconstitution disease (Antonelli et al., 2010; Murdoch et al., 2009). However, none of our participants had active immune reconstitution disease. It is therefore possible that our cohort,

which started treatment 2–3 years after having been diagnosed with HIV, with a resulting low CD4 counts at baseline, could have more spontaneously activated effector CD4 T lymphocytes, leading to higher underlying immune activation, which could then induce poor sleep quality. Conversely, Oshinaike et al. (2014) reported in their study on sleep and HIV in Nigeria that HIV diagnosis and ART were both on average 27 months, suggesting that in Nigeria, ART was started as soon as the diagnosis was made (Oshinaike et al., 2014).

4.5. Study limitations

Our study's main limitations were its cross sectional design, a possible recruitment bias whereby those who had sleep complaints may have been more likely to participate than those who did not, and the absence of more objective measurements of sleep (such as actigraphy or polysomnography). The use of questionnaires in English, which have been developed in a different context to South Africa's may have led to errors in interpretation from our participants (even with the satisfying internal consistency and construct validity). Only having CD4 counts as immune measurements was also limiting: to better understand the role of immune activation on sleep quality in treated HIV positive patients, future studies would need to include more specific immune measurements such as: 1) CRP plasma concentration, 2) flow cytometry including activation markers of CD4 T lymphocytes (in particular HLA-DR, CD38, PD-1, Ki-67), or 3) measuring plasma concentrations of IL-1 β , IL-6, TNF alpha, (cytokines with known effects on sleep).

5. Conclusion

We have shown that treated HIV positive patients in this South African cohort report lower sleep quality at comparable levels to those described elsewhere. This poor sleep quality was associated with the presence of pain, depression, daytime sleepiness and unexpectedly with higher current CD4 counts/higher CD4 change from baseline. We argue this may be related to an underlying immune activation, even in stably controlled treated HIV positive patients.

Chronic lower sleep quality has been shown to not only impact quality of life, enhance risk of depression and increase pain sensitivity but also to increase the risk of developing metabolic disease and cardiovascular disease (Gangwisch et al., 2006; Knutson et al., 2007). This may be particularly relevant in HIV positive patients as it may add to the cardiometabolic risk associated with antiretroviral therapy alone (Balt, 2013). It is therefore key to better understand the mechanisms of HIV-related sleep disturbances to both improve long term quality of life but also general health outcomes in treated HIV positive patients.

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Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.bbi.2018.02.002>.

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Supplementary material 1 – variable selection for the multivariate analysis: methods and results.**1- Methods for variable selection**

For all univariate analyses, where the association tested between Global PSQI score and the variable yielded a p value below 0.2, we included the variables in a linear multivariate analysis. The rationale for choosing a p value of 0.2 as a threshold to include in the general model was to ensure we did not exclude any potential confounding effects between covariates of interest. In addition, we had planned to adjust for age and sex regardless of the univariate analysis's p values. This model is referred to as the 'kitchen sink' model, from which we started our variables' selection.

To select variables for our model from the initial 'kitchen sink' model, we used a 1) backward elimination (with a p value for the selection to stay set at 0.1), 2) a forward selection (with a p value for the selection for entry set at 0.1 as well) and 3) the Mallow's C(p) analysis (determined by the first C(p) which met the $C(p) < (\text{number of variables} + 1)$ criteria). From the forward, backward and C(p) variable selections, we then tested non-significant covariates for collinearity and removed them if necessary from the model, keeping the significant covariates in the model. If significant univariate covariates had been stripped out of the backward/ forward/Mallow's C(p) variables selection, we tested them again by adding them back in one by one. Finally, we tested for specific interactions between the main factors.

2- Results for the variable selection

From the univariate analyses, 11 variables either met our criteria for examination in the multivariate analysis with a univariate analysis p value less than 0.2, or were included as known correlates of sleep quality (age, sex and a surrogate of socioeconomic status, employment). The 11 variables considered in the model-building process 'kitchen sink'

model were: age, sex, employment status, current CD4 count, CD4 change from baseline, ESS, BDI score, presence of pain in the past month, presence of pain at the time of the visit, rating of pain in the past month and whether they were on tenofovir or not (Supplementary Table 1). Five variables remained after both the backward elimination and forward selection: CD4 change from baseline, ESS, 'presence of pain in the past month', 'presence of pain at the time of the visit' and whether the patients were on tenofovir or not. The best model in the Mallow's $C(p)$ selection was a 5-variable model with a $C(p) = 5.26$, in which ESS was replaced by BDI.

In both 5-variable models obtained using the backward elimination/forward selection or Mallow's $C(p)$, the variables 'whether the patients were on tenofovir or not' showed an adjusted p value of 0.4 and 0.589, respectively. Again for parsimony, we decided to remove it from the final model. We also removed 'presence of pain in the past month' from the final model which had a p value of $p=0.064$ in the backward elimination/forward selection 5-variable model and a $p=0.114$ in the model resulting from the Mallow's $C(p)$ selection. Therefore we were left with 3 variables selected from the backward elimination/forward selection: CD4 change from baseline, ESS, 'presence of pain at the time of the visit' and 3 variables from the Mallow's $C(p)$; CD4 change from baseline, BDI, and 'presence of pain at the time of the visit'.

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Supplementary Table 1: univariate analysis, 'kitchen-sink' multivariate model and multivariate models after 1) forward/backward and 2) Mallow's C(p) variable selection.

	N=139	%	<u>Univariate</u>			<u>Kitchen sink</u>			<u>Forward/Backward Model</u>			<u>Mallow's C(p)</u>		
			Beta	SE	p	Beta	SE	p	Beta	SE	p	Beta	SE	p
Population characteristics														
Age (years), mean (SD)	42.7 (9.2)		0.04	0.05	0.36	0.012	0.05	0.81						
Sex=female	109	79	1.92	1.04	0.07	1.44	1.18	0.23						
Employed	36	26	-1.58	0.96	0.10	-1.30	1.14	0.25						
HIV characteristics														
Baseline CD4 count (/μL), median [IQR]	82 [33-165]		-0.003	0.003	0.24									
Baseline CD4 count <100/μL	82	59	1.14	0.85	0.18									
CD4 count (present) (/μL), median [IQR]	467 [233-604]		0.004	0.0014	0.005	-0.003	0.004	0.4488						
CD4 increase (/μL)/time (years) median [IQR]	46 [18-101]		-	0.004	0.89									
			0.0006											
Current CD4 - baseline CD4 (/μL), median [IQR]	297 [103-487]		0.0046	0.0013	0.0007	0.0049	0.004	0.22	0.004	0.001	0.0006	0.00381	0.00131	0.004
Viral load (baseline) (log/ μL), mean (SD)	4.5 (1.37)		0.246	0.325	0.45									
Viral load (present) (log/ μL), mean (SD)	2.0 (0.98)		0.004	0.436	0.99									
Time since diagnosis (years), mean (SD)	6.8 (4.3)		0	0	0.95									
Time on treatment (years), mean (SD)	4.2 (2.9)		0.0003	0.0004	0.36									
Sleep, depression and pain														
PSQI global score, mean (SD)	7.7 (5.0)													
PSQI> 5 (n, %)	84	61												
ESS global score, mean (SD)	7.9 (5.9)		0.23	0.07	0.001	0.16	0.08	0.0514	0.184	0.065	0.0056			
ESS>10 (n, %)	43	31	2.03	0.9	0.03									
BDI score, mean (SD)	17.3 (12.5)		0.127	0.032	0.0001	0.046	0.037	0.21				0.102	0.031	0.0012
BDI score > 17 (clinical depression)	57	41	2.118	0.850	0.014									
Presence of pain in the past month	96	73	2.65	0.946	0.006	8.26	4.5	0.07	1.647	0.881	0.064	1.40	0.879	0.114
Rating of pain in the past month (1-10)	6.5 (2.8)		0.338	0.183	0.007	0.023	0.181	0.90						
Presence of pain on the study day	74	55	2.82	0.82	0.0009	2.01	1.01	0.0504	1.847	0.797	0.02	1.93	0.784	0.015
Rating of pain on the study day (1-10)	5.4 (2.7)		0.009	0.209	0.96									
ARV treatment														
NRTI														
Zidovudine (AZT)	31	22.3												
Didanosine (ddI, videx)	12	8.6												
Tenofovir (TDF)	57	41.0	-1.11	0.86	0.1995	-1.94	0.98	0.05	-0.623	0.813	0.445	-0.434	0.803	0.589
Abacavir (ABC)	8	5.8												
Stavudine (d4T)	35	25.2	0.504	0.980	0.607									
Lamivudine (3TC)	112	80.6												
NNRTI														
Efavirenz (EFV)	106	76.3	-0.138	1.0008	0.89									

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Nevirapine (NVP)

1 0.7

PI

Lopinavir/ritonavir

23 16.5 1.20 1.14 0.29

Supplementary material 2: Reliability and validity of the English Version of the Pittsburgh Sleep Quality Index (PSQI), Epworth Sleepiness Scale (ESS), and Beck Depression Inventory (BDI) when completed by second language English speakers.

Methods:

One hundred and fifty-two patients living with HIV and attending the adult HIV clinic at Chris Hani Baragwanath completed the English version of the PSQI, the ESS and the BDI. All of them were fluent in English but were second-language English speakers. For factor selection, for the PSQI and the BDI, we used the scree plots obtained for each questionnaire and included the factors that appeared before the linearization of the scree plot. For the BDI, we used the scree plot but also used guidance from past publications to limit the number of factors to 2.

Results

1- PSQI:

The Cronbach's alpha was 0.77 (raw variables) and 0.78 (standardized variables) (Supplementary Table 2). Omission of any of the 7 individual components did not negatively affect the underlying construct of the questionnaire (Supplementary Table 2). The 'sleeping medications' component was the least important aspect of the PSQI, which is in keeping with the fact that sleep disturbances are usually not treated per se in the public sector in South Africa. The most important factor was component 1, reflecting subjective sleep quality.

Factor analysis revealed that the questionnaire had a three-factor structure (supplementary Figure 1). Factor 1 included the sleep quality components (component 1: overall estimate of sleep quality, component 2 which evaluates sleep onset latency, component 5 for sleep disturbances). Factor 2 included the sleep duration items (component 3 for sleep duration and component 4 for sleep efficiency), and Factor 3 included the daytime impacting components (component 6 for sleeping medications and component 7 for daytime dysfunction). The pre-rotations eigenvalues of the three factors were 2.16 for Factor 1, 0.59 for Factor 2 and 0.18 for Factor 3. The three factors explained 42% of the variance; factor loadings are shown in Supplementary Figure 1.

2- Epworth Sleepiness Scale

For the ESS, the Cronbach's alpha was 0.76 (Supplementary Table 3). Loading of the 8 items comprising the questionnaire followed 3 main factors: a 'sleepiness while physically inactive' factor (Questions 1 to 3 and Question 7) with an eigenvalue of 2.5, a 'sleepiness when passive' factor (Questions 4 and 8) with an eigenvalue of 0.54 and a 'sleepiness while

engaged' factor (Questions 5 and 6) with an eigenvalue of 0.25. These 3 factors represented 41% of the variance. Factor loadings are shown in Supplementary Figure 2.

3- Beck Depression Inventory

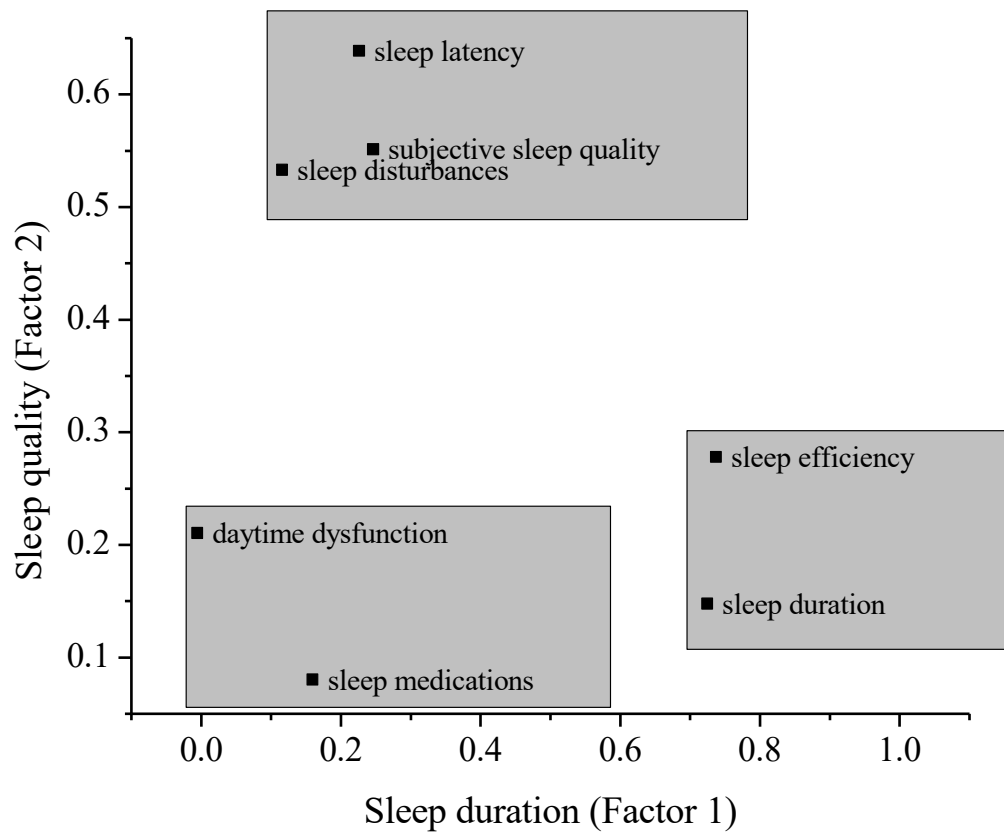
The Cronbach's alpha (both raw and standardized) was 0.90. Omission of one of the 21 questions did not affect the questionnaire's construct with Cronbach's alpha staying at 0.9 (Supplementary table 4). As observed in the original BDI validation study and subsequent validation studies of translated versions of the BDI, loading followed 2 main factors, a cognitive –affective factor (Factor 1, with an eigenvalue of 6.76) and a somatic factor (Factor 2, with an eigenvalue of 1.16) representing 37% of the variance. Factor loadings are represented in Supplementary Figure 3.

Supplementary Table 2: Cronbach's coefficient Alpha with deleted variable for the Pittsburgh Sleep Quality Index Scale – English as a second language in Johannesburg, South Africa.

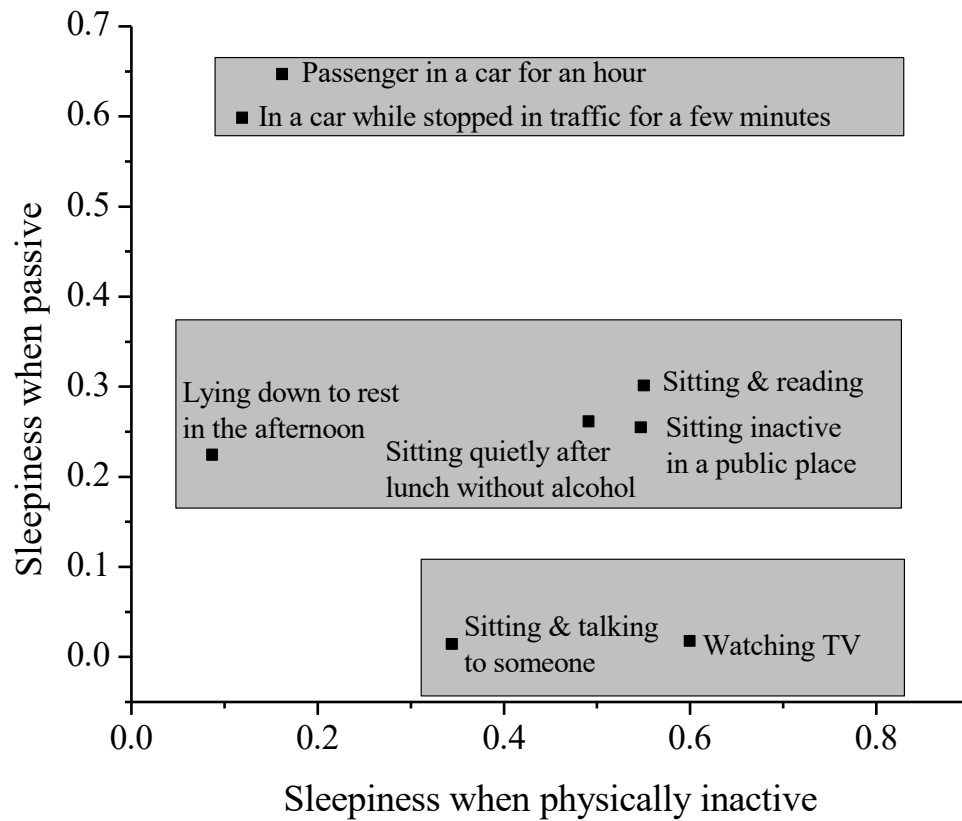
	Raw variables		Standardized variables	
	Correlation with total	Alpha	Correlation with total	Alpha
component 1: Subjective sleep quality	0.60	0.71	0.62	0.72
component 2: Sleep latency	0.55	0.73	0.57	0.73
component 3: Sleep duration	0.53	0.73	0.51	0.75
component 4: Sleep efficiency	0.53	0.73	0.51	0.75
component 5: Sleep disturbances	0.59	0.73	0.61	0.73
component 6: Sleep medications	0.32	0.77	0.33	0.78
component 7: Daytime dysfunction	0.34	0.77	0.36	0.77
Cronbach's alpha		0.77		0.78

Supplementary Table 3: Cronbach's coefficient Alpha with deleted variable for the Epworth Sleepiness Scale – English as a second language in Johannesburg, South Africa.

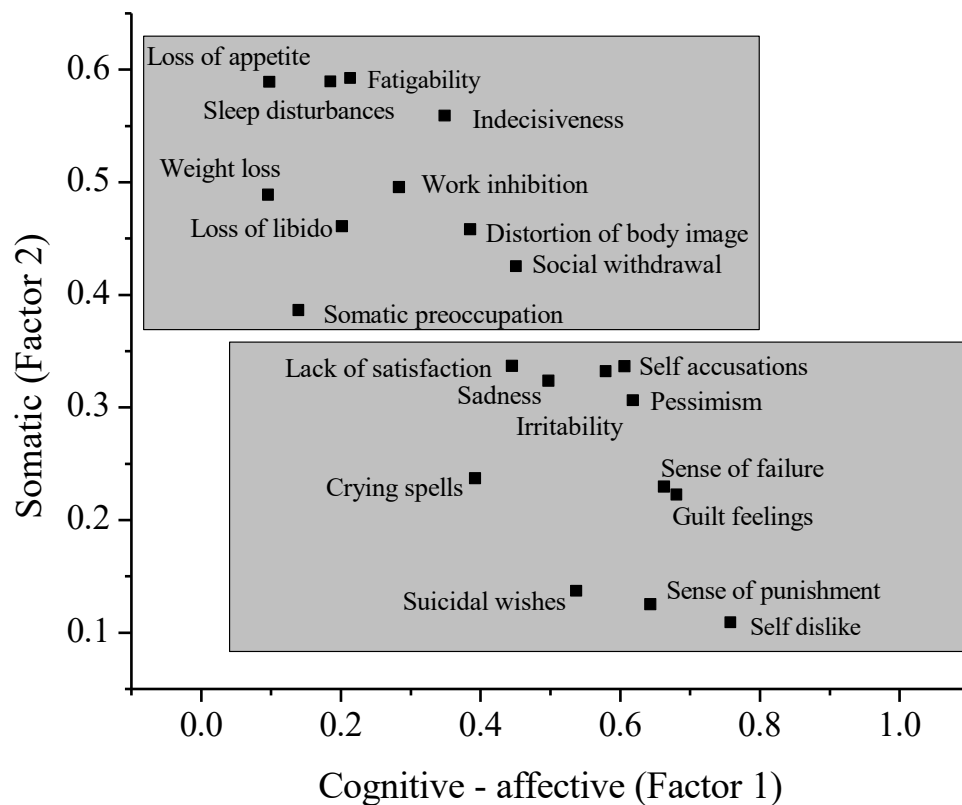
Deleted Variable <i>(In the last 30 days, how likely are you to doze off or fall asleep in the following situations?)</i>	Raw variables		Standardized variables	
	Correlation with total	Alpha	Correlation with total	Alpha
Q1 Sitting and reading	0.49	0.74	0.49	0.73
Q2 Watching TV	0.40	0.75	0.40	0.75
Q3 Sitting inactive in a public place	0.55	0.73	0.55	0.72
Q4 As a passenger in a car for an hour without a break	0.55	0.72	0.55	0.72
Q5 Lying down to rest in the afternoon	0.36	0.76	0.35	0.76
Q6 Sitting and talking to someone	0.39	0.75	0.39	0.75
Q7 Sitting quietly after lunch without alcohol	0.62	0.71	0.62	0.71
Q8 In a car while stopped for a few minutes in traffic	0.35	0.76	0.35	0.76
Cronbach's alpha		0.77		0.76



Supplementary Figure 1: Representation of the loading of the 7 PSQI components on the 2 main factors (sleep duration and sleep quality) in the rotated matrix of the factor analysis.



Supplementary Figure 2: Representation of the loading of the 8 questions comprising the ESS on the 2 main factors ('Sleepiness when physically inactive' and 'Sleepiness when passive') in the rotated matrix of the factor analysis.



Supplementary Figure 3: Representation of the loading of the 21 BDI questions on the 2 main factors (cognitive affective and somatic) in the rotated matrix of the factor analysis.

2.3 Cross-sectional study in a few people to nearly 2000 in Groblersdal

The first results chapter (Chapter 2 of this thesis) highlighted the presence of poor sleep in an HIV-positive population in South Africa. It is important to note that when these data were collected it was before the implementation of the HIV Counselling and Testing (HCT) Campaign, and when WHO guidelines required patients initiating antiretroviral therapy (ART) to have CD4 counts below 200 cells/ μ L, indicating clinical AIDS. By the end of data collection, the requirement had increased to 350 cells/ μ L. That being said, looking at the change in enrolment onto ART between 2009 at $\pm$ 300 000 people enrolled onto treatment, and over 600 000 people enrolled onto treatment as a result of the HCT campaign, South Africa really has improved on assisting PLWH in the management of their disease.

Additionally, the provision of fixed-dose combination (FDC) products in April 2013, from a pharmaceutical perspective has improved patient adherence, compliance, (Hirasen et al. 2017) and life expectancy, comparable to that of an uninfected individual (Johnson et al. 2013). Consequently, PLWH are living longer, but they may also be more susceptible to lifestyle and aging-related diseases such as cardiovascular disease and diabetes.

The first paper thus emphasized the problem of poor sleep among South Africans living with HIV and the association between CD4 counts and pain. This highlights the need for pain assessments in PLWH and further investigation into the role of CD4 in the inflammatory pathway of PLWH.

Given South Africa's significant efforts to provide access to medication for PLWH, the interaction between pain, HIV status, poor sleep, and CD4 T-cell count prompted the question of whether there was any evidence that HIV could have an impact on the circadian rhythm of PLWH. This led to the initiation of the second arm of the study, detailed in Chapter 3.

CHAPTER 3: DELAYED CIRCADIAN RHYTHMS IN OLDER AFRICANS LIVING WITH HUMAN IMMUNODEFICIENCY VIRUS (HIV)

This study was done as a collaboration between The University of the Witwatersrand (School of Public Health and the School of Physiology), The University of Cape Town, The University of Surrey, and The University of Northumberland. The study was conducted in the town of Agincourt, Bushbuckridge, Mpumalanga, South Africa.

We are grateful for the opportunity to take part in this collaborative effort to add to the knowledge base of persons living with HIV in South Africa.

Kindly note that this paper may be used by Katie O'Brien as part of her PhD thesis. Katie and I contributed equally to this paper, she focused primarily on the analysis of the actigraphy portion of the study where I was focused on the data collection, processing, and analysis of the melatonin samples that were used to create the dim light melatonin onset data, which I then analysed and used for the study.

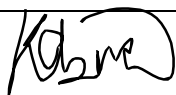
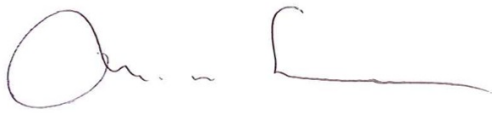
The melatonin samples were processed by Ms. Monica Gomez at the School of Physiology, while Kirsten transformed the data into a usable format and analysed it using GraphPad Prism (9) and SAS 9.4 ©. Kirsten specifically focused on analysing the melatonin data and actively participated in the creation of figures, graphs, and the manuscript for publication.

Ms. Katie O'Brien utilized the actigraphy data from the study, analysing bedtimes, wake times, and combining this information with the DLMO data to calculate the Phase Angle of Entrainment. Due to COVID-19 restrictions, Katie was unable to collect on-site data but did visit the research sites for a week to familiarize herself with the study locations. Katie also contributed in the creation of figures, graphs, and preparation of the manuscript for publication.

3.1 Permission from authors to use paper

Article 2: Title: ... Delayed circadian rhythms in older Africans living with human immunodeficiency virus (HIV)

Journal name, year, volume and page numbers: *Journal of Pineal Research*, 74(1), p.e12838

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6 th author	Prof. Malcolm Von Schantz		29/09/2023
7 th author	Prof. Karine Scheuermaier		30/03/2023

*contributed joint first author, contributed equally to this project

Comments by primary supervisor: While writing Paper 1 above, Ms Redman came across 2 intriguing papers in the literature which suggested delayed circadian rhythms induced by HIV infection. When Prof von Schantz and Dr Gomez-Olive received a Newton Fund Grant and proposed that we collaborate, Ms Redman helped supervise the dim light melatonin onset data collection between January and April 2019 at the Agincourt research centre. She then ran the melatonin assays and calculated DLMO in the participants described in the paper. She decided to compare the HIV+ and HIV- DLMO timing and found a significant delay in HIV+ participants (by nearly 1h). We decided to publish this finding along Prof von Schantz's PhD student (K O'Brien) analysis on sleep patterns in HIV+ and HIV- participants in the sample. This complete novel finding of delayed circadian rhythms in people with HIV may explain poor sleep quality and enhanced cardiometabolic risk in people with HIV

3.2 Paper in PDF format

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ORIGINAL ARTICLE



Delayed circadian rhythms in older Africans living with human immunodeficiency virus (HIV)

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Abstract

The increasing number of people living with human immunodeficiency virus, HIV, (PLWH) have an elevated incidence of risk for noncommunicable comorbidities, the aetiology of which remains incompletely understood. While sleep disturbances are often reported in PLWH, it is unknown to what extent they relate to changes in the circadian and/or sleep homeostatic processes. We studied the relationship between sleep characteristics, circadian phase, and HIV status in older adults from the HAALSI (Health and Ageing in Africa: a Longitudinal Study of an INDEPTH Community in South Africa) subsample of the Agincourt Health and Demographic Surveillance System in South Africa ($n = 187$, 36 human immunodeficiency virus positive [HIV+], age: 66.7 ± 11.5 years, range 45–93 years), where HIV prevalence is high and (in contrast to the global north) does not associate significantly with potentially confounding behavioural differences. In participants with valid actigraphy data ($n = 172$), regression analyses adjusted for age and sex indicated that HIV+ participants had slightly later sleep onset ($\beta = .16$, $p = .039$), earlier sleep offset times ($\beta = -.16$, $p = .049$) and shorter total sleep times ($\beta = -.20$, $p = .009$) compared to the HIV negative (HIV–) participants. In a subset of participants ($n = 51$, 11 HIV+), we observed a later dim light melatonin onset (DLMO) in HIV+ ($21:16 \pm 01:47$) than in HIV– ($20:06 \pm 00:58$) participants ($p = .006$). This substantial difference remained when adjusted for age and sex ($\beta = 1.21$; $p = .006$). In 36 participants (6 HIV+) with DLMO and actigraphy data, median phase angle of entrainment was -6 min in the HIV+ group and $+1$ h 25 min in the HIV– group. DLMO time correlated with sleep offset ($\rho = 0.47$, $p = .005$) but not sleep onset ($\rho = -0.086$, $p = .623$). Collectively, our data suggest that the sleep phase occurred earlier than what would be biologically optimal among the HIV+ participants. This is the first report of a mistimed circadian phase in PLWH, which has important potential

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implications for their health and well-being, especially given the well-established relationships between circadian asynchrony and sleep deprivation with poorer health outcomes.

KEYWORDS

circadian rhythm disorders, human immunodeficiency virus, neuroendocrinology

1 | INTRODUCTION

The development and global roll-out of antiretroviral therapy (ART) has dramatically increased the number of people living with human immunodeficiency virus (HIV) (PLWH). Calculated life expectancy at 20 years of age for people receiving ART ranges from 60% of normal (in Rwanda) to 89% (in Canada).¹ Improved odds for long-term survival result in a growing ageing population of PLWH. In the United States, nearly half of PLWH are now 50 years or older.² By necessity, the systems for caring for PLWH were initially designed to manage and treat primary and secondary infections. Consequently, these systems are not necessarily optimised and integrated for managing prevention and treatment of noncommunicable comorbidities in people who are living with well-controlled HIV infection for years or decades.³ Prominent among noninfectious comorbidities in PLWH are cardiometabolic disorders.^{4,5} PLWH have a twofold higher risk of developing cardiovascular disease (CVD) than uninfected individuals.⁶ While conditions such as hypertension, hypercholesterolaemia, and diabetes can be treated pharmacologically, modification of behaviours such as diet, physical activity and sleep are also important strategies to prevent and manage these disorders. Poor sleep quality is a well-established consequence of living with HIV, with an estimated prevalence of around 58%.⁷ While generalised sleep disturbances are widely reported in PLWH, the exact nature of these disturbances is scarcely discussed in the existing literature and generally lacks biological precision.⁸ Reports describe cognitive performance decrements associated with higher daytime sleepiness,⁹ symptoms associated with insomnia¹⁰ and atypical, apparently weight-independent presentations of obstructive sleep apnoea¹¹ in PLWH. Additionally, in a cohort of veterans, it was observed that PLWH with 'highly bothersome symptoms of insomnia' had a higher risk of incident CVD than PLWH without insomnia, suggesting an independent role of sleep in enhancing CVD risk in PLWH.¹²

Thus far, research on sleep in HIV has primarily focused on its homeostatic components (as measured

through sleep duration and staging) rather than testing the hypothesis that HIV may also impact circadian-related aspects of sleep (e.g., sleep-wake timing) or core measures of circadian regulation. There are some suggestions in the literature that HIV may indeed be associated with changes in circadian regulation. In a mouse model, intraparenchymal injection of HIV Tat protein into the suprachiasmatic nucleus (SCN, the master circadian pacemaker) was associated with phase delay shifts in the rest-activity rhythms.¹³ In one human study, PLWH had higher plasma melatonin levels in the morning (09:00) than HIV-negative (HIV-) individuals, suggesting a possible delay in the circadian phase, and higher morning melatonin concentration was associated with higher concomitant levels of Tat protein.¹⁴ Disrupted circadian rhythms in PLWH could at least partially explain the poor sleep quality and associated daytime dysfunction noted in this population, a likely risk factor for the increased prevalence of cardiometabolic comorbidities.

South Africa has the fourth highest prevalence of HIV globally (14%).¹⁵ It also has one of the largest ART programmes,¹⁶ which has enabled a decreased mortality and increased life expectancy. This high HIV prevalence in the community provides a unique opportunity (especially in rural areas) to investigate associated research questions within the traditional demographic composition and behavioural risk factors associated with HIV infection in the global north (e.g., gay men, other men who have sex with men and people who inject drugs, which is a well-documented disruptor of circadian rhythms¹⁷) being much less apparent. These unique circumstances enable the investigation of the biological associations between HIV infection, sleep, and circadian rhythms in a way that is not confounded by behavioural risk factors. Second, the rural setting of the study conveys the benefit of a stronger relationship with natural Zeitgebers (with lower availability of artificial lighting) and potentially reduces confounding factors associated with urban lifestyle.^{18,19}

Thus, the aim of this study was to compare objectively measured aspects of the circadian phase and actigraphy-derived sleep characteristics between older, rural individuals living with HIV to those who are HIV-.

2 | MATERIALS AND METHODS

2.1 | Participants and study design

Participants in this study were a random sample representative of the HAALSI (Health and Ageing in Africa: a Longitudinal Study of an INDEPTH Community in South Africa) study, which in turn is a representative sample of participants 40 years and above within the Agincourt Health and Socio-demographic Surveillance System.²⁰ This investigation conformed with the tenets of the Declaration of Helsinki and was approved by the University of the Witwatersrand Human Research Ethics Committee (#M180667) and the Mpumalanga Provincial Research and Ethics Committee. Written informed consent was obtained from all participants. For illiterate participants, a witness not linked to the study was present during the consenting process and signed and dated the form on behalf of themselves and the participant. This rural cohort is based in the

Bushbuckridge subdistrict, Mpumalanga Province, South Africa (24.4°S 31.5°E). We present preliminary data for a subgroup of participants from a larger study.²¹ Figure 1 displays the sample sizes for all participants along with groups that explored actigraphy, dim light melatonin onset (DLMO), and circadian phase angle data in the present study. Demographic (age, sex, employment status, wealth index), Pittsburgh Sleep Quality Index (PSQI)²² and actigraphy data were collected for 187 participants (HIV–: $n = 151$, 81%; HIV+: $n = 36$, 19%). Of these, 172 participants (age range: 45–93 years) had valid actigraphy data (HIV–: $n = 141$, 82%; HIV+: $n = 31$, 18%). A subgroup of 59 individuals participated in a protocol to determine DLMO, HIV–: $n = 47$, 80%; HIV+: $n = 12$, 20%). Valid DLMO data were available for 51 of these participants (HIV–: $n = 40$, 78%; HIV+: $n = 11$, 22%). Finally, the circadian phase angle of entrainment was calculated from a subgroup of 36 participants with both valid actigraphy and DLMO data (HIV–: $n = 30$, 84%; HIV+: $n = 6$, 16%).

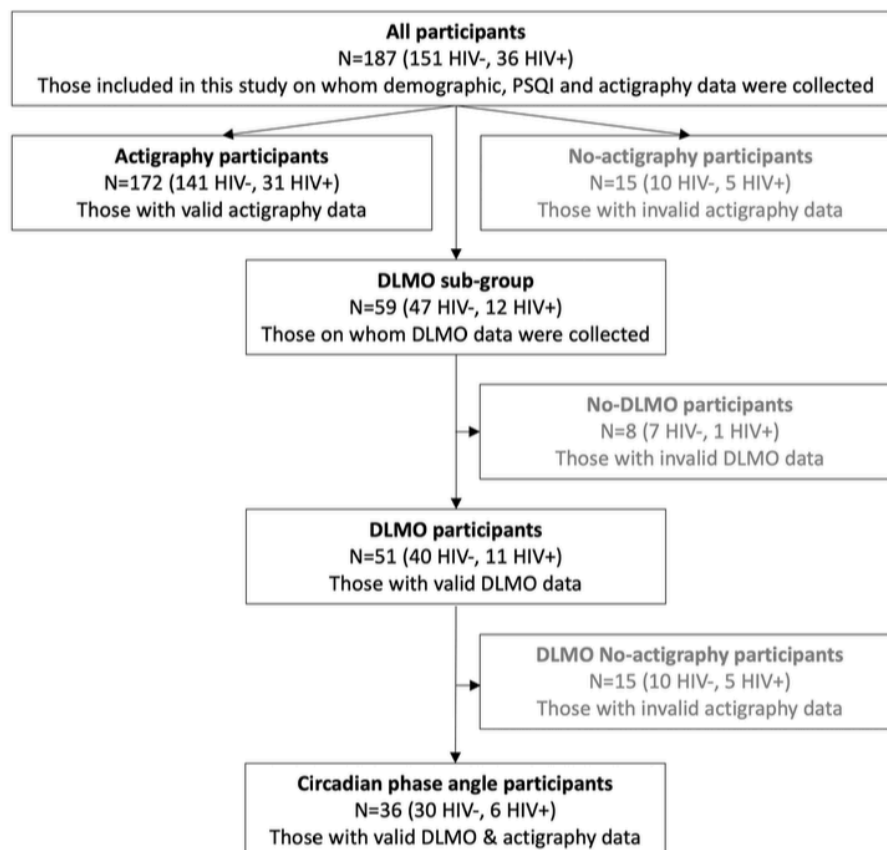


FIGURE 1 Diagram showing sample sizes and HIV status composition of all participants in this study as well as subgroups for actigraphy, DLMO and circadian phase angle analyses. DLMO, dim light melatonin onset; HIV, human immunodeficiency virus; PSQI, Pittsburgh sleep quality index.

2.2 | HIV status

HIV status was determined using the dried blood spot (DBS) assay. Five blood drops were collected from each participant using the finger prick technique on Whatman 903 TM filter paper and kept at room temperature for 1–3 weeks. The DBS was sent to Global Labs and stored at -20°C until the subsequent Vironostika Uniform 11 (Biomerieux) immunoassay. If positive, confirmation was performed using Elecsys (Roche). HIV+ status based on immunoreaction was then confirmed by reverse transcription-polymerase chain reaction-based viral load analysis.

2.3 | Actigraphy

Participants wore an accelerometer (ActTrust; Condor Instruments) on their nondominant wrist for 14 consecutive days. Files were processed in ActStudio (Condor Instruments) and visually inspected to ascertain data quality. Sleep onset (time at which sleep started) and offset (time at which sleep ended) were manually marked according to published actigraphy protocols.²³ Subsequently, application of the Cole-Kripke algorithm in ActStudio generated estimates of sleep-based variables, including sleep onset and offset, wake after sleep onset (WASO), total sleep time (time elapsed between sleep onset and sleep offset, less WASO) and sleep efficiency (percent time spent asleep between sleep onset and offset). Each of these variables was averaged across all valid days (minimum of 5 consecutive nights) of actigraphy data for each participant. Because actigraphy data were invalid for 15 participants (including 5 HIV+), we only present actigraphy data for 172 participants (HIV+: $n = 31$, 18%; Figure 1).

2.4 | DLMO

Saliva samples were collected hourly between 17:00 and 23:00 while participants sat in a dimly lit (<3 lux) room at the laboratory at Agincourt. Participants were asked to accumulate saliva in their mouths and spit directly into 15-ml collecting tubes, as described previously.¹⁸ Saliva was stored at -20°C until subsequent analysis at the University of the Witwatersrand. We were able to limit the melatonin assay to six samples per person by excluding one collected sample from the radioimmunoassay. Using the usual relationship between DLMO and bedtime (DLMO occurring ~ 2 h before bedtime), we used the participant's known bedtime as an approximation to decide whether we should rather

assay the earlier sample (17:00) or the later sample (23:00). Saliva was stored at -20°C until subsequent analysis at the University of the Witwatersrand. Samples were thawed and centrifuged to remove any debris. The supernatant was processed to determine melatonin saliva concentration using the direct saliva melatonin radioimmunoassay kit (Bühlmann Laboratories) (intraassay precision: 2.6%–20.1%; interassay precision: 6.6%–16.7%). Each sample was assayed in duplicate. The standard curve for each melatonin kit was determined using Prism 9 Software (GraphPad) and melatonin concentration for each participant was computed based on the standard curve for each kit. For participants whose measurements did not reach the 3 pg/ml cutoff for DLMO but had a clear rising slope of melatonin secretion, we extrapolated up to the 3 pg/ml limit to determine DLMO time. We only did this when the 3 pg/ml threshold was estimated to happen in the hour following the last sampled melatonin, assuming the slope remained constant between the last hour of sampling and this unsampled hour. Thus, we used the slope (change in melatonin concentration over 1 h) of the previous hour and calculated the time required to get from the last sample to reach 3 pg/ml. Of the 59 participants with melatonin data, eight had no detectable levels of melatonin during the sampling period and were labelled nonsecretors. We therefore present DLMO analysis data for 51 participants (HIV+: $n = 11$, 21.6%; Figure 1). Finally, we determined the circadian phase angle of entrainment for a subset of participants who had both valid actigraphy and DLMO data ($n = 36$, HIV+: $n = 6$; Figure 1). To do this, we subtracted DLMO time from habitual sleep onset time obtained from actigraphy. For the majority of participants, wherever possible, actigraphy immediately followed the DLMO collection.

2.5 | Data and statistical analyses

Data are presented as mean $\pm$ SD, count (percentage) and β -coefficient (95% confidence intervals). Between-group (HIV– vs. HIV+) comparisons of our demographic, actigraphy and DLMO variables of interest were made using analysis of variance (ANOVA) or chi-squared tests as appropriate. Spearman's correlation was used for bivariate associations. General linear models (GLMs) were constructed to assess the relationship between HIV status and DLMO/collected actigraphy parameters, controlling for age and sex. For statistically significant models, the effect size was established through the calculation of partial eta-squared (denoted by η^2_p). Data were analysed using RStudio.

3 | RESULTS

Descriptive demographic, health and sleep characteristics and unadjusted univariate analyses of the HIV– and HIV+ groups are presented in Table 1. HIV+ participants were younger ($p < .001$), had lower body mass index ($p = .049$), were less likely to be hypertensive ($p = .011$) and had lower total sleep time ($p = .035$) than the HIV– participants.

3.1 | Actigraphy-derived sleep characteristics

Regression models, adjusted for age and sex, assessing associations between HIV status and the sleep variables obtained from actigraphy are summarised in Table 2. We found suggestive evidence for a slightly later sleep onset (adjusted average delay of 0.16 h, i.e., 10 min; $F(3,168) = 1.63$; $p = .039$) and earlier sleep offset

(adjusted average advance of 0.16 h, i.e., 10 min; $F(3,168) = 2.65$; $p = .049$) in HIV+ participants compared to HIV– participants when adjusting for age and sex. Additionally, total sleep time in HIV+ participants was shorter compared to HIV– participants when adjusting for age and sex (adjusted average total sleep time of 0.20 h, i.e., 12 min; $F(3,168) = 2.69$; $p = .009$; observed power = 0.625, $\eta^2_p = 0.03$). No significant relationships were observed between HIV status and WASO or sleep efficiency.

3.2 | DLMO

Age (64.2 ± 7.6 years) and gender distribution (32 females, 63%) of the subset of participants for whom DLMO data were available ($n = 51$, HIV+: $n = 11$) were not different to the larger sample. There were no significant relationships between DLMO times and either age, sex, employment status, PSQI score, or wealth quintile (data not shown).

TABLE 1 Demographic, health and sleep characteristics of all participants

	HIV– (N = 151)	HIV+ (N = 36)	Total (N = 187)	p Value
Age (years)	68.2 $\pm$ 11.2	60.6 $\pm$ 10.6	66.7 $\pm$ 11.5	<.001
Sex				.693
Female	87 (59.2%)	20 (55.6%)	107 (58.5%)	
Male	60 (40.8%)	16 (44.4%)	76 (41.5%)	
BMI (kg/m ²)	26.9 $\pm$ 9.0	24.7 $\pm$ 9.8	26.5 $\pm$ 9.2	.049
Hypertension	89 (59.3%)	11 (34.4%)	100 (54.9%)	.011
Diabetes	9 (6.0%)	0 (0.0%)	9 (4.9%)	.366
Employment status				.158
Employed	16 (10.7%)	8 (22.2%)	24 (13.0%)	
Homemaker	15 (10.1%)	3 (8.3%)	18 (9.7%)	
Unemployed	118 (79.2%)	25 (69.4%)	143 (77.3%)	
Wealth quintile	3.2 $\pm$ 1.5	2.9 $\pm$ 1.4	3.3 $\pm$ 1.5	.246
PSQI global score	4.0 $\pm$ 3.2	4.7 $\pm$ 3.5	4.1 $\pm$ 3.3	.234
Actigraphy data	HIV– (N = 141)	HIV+ (N = 31)	Total (N = 172)	p Value
Sleep onset (hh:mm)	21:00 $\pm$ 01:01	21:27 $\pm$ 01:05	21:04 $\pm$ 01:03	.109
Sleep offset (hh:mm)	06:02 $\pm$ 00:56	05:40 $\pm$ 01:28	05:58 $\pm$ 01:03	.337
Total sleep time (hh:mm)	07:10 $\pm$ 01:31	06:30 $\pm$ 01:15	07:03 $\pm$ 01:29	.035
WASO (hh:mm)	01:45 $\pm$ 00:42	01:41 $\pm$ 00:36	01:45 $\pm$ 00:41	.723
Sleep efficiency (%)	78.9 $\pm$ 8.1	77.9 $\pm$ 5.9	78.7 $\pm$ 7.8	.385

Note: Data are presented as unadjusted mean $\pm$ SD or count (percentage). Between-group comparisons were performed using ANOVA or chi-squared tests. Bold values indicate statistical significance.

Abbreviations: ANOVA, analysis of variance; BMI, body mass index; HIV, human immunodeficiency virus; PSQI, Pittsburgh sleep quality index; WASO, wake after sleep onset.

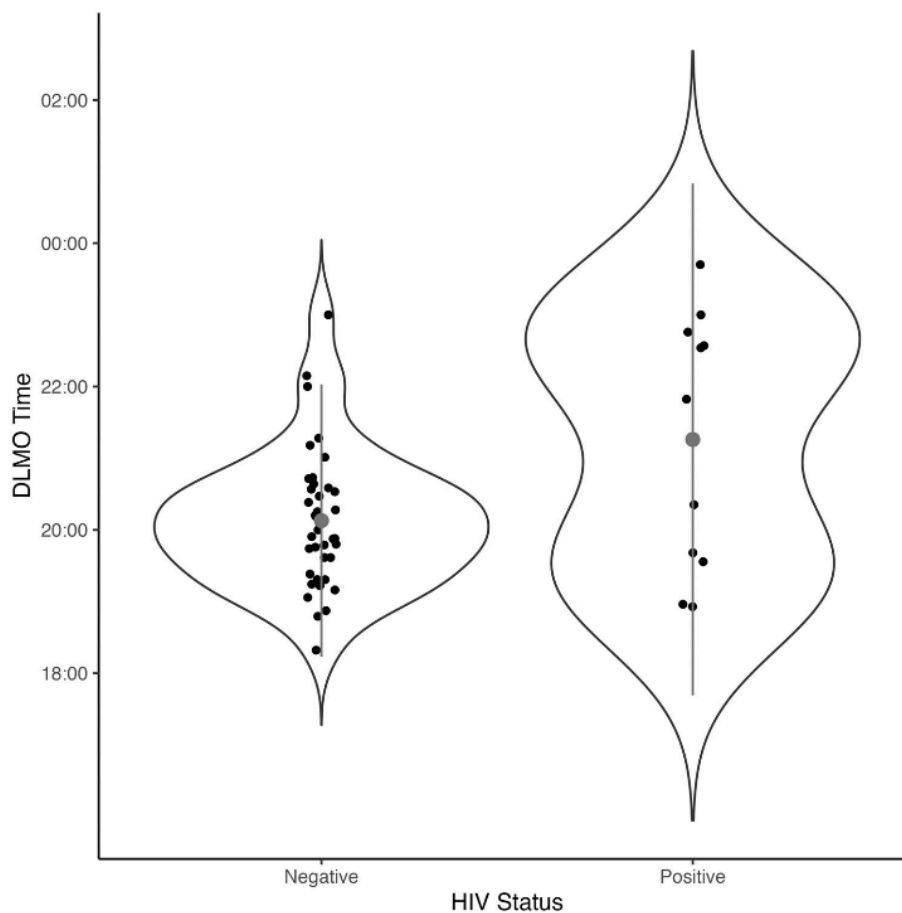
TABLE 2 Standardised β -coefficients extracted from linear regression models to investigate the relationships between actigraphy-derived sleep characteristics and HIV status ($n = 172$)

Variable	Sleep onset	Sleep offset	Total sleep time	WASO	Sleep efficiency
Model number	(1)	(2)	(3)	(4)	(5)
HIV+	0.16* (0.22)	-0.16* (0.22)	-0.20 (0.31)**	-0.01 (8.44)	-0.08 (1.59)
Age (years)	-0.01 (0.01)	-0.11 (0.01)	-0.13 (0.01)	0.11 (0.28)	-0.14 (0.05)
Sex—male	0.02 (0.16)	-0.02 (0.16)	0.00 (0.23)	-0.03 (6.37)	-0.00 (1.21)

Note: Data are presented as β -coefficients (95% confidence interval).

Abbreviations: HIV, human immunodeficiency virus; WASO, wake after sleep onset.

* $p < .05$; ** $p < .01$.

**FIGURE 2** Timing of DLMO in HIV- ($n = 40$) and HIV+ ($n = 11$) participants. Individual data points are plotted in addition to the median represented by the larger grey dot, with the dispersion displayed as a violin plot. **A statistical between-group difference ($p = .006$) determined using one-way ANOVA. ANOVA, analysis of variance; DLMO, dim light melatonin onset; HIV, human immunodeficiency virus.

HIV+ participants had significantly later DLMO times compared to HIV- participants (HIV-: $20:06 \pm 00:58$, HIV+: $21:16 \pm 01:47$; one-way ANOVA $F(1, 49) = 8.46$, $p = .006$) (Figure 2). As the average age of the HIV+ participants was lower than that of the HIV- participants, we also ran a GLM on the effect of HIV status on DLMO time when adjusting

for age and sex. This confirmed a large significant phase delay in HIV+ participants [β (95% CI) = 1.21 (1.68); $p = .006$] with no adjusted effect of age ($p = .379$) or sex ($p = .070$).

In Figure 3, we extend a previously published figure¹⁸ displaying DLMO times from 16 other studies to

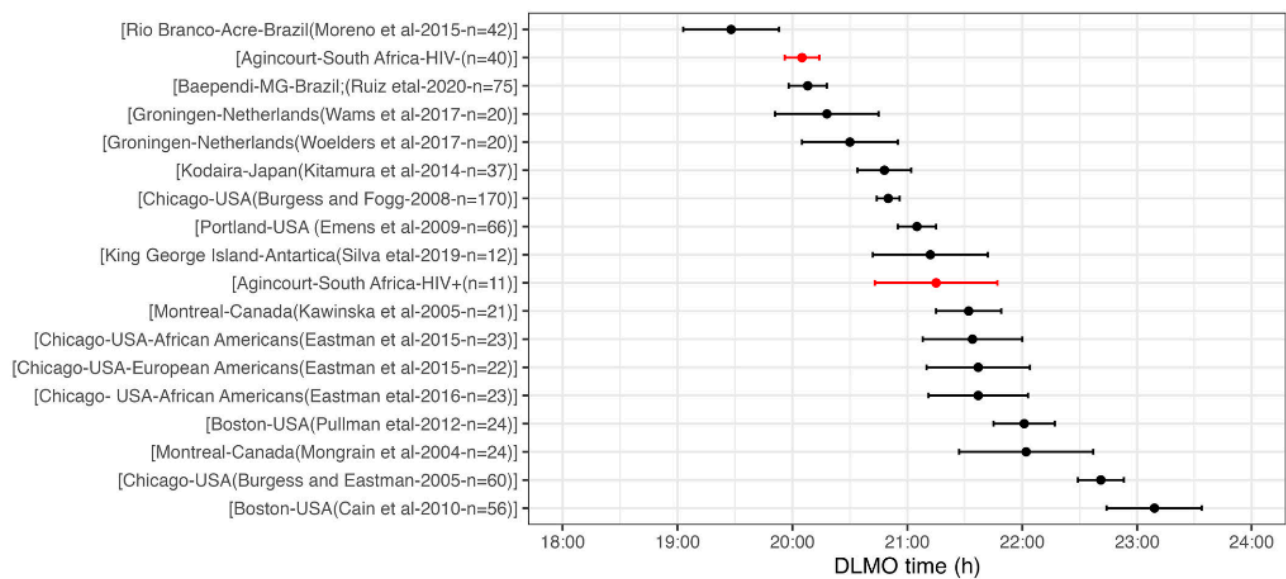


FIGURE 3 DLMO times for the HIV+ and HIV- participants from the current study (shown in red) compared to published DLMO data from other adult populations. Figure modified from Ruiz et al.¹⁸ where references are listed. Data are presented as mean $\pm$ SEM. DLMO, dim light melatonin onset; HIV, human immunodeficiency virus.

contextualise the timing of DLMO observed in the HIV+ and HIV- participants in the present study in relation to other cohorts.

3.3 | Phase angle of entrainment

Finally, we explored the relationship between DLMO time and habitual actigraphy-derived sleep onset in our participants as a measure of the phase angle of entrainment between the sleep-wake cycle and endogenous circadian rhythms. Given the relatively small sample for whom we have both valid actigraphy and DLMO data ($n = 36$, HIV+: $n = 6$, 16%), we present these data descriptively in Figure 4, which shows each participant's DLMO time relative to their habitual sleep period and Figure 5, which shows the phase angle of entrainment between the HIV- and HIV+ groups. The observed data suggest that HIV+ participants had a smaller phase angle between DLMO time and habitual sleep onset, with half of the HIV+ group having an earlier habitual sleep onset than DLMO time, suggesting a negative phase angle of entrainment. DLMO time was associated with sleep offset time (Spearman's correlation: $n = 36$, $\rho = 0.47$, $p = .005$) but not with sleep onset time ($n = 36$, $\rho = -0.086$, $p = .623$). When doing partial correlations adjusting for HIV status, we observed the same results.

4 | DISCUSSION

In this randomly selected and representative sample from an ageing rural South African population, PLWH had significantly later DLMO timing (by more than an hour) compared to those without HIV infection. To contextualise this, the early DLMO timing in the HIV- participants in this study was very close to that observed in a Brazilian Baependi cohort, another rural community in the global south (Figure 3),¹⁸ while that of the HIV+ group was very much in line with urban cohorts in the United States. In spite of their later circadian phase, however, the timing of sleep onset of PLWH was only slightly delayed (10 min in adjusted analyses) and not correlated with DLMO timing. This would suggest that PLWH are sleeping earlier in their circadian phase compared to HIV- participants, resulting in a shorter phase angle of entrainment between the circadian and the sleep-wake cycle. The possible consequences of a smaller phase angle of entrainment include difficulty in initiating and maintaining sleep, similar to what is observed in younger adults living with insomnia and in healthy older adults whose sleep is more disrupted than that of younger adults.^{24,25} This later timing of DLMO with a shorter phase angle of entrainment may to some extent explain our observation of the slightly shorter total sleep time (12 min in adjusted analyses) derived from actigraphy in PLWH, although we have no way of distinguishing to what extent this shorter total sleep time

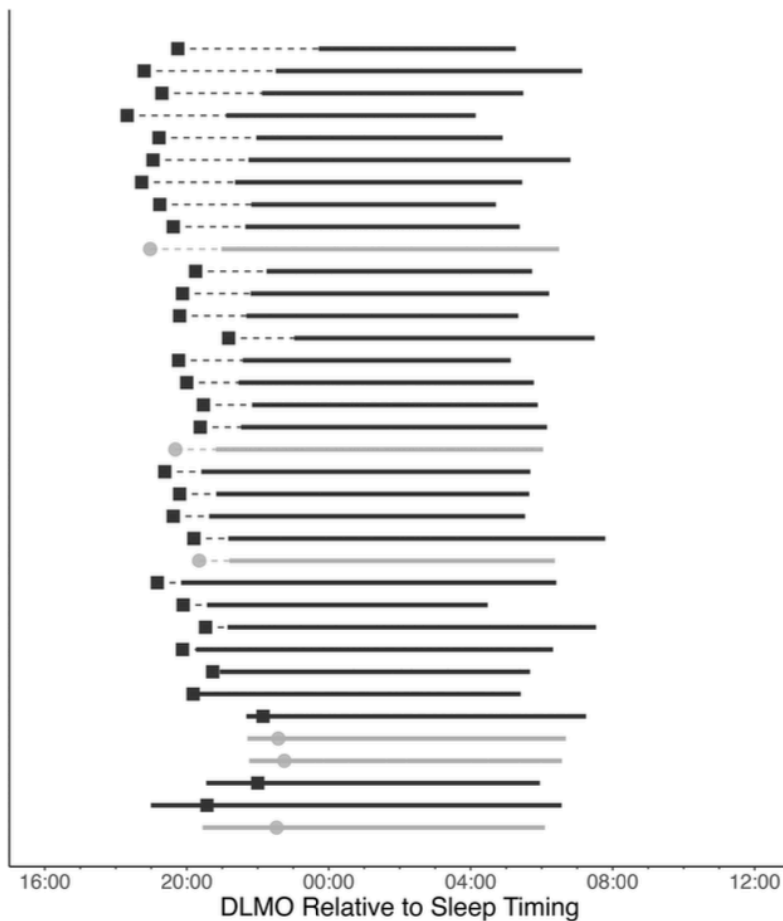


FIGURE 4 Relationship between DLMO time and habitual actigraphy-derived sleep timing ($n = 36$). DLMO time is represented by black squares for HIV $-$ participants ($n = 30$) and grey circles for HIV $+$ participants ($n = 6$). Average actigraphy-derived sleep onset and offset across the actigraphy collection period were plotted for each participant, represented by the horizontal bars. DLMO, dim light melatonin onset; HIV, human immunodeficiency virus.

may also result from inflammatory processes, the infection itself, or comorbid conditions such as pain or depression.^{8,26} Nonetheless, the shorter, potentially mistimed sleep relative to the endogenous circadian cycle observed in this study provides objectively measured evidence supporting the abundant previous subjective reports of poor sleep quality and insomnia in PLWH.^{8,26,27}

Sleep efficiency, a marker of sleep quality, was generally low in this population, regardless of HIV status, and was poorer than would generally have been expected in this age group.²⁸ This suggests that the sleep environment, in general, was not optimal (a well-documented feature of sleep in deprived areas of the global south, associated with factors such as sleeping quarters, temperature, noise and concerns about personal safety^{19,29}), with similar amounts of time spent awake during the night regardless of HIV status. The fact that PLWH had a significantly shorter sleep time, even though their sleep efficiency was not significantly different, may be partly explained by the slight delay in sleep onset and slight advance in sleep offset observed in the actigraphy data of PLWH. We hypothesise that the

advanced age of our sample may make it difficult to see true differences in sleep efficiency between the two groups, which both had age-related reduced efficiency, and therefore efficiency may not be reduced further by HIV infection. Other limitations include that the DLMO and actigraphy data were based on different (but partially overlapping) subsets of participants as well as the absence of data on napping, light exposure, sleep-wake schedules, sleep and mood disorders, all of which may influence sleep parameters and circadian phase.

Although the timing of seroconversion is not known, most of the ageing PLWH in this study will have lived with the infection for a number of years; for this reason, we cannot entirely rule out the theoretical possibility that our observation reflects some kind of survivor effect driven by delayed circadian phase. The observation does suggest multiple novel lines of research. A key element will be to confirm whether the delayed circadian phase is also present in PLWH of different ages and living in other settings. This is important to understand the biological reason for and downstream effects of this circadian phase delay and its potential roots in abnormal circadian entrainment. A biological cause for an HIV-related phase

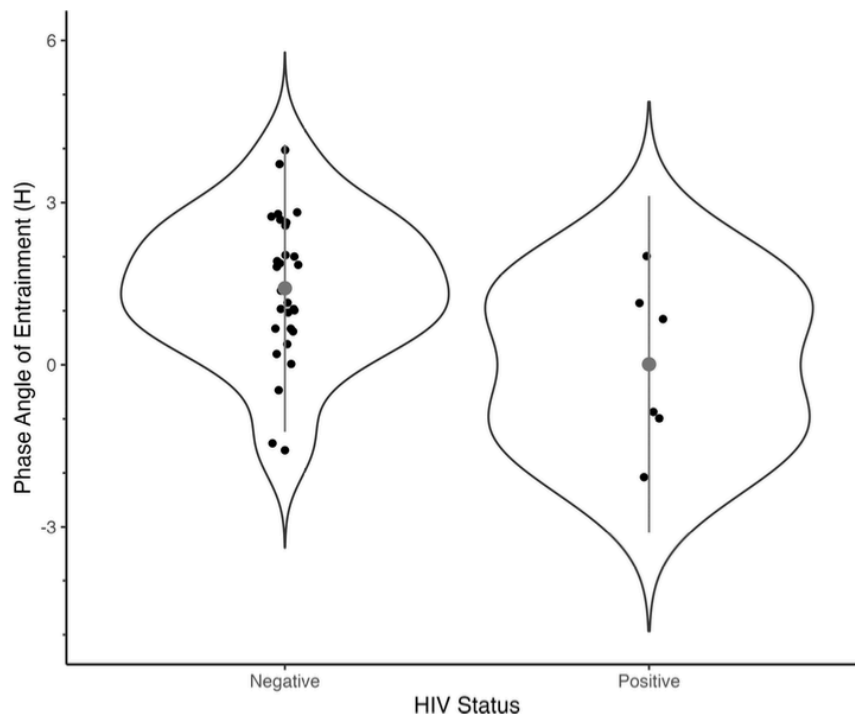


FIGURE 5 Violin plot comparing the phase angle of entrainment between the HIV– ($n = 30$) and HIV+ ($n = 6$) participants. Individual data points are plotted in addition to the median represented by the larger grey dot, with the dispersion displayed as a violin plot. HIV, human immunodeficiency virus.

delay in circadian rhythms is supported by a mechanistic animal study, which found phase delays of the rest-activity cycle of mice associated with SCN injection of the HIV protein Tat. In this study, further experiments showed that Tat injection impeded the downstream process of light entrainment in the SCN rhythms.¹³ In a human study, HIV+ participants had higher morning melatonin levels at 09:00, a finding compatible with a delay in circadian rhythms in PLWH.¹⁴ In that study, the higher morning plasma melatonin levels were associated with higher HIV protein Tat plasma levels, suggesting a direct biological effect of Tat on circadian rhythms in PLWH.

Globally, the relative risk of HIV seroconversion is more than 20 times higher in certain groups (gay men and other men who have sex with men, people who inject drugs, and sex workers) compared to adults aged 15–49 years in the general population.¹⁶ Behavioural factors associated with belonging to one or more of these groups would be strong potential confounders for studies of sleep and circadian phase. By contrast, in rural Southern Africa, the epidemic has been less demographically discriminating, as is evident from its much higher general population prevalence. This strengthens the

biological validity of the delayed circadian phase in PLWH observed in this study. There are no notable differences in lifestyle between the HIV– and HIV+ individuals in this study. The members of this ageing population are mostly beyond retirement age, living quiet, rural lives supported by government remittances and subsistence farming. Potential lifestyle differences would have been much harder to control for if we had studied a younger population or indeed one living on another continent. If the lifestyle-independent circadian misalignment observed in the current study is confirmed to be a constant feature of chronic HIV infection, then it may be a mediator both of poorer sleep health and of poorer physical health in PLWH, which could potentially be alleviated through light therapy or chronobiotic medication or supplements.

AUTHOR CONTRIBUTIONS

D. E. Rae, F. X. Gómez-Olivé, M. von Schantz and K. Scheuermaier designed the study. K. N. Redman, K. E. O'Brien, F. S. Ruiz and K. Scheuermaier developed methods and collected data. K. N. Redman, K. E. O'Brien, F. S. Ruiz, D. E. Rae, F. X. Gómez-Olivé, M. von Schantz and K. Scheuermaier analysed and interpreted

the data. K. N. Redman, K. E. O'Brien, F. S. Ruiz, D. E. Rae, F. X. Gómez-Olivé, M. von Schantz and K. Scheuermaier prepared the manuscript. All authors approved the final version of the manuscript.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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3.3 From circadian misalignment to evidence of the role of sleep in inflammation and CVD

The studies outlined in papers one and two (results sections one and two) shed light on the impact of HIV on the two aspects of sleep: homeostatic sleep in that there is evidence of poor sleep in people living with HIV (PLWH), and process C, in which there is evidence of a phase delay in the circadian rhythm of PLWH. In that there is a shift in the timing of DLMO (dim light melatonin onset) to later in the biological evening. While it was known that sleep was affected in PLWH, the extent of this impact remained unclear. According to the aims highlighted in the introduction chapter, we wanted to know if there was any impact of poor sleep / insomnia in PLWH on inflammation, using C-reactive protein.

Consequently, the study included a third and final arm, which aimed to investigate the relationship between sleep, HIV status, inflammation and any markers of cardiometabolic disease risk

Given the rising prevalence of obesity, diabetes, and hypertension in South Africa, coupled with the increasing longevity of PLWH due to treatment, it became crucial to examine the potential influence on cardiometabolic risk factors.

CHAPTER 4: INSOMNIA AND LIVING WITH HIV ARE ASSOCIATED WITH HIGHER LEVELS OF INFLAMMATION IN PEOPLE LIVING IN RURAL SOUTH AFRICA

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4.1 Abstract

Background

Poor sleep is a common complaint in people living with HIV (PLWH) and has been independently associated with inflammation and higher cardiovascular disease (CVD) risk. Therefore, this study investigated the association of sleep complaints and HIV with inflammation and CVD risk factors in a South African population of PLWH.

Methods

We used baseline data from the Ndlovu Cohort Study in Elandsdoorn, South Africa, which included 1915 participants (1040 PLWOH and 887 PLWH, including 691 on ART). We investigated the presence of poor sleep (sleep complaints) in this cohort using Question 3 of the Patient Health Questionnaire–9 (PHQ-9). We collected anthropometric measurements, lipogram data, and C-reactive protein (CRP) as a marker of inflammation and assessed levels of activity using the International Physical Activity Questionnaire (IPAQ). We first compared patients with and without sleep complaints. We then ran univariable and multivariable analyses of the association of inflammation with HIV status, sleep complaints, and CVD risk factors with adjustment for confounders. Finally, we tested the association of CVD risk (Framingham risk score, FRS) with HIV status, inflammation and sleep complaints in an adjusted multivariable analysis.

Results

People with sleep complaints (n=290) were more likely to be HIV-positive, and have higher total and LDL cholesterol, higher levels of depression, and were less likely to be on ART than those without sleep complaints and had higher levels of CRP ($p<0.001$).

In univariable analysis, sleep complaints ($\beta=0.4$, $p=0.03$) and being HIV positive ($\beta=0.2$, $p<0.001$) were both associated with CRP. In multivariable analyses, we found an interaction between sleep complaints and HIV status, whereby having sleep complaints and being HIV positive was associated with the highest levels of CRP ($\beta=0.292$, $p<0.001$). Being an HIV-positive male was associated with higher levels of CRP ($\beta=0.208$, $p<0.001$). Having sleep complaints, higher inflammation and being HIV negative were independently associated with higher FRS (all $p<0.01$).

Conclusions:

We found that being HIV positive and having sleep complaints together enhance inflammation in a South African population. Higher inflammation and sleep complaints were associated with higher cardiovascular risk. Detecting and treating sleep issues early should become a focus of care in PLWH.

Keywords: Inflammation, HIV, cardiometabolic risk, sleep complaints, Ndlovu

Abbreviations: ART= anti-retroviral therapy, CRP = C-reactive protein, CVD = cardiovascular disease, HIV = Human Immunodeficiency Virus, FRS = Framingham Risk Score, HDL=High Density Lipoprotein, LDL=Low Density Lipoprotein, PLWH = people living with HIV, PLWOH = people living without HIV.

4.2 Introduction

Inflammation is present in people living with HIV (PLWH) (Hileman and Funderburg, 2017; Lv, Cao and Li, 2021), and, along with traditional cardiovascular disease (CVD) risk factors and the use of antiretroviral therapy (ART), may contribute to increased CVD risk in PLWH compared to people living without HIV (PLWOH) (Friis-Møller *et al.*, 2003). Poor sleep and sleep complaints have been reported in many HIV studies and have been associated with higher inflammation (O'Brien *et al.*, 2021; Bruno *et al.*, 2023).

Inflammation has been hypothesised to mediate the higher cardiovascular risk observed in well-controlled PLWH. It causes structural changes to the vascular endothelium, affects cardiomyocyte function, and is associated with an increased risk of diabetes and metabolic syndrome (Wolf and Ley, 2019). There are complex interrelationships between inflammation, sleep, depression, and physical activity. In PLWH, HIV infection itself, the use of antiretroviral therapy (ART), lower physical activity, poor sleep and higher depression can modify levels of inflammation (Hileman and Funderburg, 2017).

Systemic inflammation affects the motor cortex and limbic system, causing lethargy and fatigue (K. A. Lee *et al.*, 2014). Depression, which is associated with poor sleep quality, has been linked to decreased ART adherence in PLWH (Yen *et al.*, 2022). Poor ART adherence, in turn, is associated with higher inflammation (Castillo-Mancilla *et al.*, 2021) in PLWH. Finally, higher physical activity levels correlate with lower inflammation (Bernal *et al.*, 2021) and better sleep overall (Alnawwar *et al.*, 2023).

Poor sleep, which affects between 52 and 58% of PLWH (O'Brien *et al.*, 2021; Mousavi *et al.*, 2023), leads to increased inflammation, as indicated by elevated levels of circulating C-reactive protein (CRP; Chiang, 2014). The mechanisms may involve modifications to regulatory T-cell function (Besedovsky, Lange and Haack, 2019). In PLWH, who are already at risk of chronic inflammation, poor sleep may thus further exacerbate the inflammatory environment and increase cardiovascular risk.

On treatment, the life expectancy of PLWH is comparable to that of PLWOH (Johnson *et al.*, 2013; Trickey *et al.*, 2023), and the effects of ageing with HIV as a chronic condition now need to be considered (Nasi *et al.*, 2016). Older people have a higher risk of CVD and have higher levels of inflammation than the general population (Rodgers *et al.*, 2019). Sleep

quality also tends to get worse with older age (Dijk *et al.*, 1999; Gadie *et al.*, 2017; Casagrande *et al.*, 2022). In addition to its bidirectional association with inflammation, poor sleep quality has a range of negative consequences, including an increased risk of developing cardiometabolic diseases (Irwin, Olmstead and Carroll, 2016).

There is evidence of an association between short sleep duration and dyslipidaemia (Smiley *et al.*, 2019; Tsiptsios *et al.*, 2023; Zhu *et al.*, 2023), and dyslipidaemia itself is known to contribute to inflammation, thereby increasing the risk of developing CVD (Lechner *et al.*, 2020; Hong *et al.*, 2022). Dyslipidaemia, an established risk factor for CVD (Powell-Wiley *et al.*, 2021; Du and Qin, 2023), describes various lipid irregularities such as elevated low-density lipoprotein cholesterol (LDL), reduced high-density lipoprotein cholesterol (HDL), and increased triglyceride (TG) levels. These lipid irregularities increase the risk of the formation of atherosclerotic plaques in artery walls (Powell-Wiley *et al.*, 2021). Lipid levels and lipid metabolism are altered during HIV infection and ART – specifically non-nucleoside reverse transcriptase inhibitors (NNRTIs), nucleoside reverse transcriptase inhibitors (NRTIs), and protease inhibitors (PIs) – is associated with less favourable lipid profiles (Dave *et al.*, 2016; Russell *et al.*, 2020). However, iterations of drug regimens, such as integrase inhibitor or rilpivirine-based therapy, have been shown to have more favourable effects on lipid profiles (Taramasso *et al.*, 2018; Valenzuela-Rodriguez *et al.*, 2023). The association of lipid levels with inflammation and poor sleep or sleep complaints in a South African cohort in the context of HIV infection has yet to be investigated.

One study has shown that severe insomnia was associated with higher odds of developing cardiovascular events in PLWH compared to PLWOH or compared to PLWH without severe insomnia (Polanka *et al.*, 2019), but without elucidating the underlying mechanism that would drive this relationship.

Thus, the relationship between poor sleep, HIV, inflammation and CVD risk remains to be further elucidated, especially in South Africa, where the prevalence of HIV infection is 13,7% (8,2 million people), and in adults aged 15–49, this increases to 19,5% (Maluleke, 2022).

Therefore, the present study aimed to investigate the association between sleep complaints, traditional CVD risk factors, inflammation and HIV infection within a South African population. We used baseline data collected in the Ndlovu cohort study to assess the prevalence of sleep complaints and compare traditional CVD risk factors and inflammation between those with and without sleep complaints. Secondly, the study aimed to assess the association between sleep complaints, HIV status, and inflammation using multivariable analyses. Finally, we tested the association between inflammation and the risk of developing cardiovascular events as measured by the Framingham risk score.

4.3 Methods:

4.3.1 Study setting:

The details of the Ndlovu cohort study are outlined in existing material (Vos, *et al.* 2017). The study enrolled participants from the rural region of Elandsdoorn, Limpopo, South Africa.

Study approval was obtained from the Human Research Ethics Committee at the University of Pretoria, Pretoria, South Africa (Ethics number 227/2014); the Human Research Ethics Committee at the University of the Witwatersrand, (Ethics number: M230466) Johannesburg, South Africa, and the Limpopo Department of Health Ethics Committee, and written informed consent was obtained from all participants prior to study participation.

4.3.2 Participant enrolment and retention

Over 2 years, participants meeting the enrolment criteria described in Vos *et al.* (2017) were recruited from the local communities and the Ndlovu Medical Centre by community liaison officers and a team of dedicated counsellors at the Ndlovu Medical Centre.

People were invited to participate in the study through community campaigns, at local events, and in shopping areas. In total, 1,080 PLWOH and 887 PLWH were recruited at baseline. The data and analyses presented in this study are from participants who were enrolled in the Ndlovu Cohort Study at baseline and had data for the sleep measurement we used (see below).

The PLWH who were not on treatment (naïve) were either newly diagnosed and had not initiated treatment by the time they enrolled into the study or were not on treatment for more than 4 weeks upon enrolling into the study (Vos *et al.*, 2017).

4.3.3 Physical measurements

We measured height, weight and hip and waist circumference, as described previously (Vos *et al.*, 2017). We categorised people in as per WHO guidelines BMI –18.5 - 24.9kg/m² 25 - 29.9kg/m² (Overweight) and BMI $\geq$ 30kg/m². We defined hypertension as classic hypertension of >140 mmHg systolic and diastolic blood pressure >90 mmHg, or being treated for hypertension.

As mentioned in a publication by Vos *et al.* 2020, the Monte-Carlo multiple imputation model was used to address the 45% missing blood pressure data in this cohort. Blood pressure BP measurements were changed halfway through recruitment, first by a validated device upper arm blood pressure, (55%) and then afterwards with a non-validated device (wrist BP).

4.3.4 Ten-year risk of cardiovascular events

The Framingham risk score (FRS) for the estimation of the 10-year CVD risk was calculated using sex, age, total cholesterol, HDL cholesterol, systolic blood pressure, smoking status and diabetes status for people between the ages of 30–74 (Jahangiry, Farhangi and Rezaei, 2017). Thus, we present data for 1,331 participants' 10-year risk score for cardiovascular events.

4.3.5 Blood measurements

A 50 ml sample of blood was taken from participants. Total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, random glucose, and C-reactive protein (CRP) were measured in all individuals as described previously (Vos *et al.*, 2017). The presence of diabetes based on either random, non-fasting glucose > 11.1 mmol/L, HbA1c $\geq 6.5\%$ or taking diabetes medication.

Participants for whom HIV status was unknown at study enrolment underwent HIV testing. Testing was performed with an antibody-based point-of-care test and then confirmed using an enzyme-linked immunosorbent assay (ELISA) as described previously (Vos *et al.*, 2017). For participants who tested positive, disease markers, including CD4 count and viral load, were further assessed. The CD4 count was performed by flow cytometry, and the viral load was performed by a PCR test (Vos *et al.*, 2017).

4.3.6 Questionnaires

Two questionnaire packs were used to collect the information in this study, one of which was the Ndlovu Cohort Study Baseline Medical Assessment Form (NCS-BMAF), and another was the Ndlovu Cohort Study Baseline Questionnaire (NCS-BQ). The NCS-BMAF includes questions on overall health, lab based information, smoking status (current or ever), diabetes, hypertension, etc. and the treatment thereof. These are included as Appendix C.

We used Question 3 of the Patient Health Questionnaire – 9 (PHQ-9) (Kroenke, Spitzer and Williams, 2001) to assess sleep ('Over the last 2 weeks, how often have you been bothered by any of the following problems: trouble falling asleep, staying asleep or sleeping too much?'), which is scored on a 4-point Likert scale with 0 for 'Not at all', 1 for 'Several Days', 2 for 'More than half the days' and 3 for 'Nearly every day'.

We dichotomised the PHQ-9 Question 3 score as the presence of sleep complaints = 1 (scores of 2 or 3) and absence of sleep complaints = 0 (scores of 0 or 1). Polanka *et al.* (2019) used a similar 5-point Likert scale for insomnia 'trouble falling asleep or staying asleep' to assess the relationship between insomnia severity and cardiovascular events (Polanka *et al.*, 2019).

Because we were using Question 3 of the PHQ-9 to generate the sleep complaints data, we used the Patient Health Questionnaire 2 (PHQ-2) from the first two questions 1 and 2 of the PHQ-9 to give a snapshot of depression in the cohort. Scores of the PHQ-2 range from 0-6, with scores of 4 or more indicating the likelihood of a depressive disorder (Kroenke, Spitzer and Williams, 2001). We used the full range of the PHQ-2 (scores from 0 to 6) and not the dichotomous tool.

The International Physical Activity Questionnaire (IPAQ; Craig *et al.*, 2003) was used to measure physical activity, which was categorised into 1 (low activity), 2 (moderate activity), and 3 (high activity). The IPAQ was used as an ordinal categorical variable and was entered as a linear variable in the multivariable models.

4.3.7 Statistical analyses

Statistical analyses were performed using SAS 9.4© (SAS Institute Inc., Cary, NC, USA). Continuous variables were tested for normality. Normally distributed data was presented as means and standard deviations (SD), while medians and interquartile ranges (IQR) were used for non-normally distributed data. As expected, C-reactive protein (CRP) data were skewed and were thus log-transformed ($\log(\text{CRP})$) as the dependent variable for univariable and multivariable analyses using general linear models (GLM). Framingham risk score was also skewed; thus, the descriptive statistics shown are median [interquartile range]. For the correlation analysis with $\log(\text{CRP})$ to test the association between the two variables, we used $\log(\text{FRS})$.

Categorical data were presented as frequencies and percentages. Continuous data was compared between groups using Student's t-test or Mann-Whitney U test for variables with Gaussian or non-Gaussian distribution, respectively. Percentages were compared between groups using the Chi-squared test.

We separated the sample population into 1) those with and without sleep complaints in the whole population, 2) those with and without sleep complaints in only PLWH, and 3) HIV status – positive or negative.

To assess the association of inflammation with the presence or absence of sleep disturbance symptoms and HIV status, we first tested the univariable association of $\log(\text{CRP})$ with sleep complaints and $\log(\text{CRP})$ with HIV status and then tested other known variables associated with $\log(\text{CRP})$, to include as potential confounders in our model. Those confounders included age, sex, BMI, waist and hip circumference, plasma glucose concentration, lipid profile and physical activity using the IPAQ (Roche, Rae, and Redman, 2021). Variables with a significance of $p \leq 0.2$ in the univariable analysis were included in the multivariable analysis using general linear models with $\log(\text{CRP})$ as the dependent variable. These models were run in both the total population and in the PLWH group. In the model for the PLWH group, we also tested markers of HIV disease, i.e. CD4 counts, viral loads (in 3 categories: undetectable; low viraemia, i.e. 50 to 1000 copies/mL; high viraemia, i.e. above 1000 copies/mL), HIV duration and ART duration. Again, only variables with $p \leq 0.2$ in the univariable analysis against $\log(\text{CRP})$ were included in the final multivariable regression model.

As previous studies reported interactions between HIV and markers of sleep quality on cardiovascular events (Polanka *et al.*, 2019) and on CRP (Roche, Rae, and Redman, 2021), we added an HIV-by-sleep complaints interaction term into the log(CRP) multivariable regression model in the total population. We also tested for an interaction between HIV and sex. We then ran post-hoc analyses using a Tukey adjustment for multiple comparisons on the significant interactions of the general linear model (Proc GLM in SAS 9.4®). Finally, to test the association between inflammation and 10-year risk of cardiovascular events, we ran a Pearson's correlation between log(CRP) and log(FRS).

4.4 Results

In total, 1,915 participants were included in this study (1,033 PLWOH and 882 PLWH, with 690 on ART), as only 1,915 of the total study participants at baseline (N=1927) had full PHQ-9 data. The sample population were 55% females. We present two tables, one for the full sample (N=1915, Table 1) and one for only PLWH (N=882, Table 2). In both tables, we compare variables between those with and those without sleep complaints.

Table 1: The demographic and clinical characteristics for all participants (N=1915) comparing those with sleep complaints to those with no sleep complaints.

Group characteristics	No sleep complaints N = 1626	Sleep complaints N = 289	P
Demographics			
PLWH N (%)	723 (44%)	159 (55%)	0.001
Female N (%)	886 (55%)	160 (55%)	0.800
Age (years)	38.1 (±12.6)	42.3 (± 13.3)	< 0.001
Blood analyses			
Glucose (mmol/L) [^]	4.6 (4.2;5.0)	4.7 (4.2;5.2)	0.488
Total cholesterol (mmol/L) [*]	4.19 (±0.99)	4.39 (±1.06)	0.002
LDL cholesterol (mmol/L) [*]	2.33 (±0.88)	2.51 (±0.93)	0.001
HDL cholesterol (mmol/L) [*]	1.41 (±0.39)	1.39 (±0.38)	0.381
Triglycerides (mmol/L) [*]	0.9 (0.7;1.4)	1.0 (0.7;1.4)	0.161
Dyslipidaemia (%) [*]	623 (38)	115 (40)	0.593
CRP (mmol/L) [*]	3 (2;8)	5 (2;11)	< 0.001
Anthropometric measurements			
SBP (mmHg) Med(IQR)	114 (102; 129)	117 (102;135)	0.046
DBP (mmHg) Med(IQR)	73 (65; 82)	73 (66; 85)	0.062
Body Mass Index (kg/m ²) ^{**}	24.1 (±5.9)	24.4 (±6.6)	0.392
Waist: Hip Ratio	0.84 (±0.09)	0.85 (±0.09)	0.411
Hip circumference (cm) ^{**}	99 (±14)	101 (±14)	0.060
Waist circumference (cm) ^{**}	83 (±13.1)	85 (±14)	0.022
Diabetes (N(%)) ^{***}	73 (4.5)	16 (5.6)	0.430
Questionnaire result			
Smoking: ever smoked N(%)	645 (39.7)	140 (48.4)	0.006
Depression score (PHQ2)	0.96 (±1.3)	2.5 (1.6)	< 0.001
Physical activity (IPAQ)			
Low N(%)	615 (37.8)	126 (43.6)	0.057
Moderate N(%)	599 (36.8)	86 (29.8)	
High N(%)	412 (25.3)	77 (26.6)	
Ten-year CVD risk (Age 30 – 74)	N = 1117	N = 226	
Framingham risk score (%)	3.31 (1.74; 7.26)	4.67 (1.93, 11.04)	0.003

Data presented as mean (±SD), median (IQR) or N (%); ^{*}no sleep complaints N=1612, sleep complaints N=285; ^{**}no sleep complaints N=1623, sleep complaints N=288; [^]no sleep complaints = 1614 sleep complaints=286, ^{***}no sleep complaints N=1625, sleep complaints N=288. //SBP = systolic blood pressure, DBP = diastolic blood pressure, CRP = C-reactive protein, IPAQ = International Physical Activity Questionnaire, SDs = Sleep complaints, PHQ2 = Patient Health Questionnaire-2, BMI = Body Mass Index.

In the full sample, 15.1% (N=289 of 1915) reported sleep complaints. There was a higher percentage of PLWH in the participants with sleep complaints (55% vs 44%; $p=0.001$). The participants with sleep complaints had higher total cholesterol ($p=0.002$), higher LDL cholesterol ($p=0.001$), higher CRP (<0.001) and were older (<0.001) than the participants with no sleep complaints. Participants with sleep complaints had slightly higher systolic blood pressure than those without sleep complaints (117mmHg vs 114mmHg respectively), but both are lower than the “normal” systolic blood pressure of 120mmHg (Chobanian et al., 2003). The 10-year risk of cardiovascular events is higher in people with sleep complaints ($p=0.003$) between the ages of 30 and 74. Smoking was also higher in people who reported sleep complaints than those who did not (48.4% vs 39.7% respectively, $p=0.006$).

After assessing the overall demographic and clinical characteristics of the full cohort, we investigated the difference between participants with sleep complaints and participants without sleep complaints in only the PLWH.

Table 2: The demographics and clinical characteristics of PLWH, comparing the participants with sleep complaints to those with no sleep complaints.

Group characteristics	No sleep complaints N = 723	Sleep complaints N = 159	P
Characteristics			
Female N (%)	434 (60%)	91 (57.2%)	0.5
Age (years)	40.95 (±10.2)	44.13 (±10.6)	<0.001
Blood analyses			
Glucose (mmol/L)	4.7 (4.3;5.1)	4.7 (4.2;5.2)	0.78
Total cholesterol (mmol/L)	4.24 (±0.99)	4.38(±1.0)	0.11
LDL cholesterol (mmol/L)	2.33(±0.9)	2.43 (±0.8)	0.19
HDL cholesterol (mmol/L)	1.44 (±0.4)	1.41 (±1.4)	0.66
Triglycerides (mmol/L)	1.18 (±0.8)	1.24 (±0.9)	0.48
Dyslipidaemia N (%)	286 (39%)	58 (36%)	0.53
CRP (mmol/L)	4 (2;10)	7.5 (2;14)	<0.001
Anthropometric measurements			
SBP (mmHg) Med (IQR)	112 (100; 125)	114 (101; 132)	0.04
DBP (mmHg) Med (IQR)	72 (64; 81)	73 (66; 84)	0.10
Body Mass Index (kg/m ²)	23.55 (±5.7)	23.95 (±6.7)	0.48
Waist : Hip ratio	0.85 (±0.09)	0.85 (±0.087)	0.77
Waist circumference (cm)	84.2 (±12.2)	86.2 (±14)	0.09
Hip circumference (cm)	99.2 (±13.6)	101.6 (±14.1)	0.06
Questionnaire results			
Smoking: ever smoked N(%)	254 (35.2)	73 (45.9)	0.014
Depression score (PHQ2)	1.02 (±1.3)	2.65 (±1.7)	<0.001
Physical activity (IPAQ)			
Low N (%)	335 (46.3)	73 (45.9)	0.03
Moderate N (%)	245 (33.9)	41 (25.8)	
High N (%)	143 (19.8)	45 (28.3)	
Ten-year CVD risk (Age 30 – 74)	N=622	N= 146	
Framingham Risk Score (%)	2.77 (1.49; 5.80)	3.38 (1.87; 8.09)	0.011
HIV specific information			
Time since diagnosis (months)	58 (13; 99)	53 (7;98)	0.40
Time on ART (months)	44 (4;90)	40 (0; 95)	0.42
CD4 count (cells/μL)	502 (±253.7)	479 (±257.8)	0.31
Viral load (copies/ml)	2406 (88; 21518)	4588 (440; 29483)	0.98
LogVL	3.2 (±1.2)	3.5 (±1.2)	0.08
ART	578 (79.4%)	112 (70.4%)	0.01
First line N (%)	514 (70.6%)	99 (62.2%)	
Second line N (%)	64 (8.8%)	13 (8.2%)	
No ART N(%)	150 (20.6%)	47 (29.6%)	

Data are presented as mean (±SD), median (IQR) and N (%). *Note, all N are as above (No sleep complaints, N = 723 and Sleep complaints, N = 159). SBP = systolic blood pressure, DBP = diastolic blood pressure, CRP = C-reactive protein, IPAQ = International Physical Activity Questionnaire, SDs = Sleep complaints, PHQ2 = Patient Health Questionnaire-2, BMI = Body Mass Index, VL = Viral load, ART = Antiretroviral therapy.

In the PLWH, 18.0% (n=159 of a total sample of 882) reported sleep complaints. Those with sleep complaints had higher inflammation ($p<0.001$), were older ($p<0.001$), had higher levels of depression ($p<0.001$) and were less likely to be on ART ($p=0.01$) than those without sleep complaints. The 10-year risk of cardiovascular events is higher in PLWH with sleep complaints ($p=0.011$) between the ages of 30 and 74. Similar to the whole cohort presented in Table 1, participants with sleep complaints had slightly higher systolic blood pressure than those without sleep complaints (114mmHg vs 112mmHg respectively), and smoking (ever) was > 10% higher in people who reported sleep complaints than those who did not (45.9% vs 35.2% respectively, $p=0.006$).

In PLWH, there was no association between any of the anthropometric measurements and inflammation ($p>0.05$), even though hip circumference was close to significance ($p=0.06$). We then split the group by HIV status and report these results in Table 3.

Table 3: The demographics of PLWOH (people living without HIV) and PLWH (people living with HIV).

Group characteristics	PLWOH N = 1033	PLWH N = 882	P
Demographics			
Female N (%)	521 (50.4)	525 (59.5)	<0.001
Age (years)	36.3 (±14.1)	41.5 (±10.3)	<0.001
With sleep complaints N (%)	130 (12.6)	159 (18.0)	<0.001
Blood analyses			
Glucose (mmol/L)*	4.5 (4.1; 5.0)	4.7 (4.3; 5.1)	<0.001
Total cholesterol (mmol/L)**	4.19 (±1.00)	4.26 (±1.00)	0.127
LDL cholesterol (mmol/L)**	2.34 (±0.89)	2.38 (±0.88)	0.216
HDL cholesterol (mmol/L)**	1.38 (±0.35)	1.43 (±0.43)	0.003
Triglycerides (mmol/L)	0.9 (0.6; 1.3)	1.0 (0.7; 1.4)	<0.001
Dyslipidaemia N (%)	394 (38.67)	344 (39.18)	0.819
CRP (mmol/L)	3 (2;6)	5 (2; 11)	<0.001
Anthropometric measurements			
Body Mass Index (kg/m ²)	24.53 (±6.22)	23.62 (±5.86)	0.001
Waist: Hip Ratio	0.83 (±0.9)	0.85 (±0.09)	<0.001
Hip circumference (cm)	99.35 (±14.03)	99.65 (±13.7)	0.6
Waist circumference (cm)	82.68 (±13.85)	84.52 (±12.59)	0.002
Diabetes (N, %)**	58 (5.6)	31 (3.5)	0.030
Questionnaire result			
Depression score (PHQ2)	1.09 (±1.35)	1.32 (±1.5)	<0.001
Physical activity (IPAQ)			
Low N(%)	333 (32.4)	408 (46.3)	<0.001
Moderate N(%)	399 (33.9)	286 (32.4)	
High N(%)	301 (29.1)	188 (21.3)	
Ten-year CVD risk (Age 30 – 74)	N = 575	N = 768	
Framingham Risk Score (%)	4.68 (2.10; 11.08)	2.89 (162; 6.19)	<0.001
HIV treatment			
Naive N (%)		185 (21%)	
On antiretroviral therapy N (%)		697 (79%)	

Data are presented as mean (±SD), median (IQR) and N (%); *N=1024 non-HIV; N=876 HIV; **N=1019 non-HIV, N=878 HIV, ***N=1033 non-HIV, N=880 HIV. IPAQ = International Physical Activity Questionnaire, SDs = Sleep complaints, PHQ2 = Patient Health Questionnaire-2.

The PLWH were older ($p<0.001$) and had a higher prevalence of sleep complaints ($p<0.001$) than the PLWOH. When adjusting for age and sex using logistic regression, PLWH were more likely to report having sleep complaints than PLWOH (OR [95%CI]=1.38 [1.07–1.78]). There was no difference between the two groups in terms of glucose levels, total cholesterol, LDL cholesterol, and percentage of dyslipidaemia. However, the PLWH had higher HDL cholesterol ($p=0.003$), and higher triglycerides ($p=0.004$), and presented with higher plasma CRP concentration ($p<0.001$) than the PLWOH group. In addition, the HIV-negative group had higher BMI ($p=0.001$), but PLWH reported higher waist-hip ratio ($p<0.001$), and higher waist circumference ($p=0.002$). There was no difference between the two groups in terms of hip circumference. The PLWH also presented with higher depression as assessed using the PHQ2 ($p<0.001$). The 10-year risk of cardiovascular events is lower in PLWH ($p<0.001$) for people between the ages of 30 and 74 when using the FRS.

For the models of inflammation and sleep, all variables that had a significance of $p<0.2$ in the univariable analyses, as well as known contributors to inflammation (age, sex, HIV status), were placed in the final regression models detailed in Tables 4 (total population) and 5 (only PLWH). The results from the univariable analyses are displayed in Supplementary Table A, included in the supplementary material.

SBP and smoking were initially included in the model, while DBP was excluded due to collinearity with SBP. However, during both backward and forward regression for variable selection, we found that the inclusion of SBP and smoking—despite their relevance to the calculation of the Framingham Risk Score (FRS)—introduced model complexity and penalisation due to overfitting. SBP and smoking contributed most significantly to this issue – signified by inflated variance. As a result, the final model (Table 4) excludes SBP and smoking to ensure optimal model performance and parsimony. We do however display this model in Appendix C, Table 2.1.

Table 4: Multivariable linear regression model investigating the association between inflammation (log(CRP)), presence of sleep complaints and HIV in the full cohort (N=1,687).

Parameter	β - Estimate	Standard Error	t Value	Pr > F
Sleep complaints	-0.013	0.040	-0.32	0.083
HIV status	0.292	0.034	8.56	<0.001
Sex (male compared to female)	-0.059	0.029	2.02	0.043
Age	0.004	0.001	4.71	<0.001
Glucose	0.016	0.005	3.47	<0.001
LDL Cholesterol	-0.001	0.012	-0.07	0.942
HDL Cholesterol	-0.134	0.029	-4.68	<0.001
Triglycerides	-0.058	0.015	-3.95	<0.001
BMI	0.013	0.003	4.74	<0.001
Waist circumference	0.003	0.001	2.76	0.006
Depression (PHQ2)	0.007	0.008	0.96	0.336
Activity (IPAQ)	-0.048	0.013	-3.56	<0.001
Interaction between HIV and Sleep complaints (HIV+, SDs+ vs. all others) *	0.130	0.055	2.36	0.018
Interaction between HIV and Sex (male and HIV+ compared to others) **	0.208	0.041	4.97	<0.001

IPAQ = International Physical Activity Questionnaire, SDs = Sleep complaints, PHQ2 = Patient Health Questionnaire-2, BMI = Body Mass Index. *Interaction is explained in Figure 1 below, ** Interaction is explained in Figure 2 below.

In the full cohort, as described in Table 4, being HIV positive ($\beta=0.292$; $p<0.001$) was independently associated with higher CRP when adjusting for age, biological sex, and known variables that affect inflammation. Higher CRP was also associated with older age ($\beta=0.004$, $p<0.001$), higher BMI ($\beta=0.013$, $p<0.001$) whereby for every increase in age by ten years, the effect on log(CRP) increases by 0.4, and for every 10kg/m² increase in BMI, the effect increases by 0.13.

As per Table 4, there was a negative association between HDL cholesterol and log(CRP) HDL being protective against higher inflammation ($\beta=-0.134$; $p<0.001$), higher glucose ($\beta=0.013$, $p=0.016$, $p<0.001$), and being male (main effect of biological sex ($\beta=-0.059$, $p=0.043$) and significant interaction between biological sex and HIV whereby HIV+ males had higher CRP than all other groups (Table 4 and Figure 2) ($p<0.001$). There was a main effect of physical activity on CRP, whereby being more active was associated with lower CRP ($\beta=-0.048$, $p=0.001$). There was no impact of LDL cholesterol on CRP levels in the final model ($p=0.942$), but triglycerides were associated with lower inflammation ($p<0.001$).

We found a significant interaction between HIV and sleep complaints ($p=0.018$), where people with HIV and sleep complaints had significantly higher log(CRP) concentration than people with HIV and no sleep complaints and people without HIV with or without sleep complaints, while sleep complaints in people without HIV with or without sleep complaints had non-significant differences in log(CRP) (posthoc analysis with Tukey adjustment; Figure 1).

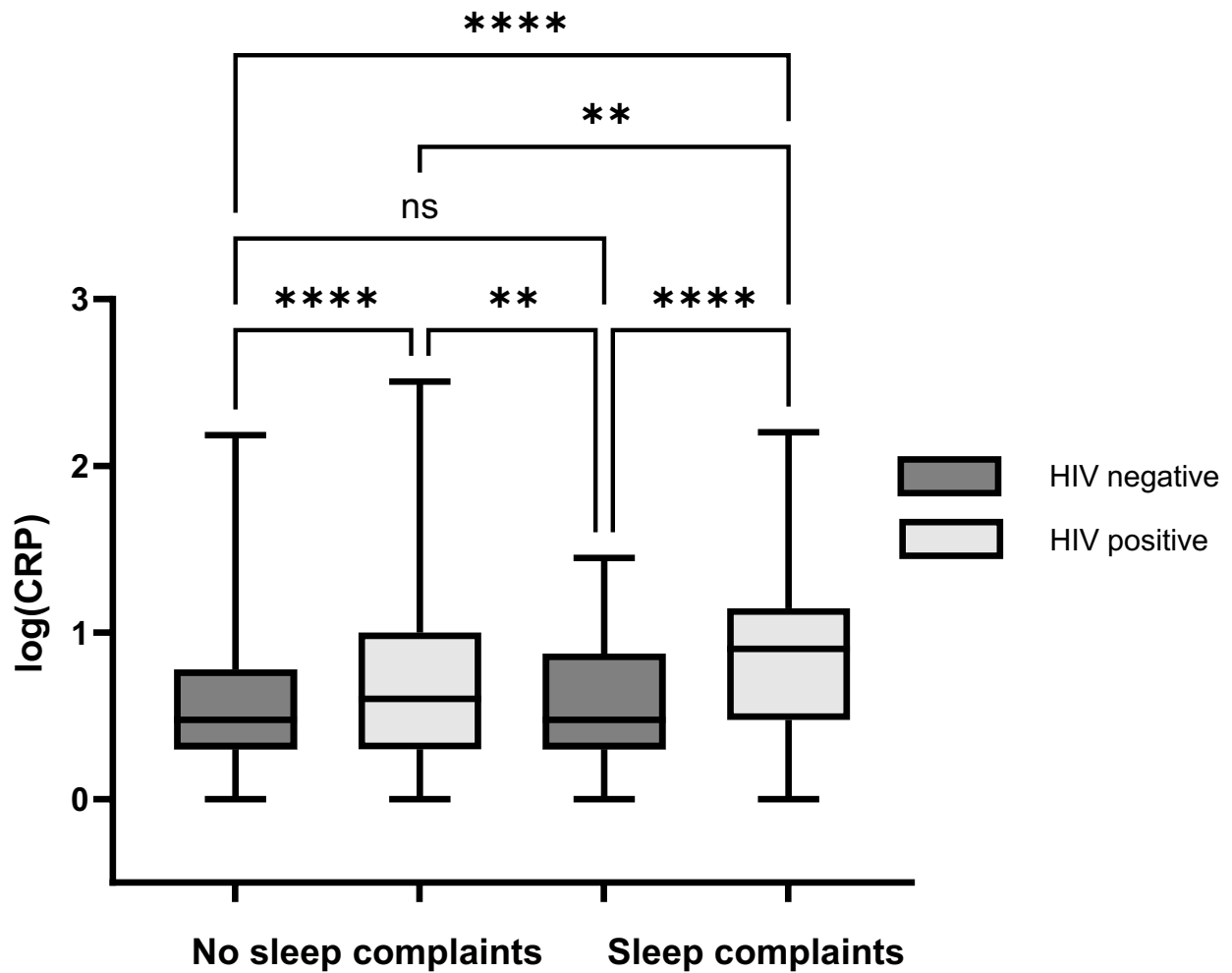


Figure 1: Boxplots showing log(CRP) in PLWH (light grey) and PLWOH (dark grey), with and without sleep complaints. No difference in log(CRP) between PLWOH, with or without sleep complaints.

** p<0.01, *** P<0.001, ****P<0.0001 in post-hoc analyses.

The next step was to assess the factors associated with inflammation in only PLWH, which is presented in Table 5. We assessed the effect of viral load, CD4 counts and ART treatment regimen in only PLWH. As there was no association between time on ART ($p=0.762$) and time since HIV diagnosis ($p=0.708$) on $\log(\text{CRP})$, we did not include those variables in the multivariable analysis.

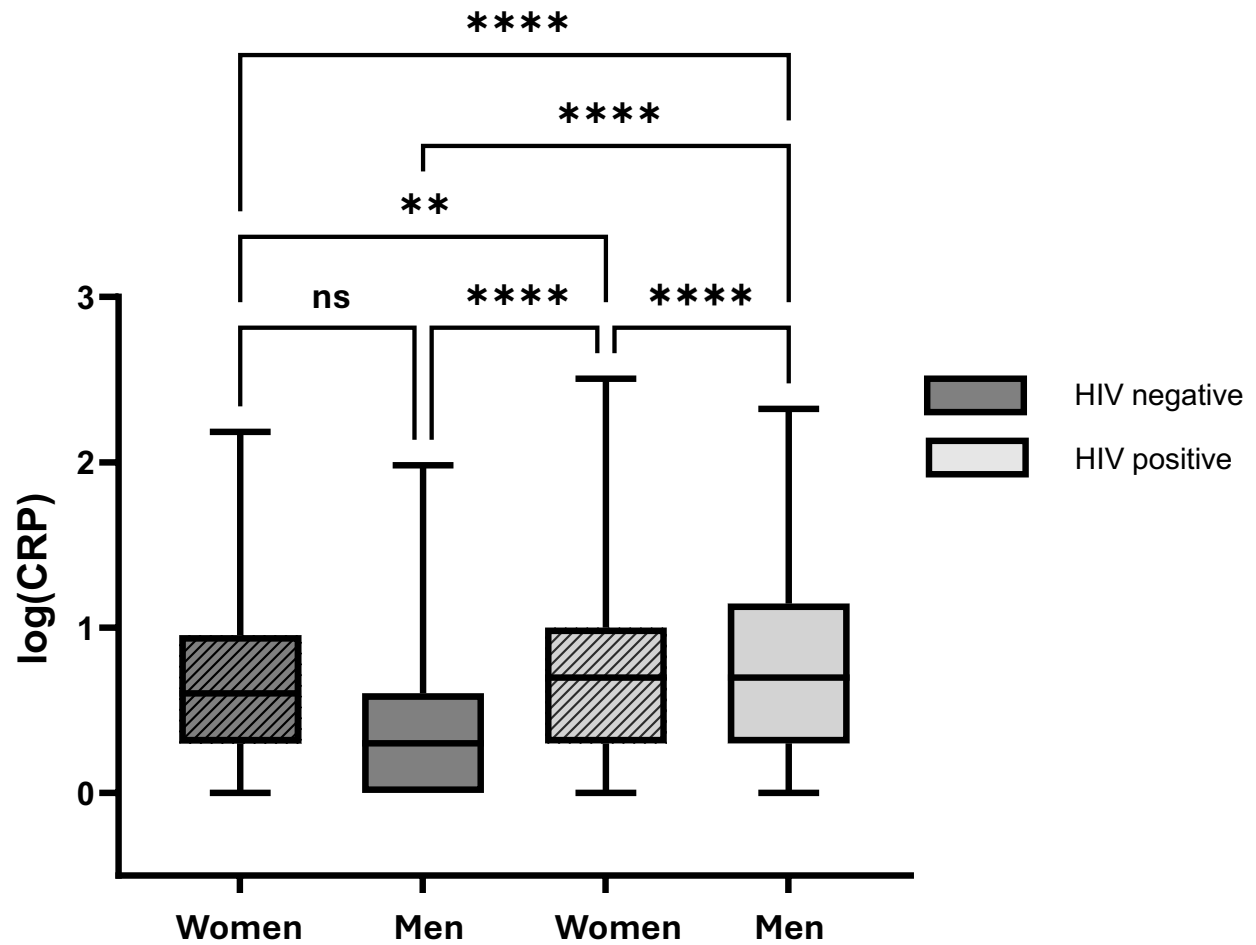


Figure 2: Log(CRP) in HIV-positive men and women compared to HIV-negative men and women. ns= p=0.180, ** p<0.01, *** P<0.001, ****P<0.0001 in post-hoc analyses. Grey lines indicate women, blank boxes indicate men.

As seen in Table 4, there was an interaction between HIV, biological sex, and CRP, where men living with HIV had the highest CRP. This is expanded upon in Figure 2, above, where we see significant differences between men and women living with HIV, and men and women living with HIV vs men and women living without HIV. Higher CRP is noted across the board in PLWH.

We modelled the variables affecting $\log(\text{CRP})$ in only the PLWH and found that sleep complaints, BMI and waist circumference all increased $\log(\text{CRP})$. Factors associated with reducing $\log(\text{CRP})$ were HDL cholesterol, CD4 count, and being female (Table 5).

Table 5: Multivariable linear regression results: Variables associated with log(CRP) in PLWH.

Parameter	Estimate	Standard Error	t Value	Pr > t
Sleep complaints	0.133	0.04	3.06	0.003
Age	0.003	0.002	1.65	0.099
Sex (female)	0.124	0.04	3.10	0.003
BMI	0.009	0.004	2.11	0.036
Waist circumference	0.004	0.002	2.19	0.029
Triglycerides	-0.068	0.022	-2.99	0.003
HDL cholesterol	-0.111	0.045	-2.45	0.015
LDL cholesterol	0.011	0.021	0.50	0.614
Glucose	0.012	0.014	0.84	0.403
CD4 count	-0.003	0.0001	-3.92	<0.001
Physical activity (IPAQ)	-0.075	0.022	-3.43	<0.001

IPAQ = International Physical Activity Questionnaire, BMI = Body Mass Index.

We also found an association between physical activity and CRP. When comparing very active people (IPAQ Category 3) to mostly sedentary people (IPAQ Category 1), lower levels of activity are associated with higher inflammation ($p < 0.001$). This was also seen when comparing highly active people (IPAQ Category 3) to moderately active people (IPAQ Category 2), whereby being moderately active was associated with increased CRP ($p = 0.052$).

In PLWH (Table 5), age did not influence CRP, but the presence of sleep complaints did ($\beta = 0.14$; $p = 0.002$). Although glucose was a significant predictor of higher log(CRP) in the overall cohort ($p = 0.0005$), this significance was not seen in the HIV group ($p = 0.416$). HDL cholesterol ($\beta = -0.11$; $p = 0.004$) and triglycerides ($\beta = -0.065$; $p = 0.002$) were associated with lower log(CRP) levels, as were CD4 counts ($\beta = -0.003$; $p = 0.002$).

Table 6: The association of sleep complaints with CVD risk factors when adjusting for HIV status and age.

Variable	Sleep complaints		HIV status		Age	
	β	<i>P</i>	β	<i>P</i>	β	<i>P</i>
LDL cholesterol	0.11	0.050	-0.068	0.11	0.017	<0.001
Total cholesterol	0.95	0.122	-0.07	0.11	0.026	<0.001
Glucose	0.087	0.521	-0.33	<0.001	0.031	<0.001
Triglycerides	-0.004	0.925	0.01	0.77	0.018	<0.001

LDL = Low-density lipoprotein.

To assess the impact of sleep complaints on CVD risk factors (triglycerides, glucose, LDL cholesterol and total cholesterol), we ran general linear models and adjusted for age and HIV status. Biological sex was excluded as there was no difference in sex between the group with sleep complaints and those without. For LDL cholesterol, we found a positive association with both sleep complaints ($p=0.0501$) and age ($p<0.001$). There was no association of sleep complaints with total cholesterol ($p=0.122$) or triglycerides ($p=0.925$), but age was positively associated with both ($p<0.001$ for both). Finally, there was no association of sleep complaints with glucose ($p=0.53$); however, the presence of HIV was negatively associated ($p<0.001$) whilst age was positively associated ($p<0.001$) with glucose levels.

Having assessed that sleep complaints were associated with higher inflammation, we evaluated whether inflammation was associated with an increased 10-year risk of CVD events. In a univariable general linear model modelling $\log(\text{FRS})$ as a function of $\log(\text{CRP})$, we found a positive association between inflammation and the Framingham risk score ($\beta=0.08$, $\text{SE}=0.02$; $p=0.003$, Table 7).

Table 7: Factors associated with 10-year cardiovascular disease risk using log(FRS) in a sub-sample of the population (N=1,331).

Parameter	β - Estimate	Standard Error	t Value	Pr > F
Sleep complaints	0.088	0.032	2.76	0.006
HIV status	-0.208	0.023	-8.98	<0.001
Log(CRP)	0.091	0.025	3.60	<0.001
Triglycerides	0.149	0.014	10.84	<0.001
BMI	-0.019	0.003	-7.26	<0.001
Waist circumference	0.009	0.001	6.81	<0.001
Depression (PHQ2)	-0.001	0.008	-0.17	0.866
Activity (IPAQ)	-0.045	0.014	-3.12	0.002

CRP = C-reactive protein, IPAQ = International Physical Activity Questionnaire, PHQ2 = Patient Health Questionnaire-2, BMI = Body Mass Index.

Factors associated with increased risk are people who have sleep complaints, higher log(CRP), triglycerides, and waist circumference. HIV status, BMI, and IPAQ were associated with lower log(FRS). Thus, higher physical activity associated with lower Framingham Risk Score is expected, though we found no association of the FRS with depression.

4.5 Discussion

In this study of 1,915 participants (882 PLWH), sleep complaints were reported in 15% of the participants overall. Sleep complaints were more prevalent in those living with HIV, with HIV being independently associated with higher odds of having sleep complaints in adjusted analysis. Sleep complaints augmented the association of HIV with plasma CRP concentration, as did being male. Similar associations with inflammation were observed in the models focusing on PLWH, where CD4 counts were negatively associated with plasma CRP concentration – suggesting more inflammation in people with lower CD4 counts. In contrast, when adjusting for age and HIV status, we found no relationship between sleep complaints and plasma lipids, and between sleep complaints and glucose concentrations. We found that higher inflammation and sleep complaints were independently associated with a higher 10-year risk of cardiovascular events, as measured by the Framingham risk score.

Our findings show that sleep complaints are present in this rural population. We found that sleep complaints are more prevalent in PLWH (18%) compared to PLWOH (12,6%), which is lower than what was found previously where the prevalence of sleep complaints in PLWH was between 40–60% (Crum-Cianflone *et al.*, 2012; Lee *et al.*, 2012; Low *et al.*, 2012). In an urban cohort, the prevalence of sleep complaints was 61% – far higher than what we report in this present study (Redman, Karstaedt and Scheuermaier, 2018). Question 3 of the PHQ-9 ('Over the last 2 weeks, how often have you been bothered by any of the following problems: trouble falling asleep, staying asleep or sleeping too much?') focuses primarily on insomnia and hypersomnia, still under the banner of sleep complaints. It may be that this tool is not sensitive enough to encapsulate all the potential sleep problems experienced by PLWH. Therefore, we are likely witnessing an underreporting of the sleep complaints in PLWH in this cohort. However, even with this snapshot tool, we are seeing the impact of sleep complaints in PLWH.

Sleep complaints among people living with HIV (PLWH) may arise from multiple causes, including the direct effects of HIV infection itself (Wiegand *et al.*, 1991; White *et al.*, 1995) and side effects associated with antiretroviral therapy (Núñez *et al.*, 2001; Moyle *et al.*, 2006; S. S. Lee *et al.*, 2014). More recently, disruptions in the circadian system have also been implicated (DLMO, Redman *et al.*, 2023). Evidence of circadian misalignment in PLWH was demonstrated in a rural cohort of ageing South Africans, where dim light melatonin onset (DLMO) - the gold standard for assessing circadian phase (Lewy & Sack, 1989) - was used to evaluate circadian timing (Redman *et al.*, 2023). In that study, actigraphy was also employed, and the phase angle of entrainment (i.e., the relationship between habitual sleep timing and circadian phase) was calculated. PLWH exhibited DLMO times that occurred very close to their bedtimes, whereas PLWOH had DLMO approximately 1–2 hours before bedtime. This suggests a misaligned or shortened phase angle of entrainment in PLWH, indicative of circadian misalignment. Such misalignment between endogenous circadian rhythms and behavioural sleep patterns may contribute to the poorer sleep quality observed in PLWH, potentially due to sleep occurring at a biologically suboptimal time. Importantly, circadian misalignment has also been associated with increased cardiovascular disease risk (Morris *et al.*, 2015; Polanka *et al.*, 2021).

Circadian misalignment and poor sleep have been shown in non-HIV cohorts to have an impact on CVD risk (Morris *et al.*, 2015; Scheer *et al.*, 2009). In our study, sleep complaints were not associated with cardiometabolic risk factors (triglycerides, glucose) when adjusting for age and HIV, and just missed significance for an association with LDL cholesterol ($p=0.05$). A study analysing the association between insomnia and CVDs in PLWH was conducted using the Veterans Aging Cohort Study Survey Cohort (Polanka *et al.*, 2019). This study evaluated the incidence of CVD events in veterans with and without HIV for a median follow-up of 11 years. The sample was separated into those with insomnia and those without, and there was a significantly higher incidence of CVD events in the group with HIV and severe insomnia than in those with HIV but without insomnia or the HIV-negative groups with or without insomnia (Polanka *et al.*, 2019). A later paper (Polanka *et al.*, 2021) by the same group found no association between insomnia severity, HIV and markers of sCD14, IL-6 and D-Dimers. However, the association with CRP (inflammation) was not tested.

In this cohort, sleep complaints were strongly associated with inflammation in PLWH. In studies of chronic sleep deprivation, inflammatory markers are elevated, which could be a result of regulatory T-cell dysfunction during sleep deprivation (Meier-Ewert *et al.*, 2004; Bollinger *et al.*, 2009). Immune activation increases inflammatory mediators such as cytokines – some of which modify sleep, resulting in sleep complaints, including sleep deprivation – indicating a bi-directional relationship between inflammation and sleep deprivation.

In the total population, there was an interaction between HIV and sleep complaints, whereby PLWH with sleep complaints had higher plasma CRP concentration than PLWH without sleep complaints or PLWOH with or without sleep complaints. Previous studies investigating CVD risk in PLWH detail how even when PLWH have lower traditional markers of CVD risk (lower BMI, waist circumference, waist: hip ratios, etc.), there still was an elevated risk of developing or dying from CVD (Pinto and da Silva, 2018). In the context of HIV infection, it is understood that PLWH – whether on treatment or not – are exposed to higher levels of chronic inflammation, which in turn may increase atherosclerosis.

Indeed, during inflammation, the liver increases the production of CRP (Sproston and Ashworth, 2018), along with proinflammatory serum amyloid A (Siegmund *et al.*, 2016). Serum amyloid protein A (SAA) is an exchangeable apolipoprotein associated with HDL (Cho, 2022) and can be exchanged with apolipoprotein-A-1 – the main lipoprotein component of HDL cholesterol particles (Han *et al.*, 2016; Reisinger *et al.*, 2021). Furthermore, CRP is a marker of HDL dysfunction (Tang *et al.*, 2021). The presence of SAA and CRP during inflammation reduces the cholesterol efflux capacity and anti-inflammatory properties associated with HDL (Ebtehaj *et al.*, 2019). Furthermore, monocytes from PLWH show impaired cholesterol efflux and increased foam cell formation, specifically within the vascular endothelium (Maisa *et al.*, 2015), contributing to the development of dyslipidaemia and laying the foundation for atherosclerotic disease.

As shown elsewhere, our study suggests inflammation plays a role in the pathogenesis of CVD in PLWH. We used the Framingham risk score (FRS) to evaluate the 10-year CVD risk in participants aged 30–74. In this study, PLWH had a significantly lower FRS score than PLWOH, but higher HDL cholesterol. This could mean that even though there are higher HDL cholesterol levels in PLWH, the cardioprotective benefits usually associated

with elevated HDL under normal circumstances may not be in play in PLWH who are experiencing higher levels of inflammation, increasing the CVD risk in this population.

There was a significant association between inflammation and FRS, confirming that higher inflammation may lead to higher CVD risk. Since we found a positive association between sleep complaints and inflammation, particularly in PLWH, this suggests that PLWH with sleep complaints may have an increased risk of CVD events mediated by increased inflammation. Indeed, within PLWH, we found a higher FRS in those with sleep complaints. Though, as previously discussed, the use of FRS may not be as robust as required in PLWH, as PLWH exhibit higher rates of CVD even with lower traditional risk factors (Freiberg *et al.*, 2013; Shah *et al.*, 2018; Eyawo *et al.*, 2019).

In a model adjusting for BMI, triglycerides, waist circumference and physical activity, having sleep complaints was independently associated with a higher 10-year risk of cardiovascular disease. Higher CRP was also independently associated with a higher 10-year risk of cardiovascular disease. HIV-positive status was associated with a lower 10-year cardiovascular disease risk, which can be further investigated in Vos *et al.* (2020). Higher physical activity is known to reduce cardiovascular disease risk (Zhang *et al.*, 2020), thus, the finding of higher physical activity being associated with lower FRS score in this population tracks with existing research.

In South Africa, the criteria used to evaluate Framingham risk score as outlined by the National Department of Health include age, sex, total cholesterol, HDL cholesterol, systolic blood pressure, and whether or not a person is a smoker or diabetic. The Framingham Risk Score has been used in other papers. Thus, a 2015 study showed that higher Carotid Intima Media Thickness (CIMT) associated with higher FRS in South Africa (Schoffelen *et al.*, 2015). Whereas Nyirenda *et al.* in 2021 showed that PLWOH had higher FRS than PLWH (Nyirenda *et al.*, 2021) – suggesting that there may be elements that are not included in the FRS that contribute to increased CVD risk in PLWH, or that we need to be investigating other avenues that could explain the increased CVD risk.

Low physical activity was associated with increased inflammation across the cohort, similar to what has been reported in previous studies (Wiegand *et al.*, 1991; Nokes and Kendrew, 2001; Low *et al.*, 2012). Leading a sedentary lifestyle increases inflammation and is associated with increased cardiometabolic disease risk (Chau *et al.*, 2015; Jaggars and

Hand, 2016), while a previous study has shown that higher physical activity in PLWH is associated with lower cardiometabolic disease risk (Wirth *et al.*, 2015). Higher physical activity also improves sleep quality and reduces fatigue (Webel *et al.*, 2016). Whether the association of physical activity with attenuated disease risk is mediated by inflammation is unknown and requires further investigation.

Possible mechanisms contributing to both poor sleep and inflammation seen in PLWH, include the effects of the viral peptide, Tat. During primary infection with HIV, a viral reservoir is established that is difficult to eradicate (Vanhamel, Bruggemans and Debyser, 2019). The viral reservoir still secretes low levels of HIV proteins – namely Gp120, Nef and Tat. Studies have shown that even with successful antiretroviral treatment and undetectable viral loads, Tat has been found in the plasma (Mediouni *et al.*, 2012) and cerebrospinal fluid of PLWH (Henderson *et al.*, 2019). These viral proteins can lead to inflammation and contribute to endothelial dysfunction (Anand, Rachel and Parthasarathy, 2018). A murine study showed that Tat directly affected the light entrainment pathway, causing circadian phase delays (Anand, Rachel and Parthasarathy, 2018), similar to those seen in PLWH not on treatment. The hypothesis that Tat was associated with circadian phase delays was tested in PLWH, where it was found that higher levels of Tat were associated with higher levels of melatonin in the biological morning – compatible with a phase delay shift in melatonin expression in PLWH (Wang *et al.*, 2014).

An interesting feature of this study was the negative association of triglyceride levels with CRP, a finding that contradicts data from previous studies (Aronson *et al.*, 2004; Firdous, 2014). However, one study has shown that in response to inflammatory mediators, macrophages store more triglycerides, leading to the production of large intracellular fat droplets. This leads to the downregulation of the production of inflammatory proteins such as IL-6 and prostaglandin-E2 by macrophages (van Dierendonck *et al.*, 2022). IL-6 is known to be a prime stimulator of the hepatic production of CRP (Castell *et al.*, 1989), which could explain the lower triglycerides in the presence of higher inflammation, as seen in our study. However, further studies are required to gain a better understanding of the relationship between serum triglycerides and inflammation in this study population.

One multi-centre study of 465 people conducted across the United Kingdom and Ireland found no association between sleep and inflammation in PLWH (Bakewell *et al.*, 2022).

This study used actigraphy and various biomarkers to assess sleep and specific aspects of inflammation, and clustered various immune markers to create a gut/immune profile or neurovascular profile and a reference profile for people without immune activation. Several areas of sleep were investigated, namely assessed by actigraphy over 7 days. PLWH represented 73.8% of the study population and were highest in the neurovascular inflammation cluster. The study found that people with higher variability of WASO (wake after sleep onset) and of length of awakenings, and higher length of awakenings were more likely to have a neurovascular profile (Bakewell *et al.*, 2022).

Some of the strengths of this study include the large sample size (~2,000 participants) and that this study adds to the knowledge base of sleep quality and inflammation in an understudied and resource-limited population. Some limitations of this study include the cross-sectional design, which only allows us to present statistical associations between variables rather than determining causative relationships. The overall NCS study is a longitudinal cohort study, and repeated measures can be investigated in future.

To conclude, the impact of higher levels of inflammation in PLWH could be a major risk factor for cardiometabolic disease and should be monitored yearly with routine measurements, such as CD4 count and viral load. Furthermore, the role of physical exercise should be highlighted as a benefit to PLWH, as it clearly attenuates inflammation. Treating sleep issues early should become a focus of care in PLWH, as neglecting sleep complaints could lead to increases in cardiovascular disease later in life.

4.6 Limitations

This study has several limitations. First, the assessment of systemic inflammation was limited to C-reactive protein (CRP) as the sole inflammatory marker. Including additional cytokines such as TNF- α , IL-6, ICAM, and VCAM would have provided a more comprehensive picture of inflammatory processes. Second, the data presented are cross-sectional, based solely on baseline measurements, offering only a snapshot in time. However, the Ndlovu Cohort Study has stored plasma samples and follow-up data available, which could facilitate longitudinal analyses.

The use of antiretroviral therapy (ARVs), as described by Vos *et al.* (2020), may influence sleep patterns, lipid profiles, and consequently affect the Framingham Risk Score (FRS).

Evaluating these effects would require follow-up data from the cohort. Moreover, the FRS may lack sensitivity in detecting cardiovascular disease (CVD) risk among people living with HIV (PLWH), potentially underestimating their true risk.

Longitudinal analyses would offer valuable insights into the progression of inflammation, sleep disturbances, and CVD risk over time. Additionally, more complete blood pressure data across participants would strengthen the findings, as would information on statin therapy use for dyslipidaemia or hypercholesterolaemia.

4.7 References

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4.8 Supplementary material

Supplementary Table A: Univariable testing of variables' association with inflammation

Variable	Beta-coefficient	P value	Numbers (N)
HIV	0.21	<0.001	1879
Age	0.008	<0.001	1879
Sleep disturbances	0.11	<0.001	1879
Gender	-0.09	<0.001	1879
BMI	0.02	<0.001	1879
Total cholesterol	0.03	0.002	1878
LDL Cholesterol	0.06	<0.001	1688
HDL Cholesterol	-0.14	<0.001	1879
Triglycerides	0.05	<0.001	1879
Waist Circumference	0.01	<0.001	1877
IPAQ category	-0.10	<0.001	1879
PHQ2	0.03	<0.001	1879
Glucose	0.02	<0.001	1879
CD4 T-cell count*	-0.0002	<0.001	858
Viral Load categories*	0.006	0.74	859

* = HIV only

Viral load categories are: 0-50, 50 - 1000, >1000 parts per mL.

CHAPTER 5: DISCUSSION AND CONCLUSION

In this chapter, we will consolidate the findings of the results chapters and compare them to existing bodies of work. We will also contextualise the impact of these studies in a South African context. Finally, we will identify gaps the research has filled and highlight areas for further investigation.

5.1 Prevalence of poor sleep in PLWH in South Africa

With the study conducted at Christ Hani Baragwanath Hospital, presented in the first results chapter, we accomplished the first aim of this thesis. We found that poor sleep was present in PLWH in South Africa, and the prevalence of poor sleep quality was ~60%, which is consistent with other studies (Wu *et al.*, 2015; O'Brien *et al.*, 2021). In our second paper, which focused on older PLWH and PLWOH living in rural Mpumalanga (average age 60 and 68, respectively), using objectively measured sleep with an actigraph, we found that PLWH had shorter sleep duration than PLWOH. Finally, in our last paper in a large cohort of younger PLWH and PLWOH (average age 41.5 and 36.3 years, respectively), using a one-question tool about sleep, we found a significantly higher prevalence of 18% of sleep complaints in PLWH vs 12% in PLWOH. Those lower prevalence numbers in this study compared to the first study (Redman *et al.*, 2018) of people with HIV of comparable age are certainly caused by the instrument used (a single question), which is likely to have not detected other sleep issues detected through the PSQI. The wording of Question 3 from the PHQ-9 is very close to that of the Insomnia Severity Index, which focuses on insomnia symptom detection rather than sleep quality as a whole.

Sleep health is assessed using multiple dimensions, which include sleep timing, sleep duration, sleep quality/satisfaction, sleep regularity, sleep efficiency (time asleep of the sleep opportunity), and daytime alertness (Buysse, 2014; Ravyts *et al.*, 2021). The PSQI addresses most of those dimensions except for sleep regularity, while we used Question 3 from the PHQ-9 to determine the presence of sleep complaints, which only focuses on the sleep duration and sleep efficiency aspects of sleep health. As such, it is unlikely to be as sensitive a tool to detect sleep difficulties compared to the PSQI. New research investigating sleep complaints in PLWH would do well to use more sensitive tools now that the presence of sleep complaints in PLWH in South Africa has been established using multiple methodologies.

5.2 Associated factors with poor sleep quality in people living with HIV

In the first results chapter, poor sleep quality was associated with the presence of pain and higher CD4+ T-cell counts (Redman *et al.*, 2018). Furthermore, we found an interaction between the presence, not the intensity, of pain, and higher CD4+ T-cell counts and sleep quality, whereby sleep quality became worse in people without pain and with higher current CD4+ T-cell counts, while people who reported experiencing pain had no effect of higher current CD4+ T-cell counts. Due to the unexpected association of poorer sleep and high CD4+ T-cell counts, this led to the hypothesis that the immune system may play a role in the pathogenesis of poor sleep in PLWH (which will be discussed later in this chapter)

Soweto is an urban area in the South West of Johannesburg, Gauteng, home to ~1,3 million people as per 2011 census (Statistics South Africa, 2011; 2022 data has not yet been released to an area level). Chris Hani Baragwanath Academic Hospital (CHBAH) is South Africa's largest hospital, the largest in Africa, and the 7th largest hospital in the world (Diptarka Ghosh, 2022). Annually, CHBAH services 150,000 inpatients and 592,000 outpatients across its various departments and disciplines. People across Gauteng travel to CHBAH to receive healthcare.

The Nthabiseng HIV Clinic at CHBAH in Soweto was where the study reported in the first results chapter was based. Soweto is an urban area, and the people who live there experience different pressures than those experienced by people in rural areas such as Agincourt, Mpumalanga and Elandsdoorn, Limpopo. Soweto is known to be a hub of vibrant activity, with CHBAH being the major hospital that services the area as well as the greater Gauteng area.

The urban experience of Soweto is in vast contrast to the experience in the rural areas of Agincourt, Mpumalanga and Elandsdoorn, Limpopo, the sites of Chapters 3 and 4, respectively.

Elandsdoorn is located in the Sekhukhune District of Limpopo, with a population of 120,000 – 130,000 people. It is a village off the main R25 road and is surrounded by small bushy hills on private farms, while the northern part of the village ushers into a fertile valley of orchards and maize crops.

The Agincourt health and socio-demographic surveillance system, established in 1992, comprises 31 villages and ~120,000 people (Tollman and Kahn, 2024) just west of the large Kruger National Park, with mainly uncultivated bushland, subsistence farming and the occasional large mall surrounding the villages. Both Elandsdoorn and Agincourt at ~120,000 people, would encompass only 6% of the population of Soweto. The lifestyles in the urban area vs the rural areas are different. The foods they eat are different, with rural communities relying less on fast-food outlets / processed foods. Violence is present everywhere in South Africa, and urban areas are characterised by higher crime rates than in rural areas (Maluleke, 2022). The fear of the potential harm that could befall one during sleep is impacting people in South Africa. A study in Khayelitsha, an urban area in South Africa, reveals that South Africans (n=411, 25–50 y, 57% women) who reported feeling unsafe during sleep were more likely to report poor sleep quality (Correia *et al.*, 2024).

The second paper (Chapter 3), based in Agincourt, is nested within the Health and Aging in Africa: A Longitudinal Study of an INDEPTH Community in South Africa (HAALSI). As mentioned, Agincourt is a rural area. A study investigating sleep in the same population in 2018 showed that in 5,059 older participants (average age of 61, 54% female), ratings of insufficient sleep were associated with unemployment, lack of formal education, obesity and older age (Gómez-Olivé *et al.*, 2018). This paper also highlighted that short sleep duration, higher incidence of night awakenings and cessation of breathing were all associated with hypertension. Thus, in both urban and rural settings within South Africa, there is evidence of poor sleep, and we took these findings further by investigating which factors are associated with poor sleep in urban and rural communities of PLWH.

This first study was used as a springboard to motivate further investigations of the presence of poor sleep quality and/or sleep complaints in PLWH. In Elandsdoorn, we also had the opportunity to investigate the prevalence of sleep complaints in PLWH compared to PLWOH.

Overall, we found poor sleep quality/ lower sleep duration/ higher sleep complaints in PLWH, which tracks what is shown in other parts of the world (Adane *et al.*, 2022; Bruno *et al.*, 2023; Melese *et al.*, 2024). In Nigeria, a study compared PLWH (N=300, 100% on treatment) with poor sleep quality (PSQI>5) to those with good sleep quality (defined as those with a PSQI≤5). Poor sleep quality was associated with lower CD4+ T-cell counts

(Oshinaike *et al.*, 2014). Similarly, in the United States of America, PLWH (N=290, 70% treated) who reported symptoms of insomnia, as evaluated by a self-report questionnaire (time in bed, time spent asleep, and any weekend oversleep), were more likely to have lower CD4+ T-cell counts (Lee *et al.*, 2012). In a study investigating exclusively women living with HIV in the United States of America (N=139), lower sleep quality (i.e., higher PSQI scores) was associated with lower CD4+ T-cell count (Seay *et al.*, 2013). All these studies show that lower CD4+ T-cell counts, a biomarker of uncontrolled HIV infection, were associated with poorer sleep, in contrast to what we found in the first study, whereby higher CD4+ T-cell counts were associated with poorer sleep (Redman *et al.*, 2018). Our population had low CD4+ T-cell counts when they started their treatment, which is associated with a reconstitution of CD4+ T-cells, which express more markers of activation (Okulicz *et al.*, 2015). It is possible that the association we found between poorer sleep quality and higher CD4 counts reflected a higher level of immune activation, which has been shown to disrupt sleep. Conversely, poor sleep quality is associated with increased immune activation. Higher CD4 counts could reflect better antiretroviral therapy adherence, and, as mentioned in the introductory chapter to this thesis, antiretroviral drugs could also be responsible for the evident poor sleep quality. Furthermore, as we expand our search for the prevalence of poor sleep quality to the Elandsdoorn study, we see that poor sleep is prevalent in the HIV group, of which most were on ARVs. However, the data for the study conducted in Soweto was collected a few years before the Elandsdoorn study, and both sites were adhering to WHO antiretroviral treatment guidelines at the time. For example, in the Soweto study, some people were still on Stavudine, whereas by the time data collection started in the Elandsdoorn study, Stavudine had been completely phased out. Moreover, in the Agincourt study, efavirenz was being phased out in favour of dolutegravir, which may also have affected sleep quality. As seen in the Agincourt study, poor sleep quality in PLWH is also associated with increased non-adherence (Saber, Neilands and Johnson, 2011), which would impact the efficacy of the ART.

Due to the counterintuitive finding that poorer sleep is associated with a higher CD4+ T-cell count, one may feel compelled to explore what is occurring at a cellular level during HIV infection and treatment with antiretroviral therapy, and how this may affect sleep quality and sleep architecture.

As mentioned in the literature review, antiretroviral therapy affects sleep. However, in our studies, we found no definitive impact of antiretroviral therapy on sleep. In Chapter 2, most of the participants were treated with drugs like efavirenz, or stavudine, and various other antiretrovirals, so the impact of any one drug may have been diluted in the presence of the other drugs. In Chapter 4, multivariable analysis did not show any impact of antiretroviral therapy on sleep either. Most of the sleep effects of antiretroviral therapy typically normalise in the first few weeks of therapy and therefore, the timing of the investigation may have missed the early impact of antiretrovirals on sleep in this population.

In contrast to the study done at Soweto (Chapter 2), the study conducted in Elandsdoorn (Chapter 4) had a much larger sample size ($N=1,933$ vs $N=139$) and a group of PLWOH for comparison. This added a significant amount of power to assess both the presence of sleep complaints within a rural cohort and the impact of HIV infection on sleep complaints.

A study conducted in Turkey supports the role that the immune system plays in sleep in PLWH. This study of 131 PLWH (116 on treatment, 15 newly diagnosed and untreated) found that 60% of participants reported PSQI >5 , indicating poor sleep. This study also found a high level of depression in PLWH with poor sleep, and, aligning with our study, that poor sleep was associated with higher CD4+ T-cell counts. In addition, this study was able to calculate CD4+ T-cell reconstitution (current–baseline CD4+ T-cell count) and CD4+ T-cell reconstitution rate [(current–baseline CD4+ T-cell count)/duration of HIV infection in years]. This study found that both the amount of CD4+ T-cell count reconstitution and the rate of reconstitution were associated with worse sleep (Korkmaz and Aydoğmuş, 2024), aligning with our Soweto study.

This further emphasises the impact of the immune system on sleep. When PLWH reconstitute from lower baseline CD4+ T-cell counts (i.e. <100 – 200 cells/ μ L) to higher CD4+ T-cell counts, there is a risk of developing immune reconstitution inflammatory syndrome (IRIS) or immune reconstitution inflammatory disease (Manabe *et al.*, 2007; Barber *et al.*, 2012). The threat of developing IRIS is usually reduced when antiretroviral therapy is initiated at higher CD4+ T-cell counts (Walker *et al.*, 2015), which is why it is important to maintain the universal test-to-treat campaigns to ensure PLWH get access to ART early on in their disease progression before CD4+ T-cell numbers deplete.

Thus, we have established that poor sleep quality is present in PLWH in South Africa. Our next objective was to understand the factors associated with poor sleep quality in PLWH.

5.3 Factors associated with poor sleep in PLWH in South Africa

In the introduction to this thesis, we discussed the role that the immune system plays in sleep and how the human immune system is affected by HIV infection and HIV treatment. In this section, I will first discuss the effects of treatment in PLWH in South Africa as evidenced by the three results chapters, including evidence of the effects of HIV both on sleep process-S (Chapters 2 and 4) and process-C (Chapter 3). I will then discuss depression and the presence of pain and conclude with an extensive discussion of the role of the immune system in sleep disturbances.

5.3.1 Sleep and ARVs in South African PLWH

Over the course of the Soweto study, there were many different types of antiretrovirals, including stavudine (which has since been phased out) and efavirenz (which is currently being used less in favour of dolutegravir). Due to the vast array of antiretrovirals that people were taking, we did not have the power to analyse the effects of different types of antiretrovirals on sleep in PLWH. Of the Soweto study participants, 76% were being treated with efavirenz. This may have limited our ability to investigate the relationship between efavirenz and sleep quality. In addition, participants who had more severe sleep or depression side effects of efavirenz may have already been switched to another antiretroviral treatment.

In the Agincourt study, we did not have the ARV information at the time, as it was only ARV information from dry blood samples, which was not yet available but could now be analysed and investigated with the actigraphy and PSQI components.

In the Groblersdal study, we only assessed the difference in sleep complaints amongst those without ART, on first-line ART, and on second-line ART and found a higher percentage of people not on ART in the sleep complaints group, while there was no effect of first-line vs. second-line ART. Overall, we could not detect any definitive impact of antiretroviral treatment (regimen or drug class) on sleep.

5.3.2 *Sleep complaints and depression in PLWH*

Depressive symptoms in PLWH are known to negatively impact patient adherence to therapy (Uthman *et al.*, 2014; Yen *et al.*, 2022; Oh *et al.*, 2023). Thus, we included depression in two of our studies (Chapters 2 and 4). Depression is a well-reported mental health complaint in people living with HIV (Bhatia and Munjal, 2014; Desta *et al.*, 2022; Gebru *et al.*, 2024). The Soweto study (Chapter 2) reported that clinical depression (defined as using a Beck Depression Inventory score above 17) was present in 41% of the sample population and was associated with worse sleep quality in unadjusted and adjusted analyses. In the Elandsdoorn study, using the PHQ-2, depression levels were consistently higher in people who reported sleep complaints ($p < 0.001$). This was true for PLWH and PLWOH. However, PLWH had higher depression scores than PLWOH ($p < 0.001$). Other studies have shown that the rate of depression in PLWH is between 40–60%, indicating that depression is a common complaint, affecting a large percentage of PLWH. Our studies support these findings.

In other studies, depression has been linked to insomnia (Staner, 2010; Riemann *et al.*, 2020) and hypersomnia (Dauvilliers *et al.*, 2013; Lopez *et al.*, 2017), but overall, depression is associated with sleep disturbance (Mayers and Baldwin, 2006; Murphy and Peterson, 2015; Fang *et al.*, 2019). For PLWH, who are up to three times more likely to have depression compared to people living without HIV (Bhatia and Munjal, 2014), depression could contribute to negative health outcomes (Deshmukh *et al.*, 2017). It is evident from our study that in South Africa, PLWH experience sleep disturbances and depression, which may be contributing to a lower quality of life. This is within the context of South Africa's contracting economy (Stats SA, 2024a), high unemployment rate (Stats SA, 2024b), and record high dissatisfaction with the government (Chironda, 2024), contributing to a lower quality of life for many South Africans.

For PLWH, monitoring depression in the absence of checking for sleep disturbance might be falling short of full patient care. Thus, several articles have suggested that diagnosing depression without first checking for sleep disturbances, such as short sleep duration, insomnia, hypersomnia, and problems with sleep maintenance, is doing a disservice to the patient (Franzen and Buysse, 2008). The DSM-5 clearly indicates that insomnia disorder (and sleep disturbances may or may not fall within this definition) must be treated independently from depression when the two coexist.

Therefore, having highlighted the impact that sleep disturbance has on cardiometabolic disease risk, inflammatory pathways, and overall quality of life, addressing sleep disturbances in people living with HIV is essential to improve their quality of life and wellbeing.

5.3.3 Poor sleep in PLWH and the presence of pain

Chapter 2 is the first study to assess pain and sleep together in PLWH and to show that there is a relationship between sleep, HIV and immunity (CD4+ T-cell counts; Redman *et al.*, 2018). Poor sleep experienced by PLWH can be attributed both to infection with HIV (O'Brien *et al.*, 2021) and treatment of HIV (Omonuwa *et al.*, 2009; Xiao *et al.*, 2022).

Previous studies have described the prevalence of pain (neuropathic pain or deep somatic pain) reported in people living with HIV and the impact of pain on overall wellbeing and quality of life (Keltner *et al.*, 2012; Mphahlele, Mitchell and Kamerman, 2012; Namisango *et al.*, 2012).

HIV-associated neuropathic pain occurs due to neuronal degradation, typically beginning in the extremities, either as a result of progression of infection with HIV to AIDS or with certain antiretroviral medications (de Souza *et al.*, 2018; Addis *et al.*, 2020). Specific ART drugs, such as protease inhibitors or non-nucleoside reverse transcriptase inhibitors, can exacerbate neuropathic pain and peripheral sensory neuropathy (Wadley *et al.*, 2011). The development of neuropathy, whether due to HIV infection or its treatment, can influence the perception of pain in PLWH and may also impact their sleep patterns.

The Soweto study takes these results further and shows the impact of pain on sleep quality. Though South Africans have a history of underreporting pain, this study hopefully allows a more in-depth view of the pain experience of PLWH in South Africa.

5.3.4 The role of the immune system, HIV status on sleep

The literature review chapter of this thesis discussed the intricate, bidirectional relationship between poor sleep and the role of the immune system in modulating sleep. This chapter highlighted how inflammatory pathways and inflammatory mediators affect sleep architecture and timing of sleep. Real-world evidence of these relationships for PLWH was evident in the Soweto study, demonstrating the interrelatedness between sleep, pain, and

the immune system. Being a cross-sectional study, we were unable to tease apart the complex relationships between the three factors. However, this study does provide an important snapshot of the experiences of PLWH in South Africa.

As mentioned previously, the study conducted in Turkey highlighted the impact of the reconstituting immune system affecting sleep (Korkmaz and Aydoğmuş, 2024). Similarly, higher CRP has been shown to be present in PLWH when compared to PLWOH (Ndlovu, Thembane and Jaya, 2025) and when sleep disturbances are present (Imani et al., 2025)

PLWH at greatest risk for developing IRIS are those with advanced immunosuppression (CD4+ T-cell counts <100 cells/μL), who fall into the category of being clinically defined as having AIDS (CD4+ T-cell counts <200 cells/μL). One study hypothesised that there is a hyper-responsiveness of the innate immune system to the reconstituting CD4+ T-cells during the reconstitution process, resulting in the destruction of tissues and widespread inflammation seen in IRIS patients (Barber *et al.*, 2012).

CD4+ T-cells, the helper T-cells, play a critical role in the communication between the innate and adaptive immune systems. The loss of which is what causes the immunodeficiency seen in untreated PLWH that results in AIDS, and eventually, death in the absence of treatment. Critical to their function is the release of cytokines that result in the production of other inflammatory mediators (Liu et al., 2017; Perkins et al., 2023). Some cytokines released during the inflammatory process include IL-6, which, as mentioned in the literature review, can lead to sleep disturbances. We postulate that factors that increase inflammation or stimulate inflammatory pathways negatively impact sleep or sleep quality in PLWH.

This is evidenced by the Elandsdoorn study (Chapter 4), showing that PLWH had higher levels of sleep complaints and higher levels of inflammation when sleep complaints were present. This study used C-reactive protein as a marker of inflammation. During the innate immune response, macrophages release IL-6, which leads to the larger production of C-reactive protein (and other inflammatory proteins) in the liver; thus, CRP levels are a good indicator of the innate immune response.

C-reactive protein (CRP) and interleukin-6 (IL-6) are commonly used biomarkers to assess chronic inflammation in people living with HIV (PLWH) (Perkins *et al.*, 2023). In response to injury, infection, or other inflammatory stimuli, adipocytes and macrophages release cytokines that activate the NF- κ B signaling pathway. This leads to the production of IL-6, tumor necrosis factor-alpha (TNF α), and other inflammatory mediators, including chemokines and adhesion molecules.

IL-6 and TNF α , both products and activators of the NF- κ B pathway, stimulate hepatocytes to synthesise CRP. As such, IL-6 and TNF α are considered upstream markers of inflammation, while CRP serves as a downstream indicator (Jhian, *et al.*, 2023). Elevated levels of IL-6, TNF α , and CRP have been linked to type 2 diabetes in women with abdominal obesity (Tangvarasittichai, Pongthaisong and Tangvarasittichai, 2016).

Additionally, adhesion molecules such as ICAM-1 and VCAM-1 are produced following NF- κ B activation, which are associated with various inflammatory conditions, including cardiovascular disease (CVD) and certain cancers (Singh *et al.*, 2023). In women, oestrogen has been shown to suppress IL-6 signalling, which may explain the increased inflammatory burden observed in postmenopausal women when oestrogen levels are reduced (Rachoń *et al.*, 2002). In obesity, dying adipocytes become encircled by macrophages, which are then activated to secrete IL-6 and TNF α . This process recruits B-cells and T-cells, further amplifying inflammatory pathways and increasing CRP levels as a downstream marker (Liu *et al.*, 2017; Guo *et al.*, 2024).

By design, the study used in Chapter 4 included only CRP – samples are stored at the Ndlovu Research Centre, and can be used moving forward to investigate the cytokines and adhesion molecules mentioned in this body of work. That being said, using CRP as a marker of inflammation we found that, in a multivariable model, we found that sleep complaints were an independent, significant predictor of higher inflammation in PLWH, aligning with the hypothesis that sleep complaints in PLWH are associated with higher inflammation.

At this point, we've established that PLWH have sleep complaints, that these sleep complaints are associated with depression, pain, and inflammation. However, we've been focusing mainly on the homeostatic aspect of sleep, as evidenced in both the Soweto study

and Elandsdoorn study. The underlying role of disruption of the circadian rhythm may be contributing to the sleep complaints experienced by PLWH, which we will explore next.

5.4 A possible mechanism to explain poor sleep experienced by PLWH in South Africa

As per the second results chapter (Chapter 3), the Agincourt study addressed the potential impact that HIV has on the circadian rhythm (Redman *et al.*, 2023). At the time of publication, there had been no definitive study investigating DLMO in PLWH since 2014 (Wang *et al.*, 2014). This study found that PLWH with higher plasma Tat concentrations (indicating viral replication) had higher melatonin levels in the morning. Usually, by the wake period, melatonin production has stopped. In this study, melatonin production was higher in PLWH at 8 am, suggesting a possible delay in circadian rhythms (Wang *et al.*, 2014).

Under normal circumstances in aligned individuals, and as explained in the literature review, the onset of melatonin release typically occurs 2 hours before bedtime (Dijk and Cajochen, 1997; Burgess *et al.*, 2003). As we age, our ability to produce melatonin and maintain sleep reduces (Dijk *et al.*, 1999), resulting in a higher risk for circadian misalignment. In the Agincourt study, which had a much older population (67 years) than Soweto (43 years) and Elandsdoorn (38 years in people without sleep complaints and 42 years in people with sleep complaints) it was found that PLWH had bedtimes much closer to their DLMO than was usual, indicating a negative phase angle of entrainment (PAE), thus circadian misalignment (Redman *et al.*, 2023).

Circadian misalignment is an independent risk factor for cardiovascular disease (Leproult *et al.*, 2014; Morris *et al.*, 2016). In PLWH, who are already at risk for cardiovascular diseases, the circadian misalignment could pose a further risk. This was the first study in PLWH to use DLMO to measure the circadian phase, and there exists only one study of circadian phase in PLWH (Wang *et al.*, 2014).

In a murine model, mice were injected intracerebroventricularly with HIV Tat protein, which led to phase delay shifts in circadian phase in that the higher the concentration of Tat injected into the cultures of SCN cells, the further the phase delays observed in the tissue (Clark *et al.*, 2005). The study postulated that Tat modified circadian phase by potentiating

N-methyl-D-aspartate (NMDA) receptor currents through the enhancement of glutamate release within the suprachiasmatic nucleus (SCN). This enhanced glutamate release causes the enhancement of excitatory nuclei in the SCN, inducing the circadian phase delay (Clark *et al.*, 2005). This study laid the groundwork for the assessment of the effects of Tat in humans.

In the Wang study mentioned previously, 96 PLWH (mean age of 38 ± 10) had cerebrospinal fluid sampled between 07:00 and 08:00 in the morning. Both melatonin and Tat were measured in blood and cerebrospinal fluid, respectively. This study found that there were higher blood levels of melatonin present in the HIV-positive participants who had higher levels of Tat in the CSF (Wang *et al.*, 2014). This finding is consistent with a phase delay shift in circadian rhythms of those who had higher Tat. However, in the same study, there was a negative relationship between the Pittsburgh Sleep Quality Index (PSQI) score and Tat concentration, suggesting that sleep quality improved with higher Tat concentration. Several aspects of HIV infection were not shown in this study (presence of inflammation, antiretroviral therapy used), and some of the relationships presented seemed to be biased by extreme inflection points, thus limiting its interpretability.

In the Agincourt study (Chapter 3), the average PSQI was <5 for both the HIV-negative and HIV-positive groups, indicating overall good quality sleep. Even for the PLWH who experienced circadian misalignment, subjective sleep quality was still PSQI <5 , indicating no sleep complaints; and DLMO time was not associated with PSQI but was associated positively with sleep onset and negatively with sleep offset and total sleep time using actigraphy. Having more granular measures of sleep, such as actigraphy, may be important to assess the relationship between HIV, DLMO time and effects on sleep quality.

With the data discussed thus far, we see how HIV infection, treatment, and underlying mechanisms negatively impact quality of sleep and circadian phase, contributing to the experience of sleep complaints experienced by PLWH.

5.5 Sleep and cardiovascular risk in PLWH

In the Elandsdoorn study, we found a possible mechanism relating sleep and cardiovascular risk. We first investigated the relationship between immune activation and sleep complaints and found that PLWH and sleep complaints had higher levels of

inflammation. Then, using the Framingham risk score as an indicator of the 10-year risk for cardiovascular events, we found that higher CRP and sleep complaints were independently associated with higher cardiovascular risk in PLWH. This supports the hypothesis that inflammation is associated with increased cardiovascular disease risk.

5.6 Sleep and circadian misalignment and potential impact on cardiovascular risk in PLWH

What we presented in the Agincourt study was a potential mechanism by which sleep is impacted in PLWH that could contribute to cardiometabolic disease. This is the first study that shows that DLMO is modified in PLWH, resulting in the negative phase angle of entrainment and circadian misalignment. The consequences of circadian misalignment in PLWH were outlined in the introduction, and for PLWH, circadian misalignment may be an additional contributor to cardiometabolic diseases (Scheer *et al.*, 2009; Morris *et al.*, 2016)

We have, in the literature review, described the inflammatory (Section 1.8) and cardiometabolic (Section 1.5) consequences associated with circadian misalignment. Higher HIV viral load indicates higher replication of the virus, and during replication, several proteins are secreted by the replicating virus that have negative impacts on the host's body. The two main proteins that I will be discussing are Tat and Nef, and their impact on circadian misalignment and inflammation.

Antiretroviral therapy keeps viral replication low and thus prevents viraemia (Byrd *et al.*, 2019). If ART is correctly adhered to, viral loads become undetectable, at which point an individual is unable to transmit HIV (Siedner and Triant 2019). However, as mentioned previously, ART does not eradicate the virus (Scholz and Kashuba, 2021; Banga and Perreau, 2024), meaning that Tat may still be secreted. This was shown in a study investigating the presence of Tat while on ART, and Tat was found in the cerebrospinal fluid of PLWH (Mediouni *et al.* 2012).

TNF- α is a pro-inflammatory cytokine associated with several diseases, including rheumatoid arthritis and ulcerative colitis (Bradley, 2008) and has effects on sleep and sleep quality (Krueger *et al.*, 1998). During viral replication and immune activation, higher levels of pro-inflammatory cytokines are present, affecting sleep.

Tat stimulates the production of pro-inflammatory cytokines, such as TNF- α and IL-6, by immune cells, including macrophages, dendritic cells and monocyte (Chen *et al.* 1997, Ben Haij *et al.*, 2015). Tat activates the NF-kB pathway (Ben Haij *et al.*, 2015), which is a key regulator of inflammation and immune responses. The proposed mechanism by which Tat affects the NF-kB pathway is by engaging the TLR4-MD2 and CD14 complex, eliciting a response similar to a lipopolysaccharide infection (Kim and Kim, 2017). This causes the release and overproduction of pro-inflammatory cytokines including TNF- α , IL-1b, IL-6 and IL-8 (Haij *et al.*, 2015; Kim and Kim, 2017). As mentioned, there was no association between ART and inflammation in our cohort.

Aside from the indirect effect on inflammation by Tat via TNF- α levels, there is evidence that Tat has deleterious effects on neuronal and connective tissue. Increased levels of circulating Tat are associated with vascular damage (Anand *et al.*, 2018), phase delay shifts in murine models (Clark *et al.*, 2005) and possibly also in humans (Wang *et al.*, 2014), and neurotoxicity (in animal models) (Bruce-Keller *et al.*, 2003; Tang, Lu and Ramratnam, 2020).

In the Elandsdoorn study, higher levels of inflammation were associated with sleep disturbances, increased LDL-cholesterol, and low physical activity in PLWH. Inflammation also increased the 10-year risk of cardiovascular disease (Cha.

5.7 Potential consequences of sleep complaints and inflammation on cardiometabolic disease risk for PLWH in South Africa

A review conducted by Anand *et al.* in 2018 highlighted the role of HIV proteins in contributing to endothelial dysfunction (Anand *et al.*, 2018). They focus on GP120, Nef, and Tat as contributors to endothelial dysfunction, and highlight endothelial dysfunction as the link between HIV infection and CVD seen in PLWH (Deshmukh *et al.*, 2017). The review puts forward that viral proteins are direct contributors to the inflammation that leads to endothelial dysfunction, and that even basal levels of viral proteins are enough to drive the inflammatory pathway. Although ART is effective in keeping viral loads low, there is evidence that viral proteins are still secreted (Mediouni *et al.*, 2012) and that these viral proteins are leading to the increased inflammation, endothelial dysfunction, sleep disturbances and increased CVD seen in PLWH.

PLWH, whether on treatment or not, are exposed to higher levels of chronic inflammation and chronic immune activation (Lv, Cao and Li, 2021). CRP is produced in the liver during inflammatory reactions, along with proinflammatory serum amyloid A. Serum amyloid protein A and CRP can replace apolipoprotein-A-1, the main lipoprotein associated with HDL cholesterol particles (Han *et al.*, 2016; Reisinger *et al.*, 2021). This reduces the cholesterol efflux capacity and anti-inflammatory properties associated with HDL (Ebtehaj *et al.*, 2019). Furthermore, monocytes from PLWH show impaired cholesterol efflux and increased foam cell formation, specifically at the transendothelial border (Maisa *et al.*, 2015), contributing to the development of dyslipidaemia and laying the foundation for atherosclerotic disease. The third results chapter showed that PLWH have lower HDL levels, and the changes to the building blocks of HDL may be the causative factor.

It is well known that lower HDL levels are associated with increased risk for cardiovascular disease, and this could serve as an investigative point in future studies.

5.7.1 The role of physical activity

Sedentary behaviour is a risk factor for cardiovascular disease, and in PLWH, it could increase the risk of developing CMD (Wirth *et al.*, 2015). Other studies from South Africa on the effect of exercise in PLWH have shown that exercise improves overall health and well-being (Jackson *et al.*, 2021). Chapter 3 showed that low physical activity is associated with higher inflammation, and from the literature, we know that a more sedentary lifestyle is associated with poorer cardiovascular health (Webel *et al.*, 2016). The role of exercise in the general population in South Africa (of which 51% is overweight/obese) (Maluleke, 2022) cannot be undervalued, as it is not just an issue for PLWH.

PLWH in South Africa with lower physical activity are at higher risk for developing ischaemic heart disease (Roos *et al.*, 2016). A study done in Umlazi, an urban area in KwaZulu-Natal, South Africa, in 2019, revealed that 67,5% of adults were classified as obese, with a BMI > 25 kg/m². This cohort of adults (median age 35 years) also had a 17,6% prevalence of metabolic syndrome, low HDL (43,2%), and elevated CRP (59,5%). Although this cohort had a high level of obesity and metabolic syndrome, 75% reported being happy with their body shape (Hanley *et al.*, 2021). However, it is well known that obesity also contributes to inflammation, CMD risk and poorer CVD outcomes. Exercise and weight management should become more integrated into the South African lifestyle.

All three projects described in the results chapters identified different issues with sleep in PLWH. The first study highlighted the presence of poor sleep in an urban setting (process-S of sleep, homeostatic), the second study identified circadian misalignment present in PLWH (process-C, circadian), and the third study showed that poor sleep, especially in PLWH, is associated with inflammation. As depicted in Figure 1 above, infection with HIV is known to cause disturbances in various tissues and cell types, including immune cells and neural tissue, and then lead to endothelial damage and further inflammation, leading to sustained and progressive damage over a long period. HIV infection contributes to poor sleep quality, which can directly influence cardiovascular disease.

With the advent of ART, however, the effects of the virus may be mitigated quite substantially.

IMMUNE ACTIVATION, SLEEP AND CIRCADIAN TIMING IN HIV PATIENTS

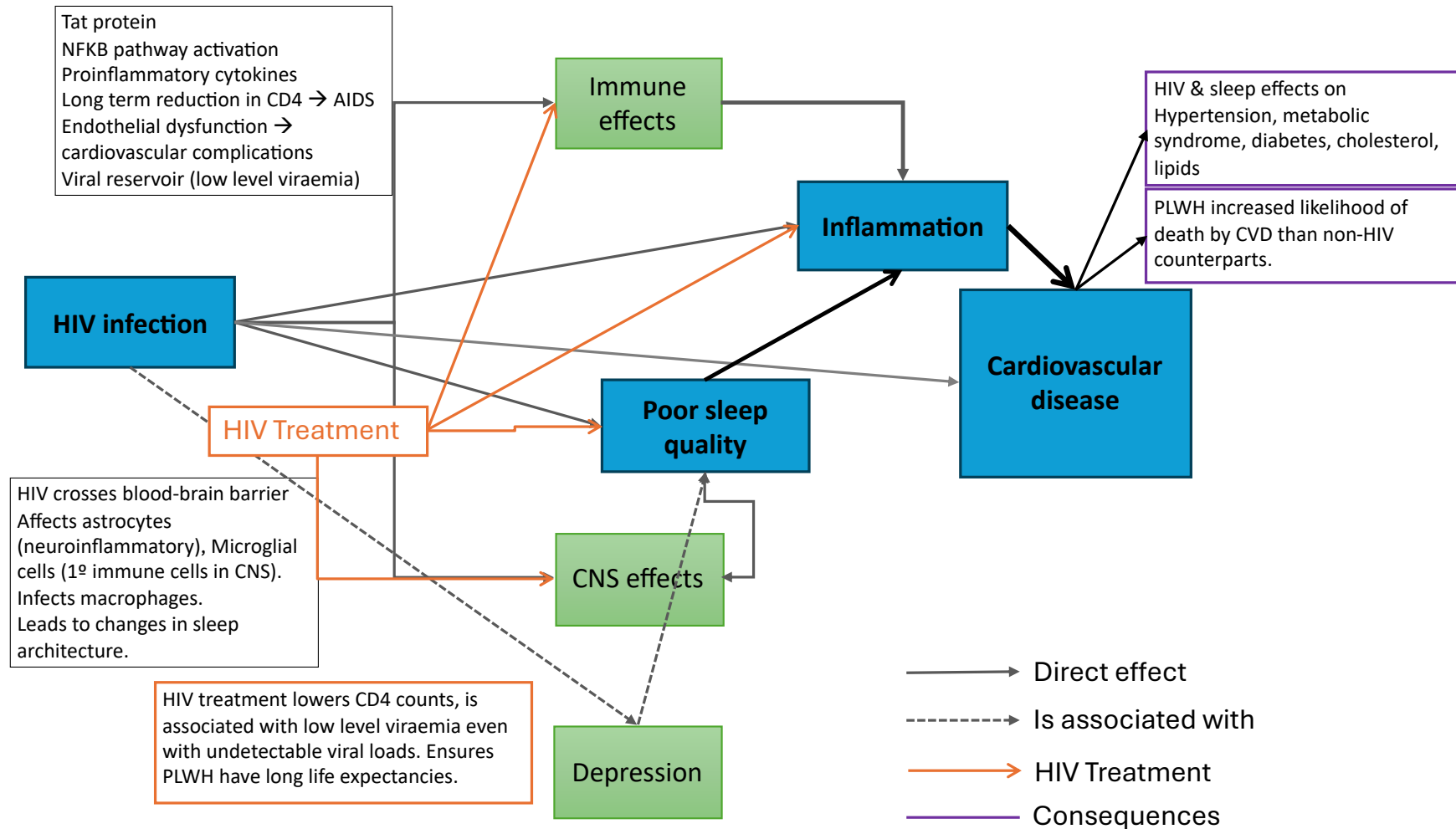


Figure 1. A summary of the factors affecting sleep and cardiometabolic disease risk mentioned in this review.

As seen in Figure 1, infection with HIV contributes directly to cardiovascular disease, but the mechanism is still unknown. What is known is that PLWH have higher levels of CVD than HIV-negative counterparts, suggesting the involvement of HIV – but whether there is a direct or indirect effect is unknown. We postulate the role of the chronic immune activation causing knock-on effects that result in changes to fat metabolism, endothelial dysfunction, and sleep complaints.

In the introductory chapter, we discussed the metabolic and inflammatory consequences of poor sleep and circadian misalignment. This thesis highlights the potential for sleep interventions in PLWH in both the rural and urban settings to improve overall sleep quality in PLWH. By doing so, we hope to reduce the negative impact of poor sleep on the health of PLWH and reduce at least one contributing factor to cardiometabolic disease.

Furthermore, these findings highlight the need for increased awareness and support for PLWH who may be experiencing sleep disturbances and associated health complications. Healthcare providers who care for PLWH should consider incorporating sleep assessment and management into routine care and work with patients to develop individualised plans for addressing sleep and circadian disruptions.

Overall, these findings have important implications for the health and well-being of PLWH and highlight the need for a holistic approach to care that addresses not only viral suppression but also the assessment and management of lifestyle factors that could impact the overall health of ageing PLWH in South Africa. The National Department of Health has ensured access to and administration of antiretroviral therapy to the nearly six million HIV-infected individuals in South Africa, and we, as researchers within the public healthcare system, should now advocate for better monitoring of and provision of education on lifestyle factors that improve health outcomes in PLWH.

5.8 Further areas for study

As PLWH are experiencing longer lives while on ART, inflammatory markers, such as C-reactive protein and proinflammatory cytokines, such as TNF- α and IL-6, should be monitored along with the usual disease markers CD4+ T-cell counts and viral load. This will ensure the detection of potential contributing factors to cardiometabolic disease and could pre-empt the development of risk factors like endothelial dysfunction and subclinical

atherosclerosis. Further research could also be done to assess levels of Tat protein within participants and, using DLMO, create a strategic framework to understand the relationship between Tat, melatonin and sleep architecture.

5.8.1 *Using a snapshot sleep questionnaire to assess sleep quality in PLWH*

Throughout this thesis, we've used various scales to assess the different dimensions of sleep health, including duration, efficiency, timing, regularity, satisfaction and alertness. We've used the Pittsburgh Sleep Quality Index (efficiency, timing, satisfaction, duration, alertness), actigraphy (regularity, timing, efficiency, duration), polysomnography (efficiency, timing, duration), and PHQ-3 (satisfaction). All of these scales may be too long or labour-intensive for use in a clinical setting, so a snapshot questionnaire that covers all of the dimensions of sleep health, such as the RU-SATED (Buysse, 2014), may be more beneficial. The RU-SATED questionnaire, developed by Buysse (2014), assesses sleep health by evaluating regularity, satisfaction, alertness, timing, efficiency, and duration. This allows clinicians and researchers to determine an individual's sleep health and make necessary assessments.

5.8.2 *HIV proteins and inflammation*

Further studies will have to assess Tat levels and their relationship to markers of inflammation such as C-reactive protein, TNF- α and IL-6. As we described previously, inflammation is linked to an increased risk of CVD. One of the ways to test CVD risk, specifically atherosclerosis, is to assess the carotid intima-media thickness (CIMT). These data are being collected in the Ndlovu study and could be a potential point to investigate in further research. Tat proteins could also be measured against the presence of sleep disturbances – both cross-sectional and in a longitudinal capacity – again, in the context of inflammatory markers. This can be done in both the Ndlovu and Agincourt research sites. We do not yet know the direct role that Tat plays in lipid metabolism, which may also be an avenue to explore in future studies. Assessing levels of viral activity per plasma concentrations of Tat and the activity of the reconstituting immune system upon initiating antiretroviral therapy would paint a picture of the trajectory of immune activation (if any) over 1–2 years of taking antiretroviral therapy.

5.8.3 *Impact and future studies*

The lack of generalisability: South Africa locked

All three of the results chapters incorporated studies from South Africa. Though all three served as strong evidence for poor sleep in PLWH in rural and urban regions, one cannot extend the findings to the rest of the world.

These studies were all conducted in PLWH in South Africa, which represents a different population to not only the global north, but also to the whole of Africa, since most people of African ancestry in South Africa are of admixed origins (Swart *et al.*, 2022). In other parts of the world, it may be worthwhile investigating the presence of these relationships of sleep complaints (of which there are already studies), but more so the presence of modifications to the circadian rhythm, as explained in the Agincourt study.

Factors such as HIV subtype C accounting for ~100% of all infections in South Africa (Musyoki *et al.*, 2015), whereas subtype B is more prominent in Europe, the Americas, and in Australia, followed by subtype A, which is prevalent in Eastern Europe and Asia (Magiorkinis *et al.*, 2016; Hemelaar *et al.*, 2019). Other parts of the world have different levels of access to different antiretroviral drugs, and said drugs suppress HIV viral replication with varying degrees of success. Further studies will have to investigate the kind of sleep disruption present in PLWH on different types of ARVs and investigate the impact on both the homeostatic and circadian control of sleep. Assessing dim light melatonin onset in PLWH would be vital in understanding the effect that HIV has on the circadian rhythm, and investigating the impact that any proposed modifications could have on CVD risk in PLWH would be important.

The average age of participants in the Soweto study was 43 years, and in Elandsdoorn, 41 years in PLWH. In Agincourt, the average age was 67 years, already considered an older population with existing modifications to sleep (Kim and Duffy, 2018; Casagrande *et al.*, 2022; Li *et al.*, 2022). It may be worthwhile exploring if the circadian misalignment demonstrated in this older population is reflected in a younger population.

The potential impact of melatonin therapy or light exposure therapy in PLWH with circadian misalignment

As shown in the Agincourt study, PLWH experience circadian misalignment as per the timing of DLMO and sleep onset. As described, circadian misalignment is associated with increased levels of inflammation and cardiometabolic risk. Designing an intervention study using a 0.5 mg dose of melatonin up to 3 mg of melatonin, (depending on age) 2–5 hours before bedtime (Burgess *et al.*, 2003) for 1 month to see if there are any improvements in sleep.

Increasing the number of inflammatory markers analysed: cytokines TNF α and IL-6.

Chapter 4 of this thesis utilised CRP as a marker of inflammation within the Ndlovu cohort, in alignment with the original study design, as previously explained. However, additional plasma samples remain frozen in storage and could be used to analyse other cytokines, such as TNF- α and IL-6, which were discussed in earlier sections. Expanding the analysis to include these markers would provide a more comprehensive understanding of inflammation among PLWH and PLWOH in this cohort. Furthermore, integrating these findings with follow-up questionnaire data from the Ndlovu Study would allow for longitudinal monitoring of inflammation and its progression over time. This approach could also help identify the onset of cardiometabolic conditions and explore their association with inflammatory markers.

Introducing exercise protocols into the daily routine of PLWH

As we mentioned, we noted a reduction in sleep quality in the first results chapter, although the amount of sleep was not a principal objective. In the second and third results chapters, we found that the amount of sleep that PLWH had was not different from that of uninfected counterparts. Sleep quality, and not quantity, was the issue. Thus, if there were modifications to sleep to be investigated in this cohort, it should be focused on improving sleep quality, rather than investigating the impact of more sleep. One area that could enhance the quality of sleep is increasing levels of activity – i.e., more could be done to ensure that PLWH exercise more to reduce inflammation and the risk of CVD.

As seen in Chapter 3, the Elandsdoorn study, higher levels of inflammation are associated with sedentary behaviour. As described, Elandsdoorn is a rural area in Limpopo, with the Ndlovu Care Group hosting a sports ground and various sporting activities for the

community. Agincourt, similarly, is a large rural area with an ageing population. There is no evidence that there are community centres for exercise in Agincourt, but there are neighbouring areas, such as Acornhoek, that have gym facilities. These may, however, not be accessible to the general public.

Introducing exercise interventions for people in these studies (both for PLWH and PLWOH) would allow researchers to view the impact of exercise and highlight the potential benefits for people in South Africa. In 2016, 31% of men and 68% of women were either overweight or obese (Lehola, 2017) – showing that people in South Africa already have an issue with increased cardiovascular disease risk. Adding exercise interventions may benefit the health of South Africans as a whole, as per the goals of the national development plan to improve wellness through eating healthy food, growing your own food, and exercise (National Planning Commission, 2024)

5.9 Study limitations

5.9.1 Older data

Older data from the Chapter 2 study at a different time for antiretroviral therapy, where pain was more prominent. As seen in Chapter 1, Table 1, there were a plethora of changes in HIV treatment in South Africa from 2012 to 2019. However, even with the older antiretroviral therapy and the longer wait times to initiate treatment, our study still revealed that immune activation could drive the relationship that is evident between sleep and HIV.

5.9.2 Cross-sectional vs longitudinal data from the Ndlovu Cohort Study

The data used in the paper from Chapter 3 represents only the baseline measurements, whereas there are 3 years' worth of six follow-up measurements. This longitudinal data can provide valuable insights into changes over time, such as in variables like CRP, CIMT, and lipids. Longitudinal analyses can also tease apart some of the relationships highlighted in this project and reveal cardiometabolic risks and the development of cardiovascular diseases. Examining the complete dataset over time can also shed light on the relationships between depression, sleep disturbance, HIV disease progression, and antiretroviral therapy.

5.9.3 Lack of inflammatory markers for the DLMO portion of the study

The study population selected for this group is a subset of the Health and Ageing in Africa: A Longitudinal Study of an INDEPTH Community in South Africa (HAALSI) population of Agincourt. We did not have access to data on blood levels of markers of inflammation in this sub-study. In taking PSG measurements, we also collected blood samples from which we isolated PBMCs and plasma for analysis in other projects. We aim to assess the levels of plasma cytokines within the DLMO study.

5.9.4 The need for more tailored CVD assessment in PLWH

Although PLWH in our cohort presented with lower traditional CVD risk factors than PLWOH, they still exhibited elevated inflammation and potential cardiovascular risk. Although Framingham Risk Score (FRS) in this study was more exploratory, the data suggests that conventional tools like the FRS may underestimate true CVD risk in this population (Triant *et al.*, 2024). Traditional CVD scores underestimate risk in PLWH, likely due to the unique contributions of HIV-related inflammation and immune activation, which are not captured by standard risk algorithms. Our findings support growing evidence that HIV-related inflammation—exacerbated by sleep disturbances—may contribute to subclinical cardiovascular disease (Vos *et al.*, 2016; Simkevich, 2025), highlighting the need for more tailored risk assessment approaches in PLWH.

5.10 Final Conclusion

In conclusion, PLWH in South Africa experience poor sleep and higher rates of insomnia than age and sex-matched controls and show evidence of circadian misalignment. Insomnia in this population is also characterised by high levels of inflammation, which is a recognised risk factor for the development of cardiometabolic and cardiovascular diseases. As PLWH are already at higher risk for these diseases, this thesis identifies inflammation and insomnia as key potential mediators of CMD and CVD risk in PLWH. To improve the health outcomes of PLWH in South Africa, greater efforts are needed to monitor inflammation and to improve individual health in this population. Longitudinal analysis of existing data could further tease apart the relationships between ageing, poor sleep, inflammation and cardiovascular disease risk in PLWH.

5.11 References

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APPENDIX A

- 1) Ethics for Sleep Study at the Chris Hani Baragwanath Academic Hospital
- 2) Ethics for Sleep Study at Agincourt
- 3) Amendment adding Kirsten Redman
- 4) Ethics from the University of Pretoria – Ndlovu Cohort Study
- 5) Ethics for Sub-Study at the Ndlovu Research Centre
- 6) Newspaper report of riots at Elandsdoorn over the closure of the ARV programme at the Ndlovu clinic
 - a) *Sekhukhune Dispatch*. “Termination of Contract Causes Tension Between Government and Community.” April 16, 2018, 220th edition.
 - b) Janine van Staden. “Public Outcry after Limpopo HIV and TB NGO Denied Funding.” *The Citizen*, April 26, 2018.
<https://www.citizen.co.za/news/south-africa/local-news/public-outcry-after-limpopo-hiv-tb-ngo-denied-funding/>

1. Ethics for Sleep Study at the Chris Hani Baragwanath Academic Hospital



UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

R14/49 Dr Karine Scheuermaier

CLEARANCE CERTIFICATE

M120411

PROJECT

Sleep, Mood and Morningness-Eveningness
Preference in Treated and Untreated HIV
Patients

INVESTIGATORS

Dr Karine Scheuermaier.

DEPARTMENT

School of Physiology

DATE CONSIDERED

04/05/2012

+DECISION OF THE COMMITTEE*

Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE

17/05/2012

CHAIRPERSON


(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor :

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.

I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

2. Ethics for the Sleep Study at Agincourt



R14/49 Dr F. Xavier Gomez-Olive and Prof Malcolm von Schantz et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M180667

NAME: Dr F. Xavier Gomez-Olive and Prof Malcolm von Schantz et al
(Principal Investigator)
DEPARTMENT: School of Public Health
MRC/Wits University Rural Public Health and Health Transitions
Research Unit (Agincourt), Acornhoek

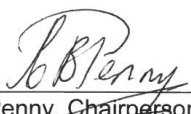
PROJECT TITLE: Timing, quality, and physiology of sleep in a deprived
community cohort in South Africa and their relationship
with chronic disease

DATE CONSIDERED: 29/06/2018

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR:

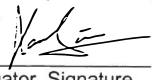
APPROVED BY: 
Professor CB Penny, Chairperson, HREC (Medical)

DATE OF APPROVAL: 23/08/2018

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

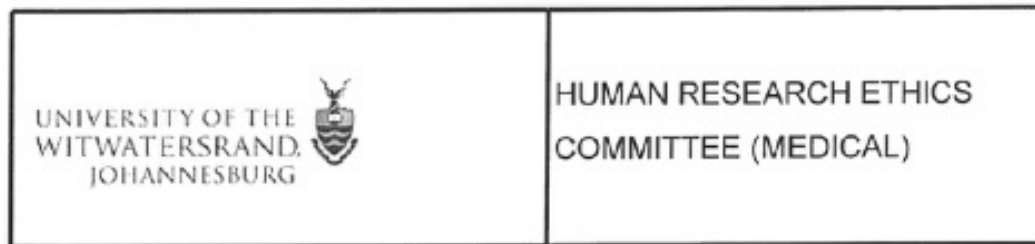
To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.** The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **June** and will therefore be due in the month of **June** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).


Principal Investigator Signature

Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

3. Ethics for Amendment Adding Kirsten Redman to the Sleep Study at Agincourt



30/01/2019

Dr F Gomez-Olive Casas, Prof M von Schantz, et al
School of Public Health
Rural Public Health & Health Transitions Research
Unit

Sent by e-mail to: F.Gomez-OliveCasas@wits.ac.za

Dear Dr Gomez-Olive

Re: **Protocol Ref No: M180667**
Protocol Title: *Timing, quality, and physiology of sleep in a deprived community cohort in South Africa and their relationship with chronic disease*
Principal Investigator: Dr F Gomez-Olive Casas, Prof M von Schantz, et al

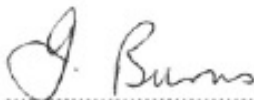
Thank you for your letter of 21/01/2019.

I confirm that the three amendments have been noted and approved. For the record, they are:

1. the addition of a named fieldworker (Ms A Khoza)
2. the addition of a PhD student to the protocol (Ms K Redman)
3. an increase in the volume of the blood sample to 30 ml

Thank you for keeping us informed.

Yours Sincerely



.....
Mr I Burns
For the Human Research Ethics Committee (Medical)

Works2000\lain0007\Acknowledge.docx

4. Ethics from the University of Pretoria – Ndlovu Cohort Study

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 22 May 2002 and Expires 20 Oct 2016.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Expires 22/04/2017.



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee

1/07/2014

Approval Certificate New Application

Ethics Reference No.: 227/2014

Title: A multidisciplinary cohort study comparing HIV-positive and HIV-negative subjects to investigate the epidemiology, pathogenesis and social factors determining the interaction between HIV and (lifestyle-related) chronic conditions, with a special focus on cardiovascular disease – The Ndlovu Cohort Study (NCS)

Dear Dr Hugo Templeman

The **New Application** as supported by documents specified in your cover letter for your research received on the 30/05/2014, was approved, by the Faculty of Health Sciences Research Ethics Committee on the 1/07/2014.

Please note the following about your ethics approval:

- Ethics Approval is valid for 11 years.
- Please remember to use your protocol number (**227/2014**) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics approval is subject to the following:

- The ethics approval is conditional on the receipt of 6 monthly written Progress Reports, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Dr R Sommers; MBChB; MMed (Int); MPharMed.
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

♦ Tel: 012-3541330 ♦ Fax: 012-3541367 Fax2Email: 0866515924 ♦ E-Mail: fhsethics@up.ac.za
♦ Web: <http://www.healthethics-up.co.za> ♦ H W Snyman Bld (South) Level 2-34 ♦ Private Bag x 323, Arcadia, Pta, S.A., 0007

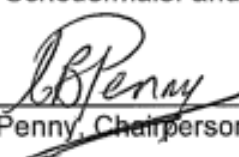
5. Ethics for the Sub-Study at the Ndlovu Research Centre



R14/49 Mrs Kirsten Redman-Vira

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M230466

NAME: Mrs Kirsten Redman-Vira
(Principal Investigator)
DEPARTMENT: School of Physiology
PROJECT TITLE: *Interaction between immune activation, sleep disturbances and circadian timing on cardio-metabolic risk in HIV positive patients*
DATE CONSIDERED: Ad hoc
DECISION: Approved unconditionally
CONDITIONS:
SUPERVISOR: Prof K. Scheuermaier and Prof N. Crowther
APPROVED BY: 
Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL: 11/07/2023 **EXPIRY DATE:** 11/07/2028

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.** The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **April** and will therefore be due in the month of **April** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).


Principal Investigator Signature

11/07/2023
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

6. Sekhukhune Dispatch. "Termination of Contract Causes Tension Between Government and Community." April 16, 2018, 220th edition.



Termination of contract causes tension between government and community

Community members holding placards demonstrating about the termination of the Ndlovu Medical Centre contract by the Limpopo Department of Health.



ELNA KERLING
OOGKUNDIGE / OPTOMETRIST

Medical Centre
14 Kruger Street
Groblersdal
Tel: 013 262 2132

WE CARE FOR EYECARE

After Limpopo Department of Health announced that it is terminating its contract with the Ndlovu Medical Centre in Elandsdoorn, Dennilton, concerned Moutse residents, are up in arms opposing and demanding the Limpopo Department of Health to reverse the decision.

The Health Department has recently instructed the Ndlovu Medical Centre to refer its HIV patients to government health institutions. According to concerned residents, 3800 HIV patients that the Ndlovu Medical Centre is currently treating will suffer under the care of government health institutions. A number of meetings were convened between the Ndlovu Medical Centre, Limpopo Department of Health and community members, where the public made direct complains to the Health Department officials but their demands were not considered.

To show their dissatisfaction, during the meetings, residents were holding placards some written 'Hands Off Tempelman', protesting against the Health Department's decision. Residents took it to the streets after the Health Department officials failed to attend a meeting on Wednesday 11 April.

The community blocked the R25 Road with rubble and burning tyres, demanding the Health officials to come and attend the meeting as they promised. Bolsheviks Party of South Africa leader, Seun Mogotji said they do not want the contract to be terminated because government health institutions have no capacity to accommodate these patients.

Continues on page 3...

Termination of contract causes tension between government and community

... *Continues from page 1*

"It is a common knowledge that these government health facilities cannot cope with their current number of patients. It is going to be worse when Ndlovu Medical Centre refer its patients to those institutions," he said. Seun Mogotji, who is also a board member at Philadelphia Hospital, claims some of patients in the hospital and clinics are at times turned away without their HIV medication. "The government does not have the capacity to treat these patients because they are sent back without given ARVs at some days. It is clear that this will also affect patients that the government demands that Ndlovu sends to public institutions," he said. Mogotji indicated that it is evident that the Ndlovu Medical Centre is among the best private health institution in the country. "The Ndlovu Medical Centre has initiated the third free ARV roll-out program in South Africa in 2003 and has treated over 20 000 HIV patients in different treatment sites. Currently it has over 3800 patients on ARVs who are satisfied with the treatment and the continuous follow up they receive from the centre," he said. According to Mogotji, the reason that the Health Department is aiming to reduce costs by terminating the contract with Ndlovu is not making any sense. "The decision will make patients suffer while the department claims they are reducing costs. These patients will not get proper care in government health institutions. Millions are spent on useless things such as corruption while government is risking lives of South Africans," he said. According to EFF Councillor in Elias Motsoaledi Local Municipality Ali Maloba, termination of the contract can lead to the collapse of the centre. "The main focus of the centre is based on HIV treatment where thousands of lives were saved by the institution. Termination of the contract will lead to many losses of lives and many people who are currently working at the centre will lose their jobs," he said. He added that the department is terminating the Ndlovu contract to fund some of the private health facilities. "They terminate the contract and recently started funding some of the private facilities such as Unjani Clinic.

It is clear that some health officials are beneficiaries from these private institutions. We demand a permanent contract between the Limpopo Department of Health and the Ndlovu Care Group," said Maloba. MEC for Health Department in Limpopo Dr Phophy Ramathuba said the department is terminating the contract because it is expensive and they want to cut the costs. "We are funding the institution with R14 Million per month where our public health facilities that render the same service receive between R4 Million to R7 Million," she said. Ramathuba added that the department is spending a lot in treating HIV patients at Ndlovu, compared to government health institutions."



Residents burning tyres on the R25 Road opposite Moutse Mall demanding Limpopo MEC for Health Dr Phophy Ramathuba to reverse the decision.

7. Janine van Staden. “Public Outcry after Limpopo HIV and TB NGO Denied Funding.” *The Citizen*, April 26, 2018

The Citizen

Public outcry after Limpopo HIV and TB NGO denied funding

By [Janine van Staden](#)

4 minute read

26 Apr 2018

12:00 pm

HIV and TB patients were up in arms and launched a protest after the Limpopo health department announced they would no longer fund the NGO.



File photo. For illustrative purposes.

The department gave the NGO three months to hand over the list of patients and transfer all patients to local clinics, provide treatment for the next two months and prepare admin for clinics who will be taking over.

HIV and TB patients that receive healthcare services from the Ndlovu Care Group want their voices to be heard, saying that they refuse to be transferred to government clinics that treat them arrogantly, have poor service and care for patients in dilapidated clinics, [Middelburg Observer](#) reports.

The Limpopo department of health announced this during their budget speech for 2018/19 on Tuesday, 10 April, saying that they will prioritise equipment maintenance, medical supplies and increasing human resources at the department with the allocated budget for this financial year, which is R19.5 billion – an increase of 8.1% from 2017/18.

They also announced that it will no longer fund centres and nonprofit organisations providing services to HIV patients across the province, saying that the system is costing the department millions of rand a year.

More than 3 700 TB and HIV patients are currently receiving treatment at Ndlovu Care Group facilities, but the department wants to part ways with the NGO in order to cut costs, and will result in the closing of the HIV and TB programme.

READ MORE

SA court upholds decision on free healthcare for foreign pregnant women and children

The patients say that the public health care system is not fully equipped, understaffed and not capacitated to look after their health needs and warned that the decision to stop funding the NGO will result in deaths.

The Ndlovu Medical Centre has been in operation since 1994 and Dr Hugo Tempelman, the founder of Ndlovu Care Group, was the first in the province to set up a free anti-retroviral programme site for HIV/Aids patients in 2003 while the Department of Health was still in denial.

Ndlovu Care Group later partnered with the department to treat HIV and TB patients, as from 2008.

Tempelman said that he regrets the decision of the Limpopo department of health, and is very disappointed that they finalised the decision without engaging properly with the community, patients and local clinics concerned.

Ndlovu Care Group does not only provide the TB/HIV/Aids treatment programme, but also provides other related services such as a cervical cancer treatment programme, audiology and innovating HIV prevention research.

He said that closing the HIV and TB programme at Ndlovu Care Group will not only result in patients having to move to other under-capacitated, understaffed, overburdened clinics, but will result in defaulters and treatment interruptions through which the health of the patients will be affected.

He does not understand the position of the department of health at a time that service delivery and employment are such important issues on the political agenda.

Closing the ARV roll-out programme at Ndlovu Care Group will lead to approximately 35-42 job losses according to the HR manager, Mrs Poppy Mashamaite. Of these employees, 85% are local residents who will be unable to find new jobs in an area with over 55% of unemployment.

Tempelman said that he cares too much about the 3 700 HIV and TB patients of the Ndlovu Care Group to accept the department's decision.

"If the Ndlovu HIV and TB programme closes, the local clinics will receive double the amount of patients they have now. The question is how will the local clinics handle double the patients if they can't even handle the amount they have now? The clinics will sink and swamp and this will create chaos," said Tempelman.

He further explained that the community, Ndlovu Care Group and the MEC of the department of health, Phophi Ramathuba, had a meeting and that Ndlovu is still fighting hard for the patient's care at the Ndlovu Care Group, and continues to negotiate with the department about their decision.

"I won't stop fighting for the patients. This is not about the Ndlovu Care Group, but about the 3 700 patients that now need to be transferred to clinics that will not be able to absorb enormous amounts of patients on top of the current workload they have.

"Ndlovu Care Group is also very concerned about the almost 42 employees that serve the community and whom the department is not willing to take over.

"Ndlovu Care Group has another meeting with the department on Friday and will continue to engage with the department on the matter," said Tempelman.

Fears have been sparked that this could be another Life Esidimeni tragedy, in which ninety-four people died after the Gauteng Health Department moved patients from Life Esidimeni and other facilities. Police are investigating several cases of murder and negligence.

APPENDIX B

1. Pittsburgh Sleep Quality Index Questionnaire
2. Epworth Sleepiness Scale
3. Beck's Depression Inventory
4. Ndlovu Cohort Study Baseline Questionnaire
5. Ndlovu Cohort Study Baseline Medical Assessment Form

1. Pittsburgh Sleep Quality Index

Subject's Name _____
Score _____

Date: _____

PITTSBURGH SLEEP QUALITY INDEX

INSTRUCTIONS: *The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.*

During the past month,

1. When have you usually gone to bed? _____
2. How long (in minutes) has it taken you to fall asleep each night? _____
3. When have you usually gotten up in the morning? _____
4. How many hours of actual sleep did you get that night? (This may be different than the number of hours you spend in bed.) _____

5. During the past month, how often have you had trouble sleeping because you . . .	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
a. Cannot get to sleep within 30 minutes				
b. Wake up in the middle of the night or early morning				
c. Have to get up to use the bathroom				
d. Cannot breathe comfortably				
e. Cough or snore loudly				
f. Feel too cold				
g. Feel too hot				
h. Had bad dreams				
i. Have pain				
j. Other reason(s), please describe, including how often you have had trouble sleeping because of this reason(s):				
6. During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?				
7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?				
8. During the past month, how much of a problem has it been for you to keep up enthusiasm to get things done?				
	Very good	Fairly good	Fairly bad	Very bad
9. During the past month, how would you rate your sleep quality overall?				

Reprinted from: Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ: Journal of Psychiatry Research, 28:193-213, 1989.

2. Epworth Sleepiness Scale

DAYTIME ACTIVITY (Epworth Scale)

- | | HIGH
CHANCE | MODERATE
CHANCE | SLIGHT
CHANCE | NEVER
DOZE |
|--|--------------------------|--------------------------|--------------------------|--------------------------|
| 1. In the last 30 days, how likely are you to doze off or fall asleep in the following situations (in contrast to feeling just tired)? | | | | |
| • Sitting and reading. | ☐ | ☐ | ☐ | ☐ |
| • Watching TV. | ☐ | ☐ | ☐ | ☐ |
| • Sitting inactive in a public place (e.g. theater, Church) | ☐ | ☐ | ☐ | ☐ |
| • As a passenger in a car for an hour without a break. | ☐ | ☐ | ☐ | ☐ |
| • Lying down to rest in the afternoon when circumstances permit. | ☐ | ☐ | ☐ | ☐ |
| • Sitting and talking to someone. | ☐ | ☐ | ☐ | ☐ |
| • Sitting quietly after lunch without alcohol. | ☐ | ☐ | ☐ | ☐ |
| • In a car while stopped for a few minutes in traffic. | ☐ | ☐ | ☐ | ☐ |
| | | | | |
| 2. Approximately how many cups (250mL) of caffeinated beverages do you drink daily (coffee, tea, chocolate, soda like coca-cola, pepsi)? | | | | CUPS |
| | _____ | | | |
| | | | | |
| 3. What time do you typically drink your last cup of caffeinated beverage? | | | | AM / PM |

3. Beck's Depression Inventory

This depression inventory can be self-scored. The scoring scale is at the end of the questionnaire.

1.
 0. I do not feel sad.
 1. I feel sad
 2. I am sad all the time and I can't snap out of it.
 3. I am so sad and unhappy that I can't stand it.
2.
 4. I am not particularly discouraged about the future.
 5. I feel discouraged about the future.
 6. I feel I have nothing to look forward to.
 7. I feel the future is hopeless and that things cannot improve.
3.
 8. I do not feel like a failure.
 9. I feel I have failed more than the average person.
 10. As I look back on my life, all I can see is a lot of failures.
 11. I feel I am a complete failure as a person.
4.
 12. I get as much satisfaction out of things as I used to.
 13. I don't enjoy things the way I used to.
 14. I don't get real satisfaction out of anything anymore.
 15. I am dissatisfied or bored with everything.
5.
 16. I don't feel particularly guilty
 17. I feel guilty a good part of the time.
 18. I feel quite guilty most of the time.
 19. I feel guilty all of the time.
6.
 20. I don't feel I am being punished.
 21. I feel I may be punished.
 22. I expect to be punished.
 23. I feel I am being punished.
7.
 0. I don't feel disappointed in myself.
 1. I am disappointed in myself.
 2. I am disgusted with myself.
 3. I hate myself.
8.
 0. I don't feel I am any worse than anybody else.
 1. I am critical of myself for my weaknesses or mistakes.
 2. I blame myself all the time for my faults.
 3. I blame myself for everything bad that happens.
9.
 0. I don't have any thoughts of killing myself.
 1. I have thoughts of killing myself, but I would not carry them out.
 2. I would like to kill myself.

3. I would kill myself if I had the chance.
- 10.
0. I don't cry any more than usual.
 1. I cry more now than I used to.
 2. I cry all the time now.
 3. I used to be able to cry, but now I can't cry even though I want to.
- 11.
0. I am no more irritated by things than I ever was.
 1. I am slightly more irritated now than usual.
 2. I am quite annoyed or irritated a good deal of the time.
 3. I feel irritated all the time.
- 12.
0. I have not lost interest in other people.
 1. I am less interested in other people than I used to be.
 2. I have lost most of my interest in other people.
 3. I have lost all of my interest in other people.
- 13.
0. I make decisions about as well as I ever could.
 1. I put off making decisions more than I used to.
 2. I have greater difficulty in making decisions more than I used to.
 3. I can't make decisions at all anymore.
- 14.
0. I don't feel that I look any worse than I used to.
 1. I am worried that I am looking old or unattractive.
 2. I feel there are permanent changes in my appearance that make me look unattractive
 3. I believe that I look ugly.
- 15.
0. I can work about as well as before.
 1. It takes an extra effort to get started at doing something.
 2. I have to push myself very hard to do anything.
 3. I can't do any work at all.
- 16.
0. I can sleep as well as usual.
 1. I don't sleep as well as I used to.
 2. I wake up 1-2 hours earlier than usual and find it hard to get back to sleep.
 3. I wake up several hours earlier than I used to and cannot get back to sleep.
- 17.
0. I don't get more tired than usual.
 1. I get tired more easily than I used to.
 2. I get tired from doing almost anything.
 3. I am too tired to do anything.
- 18.
0. My appetite is no worse than usual.
 1. My appetite is not as good as it used to be.
 2. My appetite is much worse now.

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3. I have no appetite at all anymore.
- 19.
0. I haven't lost much weight, if any, lately.
1. I have lost more than five pounds.
2. I have lost more than ten pounds.
3. I have lost more than fifteen pounds.
- 20.
0. I am no more worried about my health than usual.
1. I am worried about physical problems like aches, pains, upset stomach, or constipation.
2. I am very worried about physical problems and it's hard to think of much else.
3. I am so worried about my physical problems that I cannot think of anything else.
- 21.
0. I have not noticed any recent change in my interest in sex.
1. I am less interested in sex than I used to be.
2. I have almost no interest in sex.
3. I have lost interest in sex completely.

INTERPRETING THE BECK DEPRESSION INVENTORY

Now that you have completed the questionnaire, add up the score for each of the twenty-one questions by counting the number to the right of each question you marked. The highest possible total for the whole test would be sixty-three. This would mean you circled number three on all twenty-one questions. Since the lowest possible score for each question is zero, the lowest possible score for the test would be zero. This would mean you circles zero on each question. You can evaluate your depression according to the Table below.

Total Score	Levels of Depression
1 – 10	These ups and downs are considered normal
11 – 16	Mild mood disturbance
17 – 20	Borderline clinical depression
21 – 30	Moderate depression
31 – 40	Severe depression
Over 40	Extreme depression

A PERSISTENT SCORE OF 17 OR ABOVE INDICATES THAT YOU MAY NEED
MEDICAL TREATMENT.

4. Ndlovu Baseline Questionnaire

Date of visit Initials Investigator

Participant Initials Participant ID **NCS**

BASELINE QUESTIONNAIRE**Demographics**

Q1	Gender	☐ Female ☐ Male
Q2	Date of Birth	DD/MM/YYYY: _____
Q3	Education	☐ None ☐ Primary school completed ☐ Secondary school completed ☐ Matric ☐ Technicon/ College ☐ University
Q4	Partnership status (Current situation; Please only select one)	☐ Married (since: _____ year) ☐ Life partner (since: _____ year) ☐ Living together >50% of the time (since: _____ year) ☐ Single (since: _____ year) ☐ Divorced (since: _____ year) ☐ Widowed (since: _____ year) ☐ Multiple partners (since: _____ year) ☐ Other (specify: _____)

Date of visit Initials Investigator

Participant Initials Participant ID **NCS**

Employment

- Q5** Are you currently employed?
- ☐ Yes, employed
 - ☐ Yes, self employed
 - ☐ No, unemployed →Go to Q9
 - ☐ No, student →Go to Q9
 - ☐ No, retired →Go to Q9
 - ☐ No, disabled →Go to Q9
 - ☐ No, other →Go to Q9
 - ☐ Specify: _____
 - ☐ Refused →Go to Q9
 - ☐ Don't know →Go to Q9

- Q6** In which economic sector do you work?
- ☐ Agriculture, fishing, forestry
 - ☐ Mining and quarrying
 - ☐ Manufacturing (e.g. clothing, food)
 - ☐ Electricity, gas, water
 - ☐ Construction
 - ☐ Wholesale/ retail
 - ☐ Catering and accommodation
 - ☐ Transport, storage and communication
 - ☐ Finance, real estate and business services
 - ☐ Public administration (local, provincial) government
 - ☐ Community, social and personal services
 - ☐ Security, police
 - ☐ Education
 - ☐ Private household
 - ☐ Other - Specify : _____
 - ☐ Don't know
 - ☐ Refused

- Q7** How many hours do you work at this job in a typical week? _____ Hours
- ☐ Refused
 - ☐ Don't know

IMMUNE ACTIVATION, SLEEP AND CIRCADIAN TIMING IN HIV PATIENTS

Date of visit Initials Investigator

Participant Initials Participant ID **NCS**

Q8 Would you say last month's take home pay was:	☐ Less than or equal to R600
	☐ R600- R1300
	☐ R1300 – R3100
	☐ R3100 – R5900
	☐ R5900 – R11 000
	☐ R11 000 – R18 000
	☐ More than R18 000
	☐ Refused
	☐ Don't know

Income composition

Q9 What are the sources of income for this household? (select all applicable)	☐ Salaries/wages/commission
	☐ Income from a business
	☐ Remittance (money received from people living elsewhere)
	☐ Pensions (Amount received per month: _____)
	☐ Grants (include old age grant here) (Amount received per month: _____)
	☐ Other income sources e.g. rental income, interest
☐ No income → Go to Q12	

Q10 What is the main source of income? (select only one)	☐ Salaries/wages/commission
	☐ Income from a business
	☐ Remittance (money received from people living elsewhere)
	☐ Pensions
	☐ Grants (include old age grant here)
	☐ Other income sources e.g. rental income, interest

Date of visit Initials Investigator

Participant Initials Participant ID **NCS**

Q11

Do you receive any of the listed financial support? (select all that apply)

- ☐ No
- ☐ Old-age grant (60-74, R1200; 75+, R1220)
- ☐ Disability grant (<60, R1200)
 - ☐ Permanent disability
 - ☐ Temporary disability
 - ☐ Don't know
- ☐ Child support grant (0-16, R280)
- ☐ Care dependency grant (0-17, R1200)
- ☐ Foster child grant (<22, R770)
- ☐ War veterans grant (60+, R1220)
- ☐ Grant-in-aid (R250 and should have another grant)
- ☐ Social relief of distress
- ☐ Refused
- ☐ Don't know

Household support

Q12 Who is the head of the household?

- ☐ Self
- ☐ Spouse/partner
- ☐ Parent
- ☐ Other, specify _____

Q13

Do other members of your household earn an income? (select those that are applicable)

- ☐ Partner (amount: _____ ☐ Don't know)
- ☐ Brother (amount: _____ ☐ Don't know)
- ☐ Sister (amount: _____ ☐ Don't know)
- ☐ Parent(s) (amount: _____ ☐ Don't know)
- ☐ Non relatives (amount: _____ ☐ Don't know)
- ☐ No
- ☐ Refuse
- ☐ Don't know

IMMUNE ACTIVATION, SLEEP AND CIRCADIAN TIMING IN HIV PATIENTS

Date of visit Initials Investigator

Participant Initials Participant ID **NCS**

Household composition

Q14 How many persons live under your roof? (excluding yourself) _____ Number

- Q15** How do these people relate to you?
- ☐ Partner
 - ☐ Biological children (number _____)
 - ☐ Non biological children (number _____)
 - ☐ Parent(s) (number _____)
 - ☐ Parent(s) in law (number _____)
 - ☐ Brothers/sisters (number _____)
 - ☐ Non-relatives (not non-biological children), (number _____)
 - ☐ I live alone
 - ☐ Refused
 - ☐ Don't know

- Q16** Have you ever had biological children? (please include those that have passed away)
- ☐ Yes, living with me (number _____)
 - ☐ Yes, not living with me (number _____)
 - ☐ Yes, but the child died (number _____)
 - ☐ No
 - ☐ Refused
 - ☐ Don't know

- Q17** Are you currently expecting a biological child (yourself or your partner)? *
- ☐ Yes (number _____)
 - ☐ No
 - ☐ Refused
 - ☐ Don't know

Date of visit Initials Investigator

Participant Initials Participant ID **NCS**
Mental Health Questionnaire

Over the last 2 weeks, how often have you been bothered by any of the following problems?

		Not at all	Several days	More than half the days	Nearly every day	Refuse
Q18	Little interest or pleasure in doing things	☐	☐	☐	☐	☐
Q19	Feeling down, depressed, or hopeless	☐	☐	☐	☐	☐
Q20	Trouble falling or staying asleep, or sleeping too much	☐	☐	☐	☐	☐
Q21	Feeling tired or having little energy	☐	☐	☐	☐	☐
Q22	Poor appetite or overeating	☐	☐	☐	☐	☐
Q23	Feeling bad about yourself — or that you are a failure or have let yourself or your family down	☐	☐	☐	☐	☐
Q24	Trouble concentrating on things, such as reading the newspaper or watching television	☐	☐	☐	☐	☐
Q25	Moving or speaking so slowly that other people could have noticed? Or the opposite — being so fidgety or restless that you have been moving around a lot more than usual	☐	☐	☐	☐	☐
Q26	Thoughts that you would be better off dead or of hurting yourself in some way	☐	☐	☐	☐	☐

Date of visit Initials Investigator

Participant Initials Participant ID **NCS**

HIV Testing

- Q27** What is your opinion of HIV testing:
- ☐ Getting tested for HIV helps people feel better
 - ☐ Getting tested for HIV helps people from getting HIV
 - ☐ People in my life would leave me if I had HIV
 - ☐ People who tested positive should hide it from others
 - ☐ I would rather not know if I have HIV
- Q28** Have you ever had an HIV test?
- ☐ Yes, within the past 6 months
 - ☐ Yes, more than 6 months ago
 - ☐ No
 - ☐ Refuse
- Q29** Did you tell anyone that you went to the clinic for HIV testing? (mark all applicable)
- ☐ Yes, I told it to my partner
 - ☐ Yes, I told it to my parent(s)
 - ☐ Yes, I told it to my friend(s)
 - ☐ Yes, I told it to my child
 - ☐ Yes, I told it to another person
 - ☐ No
 - ☐ Not applicable
- Q30** Did you tell anyone about the test result? (mark all applicable)
- ☐ Yes, I told it to my partner
 - ☐ Yes, I told it to my parent(s)
 - ☐ Yes, I told it to my friend(s)
 - ☐ Yes, I told it to my child
 - ☐ Yes, I told it to another person
 - ☐ No
 - ☐ Not applicable
- Q31** Did you go for HIV testing at your own initiative? (mark all applicable)
- ☐ Yes
 - ☐ No, I was stimulated/ enforced to go for testing by my partner
 - ☐ No, I was stimulated/ enforced to go for testing by my parent(s)
 - ☐ No, I was stimulated/ enforced to go for testing by my friend(s)
 - ☐ No, I was stimulated/ enforced to go for testing by my child
 - ☐ No, I was stimulated/ enforced to go for testing by another person
 - ☐ No, I did the test to be eligible to join the study

Date of visit Initials Investigator

Participant Initials Participant ID **NCS**

Q32 Was it likely that any of your family members was HIV infected at the time that you went for testing?*	☐ Definitely not ☐ Probably not ☐ Not sure ☐ Probably yes ☐ Definitely yes
Q33 I got HIV tested because:	☐ If I am infected, I want to know so that I will not infect others ☐ I was concerned that I might have been sexually exposed ☐ If I am infected I want to know so that I can get treatment ☐ I believe there is more hope with treatment these days ☐ I was concerned that I might have been exposed through blood or needles ☐ My sex partner wanted me to ☐ My friends wanted me to ☐ My family wanted me to ☐ I was in a research study ☐ I was in a drug treatment program

IMMUNE ACTIVATION, SLEEP AND CIRCADIAN TIMING IN HIV PATIENTS

Date of visit Initials Investigator

Participant Initials Participant ID **NCS**

STIGMA

What is your opinion on the following statements?		Never	Sometimes	Often	Always
Q34	People who have AIDS are dirty	☐	☐	☐	☐
Q35	People who have AIDS are cursed	☐	☐	☐	☐
Q36	People who have AIDS cannot be trusted	☐	☐	☐	☐
Q37	People who have AIDS are like everybody else	☐	☐	☐	☐
Q38	People who have AIDS should be ashamed	☐	☐	☐	☐
Q39	People who have AIDS have nothing to feel guilty about	☐	☐	☐	☐
Q40	Most people become HIV positive by being weak or foolish	☐	☐	☐	☐
Q41	It is safe for people who have AIDS to work with children	☐	☐	☐	☐
Q42	People who have AIDS must expect restrictions on their freedom	☐	☐	☐	☐
Q43	A person with AIDS must have done something wrong and deserves to be punished	☐	☐	☐	☐
Q44	People who have HIV should be isolated	☐	☐	☐	☐
Q45	People who have AIDS should not be allowed to work	☐	☐	☐	☐
Q46	Would you care for a family member with HIV/AIDS?	☐ Yes ☐ No			
Q47	Would you mind if people knew if your family member had HIV/AIDS	☐ Yes ☐ No			

IMMUNE ACTIVATION, SLEEP AND CIRCADIAN TIMING IN HIV PATIENTS

Date of visit Initials Investigator

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Sexual Partners in the last 12 months

Q48 How many sexual partners have you had in the last 12 months? _____ Number

Q49 What is your sexual partner's age compared to you?

☐ ≥5 years older

☐ ≥5 years younger

☐ About the same age

☐ Not applicable

Q50 How many sexual partners do you have at the same time? _____ Number

Q51 Do you ever have sex in exchange for money or goods?

☐ Never

☐ Sometimes

☐ Often

☐ Always

IMMUNE ACTIVATION, SLEEP AND CIRCADIAN TIMING IN HIV PATIENTS

Date of visit Initials Investigator

Participant Initials Participant ID **NCS**

Diet

Q52 Did your household run out of money to buy food during the past 12 months? ☐ Yes
☐ No → Go to Q59
☐ Don't know

Q53 Has it happened in the past 30 days? ☐ Yes
☐ No → Go to Q59
☐ Don't know

Q54 Has it happened 5 or more days in the past 30 days? ☐ Yes
☐ No
☐ Don't know

Q55 In the past 12 months, were there times when members of your household went hungry because there was not enough food in the house to eat? ☐ Yes
☐ No
☐ Don't know

Q56 Which were the months (in the last 12 months) in which you experienced a lack of food or money such that one or more members of your household had to go hungry?
☐ January
☐ February
☐ March
☐ April
☐ May
☐ June
☐ July
☐ August
☐ September
☐ October
☐ November
☐ December
☐ Not applicable
☐ Don't know

Q57 Did you cut the size of meals during the past 12 months because there was not enough food in the house? ☐ Yes
☐ No → Go to Q60
☐ Don't know

Q58 Has it happened 5 or more days in the past 30 days? ☐ Yes
☐ No
☐ Don't know

IMMUNE ACTIVATION, SLEEP AND CIRCADIAN TIMING IN HIV PATIENTS

Date of visit Initials Investigator

Participant Initials Participant ID **NCS**

Q59	Did you skip any meals during the past 12 months because there was not enough food in the house?	☐ Yes ☐ No → Go to Q62 ☐ Don't know
Q60	Has it happened 5 or more days in the past 30 days?	☐ Yes ☐ No ☐ Don't know
Q61	Did you eat smaller variety of foods during the past 12 months than you would have liked to, because there was not enough food in the house?	☐ Yes ☐ No → Go to Q64 ☐ Don't know
Q62	Has it happened 5 or more days in the past 30 days?	☐ Yes ☐ No ☐ Don't know
Q63	In a typical week, on how many days do you eat fruit?	_____ Days ☐ Don't know
Q64	How many servings of fruit do you eat on one of those days?	_____ Servings ☐ Don't know
Q65	In a typical week, on how many days do you eat vegetables?	_____ Days ☐ Don't know
Q66	How many servings of vegetables do you eat on one of those days?	_____ Servings ☐ Don't know

Physical activity

If you work, please answer all questions, if you are unemployed, please start from Q73

Q67	Does your work involve vigorous-intensity activity that causes large increases in breathing or heart rate like carrying or lifting heavy loads, digging or construction work for at least 10 minutes continuously?	☐ yes ☐ no
Q68	In a typical week, on how many days do you do vigorous-intensity activities as part of your work?	_____ Days
Q69	How much time do you spend doing vigorous-intensity activities at work on a typical day?	 Hours: Minutes
Q70	Does your work involve moderate-intensity activity that causes small increases in breathing or heart rate like brisk walking, carrying light loads, cleaning, cooking, washing clothes for at least 10 minutes continuously?	☐ yes ☐ no
Q71	In a typical week, on how many days do you do moderate-intensity activities as part of your work?	_____ Days

IMMUNE ACTIVATION, SLEEP AND CIRCADIAN TIMING IN HIV PATIENTS

Date of visit Initials Investigator

Participant Initials Participant ID **NCS**

Q72	How much time do you spend doing moderate-intensity activities at work on a typical day?	
		Hours: Minutes
Q73	Do you walk or use a bicycle for at least 10 minutes continuously to get to and from places?	☐ yes ☐ no → Go to Q77
Q74	In a typical week, how many times do you walk or cycle for at least 10 minutes continuously to get to and from places?	Days
Q75	How much time do you spend walking or cycling for travel on typical days?	
		Hours: Minutes
Q76	Do you do any vigorous-intensity sports, fitness or recreational (leisure) activities that cause large increases in breathing or heart rate like running or football for at least 10 minutes continuously?	☐ yes ☐ no → Go to Q80
Q77	In a typical week, how many times do you do vigorous-intensity sports, fitness or recreational (leisure) activities?	Days
Q78	How much time do you spend doing vigorous-intensity sports, fitness or recreational (leisure) activities on typical days?	
		Hours: Minutes
Q79	How much time do you spend sitting or reclining on typical days?	
		Hours: Minutes

Smoking habits

Q80	Have you ever smoked any tobacco product such as cigarettes, cigars or pipes?	☐ yes ☐ no → Go to Q86
Q81	How old were you when you first started smoking?	☐ years old ☐ unknown
Q82	Do you currently smoke any tobacco products such as cigarettes, cigars, or pipes?	☐ yes → Go to Q84 ☐ no
Q83	How long ago did you stop smoking? (Choose either years, months or weeks)	☐ Years ago → Go to Q85 ☐ Months ago → Go to Q85 ☐ Weeks ago
Q84	During the last 30 days, how frequently have you smoked your tobacco products	☐ Daily ☐ 5-6 days per week ☐ 1-4 days per week ☐ 1-3 per month

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Q85	On average how many of the following do you smoke each day?	☐ Manufactured cigarettes (amount _____)
		☐ Hand rolled cigarettes (amount _____)
		☐ Pipes full of tobacco (amount _____)
		☐ Cigars (amount _____)
		☐ Dagga/ marijuana (amount _____)
		☐ Other, please specify _____ (amount _____)

Alcohol consumption

Q86	Have you ever consumed an alcoholic drink such as beer, wine, spirits, fermented cider, <i>thothotho</i> or traditional beer?	☐ yes ☐ no → Go to Q92
Q87	Have you consumed an alcoholic drink within the past 12 months	☐ yes ☐ no
Q88	Have you consumed an alcoholic drink within the past 30 days?	☐ yes ☐ no → Go to Q92
Q89	During the past 30 days, how frequently have you had at least one alcoholic drink?	☐ Daily ☐ 5-6 days per week ☐ 1-4 days per week ☐ 1-3 days per month ☐ Don't know
Q90	During the past 7 days, how many drinks of any alcoholic beverage did you have each day?	☐ on Monday (amount _____) ☐ on Tuesday (amount _____) ☐ on Wednesday (amount _____) ☐ on Thursday (amount _____) ☐ on Friday (amount _____) ☐ on Saturday (amount _____) ☐ on Sunday (amount _____) ☐ Don't know
Q91	During the past 30 days, when you consumed an alcoholic drink, how often was it with meals? Do not count snacks	☐ Usually with meals ☐ Sometimes with meals ☐ Rarely with meals ☐ Never with meals

IMMUNE ACTIVATION, SLEEP AND CIRCADIAN TIMING IN HIV PATIENTS

Date of visit Initials Investigator

Participant Initials Participant ID **NCS**

HIV Status

- Q92** What is your HIV Status?
- ☐ Positive, on treatment
 - ☐ Positive, not on treatment
 - ☐ Negative → **END QUESTIONNAIRE**
 - ☐ I don't know → **END QUESTIONNAIRE**

- Q93** If you are HIV positive, do you often talk about being HIV positive?*
- ☐ Never
 - ☐ Sometimes
 - ☐ Regularly
 - ☐ Often
 - ☐ All the time
 - ☐ Not applicable

HIV Positive, on-treatment cohort ONLY - Adherence

How sure are you that:		Not at All	Somewhat	Very	Extremely
Q94	You will be able to take all or most of the medication as directed?	☐	☐	☐	☐
Q95	The medication will have a positive effect on your health	☐	☐	☐	☐
Q96	If you do not take this medication exactly as instructed, the HIV in your body will become resistant to HIV medication	☐	☐	☐	☐

The following questions asks about your social support

- Q97** In general, how satisfied are you with the overall support you get from your friends and family members?
- ☐ Very Dissatisfied
 - ☐ Somewhat Dissatisfied
 - ☐ Somewhat Satisfied
 - ☐ Very Satisfied
- Q98** To what extent do your friends or family members help you remember to take your medication?
- ☐ Not At All
 - ☐ A Little
 - ☐ Somewhat
 - ☐ A Lot
 - ☐ Not Applicable

IMMUNE ACTIVATION, SLEEP AND CIRCADIAN TIMING IN HIV PATIENTS

Date of visit Initials Investigator

Participant Initials Participant ID **NCS**

People may miss their medication for various reasons. Here is a list of possible reasons why you may have missed taking any medications within the **past month**.

In the past month, how often have you missed taking your medication because you:	Never	Rarely	Sometimes	Often
Q99 Were away from home?	☐	☐	☐	☐
Q100 Were busy with other things?	☐	☐	☐	☐
Q101 Simply forgot?	☐	☐	☐	☐
Q102 Had too many pills to take?	☐	☐	☐	☐
Q103 Wanted to avoid side effects?	☐	☐	☐	☐
Q104 Did not want other notice you taking medication?	☐	☐	☐	☐
Q105 Had a change in daily routine?	☐	☐	☐	☐
Q106 Felt like drug was toxic/harmful?	☐	☐	☐	☐
Q107 Fell asleep/slept through dose time?	☐	☐	☐	☐
Q108 Felt sick or ill?	☐	☐	☐	☐
Q109 Felt depressed/overwhelmed?	☐	☐	☐	☐
Q110 Had problem taking pills at a specified times (with meals, on empty stomach etc)?	☐	☐	☐	☐
Q111 Ran out of pills?	☐	☐	☐	☐
Q112 Felt good?	☐	☐	☐	☐

- Q113** When was the last time you missed taking any of your medications? Check one box.
- ☐ 5: within the past **week**
 - ☐ 4: 1-2 **weeks** ago
 - ☐ 3: 2-4 **weeks** ago
 - ☐ 2: 1-3 **months** ago
 - ☐ 1: More than 3 **months** ago
 - ☐ 0: **Never** skip medication or **not applicable**

****END QUESTIONNAIRE ****

5. Ndlovu Cohort Study Baseline Medical Assessment Form

A multidisciplinary longitudinal study comparing HIV positives and HIV negatives to investigate the epidemiology, pathogenesis and socio-scientific factors determining the interaction between HIV and (lifestyle-related) chronic conditions, with a special focus on cardiovascular disease

Date of visit -

Subject Initials -

Subject ID -

Physical Examination		
Q1	Weight	kg (to one decimal point)
Q2	Height	cm
Q3	BMI calculation	
Q4	Waist Circumference	cm (to one decimal point)
Q5	Hip Circumference	cm (to one decimal point)
Q6	Blood Pressure	/ mmHg
Q7	Pulse rate	/ min

General health		
Q8	Do you mind if I ask specific questions about your health?	☐ Yes ☐ No ☐ Refuse
Q9	How would you rate your health today?	☐ Very good ☐ Good ☐ Bad ☐ Very bad
Q10	Overall in the last 30 days, how much difficulty did you have with work or household activities?	☐ None ☐ Mild ☐ Severe ☐ Extreme
Q11	Are you covered by a medical aid or medical benefit scheme or private health insurance?	☐ Yes ☐ No ☐ Don't know ☐ Refuse
Q12	During the past month, did you suffer from any illnesses or injuries?	☐ Yes ☐ No → Go to Q14 ☐ Don't know ☐ Refuse
Q13	Did you consult a health worker such as a nurse,	☐ Yes → Go to Q15

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	doctor or traditional healer as a result of this illness or injury?	☐ No ☐ Don't know	
Q14	If "No" in Q12, what is the main reason, why did you not consult any health worker?	☐ Too expensive ☐ Too far ☐ Not necessary/the problem was not serious enough ☐ Self-medicated/treated myself ☐ Fear of stigmatization ☐ Don't know ☐ Other	
Q15	Have you been informed by a medical practitioner or nurse that you suffer from any of the following chronic illnesses or conditions? Asthma? Diabetes? Cancer? HIV? TB? Hypertension/high blood pressure? Arthritis? Other? (please specify in the box below)	☐ Yes ☐ Yes ☐ Yes ☐ Yes ☐ Yes ☐ Yes ☐ Yes	☐ No ☐ No ☐ No ☐ No ☐ No ☐ No ☐ No (if all "no" → Q 17)
Q16	If "yes" for question X, are you taking medication for the chronic illness(es)?	☐ Asthma? ☐ Diabetes? ☐ Cancer? ☐ HIV and AIDS? ☐ Hypertension/high blood pressure? ☐ Arthritis? ☐ Other? (please specify in the box below) ☐ Don't know	
Q17	Has one or both of your parents ever suffered from any of the following medical problems below the age of 60 years?	☐ Heart attack ☐ Stroke ☐ Angina / angina pectoris ☐ High blood pressure / hypertension ☐ Diabetes ☐ Other heart problems, please specify	
Q18	Has one or more of your siblings* ever suffered from any of the following medical problems below the age of 60 years? *sibling: brother or sister, having at least one parent in common	☐ Heart attack ☐ Stroke ☐ Angina / angina pectoris ☐ High blood pressure / hypertension ☐ Diabetes	

		☐ Other heart problems, please specify
	Do you have difficulty in doing any of the following?	
Q19	Seeing (even with glasses if you wear them)?	☐ No difficulty ☐ Some difficulty ☐ A lot of difficulty ☐ Unable to do ☐ Do not know ☐ Cannot yet be determined
Q20	Hearing (even with a hearing aid if you wear one)?	☐ No difficulty ☐ Some difficulty ☐ A lot of difficulty ☐ Unable to do ☐ Do not know ☐ Cannot yet be determined
Q21	Walking a kilometre or climbing a flight of steps?	☐ No difficulty ☐ Some difficulty ☐ A lot of difficulty ☐ Unable to do ☐ Do not know ☐ Cannot yet be determined
Q22	Remembering and concentrating?	☐ No difficulty ☐ Some difficulty ☐ A lot of difficulty ☐ Unable to do ☐ Do not know ☐ Cannot yet be determined
Q23	With self-care, such as washing or dressing?	☐ No difficulty ☐ Some difficulty ☐ A lot of difficulty ☐ Unable to do ☐ Do not know ☐ Cannot yet be determined
Q24	In communication in your usual language including sign language (understanding others and being understood by others)?	☐ No difficulty ☐ Some difficulty ☐ A lot of difficulty ☐ Unable to do ☐ Do not know ☐ Cannot yet be determined

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Relevant medical history	
Diagnosis	Stop date
	____/____/____ (MM/YYYY)
	____/____/____ (MM/YYYY)
	____/____/____ (MM/YYYY)
	____/____/____ (MM/YYYY)
	____/____/____ (MM/YYYY)

Current medical conditions	
Diagnosis	Diagnosed since
	____/____/____ (DD/MM/YYYY)
	____/____/____ (DD/MM/YYYY)
	____/____/____ (DD/MM/YYYY)
	____/____/____ (DD/MM/YYYY)
	____/____/____ (DD/MM/YYYY)

Chronic medication use (including contraceptives) , including herbal and traditional medication	
ART information is not collected here	
Name	Characteristics
	Start date: ____/____/____ (DD/MM/YYYY) Stop date: ____/____/____ (DD/MM/YYYY) Dosage: Frequency: Route:
	Start date: ____/____/____ (DD/MM/YYYY) Stop date: ____/____/____ (DD/MM/YYYY) Dosage: Frequency: Route:
	Start date: ____/____/____ (DD/MM/YYYY) Stop date: ____/____/____ (DD/MM/YYYY) Dosage: Frequency: Route:
	Start date: ____/____/____ (DD/MM/YYYY) Stop date: ____/____/____ (DD/MM/YYYY) Dosage: Frequency: Route:
	Start date: ____/____/____ (DD/MM/YYYY) Stop date: ____/____/____ (DD/MM/YYYY) Dosage: Frequency: Route:
	Start date: ____/____/____ (DD/MM/YYYY) Stop date: ____/____/____ (DD/MM/YYYY) Dosage: Frequency: Route:

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Laboratory		
Were blood samples taken?	☐ EDTA (2 tubes) ☐ SST (1 tube) ☐ Grey top (1 tube) ☐ Heparin (1 tube) ☐ Other: ☐ no	____/____/____ (DD/MM/YYYY) ____/____/____ (DD/MM/YYYY) ____/____/____ (DD/MM/YYYY) ____/____/____ (DD/MM/YYYY)
Was a urine sample taken?	☐ yes ☐ no	
Full blood count (FBC)	☐ yes ☐ no	
CD4 (HIV positives only)	☐ yes ☐ no ☐ not applicable	
Viral Load (HIV positives only)	☐ yes ☐ no ☐ not applicable	
Total Cholesterol requested	☐ yes ☐ no	
LDL Cholesterol requested	☐ yes ☐ no	
HDL-cholesterol requested	☐ yes ☐ no	
Triglycerides requested	☐ yes ☐ no	
CRP requested	☐ yes ☐ no	
HbA1c	☐ yes ☐ no	
Blood glucose requested	☐ yes ☐ no	
Urine creatinine and micro albumin	☐ yes ☐ no	

Tuberculosis questionnaire			
Please indicate if you had any of the following problems for 24hrs, two weeks or more.			
Symptoms	Yes	No	Symptom Score*
Cough >2 weeks	☐	☐	1
Cough >24hrs (If HIV Positive)	☐	☐	2
Loss of weight	☐	☐	3
Fever	☐	☐	4
Night sweats	☐	☐	5
Sputum production / Blood streaked sputum	☐	☐	6
Chest pain	☐	☐	7
Shortness of breath	☐	☐	8

* NOTE: Use codes in the referral card (criteria)

**IF ONE OR MORE RESPONSES TO THE ABOVE QUESTIONS IS "YES" SUSPECT TB AND INVESTIGATE FOR TB
(COLLECT SPUTUM FOR TB MICROSCOPY)**

Complementary Questions		
Have you been treated for TB before?	☐ Yes	☐ No
When were you treated for TB?	____/____/____ (DD/MM/YYYY)	
Was your TB treatment completed?	☐ Yes	☐ No
Have you been in contact with someone diagnosed with TB in the past year, e.g. Same household/school/work?	☐ Yes	☐ No

Only for HIV positives: HIV-Status & antiretroviral therapy		
Date of HCT/HIV-diagnosis	____/____/____ (DD/MM/YYYY)	
Laboratory history		
Date latest CD4-count	____/____/____ (DD/MM/YYYY) ☐ Unknown	
Result latest CD4-count	_____ cells/mm ³	
Date latest HIV-viral load	____/____/____ (DD/MM/YYYY)	
Result latest HIV-viral load	____/____/____ (DD/MM/YYYY)	
On cART	☐ Yes ☐ No	
Date of Start 1 st line Art	____/____/____ (DD/MM/YYYY)	
1st line ART-regimen		
Group	Code	Medicine
NRTI		
	11	☐ Lamivudine/3TC
	12	☐ Zidovudine AZT/ZDV
	13	☐ Stavudine/d4T
	14	☐ Didanosine/ddl
	15	☐ Emtricitabine /FTC
	16	☐ Tenofovir/TDF
	17	☐ Abacavir/ABC
NNRTI		
	21	☐ Efavirenz/EFV
	22	☐ Nevirapine/NVP
PI		
	31	☐ Lopinavir/ritonavir/DRV/r
	32	☐ Atazanavir/ritonavir/DRV/r
	33	☐ Darunavir/ritonavir/DRV/r
	34	☐ Fosamprenavir/ritonavir/FPV/r

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35	☐ Saquinavir/ritonavir/SQV/r
Start 2 nd line ART	☐ Yes ☐ No
Date of Start 2 nd line ART	____/____/____ (DD/MM/YYYY)
1 st line failure reason for switch	☐ Yes ☐ No
HIV-viral load at switch to 2 nd line	
Date of HIV-viral load switch to 2 nd line	____/____/____ (DD/MM/YYYY)
Dry Blood Spot Genotype Resistance testing performed	☐ Yes ☐ No
2 nd line ART-regimen (if applicable)	
Group	Code Medicine
NRTI	
11	☐ Lamivudine/3TC
12	☐ Zidovudine AZT/ZDV
13	☐ Stavudine/d4T
14	☐ Didanosine/ddI
15	☐ Emtricitabine /FTC
16	☐ Tenofovir/TDF
17	☐ Abacavir/ABC
NNRTI	
21	☐ Efavirenz/EFV
22	☐ Nevirapine/NVP
PI	
31	☐ Lopinavir/ritonavir/DRV/r
32	☐ Atazanavir/ritonavir/DRV/r
33	☐ Darunavir/ritonavir/DRV/r
34	☐ Fosamprenavir/ritonavir/FPV/r
35	☐ Saquinavir/ritonavir/SQV/r

APPENDIX C

1. Univariate analysis:
 - 1.1. **Table 1.1.** Effect of individual variables on log(CRP) – bivariate analysis.
 - 1.2. Variable selection
2. Multivariate analysis:
 - 2.1. **Table 2.1.** Multivariable linear regression results: All variables associated with log(CRP) in PLWH, including SBP and smoking.

1.1. Univariate analyses:

Table 1.1. Effect of individual variables on log(CRP) – bivariate analysis

	β-estimate	Standard Error	t-value	p-value
<i>Demographics</i>				
Biological sex	-0,091	0,021	-4,37	<0,001
Age (years)	0,008	0,001	10,52	<0,001
Sleep complaints (SD)	0,113	0,029	3,86	0,001
HIV status	0,207	0,020	10,13	<0,001
Smoking (ever)	-0,051	0,021	-2,38	0,017
<i>Blood analyses</i>				
Glucose (mmol/L)	0,023	0,005	4,74	<0,001
LDL cholesterol (mmol/L)	0,058	0,012	4,65	<0,001
HDL cholesterol (mmol/L)	-0,139	0,027	-5,19	<0,001
Triglycerides (mmol/L)	0,046	0,014	3,33	<0,001
<i>Anthropometric measurements</i>				
Body Mass Index (kg/m ²)	0,019	0,002	11,52	<0,001
Waist circumference (cm)	0,010	0,001	13,02	<0,001
Systolic blood pressure (mmHg)	0,001	0,0005	2,64	0,008
Diastolic blood pressure (mmHg)	0,003	0,001	4,13	<0,001
<i>Questionnaire results</i>				
Depression score (PHQ2)	0,029	0,007	4,03	<0,001
IPAQ category	-0,099	0,013	-7,55	<0,001

IPAQ = International Physical Activity Questionnaire, SDs = Sleep Complaints, PHQ – Patient Health Questionnaire.

1.2. Methods for variable selection (similar to the format used in Chapter 2)

For all univariate analyses, where the association tested between log(CRP) and the variable yielded a p-value less than 0.2, we included the variables in a multivariate linear regression. The rationale for choosing a p value of 0.2 as a threshold to include in the general model was to ensure we did not exclude any potential confounding effects between covariates of interest. In addition, we had planned to adjust for age and sex regardless of the univariate analysis's p values. This model is referred to as the 'kitchen sink' model, from which we started our variables' selection.

To select variables for our model from the initial 'kitchen sink' model, we used a 1) backward elimination (with a p-value for the selection to stay set at 0.1), 2) a forward selection (with a p-value for the selection for entry set at 0.1 as well) and 3) the Mallow's $C(p)$ analysis (determined by the first $C(p)$ which met the $C(p) < (\text{number of variables} + 1)$ criteria). From the forward, backward and $C(p)$ variable selections, we then tested non-significant covariates for collinearity and removed them if necessary from the model, keeping the significant covariates in the model. If significant univariate covariates had been stripped out of the backward/ forward/Mallow's $C(p)$ variables selection, we tested them again by adding them back in one by one. Finally, we tested for specific interactions between the main factors.

2. Multivariate Analyses

2.1. Table 2.1. Multivariable linear regression results: All variables associated with log(CRP) in PLWH, including SBP and smoking.

Parameter	Estimate	Standard Error	t Value	Pr > t
Sleep complaints	-0.013	0.040	-0.33	0.739
HIV	0.077	0.028	2.70	0.007
Age	0.005	0.001	5.07	<0.001
Depression	0.007	0.008	0.94	0.350
Sex (female)	-0.059	0.032	-1.86	0.063
BMI	0.014	0.003	4.96	<0.001
SBP	-0.001	0.001	-1.90	0.058
Smoking (ever)	0.014	0.024	0.57	0.569
Waist circumference	0.003	0.001	2.84	0.005
Triglycerides	-0.056	0.015	-3.82	<0.001
HDL cholesterol	-0.056	0.015	-3.79	<0.001
LDL cholesterol	0.000	0.012	0.01	0.996
Glucose	0.017	0.005	3.53	<0.001
Physical activity (IPAQ)	-0.048	0.013	-3.53	<0.001
Interaction between HIV and Sleep complaints (HIV+, SDs+ vs. all others) *	0.133	0.055	2.41	0.016
Interaction between HIV and Sex (male and HIV+ compared to others) **	0.210	0.042	4.99	<0.001

IPAQ = International Physical Activity Questionnaire, BMI = Body Mass Index, SDs = Sleep Complaints, SBP = Systolic Blood Pressure

Adjusted R-square for this model – 0.1991