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The role of obstructive sleep apnoea on chronic inflammation and cardiometabolic risk in people living with HIV

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Ezintsha

I, Johanné Marais, declare that this Dissertation is my own, unaided work.

It is being submitted for the Degree of Master of Science (Medicine) in Physiology at the Faculty of Health Sciences,

University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University

Signature: 

Signed on this 17th Day of June 2025 in Johannesburg

Presentations arising from this research:

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Abstract:

Obstructive sleep apnoea (OSA) is a proinflammatory respiratory disorder characterised by interruptions to breathing during sleep. OSA pathophysiology potentiates cardiometabolic risk (CMR) factors. Cohort follow-up studies support that with early OSA detection and intervention, CMR variables like hypertension status, circulating cholesterol levels, and certain peripheral markers of systemic inflammation can be reduced. Despite often showing more favourable cardiometabolic risk profiles using traditional risk factors of CMR, more people living with HIV (PLWH) develop cardiovascular disease and at a younger age than people without HIV. OSA prevalence in the general population can be up to 30%, yet there are no data available on OSA prevalence for PLWH in South Africa, nor whether OSA prevalence is associated with increased traditional (hypertension, insulin resistance, dyslipidaemia) and non-traditional (systemic and endothelial inflammation) CMR factors.

Aim: This project intended to characterise the prevalence of high risk of OSA using the Berlin Questionnaire in South African people living with HIV, and to investigate the association between high risk OSA and CMR using traditional CMR factors and inflammatory markers.

Methods: This is a cross-sectional, observational study of a well-characterised cohort of antiretroviral treatment-experienced people living with HIV residing in Johannesburg. Between September 2021 and November 2022, 210 participants previously enrolled in ADVANCE were recruited into the CHARACTERISE trial. Participants were excluded if pregnant or unable to complete the Berlin Questionnaire (BQ), a screening tool to detect OSA validated in “Global North” populations. All participants had been transitioned onto dolutegravir-based ART at least two years before recruitment. We measured blood pressure, body mass index, waist circumference and assayed blood for fasting lipid profiles and glucose concentration. A CMR score was calculated as a composite of calculated z-scores of waist circumference (WC), fasting glucose, high-density lipoprotein cholesterol (HDL-C), triglycerides, and mean arterial blood pressure. OSA risk was evaluated using the BQ. In 95 participants (24 with high risk OSA), we then assayed stored plasma for markers of systemic inflammation (IL-1 beta, IL-6, IL-8, TNF alpha) and endothelial activation (ESM, MCP1).

Results: In a relatively young (mean age (SD) = 38.4 years (± 7.5), 62% female) cohort of PLWH, 43 (20.5%) scored high risk of OSA. All participants self-identified with Black African ancestry, and 68% were employed at the time of enrolment. Three-quarters of the participants had hypertension or obesity (75%). Median CMR score ($p = 0.0024$; 95% CI: 0.090 - 0.437), body mass index ($p < 0.0001$; 95% CI: 2.318 - 7.708), WC ($p < 0.001$; 95% CI: 3.446 - 12.062), and triglyceride concentration ($p = 0.0385$; 95% CI: -0.059 - 0.272) were greater in patients at high risk of OSA than those at low risk for OSA. Five of the six markers of inflammation and endothelial activation were detectable in all participants for whom we had plasma (TNF alpha only in 35 participants, including 11 with high risk OSA). We found no significant difference between inflammation/endothelial activation in the high risk and low risk OSA groups.

Conclusion: Individuals with high risk OSA had significantly higher cardiometabolic risk scores using traditional markers of CMR. No difference in systemic inflammation nor endothelial activation was found. However, the cohort-wide obesity and hypertension prevalence, and overall detectable rates of inflammation are concerning for the growing contribution of cardiometabolic diseases to mortality of PLWH in South Africa. A case can be made for the necessity of point-of-care OSA screening in HIV primary care to manage progression of cardiometabolic diseases. These findings further impress upon the potentials of monitoring impaired sleep quality from ART side-effects as a public health imperative.

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ART – antiretroviral therapy

ARV – antiretroviral

BQ – Berlin Questionnaire

CMD – cardiometabolic disease

CMR – cardiometabolic risk

DBP – diastolic blood pressure

DTG – dolutegravir

GLU – glucose

HDL-c – high-density lipoprotein cholesterol

HIV – human immunodeficiency virus

LDL-c – low-density lipoprotein cholesterol

MAP – mean arterial pressure

OSA – obstructive sleep apnoea

PLWH – people living with HIV

SBP – systolic blood pressure

SNS – sympathetic nervous system

TGL - triglyceride

ROS – reactive oxygen species

WC – waist circumference

1 INTRODUCTION

South Africa has the largest population of people living with HIV globally, with approximately 8.45 million individuals estimated to be living with HIV in 2022; 5.7 million of whom are on antiretroviral therapy (ART) (Statistics South Africa, 2022). This translates to around 14% of the South African population to be living with HIV (Zuma et al., 2022), with around 86% estimated to be virally suppressed taking ART (Pillay et al., 2020). Before the widespread use of ART, serious AIDS- defining illnesses such as tuberculosis, pneumonia and diarrhoea were leading causes of mortality among people living with HIV in South Africa (Gona et al., 2020). Now, however, with the remarkable success of ART, HIV is a manageable chronic disease, where people living with HIV are expected to have similar lifespans (Payne et al., 2022) though they may experience up to 16 years fewer years in good health compared to persons without HIV (Marcus et al., 2020).

Chronic complications observed among people ageing on ART have since become an important focal point in HIV care (Chandiwana et al., 2023). Cardiometabolic diseases (CMD) such as type diabetes mellitus and hypertension contribute to nearly one-fifth of death in the South African population (World Heart Federation, 2019). Between 2008 and 2018, diabetes-specific mortality rates increased by over 30% (Statistics South Africa, 2022). The World Obesity Atlas suggests that the portion of the South African population with a high body mass index is increasing annually by 2.3 % (World Obesity Federation, 2024).

Another important example of the growth of CMD prevalence is the rise of stroke incidence by at least 5% in South Africa over 20 years (Owolabi et al., 2015). People living with HIV are at an increased risk of cardiovascular disease compared to people without HIV (Triant, 2013). Specific early indicators of cardiometabolic risk (CMR) like obesity, high systolic blood pressure and dyslipidaemia are most often progressive (Roth et al., 2020), yet hypertension and dyslipidaemia remain underdiagnosed and should therefore be strong considerations of the national health services; health policies; and treatment implementation plan for treating people living with HIV (South African National AIDS Council, 2023).

There is considerable evidence that PLWH are at a higher risk for myocardial infarction (Eyawo et al., 2019), where the mechanism is closely linked to various indicators of increased inflammation. One sample of 55 PLWH with first-time myocardial infarction - in a nested case-controlled study - presented with higher concentrations of IL-1 activation markers like IL-1 receptor antagonist (IL-1Ra) than PLWH who did not have cardiovascular disease (Hoel et al., 2020). Another study suggests that inflammatory indicators like higher lipopolysaccharide-binding protein and C-Reactive Protein (CRP) are associated with future myocardial infarctions among PLWH (Trevillyan et al., 2020). Finally, alterations to indicators of endothelial function and platelet function were noted in PLWH who have a greater risk for cardiovascular disease (Gresele et al., 2012). The underlying aggravation of inflammatory marker concentrations commonly reported in cases of cardiovascular disease in PLWH clearly suggests that inflammation is closely linked with higher CMD risk.

Polanka and colleagues found an association between sleep disturbances at baseline and higher risk of developing a cardiovascular eventamong PLWH(Polanka et al., 2019). This type of associated risk between cardiovascular disease and sleep disorders which worsen cardiometabolic health outcomes need to be further investigated in PLWH.

Obstructive sleep apnoea (OSA) is a pro-inflammatory respiratory sleep disorder independently associated with higher cardiometabolic risk . The contribution of OSA, the most common sleep disorder, to the cardiometabolic risk in people living with HIV who are taking ART requires further investigation.

The dissertation will elaborate on some of the intricacies of the cardiometabolic risk of PLWH, specifically through a lens of OSA risk and consequences. The literature review first texamines the role of ART in CMR progression, what associations can be made between CMR risk of PLWH and inflammatory profiles that may be connected to a risk for obstructive sleep apnoea. Attention is then drawn to the novelty of clinical sleep research in South Africa, and why this may be important for primary health care services relating to cardiometabolic- and HIV-related pandemics in South Africa.

Some proposed mechanisms of OSA-associated immune activation and chronic inflammation are expanded upon in this work, to emphasize how biochemical and clinical indicators of CMD among PLWH may additionally be indicators for the presence of a relative risk for OSA. Through these focal points, a foundational case will be built for the necessity of screening for obstructive sleep apnoea in primary HIV-care. This project dissertation investigates the impact of OSA risk by self-reported questionnaire data on constructed, continuous cardiometabolic risk scores in a cohort of people living with HIV in Johannesburg, South Africa.

2 Literature Review

2.1 HIV has become a chronic disease associated with higher cardiometabolic risk (CMR)

Modern ART has dramatically changed the treatment landscape in South Africa and around the world over the last 20 years (Fauci and Lane, 2020; Vella et al., 2012). People living with HIV and taking ART are now reported to have similar life expectancy to those who do not have HIV. Pooled data from 6 cohorts in South Africa showed that patients with HIV had life expectancies around 80% of people living without HIV's life expectancy (Johnson et al., 2013), though in more recent years the life expectancy of virally-suppressed PLWH is comparable to people without HIV (Payne et al., 2022). Throughout the years modern ART has evolved to once-a-day, easy-to-take regimens. By 2019, dolutegravir – a second-generation integrase strand transfer inhibitor which was a World Health Organization-recommended first line ART option – was prescribed in combination with other antiretroviral (ARV) medications (Kandel & Walmsley, 2015) in South Africa. Some of its many advantages included rapid and sustained viral suppression; for example, a suppression rate of 91.3% in a sample of 7117 PLWH who are treatment-experienced in Cameroon (Fokam et al., 2023). The national transition to dolutegravir -based ART has been well described in some local South African clinical trials, namely ADVANCE (Venter et al., 2020) and CHARACTERISE (Bosch et al., 2022) - the latter study is where this masters project is nested in.

2.1.1 Higher CMR in South African PLWH may be mediated through an increase in traditional risk factors (obesity, dyslipidaemia, insulin resistance)

The cardiometabolic class of diseases (CMD) includes coronary heart disease, stroke, chronic hypertension, atherosclerosis, and metabolic syndrome (Kirk & Klein, 2009). Evidence from the past decade suggests that PLWH are more affected by CMD than people without HIV. In the next paragraphs, we will examine evidence of higher CMD risk in PLWH and how the ART-associated pathophysiology for increased CMD in PLWH has been progressively moving from dyslipidaemic profiles induced by stavudine and protease inhibitors to a more classic weight gain observed with the new integrase strand transfer inhibitors.

i- HIV has become a chronic disorder, with PLWH having higher CVD risk than matched HIV negative people.

For people living with HIV, the historical presentation – prior to or with early ART – of pathophysiology symptoms associated with HIV itself included muscular dystrophy, viral or bacterial co-infections, skin lesions, weight loss/wasting, and fatigue (Justiz & Gulick, 2022).

The success of modern antiretroviral therapy (ART) has since prolonged life expectancy and improved the quality of life of PLWH. Despite these improvements, the rising cardiometabolic disease (CMD) variables presenting in PLWH suggest a changing phenotype of HIV-specific symptoms and side-effects, as supported by numerous studies which have impressed upon the high risk of CMD for PLWH (Alonso et al., 2019; Feinstein et al., 2019; Triant, 2013).

A large meta-analysis of twenty studies published before 2010 showed that PLWH on ART were twice as likely to present with cardiovascular disease than people living without HIV (Islam et al., 2012). This is further supported by later studies which similarly suggest that people living with HIV have a doubled risk than the general population of cardiovascular disease (Shah et al., 2018).

ii- HIV-related CMR has been mediated by older antiretroviral medications

Before the phasing out of older antiretroviral medications (stavudine and protease inhibitors), and as early as the year 2000 (Max & Sherer, 2000) there was evidence

that the body shape changed on ART, and this was associated with cardiometabolic diseases. Notable changes included what is termed lipodystrophy, or the abnormal distribution of fat tissues in the body where excess weight typically accumulates in areas like the abdomen, upper back (sometimes referred to as a "buffalo hump"), and breasts. Lipodystrophy can also encompass metabolic changes, including insulin resistance, dyslipidaemia, and an increased risk of cardiovascular diseases (Rossouw et al., 2013). A 2024 review (Capeau et al., 2024) has ascribed lipodystrophies to previous ART regimens (stavudine, protease inhibitors (Molina-Flores et al., 2024)) but not current ones (tenofovir, efavirenz, lamivudine, or dolutegravir).

iii- Current INSTI-based ART regimens and weight gain

A new class of ART (integrase strand transfer inhibitor – INSTI, including dolutegravir) was developed and South African national standards for ART shifted to dolutegravir-based ART in 2017. Dolutegravir-associated weight gain has been widely reported (Bakal et al., 2018; Bourgi et al., 2019; Venter et al, 2019; Brennan et al., 2023; Koethe et al., 2016; Menard et al., 2017; Rizzardo et al., 2019). Specific to a South African context, the landmark South African ADVANCE study, a 192-week randomized clinical trial, documented substantial weight gain in PLWH treated with dolutegravir-based ART regimens (Sokhela et al., 2024; Venter et al., 2020). Over the course of 192 weeks, patients in the dolutegravir-based ART groups (TAF/FTC + DTG arm) experienced the highest mean weight gain of 8.9 kg, followed by 5.9 kg (in the TDF/FTC + DTG group). The greatest increases were observed among women, individuals on TAF, and those with lower baseline CD4 counts (Sokhela et al., 2024). Further, a study comparing a Ugandan and South African sample of PLWH taking dolutegravir showed that weight gain was observed in the South African population but not in the Ugandan population, suggesting that there is some developing relationship between weight gain on dolutegravir and the South African contexts (Migisha et al., 2024).

Brennan and colleagues conducted another prospective cohort study in Johannesburg (Brennan et al., 2023) and noted an increased mean higher inrease in weight of 1.78kg across 12 months, observed for over 794 patients who initiated dolutegravir, when compared to 794 patients who remained on efavirenz; they also observed a 14.2 % increase in the risk of hypertension in the group which switched to

dolutegravir compared to those who stayed on efavirenz. Despite the internationally documented evidence for weight gain of PLWH who take INSTI-based ART (from above, respective countries include Brazil, USA, two South African studies, Canada, France and Italy) there is underwhelming emphasis on the importance of screening for cardiometabolic risk among PLWH who take INSTIs.

iv- Mechanisms for weight gain in the INSTI-based ART regimen

The underlying mechanism for excessive weight gain and increased obesity in people living with HIV on ART remains not well understood, though weight gain on ART has been reported more frequently for women, Black patients, and those who had advanced HIV disease when they started ART (Sax et al., 2019). Interestingly, another recent study has suggested that weight gain after switch to dolutegravir seems to be context dependent since PLWH in Uganda did not gain weight while those in South African gained weight (Migisha et al., 2024).

v- Cardiometabolic risk in PLWH

The drivers of CMD in people living with HIV, while not fully understood, are thought to be the same as in the general population. Those would include a sedentary lifestyle; increased blood pressure; having elevated blood lipids and cholesterol (Roth et al., 2020); showing elevated blood glucose levels or being insulin resistant; and importantly a systemic state of chronic inflammation (Luyster et al., 2014).

Taken together, it is important to recognize that the literature (though predominantly produced in “Global North” studies) suggests a potential role of ART in aggravating the cardiometabolic disease risk profile of PLWH. The mechanism through which this occurs remains uncertain, and points to an absence of relevant data from “Global South” contexts.

2.1.2 Higher CMR in PLWH may also be mediated by chronic inflammation

Higher risk of CMD in PLWH may also be caused in part by the chronic inflammatory state of the body with persistent immune activation seen in HIV (Feinstein et al., 2019; Nou, Lo and Grinspoon, 2016). The Ndlovu Cohort study, based in rural Limpopo, South Africa, recorded carotid-intima medial thickness (CIMT) (a marker of atherosclerotic disease) in participants with and without HIV (n=1080 HIV- and n=887 PLWH) (Vos et al., 2020). There was a significant interaction between HIV status and age whereby being HIV positive on treatment (majority on first line tenofovir,

emtricitabine and efavirenz ART) and between 30 and 49 years old was associated with a CIMT 0.015 cm thicker than in the HIV negative participants while those aged more than 49 had a 0.050 cm thicker than the HIV negative participants in adjusted multivariable analyses. While PLWH had lower traditional markers of cardiometabolic risk (lower glucose, LDL, triglycerides, blood pressure), C reactive protein (CRP) concentration, a marker of inflammation. was significantly higher in all HIV groups (naïve, treated on first line ART and treated on second line ART) compared to people without HIV. This increased cardiovascular risk among PLWH who take ART is of concern as they are also aging and gaining weight (van Ginkel et al., 2024).

2.1.3 Metabolic syndrome and obesity phenotypes among people living with HIV

Metabolic syndrome describes a collection of clinical presentations that place an individual at greater risk for cardiometabolic diseases and disorders (Grundy et al., 2005). Typically, this is categorized from a set of criteria rather than measured as a continuous score; three or more of five criteria must be met for metabolic syndrome to be reported. These criteria are: triglycerides ≥ 1.7 mmol.L^{-1} or; HDL-C < 1.03 mmol.L^{-1} for men and < 1.3 mmol.L^{-1} for women; fasting glucose ≥ 5.5 mmol.L^{-1}; WC ≥ 102 cm for men and ≥ 88 cm for women; and SBP ≥ 130mmHg or DBP ≥ 85mmHg or when the participant reported current use of medication to treat hypertension (Grundy et al., 2005).

Although metabolic syndrome surfaces in earlier research among PLWH who have a supposedly high risk for cardiometabolic disease, what this categorization does not capture is the critical distinctions between various weight distributions on the body for the risk of CMD – called obesity phenotypes (Swartz et al., 2024). Swartz and colleagues build a strong case for understanding CMR by looking at obesity phenotypes among PLWH, where discrete biochemical indicators of metabolic syndrome may be absent.

In total, PLWH have higher CMR, recently mediated by weight gain and chronic inflammation under ART treatment. Weight gain has also been associated with higher risk of having obstructive sleep apnoea (OSA), which constitutes an independent additional risk factor for cardiometabolic disease. In the following section, we will explore how during sleep apnoea, the multiple awakenings, and hypoxia events lead

to SNS activation and chronic inflammation, leading to hypertension and endothelial stress, and thus contribute to higher cardiometabolic risk..

2.2 Obstructive sleep Apnoea: pathophysiology and association with CMR

2.2.1 Pathophysiology of OSA

Obstructive sleep apnoea (OSA) is a respiratory sleep disorder with systemic, pro-inflammatory consequences which are associated with increased risk for cardiometabolic system pathologies (Arnaud et al., 2020). Sleep is a state of decreased metabolic functions, notably dominated by the parasympathetic nervous system regulation, which leads to lower respiratory rate, heart rate, lower blood pressure, lower muscle tone and decrease in core body temperature. This toning down of metabolic functions results in a dipping of blood pressure during a normal sleep episode. Higher CVD has been associated with the absence of periods of lowered nocturnal blood pressure (Fagard et al., 2009)

During OSA, due to the supine posture which allows gravity to pull down tissues around the upper airways and due to the sleep-associated lower muscle tone, short-term collapses of upper airways happen, leading to a partial (hypopnea) or complete (apnoea) closing of the upper airways. The severity of these partial or complete "obstructive" events is measured by an apnoea/hypopnea index (AHI) (Berry et al., 2012). Mild hypopnea (partial obstruction) or apnoea (complete obstruction) is defined by the presence of five to 14 ten-second-long obstructive events per hour of sleep. Moderate OSA is defined by an apnoea/hypopnoea index (AHI) of comprised between 15 and 30 and severe OSA by an AHI ≥ 30 events.h^{-1}. Those events are associated with hypoxia and respiratory efforts to open the airways, which result in multiple arousals. Both hypoxia and frequent arousals result in sympathetic nervous system activation while hypoxia results also in endothelial stress due to higher presence of free radicals.

Traditional risk factors for developing OSA – those traditionally observed in studies of the higher income countries - include male sex, having obesity, and being of older age (Young, 2004). Treatment of OSA using continuous positive airway pressure (CPAP, a mask fitted to the nose and mouth connected to a device which maintains positive pressures in the airways to avoid upper airway collapse) is usually initiated from AHI ≥ 15 (Epstein et al., 2009).

The pathophysiology of OSA is marked most notably by sleep disruption and intermittent hypoxia (IH) – the negative impacts of IH extend to most organ systems of the body (Lv et al.,

2023). Extensive review of available pathophysiology literature by Lv and colleagues as well as a publication over a decade earlier (Dempsey et al., 2010) supports how repeated episodes of reduced flow of oxygen to the body impairs hormone production and regulation (higher ghrelin and lower leptin, leading to higher food intake), higher immune activation, increased risk of developing hypertension and insulin resistance.

However, despite considerable research unpacking the pathophysiological impact of OSA, the specific mechanism that causes sleep apnoea remains uncertain. Some specific risk variables that researchers can associate with sleep apnoea progression include distribution of fat around the neck; having dyslipidaemia, diabetes mellitus or other pre-existing metabolic disorders; and previous or concurrent cardiovascular events such as stroke or atherosclerosis (Slowik & Collen, 2022). Those risks associated with OSA however can either result or cause OSA, or both. In children, the most frequent cause of OSA is adenotonsillar hypertrophy (Kim et al, 2009).

Epstein et al. (1995) have shown the direct implication of adenotonsillar hypertrophy (local upper airways inflammation) rather than obesity as a mechanism for OSA in PLWH. It has thus been suggested that even without obesity, PLWH may be at higher risk of having OSA due to higher upper airway inflammation. One more recent study has attempted to revisit this mechanism of OSA in PLWH using MRI of respiratory airways in PLWH with OSA and matched HIV negative controls with OSA and did not find significant anatomical changes. However, this study (Darquenne et al., 2023) was done in the USA in a Caucasian male dominant population and with a small sample (total n=20), Thus we cannot exclude that upper airways inflammation may contribute to OSA pathophysiology in PLWH, in particular if HIV treatment was delayed; thus increasing the risk of upper respiratory infections leading to upper airway obstruction (Sittitrai et al., 2018).

2.2.2 Sympathetic activation from OSA increases cardiometabolic risk

Cardiometabolic diseases, and specifically the symptoms of metabolic syndrome, overlap with the risks and symptoms for OSA, in part due to the overall systemic inflammation associated with both these syndromes. A growing body of research at the intersection of immunology and cardiovascular pathology supports that systemic inflammation can be a mediator in developing hypertension.

Healthy sleep helps with recovery and restoration. Traditionally, the cardiorespiratory system slows at night, with cardiac rhythms slowing during sleep and blood pressure dipping, because of higher nocturnal parasympathetic tone (Grassi et al., 2010; Santilli et al., 2016). The presence of healthy nocturnal blood pressure dipping reduces daytime blood pressure and stabilizes peripheral resistance (Grassi et al., 2010), supports optimal systolic function (Woodiwiss et al., 2018), and reduces systemic inflammation indices (Gunay et al., 2021) to protect cardiovascular health.

A review of literature on sympathetic nervous system (SNS) overactivity proposes that hypoxia induced by airway obstruction during OSA is associated with cardiac chemoreceptor (chemical messenger) and baroreceptor (mechanistic pressure) activation, to cause increases in SNS activity and basal blood pressure (Fletcher, 2003; Thareja et al., 2024), suggesting that OSA may be a driver for the rise of CMR.

Increased sympathetic nervous system activity is an understood consequence of OSA. The overactive sympathetic tone shifts characteristic nocturnal blood pressure decreases to a "non-dipping" blood pressure fluctuation instead (Wolf et al., 2010). Reduced/absence of blood pressure dipping at night has been associated with higher CMR (Kim et al., 2022) and sustained elevated daytime arterial blood pressure (Weiss et al., 2015) .

In a meta-analysis of 2519 participants across Asia, Europe, and the Americas, non-dipping nocturnal blood pressure was observed in 59% of OSA-experiencing participants (Cuspidi et al., 2019). Kareem and colleagues (Kareem et al., 2018) also highlight in their review that the characteristic dipping pattern of nocturnal blood pressure shows a reduced circadian amplitude with OSA-sleep fragmentation when compared to people without OSA. . One study (Roche et al, JCSM, 2021) showed an independent association between increasing OSA severity and higher cardiometabolic risk score in older adults with and without HIV in rural South African setting. However no studies on the association between OSA and cardiometabolic risk have been done in African-based people living with HIV .

2.2.3 Sympathetic overactivity and chronic intermittent hypoxia during OSA become drivers of increased systemic inflammation:

Chronic SNS activation also increases systemic inflammation by increasing T-cell dominant immune activation of monocyte-macrophage differentiation, leukocyte recruitment, and increased production of reactive oxygen species (ROS) (Carnagarin et al., 2018). This mechanism is one that may explain how prolonged sympathetic overactivity relating to chronic hypoxia from OSA drives the proinflammatory response associated with OSA.

2.2.4 OSA induced hypoxia and the cardiovascular system

Specifically, SNS activation and associated hypoxia-inducible factors increase synthesis and circulation of reactive oxygen species (ROS) (Fletcher, 2003; Kizawa et al., 2009). Higher ROS in turn upregulates levels of angiotensin 2 and aldosterone. These two hormones facilitate remodelling of vascular architecture, increasing cardiac output and blood pressure. (Montezano and Touyz, 2014). In addition to effects on the cardiovascular system, increased ROS in OSA is associated with systemic inflammation, in particular with a rise in plasma concentration of IL-1β and IL-6 (Said et al., 2017) and endothelial dysfunction often characterized by ESM-1 and MCP-1 elevations (Mochol et al., 2021). This proinflammatory state caused by intermittent hypoxic events might offer a potential mechanism for increased CMR experienced by PLWH on ART (Malpas, 2010).

2.2.5 OSA-related hypoxia association with insulin resistance and dyslipidaemia

Hypoxia during OSA interrupts lipolysis and lipid profiles; neuroendocrine signalling of somatostatins and ghrelin (Izumi et al., 2016) and insulin-like growth factor (IGF-1) production by the liver. In a 817-participant, multi-centre cohort of people with PSG-confirmed sleep apnoea (n = 567), thigher AHI was statistically significantly associated with lower IGF-1 (Galerneau et al., 2020), which is in turn associated with higher adipose tissue deposition, lower glucose metabolism and cardiovascular function.

Thus, OSA is associated with higher cardiometabolic risk through SNS activation and ROS production which lead to hypertension, insulin resistance and endothelial stress. Hypertension, insulin resistance and endothelial stress combine into increasing the risk of atherosclerosis. Atherosclerosis leads to arterial blockage, which is associated with high risk of acute ischaemic events (myocardial hypoperfusion/ infarction, plaque

migration or build-up leading to stroke or limb claudication) which constitute cardiovascular morbidity/mortality.

In the following section I will examine the pathophysiology of atherosclerosis and how OSA contributes to its aggravation.

2.2.6 Pathophysiology of atherosclerosis, increased systemic inflammation and OSA

i- Predisposing factors for atherosclerosis

Atherosclerosis is a progressive cardiometabolic pathology which describes the accumulation of fatty deposits along the endothelium of large arteries (Lusis, 2000). The progression of atherosclerosis likely begins with some predisposing factor such as a dysregulated lipid profile: when atherogenic or so called “progressive” lipoproteins (namely lower-density lipoproteins that include triglyceride segments) are present in excess, while anti-atherogenic or “protective” (high-density) lipoproteins are reduced (Feingold and Grunfeld, 2018).

Atherosclerotic plaques start to form when lower density lipoproteins that have circulated the body via the bloodstream are transported into and oxidized within the intima of an artery, adjacent to the endothelial cells which line the lumen of the artery. At various places along blood vessels, the intima becomes a site of clumped, or aggregated, lipoproteins. Accumulation of fatty acids then disrupts the tight-junctions between endothelial cells, increasing both the presentation of adhesion molecules in the lumen of blood vessels, and recruiting monocytes to the site (Lusis, 2000).

ii- Precipitating factors for atherosclerosis

Atherosclerosis is accelerated by hypertension, hypercholesterolaemia, obesity, metabolic syndrome and chronic inflammation (Kullo & Ballantyne, 2005). As seen in previous sections of this literature review, OSA and HIV infection are both characterized by this chronic inflammation, and thus are both candidates for accelerating atherosclerotic processes. The next paragraph will focus on how chronic inflammation may lead to endothelial activation to accelerate atherosclerosis progression.

iii- Role of endothelial activation in atherosclerosis

Endothelial activation (Liao, 2013) is defined as the pro-inflammatory process that is centred around the increase of endothelial adhesion molecule receptors like vascular cell adhesion molecule (VCAM-1) and intercellular adhesion molecule (ICAM-1). This increase is frequently enhanced by increased circulating proinflammatory markers like IL-6 and TNF-alpha (Feinstein et al., 2019; Liao, 2013).

Early atherosclerosis progression is closely linked with the differentiation of monocytes into macrophages that have migrated across the endothelium to take up oxidized LDL in the intima of blood vessels (Hilgendorf et al., 2015). Higher differentiation of monocytes into macrophages is associated with higher concentration of the plasma monocyte chemoattractant protein-1 (MCP-1), a useful biomarker to capture endothelial activation (Marincowitz et al., 2018).

These fatty acid-phagocytosed macrophages form what are then described as foam cells (Lusis, 2000; Woollard and Geissmann, 2010). Foam cells progress to firmer plaques – facilitated in part by specific cytokine cascades, growth factors and additionally accumulated extracellular lipids (Gimbrone and García-Cardeña, 2016; Woollard and Geissmann, 2010).

Though there are no studies that focus on endothelial activation and vascular remodelling by immune recruitment in the context of intersecting OSA risk, cardiometabolic risk, and HIV in South Africa, there is an abundance of literature which focuses on each context individually. This is detailed below to substantiate the case for investigating endothelial activation and vascular remodelling in populations at high risk for OSA, with high CMR scores, and who take ART for viral suppression.

2.3 POOR SLEEP QUALITY, CHRONIC IMMUNE ACTIVATION AND CARDIOMETABOLIC HEALTH:

HIV infection causes chronic inflammation, even in PLWH receiving ART. Many of the inflammatory cytokines implicated in HIV infection and persistence (Obare et al., 2024) such as IL-1β, IL-6 and TNF-R1) have also been associated with partial or total sleep deprivation (O'Brien et al., 2022). We have mentioned previously that those same cytokines are increased in OSA through intermittent hypoxia-induced reactive oxygen species. Circulating cytokines

such as IL-1β, IL-6 and TNF-R1 can be measured to understand the impact of OSA on immune activation (Testelmans et al., 2013).

Circulating IL-6 and TNF-α in PLWH who present with OSA (OSA+) have been shown to be elevated regardless of ART treatment (Brigham et al., 2014) compared to PLWH without OSA living in the USA. .

Additional putative markers of immune activation and cardiometabolic disease that may reside at the intersection of OSA and HIV include monocyte chemoattractant protein-1 (MCP-1) (Ellulu et al., 2017) and endothelial cell-specific molecule-1 (ESM-1) (Sun et al., 2018; Takasawa et al., 2023). These two markers of endothelial activation may help comprehend how increased inflammation in OSA and HIV may result in higher endothelial activation and thus risk of atherosclerosis progression.

Our next section will now examine specific inflammatory/endothelial activation markers of interest which may be elevated in HIV and OSA, and how those tie into cardiometabolic risk in PLWH.

2.4 Specific plasma markers of interest that may capture an intersection of OSA, HIV, and CMR

PLWH have been shown to haveelevated plasma concentrations of IL-1beta, IL-6, and TNF-alpha to varying degrees. We expect that with comorbid OSA, these well-characterised proinflammatory markers will have increased concentration resulting from the consequences of OSA-related hypoxia and systemic inflammation. The following sections will show evidence of increased inflammation in people diagnosed with OSA, and identify the more interesting markers to investigate.

2.4.1 IL-1beta, IL-6, IL-8 and TNF-alpha during OSA

i- Higher concentration of IL-1 beta and IL-6 in OSA, association with higher apnoea/hypopnoea index, and association with specific symptoms of OSA.

Though OSA is a proinflammatory disorder, research studies which focus on quantifying specific inflammatory markers of OSA are mainly from cross sectional studies. Comprehensive systematic reviews and meta-analyses (Golshah et al., 2024; Janmohammadi et al., 2023; Yi et al., 2022) show that across more than 100 studies

in people without HIV, IL-1beta, IL-6, IL-8 and TNF-alpha are measured in higher concentrations in OSA patients than in controls.

Intermittent hypoxia has been associated with higher secretion of both IL-6 and IL-1beta (Fitzpatrick et al., 2020), due in part to the macrophage activation caused by oxygen stress. In particular, substantial literature suggests the apnoea/hypopnea index is positively correlated with higher IL-1beta secretion. (Chen et al., 2021 ; Girtman et al., 2022 ; Lal et al., 2018 ; Su et al., 2018 ; Ty et al., 2017).

Finally, there are some preliminary studies which suggest that diurnal fluctuations of certain cytokines may be associated with symptoms of OSA. For example, in a 20-person American sample (median age 51 years; 50% male; all with at least mild OSA and overweight or obesity), IL-8, IL-6 and TNF-alpha had positive associations with various OSA symptoms (Yang et al., 2020). Specifically, morning levels of TNF-alpha ($r = 0.53$) and IL-8 ($r = 0.79$) were correlated with reported post-sleep fatigue following overnight polysomnography, higher evening plasma IL-6 concentration was positively correlated ($r = 0.69$) with self-assessed sleepiness, while diurnal variations of IL-8 were negatively associated with morning energy levels. This suggests that there may be value to test the association of specific OSA symptoms with those inflammatory markers (such as snoring and daytime fatigue; rather than just its presence vs. absence).

ii- Association between OSA-related and non-OSA elevation in IL-1beta, IL-6, IL-8 and TNF-alpha with higher CMR

- OSA-related elevation:

Thirty-one articles that relate increased inflammatory marker expression to OSA and OSA-related cardiometabolic phenotypes were systematically reviewed and revealed that elevated TNF-alpha and IL-6 expression are associated with obesity and hypertension phenotypes in people with diagnosed OSA (Fei et al., 2022).

- Non-OSA related elevation:

A review by Ridker and Rane (2021) further associated IL-6 concentrations and atherogenesis. Additional studies (Grebe et al., 2018; Moriya, 2019; Libby, 2021) also impress upon the effects of chronically upregulated IL-6 and IL-1Beta as additive factors to progression of atherosclerosis and dyslipidaemia.

The elevation of IL-1beta, IL-6, IL-8 and TNF-alpha in OSA and HIV may result in higher cardiometabolic risk and thus could serve as markers of interest when investigating the relationship between HIV, OSA and cardiometabolic risk. Additional markers we have mentioned previously include markers of endothelial activation, which we will examine in more detail in the following section.

2.4.2 ESM-1 and MCP-1 as potential indicators for endothelial activation and immune recruitment in vascular tissue as considerations for increased CMR

i- Endothelial-specific marker-1 (ESM-1)

ESM-1 is an endothelial cell-produced protein, which plays a role in vascular remodelling, especially under hypoxic stress (as that seen in OSA). Elevated ESM-1 levels correlate with the severity of endothelial activation (Takasawa et al., 2023), making it a potential peripheral biomarker for the presence, severity, and improvement of OSA-induced hypoxic stress and cardiometabolic disease progression (Budhiraja et al., 2007). These two studies suggest ESM-1 concentrations are increased with proinflammatory cytokine signalling and atherogenic factors; additionally ESM-1 concentrations are responsive to interventions like CPAP therapy (Altıntaş et al., 2015).

ii- Monocyte chemoattractant protein (MCP-1)

MCP-1 is a chemokine which recruits monocytes to specific regions of inflammatory localisation within the body. As mentioned in our section on the pathogenesis of atherosclerosis, overexpressed MCP-1 drives endothelial dysfunction and atherogenesis (Tsao et al., 1997; Singh et al., 2021) in part by the surge of monocyte recruitment (and accumulation) at the inner arterial walls. MCP-1 circulation in the blood plasma may be a useful peripheral indicator of endothelial activation and accelerated atherosclerosis. A significant driver of MCP-1 circulation is oxidative stress (Zeiher et al., 1995), such as that caused by OSA-intermittent hypoxia. Despite being a thoroughly characterised chemokine, very little is known about the activity of MCP-1 in comorbid OSA and HIV.

The diagram below, **Figure 1**, summarizes the information described in 2.4.1 and 2.4.2 above, showing how specific inflammatory markers and markers of endothelial activation contribute to atherosclerosis progression.

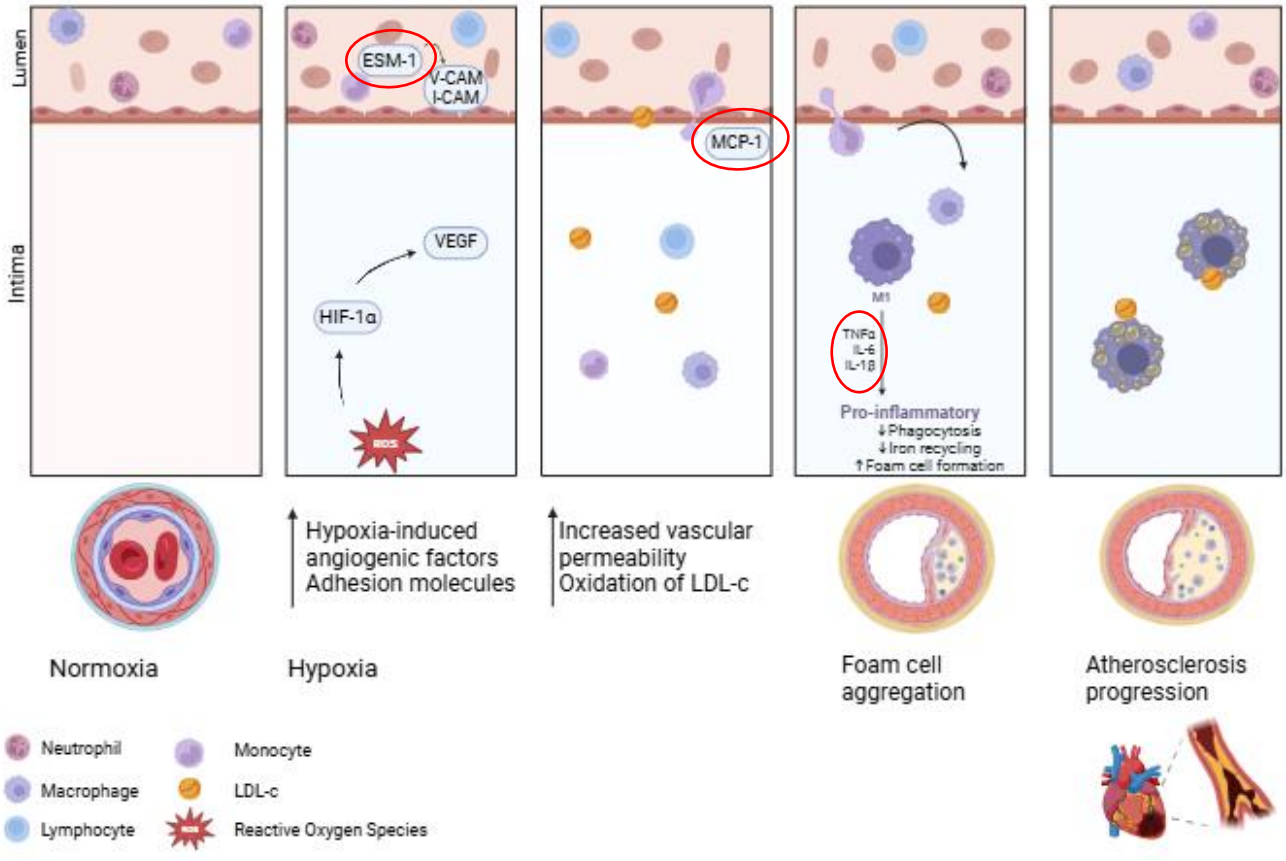

Figure 1: *Focal diagram explaining the progression of vascular remodelling from endothelial activation under hypoxic stress such as that generated during OSA. Markers of interest are encircled in red.(diagram produced by J. Marais, using Biorender?)*

2.5 Understanding the current state of Obstructive Sleep Apnoea research in people Living with HIV in South Africa

Research into the occurrence and implications of Obstructive Sleep Apnoea (OSA) in People Living with HIV (PLWH) is noticeably sparse. Some studies suggest that PLWH have higher prevalence of OSA than their seronegative counterparts in the general population. Studies from the late 1990s highlighted adenotonsillar hypertrophy as a potential cause of OSA among HIV-infected individuals (Epstein et al., 1995). In the first decade of high active ART (before 2012), researchers have considered the effects

of lipodystrophy (Dorey-Stein et al., 2008) as contributors to OSA. Finally in the past decade. ART-induced weight gain has been studied as a pertinent contributing factor to OSA presentation in PLWH.

Figure 2 has been designed in such a way to map the intricate, bidirectional relationships between the factors elaborated in the literature. There is research to support the relationships between some isolated variables, like the positive correlation between SNS activation and CMR, however the unification of concepts like systemic inflammation, CMR, SNS activity, OSA and its impacts has not been investigated in PLWH in South Africa.

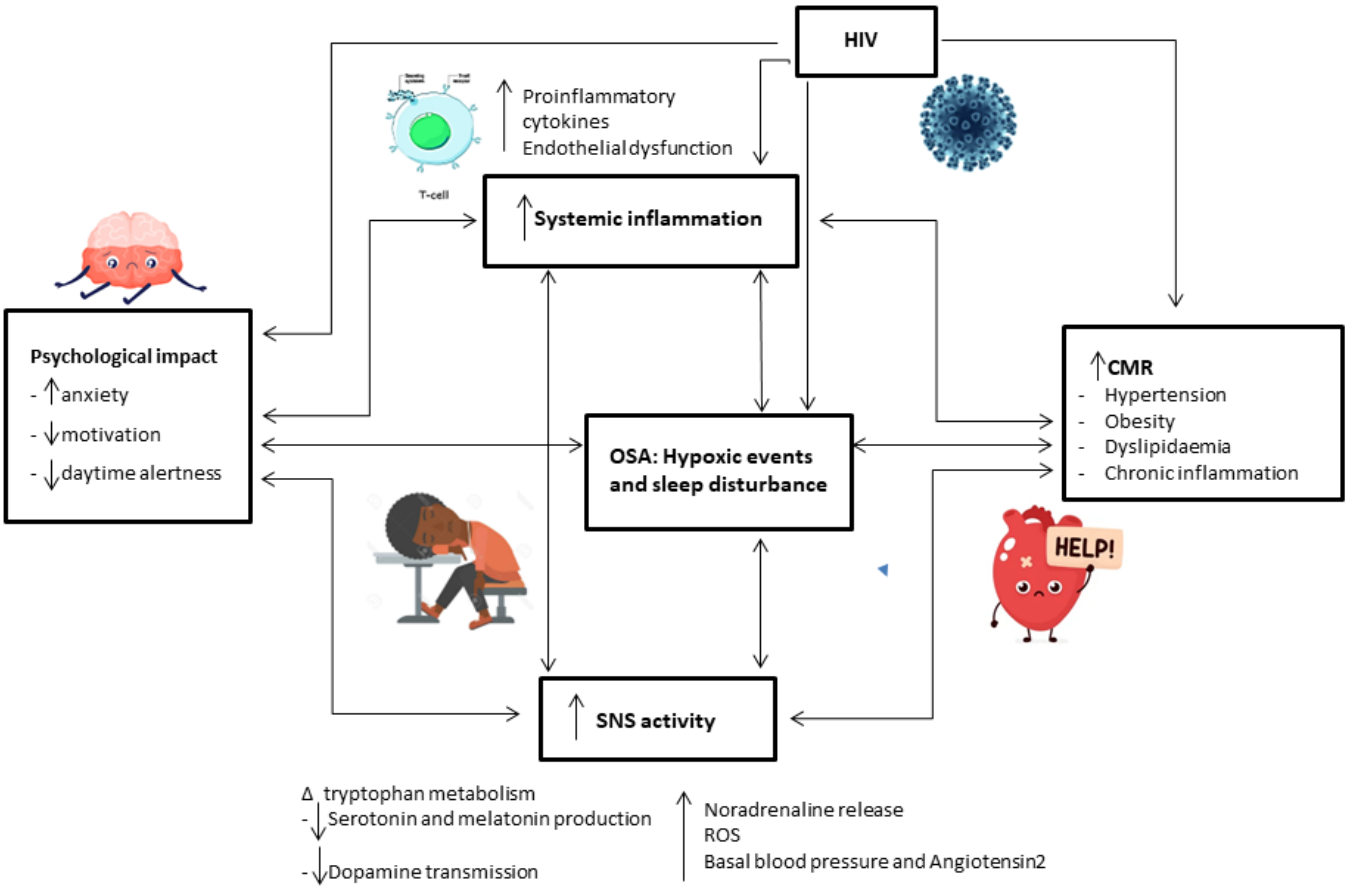

Figure 2: *The cardiometabolic, inflammatory, and nervous system relationships between obstructive sleep apnoea and HIV*

CMR: cardiometabolic risk; HIV: human immunodeficiency virus; OSA: obstructive sleep apnoea; SNS: sympathetic nervous system; ROS: reactive oxygen species

2.6 Prevalence and Characteristics of OSA among PLWH:

Despite the clear cardiometabolic implications of OSA for PLWH, diagnosis and treatment remain inadequate. Limited understanding of OSA among health care providers who care for people living with HIV, coupled with diagnostic challenges and the tendency to attribute common OSA symptoms to HIV itself, has led to low diagnosis rates (Owens and Hicks, 2018; Schmickl et al., 2022). While OSA is generally underdiagnosed, the disparity is more pronounced in PLWH, where diagnostic rates are significantly lower compared to HIV-uninfected populations.

Emerging evidence suggests that OSA is prevalent among PLWH, challenging traditional risk profiles seen in the general population. For example, data from the Multicentre AIDS Cohort Study (MACS) revealed a high prevalence (55%) of polysomnography-diagnosed OSA among HIV-infected subjects, hinting at a significant number of PLWH potentially living with undiagnosed OSA (Punjabi et al, 2023). Contrary to the general population where OSA risk increases with age and weight, PLWH diagnosed with OSA were notably younger and leaner, and some African-led research (Njoh et al., 2017) supports that the risk for OSA can be greater amongst PLWH (43.6%) than the general population (14.0%). This deviation from traditional risk factors underscores the potential influence of HIV and ART on the development of OSA, necessitating further investigation into the physiological and treatment-related drivers of OSA in PLWH. This gap is evidenced when looking at the infrequency of sleep screening asked for by HIV physicians for their patients (Chen et al., 2019; Kunisaki et al., 2015).

In South Africa we have limited data on the prevalence and risk factors of OSA in diverse populations including people living with HIV. There are no more than a handful of studies. Recent studies (Chandiwana et al., 2023, Orr et al., 2021) have only begun to shed light on the prevalence and characteristics of OSA among PLWH. Orr and colleagues (Orr et al., 2021) emphasize in particular the importance for studying OSA characteristics among cis-women living with HIV. .

To expand, prevalence of obstructive sleep apnoea syndrome (OSAS, describing a collection of symptoms and consequences arising from OSA) in South Africa in a

review of 17 studies in 16 countries has been estimated to be between 22.8 and 40.4 % (Benjafield et al., 2019). This data, though strongly built, is based substantially on inference data rather than objective measures. An observational study of OSA prevalence in South Africa – conducted in a cohort (n = 75) of older persons (66.1 ± 10.7 years) in rural South Africa – as measured by home-based polysomnography reported an OSA prevalence of 29% (Roche et al., 2021). This forms the extent of South African literature for objective measures of OSA among PLWH.

OSA's relevance to PLWH extends beyond its prevalence, as it results in systemic inflammation and higher cardiovascular risk (Maniaci et al., 2021). Studies have identified sleep apnoea as a potent predictor of fatigue in PLWH (Orr et al., 2021), an insight that emphasizes the need for healthcare providers to consider OSA in symptom management. Additionally, the presence of moderate to severe OSA in PLWH has been linked with heightened levels of inflammatory markers, suggesting that untreated OSA may exacerbate the inflammatory state inherent to HIV infection. Considering this burden on the internal state of wellbeing, undiagnosed and untreated OSAS negatively may be associated with lower quality of life and higher cardiometabolic health of the South Africans.

2.7 Using the Berlin Questionnaire to screen for Obstructive Sleep Apnoea

While polysomnography (multi-channel nocturnal sleep measurement, which includes overnight recording of the electroencephalogram (EGG), ocular movements, electrocardiogram and respiratory variables such as nasal airflow, thoracic and abdominal movement during breathing and pulse oximetry) is considered the gold-standard approach for diagnosing sleep disorders like OSA, this is a time-consuming and expensive protocol that is not available for the ~84% of the South African residents who rely on public health care (Benatar & Gill, 2020).

Other than conducting a sleep study in a clinical sleep laboratory or home-based setting, there are alternative ways to measure sleep quality – including smart devices or applications that measure data such as nocturnal blood oxygen saturation and

snoring patterns. Actigraphy measures movement in three dimensional planes; when used along with a self-reported sleep diary, actigraphy can be equally valuable for understanding sleep patterns by segmenting portions of wake from times of rest. As OSA events are characterised by fluctuations in apnoea/hypopnoea indices, the use of pulse oximetry for measing oxygen desaturation index could serve as an intermediate between full polysomnography (PSG) and qualitative methods of OSA screening. Still, this remains an expensive and inaccessible option for many South African residents.

Fortunately, questionnaires that screen for risk factors of OSA like being assigned male sex at birth; having hypertension/obesity; frequency and intensity of snoring; or levels of experienced daytime sleepiness do exist and have been validated – though, mostly in “Global North” populations. When assessing the success of a qualitative screening tool like a questionnaire against a quantitative measurement like PSG: sensitivity describes the proportion of instances when the questionnaire correctly predicts OSA presence later confirmed during PSG, while specificity describes the proportion of instances when low-risk OSA was correctly allocated to a person who doesn’t show OSA during PSG.

The Berlin Questionnaire (Netzer et al.,1999) remains one of the most widely used OSA screening tools with good sensitivity (~86%), respectable specificity (77%), and a positive predictive value of 89% (in people who are not HIV positive), when compared to the PSG gold standard. Questionnaires are easy and affordable to roll-out within point-of-care screening settings. Other questionnaires like STOP-BANG questionnaire outlined by Chung and colleagues (Chung et al., 2012) or the Epworth Sleepiness Scale (Johns, 1991) are also frequently used to screen for OSA risk. However, the Berlin Questionnaire has been well-reviewed for its sensitivity and specificity and was reported to have the greatest number of validation studies when compared to other OSA questionnaires (Senaratna et al., 2017). In one study in American PLWH (mainly male and of European ancestry), both questionnaires detected poorly mild OSA while STOP BANG kept an acceptable sensitivity for moderate severe OSA (Schmickl et al., 2022). However those have not been assessed in a South African population with HIV, essentially of African ancestry and female.

2.8 Problem statement:

There are few studies on OSA and HIV intersections in diverse populations with respect to age, sex, BMI, and ancestry - with no data available on cis-women with HIV and OSA by 2018 (Owens and Hicks, 2018). There are notably few studies done in Africa - where most people living with HIV reside - and where undetected HIV-associated comorbidities like OSA can quickly manifest as a public health crisis. The strong correlation between chronic inflammatory states and high cardiometabolic risk markers in PLWH could be explained by a rise in OSA prevalence, as OSA amplifies both increased pro-inflammatory and compromised cardiometabolic outcomes.

Understanding the relationship between inflammatory markers, HIV and ART-associated pathophysiology and OSA risk could be important to consider when making ART regimens available to people with various cardiometabolic (cardioprotective or atherogenic) and inflammatory profiles. The overall aim of this study was to describe the relationship between OSA risk and cardiometabolic disease risk in people living with HIV on dolutegravir-based ART. This will be fulfilled by completing the following objectives:

1) To determine the prevalence of high risk OSA in a cohort of 210 PLWH taking dolutegravir, by screening with the Berlin Questionnaire.
2) To investigate the association between high risk OSA and traditional markers of cardiometabolic risk (blood pressure, wasit circumference, glucose, lipid profiles) , the presence of metabolic syndrome and its association with a previously-used composite cardiometabolic risk score in a cohort of 210 PLWH enrolled in the study CHARACTERISE.
3) To characterisethe overlap between risk for OSA and risk for CMD in the cohort, by drawing attention to which components of the CMR score dichotomize low-risk and high-risk for OSA persons.
4) To quantify the plasma concentration of four well-characterised pro-inflammatory markers and two markers of endothelial activation, potentially relevant to the development and progression of OSA-associated elevated CMR.

3 METHODOLOGY:

This study was part of a larger study to investigate consequences of weight gain in patients living with HIV who were treated with the dolutegravir-based ART. The larger

study was CHARACTERISE ("A cross-sectional observational study to characterise the transition to Dolutegravir-based regimens in South Africa in terms of the emergence of obesity, viral re-suppression and integration into routine programme care") while this research formed part of a sub-study of CHARACTERISE investigating risk of sleep apnoea and association with cardiometabolic risk, entitled OPHA (Characterising Obesity and Obstructive Sleep Apnoea in People living with HIV in South Africa). For plasma analysis of inflammatory markers, stored blood samples from 103 of the PLWH who participated in CHARACTERISE were used.

3.1 Ethical Approval, Good Clinical Practice, and Informed Consent:

The South African national imperatives of good clinical practice were always maintained to ensure the righteous treatment of participants and research integrity. All clinicians and research scientists who engaged with participants or participant data were trained with a certification of Good Clinical Practice informed by the Helsinki Declaration and The Belmont Report. All participants of CHARACTERISE (NCT05502692) were at liberty to leave this study at any time or withdraw consent for their stored blood samples to be used for future analysis. Human Research Ethical Clearance (HREC) for the CHARACTERISE study was approved on 22 June 2022 (220410B), an amendment for immune-based blood tests forming part of the OPHA trial was reviewed and accepted on 30 June 2023 (clearance number 230610).

3.2 CHARACTERISE inclusion and exclusion criteria:

CHARACTERISE sought to understand how a pharmaceutical transition to dolutegravir-based ART has impacted the cardiometabolic health of PLWH – especially following the considerable weight gain seen during the ADVANCE trial (Sokhela et al., 2024; Venter et al., 2020). The participant cohort for CHARACTERISE was composed of cis-gender men and women living with HIV - of diverse ages and BMI - who were not treatment-naïve at the time of screening. The participants who were invited to participate had initiated dolutegravir-based ART more than one year prior to the start of the CHARACTERISE study, and were recruited from two previous cohorts, namely:

1. The ADVANCE trial cohort, which comprised participants who had been on the **tenofovir + lamivudine + dolutegravir** (TLD) state programme for at least one

year at the time of recruitment into the CHARACTERISE study, or,

2. part of an existing cohort of patients, at the time of recruitment on TLD, but who had initiated treatment with **tenofovir + lamivudine + efavirenz** (TLE) (since then, switched to tenofovir + emtricitabine + efavirenz) more than 9 years ago, and who had been transitioned to TLD within the national programme at least one year prior to the time of recruitment into CHARACTERISE.

To be included in this study screening, participants had to be over the age of 18 years old, and able and willing to provide informed, written, or electronic consent in English at the time of recruitment into the study CHARACTERISE. Additionally, participants had to have direct access to reliable telephonic or email-permitting devices for communication between study staff and participants.

Potential participants were excluded if they were pregnant at the time of recruitment; if there was a chance for participant relocation during the time of the study; if the participant was not in good mental, social and physical health as decided objectively by the Principal Investigator at the time of the study screening; or if the participant was involved in any other professional role at the study site at the time of the study screening.

3.3 Role of master's Student

While this project was nested within the CHARACTERISE study and extended from the ADVANCE study, I had clear deliverables that were adjacent to the clinical procedures retained within clinical trial operations. These include the points in the following section, from **3.4.3** – **3.6**. In being well-informed regarding such operations, I underwent training regarding the informed consent process and protocol of CHARACTERISE.

1) A certificate of Good Clinical Practice (GCP) was required for me to delegated onto the CHARACTERISE study staff, and in pursuing GCP certification I additionally completed Training and Resources in Research Ethics Evaluation (TRREE) modules (Appendix).

2) Raw data was collected on site (Ezintsha) and captured into REDCap, and then exported into a ~16 000 cell data file that had to be cleaned, processed, and analysed by me. I then scored the Berlin Questionnaire.

3) I calculated a continuous cardiometabolic risk score, constructing z-corrected scores per variable within the formula and calculating mean arterial pressure from blood pressure. Comparison of CMR score by BQ outcome through statistical testing was completed by me.

4) I determined whether participants had Metabolic Syndrome , using the presence of hypertension; obesity; and dyslipidaemia. Any analysis that followed with these categories was also completed by me.

5) I reviewed the literature on inflammation and cardiometabolic disease as it relates to both HIV and OSA, and selected and ordered six cytokines for analysis by multiplex technology.

6) Multiplex assays to quantify marker concentration for IL-1beta, IL-6, IL-8, TNF-alpha, MCP-1 and ESM-1 were carried out as per method described (**3.6**) and analysed by me.

3.4 STUDY DESIGN AND METHODOLOGY FOR CHARACTERISE:

3.4.1 Study description and study sites:

This dual-site observational cohort study was conducted at **Ezintsha Clinical Research Centre, Wits Health Consortium** (28 Princess Wales Avenue, Sunnyside Office Park, Parktown, Johannesburg) and the **School of Physiology, Wits Faculty of Health Sciences, University of the Witwatersrand** (7 York Road, Parktown, Johannesburg).

3.4.2 Data collected by clinicians at Ezintsha:

During the visit for the study – following telephonic, email, or in-person inclusion screening (which may have happened if the participant came for an annual visit for the ADVANCE study mentioned above) - participants who agreed to participate were invited to Ezintsha to be formally first consented into the study. A clinician guided each participant through the informed

consent and official enrolment process; through the questionnaires; and record any previous medical conditions into the REDCap database. Enrolment for CHARACTERISE was between June and December 2022.

A blood test measuring fasted glucose levels and lipid profiles was performed by a skilled phlebotomist or nurse and processed off-site. 50 mL of additional blood was taken and sent for storage at Bio Analytical Research Corporation (BARC) South Africa if full, informed consent had been given by the participant during this visit for this blood to be used in future sub-studies.

Anthropometric measurements including height, weight, and waist-circumference were taken and recorded into REDCap. Peripheral blood pressure was measured using an automatic blood pressure cuff; measurements were repeated three times at the brachial artery while participants were seated and relaxed, at two-minute intervals (Seedat et al., 2014; Whelton et al., 2018), and recorded as an average blood pressure.

Mean arterial pressure was calculated to accommodate for the frequency of diastole by $\frac{SBP + 2(DBP)}{3}$. Hypertensive status was taken as SBP $\geq 130\ mmHg$ or DBP $\geq 85\ mmHg$ (Unger et al., 2020) or if anti-hypertensive medication was taken by the participant.

3.4.3 Metabolic Syndrome determination:

The presence of metabolic syndrome (Grundy et al., 2005) was determined when a participant had at least 3 of the following criteria: triglycerides ≥ 1.7 mmol.L^{-1} or; HDL-C < 1.03 mmol.L^{-1} for men and < 1.3 mmol.L^{-1} for women; fasting glucose ≥ 5.5 mmol.L^{-1}; WC ≥ 102 cm for men and ≥ 88 cm for women; and SBP ≥ 130mmHg or DBP ≥ 85mmHg or when the participant reported current use of medication to treat hypertension.

3.4.4 Cardiometabolic Risk Score construction:

In another study on obstructive sleep apnoea and cardiometabolic risk (Roche et al., 2021), authors had constructed a continuous cardiometabolic risk (CMR) score to have higher power to detect associations with obstructive sleep apnoea status. We decided to construct a similar

CMR-score to also increase our power to detect associations with risk of sleep apnoea status. As described in Roche et al. (2021), results of available clinical anthropometric and blood tests were combined into a cardiometabolic risk (CMR)-score for each participant. The CMR-score was calculated with sample-calculated z-scores for waist circumference (WC); fasting glucose (GLU) – though this differed from Roche and colleagues who used anytime glucose; mean arterial pressure (MAP); triglycerides (TGL); and high-density lipoprotein cholesterol (HDL-c), $\left(z = \frac{(sample\ variable - sample\ mean)}{standard\ deviation} \right),\ such\ that\ CMR\ score = \frac{(zWC + zGLU + zMAP + zTGL) - zHDL}{5}$).

3.4.5 Dyslipidaemia screening:

During data analysis, presence of dyslipidaemia (Davidson and Pradeep, 2023; Grundy et al., 2018) was noted for participants who fit the criteria for dysregulated lipid levels measured in the blood. These criteria were met for individuals who had elevated total cholesterol (> 5.2 $mmol.L^{-1}$); reduced HDL-c (< 1.03 $mmol.L^{-1}$ for men and < 1.3 for women); elevated LDL-c (> 2.5 $mmol.L^{-1}$) or elevated triglyceride (> 1.7 $mmol.L^{-1}$) plasma concentrations.

3.4.6 Body mass index and waist-to-height ratios:

Body mass index was calculated for the sole purpose of screening for an objective measure of obesity that is widely used within cardiometabolic risk research. This measure is built as a ratio of $\frac{weight\ (kg)}{height\ (m)^2}$. A secondary measure of weight distribution that is strongly associated with CMR is the waist-to-height ratio, and was calculated by $\frac{waist\ circumference\ (cm)}{height\ (cm)}$.

3.4.7 Risk for obstructive sleep apnoea:

The modified Berlin Questionnaire (BQ), for screening of sleep apnoea (Netzer et al., 1999), was presented to the participants from CHARACTERISE. The only modified question related to the category scoring daytime sleepiness and asking about the participant's likelihood to fall asleep while driving. Since most of our participants never

drove, we replaced the question with likelihood to fall asleep when being a passenger in a car/ bus/ taxi. The BQ has three categories related to 1) frequency and intensity of snoring (**Appendix; Questions 1-5**) 2) daytime sleepiness (**Appendix; Questions 6-9**); and 3) presence of a hypertensive state and/or an obese BMI (**Appendix; Question 10**). Each of the three categories results in either having a 1 or a 0. A positive score (1) in at least two categories declares a high risk of OSA (BQ = 1) whereas a positive score in only one or no category suggests a low risk of OSA (BQ = 0). This is not a diagnosis, but rather a self-reported screening for risk of OSA presence.

3.4.8 Scoring criteria for the Berlin Questionnaire:

Category 1 includes questions one to five. A positive score for category 1 was taken if at least three of the questions had a score of 1. Category 2 includes questions six to nine. A positive score for category 2 was taken if at least one question had a score of 1. Category 3 includes question ten and was taken to have a positive score if the answer to question 10 was scored 1.

The full questionnaire, as modified, can be found in the Appendix.

Below is a graphic depiction (**Figure 3**) of the processes involved in the construction of the CMR score as well as the categorization of high- and low-risk OSA groups. The variables used in the CMR score were additionally used to determine metabolic syndrome presence as well as determine cohort-wide prevalence of hypertension, dyslipidaemia, and obesity.

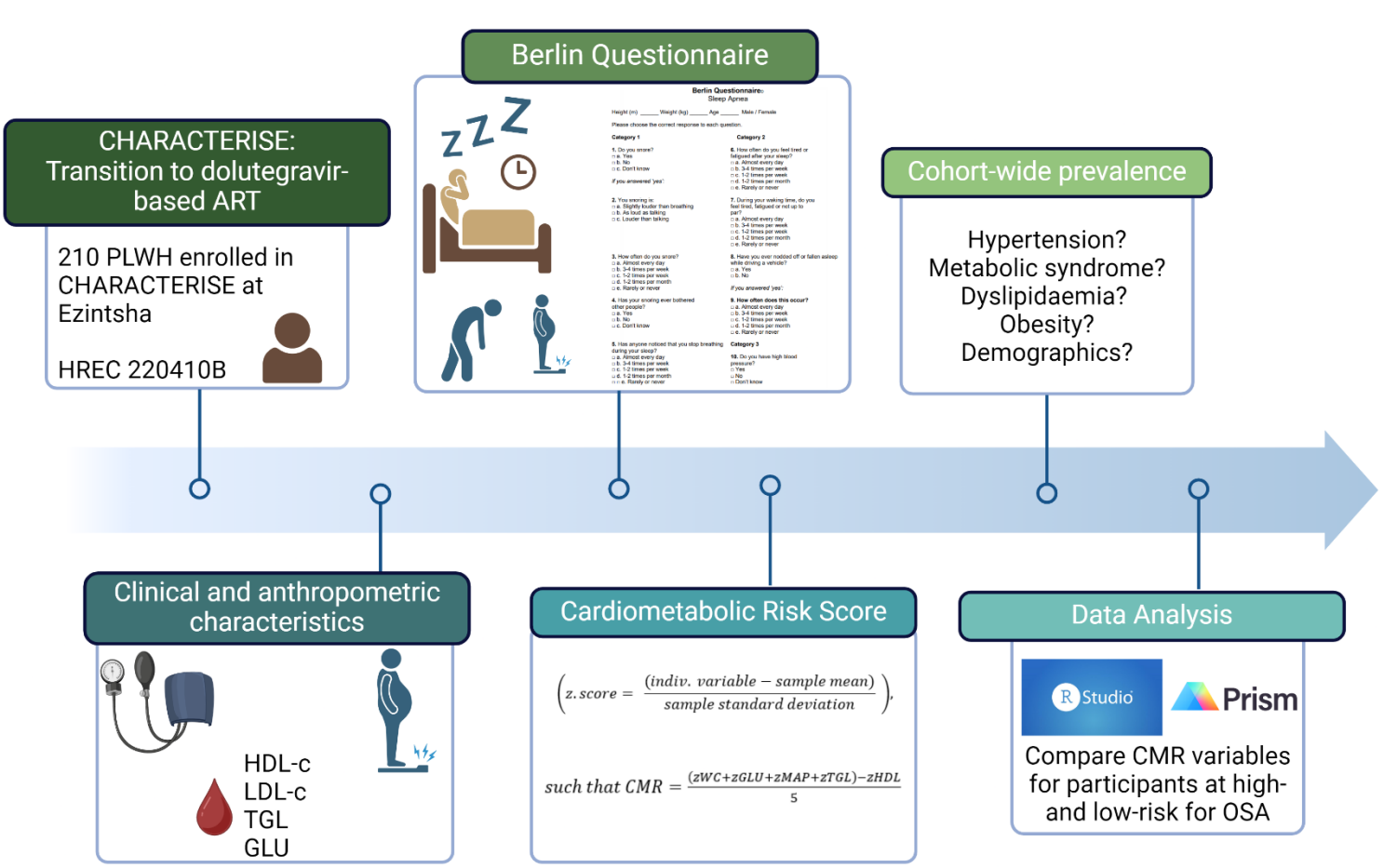

Figure 3: *Stepwise flow of methodological processes involved for this project*

CMR: cardiometabolic risk ; GLU: glucose; HDL-c : high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; TGL: triglycerides; WC: waist circumference (diagram created by J Marais, using Biorender)

3.5 Biomarker assay by Luminex™ multiplex technology

Plasma cytokine quantification was performed using high-sensitivity Magnetic Flourescence Assay Kits created by Cloud-Clone Corporation (*BIOCOM Africa, Centurion, South Africa*). The assay kits were designed by specifications for human

biological samples, after screening compatibility for collectively assaying endothelial cell-specific molecule-1 (ESM-1), monocyte chemoattractant protein-1 (MCP-1), interleukin 1-beta (IL-1β), interleukin 6 (IL-6), interleukin 8 (IL-8) and tumour necrosis factor-alpha (TNF-α) by multiplex methodology.

The protocol for quantification was followed in detail according to manufacturer instructions but can be visualised in the simplified diagram that follows (**Figure 4**).

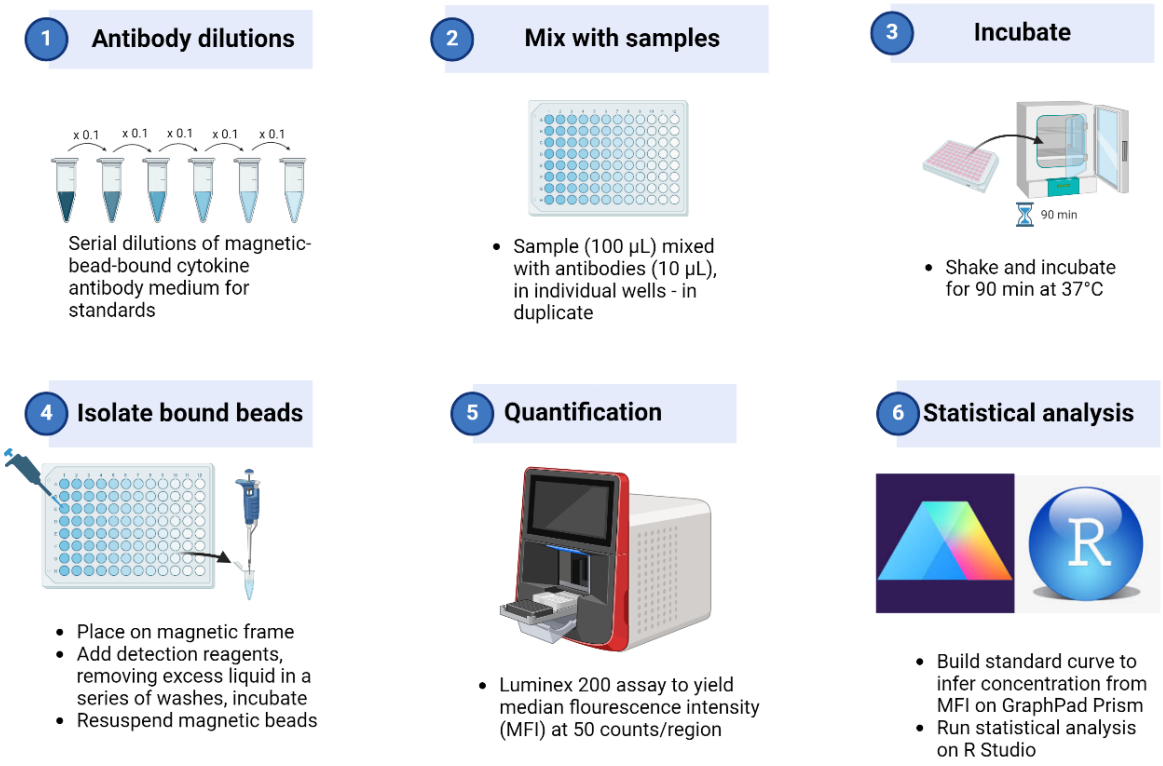

Figure 4: *Simplified visual depiction of workflow for quantifying plasma cytokine concentrations using Luminex™-facilitated multiplex technology for ESM-1, MCP-1, IL-1β, IL-6 and IL-8 (diagram created by J Marais, using Biorender)*

In brief, the cytokine antibody for each of our listed markers was bound to magnetic beads of differing sizes in one solution. Serial dilutions of antibodies in phosphate-buffered saline at a total volume of 100 µL were then made for measurement as a range of expected concentrations per manufacturer indications, from which median fluorescence intensity (MFI) correlating to a certain concentration of solute could be deduced during quantification. Plasma samples (10 µL) of each participant (n = 95) were mixed with undiluted antibody solution (90 µL), in two allocated wells on a 96-

well plate for procedural duplicates of quantification. Sequentially, the plates containing the mixed antibody solution and plasma were incubated, shaken, and washed before being quantified by Luminex 200 ™ multiplex technology. Once the median fluorescent intensity per well was reported, a standard curve was built using standards of known concentration, , and interpolation of concentrations of unknown value was made possible for each biomarker per sample.

3.6 Statistical Data Analyses

We categorised the 210 participants from CHARACTERISE according to OSA risk outcomes by the Berlin Questionnaire overall scores, where BQ = 0 will be referred to as a low risk for OSA and BQ = 1 will be referred to as a high risk for OSA.

All data were initially exported as anonymized participant profile outcomes from REDCap to Excel for data cleaning. Data from all 210 participants were further processed in R Studio 2023.12.0-369, including drawing on descriptive statistics to understand which data analyses were appropriate. Graphs were constructed using GraphPad Prism 9.2.0. Categorical data were analysed as proportions (%) of the cohort and compared by chi-squared tests between OSA lower risk and high-risk groups.

Distribution of continuous variables was tested for normality using the Shapiro-Wilk Normality test. Normally distributed (alpha = 0.05) data were presented as means and standard deviation (± SD), while median and interquartile range [IQR] were used for non-normally distributed data. Parametric Welch's two-sample t-tests – useful when variances and group size are not equal - discerned differences between means of the groups with low risk and high risk of sleep apnoea (Age, Waist circumference, LDL-c, waist/height ratio, and CMR-score), whereas non-parametric Mann-Whitney U tests (using the Mann-Whitney Wilcoxon test, by command "wilcox.test" on R Studio) were used to test differences between medians (Weight, Height, BMI, SBP, DBP, MAP, Years on ART, HDL-c, TGL, GLU).

Univariate analysis by spearman correlation sought correlations between BQ outcome and each of the other independent variables. When significant, multivariable analysis of CMR and BQ correcting for age, sex and time on ART was performed. This approach was repeated for MCP-1 and ESM-1 concentrations and BQ, age and sex.

4 RESULTS:

4.1 DEMOGRAPHICS AND CLINICAL CHARACTERISTICS:

In total, 210 people living with HIV taking ART were enrolled in the study. The collected demographic and clinical data are summarized in **Figure 5.** All participants self-identified with African ancestry, 64% of the participants held South African

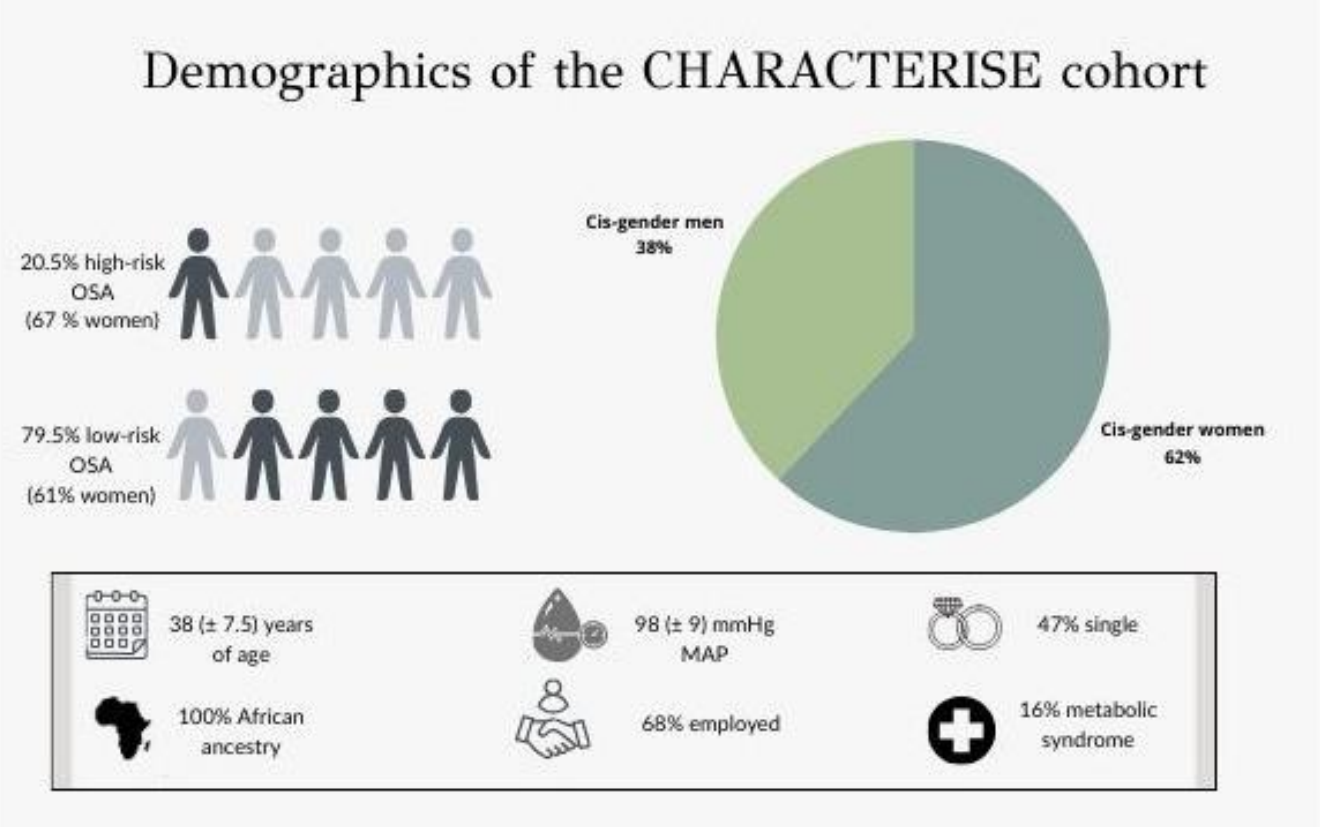

Figure 5: *Infographic reporting cohort-wide demographics data for the CHARACTERISE participants*

significance tests showed no statistical difference between OSA risk group for each variable in this figure; *MAP: mean arterial pressure; OSA: obstructive sleep apnoea*

nationality, 31% held Zimbabwean nationality, 2% had Malawian (1%) or Mozambiquan (1%) nationality; and the other 3% held unspecified African nationality. Fifty percent of the cohort resided in central Johannesburg, and 50% of the cohort lived in surrounding areas within Gauteng.

Of the 210 participants, 29% reported being unemployed at the time of enrolment into the study. Three percentof the cohort were on pension, and the remaining 68% were

employed or self-employed. The average time on dolutegravir-based (DTG-based) antiretroviral therapy across the cohort was 2.5 years, however we only had this information available for 76% of the cohort. Average mean arterial pressure was 98 ± 9 mmHg for the cohort, with 61% of the total cohort having hypertension. Average BMI across the cohort was 29 ± 6.7 $kg.m^{-2}$. Sixteen percent of the cohort fitted the profile for persons with metabolic syndrome. 38% of the total cohort were cis-gender men; 62% were cis-gender women; and no gender-diverse or transgender persons formed part of the cohort.

4.2 Berlin Questionnaire outcomes:

The 210 participants were screened for their risk of obstructive sleep apnoea using the Berlin Questionnaire (BQ). Forty-three out of 210 persons (20.5% of the cohort) scored high risk for obstructive sleep apnoea, while 79.5% (n = 167) were scored at low risk for obstructive sleep apnoea.

***Figure* 6** below shows the proportion, in each of the high- and low-risk outcome groups, for the questions answered within the Berlin Questionnaire. The ten questions of the Berlin Questionnaire have three categorical groupings. In brief, these categories collate the traditional signs in sleep apnoea patients to snoring (*Category 1*), daytime fatigue or sleepiness (*Category 2*) and being overweight or hypertensive (*Category 3*).

In this cohort, **Category 1** questions strongly dichotomized low-risk and high-risk groups ($p < 0.0001$, chi-squared test). These questions ask:

1) Do you snore?
2) … How loud do you snore?
3) … How often do you snore?
4) … Has your snoring ever bothered people?
5) Has anyone ever noticed you stop breathing during sleep?

In the high-risk group, 91% of the participants reported snoring (***Figure* 6***, Q1*); 60% of whom reported snoring at least as loud as talking (***Figure* 6***, Q2*); and 30% of these participants reported snoring at least three times a week (***Figure* 6***, Q3*). In contrast, the low-risk group reported statistically significantly lower frequency and intensity of

snoring: only 13.7% reported snoring (Chi Square test; $p < 0.0001$); 1.8% snoring at least as loud as talking (Chi Square test; $p < 0.0001$); and 1% snored at least three times a week (Chi Square test; $p < 0.0001$).

Category 2 questions ask about:

6) How often do you feel tired after sleep per week?
7) How often do you feel fatigued during the waking day per week?
8) If you ever fall asleep while being a passenger in a car?
9) … How often per week?

Outcomes were also significantly different between risk groups when comparing the frequency of feeling fatigued or sleepy during the wake part of their day (***Figure*** **6**, Q7). Twenty-three percent of the high risk OSA group reported feeling fatigued during the day at least three times per week, but only 6% of the low risk OSA group reported this (Chi Square test, $p = 0.0016$). Category 2 is significant in dichotomizing OSA risk groups ($p = 0.0017$) within this cohort, as nearly a quarter of the high-risk group experienced daytime fatigue.

Category 3 is exclusively comprised of Q10, which asks if a person has high blood pressure (corresponding to either SBP ≥ 135 mmHg or DBP ≥ 80 mmHg, or taking anti-hypertensives) or BMI (≥ 30 $kg.m^{-2}$). Using the anthropometric measurements performed during the visit, overall, 75% of the full cohort were found to have obesity or have hypertension. Within the risk groups, 95% of the high risk OSA group scored 1 on Category 3, while 70% in the low-risk group ($p = 0.0012$) scored 1.

Interestingly, we had also asked the participants to self-report on their obesity/hypertension status for category 3. When self-reporting here, only 22% of the low risk OSA group and 49% of the high risk OSA group declared being hypertensive and/or having obesity (while we found 70% with our measurements for low risk OSA group and 95% in the high-risk group), suggesting a fair number of participants were not aware of their hypertensive/obesity status. For all analyses, we used the actual visit measurements or whether they were taking anti-hypertensive medication for Category 3 classification.

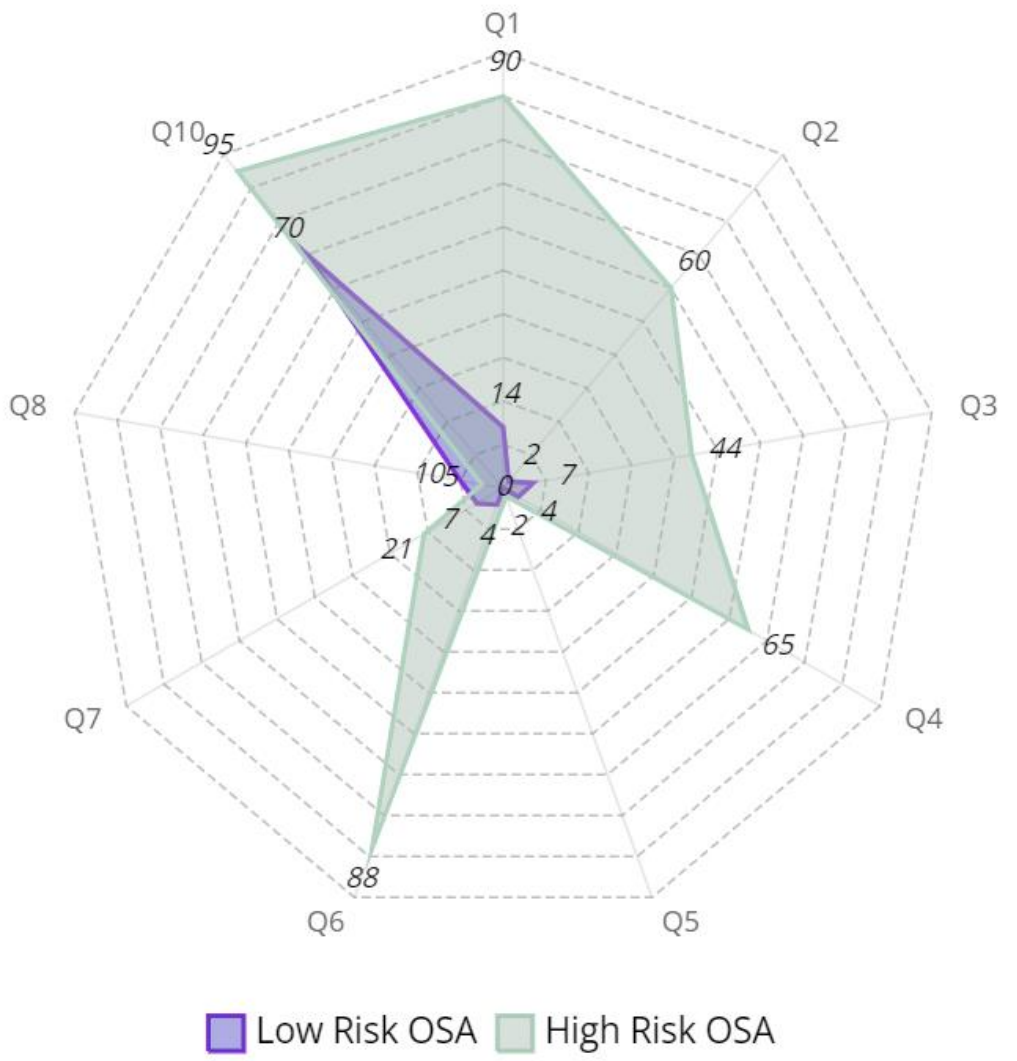

Figure 6: *Radial map of proportions (10% increments) of the low-risk and high-risk OSA groups that answered affirmatively for nine of the ten questions of the Berlin Questionnaire, where no score is allocated for question 9.*

Category 1: Questions 1-5 *Category 2: Questions 6-9* *Category 3: Question 10*

When comparing demographics between the high-risk and low-risk, we observed no difference in age, sex distribution, prevalence of hypertension, dyslipidaemia and tobacco use between the OSA risk groups (**Table 1**). Participants with high risk of OSA had shorter time on dolutegravir ($p = 0.0435$, Mann-Whitney U test), and prevalence of obesity differed between OSA risk groups ($p < 0.0001$).

Marital status was not associated with OSA risk group outcomes in the Berlin Questionnaire (p = 0.8358, chi-squared test); further, relationship status did not seem to influence the reported snoring frequency or intensity reported by **Category 1** (p = 0.7154, chi-squared test).

Table 1: *Participant demographic and clinical outcomes by OSA risk groups*

Demographic or clinical variable	Low Risk (n = 167)	High Risk (n = 43)	p-value
Age in years (± SD)	38.0 (± 7.2)	39.9 (± 8.6)	0.2676
Time on DTG in years (± SD)	2.6 (± 1.7)	1.9 (± 1.5)	**0.0435**
Female sex (%)	102 (61)	29 (67)	0.5540
Single (%)	81 (49)	17 (40)	0.8358
South African nationality (%)	104 (62)	30 (70)	0.5343
Employed (%)	98 (59)	21 (49)	0.5613
Tobacco use (%)	20 (14)	9 (21)	0.2920
Elevated blood pressure (%)	98 (57)	30 (74)	0.2487
Obesity by BMI > 30kg.m^{-2} (%)	52 (31)	29 (67)	**0.0000**
Dyslipidaemia (%)	102 (61)	29 (67)	0.5806
Metabolic syndrome (%)	25 (15)	9 (21)	0.4752

ALL COMPARISONS INVOLVING FREQUENCIES WERE TESTED STATISTICALLY USING A CHI-SQUARED (OR FISHER'S EXACT) TEST. DIFFERENCES IN MEAN (SD) WERE TESTED USING WELCH'S TWO-SAMPLE T-TESTS

4.3 METABOLIC SYNDROME:

Metabolic syndrome was inferred from the data by the criteria previously described in the Methodology. Metabolic syndrome was not associated with OSA risk outcomes (p = 0.4752, chi-squared test, **Table 1**). The low risk OSA group showed 15% prevalence of metabolic syndrome, while the high-risk group showed 21% prevalence of metabolic syndrome.

4.4 ANTHROPOMETRIC AND CLINICAL VARIABLES TAKEN AT EZINTSHA:

Some anthropometric and clinical variables taken during the primary visit to Ezintsha were measured in this study. The representation of these variables is divided into **Figures 7 to 14,** displaying the anthropometric variables, and **Figures 15 to 19** that display the clinical or biological variables.

Systolic blood pressure (**Figure 7**) was higher in the participants who were at high risk (131 mmHg [120 - 139 mmHg]) compared to those at low risk (127 mmHg [118 - 134 mmHg]) for OSA when considering the median values (Mann-Whitney U test, p = 0.0486).

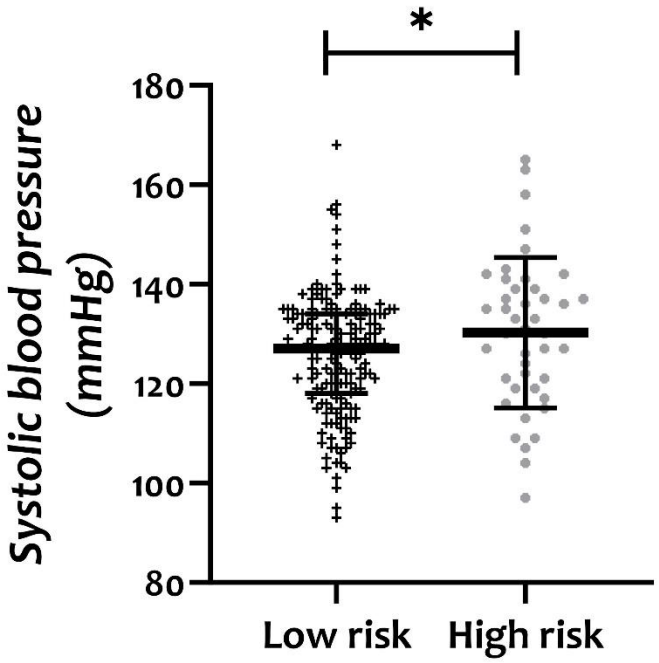

Figure 7: *Systolic blood pressure (mmHg) distribution plot for high risk and low risk OSA groups*

*Each individual measurement is represented as a point on the distribution plot. Bars indicate median ± interquartile range for each OSA risk group. Mann-Whitney U test; * p < 0.05 (p = 0.0486)*

Diastolic blood pressure (**Figure 8**), however, did not differ significantly between the two groups (p = 0.1275), with the low-risk OSA group presenting a median DBP of 83 mmHg [78 - 87 mmHg] while the high-risk OSA group had a median DBP of 86 mmHg [78 - 91 mmHg].

Mean arterial pressure (MAP) is the final of the five variables which comprise the cardiometabolic risk score. There was not a significant difference (**Figure 9**, Mann Whitney U test, p = 0.0636) between the low-risk (98 mmHg [92 - 102 mmHg]) and high-risk (101 mmHg [93 - 105 mmHg]) OSA groups regarding median MAP values.

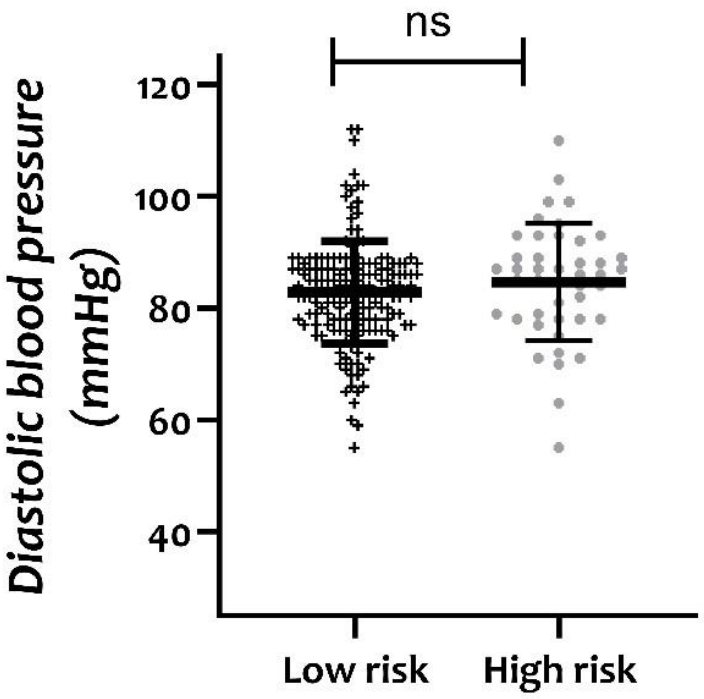

Figure 8: *Diastolic blood pressure (mmHg) distribution plot for high risk and low risk OSA groups*

Each individual measurement is represented as a point on the distribution plot. Bars indicate median ± interquartile range for each OSA risk group. Mann-Whitney U test; ns = p-value not significant (p = 0.1275)

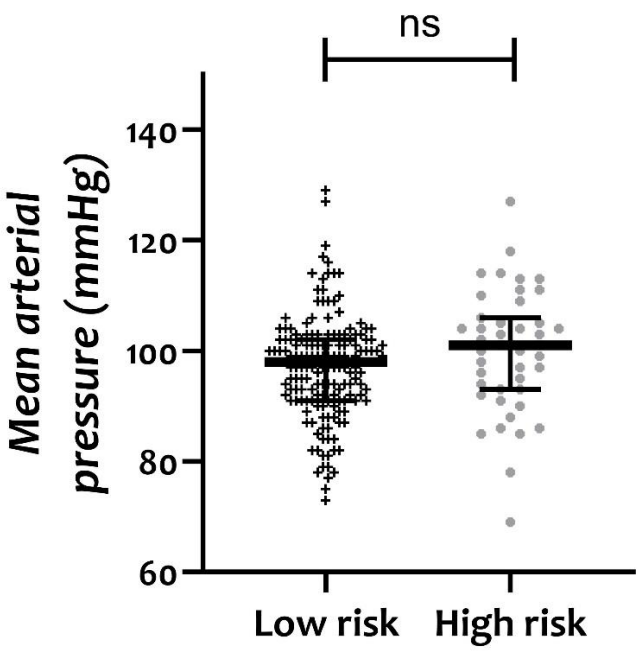

Figure 9: *Mean arterial pressure (mmHg) distribution plot for high risk and low risk OSA groups*

Each individual measurement is represented as a point on the distribution plot. Bars indicate median ± interquartile range for each OSA risk group. Mann-Whitney U test; ns = p-value not significant (p = 0.0662)

Height (**Figure 10**) was not significantly different between those at high risk for OSA and those at low risk for OSA (p = 0.5996). However, the median weight (**Figure 11**) was much greater (p < 0.0000) in the high-risk OSA group (86.9 kg [76.2 – 93.5 kg]) than in the group at low-risk for OSA (72.1 kg [63.8 – 82.9 kg]).

As expected, median BMI (**Figure 12**) in the high-risk OSA group was significantly higher in comparison to the low-risk OSA group (32.44 $kg.m^{-2}$ [27.88 – 35.67 $kg.m^{-2}$] vs. 26.46 $kg.m^{-2}$ [23.11 – 30.67 $kg.m^{-2}$], respectively, p<0.0001).

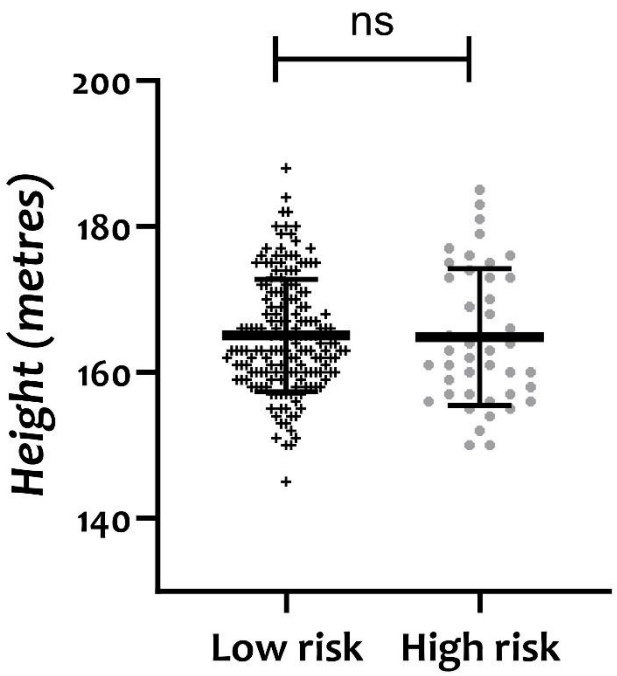

Figure 10: *Height (centimetres) distribution plot for high risk and low risk OSA groups*

Each individual measurement is represented as a point on the distribution plot. Bars indicate median (IQR) for each OSA risk group. Mann-Whitney U test; ns = p-value not significant (p = 0.5996)

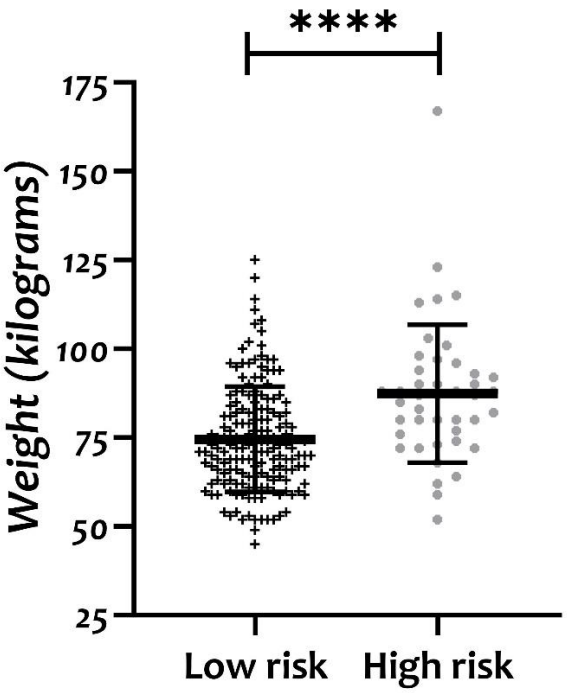

Figure 11: *Weight (kilograms) distribution plot for high risk and low risk OSA groups*

*Each individual measurement is represented as a point on the distribution plot. Bars indicate median (IQR) for each OSA risk group. Mann-Whitney U test; **** p < 0.0001 (p = 0.0000)*

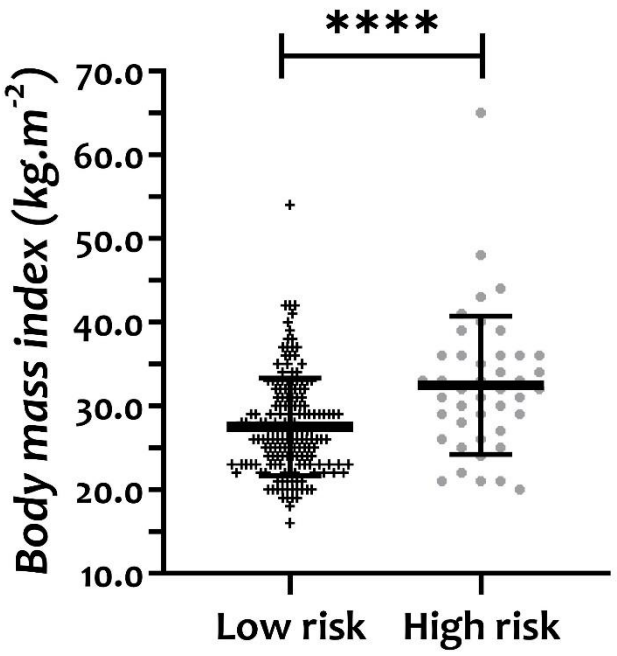

Figure 12: *Body mass index (kg.m-2) distribution plots indicating BMI distributions patterns of high risk and low risk OSA groups*

*Each individual measurement is represented as a point on the distribution plot. Bars indicate median (IQR) for each OSA risk group. Mann-Whitney U test; **** p < 0.0001 (p = 0.0000)*

Waist circumference (**Figure 13**) was much greater (mean ± SD, p = 0.0007) in the high-risk OSA group (100.2 cm ± 11.2 cm) than in the low-risk OSA group (89.2 cm ± 11.4 cm).

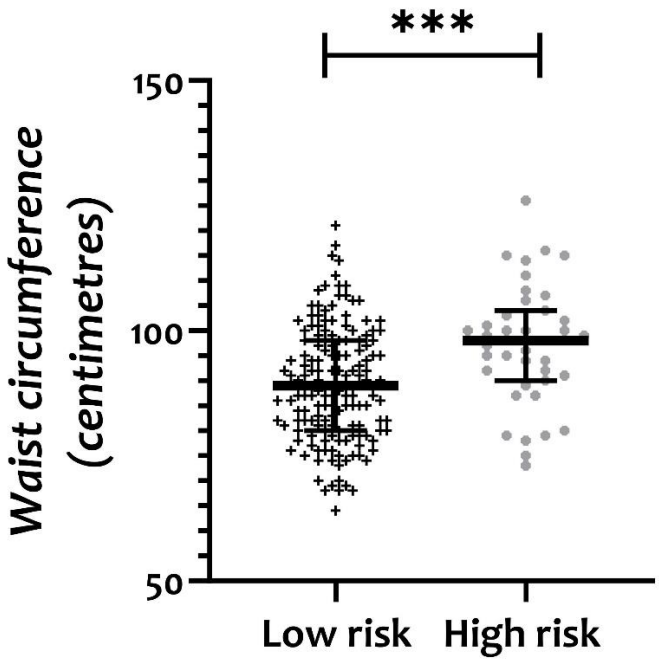

Figure 13: *Waist circumference (centimetres) distribution for high risk and low risk OSA groups*

*Each individual measurement is represented as a point on the distribution plot. Bars indicate mean ± SD for each OSA risk group. Welch's two-sample t-test; *** p < 0.001 (p = 0.0007)*

Waist circumference to height ratio (**Figure 14**) was not different between the high-risk (0.56) and low-risk OSA group (0.54), while waist-to-height ratios for both low-risk and high-risk groups were considered high risk values (> 0.49).

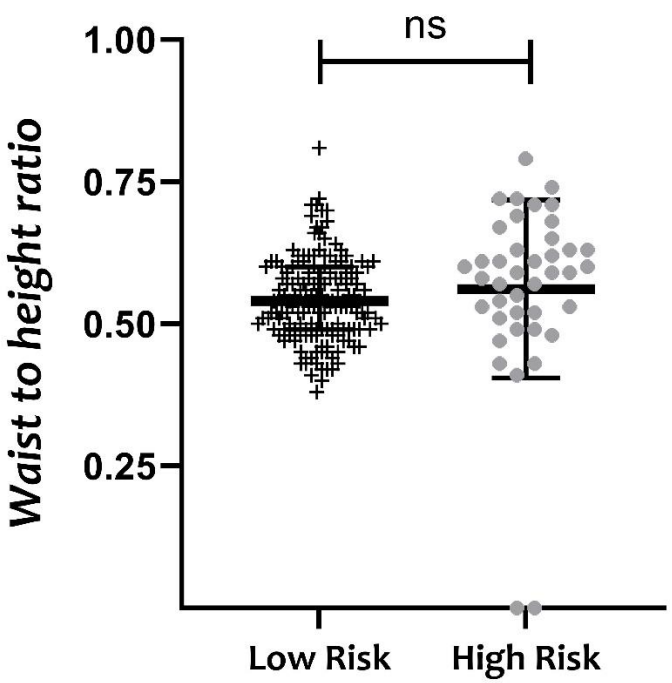

Figure 14: *Waist circumference to height ratio (centimetres) distribution for high risk and low risk OSA groups*

Each individual measurement is represented as a point on the distribution plot. Bars indicate mean ± SD for each OSA risk group. Welch's two-sample t-test (p = 0.475)

4.5 BIOLOGICAL VARIABLES:

Biological variables from blood plasma were measured and compared between high risk and low risk OSA groups (**Figs. 15 to 19**).

The plasma low-density lipoprotein cholesterol concentration (**Figure 15**) as quantified by routine blood testing was also compared between the high- and low-risk OSA groups, but the difference was not significant (p = 0.5793). The mean LDL-c for the high-risk group (2.52 ± 0.70 $mmol.L^{-1}$) was similar to the mean LDL-c concentration for the low-risk OSA group (2.42 ± 0.74 $mmol.L^{-1}$).

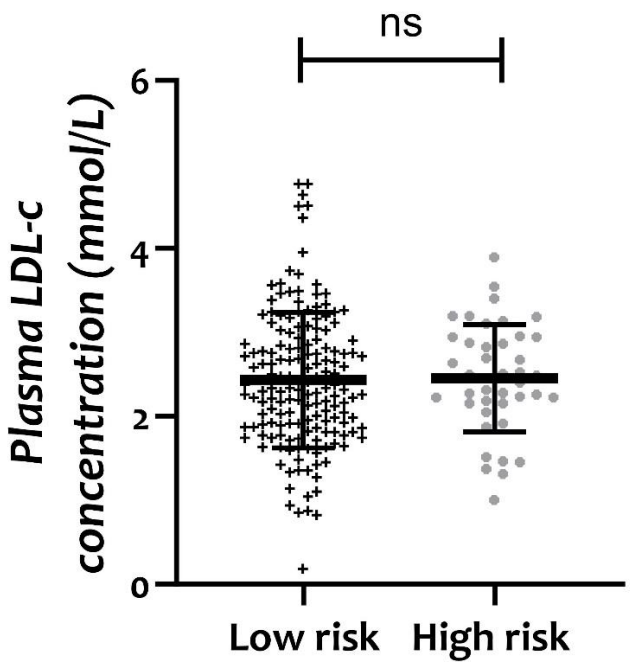

Figure 15: *Plasma low-density lipoprotein cholesterol (mmol.L^{-1}) distribution plot for high risk and low risk OSA groups*

Each individual measurement is represented as a point on the distribution plot. Bars indicate mean ± SD for each OSA risk group. Welch's two-sample t-test; ns = p-value not significant (p = 0.8474)

Plasma triglyceride concentrations (**Figure 16**) differed significantly (p = 0.0389) between participants at high risk for OSA (0.925 mmol.L^{-1} [0.75 – 1.23 mmol.L^{-1}]) and those at low risk for OSA (0.795 mmol.L^{-1} [0.61 – 1.08 mmol.L^{-1}]).

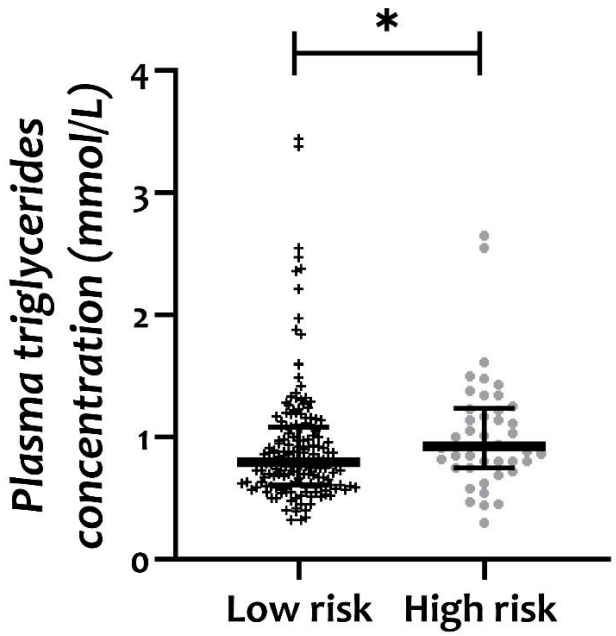

***Figure 16**: Plasma triglyceride concentration (mmol.L^{-1}) distribution plot for high risk and low risk OSA groups*

*Each individual measurement is represented as a point on the distribution plot. Bars indicate median ± interquartile range for each OSA risk group. Mann-Whitney U test; * p < 0.05 (p = 0.0389)*

Plasma high-density lipoprotein cholesterol concentrations (**Figure 17**) were not considered significantly different (p = 0.2004) for the low- and high-risk OSA groups. The median concentration in the low-risk group (n = 167) was 1.18 mmol.L^{-1} [0.99 – 1.42 mmol.L^{-1}]; the median concentration was 1.11 mmol.L^{-1} [0.96 – 1.38 mmol.L^{-1}] in the high risk group (n = 43).

Fasting glucose plasma concentration was not significantly different (p = 0.9824) between PLWH who were at high risk and low risk for OSA (**Figure 18**). The median [interquaritle range] fasting glucose plasma concentration for the high-risk was 4.70 mmol.L^{-1} [4.6 – 5.0 mmol.L^{-1}], and 4.80 mmol.L^{-1} [4.5 – 5.1 mmol.L^{-1}] in the low risk OSA groups.

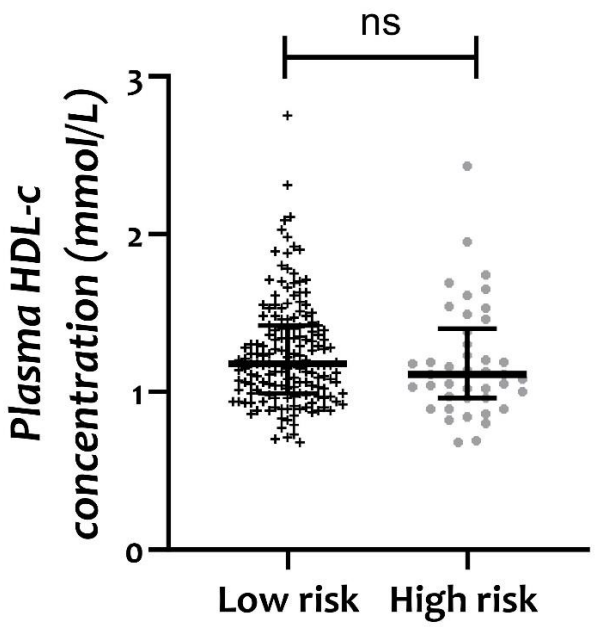

Figure 17: *Plasma high-density lipoprotein cholesterol concentration (mmol.L^{-1}) distribution for high risk and low risk OSA groups*

Each individual measurement is represented as a point on the distribution plot. Bars indicate median ± interquartile range for each OSA risk group. Mann-Whitney U test; ns = p-value not significant (p = 0.2004)

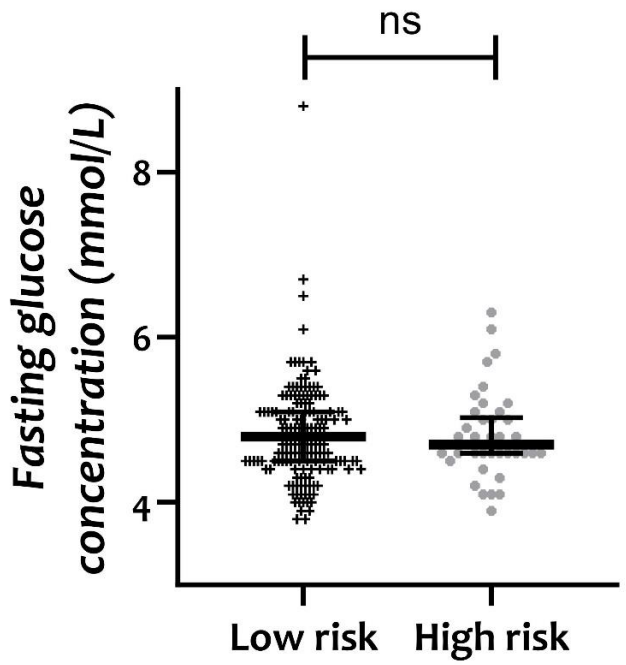

Figure 18: *Fasting glucose concentration (mmol.L-1) distribution plot for high risk and low risk OSA groups*

Each individual measurement is represented as a point on the distribution plot. Bars indicate median ± interquartile range for each OSA risk group. Mann-Whitney U test, ns = p-value not signiifcant (p =0.9824)

4.6 CARDIOMETABOLIC RISK SCORES:

A composite score of cardiometabolic risk variables was compared groupwise (Welch's two-sample t-test, alpha significance set at p = 0.05) between high risk and low risk groups (**Figure 19**, p = 0.0035). The distributions of cardiometabolic risk scores indicate that participants with low risk for OSA had a lower associated mean cardiometabolic risk score (-0.06 ± 0.53). The distribution of cardiometabolic risk scores in participants who had a high risk for OSA were higher, on average (0.20 ± 0.42).

Two of the CMR score variables (waist circumference and triglyceride concentrations) made significant contributions to the distributions of the composite CMR scores in low risk and high risk OSA groups. Three of the CMR score variables were not significantly different between the groups at high risk and low risk for OSA.

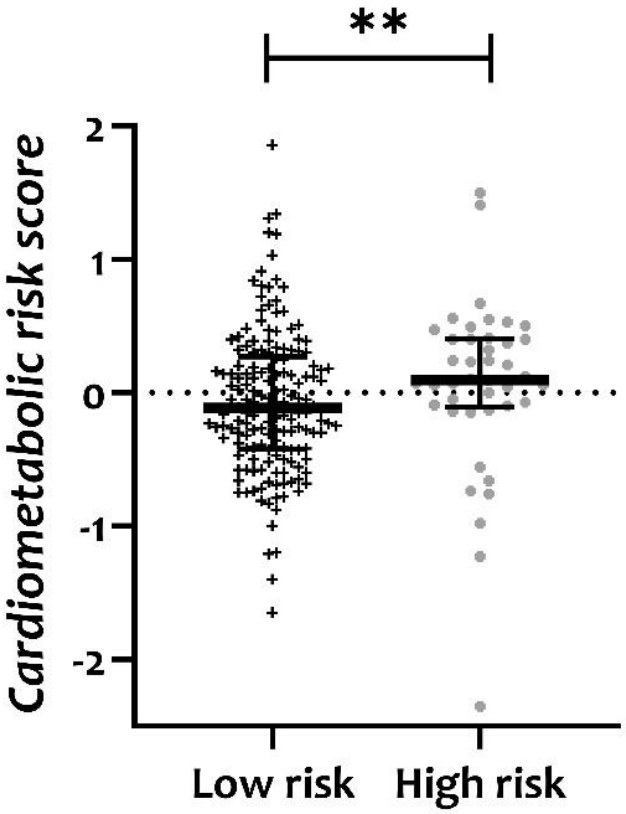

Figure 19: *Cardiometabolic risk scores as composite z-scores for participants with high risk and low risk for obstructive sleep apnoea by Berlin Questionnaire outcomes.*

*Z-scores are developed as explained in the methodology. Bars represent mean ± SD. Welch's two-sample t-test; ** p < 0.005 (p = 0.0035)*

4.7 MULTIVARIABLE ANALYSIS OF CARDIOMETABOLIC RISK SCORE:

We next investigate the association between the calculated composite cardiometabolic risk score and risk of sleep apnoea. We found higher cardiometabolic risk scores (**Figure 19**) in participants with high risk of sleep apnoea compared to those with low risk ($p < 0.0000$, Welch's two-sample t-test). We also tested the relationship between risk of sleep apneoa and metabolic syndrome status .

To further understand whether this relationship may be confounded by other variables, we tested the relationship between CMR score and Time on ART, CMR score and sex and between CMR score and age using simple bivariate analyses. Using the Pearson's correlation we found no correlation between time on ART and CMR score ($p = 0.5932$). Using an unpaired Student's t-test, we found also no association between sex and CMR score ($p = 0.8840$), but we found a positive significant correlation between age and CMR score (Pearson's correlation; $p = 0.0143$). Then, multivariable analysis using the method of least squares to fit general linear models (GLM procedure) showed that the association between CMR score and BQ outcome (high risk vs low risk sleep apnoea) remained significant, even when adjusting for age ($p = 0.0268$) and sex ($p = 0.9943$), whereby PLWH who had high risk of sleep apnoea had higher CMR score than those with low risk ($\beta=0.25$, $p=0.0085$).

Table 2: *Predictors of Cardiometabolic Risk score (outcome variable) in multivariable analyses*

	β	SE	P-value
Age, y	0.01	0.005	0.059
Sex (female)	-0.183	0.086	0.035*
Risk of OSA (high risk)	0.239	0.106	0.026*

*Multivariate analysis was performed using linear regressions (*p < 0.05)*
Pearson correlation of age and CMR was r value = 0.18, p = 0.014
SE: Standard Error

Using logistic regression (**Table 2**), we found that OSA status was not associated with the odds of having metabolic syndrome (OR = 1.059; 95% CI[0.434-2.581]). Being a woman (OR=4.875, 95% CI: 1.788 – 13.514) and of older age (1.062, 95% CI: 1.008 – 1.118) were independently associated with higher odds of having metabolic syndrome (**Table 3**). Further association tests for CMR score and metabolic syndrome

outcomes showed that there was a significant correlation between having a positive CMR score and presenting with metabolic syndrome (**Table 3**).

Table 3: *Associations with Metabolic Syndrome (outcome variable) by point estimates*

	OR	95% CI
Age, y	1.062	1.008 – 1.118
Sex (ref = male)	4.875	1.758 – 13.514
Risk of OSA (high risk)	1.059	0.434 – 2.581

CI: Confidence Interval; OR: Odds Ratio

4.8 Data arising from the plasma analysis of cytokines

We were able to assay cytokines only in a subsample of 95 participants (n=24 (25%) with high risk of OSA). Table 4 below shows the participants' characteristics and cytokine concentration results in participants in the low risk OSA vs the high risk OSA groups.

Table 4: Clinical variables and cytokine distributions in high-risk and low-risk OSA groups

Clinical and demographic outcomes	**Low-Risk OSA** ***n = 71 (75%)***	**High-Risk OSA** ***n = 24 (25%)***	**p-value***
Female sex, n (%)	45 (63%)	16 (67%)	0.811
Mean age, years (± SD)	38.9 (± 6.3)	40.8 (± 7.1)	0.253
Median CMR score [IQR]	-0.02 [-0.29 – 0.31]	0.23 [-0.02 – 0.41]	0.072
Elevated blood pressure, n (%)	44 (66%)	16 (67%)	>0.99
Dyslipidaemia, n (%)	44 (65%)	14 (61%)	0.804
Mean TGL:HDL ratio (± SD)	0.77 (± 0.05)	0.88 (± 0.11)	0.372
Mean WC:Height ratio (± SD)	0.56 (± 0.07)	0.61 (± 0.09)	**0.026**
Cytokine concentrations ESM-1 (ng/mL)	*Median [IQR]* 3.45 [2.67 – 4.76]	*Median [IQR]* 3.85 [3.07 – 6.16]	0.581

MCP-1 (pg/mL)	426 [316 – 587]	467 [358 – 761]	0.299
IL-1β (pg/mL)	761 [554 – 1064]	802 [636 – 1218]	0.516
IL-6 (pg/mL)	111 [79.1 – 163]	133 [98.5 – 218]	0.269
IL-8 (pg/mL)	436 [317 – 570]	496 [368 – 718]	0.436
TNF-α has been omitted	**27/71 quantified**	**11/24 quantified**	**Low power**

**ALL COMPARISONS INVOLVING FREQUENCIES WERE TESTED STATISTICALLY USING A CHI-SQUARED (OR FISHER'S EXACT) TEST. DIFFERENCES IN MEAN (±SD) WERE TESTED USING WELCH'S TWO-SAMPLE T-TESTS FOR NORMALLY DISTRIBUTED DATA, AND MEDIAN [IQR] DIFFERENCES TESTED USING WILCOXON RANK-SUM TESTS FOR NON-NORMALLY DISTRIBUTED DATA.*

4.8.1 Clinical variables and cytokine distributions in high-risk and low-risk OSA groups

The differences between the clinical and demographic variables of the low-risk and high-risk OSA sub-groups that were selected for cytokine analysis were minimal (**We were** able to assay cytokines only in a subsample of 95 participants (n=24 (25%) with high risk of OSA). Table 4 below shows the participants' characteristics and cytokine concentration results in participants in the low risk OSA vs the high risk OSA groups.

Table 4). The age, sex distribution, hypertension status, presence of dyslipidaemia and CMR score were not significantly different between the sub-groups.

The mean TGL:HDL ratios (**We were** able to assay cytokines only in a subsample of 95 participants (n=24 (25%) with high risk of OSA). Table 4 below shows the participants' characteristics and cytokine concentration results in participants in the low risk OSA vs the high risk OSA groups.

Table 4) did not differ, with the difference between means (± SEM) being 0.110 ± 0.122 and (95% CI: -0.137 - 0.357). We found a significant difference (p = 0.026, Welch's two-sample t-test) in mean waist circumference-to-height ratios between the low-risk and high-risk OSA subgroups, with the difference in means (± SEM) being 0.049 ± 0.021 (95% CI: 0.006 - 0.091 between the high risk OSA (higher WHR) and the low risk OSA.

All cytokines except for TNF alpha were at detectable levels in the 95 participants. Because of the low number of participants having TNF alpha values, we did not

present those results. For all other 5 cytokines, we found no difference in cytokine plasma concentration in the high risk vs. low risk OSA groups.

4.8.2 Associations of clinical data with ESM-1 and MCP-1 concentrations in high-risk and low-risk OSA groups

MCP-1 and ESM-1 have been chosen for a multivariate analysis as there are no reported South African analysis research studies which look into the relationship between these markers, OSA, HIV and CMR.

MCP-1 (**Table 5**) and ESM-1 (**Table 6**) concentrations were not correlated with nor associated with age, sex or risk of obstructive sleep apnoea.

Table 5: *Multivariate analysis for Monocyte Chemo-attractant Protein-1 (MCP-1)*

	β	SE	P-value
Age, y	0.008	0.008	0.305
Sex (female)	0.111	0.108	0.309
Risk of OSA (high risk)	0.090	0.115	0.437

Multivariate analysis was performed using GLM regression operant.
*(*p < 0.05) SE: Standard Error*

Table 6: : *Multivariate analysis for Endothelial Cell-Specific Molecule-1 (ESM-1)*

	β	SE	P-value
Age, y	0.015	0.035	0.660
Sex (female)	0.447	0.472	0.346
Risk of OSA (high risk)	0.219	0.504	0.664

Multivariate analysis was performed using GLM regression operant.
*(*p < 0.05) SE: Standard Error*

5 DISCUSSION:

5.1 SUMMARY OF SIGNIFICANCE OF RESULTS:

As an overview summary to keep in mind for understanding our results, it is necessary to contextualize our findings. The cohort of PLWH was mostly comprised of cis-gender women, of a relatively young age, where at least two thirds of the cohort had hypertension or obesity or both.

Using the Berlin Questionnaire, there was a 20.5% prevalence of high risk of obstructive sleep apnoea. Persons from the high-risk OSA group had a greater body mass index, higher WC, and higher triglycerides than low-risk OSA participants.

We also found that people with high risk of OSA had higher cardiometabolic risk, as measured with a previously developed continuous cardiometabolic risk score composed of five clinical and anthropometric measurements associated with cardiometabolic risk. As shown previously, this continuous cardiometabolic risk score was significantly higher in those with metabolic syndrome compared to those without metabolic syndrome, making the continuous cardiometabolic risk score a reliable measurement of cardiometabolic risk in this cohort. In contrast, when using the presence of metabolic syndrome as a cardiometabolic risk measurement, we found no association between high risk of OSA and risk of having metabolic syndrome. Using a continuous cardiometabolic risk score, rather than a dichotomous measurement like metabolic syndrome, may increase the instrument's sensitivity to detecting early cardiometabolic risk changes.

Finally, contrary to what we expected, we did not find differences in plasma cytokine concentration in the group with high risk vs. the group with low risk of OSA.

5.2 Obesity and hypertension: the undermined national pandemic

In our sample, we found an overall prevalence of obesity of 38.5% (62% in those with high risk OSA) and of dyslipidaemia of 62%. As early as 1998, the South African population has been reported to have a substantial sex-dependent incidence of obesity. The national Demographic and Health Survey outcomes of obesity for 1998 - as detailed in a 2002 report (Puoane et al., 2002) - showed that of 13 089 men and women surveyed, 29% of men were obese while 57% of women were obese. Despite how staggering these statistics of obesity were thirty years ago, anthropometric measurements have since been on a steady rise (with 2016 obesity rates among women of South Africa sitting at 68%, and 31% obesity rates among men (National Department of Health (NDoH) et al., 2019)). Our sample made up of 100% PLWH, of which 60% were women, thus had a prevalence of obesity similar to that of the general population where 14% live with HIV.

With the context of South African obesity prevalence in mind, we must consider the obesity rates of PLWH over the last twenty years. A meta-analysis (Gizamba et al., 2023) of obesity, diabetes, or hypertension outcomes in PLWH pooled data from 32 studies of PLWH in South Africa, published between 2004 and 2020, amounting to data from 123,951 PLWH. The authors split the period examined in two; in particular: post-2004 - when ARVs were relegalized in South Africa - and then from 2013, which corresponds to the change in ARV policy. Pre-2013, ARVs were given based on CD4 counts reaching 350 copies.mm3 for persons found to be HIV positive (Iwuji et al., 2018); the change in policy prioritized initiating treatment as soon as the patient was found to be HIV positive.

This meta-analysis included 19 studies that had measured obesity prevalence data for 13 779 PLWH and reported that the pooled prevalence of obesity amongst PLWH was 25.8% between 2013 and 2020. This prevalence had increased from the previous examined period (2004 to 2013) period (number of studies = 8), where the pooled prevalence of obesity was only 19.7%. The pooled obesity rates over the entire study period showed obesity to be higher in women (23.6%) compared to men (12.1%). The same meta-analysis publication determined pooled hypertension prevalence among PLWH over the 2000-2022 period to be 25.5% (2000-2012: 34.1% vs 2013-2022: 20%). These twenty-year averages are good indicators of the upward trends for changes in obesity and hypertension rates among PLWH. However, the hypertension data from this CHARACTERISE cohort are two times greater than the estimated prevalence. With 61% of the cohort having hypertension, an important variable must be brought to the attention of the reader: the shift from first-line ART regimens being Tenofovir, Lamivudine, Efavirenz (TLE) to the Tenofovir, Lamivudine, Dolutegravir (TLD) regimen.

The obesity and hypertension results from this study, seven years after DTG-rollout, suggest that CMD prevalence will continue to climb among PLWH. Being overweight is a strong distinguishing factor for the risk of OSA in this cohort; BMI and waist circumference were significantly higher in the high-risk OSA group, while height did not differ between the groups. The waist circumference-to-height ratio was not significantly different between the two OSA risk groups (yet both were considered within the range for concern for CMR (0.49 < waist-to-height ratio < 0.59)), but remain

potentially useful for ascertaining risk of increased cardiometabolic disease by weight distribution in this cohort, considering the high prevalence of overweight across both OSA risk groups, and the contribution of central fat distribution to cardiometabolic disease risk (Després, 2012). Refining the way in which we study the prevalence of obesity to reflect rather the distribution of fat over the body may improve the OSA screening specificity in populations that have high obesity rates.

We found an overall prevalence of hypertension (elevated blood pressure in table 1) of 61% in our population, with no difference in prevalence between those high risk and those with low risk of OSA. Untreated hypertension among PLWH takes many steps back for improving quality of life for PLWH, as the risk for atherosclerosis and thrombosis is exacerbated with high blood pressure. Sleep apnoea additionally creates a cycle of vascular endothelial stress by increased reactive oxygen species from hypoxic events that places the cardiovascular system under pressure (Budhiraja et al., 2007). In turn, blood pressure increases, and vascular tissue undergoes architectural changes (Arnaud et al., 2020); if left undiagnosed or untreated, OSA will drive an increase in hypertension rates and subsequent cardiometabolic disease risk.

The results of obesity and hypertension from CHARACTERISE are substantially greater than the national population estimates of obesity and hypertension. The Ndlovu cohort (Vos et al., 2020), nested in rural South Africa, of similar mean age and sex distribution to participants from CHARACTERISE showed a much lower prevalence (~ 30 %) of obesity, hypertension, or dyslipidaemia (Roozen et al., 2019) – though 70% of this cohort was unemployed and the majority was living below the poverty line (Vos et al., 2020). In contrast, this CHARACTERISE cohort had a 29% unemployment rate which supposes a much lower rate of persons living below the poverty line. This suggests that socioeconomic status might influence the progression of CMD when comparing urban and rural population groups of PLWH, as our peri-urban cohort seems to display higher percentage employment (68%) and hypertension or obesity (75%). This trend of rising cardiometabolic risk factors with changing demographics speaks to the theory of the epidemiological transition (Omran, 2001), whereby a rise in “man-made diseases” like cardiometabolic diseases correlates to an urbanization of persons previously living in rural areas, and the change in lifestyle that comes with this shift (Freire & Aparecida, 2020).

Hypertension across both OSA risk groups in 61% of the cohort are concerning; this distribution of PLWH with hypertension impresses upon the importance of detecting and treating hypertension early; increasing knowledge-sharing about risks for and signs of hypertension with participants; and encouraging learning about hypertension status to empower people in managing their cardiometabolic risk. Of the 128 of 210 people across the cohort who had hypertension, only 7 of 128 were taking antihypertensive medication. These data urge, then, that recognizing the possible role of obstructive sleep apnoea in exacerbating CMR factors like hypertension and central obesity among PLWH should become a national imperative by OSA screening for improving HIV point-of-care practices (Owens and Hicks, 2018).

5.3 Is there a relationship between OSA classification criteria and cardiometabolic risk amongst PLWH in South Africa?

Amongst those at high-risk for OSA by the BQ, 95% had a positive score for category 3 (high BP or obese BMI). Though this was significantly greater ($p = 0.0012$) than the 70% of participants who had a high BP or obese BMI in the low-risk OSA group, 70% of participants scoring positively here still suggests that there may be a low sensitivity and specificity for this category of the questionnaire in this cohort.

If we contrast the percentage of participants who had positive scores for category 1 – snoring frequency and intensity – in the low-risk OSA (~5%) relative to the high-risk group (~88%), we can say with more certainty ($p < 0.0001$) that the sensitivity and specificity of this category for distinguishing risk groups is much stronger in this cohort of PLWH.

While statistically significantly more participants in the high-risk group (9 of 43 participants) scored positively for category 2 than those in the low-risk group (8 of 167 participants), only one question within the category showed significant results for distinguishing low- and high-risk groups: Question 7 ($p = 0.0016$). Twenty-three participants (53.5%) reported feeling fatigued after sleeping at least three times per week in the high-risk group, while only six participants (3.6%) reported this in the low-risk group.

Categories 1 and 2 of the Berlin Questionnaire may be the real discriminators for classification into high and low risk of sleep apnoea in this cohort, considering the difference in snoring frequency and intensity between the two OSA risk groups. Category 3 of the Berlin Questionnaire is the only category that is specifically screening for cardiometabolic risk measurement as it asks for a participant's hypertension and obesity status. As such, scoring high-risk for sleep apnoea by the BQ may not align with true cardiometabolic risk, if we consider that most of both low-risk and high-risk OSA participants have hypertension or obesity. Ultimately, proper validation of this questionnaire against the gold standard, i.e., an objective overnight sleep recording using polysomnography and subsequent OSA diagnosis by apnoea/hypopnoea indices is an imperative bridging variable methodology which will be necessary prior to making suggestions on how to modify the BQ to this South African context.

In other studies, the prevalence of OSA has variability based on sex distribution, ancestry, and age. Sex distribution does not differ in this cohort, however. A South African study conducted some years ago in the North West province (Malan et al., 2016) used the BQ as a screening tool amongst 329 teachers between the ages of 20 – 65 years of age. At the baseline visit, 35.7 % screened for high risk by the BQ. Though this number is higher than the high-risk OSA proportion of the CHARACTERISE cohort, the age distribution is of a wider range, and the population size is one third bigger. The outcomes of the teacher study support our findings that increasing age plays a significant role in increasing risk for sleep apnoea, but greater population sample sizes are crucial for understanding OSA prevalence in South Africa.

In an adult Korean population (Sunwoo et al., 2018), the BQ outcomes of one study included over 2000 people – 15% of whom scored a high-risk on the questionnaire. This study also associated BQ outcome with increased body mass index and age and showed increased odds of hyperlipidaemia and hypertension with a high-risk for OSA. Finally, in a survey of truck drivers in Brazil who are very near in age to the CHARACTERISE cohort (39 ± 9.9 years), 26% of those surveyed by BQ had a high-risk for OSA. However, the size of this group was much greater (over 10 000 people surveyed) than the CHARACTERISE cohort, and associations were found between BQ outcome and both drug use and smoking cigarettes (Moreno et al., 2004). Taken together, the global understanding of risk factors for sleep apnoea that remain relevant

to a South African context include age, obesity, hypertension, and dysregulated lipid metabolism. Still, there remains a fundamental distinction between the success of BQ elsewhere and the success of BQ in South Africa (requiring validation of the questionnaire) as our population seems to display a unique OSA-risk phenotype.

5.4 Cardiometabolic risk score or Metabolic Syndrome? The strength of variables that change over time:

The continuous composite cardiometabolic risk score was only recently established with relevance to a southern African context of OSA (Roche et al., 2021). Prior to the use of this continuous score, a static score for metabolic syndrome (Grundy et al., 2005) has historically been used to frame cardiometabolic risk. The metabolic syndrome classification indicates a tally of binary outcomes; for example, having high blood pressure and high triglyceride levels means that two out of five criteria for metabolic syndrome are met. Here, the person would be declared as not having metabolic syndrome (at least three criteria must be met to have metabolic syndrome).

In contrast, a continuous composite cardiometabolic risk z-score using the same variables as those used in the metabolic syndrome classification standardizes the sample population measurement of each variable comprising the score and is sensitized to the notion of discrete obesity phenotypes (Swartz et al., 2024). By this consideration: even for an individual who has dangerously high blood pressure as their only “risky” variable, the cardiometabolic risk score will offer a more accurate indication of overall cardiometabolic risk than binary “presence/absence” tools of metabolic syndrome.

Positive cardiometabolic risk scores were associated with being at high risk for OSA in this cohort. The mean (±SD) score among the high-risk group was 0.20 ± 0.42 while the low-risk group had a significantly lower CMR score (-0.06 ± 0.53). This association of a higher cardiometabolic risk with high OSA risk is lost when comparing metabolic syndrome presence in low- and high-risk groups: 21% of high-risk participants had metabolic syndrome, and 15% of low-risk participants had metabolic syndrome. These results, taken together, suggest that while metabolic syndrome is associated with a positive continuous cardiometabolic risk score, metabolic syndrome is not a strong dichotomizing standard when seeking to group PLWH into high- and low-risk for OSA.

There is research that champions the use of waist-to-height ratio (WHtR) in South African populations (Gradidge et al., 2022) to more accurately score MetSyn than WC as a measure of obesity in the composition of MetSyn classification, so reconfiguring a contextually-relevant MetSyn make-up to South African groups may further improve the associations of MetSyn to CMR scores.

Along with the cardiometabolic risk score's relevance in OSA risk, specific components of the score are weighty contributors to outcome. Average plasma triglyceride concentrations are greater in the high-risk than in the low-risk group. Though not statistically significantly different between OSA risk groups, a total of 115 out of 210 participants had lower HDL-c levels than desired (< 1.03 mmol.L-1 for men and < 1.3 mmol.L-1 for women; Grundy et al., 2018). This remains a crucial consideration for the context of dyslipidaemia as a primary causal factor in atherosclerosis (Davidson & Pradeep, 2023), again proposing that the presentation of OSA in South African populations relies on including acute biological variables (like lipid profiles and inflammatory markers relating to vascular remodelling) rather than "traditional" risk variables such as snoring frequency, obesity, and hypertension.

5.5 Obesity phenotypes

As an extension of the strength of cardiometabolic risk variables that change over time, and applying the literature which suggests discrete obesity phenotypes exist among PLWH (Swartz et al., 2024), there is value in considering not only the CMR score differences between the high-risk and low-risk OSA groups, but also the importance of waist-to-height measures of central obesity, and the triglyceride-to-HDL cholesterol measurement for dysregulated cholesterol balance.

5.5.1 CMR by waist-to-height ratio

The downfall of using BMI-defined obesity measures is that different types of fat stores in specific areas of the body are considered variably-significant contributors to CMR of people with obesity (Ross et al., 2020). Body fat percentage, visceral (waistline) fat stores and central fat distribution (on the torso of the body) are all markedly more successful in understanding true cardiometabolic risk than simply measuring BMI as an obesity indicator (Davidson et al., 2019).

In the OSA-risk groups (**Figure 14: Waist circumference to height ratio (centimetres) distribution** for high risk and low risk OSA groups), waist circumference to height ratios (WHtR) are not statistically significant between low-risk and high-risk OSA groups, despite large significant differences being observed in BMI (**Figure 12**). What is notable here is that regardless of OSA risk, both groups are at increased risk for CMD by these ratios alone, where mean WHtR greater than 0.49 are taken to be of concern for cardiovascular and metabolic wellbeing (Browning et al., 2010).

When we look at the difference then between WHtR of the cytokine-analysis OSA-risk subgroups, reporting that statistically significant difference (p = **0.026)** exists between the low-risk (0.56 (± 0.07), Table 4) and high-risk OSA groups (0.61 (± 0.09), Table 4) is then a compelling outcome. This sub-sample has 3 out of 4 PLWH at low-risk for OSA rather than the population group which had 4 out of 5 PLWH at low-risk for OSA, which suggests that as the number of people with high-risk OSA increases, the subtle differences in CMR measures become starker. Still, it must be noted that even the low-risk OSA group has a WHtR that exceeds 0.50, which places the entire cohort population within a concerning WHtR that pre-empts early CMD risk (Ashwell & Gibson, 2016).

5.6 Specific endothelial activation and immune recruitment in high-risk and low-risk OSA groups among PLWH

5.6.1 CMR by triglyceride-to-HDL cholesterol ratios

When observing the triglyceride-to-HDL cholesterol scores as biochemical indicators of CMR, keeping the importance of the above WHtR in mind explains why there could be strong similarities between the ratios for both sub-groups (difference between means (± SEM) being 0.110 ± 0.122 (95% CI: -0.137 - 0.357), where p = 0.372). Typically, TG:HDL ratios greater than 2.5 have been considered a risk for cardiometabolic wellbeing (Salazar et al., 2013) however, there are also research papers which suggest that people of Black African ancestry who have high CMR frequently have “paradoxically” healthy cholesterol profiles . Recalling how cholesterol is implicated in the endothelial activation and monocyte infiltration pathways of the vasculature, the regulation of cholesterol metabolism among PLWH who take DTG-based ART should be investigated with greater care and specificity.

In this cohort sub-sample, TGL:HDL-c ratios remain less than 2.0 (0.77 (± 0.05) in low-risk OSA and 0.88 (± 0.11) in high-risk OSA, p = 0.372), indicating that there is indeed a necessity to create locally-relevant and nationally informed criteria for dyslipidaemia variables amongst South African residents before research studies such as this can dictate whether OSA risk is increased by cholesterol dysregulation. The elevated triglyceride concentrations and dyslipidaemia percentage across the cohort is suggestive of endothelial activation pathways being elevated among PLWH who take DTG-based ART, even before the OSA-risk and OSA-influence on CMD progression can be better considered.

5.6.2 HIV and OSA risk relating to MCP-1 and ESM-1

The outcomes of the multivariate analysis performed on two of the five chosen cytokines does not correlate ESM-1 and MCP-1 concentrations to increased OSA risk. This is not an expected result, as the literature strongly links ESM-1 and MCP-1 to inflammatory states associated with HIV, CMR, and OSA. In pursuing an explanation for this, concentrations of ESM-1 and MCP-1 in populations of PLWH and people with OSA of various severity (**Table 7**) were researched.

In the low-risk OSA cytokine sub-group (**We were** able to assay cytokines only in a subsample of 95 participants (n=24 (25%) with high risk of OSA). Table 4 below shows the participants' characteristics and cytokine concentration results in participants in the low risk OSA vs the high risk OSA groups.

Table 4), median [IQR] ESM-1 concentration was 3.45 ng/mL [2.67 – 4.76 ng/mL]. This is nearly three times greater than the highest reported concentration among people with diagnosed OSA (Bingol et al., 2016). Similarly, the high-risk OSA cytokine sub-group (**We were** able to assay cytokines only in a subsample of 95 participants (n=24 (25%) with high risk of OSA). Table 4 below shows the participants' characteristics and cytokine concentration results in participants in the low risk OSA vs the high risk OSA groups.

Table 4) has a much greater median concentration (3.85 ng/mL [3.07 – 6.16]) compared to the highest reported concentration, with a quarter of our group having concentrations nearly five times greater than in severe sleep apnoea (Bingol et al., 2016).

MCP-1 concentrations by Luminex quantification average around 137 pg/mL among PLWH (Zungsontiporn et al., 2016), while in study participants who are diagnosed with OSA the concentration ranges from 71pg/mL in moderate OSA (Kim et al., 2010) to 130pg/mL (Ohga et al., 2003) when measured by enzyme-linked immunosorbent assay (ELISA). Though this concentration increases when measured by Luminex, the mean mixed apnoea severity MCP-1 concentration (~280 pg/mL, Wen et al., 2020) remains much lower than the concentrations measured (**We were** able to assay cytokines only in a subsample of 95 participants (n=24 (25%) with high risk of OSA). Table 4 below shows the participants' characteristics and cytokine concentration results in participants in the low risk OSA vs the high risk OSA groups.

Table 4) in the median low-risk OSA cytokine subgroup (426 pg/mL [316 – 587 pg/mL]) and the high-risk OSA subgroup (467 pg/mL [358 – 761 pg/mL]. It can be thought that either the BQ has not sensitively and specifically screened for OSA in our cohort such that oxidative stress is elevated cohort-wide; or, that with increased CMR from obesity and hypertension in our cohort the burden on the vascular remodelling is independently sufficient to drive a tremendous rise in monocyte infiltration of the vasculature, causing this substantial increase of MCP-1. Alternatively, there could be another mechanism independent of OSA and ART that is elevating monocyte chemoattraction protein-1 levels.

Table 7: *Literature-based research study outcomes for the measurement of MCP-1 and ESM-1 amongst people with diagnosed OSA*

Research Study Reference	*Population*	*Method*	*Control [mean]*	*OSA [mean]*
Ohga, et al., 2003 MCP-1	20 male OSAS diagnosed / 10 male control	ELISA	~ 25 pg/mL	~ 130 pg/mL
Kim et al., 2010 MCP-1	37 diagnosed OSA / 22 control	ELISA	32 pg/mL	71 pg/mL (moderate, n = 9) and 111 pg/mL (severe, n=28)
Wen et al., 2020 MCP-1	52 control, 44 mild OSA, 44 moderate OSA, 67 severe OSA	Luminex	Around 250 pg/mL (n = 52)	~280 pg/mL for mixed apnoea severity (n = 155)
(Altıntaş et al., 2015) ESM-1		ELISA	0.0248 ng/mL (n = 40)	0.04 ng/mL (moderate, n = 14) 0.0568 ng/mL (severe, n = 26)

(Kanbay et al., 2016) ESM-1	43% women; mean age 51 years; n = 128	ELISA	0.9 ng/mL (n = 15)	3.14 ng/mL (mild, n = 22); 5.32 ng/mL (moderate, n = 22); 9.87 ng/mL (severe, n = 69)
(Kum et al., 2023) ESM-1	Patients reported daytime sleepiness but were excluded if any co-morbid CMDs	ELISA	0.214 ng/mL (n= 16)	0.242 (mild, n= 33), 0.282 (moderate, n = 13), 0.292 (severe, n= 16)
(Bingol et al., 2016) ESM-1	Control had negative STOPBANG but no PSG, experiment group confirmed OSA with PSG	ELISA	**0.93 ± 0.3 ng/ml, (n = 43)**	**1.25 ± 0.4 ng/ml (n = 63, OSA)**

Primary reasons for differences in these concentrations (**Table 7**) in comparison to our data could be the difference between ELISA and multiplex methods of quantifying biomarkers, as well as the use of the high-sensitivity kits to quantify biomarkers in our research study, where specificity is much greater than standard multiplex kits are. However, if the concentration of ESM-1 and MCP-1 were more strongly and frequently represented in OSA and HIV literature, we could have a strong case for these markers being associated with not only presence of sleep apnoea, but severity of sleep apnoea.

Potential reasoning relating to HIV treatment for why both the low-risk and high-risk OSA groups have incomparably high concentrations of ESM-1 and MCP-1 is difficult to substantiate from literature due to lack of evidence in South African contexts. However, the possibility that PLWH who take DTG-based ART are independently at greater risk for exacerbated endothelial activation and monocyte-infiltration of the vascular system than other PLWH taking different medication is a strong supposition. The possibility of poor ART adherence of the low-risk group or the possibility of insufficient research among PLWH of African ancestry also factor as potential reasons for non-differing concentrations between OSA risk groups.

5.7 Future considerations related to the context of this project

5.7.1 Dyslipidaemia suggests increased atherosclerosis risk and progression:

From our results, triglyceride concentrations are elevated in people living with HIV who have a high risk for OSA by the Berlin Questionnaire. The relationship between lipoprotein aggregation in atherosclerosis progression and types of lipoproteins in

circulation previously described suggests that, while the relative concentration of plasma LDL-c does not differ between OSA risk groups, measuring the discrete lipoprotein constituents in circulation – especially oxidized LDL rather than LDL-c (Grundy et al., 2018) – may improve our understanding of plasma lipid profiles in PLWH who are at higher risk for OSA. Triglyceride concentration - potentially supplemented with measuring LDL-particle size and LDL oxidation levels in future studies - is a relevant and necessary plasma indicator of risk for OSA in this population.

Obstructive sleep apnoea is central to the connections between obesity, cardiovascular health, and metabolic disorders. As described in the introduction, hallmark features of obstructive sleep apnoea's impact on cardiometabolic risk factors include a shift in blood lipid metabolism, increased pressure on the cardiovascular system, and chronic oxidative stress. In a healthy state, anti-inflammatory adipokine production and circulation contributes to lipid balance in the blood, maintaining healthy cholesterol profiles, insulin function and a cardioprotective blood vessel architecture (Weiss et al., 2009). Though we have not measured adipokines in the plasma of the participants in this study, the dysregulated lipid profiles across the cohort suggests that adipokine secretion will likely be impaired in PLWH who show DTG-related weight gain. Independent of the risk for sleep apnoea, compromised lipid profiles clearly perpetuate the risk of diabetes, atherosclerosis, and metabolic syndrome. Statin therapy to manage cholesterol and triglyceride levels (Stone et al., 2013) for PLWH who take dolutegravir should not be undervalued as a strong early intervention strategy to manage CMD symptoms and progression (Grinspoon et al., 2023).

5.7.2 Oxidative stress, endothelial dysfunction, coagulation, and cardiovascular health: could OSA be a catalyst?

A crucial consequence of obstructive sleep apnoea is chronic sympathetic nervous system (SNS) activation. As explained at length, this arises from reduced blood oxygen saturation, and an increased state of adrenergic tone through the body which persists through the day (Weiss et al., 2015). Increased SNS activity is associated with increased inflammation that often causes long-term health issues like increased cardiovascular disease risk and hypertension (DeLalio et al., 2020).

Although the stimulators for endothelial dysfunction include circulating inflammatory markers, hypertension, metabolic disorders, and dyslipidaemia (Gimbrone and García-Cardeña, 2016), endothelial dysfunction is hallmarked by a decreased nitric oxide presence (De Caterina et al., 1995; Liao, 2013). Vascular regulation (vasoconstriction or vasodilation) and endothelial dysfunction is highly dependent on relative levels of nitric oxide and endothelial nitric oxide synthase (eNOS) present near vascular walls and within circulation (De Caterina et al., 1995).

De Caterina and colleagues highlighted in this study that the endothelial activation processes like monocyte-endothelium adhesion and proinflammatory cytokine release (as measured with in vitro assays) are ameliorated by increased levels of nitric oxide. To recontextualize the importance of endothelial activation and dysfunction: vascular integrity is compromised independently in both HIV – with vascular dysregulation increasing risk for thrombosis and coagulation (Gresele et al., 2019) common in PLWH (Perkins et al., 2023) - and OSA (Budhiraja et al., 2007), where nitric oxide is characteristically reduced.

Oxidative stress is then a particularly important mechanism for the increased risk of atherosclerosis and cardiometabolic disease progression with decreased NO – as is seen in hypoxia from OSA (Suzuki et al., 2006). Chronic sympathetic activation contributes to reactive oxygen species generation and vascular dysfunction; continuous hypoxic or anoxic events create nocturnal cycles of oxygen desaturation and reoxygenation (Levy et al., 2008). Systemically, this quality of insult signals the activation of the sympathetic nervous system – vasoconstriction, increased blood pressure, sweating, raised heart rate and similar sympathetic consequences follow. The cardiovascular and renal systems are placed under substantial pressure here, as vasoconstriction increases both arterial pressure, peripheral resistance, and reduced blood flow (Malpas, 2010).

More acutely, by-products from ROS interactions frequently create superoxide-populated vascular environments that directly impair enzymes responsible for vascular homeostasis (Levy et al., 2008). SNS activation, associated ROS generation coupled with obesity-induced oxidative stress (Ellulu et al., 2017) clearly affects endothelial cells, their dysfunction and must contribute to cardiovascular disease progression.

If we are then observing increased blood pressure; obesity; concerning waist circumference measurements; dyslipidaemia; and simultaneously snoring is highly reported by individuals within this cohort, then I strongly impress upon the likelihood of observable markers of changing vascular architecture; coagulation; and endothelial dysfunction (Fragkou et al., 2023) may be present in not only the OSA high-risk group, but likely in the low-risk group as well. It is imperative to build our evidence-based understanding of CMR pathophysiology for PLWH in southern African contexts, and at what biomarker concentrations we may observe additional endothelial dysfunction from intermittent hypoxia caused by OSA. This call to re-investigate and re-establish updated, relevant markers of coagulation and inflammation amongst PLWH who do not immediately have cardiometabolic risk profiles exists in the literature (Louw et al., 2023).

Crucial to further investigations of the intersection between SNS-overactivity, CMR and OSA should introduce biochemical investigations of monocyte activation and coagulation in PLWH who are obese and at high risk for OSA after questionnaire screening. But, without a well-synthesized review of which biomarkers are relevant to this intersection – especially targeted to PLWH rather than isolated OSA studies – there can be little progress in understanding the context of OSA risk while living with virally-suppressed HIV.

5.7.3 Validating multiple questionnaires with overnight sleep data

The Berlin Questionnaire is one of several strong screening tools available for unpacking symptoms of sleep apnoea and has been widely used in populations where HIV prevalence is low (1% or less). In fact, a range of self-administrable questionnaires may prove most useful for understanding not only prevalence of risk for OSA, but also severity of symptoms. The Epworth Sleepiness Scale, for example, places emphasis on the daytime sleepiness effects of obstructive sleep apnoea by asking questions related to daytime functioning and energy levels. STOPBANG is valuable for sleep apnoea's "traditional" risk factor screenings, and the acronym offers a memorable mnemonic that indicates the questions within the name. STOPBANG has not,

however, been validated in an African context – what may be "traditional" in the Global North, where HIV prevalence is low, may indeed be not as relevant in populations of the Global South, in particular Southern Africa, where HIV prevalence is much higher.

5.7.4 Using gold-standard sleep disorder diagnostic parameters of polysomnography to justify HIV point-of-care implementation of routine OSA screening.

Polysomnography (PSG) is a multi-parametric diagnostic tool that assesses the quality and duration of sleep overnight, using a person's breathing and movement patterns in combination with their sleep architecture from electroencephalography to diagnose sleep disorders such as OSA. There is a necessity for the use of PSG to diagnose OSA as this tool is the gold standard for diagnosing sleep-disordered breathing (Broaddus et al., 2021). OSA-linked arousals, interruptions to air flow, and blood oxygen desaturation are crucial measurements from PSG, and only through determining specificity and sensitivity of screening questionnaires for southern African populations by validating against PSG can the Berlin Questionnaire hold true risk-screening relevance. PSG will be a pertinent step between recognizing the importance of screening for OSA and trusting the validity of OSA questionnaires in Global South populations, where the modal age is characteristically younger than Global North populations.

5.8 Limitations of this study:

Due to delays in receiving ethical clearance, the intended thoroughness of this project had to be adapted. Collecting polysomnography and additional sleep questionnaire data was not possible given the period for completion, as ethical clearance was only received in July 2023. However, the preliminary data remains accurately analysed and informs future studies that may arise in this cohort.

Unfortunately, the CHARACTERISE database for this study did not report on time on DTG-based ART for 28% of the cohort, as their data was taken from a different seeding study. Given more time and if granted access to the ADVANCE database, this could easily be traced back per participant for whom this data is missing.

5.9 Strengths of this study:

Prior to this study, no large-scale public data had been gathered on obstructive sleep apnoea risk as it relates to cardiometabolic risk in South Africa. The number of participants who took part in this study and the numerous cardiometabolic variables

gathered from the cohort situate this data to be novel and potentially valuable for improved routine screening of PLWH and their cardiometabolic health. The use of a well-validated screening tool, the Berlin Questionnaire, within this important demographic group also informs novel work on the relevance of this tool for prospective studies on sleep apnoea, particularly highlighting the relevance of categorizing obesity phenotypes, fat distribution, cholesterol profiles and hypertension as crucial CMR-OSA risk factors.

6 CONCLUSION:

The data reported in this study highlights three important associations between being at high risk for obstructive sleep apnoea and having an increased cardiometabolic risk score for a sample of people living with HIV in Johannesburg. Firstly, the risks for both OSA and CMD increase with obesity and a higher waist circumference. Secondly, having elevated plasma triglyceride concentrations is associated with a higher cardiometabolic risk amongst persons at high risk for OSA. Lastly, there is a staggering prevalence of obesity; hypertension; and reduced high-density lipoprotein-cholesterol across the cohort regardless of a relative sleep apnoea risk - and this points to a greater issue for managing chronic comorbid conditions for people living with HIV who take ART.

Obesity, high blood pressure, obstructive sleep apnoea risk and HIV independently contribute to inflammation. These drivers of persistent systemic inflammation display a synergistic effect that intensifies the inflammatory response demanded of the immune and cardiometabolic systems. The overwhelming prevalence of obesity, hypertension, and elevated triglycerides within this cohort of PLWH emphasizes an interplay between immunological and metabolic processes that should be a non-negotiable focus in future HIV research. Particularly, composite continuous cardiometabolic risk scores determined at the time of seroconversion should be monitored throughout treatment with ART.

In parallel to monitoring the cardiometabolic risk score of PLWH, screening for OSA symptoms like snoring frequency and intensity may be pertinent to monitoring cardiometabolic risk associated with oxidative stress. Biochemically monitoring oxidized-LDL levels and fibrinogen or d-dimer concentrations for persons who do

snore and have a positive cardiometabolic risk score could inform health practitioners and patients about their risk of developing CMD in “real-time”. The results from this study suggest that to slow the progression of atherosclerosis and endothelial dysfunction through reducing the time taken to intervene with dyslipidaemia and oxidative stress may benefit the quest of improving quality of life for PLWH once again.

The implications of dolutegravir on CMR should impress upon point-of-care services in the public and private healthcare spaces the importance to follow up with cardiometabolic profiles of persons taking dolutegravir-based ART. This may assist in timely management of hypertension, dyslipidaemia, insulin function, and progression of obesity. Comprehensive management strategies should encompass lifestyle modifications; thoughtful selection of antiretroviral therapies; earlier initiation of statin or anticoagulant medications where appropriate; and ongoing research that fills existing knowledge gaps by centering previously excluded population groups like women, African nationals, sex-workers, and unhoused persons.

> “… but given the intimacy of physiology and pathology, in the near future chronobiology [study of biological rhythms, such as sleep] is likely to grow in importance within diagnostics and therapeutics and then become as familiar to us as consumers of health care as the role of nutrition in health is today.”
>
> Jole Shackelford, *An Introduction to the history of chronobiology: Biological rhythms emerge as a subject of scientific research, p. 9*

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8 APPENDICES:

8.1 BERLIN QUESTIONNAIRE© SLEEP APNOEA

Height (m) _____ Weight (kg) _____ Age _____ Gender_____

Please choose the correct response to each question.

Category 1

1. Do you snore?

□ a. Yes **(1 point)**

□ b. No

□ c. Don't know

If you answered 'yes':

2. Your snoring is:

□ a. Slightly louder than breathing

□ b. As loud as talking (1 point)

□ c. Louder than talking (1 point)

Category 2

6. How often do you feel tired or fatigued after your sleep?

□ a. Almost every day **(1 point)**

□ b. 3-4 times per week

□ c. 1-2 times per week

□ d. 1-2 times per month

□ e. Rarely or never

7. During your waking time, do you feel tired, fatigued or not up to par?

□ a. Almost every day **(1 point)**

□ b. 3-4 times per week **(1 point)**

□ c. 1-2 times per week

□ d. 1-2 times per month

□ e. Rarely or never

3. How often do you snore?

□ a. Almost every day

□ b. 3-4 times per week **(1 point)**

□ c. 1-2 times per week **(1 point)**

□ d. 1-2 times per month

□ e. Rarely or never

4. Has your snoring ever bothered other people?

□ a. Yes **(1 point)**

□ b. No

□ c. Don't know

5. Has anyone noticed that you stop breathing during your sleep?

□ a. Almost every day

□ b. 3-4 times per week **(2 points)**

□ c. 1-2 times per week **(2 points)**

□ d. 1-2 times per month

□ □ e. Rarely or never

8.Have you ever nodded off or fallen asleep while driving in a vehicle?

□ a. Yes **(1 point)**

□ b. No

If you answered 'yes':

9. How often does this occur?

□ a. Almost every day

□ b. 3-4 times per week

□ c. 1-2 times per week

□ d. 1-2 times per month

□ e. Rarely or never

Category 3

10. Do you have high blood Pressure or a BMI > 30 $kg.m^{-2}$?

□ Yes **(1 point)**

□ No

□ Don't know

Category 1: "+" if the total score is 2 or more points. Category 2: "+" if the total score is 2 or more points. Category 3: "+" if the score is 1.

High Risk: + in 2 or 3 categories Low Risk: + in 1 or 0 categories

8.2 PLAGIARISM FORM AND ETHICAL CLEARANCE CERTIFICATES, INCLUDING CERTIFICATES OF GOOD CLINICAL PRACTICE AND TRREE: