Determining the role of differential expression of candidate microRNAs in cardiometabolic diseases among South African adults living with HIV

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Abstract

Background: MicroRNAs (miRNAs) are potential prognostic/diagnostic markers that have been investigated for screening of cardiometabolic disease (CMD) risk in general populations. However, little is known about their value in people living with human immunodeficiency virus (PLWH), who have an increased susceptibility to CMDs. PLWH have an increased susceptibility to CMDs because of chronic inflammation caused by a persistent immune response, metabolic complications from antiretroviral therapies, and traditional risk factors. In this study the association of differential expression of candidate miRNAs, miR-126-3p, -223-3p, and -320a with CMDs was investigated among PLWH.

Methods: Using a cross-sectional study design ≥18-year-old PLWH were recruited from 17 random HIV clinics in the Western Cape, South Africa between 2014-2015. Standard international definitions were used to diagnose CMDs. Whole blood miRNAs were isolated, and expression quantified by reverse transcription quantitative polymerase chain reaction. MiRNA expression was compared between participants with a CMD and without for each investigated outcome, using Wilcoxon rank sum/Kruskal Wallis tests. Robust correlations, robust linear regressions and logistic regressions assessed miRNAs relationships with cardiometabolic risk profiles. A p-value <0.05 was considered statistically significant.

Results: Among 675 participants (81% women), prevalence of CMDs/traits was: elevated high-sensitivity C-reactive protein (67.4%), raised waist circumference (WC) (63.3%), obesity (34.1%), insulin resistance (IR) (9.9%), and diabetes mellitus (8.6%). Target miRNAs were not significantly differentially expressed based on individual CMD statuses. However, target miRNAs were significantly correlated with glucose homeostasis variables [fasting glucose ($r \leq 0.129$, $p \leq 0.046$); fasting insulin ($r \leq 0.115$, $p \leq 0.015$); 2-hour insulin ($r \leq 0.097$, $p \leq 0.029$); and homeostatic model assessment of insulin resistance (HOMA-IR) ($r \leq 0.144$, $p \leq 0.002$)], and miR-126-3p and -223-3p were significantly correlated with alanine transaminase ($r \leq 0.128$, $p \leq 0.022$). In linear regression models after adjusted for age and gender, miR-126-3p had a borderline association with HOMA-IR ($\beta \leq 0.018$, $p \leq 0.081$), while miR-223-3p was borderline associated with glucose when adjusted for age, gender, and WC ($\beta = 0.001$, $p = 0.066$). There were no significant associations in logistic regression models.

Conclusion: The miRNAs tested in this study appear not to be important markers for CMDs in PLWH. A genome-wide approach is recommended to uncover other miRNAs with potential as biomarkers of CMDs in PLWH.

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Abbreviations and Acronyms

ALT - Alanine Aminotransferase

AST - Aspartate Aminotransferase

ART – Antiretroviral Therapy

BMI – Body Mass Index

CMD – Cardiometabolic Disease

CVD – Cardiovascular Disease

cDNA – Complementary deoxyribonucleic acid

CI – Confidence Interval

DNA – Deoxyribonucleic acid

HIV – Human Immunodeficiency Virus

HOMA-IR - Homeostatic Model Assessment of Insulin Resistance

hs-CRP – High-sensitivity C-reactive protein

LMICs - Low- and Middle-Income Countries

Mins - Minutes

miRNA - microRNA

NCD – Non-communicable Disease

OR – Odds Ratio

PLWH – People Living with HIV

Cq – Quantitation cycle

RNA – Ribonucleic acid

RPM – rotations per minute

RT-qPCR – Reverse transcription quantitative polymerase chain reaction

Secs – Seconds

WC – Waist Circumference

WHO – World Health Organisation

Operational definitions

Diseases and Traits	**Definition**
Overweight and/or Obesity	Body mass index (BMI) calculated as weight in kilograms (kg) divided by height in metres (m) squared, and categorized as normal weight <25.0 kg/m^2, overweight 25.0-29.9 kg/m^2, and obesity ≥30.0 kg/m^2 [1].
Raised waist circumference (WC)	WC ≥94 centimetres (cm) in men and ≥80 cm in women [1].
Dysglycaemia	Pre-diabetes: 1) Impaired glucose tolerance: fasting plasma glucose (FPG) <7.0 millimoles per litre (mmol/L) and 2-hour post glucose load between 7.8 mmol/L - 11.1 mmol/L, or 2) impaired fasting glycaemia: FPG between 6.1 mmol/L – 7.0 mmol/L and 2-hour post glucose load <7.8 mmol/L [2]. Diabetes mellitus: FPG ≥7.0 mmol/L or a 2-hour post glucose load ≥11.1 mmol/L or known diabetes on treatment [2].
Insulin resistance	The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as the product of insulin in milli-international units per litre (mIU/L) and glucose (mmol/L) divided by 22.5 [3]. Participants with a HOMA-IR above the data specific 90th percentile were considered to have insulin resistance.
Elevated high-sensitivity C-reactive protein (hs-CRP)	Hs-CRP levels >3 milligrams per litre (mg/L) [4].

Table of Contents

List of tables

List of figures

Chapter 1: Introduction

1.1 Introduction

Human Immunodeficiency Virus (HIV) and Acquired Immunodeficiency Syndrome (AIDS) has affected populations worldwide including in Africa, and particularly in South Africa, which has the greatest burden of people living with HIV (PLWH) globally [5–7]. Over the last four decades, HIV-related mortality has reportedly accumulated 40.4 million deaths according to the World Health Organisation (WHO) in 2022 [5]. Fortunately, the introduction of antiretroviral therapy (ART) has dramatically reduced global HIV-related mortality by more than 50% [8]. However, recent literature, has reported that with longevity in PLWH, there has been an increased incidence of non-communicable diseases (NCDs) such as cardiometabolic diseases (CMDs) and cardiovascular diseases (CVDs) [9]. The convergence of NCDs with communicable diseases has since been recognised as a major public health issue for PLWH [9]. This is because the dual disease burden puts an already vulnerable population at a greater risk for morbidity and mortality, while the presence of co-morbidities further complicates the treatment of PLWH [10–12].

This study focusses on two highly prevalent CMDs in PLWH; these include dysglycaemia and obesity [13,14]. The presence of either dysglycaemia or obesity is a serious health concern irrespective of HIV infection, as these conditions can contribute to CVDs such as heart attack or stroke, chronic kidney disease, and in some instances mortality [15,16]. There are multiple risk factors for dysglycaemia and obesity, however inflammation is a major contributor [17]. Inflammation promotes insulin resistance and metabolic disruption which can lead to dysglycaemia and obesity [18,19].

With advances in the biological sciences, changes in an organisms such as disease states, have become easier to correlate with molecular changes such as modified gene expression [20]. As an example, epigenetics in particular, has been useful in explaining how modified gene expression leads to disease outcomes [21]. Through epigenetics, microRNAs (miRNAs) have recently emerged as pivotal regulators of gene expression, and have shown immense potential as future prognostic and diagnostic markers and possibly therapeutic targets [22,23].

1.2 Study Rational

The diagnostic, prognostic, and therapeutic potential of miRNAs, could possibly contribute towards alleviating some of the burden of CMDs such as dysglycaemia and obesity in PLWH [22,24]. However, this option cannot be utilized until miRNAs associated with CMDs have fully been profiled in the target population. This is because miRNAs are sequence specific markers, therefore different miRNAs may be expressed in different populations [25]. Considering there are only two studies which have profiled miRNAs associations with dysglycaemia in PLWH [26,27], this highlights the novelty of this field as well as emphasizes its need for further assessment. Furthermore, none of these studies profiled the target miRNAs being evaluated in this study. Additionally, with South Africa having the highest burden of HIV globally [6,7], this also supports the importance of profiling miRNAs associated with CMDs in PLWH in this South African population. If found to be relevant the findings of this study may be used to validate the use of these candidate miRNAs as potential CMD risk screening markers for PLWH.

1.3 Aim

To assess and compare the expression patterns of candidate miRNAs, miR-126-3p, miR-223-3p, and miR-320a, in HIV infected adults 18 years and older, with and without CMDs of obesity, dysglycaemia and insulin resistance, in order to characterise their expression signature in this South African population.

1.4 Objectives

1. To isolate miRNAs from whole blood samples and quantify their expression using reverse transcription quantitative polymerase chain reaction (RT-qPCR).
2. To explore the association between the expression patterns of the target miRNAs with CMDs including overweight/obesity and dysglycaemia, in PLWH.
3. To assess the association between miRNAs expression and markers of inflammation such as high-sensitivity C-reactive protein (hs-CRP) and insulin resistance.

Chapter 2: Literature Review

2.1 Human Immunodeficiency Virus epidemiology

HIV and AIDS was first described in 1981; it was a substantial cause of mortality in the late 20th century [28]. According to the WHO, the estimated global population of PLWH was 39 million, and the global prevalence was 0.7% for adults aged 15-49 years in 2022 [5]. However, Africa accounts for almost two thirds of the global HIV burden with 25.7 million (65.9%) PLWH residing on the continent [6].

HIV attacks the infection-fighting cells, namely CD4+ T-lymphocytes, in infected persons thereby compromising the immune system [29]. The high mortality of PLWH usually occurs with AIDs, the most advanced stage of the HIV infection [30]. With the advent of ART, there has been more than a 50% reduction in global HIV-related mortality [8]. Prior to ART, HIV-related mortality was 45-88 deaths per 1000 person years, this was reduced to 14-46 deaths per 1000 person years following ARTs [8].

There have been concerted efforts to ensure accessibility of ARTs for all populations including those living in low- and middle-income countries (LMICs) [31–33]. South Africa has the largest ART programme in the world [34]. Fortunately, with effective administration of ARTs, infection with HIV has shifted from being a fatal infectious disease to a more manageable chronic condition [35,36]. ARTs reduce the viral load in a patient to almost undetectable amounts, this in turn has had positive implications on the life expectancy as well as quality of life for PLWH [36–39]. Furthermore, ARTs have also mitigated the transmission of HIV between people [40]. Consequently with this longevity and exposure to lifestyle factors, the morbidity and mortality of PLWH has shifted from direct HIV-related causes to NCDs such as CMDs like diabetes mellitus [41,42].

The Sixth South African National HIV Prevalence, Incidence, and Behaviour survey (SABSSM VI) conducted on South African adults 15 years and older in 2022 reported there were approximately 7.8 million PLWH in South Africa [43]. While the overall HIV prevalence is 12.7%, these rates are substantially high in women (20%) compared to men (12%) [43]. Comparatively however, HIV prevalence is disparate throughout South Africa according to factors such as location, gender, and race. For instance, HIV prevalence ranges from as low as 8.2% in the Western Cape province to as high as 21.8% in the KwaZulu-Natal province (Figure 2.1) [43]. Moreover, according to race, Black Africans (20%) have the highest HIV prevalence, followed by the Coloured population (5%), then White and Indian populations (1% each) [43].

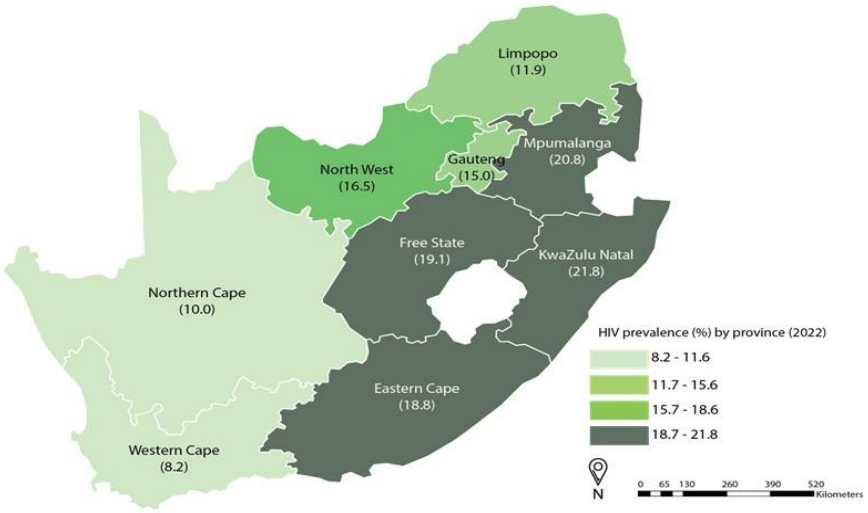

Figure 2.1: HIV prevalence across South Africa among adults aged 15 years and older in 2022. *Image taken from the Human Sciences Research Council (HSRC), November 2023, Personal communication* [43].

2.2 Cardiometabolic Disease risk factors in PLWH

PLWH have an increased susceptibility to CMDs because of chronic inflammation caused by a persistent immune response to the HIV infection [44], the effects some ART medications have on metabolic homeostasis [45,46], and general exposure to traditional risk factors operating in the general population (Figure 2.2).

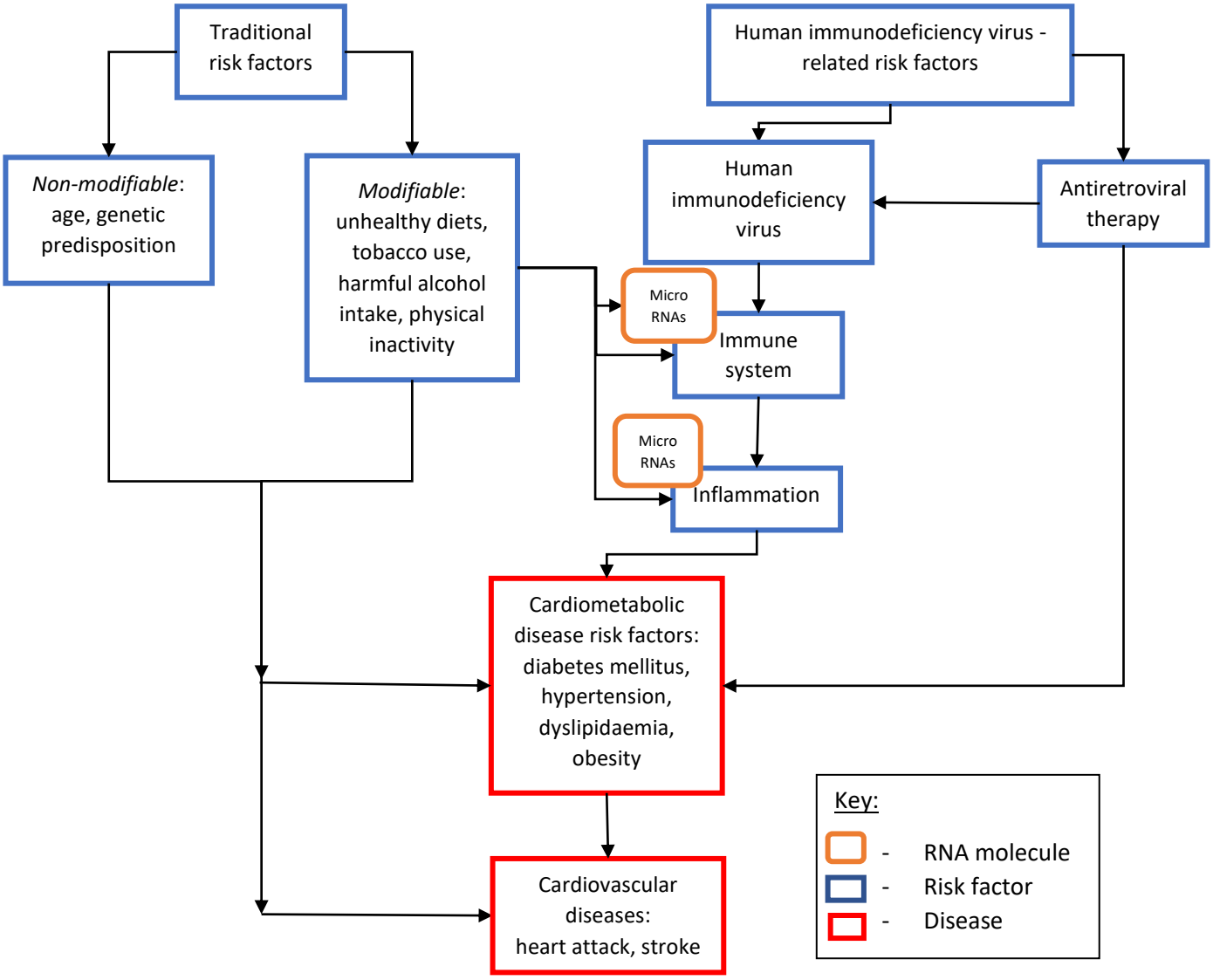

Figure 2.2: Pathways contributing to the development of cardiometabolic diseases (CMDs) and cardiovascular diseases (CVDs) in people living with human immunodeficiency virus (PLWH).

2.2.1 Traditional risk factors for Cardiometabolic Diseases in PLWH

Traditional risk factors can include non-modifiable risk factors like older age and genetic predisposition, and modifiable risk factors such as behavioural and lifestyle factors like over nutrition, a sedentary lifestyle, and tobacco and alcohol misuse [47] (Figure 2.2). Rapid industrialisation, urbanisation, and development seen in the 20th and 21st century has been accompanied by increased consumerism and subsequently a more sedentary lifestyle [48,49]. Consequently, this has led to an increased uptake of modifiable risk factors such as unhealthy diets, physical inactivity, and excessive tobacco and alcohol use [50]. The uptake of such risk factors ultimately increases the incidence of CMDs such as type 2 diabetes mellitus

(henceforth will be referred to as diabetes mellitus) and obesity, in all populations, including PLWH [50].

2.2.2 The negative effects of long-term antiretroviral therapy use on Cardiometabolic Disease development

Due to the chronic nature of ART regimes, long term use has been implicated with metabolic complications [51]. ART drugs such as protease inhibitors (PIs) and nucleoside reverse transcriptase inhibitors (NRTIs) are known to cause metabolic abnormalities by disrupting both lipid and glucose metabolism [51–55]. As such, prolonged exposure to these kinds of metabolic disruptions contribute to the development of CMDs such as dysglycaemia and insulin resistance seen in PLWH (Figure 2.2) [56–58]. Furthermore, a study on PLWH from South Africa reported dysglycaemia prevalence increased with ART use, this was 21.6% with ART-naïve, 26.0% with first-line ART, and 37.0% with second-line ART [58]. The differences in dysglycaemia prevalence were supported by the level of insulin resistance, inflammation, and weight gain associated with each regime [58].

2.2.3 Chronic inflammation in PLWH

PLWH persistently have higher levels of inflammation compared to HIV-negative controls, both before and while receiving ART [59,60]. Chronic inflammation can promote CMDs such as obesity and diabetes mellitus through increasing insulin resistance, activating atherosclerosis, and disrupting metabolic regulation [61–64]. Consequences of prolonged exposure to insulin resistance which prevents optimal glucose storage occurring in the liver [65], may lead to the occurrence of CMDs such as dysglycaemia and obesity [18]. Insulin resistance contributes to CMDs through high blood glucose levels leading to dysglycaemia, and promoting glucose uptake in fat and muscle tissue leading to obesity [19]. Hence, markers of inflammation such as hs-CRP and insulin resistance are important when investigating CMD risk. High hs-CRP has been significantly correlated with increased CVD risk [66]. In addition, high hs-CRP has also been significantly correlated with incidence of insulin resistance and obesity, and been shown to fall in parallel with weight loss and improvements in insulin resistance [67].

2.3 Burden of comorbid Cardiometabolic Diseases in PLWH

With the rising incidence of overweight/obesity in all populations significant health concerns have emerged, more especially for PLWH [68,69]. These health concerns can include CVDs such as heart attacks and stroke, CMDs such as diabetes mellitus and insulin resistance, and cancers among other co-morbidities, all of which further complicates the health management of PLWH [15,70,71]. Obesity contributes to the incidence of these conditions by promoting high levels of blood lipids and glucose, endothelial dysfunction, inflammation, and elevated blood pressure [17,72–74]. In addition, overweight/obesity have also demonstrated unfavourable effects on immune recovery following ART initiation [75]. The overall prevalence of obesity for PLWH from South Africa was reported at 23.3%, this was determined by meta-analysis in 2023 [14].

In addition to obesity, PLWH are also experiencing a high incidence of dysglycaemia [14,76]. A meta-analysis conducted in 2023 reported the prevalence of diabetes mellitus at 6.1% for PLWH in South Africa [14]. Diabetes mellitus is a serious public health concern, as it can lead to conditions such as CVD, chronic kidney disease, nerve damage; in addition to other problems concerning feet, oral health, vision, hearing, and mental health [16]. Dysglycaemia contributes to the incidence of the aforementioned conditions/problems through high blood glucose levels that damage blood vessels, thereby disenabling adequate blood supply and ultimately damaging various tissues [77].

The high CMDs observed in PLWH is concerning, because not only does it pose its own risks, but it also contributes to an increased CVD risk in an already vulnerable population (Figure 2.2) [10,11]. According to the WHO, CVDs are the leading cause of mortality globally, and is responsible for almost 18 million deaths annually [78]. Furthermore, emerging evidence from Asia [76], Europe [79], North America [80], and Africa [81,82] have reported CVD as a serious health concern and potential leading cause of mortality for PLWH. There has been more than a two-fold increase in CVD risk reported for PLWH compared to HIV negative persons [83].

In 2017, in one of the most HIV-afflicted provinces in South Africa, Kwa-Zulu Natal (Figure 2.1), it was reported that almost 30% of PLWH suffered from at least ≥one co-morbidities and/or co-infections; these included hypertension, diabetes mellitus, and tuberculosis [84]. The dual disease burden experienced by PLWH also places a significant burden on the country, by increasing the demand on healthcare systems and increasing the cost-of-care needed for

PLWH [85]. Hence, alternative methods, including molecular approaches, could be useful in identifying CMDs early through potentially more sensitive screening options, or in reducing some of the burden of CMDs through new treatment possibilities [24].

2.4 Molecular approaches currently being investigated for risk screening and treatment possibilities

Epigenetics studies changes in an organism caused by modified gene expression [21]. Gene expression is the process of translating information from a gene into a functional product such as proteins, thus enabling cell function and affecting the phenotype of an organism (Figure 2.3) [86]. Gene expression can be summarised as a gene being transcribed into messenger ribonucleic acid (mRNA) via transcription in the nucleus, followed by mRNA being translated into a protein via translation which occurs outside the nucleus (Figure 2.3) [87].

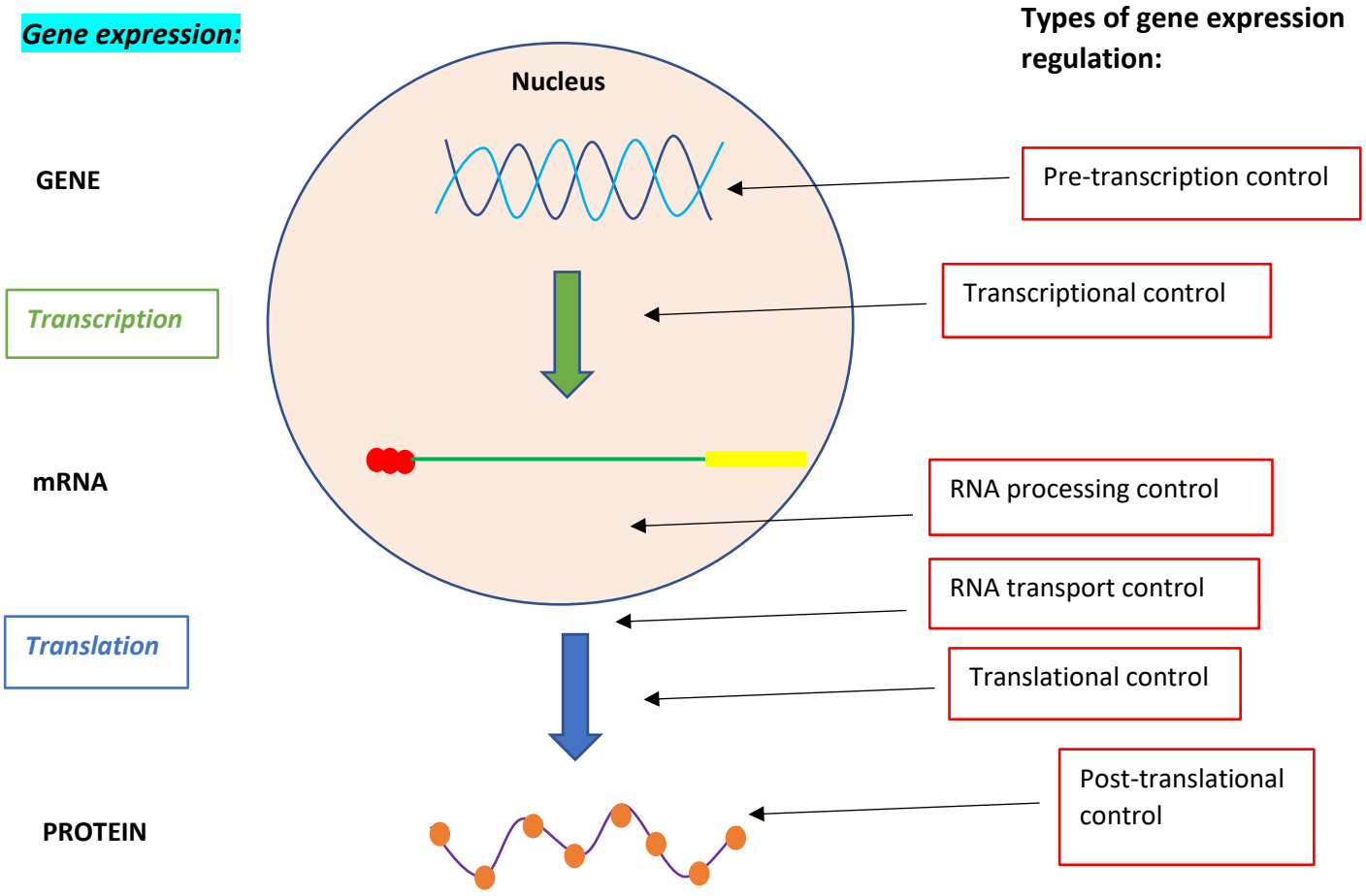

Figure 2.3: An overview of gene expression and the different types of regulation.

Through studying epigenetic modifications behind physiological and pathological changes, our understanding on the origins and progression of disease was dramatically improved [88]. For

instance, a review by Wang *et al* (2023) elaborates on the potential significance of an epigenetic modification in the diagnosis and treatment of various kidney diseases [89]. Epigenetic modifications can sometimes be beneficial, for example when potential cancerous mutations are epigenetically tagged to be destroyed, or they can have detrimental effects such as instigating cancer by silencing the expression tumour supressing genes [20,90]. As such, these pathological changes have been implicated with disease development and progression [91]. However, due to the plethora of biological mechanisms and pathways involved in disease occurrence, it is sometimes unclear whether these modifications precede disease development, or occur as a result of disease [20,92]. The influence of epigenetics on disease occurrence were first described in cancers in 1983 [93]. Later, the role of epigenetics was suspected in CVD, because of epigenetic contributions which affect vascular development and inflammation [94,95]. Since then, a number of epigenetic modifications affecting the development and progression of CVD have been identified [96]. Epigenetic modifications have even been established in CVD risk factors such as smoking [97], diabetes mellitus [98,99], obesity [100], and age [101]. Epigenetics has grown in popularity because it is able to shed light on the complex pathophysiology underlying CVD and other inflammatory diseases such as CMDs [96,102]. Furthermore, unlike mutations, epigenetic modifications are not permanent changes to the deoxyribonucleic acid (DNA) sequence [21]. Hence, they are reversible, making them ideal targets for therapeutic interventions [100]. There are several epigenetic modifications including DNA methylation, histone modification, as well as ribonucleic acid (RNA)-interference by non-coding RNA [103]. Regulation via non-coding RNA is a relatively newly characterised approach to gene regulation and is currently undergoing intensive investigation [104]. Non-coding RNA makes up the bulk of human DNA, this is about 98%, however for a long time before its regulatory function was revealed, non-coding RNA was referred to as “junk DNA” [105–108]. Of the important non-coding RNA transcripts, miRNAs which are short non-coding RNA sequences, have emerged as pivotal regulators that are easily linked with disease pathophysiology and progression [22].

2.5 MicroRNAs and their roles in HIV and Cardiometabolic Diseases

MiRNAs are small single-stranded molecules which are approximately 18-22 nucleotides in length [109]. The first miRNA lin-4 was discovered in 1993, by the Ambros and Ruvkun groups in *Caenorhabditis elegans* and since their discovery, miRNAs have been shown to have diverse roles in cell signalling and gene expression regulation [109,110]. In addition, miRNAs are freely

found and stably expressed in circulation, they can have tissue specific expression, and can be easily and reliably quantified [23].

2.5.1 MicroRNAs role in regulating gene expression

MiRNAs regulate gene expression via RNA processing control (Figure 2.3), by binding to mRNA and thereby preventing sequential protein translation or inciting targeted mRNA degradation [111]. The crucial role of miRNAs in regulating gene expression, has allowed them to operate in various pathways and play a vital role in the overall succession of biological processes [111]. As a result, miRNAs have been found to be differentially expressed according to disease status, type, and sometimes stage [23]. This has made them ideal candidates as potential prognostic or diagnostic markers in addition to being possible therapeutic targets, which can be used to predict, track, and even treat diseases [23].

2.5.2 MicroRNAs with Human Immunodeficiency Virus

Until recently miRNA research in PLWH has primarily been focused on the pathogenesis of HIV infection on miRNA expression or vice versa [112–114], as well as the potential use of miRNAs to determine ART treatment possibilities [112,115,116]. However, little research has been done on the role of miRNAs with CMDs in PLWH. As a result, candidate miRNAs chosen for evaluation in our study had to be selected based on findings from general populations.

2.5.3 MicroRNAs with Cardiometabolic Diseases

Due to their advanced ability to predict and track diseases, much effort has been dedicated into profiling the associations between miRNAs with disease states, particularly with respect to cancers, in general populations [23]. However, recent studies have highlighted the role of miRNAs in inflammatory diseases such as CMDs like diabetes mellitus and obesity [117]. This topic has since become an exponentially growing research field, with studies being done in all regions of the world, including Africa [117–119]. The information generated from such studies may ultimately be used to improve CMD profiles and reduce CVD risk, through early diagnosis and alternative treatment possibilities [120,121]. However, because miRNAs are sequence specific, genetic variation across populations would affect miRNA expression in that population [25]. Hence, miRNA profiling studies on specific populations are necessary before it can be considered in diagnostic, prognostic, and treatment possibilities.

2.6 The role of miRNA in Cardiometabolic Diseases

The following three target miRNAs, namely miR-126-3p, -223-3p, and -320a, were selected for investigation this study because of their significant associations with CVD, inflammatory disease pathophysiology, and CMDs, which were determined in general populations [122–124].

2.6.1 Target microRNAs with dysglycaemia and insulin resistance

The target miRNAs have demonstrated links with dysglycaemia and insulin resistance in general populations [117,119,122], as such dysregulation of miR-126-3p has been well characterised with dysglycaemia in most populations, including in Africa [118]. A study by Weale *et al* (2021) reported significant differential expression of miR-126-3p in the presence of diabetes mellitus and pre-diabetes, in a community-based South African population [118,122]. Similarly, with respect to miR-223-3p, significant dysregulation of the target miRNA with impaired glucose tolerance as well as diabetes mellitus was reported in a miRNA profiling study by Matsha *et al* (2018), also from a community-based South African population [117]. Contrastingly, miR-320a expression with dysglycaemia has not been characterised in a South African population. However in a study conducted on obese Egyptian women in 2022, reported miR-320a expression was significantly correlated with serum insulin, homeostatic model assessment of insulin resistance (HOMA-IR), and fasting glucose [119]. In light of the aforementioned studies, miR-126-3p, -223-3p and -320a, have been shown to be involved in glucose homeostasis, with altered expressions linked to dysglycaemia. One of the pathways in which miR-126-3p is involved in glucose regulation is through targeting an insulin receptor substrate [125]. Whereas, miR-223-3p expression is upregulated in response to high glucose, which results in increased inflammation and promotes insulin resistance [64,126]. Molecular targets for miR-320a in glucose regulation is still being established, however its critical role in glucose metabolism has been observed [127]. These findings have been demonstrated in general populations, with no data pertaining to the relationship between their expression patterns and dysglycaemia and/or insulin resistance, in HIV infected populations, which warrants further scrutiny.

2.6.2 Target microRNAs with overweight/obesity

The target miRNAs have been linked with overweight/obesity also in general populations [128–130]. With regards to miR-126-3p expression with overweight/obesity, a systematic review in 2022 reported that miR-126-3p was significantly associated with adiposity in global populations [129]. Furthermore, a meta-analysis in 2023 reported that miR-126-3p and -223-3p expression was significantly increased with weight loss in global populations [128]. For African populations, miR-126-3p expression is reportedly significantly correlated with waist circumference (WC), and miR-320a is reportedly significantly correlated with WC and body mass index (BMI) [119,130]. The molecular pathways that involve the target miRNAs related to overweight/obesity include supressing pro-inflammatory activation, metabolic regulation, and preventing the development of insulin resistance [127,131–133]. These aforementioned studies demonstrate that the target miRNAs have a role in overweight/obesity, however none of the studies considered HIV-infected participants, thus warranting further investigation.

Chapter 3: Methods

3.1 Main study

3.1.1 Study design, setting, and population

This cross-sectional study was conducted between March 2014 and February 2015 at public healthcare facilities that provided ART care to at least 325 PLWH per month in the Western Cape province of South Africa. Participants recruited were ≥18-year-old PLWH receiving care at 17 randomly selected public healthcare facilities, these included four rural facilities and 13 urban and peri-urban facilities.

Inclusion criteria was: HIV-positive adults (18 years and older) attending facilities with directed HIV clinics, and who were willing to participate and provided informed consent.

Exclusion criteria were: bedridden patients, patients with active malignancy or currently undergoing treatment for malignancy, patients on chronic corticosteroid treatment, pregnant or breastfeeding women, and patients unwilling or unable to give informed consent.

3.1.2 Participant history, physical measurements, and laboratory measurements

Data collection comprised administered questionnaires, clinical examinations and biochemical assessments. Trained interviewers collected self-reported data from 831 participants (Figure 3.1), this included socio-demographic data such as age, gender, years of education, and employment status; as well as medical history. The questionnaire was adapted from the WHO's STEPwise approach to Surveillance tool [134]. Duration of HIV diagnosis, CD4 counts, and ART regimens were obtained from participants' clinical records.

Participants' anthropometric measurements such as weight, height, WC and hip circumference were measured using standardized techniques [135]. Weight was measured to the nearest gram (g) with participants in light clothing and without shoes, using a A&D Personal Scale (Model UC-321, Toshima-Ku,Tokyo, Japan). Height was measured to the nearest centimetre (cm) with participants barefoot and in the upright position, using a Leicester Height Scale (Seca, Liverpool, UK). WC was measured at the level of the umbilicus using a non-elastic measuring tape and recorded to the nearest cm. Hip circumference was measured to the nearest cm around the largest circumference of the buttocks. Three blood pressure (BP) readings were recorded 3 minutes (mins) apart on the right arm of the

participant, using a digital automatic BP monitor (Omron, M6 Comfort, Hoofddrop, The Netherland). The average of the 2nd and 3rd BP measurements was used in the analysis.

Of the 831 participants, 754 returned for biochemical assessment (Figure 3.1). Fasting (minimum overnight fast of eight hours) venous whole blood samples were collected. A standard oral glucose tolerance test (OGTT) was conducted. Participants were given 75 grams (g) of anhydrous glucose diluted in 250-300 milliliters (mL) of water to drink in a 5 min period, after which blood samples were taken at 120 mins [136]. Following, biochemical analysis was performed by an ISO 15189 accredited pathology laboratory (PathCare, Reference Laboratory, Cape Town, South Africa), which included the following:

a) Enzymatic colorimetric methods which cleaves cholesterol esters by the action of cholesterol esterase to yield free cholesterol and free fatty acids [137], was used to measure serum **cholesterol**, **triglycerides**, **aspartate aminotransferase** (AST), and **alanine aminotransferase** (ALT). **Low-density lipoprotein cholesterol** (LDL-C) was calculated using the Friedewald formula [138].
b) Plasma **glucose** was determined using the hexokinase method, which spectrophotometrically measures oxidized glucose-6-phosphate to give nicotinamide adenine dinucleotide + hydrogen, using a Beckman Coulter AU 500 spectrophotometer [139].
c) **Glycated haemoglobin (HbA1c)** level was quantified by high-performance liquid chromatography, a technique which uses high pressurisation to separate components in a mixture [140].
d) **Insulin** concentrations were measured by the Chemiluminescence Immunoassay method [141], which uses an enzyme to convert the target substrate into a chemiluminescent signal that emits light to correlate the amount of substrate present [141].
e) **Hs-CRP** was assessed using a Beckman Coulter AU 500 spectrophotometer.

3.1.3 Definitions

Overweight and/or Obesity was defined using the WHO recommended definition, which is based on body mass index (BMI) calculated as weight in kilograms (kg) divided by height in metres (m) squared [1]. BMI was then categorized as: normal weight if <25.0 kg/m^2, overweight if 25.0–29.9 kg/m^2, and obesity if ≥30.0 kg/m^2 in the study population [1]. Raised WC was defined using WHO recommended cut-offs of ≥94 cm in men and ≥80 cm in women [1].

Pre-diabetes was defined by the presence of impaired glucose tolerance (IGT) and/or impaired fasting glycaemia (IFG). IGT was defined as fasting plasma glucose (FPG) <7.0 millimoles per litre (mmol/L) and 2-hour post glucose load between 7.8 mmol/L - 11.1 mmol/L. IFG was defined as FPG between 6.1 mmol/L – 7.0 mmol/L and 2-hour post glucose load <7.8 mmol/L [2]. Diabetes mellitus was defined as FPG ≥7.0 mmol/L or a 2-hour post glucose load ≥11.1 mmol/L or known diabetes on treatment [2].

Insulin resistance was defined as the homeostatic model assessment of insulin resistance (HOMA-IR) above the 90th percentile. HOMA-IR was calculated as the product of insulin in milli-international units per litre (mIU/L) and glucose (mmol/L) divided by 22.5 [3]. Elevated hs-CRP was defined as hs-CRP above 3 milligrams per litre (mg/L) in the study population [4].

3.2 Current study

3.2.1 Target miRNAs

The three chosen miRNAs for this research study were selected based on findings from both African and international studies. In South Africa, miR-126-3p exhibited significant associations with diabetes mellitus and pre-diabetes [118,122], while miR-223-3p showed significant associations with impaired fasting glucose [117]. Moreover, in an Egyptian population, miR-320a demonstrated significant correlations with glucose-related variables such as serum insulin, fasting glucose, and HOMA-IR [119]. Additionally, in South Africa, miR-126-3p displayed significant correlations with WC [130], and in an African population, miR-320a exhibited significant correlations with both WC and BMI [119]. To elucidate some of the gene targets of these miRNAs, online tools such as miRBase available on https://www.mirbase.org/ [142] and miRNA Pathway Dictionary Database (miRPathDB) 2.0 accessible at https://mpd.bioinf.uni-sb.de/overview.html [143], were visited on May 15 2024. For miR-126-3p, 26 noteworthy gene targets were identified [142,143], including insulin receptor substrate 1 (IRS1) which was found to be inhibited by miR-126-3p [144]; and Protein kinase B (AKT) serine/threonine kinase 1 and 2 (AKT1 and AKT2 respectively), which have suppressed expression in the presence of miR-126-3p [145,146]. These gene targets are known to have pivotal roles in glucose homeostasis and are linked with CMDs such as dysglycaemia and insulin resistance [142,143]. MiR-223-3p has up to 427 targets identified, several of which are involved in glucose homeostasis such as CF Transmembrane Conductance Regulator (CFTR) and Forkhead Box O1 and O3 (FOXO1 and FOXO3 respectively) [142,143]. These gene targets were found to be down regulated with the expression of miR-223-3p [147,148]. Lastly miR-320a has 937 noted gene targets, including SH2B adaptor protein 3 (SH2B3), mutations to which have been linked with insulin dependent diabetes mellitus [86,143,149]. Due the close relationship between dysglycaemia, insulin resistance, and obesity, coupled with the pivotal roles these miRNAs play in glucose homeostasis, these target miRNAs were deemed highly appropriate for inclusion in this study [150].

Qiagen miRCURY LNA miRNA Custom PCR Panels (Qiagen, Hilden, Germany) containing predesigned primers lyophilized in respective wells of the 96-well panel (Appendix 2), was used for RT-qPCR. The mature sequences of the target miRNAs were as follows: miR-126-3p is UCGUACCGUGAGUAAUAAUGCG; miR-223-3p is UGUCAGUUUGUCAAAUACCCCA; and miR-320a is AAAAGCUGGGUUGAGAGGGCGA.

3.2.2 Total RNA extraction

Total RNA, inclusive of miRNAs, was extracted from 754 whole blood samples (Figure 3.1) that were stored in Tempus^{TM} Blood RNA Tubes (Applied Biosystems, South Africa) at -80°C and freeze-thawed prior to extraction. The Tempus^{TM} Spin RNA Isolation Kit supplied by ThermoFisher Scientific (Applied Biosystems, South Africa) was used for extractions with modified specifications to the manufacturer's instructions.

The manufacturer's protocol recommended adding 3 mL of 1X phosphate buffered saline (PBS) [consisting 2.700 millimolar (mM) potassium chloride, 0.137 molar (M) sodium chloride, 1.760 mM potassium phosphate monobasic, and 10.100 mM sodium phosphate dibasic] to 6 mL of room temperature whole blood, however, to conserve blood samples and allow for repeat extractions, when necessary, we modified the protocol to add 2 mL 1X PBS to 4 mL of each whole blood sample. This was followed by a 30 second (sec) pulse vortex to mix the diluted whole blood samples. Following that, the diluted whole blood samples were centrifuged. The manufacturer's protocol recommended spinning the conical tubes containing whole blood and PBS, for 30 mins at 4°C at 3 000 × g, and thereafter pour out the supernatant. However, pouring out of the supernatant resulted in repeated loss of the blood cell pellets with subsequent low RNA yields downstream. As such, this was step required modification by centrifuging the conical tubes at 4°C at 4 000 rotations per minute (rpm) for 1 hour, followed by pipetting out the supernatant instead of pouring it out. This was followed by another centrifugation of the conical tubes at 4°C at 4 000 rpm for 30 mins. The conical tubes were then inverted on absorbent paper for 2 mins, after which the rims were cleaned with more absorbent paper.

Next, 400 microliters (µL) of RNA Purification Resuspension Solution was added into the conical tubes which were then briefly vortexed and left on ice, while the RNA purification filters were prepared. To prepare the RNA purification filters, they were inserted into waste collection tubes and 100 µL of RNA Purification Wash Solution 1 was added directly onto the filter. Following, ~400 µL of the resuspended RNA was transferred from the conical tubes to the RNA purification filter, and as per the manufacturer's recommendation was to be centrifuged for 30 secs at 16 000 × g. However, this centrifugation step, along with the following centrifugation steps, were optimised to a spin-time of 14 000 rpm, instead of 16 000 × g, due to the RNA purification filters being dislodged at higher speeds thus compromising the purity of the sample.

After centrifugation, the RNA purification filters were removed and the subsequent flow-through collected in the waste collection tube was discarded. The RNA purification filters were then reinserted back into the waste collection tubes, upon which 500 µL of RNA Purification Wash Solution 1 was added into the filters. Samples were then spun at the optimised 14 000 rpm for 30 secs and the resultant flow-through discarded, after which 500 µL of RNA Purification Wash Solution 2 was added to each RNA purification filter. The filters were also spun at the optimised 14 000 rpm for 30 secs, and the previous step was repeated. Thereafter, the flow through was discarded and the RNA purification filters were spun again at the optimised 14 000 rpm for 30 secs to dry the membrane.

The RNA purification filters were then transferred to new collection tubes, and 100 µL of Nucleic Acid Purification Elution Solution was added to each filter. Samples were then left to incubate at 70°C for 2 mins and centrifuged at the optimised 14 000 rpm for 30 secs. Thereafter, the resultant eluate collected in the collection tube was re-eluded back (approximately 100 µL) into the purification filters and centrifuged for another 2 mins at 16 000–18 000 × g. Following that, 90 µL of each RNA eluates was transferred into new 1.5 mL RNA Tubes (Applied Biosystems, South Africa) and stored at -20°C.

Prior to storage, quality of the RNA samples was assessed by spectrophotometry using the NanoDrop One (Nanodrop Technologies, Wilmington, USA). Samples with an RNA concentration >20 nanograms per millilitre (ng/mL) and an optical density ratio of A260/A280 >1.8 were used for further analysis.

3.2.3 Complementary DNA (cDNA) synthesis

The resultant total RNA was diluted to 5 nanograms per microliter (ng/µL) and reverse transcribed into cDNA, using the Qiagen miRCURY LNA RT Kit (Qiagen, Hilden, Germany) and MicroAmp Fast 96-Well Reaction Plates (Applied Biosystems, South Africa) according to manufacturers' instructions. Manufacturer's protocol was as follows: 10 µL reactions contained 2 µL of 5X miRCURY RT Reaction Buffer, 4.5 µL of RNase free water, 1 µL of 10X miRCURY RT Enzyme Mix, 0.5 µL of Synthetic RNA spike-ins, and 2 µL of template RNA diluted to 5 ng/µL. PCR cycling parameters included a reverse transcription step at 42°C for 60 mins, followed by an inactivation step at 95°C for 5 mins and storage at 4°C for infinity as per manufacturer's instructions. Following cDNA synthesis, random samples were selected to assess the quality of the cDNA spectrophotometrically using the NanoDrop One (Nanodrop

Technologies, Wilmington, USA). cDNA samples were then stored in 1.5 mL RNA Tubes (Applied Biosystems, South Africa) at -20°C, till further processing.

3.2.4 SYBR green based RT-qPCR

SYBR green RT-qPCR assays were performed using qPCR miRCURY LNA miRNA Custom PCR Panels (Qiagen, Hilden, Germany) and the QuantStudio 7 Flex Real-Time cycler (Applied Biosystems, South Africa), according to the manufacturer's instructions. Prior to RT-qPCR, cDNA was diluted 1:80 with RNase free H_2O, in order for normalisation and optimal quantification. Manufacturer's protocol was as follows: 10 µl reactions contained 5 µl of 2X miRCURY SYBR® Green Master Mix, 0.5 µl of ROX Reference Dye, 0.5 µl of RNase-free H2O and 4 µl of cDNA template diluted 1:80. Each PCR was run alongside two housekeeping genes, which were U6 snRNA and SNORD48, and one synthetic RNA spike-in, this was UniSp2. Endogenous control U6 snRNA is a common housekeeping gene often used for miRNA quantification, because it is well conserved and stably expressed across different tissue types [151]. Whereas, SNORD48 is a small nucleolar RNA that is highly referenced as an endogenous control in CVD studies [152]. UniSp2 is a synthetic RNA spike-in that is used to monitor RNA isolation quality [153]. PCR cycling parameters included initial heat activation at 95°C for 2 mins, following by 40 cycles of denaturation at 95°C for 10 secs and combined annealing and extension steps at 56°C for 60 secs. Acquisition on the green channel was recorded at the end of the extension step.

3.2.5 Endogenous control stability test

The standard deviation of the quantification cycle (Cq) data across all samples for the endogenous controls SNOR D48 and U6 snRNA were calculated as 0.965 and 3.230, respectively, on Microsoft excel. U6 snRNA was rejected as a control because of its unstable expression, and SNOR D48 was used as a single endogenous control to normalize miRNAs expression.

3.2.6 Realised sample size

Following RNA extraction, 698 samples were successfully extracted and subjected to RT-qPCR. Thereafter, 23 samples were removed due to the Cq being ≥35 or undetermined, resulting in a realised sample size of 675 participants. Figure 3.1 below illustrates the participants included in the analysis for this study.

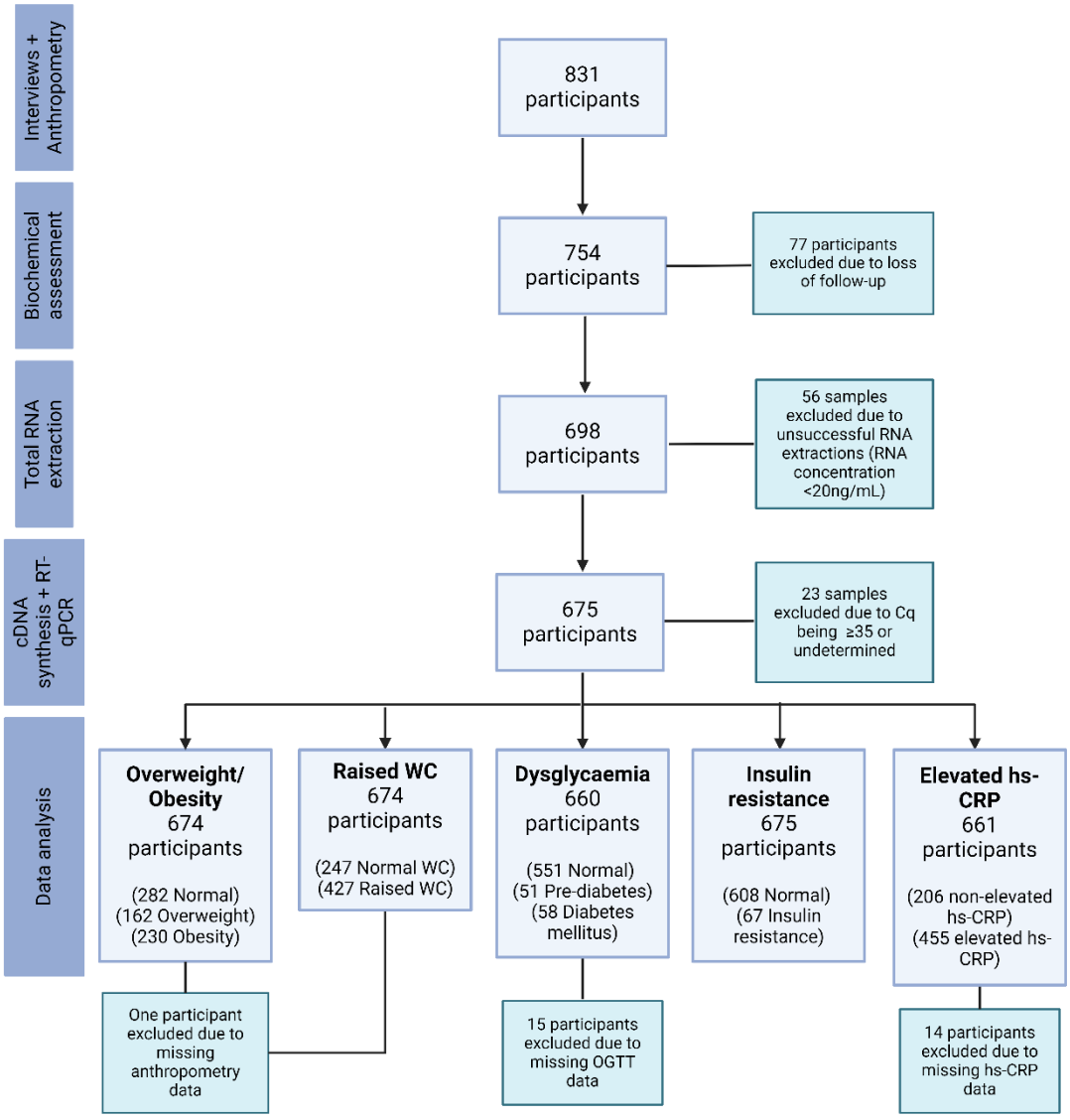

Figure 3.1: Flow diagram of the participants included in this study. *RNA, Ribonucleic acid; Cq, quantification cycle; cDNA, complementary Deoxyribonucleic acid; RT-qPCR, Reverse Transcription quantitative Polymerase Chain Reaction; WC, waist circumference; OGTT, Oral Glucose Tolerance Test; and hs-CRP, high-sensitivity C-reactive protein.*

3.2.7 Power analysis of realised sample size

A correlation power analysis was performed using R® Statistical Software, version 4.3.0 (The R Foundation for Statistical Computing Platform, Vienna, Austria) and the package "pwr" (function employed "pwr.r.test").

Where the sample size=675, effect size=0.1, and a significance level=0.05. An effect size of r=0.1 was selected from Cohens recommended effect sizes due to the unknown effect size of the target miRNAs in the study population [154]. An approximate power of 74.0% was calculated for the realised sample size (Figure 3.2).

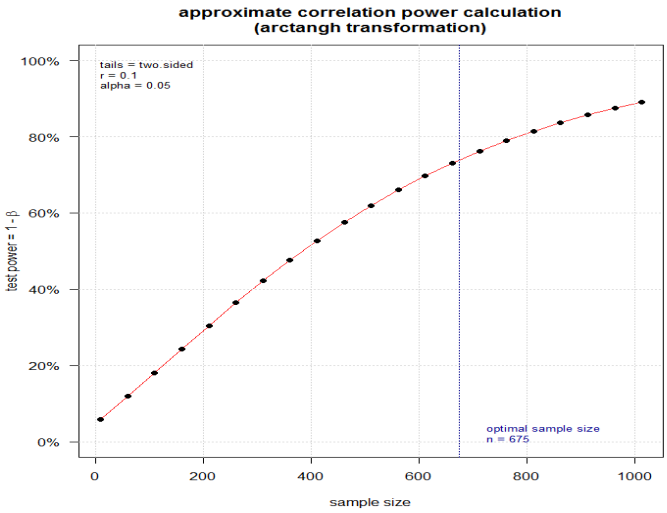

Figure 3.2: Power curve according to sample size for a correlation test with small effect size.

3.3 Statistical data analysis

Relative miRNA expression was normalised using the $2^{-\Delta Ct}$ method on Microsoft excel, which uses the average expression of the endogenous control according to the presence and absence of individual CMDs and traits [155]. Therefore, the expression of the target miRNAs was differential according to which outcome was used to normalise the target miRNAs expression. Samples with missing variables where the presence or absence of a particular CMD or trait could not be determined were excluded from the analysis for that outcome.

Data analysis was performed using R® Statistical Software, version 4.3.0 (The R Foundation for Statistical Computing Platform, Vienna, Austria). Assumption of normal distribution was assessed using the Shapiro-Wilk tests. Continuous variables are reported as medians with the 25th and 75th percentiles because of the data being not normally distributed, and categorical variables are reported as counts and percentages. Wilcoxon Rank Sum tests and chi-square

tests were used for men *vs.* women comparisons. Furthermore, comparisons were also analysed by overweight/obesity status using BMI cut-offs and dysglycaemia status, using Kruskal Wallis tests and chi-square tests. Differences in target miRNAs expression according to the presence and absence of individual CMDs and traits [i.e., overweight/obesity, raised WC, dysglycaemia, insulin resistance, and elevated hs-CRP] were assessed using the Wilcoxon Rank Sum tests or Kruskal Wallis tests. Continuous variables' correlations with target miRNAs were analysed using robust correlations that were derived from the minimum covariance determinant (MCD) estimator. MCD method is a highly robust estimator of multivariable location and scatter, and resistant to outliers [156]. In addition, Spearman Rank correlation tests were performed, as well as robust correlations stratified by gender, for comparison purposes. Thereafter, separate multivariable robust linear [equation: cardiometabolic variable = β_0 + β_{miRNA}(miRNA level) + $\beta_1(x_1)$ + … $\beta_n(x_n)$ + ε] and logistic regression models [equation: $\pi(\text{miRNA level}) = \frac{e^{\beta 0 + \beta \text{miRNA}(\text{miRNA level}) + \beta 1(x1) \ldots + \beta n(xn)}}{1+e^{\beta 0 + \beta \text{miRNA}(\text{miRNA level}) \ldots + \beta 1(x1) + \beta n(xn)}}$] (where β_0 is the intercept, β_{miRNA} is the regression coefficient for the miRNA of interest, and β_1 to β_n are the regression coefficients for covariates x_1 to x_n in the model) were used to investigate the associations of target miRNAs with individual cardiometabolic variables and CMDs. Samples with missing variables were disregarded in the corresponding tests. A significance threshold of *p*-value <0.050 was selected, and a p-value ≥0.050 but <0.095 was considered borderline significant.

3.4 Ethical considerations

Ethical clearance for the main study was obtained from the South African Medical Research Council Ethics Committee (SAMRC-EC) in November 2013 (EC021-11/2013) (Appendix 3). All participants provided signed informed consent for the questionnaire and blood sample collection (Appendix 4). Separate signed informed consent was collected separately for genetic analysis (Appendix 5). Permission to recruit participants was requested and received from the Health Research Office of the Western Cape Department of Health and the individual healthcare facilities. Ethics for the current study was provided by the University of Cape Town Human Research Ethics Committee (UCT-HREC) in December 2022 (HREC REF:555/2022) (Appendix 6). This study was conducted in accordance with the principles of the Declaration of Helsinki [157].

Chapter 4: Results

4.1 Sociodemographic and other participant characteristics

The 675 participants involved in this study comprised mostly women (81%). As indicated in Table 4.1, the median age (25^{th}–75^{th} percentile) was 38.5 years (32.0-44.0 years) overall, 40.5 years (34.0-47.0 years) for men and 37.0 years (31.0-43.0 years) for women (p<0.001 for men vs. women difference). Majority of participants (85.5%) had ≥7 years of education, with men less educated than women (76.6% *vs* 87.6%, p<0.001). Most participants (55.1%) were employed, with similar rates seen across men (49.2%) and women (56.2%) (p=0.067). Prevalence of smoking and alcohol use were higher in men (56.3% and 59.4%, respectively) than in women (14.6% and 35.5%, respectively, p<0.001). The median BMI (25^{th}–75^{th} percentiles) was 27.8 kg/m^2 (22.3-32.2 kg/m^2) overall and was significantly lower in men than women (21.3 kg/m^2 *vs* 28.4 kg/m^2, p<0.001). Men had a higher systolic BP compared to women (123.3 mmHg *vs* 115.8 mmHg, p<0.001) but similar diastolic BP levels (82.0 mmHg *vs* 82.0 mmHg, p=0.559).

As seen in Table 4.2, men compared to women had lower levels of HDL-C (1.2 mmol/L vs 1.3 mmol/L, p=0.006), LDL-C (2.3 mmol/L vs 2.5 mmol/L, p=0.006), fasting insulin (4.0 mIU/L *vs* 6.6 mIU/L, p<0.001), 2-hour insulin (15.0 mIU/L *vs* 27.8 mIU/L, p<0.001), and HOMA-IR (0.9 *vs* 1.4, p<0.001). Men had higher levels compared to women of triglycerides (1.1 mmol/L *vs* 1.0 mmol/L, p=0.045), fasting glucose (5.1 mmol/L *vs* 4.9 mmol/L, p=0.002), ALT (26.0 U/L *vs* 22.0 U/L, p<0.001), and AST (34.0 U/L *vs* 28.0 U/L, p<0.001). In the overall sample, the median CD4 count (25^{th}–75^{th} percentile) was 439.5 cells/mm^3 (240.3-623.3), with lower levels in men compared to women (282.0cells/mm^3 *vs* 417.0 cells/mm^3, p=0.008; Table 4.2).

The prevalence of individual CMDs and traits in the overall sample were as follows: 24.0% for overweight; 34.1% for obesity; 63.4% for raised WC; 9.9% for insulin resistance; 7.6% for pre-diabetes; 8.6% for diabetes mellitus; and 67.4% for elevated hs-CRP (Tables 4.1 and 4.2).

Furthermore, when analysed by overweight/obesity status using BMI cut-offs the following variables were significantly different, these included: gender (p<0.001), smoking (p<0.001), alcohol use (p=0.008), CD4 count (p<0.001), diastolic blood pressure (p=0.024), HDL-C (p=0.005), LDL-C (p<0.001), triglycerides(p=0.011), fasting glucose (p=0.004), fasting insulin

(p<0.001), 2-hour glucose (p<0.001), 2-hour insulin (p<0.001), HbA1c (p<0.001), HOMA-IR (p<0.001), AST(p<0.001), and hs-CRP(p<0.001) (Table S1).

Also, when analysed by dysglycaemia status the following variables were significantly different: age (p<0.001), alcohol use (p=0.011), ART regime (p=0.025), CD4 count (p=0.023), systolic blood pressure (p<0.001), HDL-C (p=0.005), LDL-C (p=0.003), triglycerides (p<0.001), and hs-CRP (p=0.012) (Table S2).

Table 4.1: Socio-demographic, lifestyle, anthropometry, HIV, and blood pressure levels in HIV infected adults

	Total (n=675)	Men (n=128)	Women (n=547)	*p*-value
Socio-demographic factors:				
Median age (years) (25^{th}-75^{th} percentiles)	38.5 (32.0-44.0)	40.5 (34.0-47.0)	37.0 (31.0-43.0)	**<0.001**
Education, n (%): ≥ year 7	577 (85.5)	98 (76.6)	479 (87.6)	**<0.001**
Employment status, n (%):				**0.067**
Employed	372 (55.1)	63 (49.2)	309 (56.5)	
Other[1]	171 (25.3)	29 (22.7)	142 (26.0)	
Lifestyle factors, n (%):				
Current smoker	152 (22.5)	72 (56.3)	80 (14.6)	**<0.001**
Current alcohol user	270 (40.0)	76 (59.4)	194 (35.5)	**<0.001**
Median anthropometry variables (25^{th}-75^{th} percentiles):				
Weight (kg)	71.7 (58.3-81.6)	62.4 (55.2-71.5)	71.3 (59.2-84.1)	**<0.001**
Height (cm)	160.9 (155.6-165.7)	170.5 (166.0-173.5)	158.9 (154.7-162.9)	**<0.001**
Hip circumference (cm)	104.0 (93.1-112.1)	91.8 (87.8-97.1)	105.8 (96.8-115.5)	**<0.001**
Body mass index (kg/m^2)	27.8 (22.3-32.2)	21.3 (19.6-24.4)	28.4 (23.9-33.2)	**<0.001**
Waist circumference (WC) (cm)	89.3 (77.7-98.1)	78.8 (74.0-89.6)	90.1 (79.5-100.7)	**<0.001**
Waist-hip-ratio	0.9 (0.8-0.9)	0.9 (0.8-0.9)	0.9 (0.8-0.9)	**<0.001**
Waist-height-ratio	0.6 (0.5-0.6)	0.5 (0.4-0.5)	0.6 (0.5-0.6)	**<0.001**
Prevalence, n (%):				**<0.001**
Raised WC	427 (63.4)	21 (16.4)	406 (74.4)	
Overweight/obesity:				**<0.001**
Overweight: BMI 25.0 - 29.9 kg/m^2	162 (24.0)	19 (14.8)	143 (26.2)	
Obesity: BMI ≥ 30.0 kg/m^2	230 (34.1)	7 (5.5)	223 (40.8)	
***HIV related factors*:**				
Antiretroviral therapy regime, n (%)				0.192
ART-Naïve	38 (5.6)	6 (4.7)	32 (5.9)	
First line	389 (57.6)	67 (52.3)	322 (58.9)	
Second line	68 (10.1)	11 (8.6)	57 (10.4)	
Other[2]	213 (31.6)	44 (34.4)	136 (24.9)	
Median CD4 count (cells/mm^3) (25^{th}-75^{th} percentiles)	439.5 (240.3 - 623.3)	282.0 (194.5 - 472.5)	417.0 (252.0-643.0)	**0.008**

Table 4.1 continued.				
	Total (n=675)	Men (n=128)	Women (n=547)	*p*-value
***Median blood pressure levels (25th-75th percentiles)*:**				
Systolic blood pressure (mmHg)	120.8 (107.0-129.1)	123.3, 114.6-138.8	115.8, 106.0-127.0	**<0.001**
Diastolic blood pressure (mmHg)	83.5, 75.0-90.5	82.0, 75.6-94.4	82.0, 74.5-90.1	0.559

HIV, Human Immunodeficiency Virus.

[1]Other: full-time homemaker, pensioner, on disability grant, student, self-employed, casual job, and part-time job.

[2]Other: single nucleotide reverse transcriptase inhibitors (NRTI), single non-nucleoside reverse transcriptase inhibitors (NNRTI), combined NRTI + protease inhibitors (PI), double NRTIs, combined NRTI + NNRTI, double PIs only, and unknown medications.

Current smoker: current use of tobacco products such as cigarettes, cigars or pipes.

Current alcohol use: consumption of any alcohol products within the previous year.

Raised waist circumference: ≥94 cm in men and ≥80 cm in women.

Table 4.2: Biochemical parameters in HIV infected adults

Biochemical parameters	Total (n=675)	Men (n=128)	Women (n=547)	*p*-value
Median values (25th-75th percentiles):				
Lipids (mmol/L):				
Total cholesterol	4.1 (3.5-5.0)	4.1 (3.4-5.0)	4.2 (3.5-5.0)	0.151
HDL-C	1.3 (1.0-1.5)	1.2 (1.0-1.5)	1.3 (1.1-1.5)	**0.006**
LDL-C	2.6 (2.0-3.1)	2.3 (1.7-3.0)	2.5 (2.0-3.1)	**0.021**
Triglycerides	1.1 (0.8-1.3)	1.1 (0.8-1.6)	1.0 (0.8-1.3)	**0.042**
Glucose homeostasis variables:				
Fasting glucose (mmol/L)	5.4 (4.6-5.4)	5.1 (4.8-5.6)	4.9 (4.6-5.4)	**0.002**
2-hour glucose (mmol/L)	5.6 (4.6-6.2)	5.1 (4.4-6.4)	5.4 (4.6-6.2)	0.138
Fasting insulin (mIU/L)	8.0 (4.0-9.6)	4.0 (2.4-6.4)	6.6 (4.4-10.3)	**<0.001**
2-hour insulin (mIU/L)	34.1 (13.9-41.3)	15.0 (9.0-24.2)	27.8 (15.9-45.3)	**<0.001**
HbA1c (%)	5.6 (5.2-5.8)	5.5 (5.2-5.8)	5.4 (5.2-5.7)	0.194
HOMA-IR	1.9 (0.8-2.2)	0.9 (0.5-1.7)	1.4 (0.8-2.3)	**<0.001**
Inflammatory markers:				
Alanine transaminase (U/L)	29.7 (17.0-34.0)	26.0 (19.0-42.0)	22.0 (17.0-32.0)	**<0.001**
Aspartate aminotransferase (U/L)	34.8 (24.0-38.0)	34.0 (27.0-45.0)	28.0 (24.0-36.0)	**<0.001**
hs-CRP (mg/L)	11.5 (2.4-14.0)	4.7 (1.9-14.5)	5.6 (2.5-13.8)	0.612
Prevalence, n (%):				
Insulin Resistance	67 (9.9)	7 (5.5)	60 (11.0)	**0.087**
Dysglycaemic status:				0.174
Pre-diabetes	51 (7.6)	13 (10.2)	38 (6.9)	
Diabetes mellitus	58 (8.6)	15 (11.7)	43 (7.9)	
Elevated hs-CRP	455 (67.4)	83 (64.8)	372 (68.0)	0.327

HIV, Human Immunodeficiency Virus, HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, glycated haemoglobin; HOMA-IR, homeostatic model assessment-estimated insulin resistance; and hs-CRP, high-sensitivity C-reactive protein.

Participants with a HOMA-IR above the data specific 90th percentile was considered insulin resistant.

Pre-diabetes: impaired glucose tolerance - fasting plasma glucose (FPG) <7.0 mmol/L and a 2-hour post glucose load 7.8 mmol/L-11.1 mmol/L, or impaired fasting glycaemia - FPG 6.1 mmol/L-7.0 mmol/L a with a 2-hour post glucose load <7.8 mmol/L.

Diabetes mellitus: FPG ≥7.0 mmol/L or a 2-hour post glucose load ≥11.1 mmol/L or known diabetes on medication.

Elevated hs-CRP: hs-CRP >3 mg/L.

4.2 MicroRNA expression analysis

4.2.1 The following outcomes were determined when the target microRNAs expression was normalised with SNOR D48 according to overweight/obesity status.

Due to missing anthropometric data, a single participant was excluded from this analysis. Resultantly, there were 674 participants included for the analysis (Figure 3.1).

Relative microRNA expression

There were no significant differences in the expression of target miRNAs: miR-126-3p (p=0.966), miR-223-3p (p=0.993), and miR-320a (p=0.860), between participants grouped by BMI categories as normal, overweight, and obese (Table S1 and Figure 4.1).

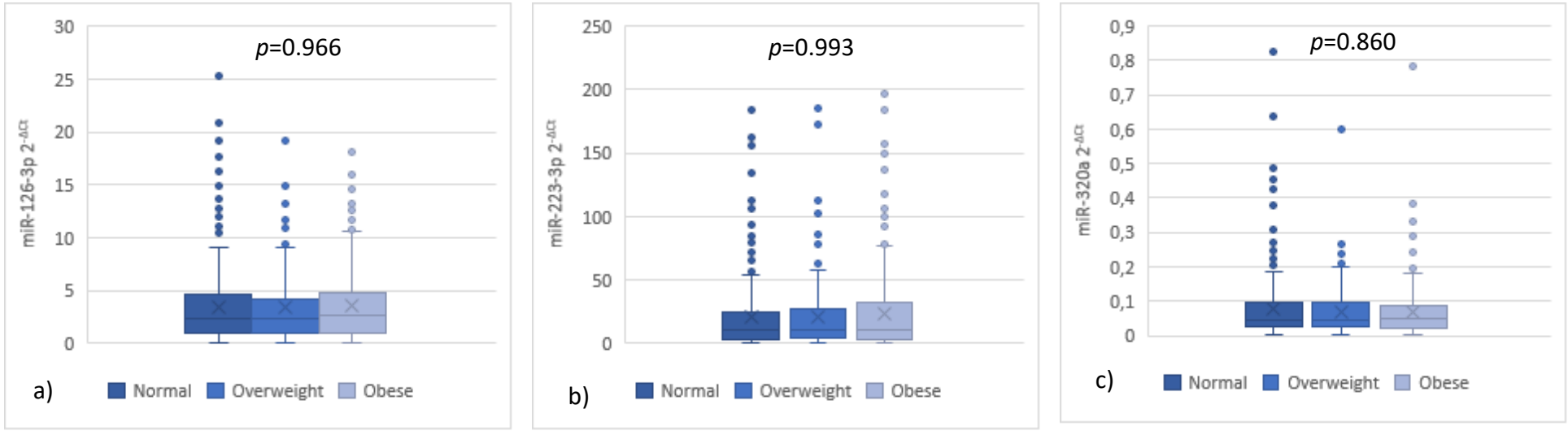

Figure 4.1: Box and whisker plots showing the relative expression of a) miR-126-3p, b) miR-223-3p and c) miR-320a normalised with SNOR D48 according to overweight/obesity status. *Overweight/obesity was defined by body mass index (BMI): normal: BMI <25.0 kg/m^2, overweight: BMI 25.0–29.9 kg/m^2, and obesity: BMI ≥30.0 kg/m^2. P-values are from the Kruskal Wallis test.*

Robust correlations to assess the associations between continuous variables and target miRNAs

MiR-126-3p was significantly correlated with fasting glucose (r=0.098, 95% CI:0.020-0.170, *p*<0.011), fasting insulin (r=0.109, 95% CI:0.030-0.180, *p*<0.005), 2-hour insulin (r=0.084, 95% CI:0.010-0.160, *p*<0.029), HOMA-IR (r=0.144, 95% CI:0.070-0.220, *p*<0.001), and ALT (r=0.123, 95% CI:0.050-0.200, *p*<0.001; Table 4.3).

MiR-223-3p was significantly correlated with fasting glucose (r=0.077, 95% CI:0.000-0.150, *p*<0.046), fasting insulin (r=0.108, 95% CI:0.030-0.180, *p*<0.005), 2-hour insulin (r=0.097, 95% CI:0.020-0.170, *p*<0.012), HOMA-IR (r=0.143, 95% CI:0.070-0.220, *p*<0.001), and ALT (r=0.089, 95% CI:0.010-0.160, *p*<0.021; Table 4.3).

MiR-320a was significantly correlated with fasting glucose (r=0.125, 95% CI:0.050-0.200, *p*<0.001), fasting insulin (r=0.094, 95% CI:0.020-0.170, *p*<0.015), and HOMA-IR (r=0.121, 95% CI:0.050-0.190, *p*<0.002; Table 4.3).

When correlations were re-assessed using the spearman rank correlation test, only miR-126-3p was significantly correlated with HOMA-IR (r=0.103, *p*=0.008). MiR-223-3p and miR-320a were not significantly correlated with any of the continuous variables (Table S3). In the additional analysis when robust correlations were stratified by gender, patterns of correlation were similar in the women only analysis compared to the analysis in the total population (Table S9). Due to these similarities, stratified correlation analyses are not reported for the other outcomes.

Table 4.3: Univariate robust correlations between continuous variables and target microRNAs expression normalized with SNOR D48 according to overweight/obesity status in HIV infected adults

	miR-126-3p			**miR-223-3p**			**miR-320a**		
	r	95% CI	*p*-value	r	95% CI	*p*-value	r	95% CI	*p*-value
Socio-demographic:									
Age (years)	-0.062	(-0.140, 0.010)	<0.110	-0.032	(-0.110, 0.040)	<0.410	0.001	(-0.070, 0.080)	<0.980
Anthropometry:									
Waist circumference (cm)	0.017	(-0.060, 0.090)	<0.660	0.026	(-0.050, 0.100)	<0.500	0.056	(-0.020, 0.130)	<0.150
Hip circumference (cm)	0.052	(-0.020, 0.130)	<0.180	0.073	(0.000, 0.150)	<0.058	0.059	(-0.020, 0.130)	<0.130
Waist-hip-ratio	-0.028	(-0.100, 0.050)	<0.470	-0.036	(-0.110, 0.040)	<0.350	0.012	(-0.060, 0.090)	<0.760
Blood pressure:									
Systolic blood pressure (mmHg)	0.018	(-0.060, 0.090)	<0.640	0.016	(-0.060, 0.090)	<0.680	-0.007	(-0.080, 0.070)	<0.860
Diastolic blood pressure (mmHg)	0.009	(-0.070, 0.080)	<0.820	-0.003	(-0.080, 0.070)	<0.940	-0.022	(-0.100, 0.050)	<0.570
HIV-related factors:									
CD4 count (cells/mm^3)	0.047	(-0.030, 0.120)	<0.220	0.009	(-0.070, 0.080)	<0.820	0.060	(-0.020, 0.130)	<0.120
Lipid levels:									
Total cholesterol (mmol/L)	-0.025	(-0.100, 0.050)	<0.520	0.025	(-0.050, 0.100)	<0.520	-0.046	(-0.120, 0.030)	<0.230
HDL-C (mmol/L)	0.032	(-0.040, 0.110)	<0.410	0.020	(-0.060, 0.100)	<0.600	-0.015	(-0.090, 0.060)	<0.700
LDL-C (mmol/L)	-0.054	(-0.130, 0.020)	<0.160	0.005	(-0.070, 0.080)	<0.900	-0.052	(-0.130, 0.020)	<0.180
Triglycerides (mmol/L)	0.002	(-0.070, 0.080)	<0.960	0.045	(-0.030, 0.120)	<0.240	0.040	(-0.040, 0.120)	<0.300
Glucose homeostasis:									
Fasting glucose (mmol/L)	0.098	(0.020, 0.170)	**<0.011**	0.077	(0.000, 0.150)	**<0.046**	0.125	(0.050, 0.200)	**<0.001**
2-hour glucose (mmol/L)	0.037	(-0.040, 0.110)	<0.340	0.069	(-0.010, 0.140)	<0.073	0.061	(-0.010, 0.140)	<0.110
Fasting insulin (mIU/L)	0.109	(0.030, 0.180)	**<0.005**	0.108	(0.030, 0.180)	**<0.005**	0.094	(0.020, 0.170)	**<0.015**
2-hour insulin (mIU/L)	0.084	(0.010, 0.160)	**<0.029**	0.097	(0.020, 0.170)	**<0.012**	0.045	(-0.030, 0.120)	<0.240
HbA1c (%)	-0.013	(-0.090, 0.060)	<0.740	0.037	(-0.040, 0.110)	<0.340	-0.040	(-0.120, 0.040)	<0.300
HOMA-IR	0.144	(0.070, 0.220)	**<0.001**	0.143	(0.070, 0.220)	**<0.001**	0.121	(0.050, 0.190)	**<0.002**
Inflammatory markers:									
Alanine Transaminase (U/L)	0.123	(0.050, 0.200)	**<0.001**	0.089	(0.010, 0.160)	**<0.021**	0.041	(-0.030, 0.120)	<0.290
Aspartate Aminotransferase (U/L)	0.065	(-0.010, 0.140)	<0.092	0.068	(-0.010, 0.140)	<0.078	0.019	(-0.060, 0.090)	<0.620
Hs-CRP (mg/L)	0.002	(-0.070, 0.080)	<0.960	0.069	(-0.010, 0.140)	<0.073	0.048	(-0.030, 0.120)	<0.210

HIV, Human Immunodeficiency Virus; CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, glycated haemoglobin; HOMA-IR, homeostatic model assessment-estimated insulin resistance; hs-CRP, high-sensitivity C-reactive protein.

Robust linear regression analyses to assess the relationship between significantly correlated variables and target miRNAs

There was a significant association between miR-320a and 2-hour insulin in the unadjusted model (β=-13.253, standard error=6.556, *p*=0.044). However, this association was no longer significant after adjusting for age and gender (*p*=0.219). Other notable observations were: miR-126-3p was borderline associated with HOMA-IR in the unadjusted model (β=0.019, standard error=0.011, *p*=0.084; Table 4.4).

Table 4.4: Robust linear regression analyses for associations between significantly correlated variables and target microRNAs expression normalized with SNOR D48 according to overweight/obesity status in HIV infected adults

	miR-126-3p			**miR-223-3p**			**miR-320a**		
	β	Standard error	*p*-value	β	Standard error	*p*-value	β	Standard error	*p*-value
Fasting glucose									
Model 1	0.005	0.006	0.457	0.001	0.001	0.185	-0.019	0.214	0.929
Model 2	0.006	0.006	0.286	0.001	0.001	0.107	-0.122	0.219	0.578
Fasting insulin									
Model 1	0.048	0.048	0.308	0.002	0.005	0.607	-2.213	1.503	0.141
Model 2	0.033	0.043	0.448	<-0.001	<0.001	0.990	-1.295	1.422	0.363
2-hour insulin									
Model 1	-0.015	0.199	0.938	0.006	0.022	0.783	-13.253	6.556	**0.044**
Model 2	-0.066	0.191	0.730	-0.007	0.021	0.750	-9.449	7.679	0.219
HOMA-IR									
Model 1	0.019	0.011	0.084	0.001	0.001	0.399	-0.370	0.352	0.293
Model 2	0.017	0.010	0.099	0.001	0.001	0.575	-0.193	0.335	0.565
Alanine Transaminase									
Model 1	-0.004	0.125	0.977	0.003	0.012	0.816	-6.246	4.383	0.155
Model 2	0.023	0.128	0.859	0.005	0.012	0.658	-6.734	4.731	0.155

Model 1: microRNA;
Model 2: model 1 + age + gender.
HIV; human immunodeficiency virus; HOMA-IR, homeostatic model assessment-estimated insulin resistance.

Logistic regression analysis to assess the associations of overweight and obesity with target miRNAs

There were no significant associations between the target miRNAs and overweight or obesity (Table S10).

4.2.2 The following outcomes were determined when the target miRNAs expression was normalised with SNOR D48 according to raised WC status.

Due to missing anthropometric data, a single participant was excluded from this analysis. Resultantly, there were 674 participants included for the analysis (Figure 3.1).

Relative miRNA expression

There were no significant differences in the expression of the target miRNAs: miR-126-3p (p=0.928), miR-223-3p (p=0.924), and miR-320a (p=0.805), between participants with and without raised WC (Figure 4.2).

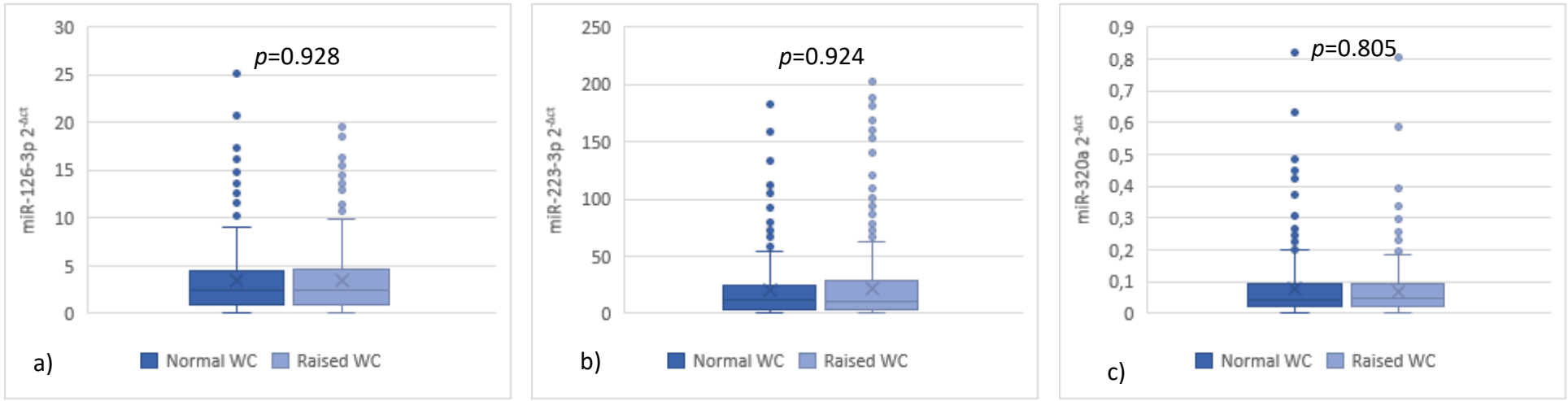

Figure 4.2: Box and whisker plots showing the relative expression of a) miR-126-3p, b) miR-223-3p and c) miR-320a, normalised with SNOR D48 according to raised waist circumference status. *Raised waist circumference (WC) was defined as ≥94 cm in men and ≥80 cm in women. P-values are from the Wilcoxon rank sum test.*

Robust correlations to assess the associations between continuous variables and target miRNAs

MiR-126-3p was significantly correlated with fasting glucose (r=0.102, 95% CI:0.030-0.180, *p*<0.008), fasting insulin (r=0.115, 95% CI:0.040-0.190, *p*<0.003), 2-hour insulin (r=0.084, 95% CI:0.010-0.160, *p*<0.029), HOMA-IR (r=0.142, 95% CI:0.070-0.220, *p*<0.001), and ALT (r=0.128, 95% CI:0.050-0.200, *p*<0.001; Table 4.5).

MiR-223-3p was significantly correlated with fasting glucose (r=0.077, 95% CI:0.000-0.150, *p*<0.046), fasting insulin (r=0.112, 95% CI:0.040-0.190, *p*<0.004), 2-hour insulin (r=0.096, 95% CI:0.020-0.170, *p*<0.013), HOMA-IR (r=0.142, 95% CI:0.070-0.220, *p*<0.001), and ALT (r=0.088, 95% CI:0.010-0.220, *p*<0.001; Table 4.5).

MiR-320a was significantly correlated with fasting glucose (r=0.129, 95% CI:0.050-0.200, *p*<0.001), fasting insulin (r=0.099, 95% CI:0.020-0.170, *p*<0.010), and HOMA-IR (r=0.124, 95% CI:0.050-0.200, *p*<0.001; Table 4.5).

When correlations were re-assessed using the spearman rank correlation test, miR-126-3p (r=0.103, *p*=0.007) and miR-320a (r=0.076, *p*=0.048) were significantly correlated with HOMA-IR. miR-223-3p was not significantly correlated with any of the continuous variables (Table S4).

Table 4.5: Univariate robust correlations between continuous variables and target microRNAs expression normalized with SNOR D48 according to raised waist circumference status in HIV infected adults

	miR-126-3p			**miR-223-3p**			**miR-320a**		
	r	95% CI	*p*-value	r	95% CI	*p*-value	r	95% CI	*p*-value
Socio-demographic characteristics:									
Age (years)	-0.061	(-0.140, 0.010)	<0.110	-0.033	(-0.110, 0.040)	<0.390	-0.002	(-0.080, 0.070)	<0.960
Anthropometry:									
Weight (kg)	0.027	(-0.050, 0.100)	<0.480	0.042	(-0.030, 0.120)	<0.280	0.046	(-0.030, 0.120)	<0.230
Height (cm)	-0.013	(-0.090, 0.060)	<0.740	0.037	(-0.040, 0.110)	<0.340	0.031	(-0.040, 0.110)	<0.420
BMI (kg/m^2)	0.026	(-0.050, 0.100)	<0.500	0.032	(-0.040, 0.110)	<0.410	0.035	(-0.040, 0.110)	<0.360
Hip circumference (cm)	0.058	(-0.020, 0.130)	<0.130	0.076	(0.000, 0.150)	**<0.049**	0.070	(-0.010, 0.140)	<0.069
Blood pressure:									
Systolic blood pressure (mmHg)	0.020	(-0.060, 0.100)	<0.600	0.018	(-0.060, 0.090)	<0.640	-0.007	(-0.080, 0.070)	<0.860
Diastolic blood pressure (mmHg)	0.012	(-0.060, 0.090)	<0.760	<0.001	(-0.070, 0.080)	<0.980	-0.021	(-0.100, 0.050)	<0.590
HIV-related factors:									
CD4 count (cells/mm^3)	0.051	(-0.020, 0.130)	<0.190	0.011	(-0.060, 0.090)	<0.780	0.061	(-0.010, 0.140)	<0.110
Lipid levels:									
Total cholesterol (mmol/L)	-0.026	(-0.100, 0.050)	<0.500	0.025	(-0.050, 0.100)	<0.520	-0.043	(-0.120, 0.030)	<0.260
HDL-C (mmol/L)	0.032	(-0.040, 0.110)	<0.410	0.021	(-0.050, 0.100)	<0.590	-0.014	(-0.090, 0.060)	<0.720
LDL-C (mmol/L)	-0.053	(-0.130, 0.020)	<0.170	0.007	(-0.070, 0.080)	<0.860	-0.051	(-0.130, 0.020)	<0.190
Triglycerides (mmol/L)	0.003	(-0.070, 0.080)	<0.940	0.044	(-0.030, 0.120)	<0.250	0.038	(-0.040, 0.110)	<0.320
Glucose homeostasis:									
Fasting glucose (mmol/L)	0.102	(0.030, 0.180)	**<0.008**	0.077	(0.000, 0.150)	**<0.046**	0.129	(0.050, 0.200)	**<0.001**
2-hour glucose (mmol/L)	0.040	(-0.040, 0.120)	<0.300	0.073	(0.000, 0.150)	<0.058	0.070	(-0.010, 0.140)	<0.069
Insulin fasting (mIU/L)	0.115	(0.040, 0.190)	**<0.003**	0.112	(0.040, 0.190)	**<0.004**	0.099	(0.020, 0.170)	**<0.010**
2-hour insulin (mIU/L)	0.084	(0.010, 0.160)	**<0.029**	0.096	(0.020, 0.170)	**<0.013**	0.051	(-0.020, 0.130)	<0.190
HbA1c (%)	-0.011	(-0.090, 0.060)	<0.780	0.035	(-0.040, 0.110)	<0.360	-0.033	(-0.110, 0.040)	<0.390
HOMA-IR	0.142	(0.070, 0.220)	**<0.001**	0.142	(0.070, 0.220)	**<0.001**	0.124	(0.050, 0.200)	**<0.001**
Inflammatory markers:									
Alanine Transaminase (U/L)	0.128	(0.050, 0.200)	**<0.001**	0.088	(0.010, 0.160)	**<0.022**	0.040	(-0.040, 0.120)	<0.300
Aspartate Aminotransferase (U/L)	0.064	(-0.010, 0.140)	<0.097	0.070	(-0.010, 0.140)	<0.069	0.017	(-0.060, 0.090)	<0.660
Hs-CRP (mg/L)	0.011	(-0.060, 0.090)	<0.780	0.071	(0.000, 0.150)	<0.065	0.051	(-0.020, 0.130)	<0.190

HIV, Human Immunodeficiency Virus; CI, confidence interval; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, glycated haemoglobin; HOMA-IR, homeostatic model assessment-estimated insulin resistance; hs-CRP, high-sensitivity C-reactive protein.

Robust linear regression analyses to assess the relationship between significantly correlated variables and target miRNAs

Only borderline associations were determined in the robust linear regression analysis, as demonstrated in Table 4.6. These were between the following: miR-320a and 2-hour insulin in the unadjusted model (β=-12.876, standard error=6.584, *p*=0.051); miR-126-3p and HOMA-IR in the unadjusted model (β=0.020, standard error=0.011, *p*=0.076); miR-126-3p and HOMA-IR in the model adjusted for age and gender (β=0.018, standard error=0.010, *p*=0.081); miR-223-3p and hip circumference in the unadjusted model (β=0.040, standard error=0.023, *p*=0.080).

Table 4.6: Robust linear regression analyses for associations between significantly correlated variables and target microRNAs expression normalized with SNOR D48 according to raised waist circumference status in HIV infected adults

	miR-126-3p			miR-223-3p			miR-320a		
	β	Standard error	*p*-value	β	Standard error	*p*-value	β	Standard error	*p*-value
Hip circumference									
Model 1	0.246	0.184	0.183	0.040	0.023	0.080	-1.777	8.198	0.829
Model 2	0.192	0.179	0.286	0.023	0.020	0.261	1.586	6.793	0.815
Fasting glucose									
Model 1	0.005	0.006	0.423	0.001	0.001	0.170	-0.013	0.215	0.950
Model 2	0.007	0.006	0.255	0.001	0.001	0.097	-0.115	0.221	0.604
Fasting insulin									
Model 1	0.055	0.048	0.259	0.003	0.005	0.549	-2.078	1.532	0.175
Model 2	0.037	0.044	0.398	<0.001	0.005	0.950	-1.202	1.443	0.405
2-hour insulin									
Model 1	-0.001	0.202	0.996	0.007	0.022	0.745	-12.876	6.584	0.051
Model 2	-0.059	0.194	0.763	-0.006	0.021	0.776	-9.224	7.716	0.232
HOMA-IR									
Model 1	0.020	0.011	0.076	0.001	0.001	0.381	-0.366	0.355	0.302
Model 2	0.018	0.010	0.081	0.001	0.001	0.528	-0.171	0.340	0.615
Alanine Transaminase									
Model 1	-0.001	0.125	0.994	0.003	0.012	0.792	-6.113	4.405	0.166
Model 2	0.026	0.128	0.836	0.006	0.012	0.630	-6.548	4.750	0.169

Model 1: microRNA;
Model 2: model 1 + age + gender.
HIV; human immunodeficiency virus; HOMA-IR, homeostatic model assessment-estimated insulin resistance.

Logistic regression analysis to assess the association of raised waist circumference with target miRNAs

There were no significant associations determined between the target miRNAs and the presence of raised WC (Table S11).

4.2.3 The following outcomes were determined when the target miRNAs expression was normalised with SNOR D48 according to dysglycaemia status.

Fifteen participants were excluded from this analysis because their OGTT data was missing. Consequently, 660 participants were included in the analysis (Figure 3.1).

Relative miRNA expression

There were no significant differences in the expression of the target miRNAs miR-126-3p (p=0.483), miR-223-3p (p=0.964), and miR-320a (p=0.807) by dysglycaemia status (Table S2 and Figure 4.3).

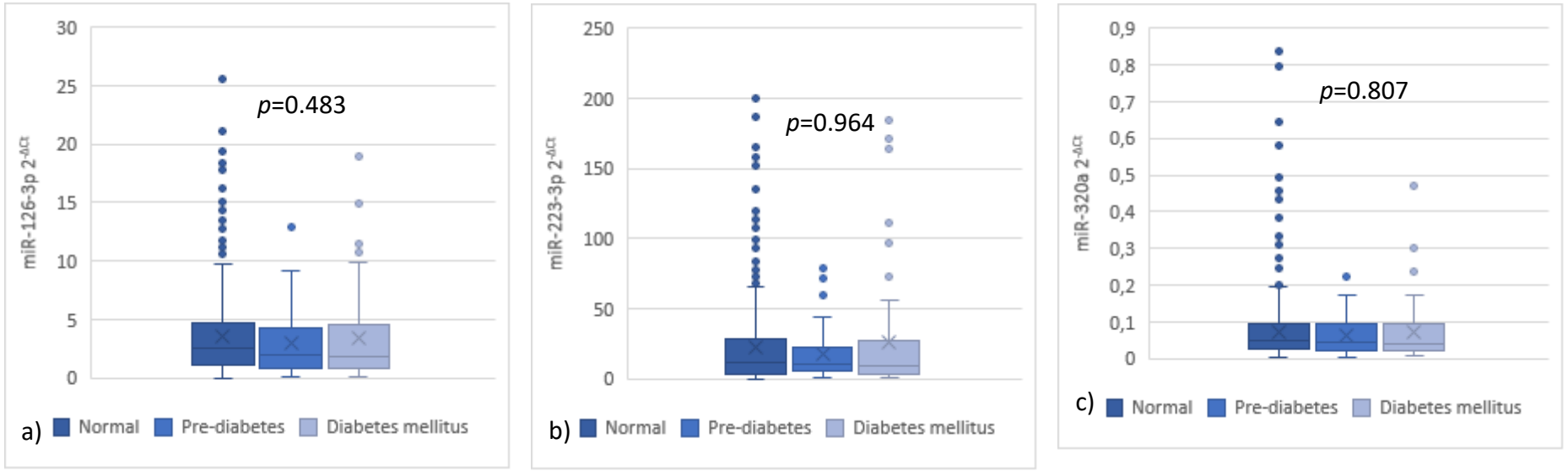

Figure 4.3: Box and whisker plots showing the relative expression of a) miR-126-3p, b) miR-223-3p and c) miR-320a normalised with SNOR D48 according to dysglycaemia status. *Pre-diabetes included impaired glucose tolerance (IGT) and impaired fasting glycaemia (IFG) defined as follows: IGT: fasting plasma glucose (FPG) <7.0 mmol/L and a 2-hour post glucose load between 7.8 mmol/L and 11.1 mmol/L; and IFG: FPG between 6.1 mmol/L and 7.0 mmol/L a with a 2-hour post glucose load <7.8 mmol/L. Diabetes mellitus was defined as: a FPG ≥7.0 mmol/L or a 2-hour post glucose load ≥11.1 mmol/L or known diabetes on treatment* [2]. *P-values are from the Kruskal Wallis test.*

Robust correlations to assess the associations between continuous variables and target miRNAs

MiR-126-3p was significantly correlated with fasting insulin (r=0.108, 95% CI: 0.030-0.180, p<0.006), 2-hour insulin (r=0.080, 95% CI: 0.000-0.160, p<0.040), and ALT (r=0.128, 95% CI: 0.050-0.200, p<0.001; Table 4.10). MiR-223-3p was significantly correlated with fasting insulin (r=0.111, 95% CI: 0.030-0.190, p<0.004), 2-hour insulin (r=0.095, 95% CI: 0.020-0.170, p<0.015), and ALT (r=0.091, 95% CI: 0.010-0.170, p<0.019; Table 4.7). MiR-320a was significantly correlated with CD4 count (r=0.082, 95% CI: 0.010-0.160, p<0.035), and fasting insulin (r=0.092, 95% CI: 0.020-0.170, p<0.018; Table 4.7). When correlations were re-assessed using the spearman rank correlation test, none of the three target miRNAs were significantly correlations with any of the continuous variables (Table S5).

Table 4.7: Univariate robust correlations between continuous variables and target microRNAs expression normalized with SNOR D48 according to dysglycaemia status in HIV infected adults

	miR-126-3p			**miR-223-3p**			**miR-320a**		
	r	95% CI	*p*-value	r	95% CI	*p*-value	r	95% CI	*p*-value
Socio-demographic:									
Age (years)	-0.056	(-0.130, 0.020)	<0.150	-0.025	(-0.100, 0.050)	<0.520	0.007	(-0.070, 0.080)	<0.860
Anthropometry:									
Weight (kg)	0.022	(-0.050, 0.100)	<0.570	0.044	(-0.030, 0.120)	<0.260	0.044	(-0.030, 0.120)	<0.260
Height (cm)	-0.008	(-0.080, 0.070)	<0.840	0.043	(-0.030, 0.120)	<0.270	0.040	(-0.040, 0.120)	<0.300
BMI (kg/m^2)	0.020	(-0.060, 0.100)	<0.610	0.029	(-0.050, 0.110)	<0.460	0.027	(-0.050, 0.100)	<0.490
Waist circumference (cm)	0.022	(-0.050, 0.100)	<0.570	0.034	(-0.040, 0.110)	<0.380	0.065	(-0.010, 0.140)	<0.095
Hip circumference (cm)	0.055	(-0.020, 0.130)	<0.160	0.074	(0.000, 0.150)	<0.057	0.066	(-0.010, 0.140)	<0.090
Waist-hip-ratio	-0.027	(-0.100, 0.050)	<0.490	-0.034	(-0.110, 0.040)	<0.380	0.013	(-0.060, 0.090)	<0.740
Waist-height-ratio	0.014	(-0.060, 0.090)	<0.720	0.015	(-0.060, 0.090)	<0.700	0.055	(-0.020, 0.130)	<0.160
Blood pressure:									
Systolic blood pressure (mmHg)	0.004	(-0.070, 0.080)	<0.920	0.004	(-0.070, 0.080)	<0.920	-0.019	(-0.100, 0.060)	<0.630
Diastolic blood pressure (mmHg)	<0.001	(-0.080, 0.080)	<0.980	-0.015	(-0.090, 0.060)	<0.700	-0.033	(-0.110, 0.040)	<0.400
HIV-related factors:									
CD4 count (cells/mm^3)	0.069	(-0.010, 0.140)	<0.076	0.038	(-0.040, 0.110)	<0.330	0.082	(0.010, 0.160)	**<0.035**
Lipids:									
Total cholesterol (mmol/L)	-0.034	(-0.110, 0.040)	<0.380	0.023	(-0.050, 0.100)	<0.560	-0.047	(-0.120, 0.030)	<0.230
HDL-C (mmol/L)	0.034	(-0.040, 0.110)	<0.380	0.020	(-0.060, 0.100)	<0.610	-0.016	(-0.090, 0.060)	<0.680
LDL-C (mmol/L)	-0.055	(-0.130, 0.020)	<0.160	0.008	(-0.070, 0.080)	<0.840	-0.053	(-0.130, 0.020)	<0.170
Triglycerides (mmol/L)	0.005	(-0.070, 0.080)	<0.900	0.048	(-0.030, 0.120)	<0.220	0.040	(-0.040, 0.120)	<0.300
Glucose homeostasis:									
Insulin fasting (mIU/L)	0.108	(0.030, 0.180)	**<0.006**	0.111	(0.030, 0.190)	**<0.004**	0.092	(0.020, 0.170)	**<0.018**
2-hour insulin (mIU/L)	0.080	(0.000, 0.160)	**<0.040**	0.095	(0.020, 0.170)	**<0.015**	0.040	(-0.040, 0.120)	<0.300
HbA1c (%)	-0.013	(-0.090, 0.060)	<0.740	0.037	(-0.040, 0.110)	<0.340	-0.036	(-0.110, 0.040)	<0.360
Inflammatory markers:									
Alanine Transaminase (U/L)	0.128	(0.050, 0.200)	**<0.001**	0.091	(0.010, 0.170)	**<0.019**	0.040	(-0.040, 0.120)	<0.300
Aspartate Aminotransferase (U/L)	0.068	(-0.010, 0.140)	<0.081	0.072	(0.000, 0.150)	<0.065	0.024	(-0.050, 0.100)	<0.540
Hs-CRP (mg/L)	0.009	(-0.070, 0.090)	<0.820	0.063	(-0.010, 0.140)	<0.110	0.046	(-0.030, 0.120)	<0.240

HIV, Human Immunodeficiency Virus; CI, confidence interval; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, glycated haemoglobin; hs-CRP, high-sensitivity C-reactive protein.

Robust linear regression analyses to assess the relationship between significantly correlated variables and target miRNAs

MiR-320a was significantly associated with 2-hour insulin in the unadjusted model (β=-13.613, standard error=6.495, *p*=0.037). However, this association was no longer significant after adjusting for age and gender (*p*=0.201; Table 4.8).

Table 4.8: Robust linear regression analyses for associations between significantly correlated variables and target microRNAs expression normalized with SNOR D48 according to dysglycaemia status in HIV infected adults

	miR-126-3p			**miR-223-3p**			**miR-320a**		
	β	Standard error	*p*-value	β	Standard error	*p*-value	β	Standard error	*p*-value
CD4 count									
Model 1	4.500	3.736	0.229	0.500	0.495	0.313	41.700	225.260	0.853
Model 2	4.106	3.747	0.274	0.349	0.494	0.480	41.955	217.468	0.847
Model 3	3.032	3.607	0.401	0.227	0.443	0.609	25.098	203.094	0.902
Model 4	3.119	3.695	0.399	0.230	0.447	0.607	23.363	209.298	0.911
Fasting insulin									
Model 1	0.046	0.047	0.327	0.003	0.005	0.604	-2.244	1.494	0.133
Model 2	0.031	0.043	0.468	<0.001	0.005	0.996	-1.313	1.414	0.353
Model 3	0.023	0.034	0.503	0.001	0.004	0.861	-0.261	1.450	0.857
Model 4	0.029	0.032	0.365	0.001	0.004	0.824	0.129	1.279	0.920
2-hour insulin									
Model 1	-0.033	0.197	0.868	0.004	0.021	0.841	-13.613	6.495	**0.037**
Model 2	-0.078	0.189	0.682	-0.008	0.021	0.716	-9.747	7.612	0.201
Model 3	-0.065	0.186	0.726	-0.008	0.020	0.708	-7.503	7.418	0.312
Model 4	-0.056	0.179	0.755	-0.007	0.019	0.705	-6.156	7.043	0.383
Alanine Transaminase									
Model 1	-0.001	0.125	0.996	0.003	0.012	0.802	-6.070	4.376	0.166
Model 2	0.023	0.128	0.858	0.005	0.012	0.659	-6.567	4.702	0.163
Model 3	0.013	0.128	0.920	0.005	0.012	0.672	-6.057	4.742	0.202
Model 4	0.013	0.128	0.917	0.005	0.011	0.681	-5.838	4.787	0.223

Model 1: microRNA;
Model 2: model 1 + age + gender;
Model 3: model 2 + BMI;
Model 4: model 2 + waist circumference.
HIV; human immunodeficiency virus; BMI, body mass index.

Logistic regression analysis to assess the association of pre-diabetes and diabetes mellitus with target miRNAs

Target miRNAs expression was not significantly associated with pre-diabetes nor with diabetes mellitus (Table S12).

4.2.4 The following outcomes were determined when the target miRNAs expression was normalised with SNOR D48 according to the presence or absence of insulin resistance.

Data were available for all 675 participants (Figure 3.1).

Relative miRNA expression

There were no significant differences in target miRNAs expression for miR-126-3p (p=0.648), miR-223-3p (p=0.541), and miR-320a (p=0.522), between participants with and without insulin resistance (Figure S4).

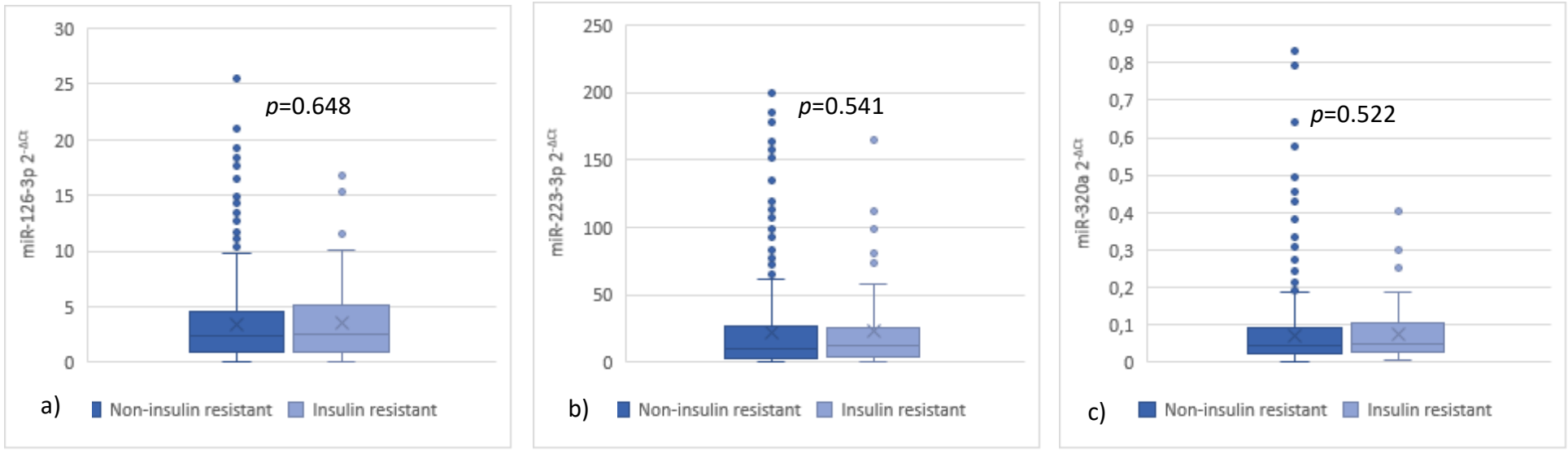

Figure *4.4: Box and whisker plots showing the relative expression of a) miR-126-3p, b) miR-223-3p and c) miR-320a normalised with SNOR D48 according to the presence or absence of insulin resistance.* *Participants with a homeostatic model assessment-estimated insulin resistance (HOMA-IR) above the data specific 90th percentile were considered insulin resistant. P-values are from the Wilcoxon rank sum test.*

Robust correlations to assess the associations between continuous variables and target miRNAs

MiR-126-3p was significantly correlated with 2-hour insulin (r=0.087, 95% CI: 0.010-0.160, p<0.024), and ALT (r=0.127, 95% CI: 0.050-0.200, p<0.001). MiR-223-3p was significantly positively correlated with 2-hour insulin (r=0.097, 95% CI: 0.020-0.170, p<0.012), and ALT (r=0.090, 95% CI: 0.010-0.160, p<0.019). MiR-320a was not significantly correlated with any of the continuous variables (Table 4.9).

When correlations were re-assessed using the spearman rank correlation test, there were no significant correlations of the three target miRNAs with any of the continuous variables (Table S6).

Table 4.9: Univariate robust correlations between continuous variables and target microRNAs expression normalized with SNOR D48 according to insulin resistance in HIV infected adults

	miR-126-3p			**miR-223-3p**			**miR-320a**		
	r	95% CI	*p*-value	r	95% CI	*p*-value	r	95% CI	*p*-value
Socio-demographic:									
Age (years)	-0.060	(-0.130, 0.020)	<0.120	-0.032	(-0.110, 0.040)	<0.410	0.001	(-0.070, 0.080)	<0.980
Anthropometry:									
Weight (kg)	0.021	(-0.050, 0.100)	<0.590	0.039	(-0.040, 0.110)	<0.310	0.040	(-0.040, 0.120)	<0.300
Height (cm)	-0.013	(-0.090, 0.060)	<0.740	0.038	(-0.040, 0.110)	<0.320	0.030	(-0.050, 0.110)	<0.440
BMI (kg/m^2)	0.020	(-0.060, 0.100)	<0.600	0.027	(-0.050, 0.100)	<0.480	0.026	(-0.050, 0.100)	<0.500
Waist circumference (cm)	0.019	(-0.060, 0.090)	<0.620	0.027	(-0.050, 0.100)	<0.480	0.058	(-0.020, 0.130)	<0.130
Hip circumference (cm)	0.053	(-0.020, 0.130)	<0.170	0.071	(0.000, 0.150)	<0.065	0.061	(-0.010, 0.140)	<0.110
Waist-hip-ratio	-0.027	(-0.100, 0.050)	<0.480	-0.035	(-0.110, 0.040)	<0.360	0.014	(-0.060, 0.090)	<0.720
Waist-height-ratio	0.013	(-0.060, 0.090)	<0.740	0.009	(-0.070, 0.080)	<0.820	0.052	(-0.020, 0.130)	<0.180
Blood pressure:									
Systolic blood pressure (mmHg)	0.017	(-0.060, 0.090)	<0.660	0.020	(-0.060, 0.100)	<0.600	-0.007	(-0.080, 0.070)	<0.860
Diastolic blood pressure (mmHg)	0.009	(-0.070, 0.080)	<0.820	<-0.001	(-0.080, 0.070)	<0.980	-0.023	(-0.100, 0.050)	<0.550
HIV-related factors:									
CD4 count (cells/mm^3)	0.053	(-0.020, 0.130)	<0.170	0.010	(-0.070, 0.090)	<0.800	0.058	(-0.020, 0.130)	<0.130
Lipids:									
Total cholesterol (mmol/L)	-0.025	(-0.100, 0.050)	<0.520	0.027	(-0.050, 0.100)	<0.480	-0.043	(-0.120, 0.030)	<0.260
HDL-C (mmol/L)	0.031	(-0.040, 0.110)	<0.420	0.023	(-0.050, 0.100)	<0.550	-0.018	(-0.090, 0.060)	<0.640
LDL-C (mmol/L)	-0.053	(-0.130, 0.020)	<0.170	0.006	(-0.070, 0.080)	<0.880	-0.052	(-0.130, 0.020)	<0.180
Triglycerides (mmol/L)	0.005	(-0.070, 0.080)	<0.900	0.046	(-0.030, 0.120)	<0.230	0.043	(-0.030, 0.120)	<0.260
Glucose homeostasis:									
2-hour glucose (mmol/L)	0.039	(-0.040, 0.110)	<0.310	0.074	(0.000, 0.150)	<0.055	0.063	(-0.010, 0.140)	<0.100
2-hour insulin (mIU/L)	0.087	(0.010, 0.160)	**<0.024**	0.097	(0.020, 0.170)	**<0.012**	0.050	(-0.030, 0.120)	<0.190
HbA1c (%)	-0.013	(-0.090, 0.060)	<0.740	0.034	(-0.040, 0.110)	<0.380	-0.034	(-0.110, 0.040)	<0.380
Inflammatory markers:									
Alanine Transaminase (U/L)	0.127	(0.050, 0.200)	**<0.001**	0.090	(0.010, 0.160)	**<0.019**	0.039	(-0.040, 0.110)	<0.310
Aspartate Aminotransferase (U/L)	0.067	(-0.010, 0.140)	<0.082	0.070	(-0.010, 0.140)	<0.069	0.022	(-0.050, 0.100)	<0.570
Hs-CRP (mg/L)	0.011	(-0.060, 0.090)	<0.780	0.068	(-0.010, 0.140)	<0.077	0.050	(-0.030, 0.120)	<0.190

HIV, Human Immunodeficiency Virus; CI, confidence interval; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, glycated haemoglobin; hs-CRP, high-sensitivity C-reactive protein.

Robust linear regression analyses to assess the relationship between significantly correlated variables and target miRNAs

There was a borderline association between miR-320a and 2-hour insulin in the unadjusted model (β=-12.880, standard error=6.567, *p*=0.050). However, when adjusted for age and gender this observation was lost (*p*=0.243, Table 4.10).

Table 4.10: Robust linear regression analyses for associations between significantly correlated variables and target microRNAs expression normalized with SNOR D48 according to insulin resistance in HIV infected adults

	miR-126-3p			**miR-223-3p**			**miR-320a**		
	β	Standard error	*p*-value	β	Standard error	*p*-value	β	Standard error	*p*-value
2-hour Insulin									
Model 1	-0.006	0.201	0.975	0.007	0.022	0.750	-12.880	6.567	0.050
Model 2	-0.058	0.193	0.764	-0.006	0.021	0.791	-9.030	7.722	0.243
Model 3	-0.047	0.190	0.802	-0.006	0.020	0.778	-6.833	7.529	0.364
Model 4	-0.038	0.183	0.835	-0.006	0.020	0.778	-5.495	7.142	0.442
Alanine Transaminase									
Model 1	-0.005	0.125	0.969	0.003	0.012	0.822	-6.171	4.390	0.160
Model 2	0.021	0.128	0.872	0.005	0.012	0.666	-6.674	4.732	0.159
Model 3	0.009	0.128	0.944	0.005	0.012	0.689	-6.219	4.770	0.193
Model 4	0.009	0.128	0.941	0.005	0.012	0.697	-6.009	4.815	0.212

Model 1: microRNA;
Model 2: model 1 + age + gender;
Model 3: model 2 + BMI;
Model 4: model 2 + waist circumference.
HIV; human immunodeficiency virus.

Logistic regression analysis to assess the association of insulin resistance with target miRNAs

There were no significant associations between the target miRNAs expression and the presence of insulin resistance (Table S13).

4.2.5 The following outcomes were determined when the target miRNAs expression was normalised with SNOR D48 according to the presence or absence of elevated hs-CRP.

Fourteen participants were excluded from this analysis because their hs-CRP levels were not evaluated. Resultantly, 661 participants were included in the analysis.

Relative miRNA expression

No significant differences were determined in the expression of miRNAs miR-223-3p (p=0.982) and miR-320a (p=0.158), between participants with and without elevated hs-CRP. Only, miR-126-3p showed a borderline difference in expression between participants with and without elevated hs-CRP (p=0.064; Figure 4.5).

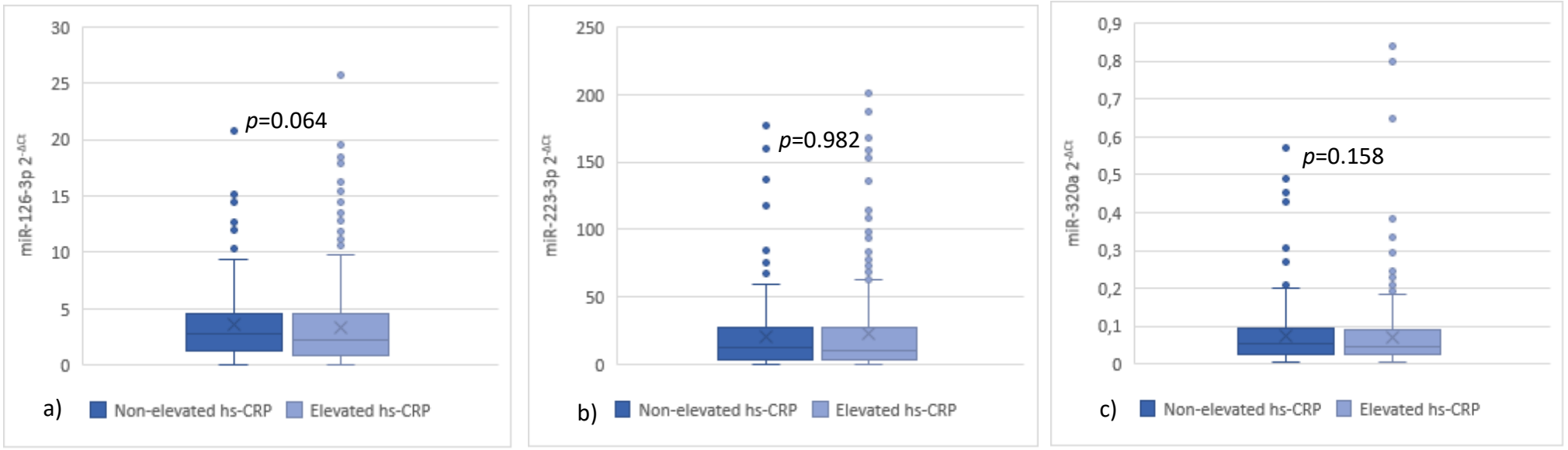

Figure 4.5: Box and whisker plots showing the relative expression of a) miR-126-3p, b) miR-223-3p and c) miR-320a normalised with SNOR D48 according to the presence or absence of elevated high-sensitivity C-reactive protein (hs-CRP). *Participants with an hs-CRP level >3 mg/L were considered to have elevated hs-CRP. P-values are from the Wilcoxon rank sum test.*

Robust correlations to assess the associations between continuous variables and target miRNAs

MiR-126-3p was significantly correlated with fasting glucose (r=0.101, 95% CI: 0.020-0.180, *p*<0.009), fasting insulin (r=0.112, 95% CI: 0.040-0.190, *p*<0.004), 2-hour insulin (r=0.086, 95% CI: 0.010-0.160, *p*<0.027), HOMA-IR (r=0.131, 95% CI: 0.060-0.210, *p*<0.001), and ALT (r=0.128, 95% CI: 0.050-0.200, *p*<0.001).

MiR-223-3p was significantly correlated with fasting glucose (r=0.078, 95% CI: 0.000-0.150, *p*<0.045), fasting insulin (r=0.113, 95% CI: 0.040-0.190, *p*<0.004), 2-hour insulin (r=0.096, 95% CI: 0.020-0.170, *p*<0.014), HOMA-IR (r=0.139, 95% CI: 0.060-0.210, *p*<0.001), and ALT (r=0.090, 95% CI: 0.010-0.170, *p*<0.021).

MiR-320a was significantly correlated with CD4 count (r = 0.084, 95% CI: 0.010 - 0.160, *p* < 0.031), fasting glucose (r = 0.127, 95% CI: 0.050 - 0.200, *p* < 0.001), fasting insulin (r = 0.096, 95% CI: 0.020 - 0.170, *p*<0.014), and HOMA-IR (r = 0.118, 95% CI: 0.040 - 0.190, *p*<0.002; Table 4.11).

When correlations were re-assessed using the spearman rank correlation test, only miR-126-3p was significantly correlated with HOMA-IR (r=0.102, *p*=0.008), while miR-223-3p and miR-320a were not significantly correlated with any continuous variable (Table S7).

Table 4.11: Univariate robust correlations between continuous variables and target microRNAs expression normalized with SNOR D48 according to hs-CRP status in HIV infected adults

	miR-126-3p			**miR-223-3p**			**miR-320a**		
	r	95% CI	*p*-value	r	95% CI	*p*-value	r	95% CI	*p*-value
Socio-demographic:									
Age (years)	-0.059	(-0.130, 0.020)	<0.130	-0.030	(-0.110, 0.050)	<0.440	0.004	(-0.070, 0.080)	<0.920
Anthropometry:									
Weight (kg)	0.023	(-0.050, 0.100)	<0.550	0.046	(-0.030, 0.120)	<0.240	0.046	(-0.030, 0.120)	<0.240
Height (cm)	-0.011	(-0.090, 0.070)	<0.780	0.040	(-0.040, 0.120)	<0.300	0.036	(-0.040, 0.110)	<0.360
BMI (kg/m^2)	0.022	(-0.050, 0.100)	<0.570	0.033	(-0.040, 0.110)	<0.400	0.031	(-0.050, 0.110)	<0.430
Waist circumference (cm)	0.023	(-0.050, 0.100)	<0.550	0.035	(-0.040, 0.110)	<0.370	0.068	(-0.010, 0.140)	<0.081
Hip circumference (cm)	0.054	(-0.020, 0.130)	<0.170	0.076	(0.000, 0.150)	<0.051	0.068	(-0.010, 0.140)	<0.081
Waist-hip-ratio	-0.021	(-0.100, 0.060)	<0.590	-0.033	(-0.110, 0.040)	<0.400	0.017	(-0.060, 0.090)	<0.660
Waist-height-ratio	0.016	(-0.060, 0.090)	<0.680	0.015	(-0.060, 0.090)	<0.700	0.058	(-0.020, 0.130)	<0.140
Blood pressure:									
Systolic blood pressure (mmHg)	0.004	(-0.070, 0.080)	<0.920	0.004	(-0.070, 0.080)	<0.920	-0.021	(-0.100, 0.060)	<0.590
Diastolic blood pressure (mmHg)	<-0.001	(-0.080, 0.080)	<0.980	-0.011	(-0.090, 0.070)	<0.780	-0.034	(-0.110, 0.040)	<0.380
HIV-related factors:									
CD4 count (cells/mm^3)	0.070	(-0.010, 0.150)	<0.072	0.043	(-0.030, 0.120)	<0.270	0.084	(0.010, 0.160)	**<0.031**
Lipids:									
Total cholesterol (mmol/L)	-0.036	(-0.110, 0.040)	<0.360	0.023	(-0.050, 0.100)	<0.550	-0.049	(-0.120, 0.030)	<0.210
HDL-C (mmol/L)	0.032	(-0.040, 0.110)	<0.410	0.022	(-0.050, 0.100)	<0.570	-0.016	(-0.090, 0.060)	<0.680
LDL-C (mmol/L)	-0.053	(-0.130, 0.020)	<0.170	0.007	(-0.070, 0.080)	<0.860	-0.053	(-0.130, 0.020)	<0.170
Triglycerides (mmol/L)	0.004	(-0.070, 0.080)	<0.920	0.044	(-0.030, 0.120)	<0.260	0.040	(-0.040, 0.120)	<0.300
Glucose homeostasis:									
Fasting glucose (mmol/L)	0.101	(0.020, 0.180)	**<0.009**	0.078	(0.000, 0.150)	**<0.045**	0.127	(0.050, 0.200)	**<0.001**
2-hour glucose (mmol/L)	0.036	(-0.040, 0.110)	<0.360	0.071	(-0.010, 0.150)	<0.068	0.059	(-0.020, 0.130)	<0.130
Insulin fasting (mIU/L)	0.112	(0.040, 0.190)	**<0.004**	0.113	(0.040, 0.190)	**<0.004**	0.096	(0.020, 0.170)	**<0.014**
2-hour insulin (mIU/L)	0.086	(0.010, 0.160)	**<0.027**	0.096	(0.020, 0.170)	**<0.014**	0.047	(-0.030, 0.120)	<0.230
HbA1c (%)	-0.013	(-0.090, 0.060)	<0.740	0.035	(-0.040, 0.110)	<0.370	-0.036	(-0.110, 0.040)	<0.360
HOMA-IR	0.131	(0.060, 0.210)	**<0.001**	0.139	(0.060, 0.210)	**<0.001**	0.118	(0.040, 0.190)	**<0.002**
Inflammatory markers:									
Alanine Transaminase (U/L)	0.128	(0.050, 0.200)	**<0.001**	0.090	(0.010, 0.170)	**<0.021**	0.039	(-0.040, 0.110)	<0.320
Aspartate Aminotransferase (U/L)	0.066	(-0.010, 0.140)	<0.090	0.069	(-0.010, 0.140)	<0.076	0.021	(-0.060, 0.100)	<0.590

HIV, Human Immunodeficiency Virus; CI, confidence interval; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, glycated haemoglobin; HOMA-IR, homeostatic model assessment-estimated insulin resistance.

Robust linear regression analyses to assess the relationship between significantly correlated variables and target miRNAs

Only miR-320a was significantly associated with 2-hour insulin in the unadjusted model (β=-13.199, standard error=6.531, *p*=0.044). However, this association was no longer significant after adjusting for age and gender (*p*=0.222).

Other notable findings include: miR-223-3p was borderline associated with fasting glucose when adjusted for age, gender, and BMI (β=0.001, standard error=0.001, *p*=0.086), and when WC replaced BMI as the measure of adiposity in the model the relationship remained borderline (β=0.001, standard error=0.001, *p*=0.066; Table 4.12).

Table 4.12: Robust linear regression analyses for associations between significantly correlated variables and target microRNAs expression normalized with SNOR D48 according to hs-CRP status in HIV infected adults

	miR-126-3p			**miR-223-3p**			**miR-320a**		
	β	Standard error	*p*-value	β	Standard error	*p*-value	β	Standard error	*p*-value
CD4 count									
Model 1	4.420	3.748	0.239	0.487	0.497	0.327	36.110	224.780	0.872
Model 2	3.988	3.760	0.290	0.335	0.496	0.501	34.079	217.045	0.875
Model 3	2.868	3.627	0.430	0.206	0.445	0.644	13.807	203.486	0.946
Model 4	2.977	3.712	0.423	0.213	0.448	0.635	12.689	209.877	0.952
Fasting glucose									
Model 1	0.005	0.006	0.439	0.001	0.001	0.173	-0.019	0.211	0.928
Model 2	0.006	0.006	0.275	0.001	0.001	0.102	-0.124	0.217	0.568
Model 3	0.006	0.006	0.281	0.001	0.001	0.086	-0.039	0.231	0.865
Model 4	0.007	0.006	0.230	0.001	0.001	0.066	-0.020	0.226	0.928
Fasting insulin									
Model 1	0.049	0.048	0.305	0.003	0.005	0.596	-2.187	1.499	0.145
Model 2	0.033	0.043	0.445	<0.001	<0.001	0.991	-1.265	1.419	0.373
Model 3	0.024	0.034	0.479	0.001	0.004	0.861	-0.224	1.462	0.879
Model 4	0.031	0.032	0.340	0.001	0.004	0.817	0.171	1.290	0.894

Table 4.12 continued.									
	miR-126-3p			miR-223-3p			miR-320a		
	β	Standard error	*p*-value	β	Standard error	*p*-value	β	Standard error	*p*-value
2-hour insulin									
Model 1	-0.016	0.199	0.934	0.006	0.021	0.791	-13.199	6.531	**0.044**
Model 2	-0.068	0.191	0.721	-0.007	0.021	0.743	-9.396	7.690	0.222
Model 3	-0.056	0.188	0.765	-0.007	0.020	0.733	-7.165	7.503	0.340
Model 4	-0.046	0.181	0.800	-0.007	0.019	0.736	-5.786	7.118	0.417
HOMA-IR									
Model 1	0.015	0.011	0.152	0.001	0.001	0.559	-0.468	0.340	0.169
Model 2	0.014	0.010	0.175	<0.001	0.001	0.795	-0.265	0.328	0.420
Model 3	0.011	0.008	0.179	<0.001	0.001	0.741	-0.059	0.339	0.862
Model 4	0.012	0.008	0.119	0.001	0.001	0.620	0.026	0.309	0.932
Alanine Transaminase									
Model 1	-0.005	0.124	0.962	0.002	0.012	0.836	-6.237	4.370	0.154
Model 2	0.019	0.127	0.879	0.005	0.012	0.680	-6.756	4.717	0.153
Model 3	0.008	0.127	0.947	0.004	0.012	0.699	-6.276	4.759	0.188
Model 4	0.009	0.127	0.944	0.004	0.012	0.706	-6.062	4.805	0.208

Model 1: microRNA;
Model 2: model 1 + age + gender;
Model 3: model 2 + BMI;
Model 4: model 2 + waist circumference.
HIV; human immunodeficiency virus; HOMA-IR, homeostatic model assessment-estimated insulin resistance.

Logistic regression analysis to assess the associations of elevated hs-CRP with target miRNAs

There were no significant associations determined between the target miRNAs and elevated hs-CRP (Table S14).

Chapter 5: Discussion

5.1 Discussion

To our knowledge this is the first study that profiled the expression patterns of miRNAs, miR-126-3p, -223-3p, and -320a, with CMDs/traits of overweight/obesity, raised WC, dysglycaemia, insulin resistance, and elevated hs-CRP in PLWH. Our findings demonstrated that although the target miRNAs were not significantly differentially expressed between participants with and without individual CMDs/traits, their expressions were significantly correlated with glucose homeostasis variables. Also, miR-126-3p and -223-3p was significantly correlated with ALT. However, there were no significant associations between the three target miRNAs expression and select CMDs/traits stated above in PLWH.

5.1.1 Target miR-126-3p expression with dysglycaemia and insulin resistance

Our results describing the role of miR-126-3p with dysglycaemia in PLWH contrasts with findings from a study conducted in a community-based South African population that reported significant associations between miR-126-3p expression and pre-diabetes and diabetes mellitus in a fully adjusted model [158]. However, the aforementioned study did find variables such as age, alcohol use, systolic blood pressure, lipids and hs-CRP, were significantly associated with dysglycaemia status, which was similar to our results [158]. Furthermore, a case control study from a general Iranian population reported results different to our findings; these included significant associations between miR-126-3p expression and diabetes mellitus, pre-diabetes and insulin resistance [159]. However, a key difference between our study and the aforementioned studies, is that our population consists only of PLWH while the other two studies were conducted in general populations. The HIV infection, together with HIV-related factors such as ART use, could be influencing these discrepant results. Our results showed that dysglycaemia status is influenced by the ART regime. A miRNA profiling study conducted on HIV-infected South African women with gestational diabetes mellitus reported differences, albeit insignificant, in miRNA expression between women who were ART-naïve *vs* ART-treated [27]. This implies a potential role ARTs might have on altering miRNA expression, and warrants further investigation. However, this was not investigated in this study as the effects of ART use on miRNA expression did not fall within the scope of our study.

5.1.2 Target miR-126-3p expression with overweight/obesity and raised WC

Our study findings describing miR-126-3p relationship with raised adiposity also contrasts with findings from a general South African population, which reported significant associations between miR-126-3p expression and WC and hip circumference [130]. Additionally, unlike our findings, miR-126-3p expression has been significantly associated with overweight/obesity in general populations globally. The latter was reported in a systematic review by Dosal *et al* (2019) and a meta-analysis by Veie *et al* (2023) [128,160]. However, as stated above, differences in HIV status between our population and the aforementioned could be the source of the discrepant findings. Though, based on our results it appears that miR-126-3p might not be a good marker for raised adiposity in PLWH.

5.1.3 Target miR-126-3p expression with inflammation

Our investigation into the relation of miR-126-3p with inflammation in PLWH reports similar findings to a community-based South African population, which reported insignificant associations between miR-126-3p expression and ultra-sensitive C-reactive protein [130]. Additionally, similar to our results, a case-control study from an elderly Italian population also found an insignificant association between miR-126-3p expression and hs-CRP [161]. The results from our study and the aforementioned studies suggest that miR-126-3p might not have an active role in managing hs-CRP, however more studies are needed to confirm this. Interestingly, our study findings support the hypothesis speculated by Coulson *et al* (2021) who used ingenuity pathway analysis to a correlate miR-126-3p expression with ALT activation [162]. Despite not being significantly associated with hs-CRP, the significant association between miR-126-3p and ALT warrants further investigation into usefulness of miR126-3p as a marker of inflammation.

5.1.4 Target miR-223-3p expression with dysglycaemia and insulin resistance

Different to our results, only one other study profiled miR-223-3p expression with diabetes mellitus in a community-based South African population [117]. Differences in age (53.4 years vs 38.5 years in our study) as well as HIV infection between our populations could be supporting the discrepant findings. Furthermore, and although not directly comparable, the lack of significant associations between miR-223-3p expression and dysglycaemia found in our study, contrasts with findings from a Swedish adolescent population, which reported significant upregulation of miR-223-3p in participants with type 1 diabetes mellitus *vs* controls

[163]. Additionally, unlike our findings, results from a general Qatar population reported significant differential expression of miR-223-3p between participants with diabetes mellitus vs controls [164]. Also contrary to our findings, a study using subcutaneous adipose tissue samples collected from an American cohort of women reported significant upregulation of miR-223-3p expression in participants with insulin resistance vs those without [165]. Unfortunately, miR-223-3p profiling studies were mostly done outside African and HIV infected populations which could not be used for comparison. However, our results indicating a significant association for variables such as gender, smoking, glucose homeostasis, and lipids, with overweight/obesity status, were similar to a study done on HIV-infected South Africans [166]. Therefore, we suspect the discrepant miRNA analysis findings between our study and the aforementioned studies conducted on global general populations, could be the result of genetic diversity in the African population [167]. Unfortunately, more often than not, our miRNA profiling studies are conceptualized off findings from populations whose genetics are very different to our own.

5.1.5 Target miR-223-3p expression with overweight/obesity and raised WC

Unlike our findings, miR-223-3p expression was significantly associated with adiposity in subcutaneous adipose tissue samples collected from obese women in New Zealand [168]. In addition, also unlike our findings, a study from a United States general population reported that miR-223-3p expression profiled in circulating serum was significantly dysregulated with obesity, and was also significantly correlated with WC [169]. The contrasting findings between our study which profiled miRNA expression in whole blood samples, compared to the aforementioned studies which used adipose tissue and circulating serum, demonstrates how differences in sample type may affect miRNA expression [170,171].

5.1.6 Target miR-223-3p expression with inflammation

Our study findings were similar to a case-control study from a general Iranian population that reported miR-223-3p expression was not significantly associated with hs-CRP. However, there were no studies done on African or HIV-infected populations for comparison, therefore further investigation is needed to confirm our study findings. Furthermore, our results supported evidence from an in vivo study done in mice, which reported that treatment with miR-223-3p reversed serum ALT levels to normal [172]. These findings suggest that miR-223-3p could also potentially be used as a marker for inflammation directly correlated to ALT.

5.1.7 Target miR-320a expression with dysglycaemia and insulin resistance

Unlike the findings from our study, a study from an Asian Indian population living in America reported that miR-320a expression was significantly associated with glycaemic impairment in fully adjusted models [173]. Also discrepant to our findings, a meta-analysis by Villard *et al* (2015) reported miR-320a was differentially expressed with diabetes mellitus in both general Caucasian and Asian populations globally [174]. Likewise, a functional analysis study done on in vitro insulin resistant adipocytes also reported findings different to our own, where miR-320a expression was increased 50-fold with insulin resistance [132]. However, there were no studies done on African or HIV-infected populations that could be used for comparison. We suspect differences in the miRNA profiling studies such as sample type, profiling method, endogenous control used, is the source for the discrepant findings between our study and the aforementioned studies [171,175].

5.1.8 Target miR-320a expression with overweight/obesity and raised WC

In addition, unlike our findings, miR-320a expression was significantly associated with BMI and WC in a general population of obese Egyptian women [119]. As previously stated, the HIV infection in our population could be potentially altering miRNA expression, and therefore should be further investigated.

5.1.9 Target miR-320a expression with inflammation

Albeit not comparable, , a German study among patients with severe respiratory failure following SARS-CoV-2 infection reported miR-320a expression was significantly associated with CRP, unlike our study findings [176]. However, similar to our study findings, a study from a general obese Italian population reported an insignificant association between miR-320a expression and CRP [177]. The inconsistency of these results, highlights how the same miRNA can be differentially expressed in different populations.

5.2 Strengths and limitations

The strength of our study lies in its novelty, there were only two other publications which looked at miRNAs and dysglycaemia in PLWH [26,27], neither of which profiled the three target miRNAs investigated in this study. Furthermore, the use of a South African population of PLWH strengthens the reliability of our findings in our setting as South Africa has the most substantial burden of HIV globally [6,7]. Additionally, using RT-qPCR to quantify the target

miRNAs expression is a highly sensitive technique of profiling miRNAs, as such also strengthening the reliability of subsequent results at an affordable rate.

A limitation of the study was the skewness of the sample towards female participants – a common observation in South African studies. Furthermore, we acknowledge the potential of chance findings from multiple comparisons in the study, and that not using a more robust cut-off for significance, or not correcting for multiple comparisons constitutes a limitation. Another limitation was the study population only consisting of HIV infected participants, having a non-HIV infected population would have added to the richness of this study. Another potential limitation is the use of traditional medication by PLWH in South Africa [178]. There could be drug interactions from the traditional medicine that may potentially having a masking effect on miRNA expression [179].

5.3 Conclusion

Key findings from the study, is that the three target miRNAs all had significant correlations with glucose homeostasis variables in the univariate analyses. However, after the effects of age, gender, and WC were accounted for, only borderline associations were established between miR-126-3p and HOMA-IR; and between miR-223-3p and fasting glucose. There were no significant associations between the target miRNAs and the select CMDs/traits being investigated. The results of this study were mostly insignificant and trended towards these target miRNAs not being important markers for the select CMDs/traits investigated for PLWH. However, given the minimal evidence on this topic, further investigation is needed for inter-study comparisons, as well as to establish miRNAs that are significantly associated with CMDs in PLWH. Our recommendations for future studies include using a genome-wide sequencing approach to identify target miRNAs, in place of using candidate miRNAs. This would be more informative and reveal more relevant miRNAs for PLWH. Future functional analysis studies are also warranted to investigate the effects of medications such as ARTs on miRNA expression. This may highlight some unknown drug-interactions that are potentially masking/altering miRNA expression.

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Appendices

A1. Supplementary tables

Table S1: Socio-demographic, clinical, and genetic factors analysed on overweight/obesity status in HIV infected adults

	Normal (n=282)	Overweight (n=162)	Obesity (n=230)	*p*-value
Socio-demographic factors:				
Median age (years) (25^{th}-75^{th} percentiles)	38 (30-45)	38 (33-45)	37 (32-43)	0.457
Gender, n (%)				**<0.001**
Men	102 (36.2)	19 (11.7)	7 (3.0)	
Women	180 (63.8)	143 (88.3)	223 (97.0)	
Lifestyle factors, n (%):				
Current smoker	108 (38.3)	22 (13.6)	22 (9.6)	**<0.001**
Current alcohol user	135(47.9)	63 (38.9)	71 (30.9)	**0.008**
***Median HIV related factors (25^{th}-75^{th} percentiles)*:**				
Antiretroviral therapy regime, n (%)				0.430
ART-Naïve	15 (5.3)	11 (6.8)	12 (5.2)	
First line	165 (58.5)	89 (54.9)	135 (58.7)	
Second line	27 (9.6)	17 (10.5)	24 (10.4)	
Other[1]	75 (26.6)	45 (27.8)	59 (25.7)	
CD4 count (cells/mm^3)	328.5 (200.5-500.0)	402.0 (236.0-634.0)	458.0 (280.5-699.8)	**<0.001**
***Median blood pressure levels (25^{th}-75^{th} percentiles)*:**				
Systolic blood pressure (mmHg)	117.0 (106.5-129.6)	118.5 (107.9-128.6)	116.3 (107.5-128.3)	0.775
Diastolic blood pressure (mmHg)	80.8 (73.0-89.5)	83.5 (75.0-92.1)	82.5 (76.5-91.5)	**0.024**
Median biochemical parameters (25^{th}-75^{th} percentiles):				
Lipids (mmol/L):				
Total cholesterol	4.1 (3.5-5.0)	4.2 (3.5-5)	4.4 (3.6-5.0)	0.758
HDL-C	1.3 (1.01-1.6)	1.2 (1.0-1.5)	1.2 (1.0-1.5)	**0.005**
LDL-C	2.3 (1.8-2.9)	2.5 (1.9-3.1)	2.7 (2.2-3.2)	**<0.001**
Triglycerides	1.0 (0.7-1.3)	1.0 (0.8-1.4)	1.1 (0.8-1.4)	**0.011**

Table S1 continued.				
	Normal (n=282)	Overweight (n=162)	Obesity (n=230)	*p*-value
Glucose homeostasis variables:				
Fasting glucose (mmol/L)	5.0 (4.6-5.3)	5 (4.6-5.3)	5.1 (4.7-5.6)	**0.004**
2-hour glucose (mmol/L)	5.1 (4.4-6.0)	5.1 (4.6-6.2)	5.7 (4.9-6.4)	**<0.001**
Fasting insulin (mIU/L)	4.2 (2.9-6.3)	6.3 (4.5-9.4)	8.9 (6.2-12.5)	**<0.001**
2-hour insulin (mIU/L)	19.4 (11.6-30.1)	26.2 (14.5-38.1)	34.4 (18.6-59.3)	**<0.001**
HbA1c (%)	5.4 (5.2-5.7)	5.4 (5.1-5.7)	5.6 (5.2-5.9)	**<0.001**
HOMA-IR	0.9 (0.6-1.4)	1.4 (0.9-2.1)	1.9 (1.3-3.0)	**<0.001**
Inflammatory markers:				
Alanine transaminase (U/L)	22.0 (17.0-34.0)	22.5 (17.0-34.0)	24.0 (18.0-34.0)	0.519
Aspartate aminotransferase (U/L)	32.0 (26.0-41.0)	28.0 (24.0-36.0)	27.0 (23.0-34.0)	**<0.001**
hs-CRP (mg/L)	4.3 (1.6-12.0)	4.4 (2.2-9.5)	8.0 (3.6-16.0)	**<0.001**
Median genetic marker $2^{-\Delta Ct}$ values (25^{th}-75^{th} percentiles):				
miR-126-3p	2.4 (1.0-4.6)	2.4 (1.0-4.3)	2.7 (1.0-4.8)	0.966
miR-223-3p	11.3 (3.3-24.5)	10.6 (3.8-27.1)	10.8 (2.9-32.6)	0.993
miR-320a	0.05 (0.02-0.10)	0.04 (0.02-0.10)	0.05 (0.02-0.09)	0.860

HIV, Human Immunodeficiency Virus; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, glycated haemoglobin; HOMA-IR, homeostatic model assessment-estimated insulin resistance; and hs-CRP, high-sensitivity C-reactive protein.

[1]Other: single nucleotide reverse transcriptase inhibitors (NRTI), single non-nucleoside reverse transcriptase inhibitors (NNRTI), combined NRTI + protease inhibitors (PI), double NRTIs, combined NRTI + NNRTI, double PIs only, and unknown medications.

Normal: body mass index (BMI) <25.0 kg/m^2, Overweight: BMI 25.0–29.9 kg/m^2, and Obesity: BMI ≥30.0 kg/m^2.

Table S2: Analysis of Socio-demographic, clinical, and genetic factors on dysglycaemia status in HIV infected adults

	Normal (n=551)	Pre-diabetes (n=51)	Diabetes mellitus (n=58)	*p*-value
Socio-demographic factors:				
Median age (years) (25^{th}-75^{th} percentiles)	37.0 (31.0-43.0)	43.0 (37.0-49.0)	45.0 (36.8-51.0)	**<0.001**
Gender, n (%)				0.174
Men	99 (18.0)	13 (25.5)	15 (25.9)	
Women	452 (82.0)	38 (74.5)	43 (74.1)	
Lifestyle factors, n (%):				
Current smoker	127 (23.0)	11 (21.6)	13 (22.4)	0.980
Current alcohol user	223 (40.5)	22 (43.1)	19 (32.8)	**0.011**
Median anthropometry variables (25^{th}-75^{th} percentiles):				
Body mass index (kg/m^2)	26.6 (22.1-31.8)	27.3 (24.1-32.5)	29.0 (24.0-34.8)	0.108
***Median HIV related factors (25^{th}-75^{th} percentiles)*:**				
Antiretroviral therapy regime, n (%)				**0.025**
ART-Naïve	26 (4.7)	3 (5.9)	9 (15.5)	
First line	329 (59.7)	30 (58.8)	21 (36.2)	
Second line	59 (10.7)	3 (5.9)	5 (8.6)	
Other[1]	137 (24.9)	15 (29.4)	23 (39.7)	
CD4 count ($cells/mm^3$)	385.0 (238.0-612.0)	395.0 (186.5-555.0)	500.0 (331.5-878.5)	**0.023**
***Median blood pressure levels (25^{th}-75^{th} percentiles)*:**				
Systolic blood pressure (mmHg)	116.0 (106.5-127.5)	118.5 (109.5-140.0)	123.3 (117.4-144.6)	**<0.001**
Diastolic blood pressure (mmHg)	81.5 (74.5-89.5)	82.0 (78.0-93.0)	86.5 (75.9-95.8)	0.072
Median biochemical parameters (25^{th}-75^{th} percentiles):				
Lipids (mmol/L):				
Total cholesterol	4.2 (3.5-5)	4.4 (3.7-5.1)	4.6 (3.5-5.5)	0.068
HDL-C	1.3 (1.1-1.6)	1.2 (0.9-1.4)	1.2 (0.9-1.4)	**0.005**
LDL-C	2.4 (1.9-3.1)	2.6 (2.1-3.1)	3.0 (2.3-3.7)	**0.003**
Triglycerides	1.0 (0.7-1.3)	1.1 (0.9-1.5)	1.4 (1.0-2.1)	**<0.001**
Inflammatory markers:				
Alanine transaminase (U/L)	23.0 (17.0-34.0)	23.0 (19.0-33.0)	25.0 (17.0-34.5)	0.799
Aspartate aminotransferase (U/L)	29.0 (24.0-38.0)	30.0 (27.0-37.0)	28.5 (23.0-33.5)	0.283
hs-CRP (mg/L)	5.2 (2.2-12.7)	6.8 (3.3-16.6)	7.9 (3.3-18.3)	**0.012**

Table S2 continued.				
	Normal (n=551)	Pre-diabetes (n=51)	Diabetes mellitus (n=58)	*p*-value
Median genetic marker $2^{-\Delta Ct}$ values (25^{th}-75^{th} percentiles):				
miR-126-3p	2.6 (1.1-4.7)	1.9 (0.9-4.4)	1.9 (0.8-4.6)	0.483
miR-223-3p	11.2 (3.2-28.6)	10.5 (5.2-22.4)	9.3 (3.5-26.7)	0.964
miR-320a	0.05 (0.02-0.09)	0.05 (0.02-0.09)	0.04 (0.02-0.10)	0.807

HIV, Human Immunodeficiency Virus; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, glycated haemoglobin; HOMA-IR, homeostatic model assessment-estimated insulin resistance; and hs-CRP, high-sensitivity C-reactive protein.

[1]Other: single nucleotide reverse transcriptase inhibitors (NRTI), single non-nucleoside reverse transcriptase inhibitors (NNRTI), combined NRTI + protease inhibitors (PI), double NRTIs, combined NRTI + NNRTI, double PIs only, and unknown medications.

Pre-diabetes: impaired glucose tolerance - fasting plasma glucose (FPG) <7.0 mmol/L and a 2-hour post glucose load 7.8-11.1 mmol/L, and impaired fasting glycaemia - FPG 6.1-7.0 mmol/L a with a 2-hour post glucose load <7.8 mmol/L.

Diabetes mellitus: FPG ≥7.0 mmol/L or a 2-hour post glucose load ≥11.1 mmol/L or known diabetes on medication.

Spearman rank correlations to assess the associations between continuous variables and target microRNAs

Table S3: Univariate spearman rank correlations between continuous variables and target microRNAs normalised with SNOR D48 according to overweight/obesity status in HIV infected adults

	miR-126-3p		**miR-223-3p**		**miR-320a**	
	r	*p*-value	r	*p*-value	r	*p*-value
Socio-demographic characteristics:						
Age (years)	-0.066	0.086	-0.034	0.385	-0.002	0.950
Anthropometry:						
Waist circumference (cm)	0.010	0.794	0.015	0.692	-0.001	0.989
Hip circumference (cm)	0.047	0.227	0.053	0.169	0.012	0.746
Waist-hip-ratio	-0.045	0.243	-0.052	0.176	-0.021	0.585
Blood pressure:						
Systolic blood pressure (mmHg)	-0.038	0.331	-0.037	0.343	-0.037	0.332
Diastolic blood pressure (mmHg)	-0.035	0.360	-0.034	0.376	-0.038	0.328
HIV-related factors:						
CD4 count (cells/mm^3)	0.023	0.670	0.003	0.952	0.008	0.884
Lipid levels:						
Total cholesterol (mmol/L)	0.018	0.639	0.065	0.092	-0.010	0.803
HDL-C (mmol/L)	0.004	0.917	0.005	0.890	-0.041	0.295
LDL-C (mmol/L)	-0.037	0.348	-0.002	0.950	-0.054	0.162
Triglycerides (mmol/L)	-0.062	0.112	-0.018	0.647	-0.013	0.749
Glucose homeostasis:						
Fasting glucose (mmol/L)	0.031	0.429	0.046	0.241	0.031	0.421
2-hour glucose (mmol/L)	0.014	0.732	0.061	0.139	0.036	0.377
Insulin fasting (mIU/L)	0.063	0.114	0.050	0.210	0.047	0.239
2-hour insulin (mIU/L)	0.005	0.913	0.033	0.422	-0.006	0.894
HbA1c (%)	-0.018	0.647	0.012	0.754	-0.006	0.886
HOMA-IR	0.103	**0.008**	0.074	0.056	0.074	0.056
Inflammatory markers:						
Alanine Transaminase (U/L)	0.049	0.206	0.028	0.475	-0.023	0.559
Aspartate Aminotransferase (U/L)	-0.002	0.958	-0.003	0.931	-0.045	0.252
Hs-CRP (mg/L)	-0.054	0.167	0.020	0.608	-0.043	0.266

HIV, Human Immunodeficiency Virus; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, glycated haemoglobin; HOMA-IR, homeostatic model assessment-estimated insulin resistance; hs-CRP, high-sensitivity C-reactive protein.
Overweight: BMI 25.0–29.9 kg/m^2.
Obesity: BMI ≥30.0 kg/m^2.

Table S4: Univariate Spearman rank correlations between continuous variables and target microRNAs normalised with SNOR D48 according raised waist circumference status in HIV infected adults

	miR-126-3p		**miR-223-3p**		**miR-320a**	
	r	*p*-value	r	*p*-value	r	*p*-value
Socio-demographic characteristics:						
Age (years)	-0.066	0.086	-0.034	0.376	-0.004	0.917
Anthropometry:						
Weight (kg)	0.011	0.770	0.015	0.706	-0.019	0.631
Height (cm)	-0.038	0.326	-0.011	0.776	0.005	0.895
BMI (kg/m^2)	0.020	0.605	0.015	0.690	-0.020	0.608
Hip circumference (cm)	0.056	0.144	0.060	0.120	0.024	0.535
Blood pressure:						
Systolic blood pressure (mmHg)	-0.037	0.331	-0.036	0.352	-0.037	0.336
Diastolic blood pressure (mmHg)	-0.034	0.373	-0.033	0.392	-0.036	0.350
HIV-related factors:						
CD4 count (cells/mm^3)	0.026	0.626	0.006	0.912	0.013	0.810
Lipid levels:						
Total cholesterol (mmol/L)	0.020	0.612	0.066	0.087	-0.010	0.800
HDL-C (mmol/L)	0.004	0.911	0.005	0.896	-0.042	0.278
LDL-C (mmol/L)	-0.033	0.398	<-0.001	0.995	-0.052	0.179
Triglycerides (mmol/L)	-0.061	0.116	-0.016	0.675	-0.011	0.775
Glucose homeostasis:						
Fasting glucose (mmol/L)	0.033	0.401	0.047	0.224	0.034	0.377
2-hour glucose (mmol/L)	0.017	0.679	0.063	0.126	0.040	0.331
Fasting insulin (mIU/L)	0.068	0.090	0.053	0.181	0.054	0.176
2-hour insulin (mIU/L)	0.009	0.833	0.036	0.382	<0.001	0.994
HbA1c (%)	-0.015	0.692	0.015	0.710	-0.002	0.965
HOMA-IR	0.103	**0.007**	0.074	0.056	0.076	**0.048**
Inflammatory markers:						
Alanine Transaminase (U/L)	0.050	0.198	0.029	0.460	-0.023	0.564
Aspartate Aminotransferase (U/L)	-0.004	0.910	-0.005	0.907	-0.048	0.223
Hs-CRP (mg/L)	-0.051	0.190	0.021	0.582	-0.039	0.319

HIV, Human Immunodeficiency Virus; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, glycated haemoglobin; HOMA-IR, homeostatic model assessment-estimated insulin resistance; hs-CRP, high-sensitivity C-reactive protein.
Raised waist circumference: ≥94 cm in men and ≥80 cm in women.

Table S5: Univariate Spearman rank correlations between continuous variables and target microRNAs normalised with SNOR D48 according to dysglycaemia status in HIV infected adults

	miR-126-3p		**miR-223-3p**		**miR-320a**	
	r	*p*-value	r	*p*-value	r	*p*-value
Socio-demographic characteristics:						
Age (years)	-0.060	0.121	-0.026	0.512	0.002	0.953
Anthropometry:						
Weight (kg)	0.006	0.880	0.011	0.773	-0.024	0.545
Height (cm)	-0.032	0.409	-0.005	0.889	0.014	0.729
BMI (kg/m^2)	0.012	0.754	0.010	0.807	-0.028	0.474
Waist circumference (cm)	0.014	0.718	0.019	0.629	0.005	0.908
Hip circumference (cm)	0.051	0.191	0.055	0.157	0.019	0.634
Waist-hip-ratio	-0.045	0.244	-0.051	0.194	-0.022	0.575
Waist-height-ratio	0.020	0.611	0.017	0.669	0.004	0.926
Blood pressure:						
Systolic blood pressure (mmHg)	-0.049	0.206	-0.050	0.204	-0.048	0.218
Diastolic blood pressure (mmHg)	-0.043	0.273	-0.045	0.248	-0.045	0.247
HIV-related factors:						
CD4 count (cells/mm^3)	0.041	0.453	0.032	0.557	0.028	0.607
Lipid levels:						
Total cholesterol (mmol/L)	0.000	0.996	0.053	0.177	-0.023	0.552
HDL-C (mmol/L)	0.006	0.874	0.006	0.881	-0.041	0.292
LDL-C (mmol/L)	-0.036	0.352	-0.001	0.980	-0.056	0.152
Triglycerides (mmol/L)	-0.062	0.114	-0.014	0.716	-0.012	0.762
Glucose homeostasis:						
Insulin fasting (mIU/L)	0.062	0.122	0.051	0.204	0.047	0.240
2-hour insulin (mIU/L)	<0.001	0.999	0.030	0.469	-0.008	0.841
HbA1c (%)	-0.015	0.703	0.014	0.720	-0.002	0.963
Inflammatory markers:						
Alanine Transaminase (U/L)	0.052	0.186	0.031	0.430	-0.021	0.595
Aspartate Aminotransferase (U/L)	-0.001	0.979	-0.000	0.991	-0.043	0.272
Hs-CRP (mg/L)	-0.056	0.154	0.017	0.665	-0.043	0.275

HIV, Human Immunodeficiency Virus; BMI, body mass index; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; HbA1c, glycated haemoglobin; hs-CRP, high-sensitivity C-reactive protein.
Pre-diabetes: impaired glucose tolerance - fasting plasma glucose (FPG) <7.0 mmol/L and a 2-hour post glucose load 7.8-11.1 mmol/L, and impaired fasting glycaemia - FPG 6.1-7.0 mmol/L a with a 2-hour post glucose load <7.8 mmol/L.
Diabetes mellitus: FPG ≥7.0 mmol/L or a 2-hour post glucose load ≥11.1 mmol/L or known diabetes on medication.

Table S6: Univariate Spearman rank correlations between continuous variables and target microRNAs normalised with SNOR D48 according to the presence or absence of insulin resistance in HIV infected adults

	miR-126-3p		**miR-223-3p**		**miR-320a**	
	r	*p*-value	r	*p*-value	r	*p*-value
Socio-demographic characteristics:						
Age (years)	-0.065	0.091	-0.033	0.389	-0.003	0.935
Anthropometry:						
Weight (kg)	0.004	0.917	0.010	0.796	-0.027	0.485
Height (cm)	-0.036	0.347	-0.010	0.802	0.007	0.856
BMI (kg/m^2)	0.012	0.755	0.010	0.790	-0.029	0.451
Waist circumference (cm)	0.012	0.749	0.018	0.648	0.002	0.961
Hip circumference (cm)	0.048	0.213	0.055	0.157	0.014	0.711
Waist-hip-ratio	-0.044	0.255	-0.051	0.183	-0.020	0.599
Waist-height-ratio	0.019	0.622	0.017	0.661	0.003	0.944
Blood pressure:						
Systolic blood pressure (mmHg)	-0.037	0.333	-0.036	0.351	-0.037	0.336
Diastolic blood pressure (mmHg)	-0.036	0.350	-0.034	0.376	-0.038	0.323
HIV-related factors:						
CD4 count (cells/mm^3)	0.024	0.662	0.005	0.932	0.010	0.853
Lipid levels:						
Total cholesterol (mmol/L)	0.020	0.608	0.067	0.084	-0.010	0.799
HDL-C (mmol/L)	0.004	0.913	0.005	0.890	-0.042	0.276
LDL-C (mmol/L)	-0.034	0.388	-0.000	0.995	-0.053	0.171
Triglycerides (mmol/L)	-0.061	0.115	-0.016	0.688	-0.012	0.761
Glucose homeostasis:						
2-hour glucose (mmol/L)	0.017	0.686	0.062	0.131	0.039	0.349
2-hour insulin (mIU/L)	0.008	0.847	0.036	0.391	-0.001	0.980
HbA1c (%)	-0.015	0.706	0.016	0.688	-0.001	0.975
Inflammatory markers:						
Alanine Transaminase (U/L)	0.051	0.194	0.029	0.462	-0.022	0.567
Aspartate Aminotransferase (U/L)	-0.002	0.952	-0.003	0.929	-0.046	0.243
Hs-CRP (mg/L)	-0.051	0.189	0.022	0.576	-0.039	0.323

HIV, Human Immunodeficiency Virus; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, glycated haemoglobin; hs-CRP, high-sensitivity C-reactive protein. Insulin resistance: participants with a homeostatic model assessment-estimated insulin resistance (HOMA-IR) above the data specific 90th percentile.

Table S7: Univariate Spearman rank correlations between continuous variables and target microRNAs normalised with SNOR D48 according to the presence or absence of elevated hs-CRP in HIV infected adults

	miR-126-3p		**miR-223-3p**		**miR-320a**	
	r	*p*-value	r	*p*-value	r	*p*-value
Socio-demographic characteristics:						
Age (years)	-0.066	0.086	-0.034	0.377	-0.004	0.925
Anthropometry:						
Weight (kg)	0.003	0.929	0.009	0.813	-0.027	0.480
Height (cm)	-0.036	0.355	-0.010	0.804	0.007	0.853
BMI (kg/m^2)	0.011	0.771	0.009	0.807	-0.029	0.445
Waist circumference (cm)	0.012	0.764	0.017	0.667	0.001	0.972
Hip circumference (cm)	0.048	0.215	0.054	0.162	0.014	0.710
Waist-hip-ratio	-0.045	0.246	-0.052	0.179	-0.021	0.587
Waist-height-ratio	0.018	0.638	0.016	0.680	0.002	0.956
Blood pressure:						
Systolic blood pressure (mmHg)	-0.038	0.326	-0.037	0.339	-0.038	0.321
Diastolic blood pressure (mmHg)	-0.036	0.352	-0.035	0.370	-0.038	0.322
HIV-related factors:						
CD4 count (cells/mm^3)	0.022	0.675	0.004	0.948	0.009	0.862
Lipid levels:						
Total cholesterol (mmol/L)	0.019	0.627	0.066	0.086	-0.010	0.789
HDL-C (mmol/L)	0.005	0.890	0.006	0.882	-0.041	0.288
LDL-C (mmol/L)	-0.036	0.362	-0.001	0.972	-0.054	0.163
Triglycerides (mmol/L)	-0.062	0.110	-0.017	0.666	-0.012	0.753
Glucose homeostasis:						
Fasting glucose (mmol/L)	0.033	0.398	0.048	0.222	0.034	0.378
2-hour glucose (mmol/L)	0.016	0.702	0.062	0.133	0.039	0.345
Insulin fasting (mIU/L)	0.063	0.114	0.050	0.210	0.048	0.231
2-hour insulin (mIU/L)	0.005	0.906	0.034	0.417	-0.003	0.935
HbA1c (%)	-0.016	0.684	0.015	0.703	-0.002	0.964
HOMA-IR	0.102	**0.008**	0.073	0.058	0.074	0.055
Inflammatory markers:						
Alanine Transaminase (U/L)	0.050	0.202	0.028	0.467	-0.022	0.564
Aspartate Aminotransferase (U/L)	-0.002	0.958	-0.003	0.941	-0.045	0.250

HIV, Human Immunodeficiency Virus; BMI, body mass index; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; HbA1c, glycated haemoglobin; HOMA-IR, homeostatic model assessment-estimated insulin resistance; hs-CRP, high-sensitivity C-reactive protein.
Elevated hs-CRP: hs-CRP >3 mg/L.

Robust correlation analysis stratified by gender

Table S8: Robust correlations between continuous variables and target microRNAs expression normalized with SNOR D48 according to overweight/obesity status in HIV infected men (128 participants)

	miR-126-3p		**miR-223-3p**		**miR-320a**	
	r	*p*-value	r	*p*-value	r	*p*-value
Socio-demographic:						
Age (years)	-0.108	<0.005	0.019	<0.620	0.048	<0.210
Anthropometry:						
Waist circumference (cm)	0.052	<0.180	0.222	**<0.001**	0.088	**<0.022**
Hip circumference (cm)	0.030	<0.440	0.139	**<0.001**	0.026	<0.500
Waist-hip-ratio	0.075	<0.052	0.173	**<0.001**	0.130	**<0.001**
Blood pressure:						
Systolic blood pressure (mmHg)	0.072	<0.062	-0.014	<0.720	0.048	<0.210
Diastolic blood pressure (mmHg)	0.161	**<0.001**	0.047	<0.220	0.148	**<0.001**
HIV-related factors:						
CD4 count (cells/mm^3)	0.196	**<0.001**	0.170	**<0.001**	0.106	**<0.006**
Lipid levels:						
Total cholesterol (mmol/L)	0.004	<0.920	0.116	**<0.003**	-0.088	**<0.022**
HDL-C (mmol/L)	0.038	<0. 320	0.014	<0.720	-0.012	<0.760
LDL-C (mmol/L)	0.007	<0.860	0.097	**<0.012**	-0.111	**<0.004**
Triglycerides (mmol/L)	0.050	<0.190	0.126	**<0.001**	0.026	<0.500
Glucose homeostasis:						
Fasting glucose (mmol/L)	0.029	<0.450	0.098	**<0.011**	0.070	<0.069
2-hour glucose (mmol/L)	0.178	**<0.001**	0.133	**<0.001**	0.186	**<0.001**
Fasting insulin (mIU/L)	0.119	**<0.002**	0.199	**<0.001**	0.136	**<0.001**
2-hour insulin (mIU/L)	0.171	**<0.001**	0.080	**<0.038**	0.145	**<0.001**
HbA1c (%)	-0.024	<0.530	-0.052	<0.180	-0.096	**<0.013**
HOMA-IR	0.143	**<0.001**	0.128	**<0.001**	0.149	**<0.001**
Inflammatory markers:						
Alanine Transaminase (U/L)	0.204	**<0.001**	0.185	**<0.001**	0.168	**<0.001**
Aspartate Aminotransferase (U/L)	0.111	**<0.004**	0.105	**<0.006**	0.121	**<0.002**
Hs-CRP (mg/L)	0.174	**<0.001**	0.204	**<0.001**	0.230	**<0.001**

HIV, Human Immunodeficiency Virus; CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, glycated haemoglobin; HOMA-IR, homeostatic model assessment-estimated insulin resistance; hs-CRP, high-sensitivity C-reactive protein.

Table S9: Robust correlations between continuous variables and target microRNAs expression normalized with SNOR D48 according to overweight/obesity status in HIV infected women (546 participants)

	miR-126-3p		**miR-223-3p**		**miR-320a**	
	r	*p*-value	r	*p*-value	r	*p*-value
Socio-demographic:						
Age (years)	-0.045	<0.240	-0.035	<0.360	-0.022	<0.570
Anthropometry:						
Waist circumference (cm)	-0.009	<0.820	-0.037	<0.340	0.042	<0.280
Hip circumference (cm)	0.029	<0.450	0.032	<0.410	0.058	<0.130
Waist-hip-ratio	-0.041	<0.290	-0.073	<0.058	-0.016	<0.680
Blood pressure:						
Systolic blood pressure (mmHg)	0.029	<0.450	0.040	<0.300	-0.012	<0.760
Diastolic blood pressure (mmHg)	-0.023	<0.550	-0.024	<0.530	-0.060	<0.120
HIV-related factors:						
CD4 count (cells/mm^3)	0.020	<0.600	-0.016	<0.680	0.040	<0.300
Lipid levels:						
Total cholesterol (mmol/L)	-0.026	<0.500	0.010	<0.800	-0.031	<0.420
HDL-C (mmol/L)	0.021	<0.590	0.013	<0.740	-0.022	<0.570
LDL-C (mmol/L)	-0.064	<0.097	-0.020	<0.600	-0.045	<0.240
Triglycerides (mmol/L)	-0.005	<0.900	0.037	<0.340	0.041	<0.290
Glucose homeostasis:						
Fasting glucose (mmol/L)	0.118	**<0.002**	0.092	**<0.017**	0.123	**<0.001**
2-hour glucose (mmol/L)	0.010	<0.800	0.044	<0.250	0.040	<0.300
Fasting insulin (mIU/L)	0.089	**<0.021**	0.082	**<0.033**	0.071	<0.065
2-hour insulin (mIU/L)	0.061	<0.110	0.089	**<0.021**	0.068	<0.078
HbA1c (%)	-0.019	<0.620	0.054	<0.160	-0.032	<0.410
HOMA-IR	0.137	**<0.001**	0.137	**<0.001**	0.118	**<0.002**
Inflammatory markers:						
Alanine Transaminase (U/L)	0.133	**<0.001**	0. 089	**<0.021**	0. 019	<0.620
Aspartate Aminotransferase (U/L)	0.081	**<0.036**	0.087	**<0.024**	-0.011	<0.780
Hs-CRP (mg/L)	-0.027	<0.48	0.028	<0.470	-0.013	<0.740

HIV, Human Immunodeficiency Virus; CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, glycated haemoglobin; HOMA-IR, homeostatic model assessment-estimated insulin resistance; hs-CRP, high-sensitivity C-reactive protein.

Logistic regression analyses

Table S10: Logistic regression analysis for associations of overweight and obesity with target microRNAs expression normalized with SNOR D48 according to overweight/obesity status in HIV infected adults

	miR-126-3p			**miR-223-3p**			**miR-320a**		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Overweight									
Model 1	0.995	(0.939, 1.051)	0.858	1.000	(0.993, 1.006)	0.915	0.275	(0.018, 2.937)	0.312
Model 2	0.990	(0.933, 1.049)	0.739	0.998	(0.990, 1.005)	0.521	0.340	(0.020, 4.107)	0.421
Obesity									
Model 1	1.012	(0.963, 1.063)	0.642	1.003	(0.997, 1.008)	0.393	0.263	(0.025, 2.192)	0.236
Model 2	0.999	(0.948, 1.054)	0.981	1.000	(0.994, 1.006)	0.974	0.423	(0.034, 4.641)	0.485

Model 1: microRNA;
Model 2: model 1 + age + gender.
HIV, human immunodeficiency virus; OR, odds ratio; CI, confidence interval.
Overweight was defined as BMI 25.0– 29.9 kg/m^2.
Obesity was defined as BMI ≥30 kg/m^2.

Table S11: Logistic regression analysis for associations between the presence of raised waist circumference and target microRNAs expression normalized with SNOR D48 according to raised waist circumference status in HIV infected adults

	miR-126-3p			**miR-223-3p**			**miR-320a**		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Model 1	0.999	(0.955, 1.045)	0.977	0.998	(0.993, 1.003)	0.477	5.139	(0.759, 37.619)	0.096
Model 2	1.008	(0.957, 1.060)	0.754	1.001	(0.995, 1.007)	0.776	4.425	(0.475, 40.666)	0.186

Model 1: microRNA;
Model 2: model 1 + age + gender.
HIV, human immunodeficiency virus; OR, odds ratio; CI, confidence interval.
Raised waist circumference: ≥ 94 cm in men and ≥ 80 cm in women.

Table S12: Logistic regression analysis for associations of pre-diabetes and diabetes mellitus with target microRNAs expression normalized with SNOR D48 according to dysglycaemia status in HIV infected adults

	miR-126-3p			**miR-223-3p**			**miR-320a**		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Pre-diabetes									
Model 1	0.950	(0.855, 1.037)	0.298	0.994	(0.981, 1.005)	0.324	0.126	(0.001, 5.900)	0.382
Model 2	0.960	(0.865, 1.048)	0.402	0.995	(0.981, 1.005)	0.383	0.096	(<0.001, 3.988)	0.313
Model 3	0.955	(0.859, 1.043)	0.347	0.994	(0.981, 1.005)	0.354	0.099	(<0.001, 4.017)	0.316
Model 4	0.956	(0.860, 1.045)	0.360	0.995	(0.981, 1.005)	0.374	0.098	(<0.001, 4.314)	0.326
Diabetes mellitus									
Model 1	1.005	(0.936, 1.093)	0.895	0.996	(0.989, 1.005)	0.348	1.036	(0.062, 45.791)	0.983
Model 2	1.002	(0.932, 1.089)	0.958	0.996	(0.988, 1.004)	0.292	1.792	(0.109, 72.365)	0.717
Model 3	1.008	(0.937, 1.097)	0.839	0.996	(0.989, 1.005)	0.380	1.872	(0.110, 74.732)	0.696
Model 4	1.008	(0.936, 1.097)	0.841	0.996	(0.989, 1.005)	0.346	1.758	(0.099, 76.695)	0.732

Model 1: microRNA;
Model 2: model 1 + age + gender;
Model 3: model 2 + BMI;
Model 4: model 2 + waist circumference.
HIV, human immunodeficiency virus; OR, odds ratio; CI, confidence interval; BMI, body mass index.
Pre-diabetes: included impaired glucose tolerance (IGT): fasting plasma glucose (FPG) <7.0 mmol/L and a 2-hour post glucose load between 7.8 mmol/L-11.1 mmol/L; and impaired fasting glycaemia (IFG): FPG 6.1 mmol/L-7.0 mmol/L and a 2-hour post glucose load <7.8 mmol/L.
Diabetes mellitus: a fasting plasma glucose (FPG) ≥7.0 mmol/L and/or a 2-hour post glucose load ≥11.1 mmol/L or known diabetes on treatment.

Table S13: Logistic regression analysis for associations of insulin resistance with target microRNAs expression normalized with SNOR D48 according to insulin resistance in HIV infected adults

	miR-126-3p			**miR-223-3p**			**miR-320a**		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Model 1	0.986	(0.924, 1.062)	0.685	0.998	(0.991, 1.007)	0.605	0.542	(0.041, 14.497)	0.675
Model 2	0.985	(0.923, 1.060)	0.666	0.999	(0.991, 1.007)	0.715	0.535	(0.040, 13.797)	0.666
Model 3	1.004	(0.936, 1.086)	0.911	1.000	(0.992, 1.009)	0.959	0.641	(0.039, 19.555)	0.773
Model 4	0.998	(0.931, 1.079)	0.961	1.000	(0.992, 1.009)	0.979	0.549	(0.032, 19.121)	0.706

Model 1: microRNA;
Model 2: model 1 + age + gender;
Model 3: model 2 + BMI;
Model 4: model 2 + waist circumference.
HIV, human immunodeficiency virus; OR, odds ratio; CI, confidence interval; BMI, body mass index.
Participants with a homeostatic model assessment-estimated insulin resistance (HOMA-IR) above the data specific 90th percentile was considered insulin resistant.

Table S14: Logistic regression analysis for associations of elevated high-sensitivity C-reactive protein (hs-CRP) with target microRNAs expression normalized with SNOR D48 according to hs-CRP status in HIV infected adults

	miR-126-3p			**miR-223-3p**			**miR-320a**		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Model 1	1.026	(0.980, 1.073)	0.271	0.999	(0.993, 1.005)	0.759	3.456	(0.486, 24.291)	0.207
Model 2	1.025	(0.979, 1.073)	0.284	0.999	(0.994, 1.005)	0.804	3.565	(0.492, 25.128)	0.198
Model 3	1.032	(0.984, 1.083)	0.188	1.033	(0.984, 1.083)	0.184	3.286	(0.428, 24.582)	0.243
Model 4	1.033	(0.984, 1.083)	0.184	1.000	(0.994, 1.005)	0.886	3.073	(0.404, 23.121)	0.269

Model 1: microRNA;
Model 2: model 1 + age + gender;
Model 3: model 2 + BMI;
Model 4: model 2 + waist circumference.
HIV, human immunodeficiency virus; OR, odds ratio; CI, confidence interval; BMI, body mass index.
Elevated hs-CRP: hs-CRP level >3 mg/L.

A2. Plate layout for RT-qPCR

	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6	
	1	2	3	4	5	6	7	8	9	10	11	12
A	hsa miR-223-3p	hsa miR-139-5p	hsa miR-223-3p	hsa miR-139-5p	hsa miR-223-3p	hsa miR-139-5p	hsa miR-223-3p	hsa miR-139-5p	hsa miR-223-3p	hsa miR-139-5p	hsa miR-223-3p	hsa miR-139-5p
B	hsa miR-125a-5p	hsa miR-133a-3p	hsa miR-125a-5p	hsa miR-133a-3p	hsa miR-125a-5p	hsa miR-133a-3p	hsa miR-125a-5p	hsa miR-133a-3p	hsa miR-125a-5p	hsa miR-133a-3p	hsa miR-125a-5p	hsa miR-133a-3p
C	hsa miR-126-3p	hsa miR-155-5p	hsa miR-126-3p	hsa miR-155-5p	hsa miR-126-3p	hsa miR-155-5p	hsa miR-126-3p	hsa miR-155-5p	hsa miR-126-3p	hsa miR-155-5p	hsa miR-126-3p	hsa miR-155-5p
D	hsa miR-92a-3p	hsa miR-145-5p	hsa miR-92a-3p	hsa miR-145-5p	hsa miR-92a-3p	hsa miR-145-5p	hsa miR-92a-3p	hsa miR-145-5p	hsa miR-92a-3p	hsa miR-145-5p	hsa miR-92a-3p	hsa miR-145-5p
E	hsa miR-33a-5p	hsa miR-181a-5p	hsa miR-33a-5p	hsa miR-181a-5p	hsa miR-33a-5p	hsa miR-181a-5p	hsa miR-33a-5p	hsa miR-181a-5p	hsa miR-33a-5p	hsa miR-181a-5p	hsa miR-33a-5p	hsa miR-181a-5p
F	hsa miR-499a-5p	hsa miR-320a	hsa miR-499a-5p	hsa miR-320a	hsa miR-499a-5p	hsa miR-320a	hsa miR-499a-5p	hsa miR-320a	hsa miR-499a-5p	hsa miR-320a	hsa miR-499a-5p	hsa miR-320a
G	UniSp2 (miRCURY LNA miRNA Custom PCR)	U6 snRNA (miRCURY LNA miRNA Custom PCR)	UniSp2 (miRCURY LNA miRNA Custom PCR)	U6 snRNA (miRCURY LNA miRNA Custom PCR)	UniSp2 (miRCURY LNA miRNA Custom PCR)	U6 snRNA (miRCURY LNA miRNA Custom PCR)	UniSp2 (miRCURY LNA miRNA Custom PCR)	U6 snRNA (miRCURY LNA miRNA Custom PCR)	UniSp2 (miRCURY LNA miRNA Custom PCR)	U6 snRNA (miRCURY LNA miRNA Custom PCR)	UniSp2 (miRCURY LNA miRNA Custom PCR)	U6 snRNA (miRCURY LNA miRNA Custom PCR)
H	SNOR D48 (miRCURY LNA miRNA Custom PCR)	NTC	SNOR D48 (miRCURY LNA miRNA Custom PCR)	NTC	SNOR D48 (miRCURY LNA miRNA Custom PCR)	NTC	SNOR D48 (miRCURY LNA miRNA Custom PCR)	NTC	SNOR D48 (miRCURY LNA miRNA Custom PCR)	NTC	SNOR D48 (miRCURY LNA miRNA Custom PCR)	NTC

A3. Ethics approval letters received from the South African Medical Research Council Ethics Committee for main study

SOUTH AFRICAN MEDICAL RESEARCH COUNCIL

ETHICS COMMITTEE

PO Box 19070, Tygerberg 7505, Cape Town, South Africa
Francie van Zijl Drive, Parow Valley 7500
Tel: +27 (0)21 938 0341; Fax: +27 (0)21 938 0201
E-mail: adri.labuschagne@mrc.ac.za
http://www.sahealthinfo.org/ethics/ethics.htm

10 December 2013

Prof AP Kengne
Director: Non-communicable Disease Research Unit
MRC Cape Town

Dear Prof Kengne

Protocol ID: EC021-11/2013
Protocol title: Utilizing HIV/AIDS infrastructure as a gateway to chronic care for hypertension in Africa
Meeting date: 25 November 2013

Thank you for your response to the Ethics Committee, dated 3 December 2013. The response was found to be acceptable. I am pleased to inform you that ethics approval is now granted for the study.

Please note that the approval is valid for 1 year, i.e. from 25 November 2013 to 24 November 2014. Any changes to the research protocol must be submitted as an amendment. Any protocol deviations have to be reported.

Wishing you well with your research.

Yours sincerely

Signed by candidate

PROF. D DU TOIT
CHAIRPERSON: MRC ETHICS COMMITTEE

HUMAN RESEARCH ETHICS COMMITTEE

9 November 2018

Prof AP Kengne
Director: Non-communicable Disease Research Unit
SAMRC Cape Town

Dear Prof Kengne

Protocol ID:	EC021-11/2013
Protocol title:	Utilizing HIV/AIDS infrastructure as a gateway to chronic care for hypertension in Africa
Meeting date:	30 October 2018

Thank you for your progress report and application to the Committee for renewal, dated 3 October 2018. The Committee noted the report and granted ethics approval for the study for another year.

Please note that the renewal is valid for 1 year, i.e. from 30 October 2018 to 29 October 2019. Any changes to the research protocol must be submitted as an amendment. Any adverse events must be reported within 48 hours. Any protocol deviations have to be reported.

Wishing you well with your research.

Yours sincerely

Signed by candidate

Prof Danie du Toit
Chairperson: SAMRC Human Research Ethics Committee

Members present at the meeting: Prof D du Toit (Chairperson), Adv J Early, Dr H Etheredge, Prof AP Kengne, Ms M Ledwaba, Prof C Lombard, Dr AG Loxton, Mr G Makanda, Prof N Morojele, Prof C Wiysonge

HUMAN RESEARCH ETHICS COMMITTEE

9 December 2019

Prof AP Kengne
Director: Non-communicable Disease Research Unit
SAMRC Cape Town

Dear Prof Kengne

Protocol ID: EC021-11/2013
Protocol title: Utilizing HIV/AIDS infrastructure as a gateway to chronic care for hypertension in Africa
Meeting date: 25 November 2019

Thank you for your close-out report to the Committee, dated 23 October 2019. The Committee noted the report and that the study is now closed.

Yours sincerely

Signed by candidate

Prof Danie du Toit
Chairperson: SAMRC Human Research Ethics Committee

Members present at the meeting: Prof D du Toit (Chairperson), Ms S Behardien, Adv J Early, Dr H Etheredge, Prof A Kengne, Ms M Ledwaba, Prof C Lombard, Mr G Makanda, Prof C Wiysonge, Dr W Zembe

THE SOUTH AFRICAN MEDICAL RESEARCH COUNCIL
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Tel: +27 (0)21 938 0687 | Fax: +27 (0)86 685 4023 | Email: adri.labuschagne@mrc.ac.za | Web: www.samrc.ac.za/ethics/ethics.htm

A4. Main study's informed consent form

PLACE BARCODE STICKER HERE

Declaration by the participant

By signing this form, I …….. agree to take part in a research study titled: Utilizing HIV/AIDS infrastructure as a gateway to chronic care of hypertension in Africa. I also give permission that the interview may be recorded.

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I am older than 18 years of age and have been employed in my current position for longer than 3 months.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I know that taking part in this study is voluntary and I have not been forced to take part. I may choose to leave the study at any time without any problems.

Signed at :	_____	on (date)	_____
Name of participant		Signature of participant	
Name of witness	_____	Signature of witness	_____

I, …… agree/disagree to having my name or designation published/publicised as part of this research study.

Signed at :	_____	on (date)	_____
Name of participant		Signature of participant	
Name of witness	_____	Signature of witness	_____

Declaration by investigator (or person designated)

I (name) ……. declare that I have explained the information in this document to …… I have encouraged him/her to ask questions and took adequate time to answer them. I am satisfied that he/she adequately understands all aspects of the research, as discussed above.

Signed at :	_____	on (date)	_____

Name of investigator		Signature of investigator	

A5. Main study's genetics informed consent form

INFORMED CONSENT FOR RESEARCH INVOLVING GENETIC TESTING

TITLE OF THE RESEARCH PROJECT: Investigating the prevalence, awareness, treatment and control of chronic non-communicable disease risk factors, particularly hypertension, in patients attending HIV-treatment centres in the Western Cape Province of South Africa

Dear Participant

We are scientists from the Non-Communicable Disease Research Unit, Medical Research Council (MRC) and require your written, informed consent to perform genetic testing for this specific research study [and any possible future research related to heart disease or diseases affecting the metabolic system]. Genetic material, also called DNA or RNA, is usually obtained from a small blood sample. Genes are found in every cell in the human body. Our genes determine what we look like and sometimes what kind of diseases we may develop. Worldwide, researchers in the field of genetics are continuously discovering new information that may be of great benefit to future generations and also that may benefit people today, who suffer from particular diseases or conditions. Please read this consent form carefully before making a decision about whether you want to participate or not.

Taking part in this study is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part initially.

By signing this consent form, you agree to give these blood samples to the MRC for research purposes.

II. TEST PURPOSE

This testing is being conducted to determine: the presence of genes known to increase the risk of heart and/or metabolic diseases, or known to protect against the same diseases.

III. POLICIES AND PROCEDURES TO PROTECT YOUR CONFIDENTIALITY

Your information will be kept confidential as described to you in the first information sheet. A coding system that protects the identity of individuals who provide blood samples will be used when storing these.

VI. FUTURE GENETIC TESTING

These blood samples that you give to the MRC could possibly lead to discoveries using methods and tests have not yet been developed. Therefore, the MRC would like to keep the samples for as long as they are considered useful for research purposes. This research could potentially be used for purposes not specified above. However, you may specify a shorter period of time for the MRC to keep the samples. Please check the relevant box below indicating the period of time for the MRC to keep the sample.

Signed consent

☐ I agree that my blood can be stored **indefinitely/stored for ..... years,** but I can choose to request at any time that my stored sample be destroyed. My sample will be identified with a special study code that will remain linked to my name and contact details. I have the right to receive confirmation that my request has been carried out.

OR

☐ I agree that my blood sample can be stored **indefinitely/stored for ..... years** after the project is completed but that all possible links to my identity be removed, and that the researchers may then use it for additional research in this or a related field. Once my sample is anonymised, my rights to that sample are waived. My sample may be shared with other investigators in other research projects in this or a related field.

OR

☐ Please destroy my blood sample as soon as the current research project has been completed.

Signed at (*place*) ..... on (*date*) .....

.....
Signature of participant

Declaration by investigator

I *(name)* ..... declare that:

- I explained the information in this document to .....
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research as discussed above.

.....
Signature of investigator

.....
Signature of witness **(*date*)** **.....**

Place barcode here

A6. Ethics approval letter received from the University of Cape Town Human Research Ethics Committee for current study

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room 45 E-52-E-Floor- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-submissions@uct.ac.za
Website: www.health.uct.ac.za/home/human-research-ethics

06 December 2022

HREC REF: 555/2022

Prof C Dandara
Division of Human Genetics
IDM-FHS
Email: collett.dandara@uct.ac.za
Student: GVNLEE008@myuct.ac.za

Dear Prof Dandara

PROJECT TITLE: DETERMINING THE ROLE OF DIFFERENTIAL EXPRESSION OF CANDIDATE MICRORNAS IN CARDIOMETABOLIC DISEASES AMONG SOUTH AFRICAN ADULTS LIVING WITH HIV-
(MASTERS CANDIDATE-MISS LEEGAN GOVENDER)

Thank you for your response letter, addressing the issues raised by the Faculty of Health Sciences Human Research Ethics Committee (HREC).

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 December 2023.

Please submit a progress form, using the standardised Annual Report Form (FHS016) if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: - Miss Leegan Govender will also be involved in this study.

Please quote the HREC REF 555/2022 in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

Signed by candidate

PROFESSOR M BLOCKMAN
CHAIRPERSON, FACULTY OF HEALTH SCIENCES HUMAN RESEARCH ETHICS COMMITTEE

HREC/ref 555.2022