Metabolic complications resulting from the use of antiretroviral therapy in HIV-infected patients

By

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This thesis is presented in fulfilment of the requirements for the degree of Doctor of Philosophy (PhD) in the Department of Medicine, Faculty of Health Sciences, University of Cape Town. The work included in this thesis is original research and has not, in whole or in part, been submitted for another degree at this or any other university. The contents of this thesis are entirely the work of the candidate, or, in the case of multi-authored published papers, constitutes work for which the candidate was the lead author. The contribution of the candidate to multi-authored papers is outlined in the preface to the thesis.

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Abstract

While antiretroviral therapy (ART) has extended the life expectancy of those infected with HIV, it is also associated with a number of metabolic complications, such as dysglycaemia, insulin resistance and lipodystrophy. Lipodystrophy is characterised by an increase in trunk fat (lipohypertrophy) and/or a decrease in limb fat (lipoatrophy). This thesis investigates the metabolic complications associated with ART, and develops simple anthropometric cut-points for identifying those with lipodystrophy.

Data for this thesis comes from three datasets (a cross sectional study and two longitudinal studies), collected between 2007 and 2013. All datasets consisted of black HIV-infected men and women presenting to ART clinics in Cape Town. The same measurements were collected in all studies: anthropometry, self-reported lipodystrophy, dual-energy X-ray absorptiometry (DXA), blood pressure (BP), cholesterol and glucose. Longitudinal data were used to assess long-term ART exposure on BP, glycaemia, insulin secretion and anthropometric measures in women on ART. We found that weight, waist circumference, and waist-hip ratio increased, limb skinfold thickness decreased, and the proportion of participants with hypertension and diabetes increased. Longitudinal data was used to describe changes in body fat distribution over a 24 month period. We found that women gained more overall weight and more regional fat in all areas compared to men. The risk of lipoatrophy was two-fold greater in men than in women.

Simple, objective measures to define lipoatrophy and lipohypertrophy by comparing patient report to anthropometric and DXA-derived variables, were developed using cross sectional data. In women, the best predictors of lipoatrophy were triceps and thigh skinfold thicknesses, and for lipohypertrophy it was waist/hip ratio. Longitudinal data was used to validate the objective measures that were developed by comparing change in limb fat and trunk fat as measured by DXA to anthropometric variables. We showed that the best predictors of lipoatrophy in women were hip and mid-thigh circumference, and mid-arm circumference in men.

The thesis findings highlight the importance of early identification of these cardiometabolic risks in Africa. The development of anthropometric measures are of particular relevance in resource limited settings, where health professionals need simple and inexpensive methods for diagnosing patients with lipoatrophy and lipohypertrophy.

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After a demanding three and a half years, I find myself adding the finishing touches to my thesis. It has been a period of intense learning for me, and so I would like to reflect on the people who have supported and helped me so much throughout this period.

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Preface

This thesis includes published papers, as per general provision 6.7 in the General Rules for the Degree of Doctor of Philosophy (PhD) of the University of Cape Town, and with the approval of the University Doctoral Degrees Board. The following four papers are included as part of the thesis and are presented as self-contained chapters in the following order:

Chapter 4

Abrahams Z, Dave J, Maartens G, Levitt N. Changes in blood pressure, glucose levels, insulin secretion and anthropometry after long term exposure to antiretroviral therapy in South African women. AIDS Res Ther 2015;12:24. (**Appendix 1**)

Chapter 5

Abrahams Z, Levitt N, Lesosky M, Maartens G, Dave J. Changes in body fat distribution on dual-energy x-ray absorptiometry in black South Africans starting first-line antiretroviral therapy. AIDS Patient Care STDs 2016;30(10);455-462. (**Appendix 2**)

Chapter 6

Abrahams Z, Dave J, Maartens G, Lesosky M, Levitt N. The development of simple anthropometric measures to diagnose antiretroviral therapy-associated lipodystrophy in resource limited settings. AIDS Res Ther 2014;11:26. (**Appendix 3**)

Chapter 7

Abrahams Z, Maartens G, Levitt N, Dave JA. Anthropometric definitions for antiretroviral-associated lipodystrophy derived from a longitudinal South African cohort with serial dual-energy X-ray absorptiometry measurements. (In review – Int J Infect Dis)

The contribution of the candidate to each paper is outlined in the acknowledgements section of each paper. The candidate was the lead and corresponding author for each paper, prepared all the data for the analyses, conceptualised and conducted all analyses and drafted all

versions of the manuscripts during the period of the doctoral degree registration. All co-authors critically reviewed and approved the submitted manuscripts, and the candidate reviewed co-author comments and suggestions and integrated them into the manuscript as appropriate. All supervisors have separately confirmed to the University of Cape Town Doctoral Degrees Board that the included papers overwhelmingly reflect the independent and original thinking of the candidate and her own scientific work.

List of Abbreviations

Abbreviations	Full name
3TC	Lamivudine
AIC	Aikaike information criterion
AIDS	Acquired immune deficiency syndrome
ART	Antiretroviral therapy
AUC	Area under the curve
AZT	Zidovudine
BMI	Body mass index
BP	Blood Pressure
CT	Computerized tomography
d4t	Stavudine
DAD	Data collection on adverse events of anti-HIV drugs study
Dio	Oral disposition index
DoH	Department of Health
DXA	Dual-energy X-ray absorptiometry
EFV	Efavirenz
FMR	Fat mass ratio
HDL	High density lipoprotein cholesterol
HIV	Human immune deficiency virus
HOMA-IR	Homeostatic model assessment of insulin resistance
HOMA-β	homeostatic model assessment of beta cell function
HOPS	HIV Outpatient Study
IFG	Impaired fasting glucose
IGI	Insulinogenic index

IGT	Impaired glucose tolerance
IL-6	Interleuken-6
IL-8	Interleuken-8
LDCD	Lipodystrophy case definition
LDL	Low density lipoprotein cholesterol
LPV/r	Lopinavir/ritonavir
MACS	Multicenter AIDS Cohort Study
MRI	Magnetic resonance imaging
mtDNA	Mitochondrial DNA
NNRTI	Non-nucleotide reverse transcriptase inhibitor
NRTI	Nucleotide reverse transcriptase inhibitor
NVP	Nevirapine
OGTT	Oral glucose tolerance test
PI	Protease inhibitor
QUICKI	Quantitative insulin sensitivity check index
ROC curves	Receiver operating characteristic curves
SAD	Sagittal abdominal diameter
SAT	Subcutaneous adipose tissue
TAT	Total adipose tissue
TDF	Tenofovir
TNF-α	Tumour necrosis factor alpha
VAT	Visceral adipose tissue
WHO	World Health Organisation

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Chapter 1. Introduction

1.1 Context

In 1981, acquired immune deficiency syndrome (AIDS) was first described [1] and by 1983 the human immune deficiency virus (HIV) was discovered [2]. Since the start of the HIV epidemic, 78 million people have been infected with the virus. By the end of 2015, 36.7 million people were estimated to be living with HIV globally, and just under half of them (17 million) were accessing antiretroviral therapy (ART). Eastern and Southern Africa carry the greatest burden, with 19 million people estimated to be positive, and 10.3 million accessing ART [3].

ART has improved the prognosis of people with HIV by suppressing HIV replication and restoring the patient's immune system. In April 2004, as part of the South African Department of Health's Operational Plan for Comprehensive HIV and AIDS Care, Management and Treatment [4], ART was made available to HIV-infected patients in the public health sector of South Africa, based on the following eligibility criteria: a CD4 ≤200 cells/mm^3 or symptomatic, irrespective of stage, or with the World Health Organisation (WHO) stage IV. ART eligibility has since changed with the CD4 cut-point increasing to ≤350 cell/mm^3 in 2011 and to ≤500 cell/mm^3 in 2015 [5]. The CD4 threshold was removed in September 2016. The adult first-line ART regimen comprises two nucleoside reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitor (NNRTI), while the second-line ART regimen comprises two NNRTIs and a protease inhibitor (PI). Patients were changed to the second-line ART treatment regimen if they experienced virologic failure as defined by two consecutive viral loads >1000 copies/mL [4]. In South Africa, the initial first-line regimen included stavudine, which was changed to tenofovir in 2010 due to stavudine's toxicity [6]. Since those early days, ART coverage has increased to such an extent that by the end of 2015, 3.4 million South Africans were receiving ART [3].

Metabolic complications of ART

ART has undoubtedly improved the quality of life and extended the life expectancy of those infected with HIV [7]. It is estimated to have prevented the death of 7.6 million people globally

[8]. However, the use of ART is also associated with a number of adverse effects which compromise adherence, leading to drug resistance, and can cause considerable morbidity and even mortality [9]. As HIV-infected patients on ART are living longer, the prevalence of a number of metabolic complications have increased [10].

Several metabolic complications, including dyslipidaemia, dysglycaemia, insulin resistance, changes in body fat distribution, hypertension and hyperlactatemia are found in patients on ART [11-14]. These metabolic complications are thought to be related to the use of specific antiretroviral agents or to classes of antiretrovirals [13,15], and have been well documented in high-income countries. Limited data is available from low- and middle-income countries [16].Dyslipidaemia is commonly found in HIV infection, with and without the use of ART. In the Multicenter AIDS Cohort Study (MACS), HIV-infected patients, prior to the introduction of ART, exhibited lower levels of total cholesterol, low-density lipoprotein (LDL) cholesterol and high density lipoprotein (HDL) cholesterol. After initiation of ART, total cholesterol and LDL cholesterol increased, while HDL cholesterol remained low [17]. Other studies have similarly found that untreated HIV-infected patients have an increased risk of low total, LDL and HDL cholesterol compared to HIV negative controls [18]. However, a systematic review by Dillon *et al.* [16], of studies from sub-Saharan Africa, found that HIV-infected individuals who were ART-naive had higher triglyceride levels and lower HDL levels that those who were uninfected, while ART treatment was associated with higher levels of LDL and HDL cholesterol. Changes in lipid profiles have been linked to specific antiretrovirals, which include PIs, thymidine analogue NRTIs, as well as NNRTIs [16,19,20]. PI-based regimens have been associated with persistent low levels of HDL cholesterol, as well as severely increased triglyceride levels [21] (though very uncommon), while the NRTIs stavudine and zidovudine are associated with increased levels of total and LDL cholesterol [22], and modestly elevated triglyceride levels [21].Regarding the NNRTI drugs, nevirapine has been shown to have a greater increase in HDL cholesterol and a decrease in the ratio of total cholesterol to HDL cholesterol than efavirenz [23].

Hyperlactatemia, characterized by mild to moderate increases in blood lactate levels, has been reported in patients receiving NRTIs, and stavudine in particular [24,25]. Evidence from

high-income countries suggest that the majority of cases are either asymptomatic, or have only mild symptoms. The most severe form of hyperlactatemia, namely lactic acidosis, develops in a small proportion of patients and can be fatal [24,25]. As stavudine has been phased out, hyperlactatemia is no longer commonly found in patients treated with antiretroviral drugs.

The relationship between hypertension and ART is inconsistent. Some early studies from high-income countries reported an association between increased systolic blood pressure and duration of ART [26,27], while others reported a decreased risk of hypertension [28]. Evidence from smaller studies in low- and middle-income countries were equally inconsistent. A Nigerian study found no association between ART and hypertension [29], while studies from Tanzania [30], Nigeria [31], and Cameroon [32,33] found that HIV-infected adults on ART had an increased risk of developing hypertension when compared to HIV negative controls or ART-naïve patients.

Insulin resistance and diabetes mellitus are well-documented complications of ART [34]. Diabetes is preceded by insulin resistance and β-cell dysfunction and is characterised by a state of high blood glucose (hyperglycaemia) that develops when the pancreatic β-cell insulin secretion is unable to meet the insulin needs as determined by insulin resistance and carbohydrate intake [35]. Risk factors for insulin resistance include the pro-inflammatory effect of the HIV infection, the direct effect of some antiretrovirals, and the indirect consequences of HIV treatment, such as lipodystrophy [36]. The development of insulin resistance does not appear to be a class effect, but appears to be linked to specific drugs within the class of PIs [37], NRTIs [38,39] and NNRTIs [40]. Reports on the association between ART and dysglycaemia has been inconsistent. While studies from high-income countries found an increased risk of diabetes in patients on ART [39,41], a systematic review by Dillon *et al.*, based on 52 datasets from 14 countries in sub-Saharan Africa, found no association between ART and fasting blood glucose levels [16]. The metabolic response to ART may be different in sub-Saharan Africa, where the majority of HIV-infected patients are black women in comparison to middle-aged white men in high-income countries [42]. Black women, when

matched to white women for body fatness, were more insulin resistant and had less visceral adipose tissue, which may indicate different pathophysiological processes [42,43].

Lipodystrophy, also known as fat redistribution, is a well-documented metabolic complication of ART [44]. It is characterized by either subcutaneous fat loss, which is most noticeable in the face, limbs, and buttocks (lipoatrophy), central fat accumulation (lipohypertrophy) seen in the abdomen, breast or posterior neck (known as 'buffalo hump'), or a combination of both [19,45,46]. The reported prevalence of lipodystrophy is varied as a result of differences in study designs, gender, ART regimens and duration of therapy [14]. Lipodystrophy has been shown to develop within four to six months of ART initiation, and continue to worsen over time [47]. There is limited research from low- and middle-income countries. However, the incidence is estimated to be high as these countries bear the greatest HIV burden, have the largest ART programmes in the world, and have only recently phased out stavudine which is a well-known cause of lipoatrophy.

A systematic review by De Waal *et al.* [48] concluded that while lipoatrophy is an antiretroviral adverse drug reaction, lipohypertrophy appears not to be. Studies have shown that patients on ART have considerably less subcutaneous fat than those who are not on ART, and that fat loss increases with time on ART and can partially be reversed by switching medication [48]. Lipoatrophy is strongly associated with the use of the NRTIs, stavudine and zidovudine [48-51], which has been shown to partially reverse after switching to tenofovir, abacavir or an NRTI-sparing regimen [48]. Lipoatrophy is thought to result from the impairment of mitochondrial function by NRTIs [52].

Central fat accumulation or lipohypertrophy, occurs with equal prevalence in patients on ART, irrespective of their ART regimen, and does not reverse after switching to a different antiretroviral [48]. This suggests that central fat gain may be a consequence of treating the HIV infection, as the concentrations of inflammatory markers which cause wasting is normalized with treatment [48]. The development of lipohypertrophy is thought to be related to elevated levels of inflammatory cytokines [53], or to a surplus of energy that cannot be stored in atrophic subcutaneous depots [54].

The development of lipodystrophy has psychosocial and lifestyle implications. Psychological effects reported include feelings of depression, poor self-confidence and loss of self-esteem [55]. Due to the stigma of HIV, patients may not want to disclose their status, yet their changing body shape acts as an HIV identifier.

The use of ART has rapidly increased in low- and middle-income countries during the last decade, and will continue to rise in these countries as they (1) follow the WHO's recommendation to 'test and treat' [56] and, (2) continue to use zidovudine as part of their second-line ART treatment regimen [6]. Thus, the early identification of patients with these metabolic complications is becoming increasingly important.

The diagnosis of lipodystrophy has been problematic, as both subjective and objective methods have been used, resulting in a number of varying definitions. Subjective methods of diagnosis include using patient perception and report [57,58], physician examination and report [59,60], and physician confirmation of patient report [45,61]. Objective measures often involve comparing measurement differences over time. Commonly used methods in research settings include computerized tomography (CT) scans [62,63], magnetic resonance imaging (MRI) [64], and dual-energy X-ray absorptiometry (DXA) scans [49,50,65]. These objective measures, while often used in research settings, is impractical in a clinical environment. Anthropometric and DXA-derived variables, such as fat mass ratio, have also been developed, in an attempt to provide standard measures for defining lipodystrophy [66-68].

In low- and middle-income countries, where resources are limited, lipodystrophy is commonly diagnosed using self-report. However, results from studies comparing patient report to clinical examination have been contradictory as both high levels of agreement [46,69] and poor agreement [70-72] between these two methods have been reported. Moreover, when using different severity score ratings, the prevalence was vastly different [57,72].

The literature review (Chapter 2) will focus on specific metabolic complications of ART relevant to the objectives of this thesis, namely hypertension, dysglycaemia and lipodystrophy.

1.2 Problem statement and rationale

Much of the information regarding the metabolic complications associated with the use of ART comes from high-income countries. There is limited data from sub-Saharan Africa. The frequency and prevalence of metabolic complications may be different in sub-Saharan Africa due to (1) poorer nutritional states resulting from poverty, malnutrition and chronic infections; (2) HIV-infected patients are predominantly young black women instead of middle-aged men; and (3) black South African women are more insulin resistant and have less visceral adipose tissue and more subcutaneous adipose tissue than white women. As findings show that lipoatrophy is an adverse antiretroviral drug reaction, while central fat gain may not be, further analysis is needed. Moreover, lipoatrophy is associated with the use of stavudine which has only recently been phased out of South Africa, and zidovudine, which is still being used.

Criteria that were established to define lipodystrophy, did not include data from any African country and may therefore not be generalizable to South Africa. Many of these criteria also require access to laboratories and the use of expensive scanning equipment which is not available in South African Primary Healthcare facilities. As a result, there is a great need for a definition and measurement of lipoatrophy and lipohypertrophy that can be used with confidence in a South African setting. This measurement should be based on a simple, cheap tool that can be applied by health care staff at a community-based centre.

1.3 Aims and Objectives

The aim of this thesis is firstly to investigate metabolic complications associated with the use of ART which include hypertension, dysglycaemia, insulin resistance and lipodystrophy, and

secondly, to develop simple anthropometric cut-points to assist in identifying those with lipodystrophy.

Specific objectives are:

1. To assess the effects of long-term antiretroviral exposure on blood pressure, glycaemia, and anthropometric measures
2. To describe changes in fat distribution over a 24 month period following ART initiation
3. To investigate factors associated with changes in fat distribution
4. To develop simple, objective measures to define lipoatrophy and lipohypertrophy using patient report as the reference standard
5. To develop simple, objective measures to define lipoatrophy and lipohypertrophy using change in DXA measures as the reference standard

1.4 Overview, context and structure of this thesis

When data for this thesis was first collected in 2007, the public health ART programme in South Africa was in its third year. As per the National Department of Health Guidelines, the first-line ART treatment regimen consisted of stavudine, lamivudine and efavirenz or nevirapine. A second-line treatment regimen was available to patients who had failed on the first-line treatment. The second-line treatment regimen consisted of zidovudine, didanosine and lopinavir/ritonavir [6]. Routine monitoring of metabolic complications was only recommended for patients on a second-line treatment regimen. However, emerging evidence suggested that metabolic complications were occurring on non-PI-based treatment regimens as well. The prevalence of metabolic complications had been reported in studies from industrialised countries, but no large prevalence studies had been reported from sub-Saharan Africa, partly due to the recent access to ART.

In addition to this chapter, this thesis includes a literature review, a methods chapter, four results chapters and a summary chapter. The literature review (**Chapter 2**) provides an

overview of specific metabolic complications of ART that are relevant to this thesis, namely hypertension, dysglycaemia and lipodystrophy. It focuses on African studies drawing special attention to gaps or ambiguity in our knowledge and understanding.

The results chapters 4-7 are directly from manuscripts published or submitted for publication, which are included as appendices 1 and 2. Minor changes to the published versions have been made to ensure consistency throughout the thesis. Chapters 4 and 5 focus on the metabolic complications of ART. **Chapter 4** (Appendix 1) addresses the first objective of the thesis, i.e. to assess the effects of long-term antiretroviral exposure on blood pressure, glycaemia, and anthropometric measures, using a sample of HIV-infected women on ART for a median of 16 months at baseline. Measurements are collected at baseline and again approximately 5 years later. **Chapter 5** addresses the second objective, i.e. to describe the changes in fat distribution over a 24 month period as well as the third objective, i.e. to investigate factors associated with changes in fat distribution. We use clinical anthropometry and DXA to describe the changes in body fat distribution. Measurements are collected at baseline, 3 months, 6 months, 12 months, 18 months, and 24 months after commencing ART.

In chapters 6 and 7 we develop simple, objective measures for defining lipodystrophy. **Chapter 6** (Appendix 2) addresses the fourth objective of the thesis, i.e. to develop simple, objective measures to define lipoatrophy and lipohypertrophy using patient report as the reference standard. DXA and anthropometric measures are obtained from a cross sectional sample of black HIV-infected South Africans on ART for a median of 16 months. **Chapter 7** addresses the fifth and final objective of the thesis, i.e. to use change in DXA measures as the reference standard to develop simple, objective measures to define lipoatrophy and lipohypertrophy. We compare change in limb fat and trunk fat as measured by DXA, to anthropometric variables in a sample of black South Africans on ART. Anthropometry and DXA measurements are collected at baseline, 3 months, 6 months, 12 months, 18 months, and 24 months on ART.

The summary in **Chapter 8** synthesises key findings from the thesis as a combined body of work. The strengths and limitations of the studies are considered as well as the implications for clinical practice, policy change and future research.

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Chapter 2. Literature Review

2.1 Overview

This literature review forms the theoretical framework of the thesis. It starts by providing an overview of specific metabolic complications of ART, namely hypertension, dysglycaemia and lipodystrophy, before focusing on the assessment of lipodystrophy. It defines key terms, definitions and terminology as well as summarising key features of existing knowledge that are most relevant to the focus of the thesis. The review is organised around the thesis objectives and addresses themes relevant to the results chapters. This review is not intended to be systematic but rather to represent key points in the literature. It focuses on African studies drawing specific attention to gaps or ambiguity in our knowledge and understanding and is divided into 3 sections.

Section 2.3 addresses the association between ART and hypertension, which is relevant to Objective 1 and provides background to Chapter 4. This section addresses the relationship between ART and hypertension as well as the proposed biological mechanisms. **Section 2.4** addresses the association between ART and dysglycaemia, which is relevant to Objective 1 and provides background to Chapter 4. Section 2.4.1 describes the measures of insulin resistance and β-cell function, while Section 2.4.2 investigates the relationship between HIV and insulin resistance, and ART and insulin resistance.

Section 2.5 is the largest section and addresses the association between ART and lipodystrophy. It is relevant to Objectives 2-5, and provides background to Chapters 4-7. Section 2.5.1 provides an overview of the prevalence of lipodystrophy in high-income countries as well as in sub-Saharan Africa, while Section 2.5.2 provides the motivation for diagnosing lipoatrophy and lipohypertrophy separately. Section 2.5.3 investigates risk factors associated with lipodystrophy, while Section 2.5.4 describes the occurrence of other metabolic complications often seen with lipodystrophy. These sections provide background to Chapters 4 and 5.The final section, Section 2.5.5, provides an overview of subjective and

objective methods that have been used to assess lipodystrophy, lipoatrophy and lipohypertrophy, and provides the background to Chapters 6 and 7.

2.2 Methodology of the literature review

PUBMED was searched for work published during the last 10 years, i.e. between 2006 and 2016. Results were limited to studies on adults that reported on hypertension, glucose dysregulation and changes in fat distribution resulting from exposure to ART. The search terms included 'HIV' or 'human immunodeficiency virus' and 'ART' or 'HAART' or 'antiretroviral' or 'ARV'. The search terms that were excluded were 'child' OR 'children' OR 'adolescent' OR 'infant'. Based on the section of the literature review that was being researched, additional terms were added, and specified to appear in the abstract. The additional terms added for Section 2.3 included 'hypertension' or 'blood pressure' or 'systolic' or 'diastolic' or 'BP', and additional terms for Section 2.4 included 'glucose' or 'dysglycaemia' or 'dysglycemia' or 'diabetes' or 'insulin'. The additional terms added for Section 2.5 included 'lipoatrophy', 'lipohypertrophy', 'lipodystrophy', 'fat redistribution', 'fat distribution', and 'body composition'. References listed in papers identified were also checked for further publications to include. The intention of this literature review is to represent key points in the literature and is not systemic or exhaustive.

2.3 Antiretroviral therapy and Hypertension

The relationship between ART and hypertension has been investigated in small and large studies from high-income countries. The results were inconsistent. A Norwegian study of 380 patients who were mostly men, found that patients on ART had a higher prevalence of hypertension than those who were ART-naïve. However the association was not statistically significant [1]. In 2003, the Data Collection on Adverse Events of Anti-HIV Drugs (DAD) study, using pooled data from 10 cohorts comprising 17170 participants, investigated the role of ART on changes in blood pressure and the development of hypertension. The DAD study found no association between hypertension and ART. Instead, they reported that cumulative exposure to non-nucleoside reverse transcriptase inhibitors (NNRTIs) was associated with a

slight decreased risk of hypertension, which varied according to the threshold used to define hypertension [2]. In 2005, the Multicenter AIDS Cohort (MAC) study, using a cohort of 5578 men that were followed between 1984 and 2003, investigated the association of hypertension with HIV infection and ART. The MAC study found that prolonged use of ART was associated with an increase in the prevalence of hypertension. After 2 or more years on ART, the odds of systolic hypertension (defined as systolic blood pressure > 140 mmHg) increased significantly, while diastolic blood pressure remained the same [3].

Evidence from smaller studies in low- and middle-income countries was equally inconsistent. A Nigerian study of 403 men and women, found no association between ART and hypertension [4], while cross sectional studies from Tanzania [5], Nigeria [6], and Cameroon [7,8] found that HIV-infected adults on ART had an increased risk of developing hypertension. All studies defined hypertension similarly.

Two systematic reviews [9,10] examined the association between ART and hypertension. In 2013, Dillon *et al*. [9] assessed possible associations between HIV, ART and cardiometabolic traits in sub-Saharan Africa. Using data from 14 countries and 2087 participants, the systematic review found no association between ART use and systolic or diastolic blood pressure, in both adjusted and unadjusted models. More recently, Nduka *et al*. [10], using data from 21 countries and 44903 HIV-infected patients from high-, low- and middle-income countries, concluded that ART exposure was associated with higher systolic and diastolic blood pressure levels and an increased risk of hypertension, regardless of ART regimen or duration.

The biological mechanism explaining the association between ART and hypertension is unclear. Increased blood pressure levels among HIV-infected patients on ART have been linked to damage to the endothelial lining of blood vessels [10]. As the endothelial cells in blood vessels play a key role in cardiovascular regulation by producing vasoactive agents which include vasodilators, dysfunction of the epithelial lining could result in hypertension [11].

An alternative theory [12] proposes that antiretrovirals such as nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors (PIs) have a mediating effect on blood pressure by causing changes in body fat distribution [13,14], which in turn leads to increased

blood pressure levels [15]. This theory appears to have merit as studies have found that hypertension was independently associated with both lipoatrophy [16] and an increased waist circumference in HIV-infected patients on ART [12,16-18]. However, a systematic review by De Waal *et al.* [14] concluded that central fat gain seems not to be an antiretroviral adverse drug reaction, but rather the result of treating the HIV infection, which normalises the concentrations of inflammatory markers that cause wasting (refer to section 2.5.2). While central fat gain seems to signify a return to health, it is nonetheless associated with an increased risk of hypertension, which in turn is associated with an increased risk of cardiovascular disease [19].

As ART coverage rates increase in low- and middle-income countries during the coming years, so may the burden of antiretroviral-associated hypertension. Even small increases in blood pressure may result in considerable public health impact on the prevalence of cardiovascular disease [19]. There is thus a need for risk factor assessment before ART initiation, as well as regular monitoring while on treatment. The early detection and management of hypertension is essential for the prevention of cardiovascular events.

2.4 Antiretroviral therapy and Dysglycaemia

Dysglycaemia refers to any disorder in the metabolism and regulation of blood glucose levels, and includes insulin resistance and β-cell dysfunction. Type 2 diabetes mellitus is preceded by both these disorders and is characterised by a state of high blood glucose (hyperglycaemia) that develops when the pancreatic β-cell insulin secretion is unable to meet the insulin needs as determined by insulin resistance and carbohydrate intake [20]. Insulin resistance and diabetes are well-documented complications of ART [21].

2.4.1 Measures of insulin resistance and β-cell function

The hyperinsulinaemic euglycaemic clamp is considered the gold standard diagnostic test to assess insulin resistance [22]. It involves the simultaneous infusion of insulin and a glucose solution into a peripheral vein, while frequently monitoring the blood glucose levels. The amount of glucose solution required to maintain normal blood glucose levels at a steady state

is measured. Both hypo- and hyperglycaemia can be detected. As this technique is time consuming and costly, it is typically used in research settings or small studies [23].

The oral glucose tolerance test (OGTT), with a fasting blood sample drawn immediately before ingesting the glucose solution and another sample drawn 120 minutes after ingestion [24], is predominantly used in large epidemiological studies as a tool to diagnose diabetes, but is also used to identify impaired glucose tolerance in non-diabetic populations. An OGTT can also be used to evaluate both β-cell function and insulin resistance, as the blood glucose and insulin responses obtained from this test are able to reflect the ability of pancreatic β-cells to secrete insulin as well as to assess the sensitivity of tissues to insulin [25]. Criteria for the diagnosis of diabetes and impaired glucose regulation have been developed by the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus [26,27]. The Expert Committee acknowledged that some individuals had glucose levels that did not meet the criteria for diabetes, but were higher than normal, and were thus considered to be at risk of developing diabetes. Both the fasting blood glucose as well as the 120 minute glucose measure can be used to diagnose diabetes. In addition, fasting blood glucose is used to diagnose impaired fasting glucose (IFG), while the 120 minute glucose is used to diagnose impaired glucose tolerance (IGT) (**Table 1**). In epidemiological studies, patients that are diagnosed with diabetes using the OGTT, are referred to healthcare facilities for assessment, confirmation of diagnosis and management.

Table 1. Diagnostic criteria for glucose abnormalities

Glucose abnormality	**Diagnostic measure**	**Range**
Impaired fasting glucose (IFG)	Fasting plasma glucose	5.6-6.9 mmol/l
Impaired glucose tolerance (IGT)	2hr plasma glucose during OGTT	7.8-11.0 mmol/l
Diabetes	Fasting plasma glucose	≥ 7mmol/l
	2hr plasma glucose during OGTT	≥ 11.1 mmol/l

A number of indices have been developed to measure β-cell function and insulin sensitivity/resistance using either fasting samples or other measures of insulin and glucose taken at various time points during the OGTT [24,28,29]. In addition to the insulin and glucose measures, the McAuley index uses the fasting triglyceride and the Stumvoll index uses BMI and age [30,31]. **Table 2** shows some of the indices used to assess β-cell function and insulin sensitivity/resistance obtained from results of an OGTT, as well as the calculation and description of each index.

High homeostatic model assessment of insulin resistance (HOMA-IR) and low homeostatic model assessment of beta cell function (HOMA-β) are associated with an increased prevalence of insulinogenic index (IGI) and risk of developing diabetes [28], while a low oral disposition index (DI_o) level is considered an early marker of inadequate β-cell function [29].

Table 2. Some of the indices used to measure β-cell function and insulin sensitivity/resistance [30-32]

Measure	Index	Calculation	Description
β-cell function	IGI	$\Delta Insulin_{0\text{-}30}$/ $\Delta Glucose_{0–30}$	A measure of the insulin response to glucose
	HOMA-β	(20 x fasting insulin)/(fasting insulin-3.5)	A measure of β-cell function
	DI_o	($\Delta Insulin_{0\text{-}30}$/ $\Delta Glucose_{0–30}$) × (1/fasting insulin)	A measure of the rise in insulin secretion in relation to insulin resistance
	Stumvoll Index (1st phase)	$1283 + 1.829 X I_{30} – 138.7 x G_{30} + 3.772 I_0$	A measure of insulin sensitivity
Insulin sensitivity/ resistance	Fasting $Insulin^{-1}$	1/ fasting insulin	A measure of insulin sensitivity
	Fasting glucose to insulin ratio	fasting glucose/fasting insulin	A measure of insulin sensitivity
	Insulin 120 min^{-1}	1/ 120 min insulin	A measure of insulin sensitivity
	HOMA-IR	(fasting glucose × fasting insulin)/22.5	A measure of insulin resistance
	QUICKI Index	1/(log fasting insulin + log fasting glucose)	A measure of insulin sensitivity
	Matsuda Index	10000/√fasting glucose x fasting insulin x G_{mean} x I_{mean}	A measure of insulin sensitivity
	Raynaud Index	40/fasting insulin	A measure of insulin sensitivity
	McAuley Index	$e^{2.63\text{-}0.28 \ln(\text{fasting insulin}) \ln(\text{fasting triglycerides})}$	A measure of insulin resistance
	Fasting Belfiore Index	2/ [(fasting insulin x fasting glucose) + 1]	A measure of insulin resistance
	Avignon Index	Sib = 10^8/ (fasting insulin x fasting glucose x VD) Si2h = 10^8/ (120 min insulin x 120 min glucose x VD) SiM = (0.137 x Sib) + Si2h/2	3 measures of insulin sensitivity
	Stumvoll Index	0.222 – 0.00333 x BMI – 0.0000779 x 120 min insulin – 0.000422 x age	A measure of insulin sensitivity that utilises demographic data

IGI – insulinogenic index; HOMA-β – homeostatic model assessment of beta cell function; DI_o – oral disposition index; HOMA-IR – homeostatic model assessment of insulin resistance; QUICKI – quantitative insulin sensitivity check index; G_{mean} – mean plasma glucose concentration during OGTT; I_{mean} – mean plasma insulin concentration during OGTT; VD – glucose distribution volume calculated using a monocompartmental model

2.4.2 Development of insulin resistance

Insulin resistance was one of the first metabolic complications of ART to be reported [21]. In HIV-negative individuals, factors contributing to the development of insulin resistance include genetic factors, physical inactivity and obesity. HIV-infected individuals on ART have the same risk factors in addition to HIV-related risk factors, which include the pro-inflammatory effect of the HIV infection, the direct effect of antiretrovirals, and the indirect consequences of HIV treatment, such as lipodystrophy [33].

Effect of HIV on insulin resistance

Before the introduction of ART, insulin resistance was rarely reported in HIV-infected individuals. Initially, small cross sectional studies from high-income countries demonstrated similar or slightly increased insulin sensitivity in HIV-infected individuals compared to uninfected controls [34,35]. However, later studies found that HIV-infected individuals were developing insulin resistance in the absence of, or prior to initiation of ART [36-38]. The MAC Study reported a lower QUICKI index (indicating decreased insulin sensitivity or insulin resistance) and higher fasting insulin levels in HIV-infected men who were not exposed to ART, and proposed an association between HIV infection and hyperglycaemia [39]. The mechanism responsible for altering insulin sensitivity in HIV-infection is thought to be related to an overproduction of pro-inflammatory cytokines, which are known to play a role in the development of insulin resistance in other conditions involving inflammation such as obesity and diabetes. In HIV-infection, infected T-cells and adipose tissue produce increased amounts of tumour necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and interleukin-8 (IL-8), which cause inflammatory insulin resistance and have been linked to increased levels of HOMA-IR [38].

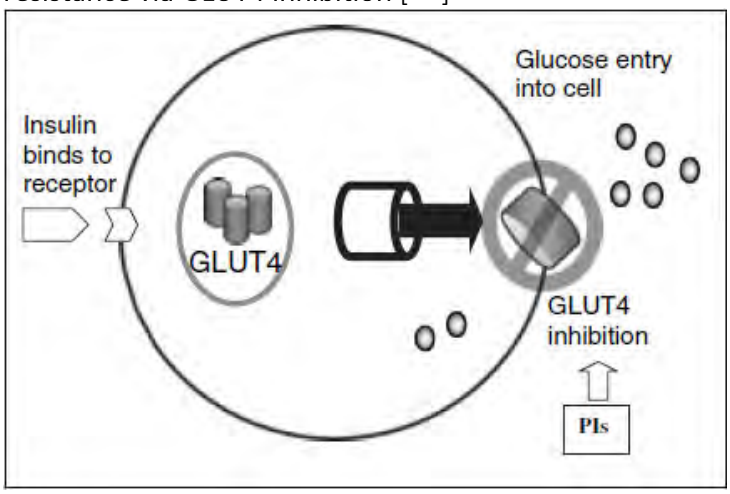
Effect of ART on insulin resistance

The association between ART and insulin resistance is well documented, and is known to affect the organs and tissues that are normally involved in glucose regulation, namely adipose tissue, skeletal muscle and the liver, resulting in insulin resistance [40,41]. The development

of insulin resistance does not appear to be a class effect, but appears to be linked to specific drugs within the class of PIs [42], NRTIs [39,43] and more recently NNRTIs [44].

The first antiretrovirals found to be associated with insulin resistance, were the first-generation PIs, indinavir [42,45] and ritonavir [46]. These PIs are thought to cause insulin resistance by blocking insulin-stimulated glucose uptake in adipocytes via inhibition of the glucose transporter GLUT4 [33,47] (**Figure 1**). While indinavir is no longer used, ritonavir remains widely used with other PIs, such as the commonly prescribed co-formulated lopinavir/ritonavir combination [23]. Whereas the previously prescribed full dose ritonavir induced insulin resistance [48], the far lower doses prescribed for boosting have been shown to have little effect on insulin sensitivity [49]. The relationship between lopinavir and insulin resistance is still unclear as Dube *et al.* [50] reported that lopinavir/ritonavir did not induce insulin resistance, while Taylor *et al.* [49] suggested that lopinavir was responsible for the insulin resistance observed in short term studies of lopinavir/ritonavir. New generation PIs, atazanavir, tipranavir and darunavir appear to have little or no effect on insulin sensitivity [23,51,52]. Although inhibition of glucose transport is considered the main mechanism for insulin resistance, prolonged exposure to PIs affect cellular insulin signalling, leading to a change in insulin sensitivity and indirectly changing glucose uptake [41].

Figure 1. Diagrammatic representation of mode of action of insulin, and mechanism of insulin resistance via GLUT4 inhibition [33]



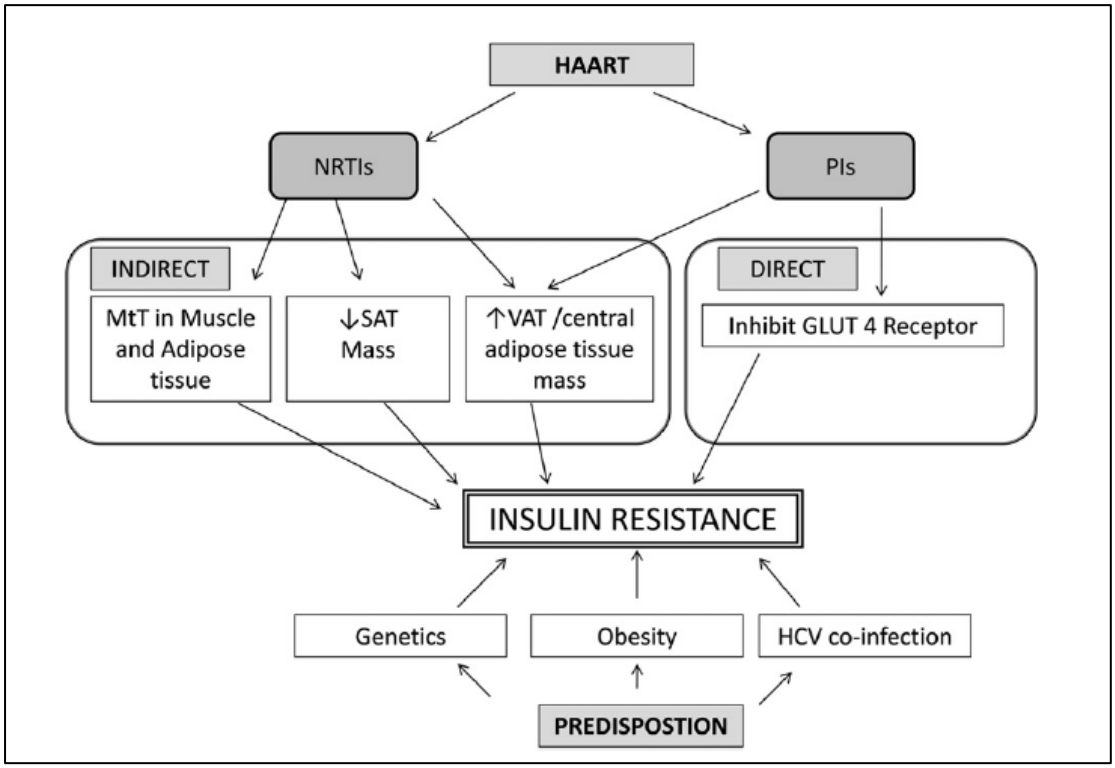
Indirect effect of ART on insulin resistance

NRTIs, particularly stavudine and zidovudine, have been implicated in the development of insulin resistance and diabetes. The MAC study [39], reported a strong association between stavudine and insulin resistance, while the DAD study [43] did not measure insulin resistance but reported that stavudine and zidovudine were associated with the development of diabetes. Findings from sub-Saharan Africa have been contradictory. In a South African population, Dave *et al*. [53] found that the incidence of dysglycaemia in ART and ART-naïve patients were similar, while a study from Ethiopia [54] reported that diabetes was associated with increasing age and duration on ART. A Ugandan study [55] reported that stavudine had a protective effect on hyperglycaemia, while also reporting that the proportion of patients with borderline hyperglycaemia and diabetes were similar on stavudine and zidovudine. More recently Karamchand *et al*. [44], based on a cohort of 56298 HIV-infected South Africans on ART, reported an increased incidence of diabetes in patients on stavudine, and to a lesser extent, zidovudine.

The NRTIs stavudine and zidovudine are linked to the development of subcutaneous lipoatrophy, thought to be mediated by inhibiting mitochondrial polymerase-gamma in adipose tissue. Apoptotic adipocytes release free fatty acids which accumulate in the liver and skeletal muscle, resulting in peripheral insulin resistance [39]. Independent of changes in body composition, NRTIs cause mitochondrial toxicity in skeletal muscle, which affects glucose regulation and causes secondary insulin resistance [23,41].

Historically, NNTRIs such as efavirenz and nevirapine, were not associated with the development of insulin resistance or diabetes as reports from high-income countries [23,43] suggested minimal changes with little clinical significance. However, in sub-Saharan Africa, reports of an association between efavirenz and dysglycaemia have emerged. In 2011, Dave *et al*. [53] reported a significant association between efavirenz use and dysglycaemia. In a recent study, Karamchand *et al.* [44] reported an increased risk of developing diabetes in a large cohort of South African patients treated with efavirenz compared with nevirapine. The mechanism whereby efavirenz results in insulin resistance is unknown, but has been postulated to involve mitochondrial toxicity, as well as being mediated though changes in fat distribution [56].

Figure 2. Potential causes of insulin resistance in HIV-infected patients on ART [23]



The development of insulin resistance in HIV-infected individuals on ART results from direct and indirect mechanisms, as well as other predisposing factors related to genetics, obesity and inactivity (**Figure 2**) [23]. The PI co-formulated lopinavir/ritonavir, the NRTI zidovudine and NNRTI efavirenz are still being used in low- and middle-income countries such as South Africa [57], while stavudine has only recently been phased out. The continued use of drugs that are directly and indirectly associated with the development of insulin resistance and diabetes, is predicted to increase the incidence of dysglycaemia and diabetes as the number of HIV-infected treated with ART continues to grow, and the time spent on ART continues to increase.

2.5 Antiretroviral therapy and Lipodystrophy

Lipodystrophy, also known as fat redistribution, is commonly reported in HIV-infected patients taking ART. It is characterized by either subcutaneous fat loss (lipoatrophy), which is most noticeable in the face, limbs, and buttocks, or fat accumulation (lipohypertrophy) seen

in the abdomen, breast or posterior neck, or a combination of both [58]. HIV-related lipodystrophy was first described after the introduction of PIs in the late 1990's [21], but was later found to occur in patients receiving non-PI regimens as well [59,60].

2.5.1 Prevalence of lipodystrophy

High-income countries

The relationship between ART and changes in fat distribution has been well studied in high-income countries. The first studies to report on lipodystrophy were based on cross sectional study designs and mostly used patient report or clinical examination to diagnose lipodystrophy, lipoatrophy and lipohypertrophy (**Table 3**). Drug regimens used were varied, with the majority of patients taking a PI-based ART regimen. The prevalence of lipodystrophy reported in these studies were wide-ranging, possibly due to different diagnostic criteria, drug regimens and demographic factors [61]. In 2001, based on 868 men-who-have-sex-with-men, the MAC study reported that 25-30% of HIV-infected men had lipoatrophy, and that 40% had a combination of lipoatrophy and central fat gain [62]. The CISAI study, consisting of 1480 patients recruited from 10 infectious disease departments in Italian hospitals between 1997 and 1999, reported a relatively low lipodystrophy prevalence of 7.5-13.6%, depending on the ART regimen used [63], while the HIV Outpatient Study (HOPS), consisting of 1077 patients recruited from 8 HIV treatment clinics in 7 cities in the USA in 1998 [60], reported that 49% of patients showed one or more signs of fat redistribution. Between 1997 and 1998, 614 HIV-infected patients were recruited from French health care centres [64]. Lipodystrophy was reported in 62% of participants, with 21% diagnosed with only lipoatrophy and 17 % with only lipohypertrophy. In a large Australian study [65] consisting of 1348 participants recruited between 1998 and 1999, lipodystrophy was diagnosed in 53% of participants.

Sub-Saharan Africa

In sub-Saharan Africa, ART slowly became available in the early 2000s. By 2004, when the cost of antiretrovirals started to decrease [66], ART become more readily available, but was still governed by eligibility criteria. The most frequently used ART regimen consisted of stavudine, lamivudine and nevirapine, with very few patients initiating ART with a PI-based regimen [67],

partly due to its cost [68]. Limited research (**Table 4**) is available on the association between ART and lipodystrophy in sub-Saharan Africa, with the largest studies coming from South Africa [69-71] and Rwanda [72]. The majority of studies used a cross sectional design and, unlike studies from high-income countries, consisted mostly of black women in their mid-thirties. Most studies reported on the prevalence of lipodystrophy which ranged from as low as 7.2% in a large Rwandan sample (n=2190) using a longitudinal study design [72] to as high as 68% in a small Ethiopian study (n=313) [73]. A few studies reported on the prevalence of lipoatrophy which ranged from less than 2% in a large South African study consisting of 2835 men and women [71] to 26% in a small Nigerian study of 288 men and women on ART for more than 6 months [74]. Even fewer studies reported on lipohypertrophy which ranged from 11% to 36% [69,75,76].

Table 3. Summary of selected studies from high-income countries measuring change in fat distribution

Country	Study design	n	Population	Assessment method	ART regimen and duration	Fat distribution
Australia [21]	Cross sectional study	195	Majority men, mean age = 40 years	Patient report and clinical examination	PI-based ART regimen	Lipodystrophy = 64%
Australia [77]	Cross sectional study	159	Only men	Patient report; DXA measures and lipid profile	PI-based ART regimen with d4T or AZT	Patient reported lipodystrophy (fat loss in arms and legs with abdominal fat gain) = 18.2%; Patient reported lipoatrophy = 20%; DXA and lipid based lipodystrophy = 54.7%
Australia [78]	Cross sectional study	1348	Majority men, mean age = 40 years	Clinical examination	Various ART regimens including PI-based	Lipodystrophy = 53%
Denmark [79]	Cross sectional study	168	Only men, median age = 43 years	Patient report and clinical examination	2 NRTIs + ritonavir or ritonavir + sequinavir or nelfinavir	Lipoatrophy = 10%; Abdominal obesity = 43%
France [64]	Cross sectional study	614	Majority men	Clinical examination	Various ART regimens, median time = 16.7 months	Lipodystrophy = 61-63%
Italy [63]	Cross sectional study	1480	Majority men, mean age = 37 years	Patient report and clinical examination	Indinavir, nelfinavir, saquinavir-HCG, ritonavir, ritonavir + saquinavir and 9 other combinations; mean	Lipodystrophy =7.5-13.6%; Lipoatrophy = 3.4-7.4%; Lipohypertrophy = 2.7-6.1% depending on drug regimen

					observation time = 22 months	
USA [60]	Cross sectional study	1077	Majority white men, mean age = 41 years	Patient report and clinical examination	Various ART regimens	1 or more signs of fat redistribution = 49%; Lipoatrophy = 15.9%; Lipohypertrophy = 9.7%
USA [62]	Cross sectional	868	Only men	Patient report and anthropometry	PI-based ART regimen	Lipoatrophy = 25-30%; Combination of lipoatrophy and lipohypertrophy = 40%

DXA - Dual energy X-ray absorptiometry; d4T – stavudine; AZT - zidovudine

Table 4. Summary of selected studies from sub-Saharan Africa measuring changes in fat distribution

Country	Study design	n	Population	Assessment method	ART regimen and duration	Fat distribution
Cameroon [75]	Cross sectional study	243	Majority women, mean age = 39 years	Patient report with 2 definitions based on severity	d4T/AZT + 3TC + NVP/EFV for >6 months	Lipodystrophy = 18.6%; Lipoatrophy = 2-9%; Lipohypertrophy = 11=36%
Ethiopia [73]	Cross sectional study	356	Majority women	Patient report and clinical examination	d4T-based ART regimen > 1 year	Lipodystrophy =68.3%
Ethiopia [80]	Cross sectional study	313	Majority women, mean age = 41 years	Patient report and clinical examination	ART > 6 months	Lipodystrophy = 12.1%
Nigeria [74]	Cross sectional study	288	Majority women, mean age = 39 years	Patient report and clinical examination using a standard questionnaire	d4T- or AZT- or TNF-based ART regimen for > 6 months	Lipodystrophy = 26%
Rwanda [72]	Longitudinal study with 1.5 years median follow-up time	2190	Majority women, mean age = 38 years	Patient report and clinical examination using a standard questionnaire	d4T + 3TC + NVP	Lipodystrophy = 7.2%
Rwanda [76]	Cross sectional study	409	Majority women, mean age = 38 years	Patient report and clinical examination using a standard questionnaire	d4T + 3TC + NVP > 1 year	Lipodystrophy = 34.2%; Lipoatrophy = 9.8%; Lipohypertrophy = 19.6%
Rwanda [81]	Cross sectional study	571	Majority women, mean age = 38 years	Patient report and clinical examination	d4T + 3TC + NVP > 6 months	Lipodystrophy = 34%
Rwanda [82]	Cross sectional study	609	Majority women, mean age = 35 years	Patient report and clinical examination using a standard questionnaire	d4T-based regimen for > 12 months	Lipoatrophy = 12.2%

Senegal [83]	Case control study	180	Majority women	Clinical examination	d4T-, AZT- and PI-based regimens for 4-9 years	Lipodystrophy = 31.1%; Lipoatrophy = 13.3%; Lipohypertrophy = 14.5%
South Africa [69]	Cross sectional study	9040	66% women	Patient report and physician conformation	d4T-based regimen; median time on ART = 19 months	Lipoatrophy = 7%
South Africa [71]	Cross sectional study	3910	58% women	Physician report	d4T + 3TC + NVP/EFV > 12 months	Lipoatrophy < 2%
South Africa [70]	Cross sectional study	2835	70% women, > 15 years	Physician report	d4T + 3TC + EFV > 15 months	Lipodystrophy = 23.9%
South Africa [84]	Cross sectional study	479	Majority women, mean age = 35 years	Patient report and clinical examination	d4T-based regimen > 12 months	Lipodystrophy = 11.7%
South Africa [85]	Longitudinal study with 1 year follow-up time	230	64% women, mean age = 35 years	Patient report	d4T + 3TC + EFV > 12 months	Lipodystrophy = 11.7%

d4T – stavudine; AZT – zidovudine; 3TC – lamivudine; EFV – efavirenz; NVP – nevirapine; TNF - tenofovir

Differences between high-income countries and sub-Saharan Africa

The prevalence of lipodystrophy is wide-ranging in both high-income countries and sub-Saharan Africa (**Table 3** and **4**). Some of this can be attributed to the subjectivity of self-report and clinical examination (refer to section 2.5.5). However, differences in populations and drug regimens play a major role when comparing high-income countries to sub-Saharan Africa, as the HIV-infected populations in these two regions are different. In high-income countries the majority of HIV-infected are men-who-have-sex-with-men, while the majority of HIV-infected in sub-Saharan Africa are black women. As black South African women have less visceral fat than white South African women [86], the prevalence of fat redistribution in high-income countries should not be expected to be similar to the prevalence in sub-Saharan Africa. Furthermore, ART drug regimens also differed – high-income countries primarily used PI-based regimens, while in sub-Saharan Africa, due to the higher cost of PIs compared to NRTIs [68], the majority of ART regimens were NRTI-based, including stavudine. As stavudine is without doubt responsible for the development of lipoatrophy [14], and stavudine has only recently been phased out in sub-Saharan Africa [87], the prevalence in sub-Saharan Africa is expected to be high.

2.5.2 Lipodystrophy versus lipoatrophy and lipohypertrophy

While the majority of studies reported on the prevalence of lipodystrophy, which referred to peripheral lipoatrophy, or central fat gain (lipohypertrophy), or a combination of both, recent findings have highlighted the need to differentiate between the two syndromes. In 2013, a systematic review by De Waal *et al.* [14] concluded that lipoatrophy was an adverse antiretroviral drug reaction, while lipohypertrophy appeared to be a consequence of treating the HIV infection. Based on 27 prospective studies that used objective methods [dual energy X-ray absorptiometry (DXA), computerised tomography (CT), or magnetic resonance imaging (MRI)] to assess fat distribution over a minimum of 24 weeks, the authors concluded that lipoatrophy was strongly associated with the use of NRTIs, specifically stavudine and zidovudine, and that subcutaneous adipose tissue (SAT) was lower in patients on ART compared to controls and decreased with decreasing time on ART. In addition, they found that when efavirenz was combined with NRTIs, the risk of limb fat loss was greater than when

combined with PIs [14]. Lipoatrophy is thought to occur as a result of mitochondrial toxicity in adipose tissue. NRTIs inhibit DNA polymerase gamma, which is needed for the replication of mitochondrial DNA (mtDNA). The subsequent depletion of mtDNA results in mitochondrial dysfunction in adipose tissue [88-90].

The results of the systematic review by De Waal *et al.* [14] suggested that lipohypertrophy was a consequence of treating the HIV infection as visceral adipose tissue (VAT) and trunk fat were no different in participants on ART compared to controls, nor was central fat gain associated with specific antiretrovirals. The authors hypothesised that lipohypertrophy developed when the concentrations of inflammatory markers that are known to cause wasting were normalised, as a consequence of treating the HIV infection.

2.5.3 Risk factors associated with lipodystrophy

Several risk factors for lipodystrophy have been identified. The most commonly reported include the use of specific antiretrovirals, duration of ART, sex and BMI. Early studies that did not differentiate between lipoatrophy and central fat gain, reported an association between PI use and fat redistribution. However, it soon became evident that lipoatrophy occurred in patients who had never been exposed to a PI-based regimen. The HOPS study [60] reported a strong association between lipoatrophy and both stavudine and indinavir, as well as more than 2 years of PI exposure. The CISAI study in Italy [63] concluded that lipodystrophy was associated with the PIs ritonavir and indinavir, as well as noting an association between stavudine and lipoatrophy. A number of smaller studies from high-income countries also reported associations between lipodystrophy and PI use [21,64], and stavudine and peripheral fat loss [79,91].

In sub-Saharan Africa, where PIs were rarely used, several studies reported an association between stavudine and lipodystrophy, especially lipoatrophy [69-72,76]. In Rwanda, patients on a stavudine-containing regimen had a three times higher prevalence of lipoatrophy compared to those on a zidovudine-containing regimen [76], while in Nigeria, 46% of patients taking stavudine developed lipoatrophy compared to 31% of those on zidovudine [74]. Studies have also shown that lipoatrophy increases with increasing duration [76] and dose of stavudine [71], and is partially reversed after switching to abacavir, tenofovir or an NRTI-

sparing regimen [14]. As evidence of the toxic effect of stavudine increased, the World Health Organisation recommended that the dosage for adults and children be reduced from 40mg to 30mg [92]. Studies comparing the effects of the 30mg versus the 40mg stavudine dosage, found that the 30mg dosage resulted in fewer toxic effects, with similar viral load suppression, when compared to the 40mg dosage [71,75]. In 2010 the World Health Organisation recommended that stavudine be discontinued and replaced with tenofovir. However, due to the higher cost of tenofovir and stockpiles of stavudine, low- and middle-income countries continued to use stavudine for several more years [92].

The relationship between fat redistribution and gender has been contradictory. While several studies in sub-Saharan Africa found a higher prevalence of lipoatrophy in women compared to men [69,76], a study from Nigeria [74] reported a higher prevalence in men, and a study from Cameroon reported an equal prevalence in men and women [75]. The relationship between BMI and lipoatrophy is also contradictory. Studies from Rwanda [76,82] reported that a higher baseline BMI was associated with lipoatrophy, while a Nigerian study [74] reported an association between lower baseline BMI and lipoatrophy.

2.5.4 Implications of lipodystrophy

The development of lipodystrophy is associated with cardiometabolic risk factors such as dyslipidaemia and dysglycaemia [93]. Patients with lipodystrophy may develop impaired glucose tolerance and insulin resistance [43,53,94] as well as increased levels of triglycerides [95] and decreased levels of HDL cholesterol [96]. As insulin resistance is often reported before lipodystrophy, it is thought to be involved in the development of lipodystrophy. However, others have suggested that lipodystrophy, particularly central fat gain, is partly responsible for the development of insulin resistance [39,97].

The development of lipodystrophy has psychosocial and lifestyle implications as well. As a result of the stigma associated with HIV-infection, patients may not want to disclose their status, yet their changing body shape acts as an HIV identifier. Patients have reported psychological effects which include feelings of depression, poor self-confidence and loss of self-esteem [98].

The prevalence of lipodystrophy, specifically lipoatrophy, is high in low- and middle-income countries as stavudine has only recently been phased out, and zidovudine continues to be used.

2.5.5 Assessment of lipodystrophy

Lipodystrophy has been diagnosed using both subjective and objective methods, resulting in challenges in the diagnosis and assessment of its prevalence and incidence. Subjective methods of diagnosis include using patient perception and report, physician examination and report and physician confirmation of patient report. Objective measures often involve comparing measurement differences over time. Commonly used methods include CT scans, MRI, and DXA scans. Anthropometric and DXA-derived variables, such as fat mass ratio, have also been developed, in an attempt to provide standard measures for defining lipodystrophy.

Subjective assessment methods

The majority of studies, both from early high-income countries as well as later studies from sub-Saharan Africa (**Table 3** and **4**), used subjective measures to diagnose lipodystrophy. Studies generally used one of the following methods: (1) only patient self-report, (2) patient self-report and examination by a clinician, (3) only examination by a clinician or (4) patient self-report confirmed by examination by a clinician. Questionnaires were commonly used to capture the change in fat distribution. The most frequently used questionnaire was developed for the HOPS study [60], in which 1077 patients completed a survey and were examined by their physician. Body areas evaluated included the abdomen, dorso-cervical spine, arms, legs, hips, buttocks and face. Changes were graded as 'subtle', 'moderate' or 'severe', and based on agreement between the patient and physician. Patients were diagnosed with moderate/severe lipodystrophy if they had 1 or more sites with a 'severe' score or 2 or more sites with a 'moderate' score. Patients were diagnosed with mild/moderate lipodystrophy if they had no signs of lipodystrophy or the signs were too mild for the moderate/severe group.

Using the HOPS questionnaire, the HIV lipodystrophy case definition group developed the HIV lipodystrophy case definition (LDCD) score [99], and later developed the lipodystrophy

severity grading scale [100]. A case-control study design was used, and 1081 HIV-infected patients enrolled from sites in North America, Europe, Australia, Asia and South America. Questions on fat gain or fat loss in 8 areas were recorded (face, neck, dorso-cervical spine, arms, breast, abdomen, buttocks and legs). The degree of fat accumulation or fat loss at each site was rated as absent (score = 0), mild (score = 1), moderate (score = 2), or severe (score =3) [100]. A lipodystrophy grade was proposed based on the severity score; grade 0 (zero score), grade 1 (score of 1-6), grade 2 (score of 7-12), grade 3 (score of 13-18) and grade 4 (score of >19) [100]. However, the HOPS and LDCD scores do not differentiate between lipoatrophy and lipohypertrophy.

Results from studies comparing patient report to clinical examination have been contradictory as both high levels of agreement [101,102] and poor agreement [83,103,104] between these two methods have been reported. In addition, when using different severity score ratings, the prevalence was vastly different [75,83].

Objective assessment methods

Objectives measures used to diagnose lipodystrophy, lipoatrophy and lipohypertrophy in high-income countries include DXA, CT and MRI scans (**Table 5**). Lipoatrophy measured by DXA has been defined as ≥ 20% loss in limb fat [13,86,89,105-108], and at times as ≥ 10% loss in limb fat [89,109]. When measured by MRI, lipoatrophy was defined as leg SAT below the 10th percentile [110], and when diagnosed by CT scan lipoatrophy was defined as a decrease in SAT [108,111,112].

Lipohypertrophy measured by DXA has been defined as ≥ 20% gain in trunk fat [105,106], as well as a change in the percentage trunk fat [89]. When measured by CT scan, lipohypertrophy was defined as an increase in VAT [89,111,112]. One study used MRI to measure change in VAT [110].

In an attempt to standardise measures of lipodystrophy, Bonnet *et al.* [113], developed an objective measure and defined standard values for defining lipodystrophy based on DXA. The authors used a cross sectional study design and 403 French men (241 HIV-uninfected; 34 HIV-

infected ART-naïve; 128 HIV-infected on ART). Fat mass ratio (FMR), defined as the ratio of percentage trunk fat over the percentage lower limb fat, was proposed as an objective lipodystrophy measure. Lipodystrophy was defined as FMR ≥ 1.5. In 2010, Freitas *et al.* [114], using a population of HIV-infected Portuguese men and women on ART, proposed separate reference values for men and women (men: FMR ≥1.961; women: FMR ≥1.329). In 2012, Asha *et al.* [115] reported that a FMR of >2.28 identified 54.3% of HIV-infected Indian men on ART. However, Asha *et al.* defined FMR as the ratio of trunk fat mass in grams to lower limb fat mass in grams.

To date studies reported from sub-Saharan Africa have not used objective measures such as DXA, MRI or CT scans to define lipodystrophy. As patient report and clinical examination are the most commonly used diagnostic methods in sub-Saharan Africa, and studies have shown poor correlation between the two methods, it emphasises the need for the development of an objective diagnostic tool.

Table 5. Objectives measures used for defining lipoatrophy, lipohypertrophy and lipodystrophy

Country	Study design	n	Measurement method	Lipoatrophy measure	Lipohypertrophy measure	Other
Canada [105]	RCT	155	DXA	≥ 20% limb fat loss	≥ 20% trunk fat gain	
France [113]	Cross sectional study	403	DXA			FMR (ratio between % trunk fat and % lower limb fat)
France [116]	RCT	100	CT scan	SAT	VAT	SAT:TAT ratio
India [115]	Cross sectional study	105	DXA			FMR (ratio between trunk fat and lower limb fat)
Multi-country [112]	RCT	48	DXA CT scan	Limb fat (g) SAT	VAT	VAT:SAT ratio
Multi-country [86]	RCT	563	DXA	≥ 20% limb fat loss		
Multi-country [106]	RCT	136	DXA	≥ 20% limb fat loss	≥ 20% trunk fat gain	
Portugal [94]	Cross sectional study	345	DXA			FMR (ratio between % trunk fat and % lower limb fat)
Spain [107]	RCT	54	DXA	≥ 20% limb fat loss ≥ 30% limb fat loss		

Spain [117]	Cross sectional study	168	DXA			FMR (ratio between trunk fat and lower limb fat)
USA [109]	RCT	334	DXA	≥ 10% limb fat loss		
USA [13]	RCT	753	DXA	≥ 20% limb fat loss		
USA [108]	RCT	200	DXA CT scan	≥ 20% limb fat loss Limb fat (g) SAT	Trunk fat (g) VAT	
USA [118]	Cross sectional study	154	DXA	Limb fat (g)		
USA [110]	Longitudinal	691	MRI	Leg SAT < 10^{th} percentile	VAT	
USA and Puerto Rico [89]	RCT	269	DXA CT scan	≥ 10% limb fat loss ≥ 20% limb fat loss % change in limb fat	% change in trunk fat % change in VAT	
USA [119]	Cross sectional study	66	DXA			FMR (ratio between trunk fat and lower limb fat)

RCT – randomised control trial; DXA - Dual energy X-ray absorptiometry; CT – computerised tomography; MRI – magnetic resonance imaging; VAT – visceral adipose tissue; SAT – subcutaneous adipose tissue; TAT – total adipose tissue; FMR – fat mass ratio

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Chapter 3. Methods

Data for this thesis came from three datasets, collected between 2007 and 2013. All datasets consisted of black HIV-infected men and women presenting to ART clinics in Cape Town. The first dataset is a cross-sectional study, while the second and third datasets are longitudinal studies (**Table 1**). These three datasets form the Metabolic Complications of HAART (McHAART) study, and was aimed at determining the prevalence of metabolic complications in HIV-infected patients receiving ART.

Table 1. Summary of thesis results chapters by data source, objective and publication title

Chapter	Dataset	Objective	Publication title
4	Longitudinal dataset A	To assess the effects of long-term antiretroviral exposure on blood pressure, glycaemia, insulin secretion and anthropometric measures in black South African women	Changes in blood pressure, glucose levels, insulin secretion and anthropometry after long term exposure to antiretroviral therapy in South African women
5	Longitudinal dataset B	To use DXA scans to describe the changes in fat distribution over a 24 month period in a population of black South African men and women initiated on ART	Changes in body fat distribution on dual-energy x-ray absorptiometry in black South Africans starting first-line antiretroviral therapy
		To investigate factors associated with changes in fat distribution	
6	Cross sectional dataset	To develop simple, objective measures to define lipoatrophy and lipohypertrophy using patient report as the reference standard	The development of simple anthropometric measures to diagnose antiretroviral therapy-associated lipodystrophy in resource limited settings
7	Longitudinal dataset B	To use change in DXA measures to validate the newly developed measures of lipoatrophy and lipohypertrophy	Anthropometric definitions for antiretroviral associated lipodystrophy derived from a longitudinal South African cohort with dual-energy x-ray absorptiometry data

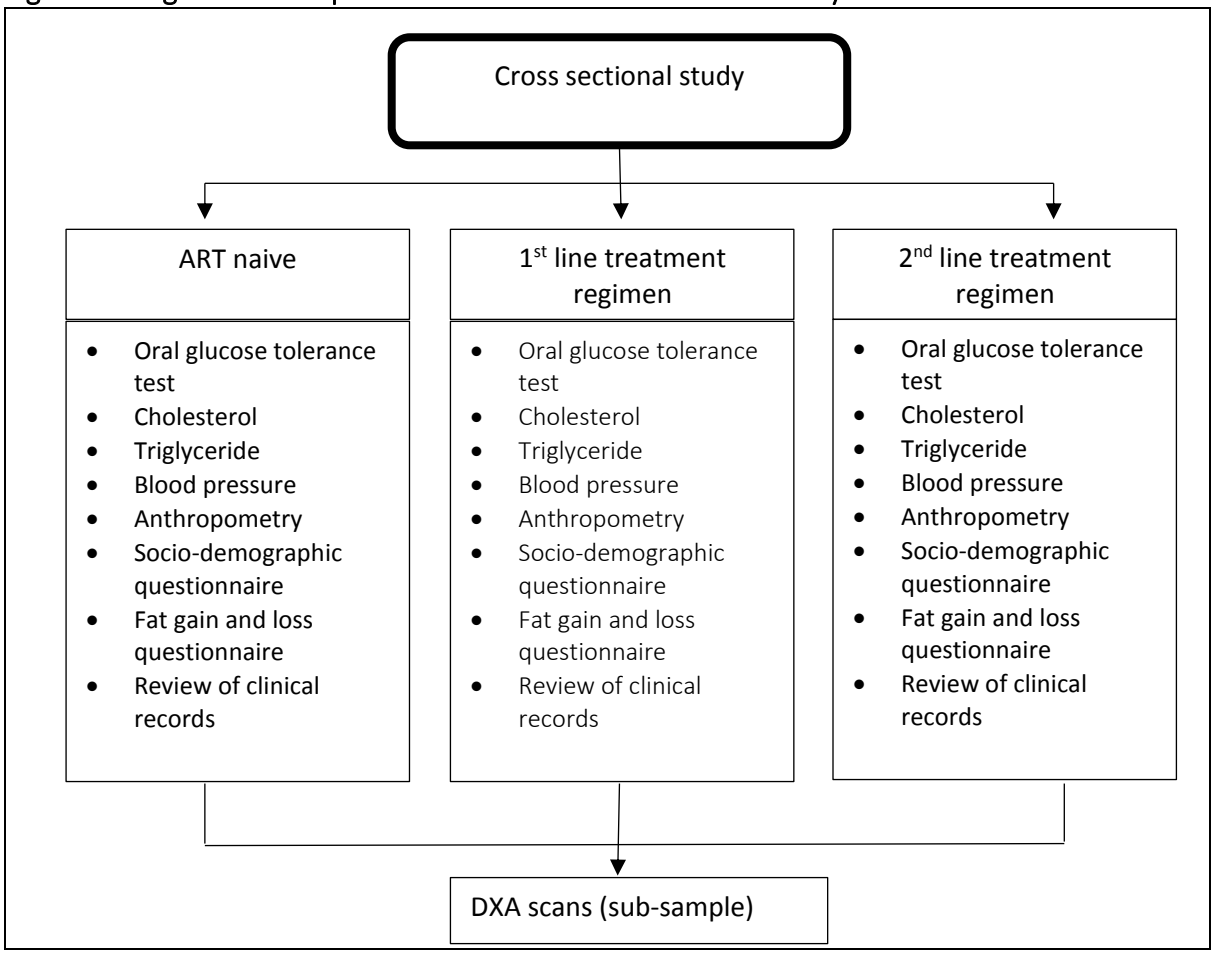
3.1 Ethical approval

The McHAART study was approved by the Research Ethics Committee of the Faculty of Health Sciences at the University of Cape Town. All procedures and risks were explained to participants, after which written, informed consent to participate in the study was obtained. Participants provided additional consent for the storage of genetic samples and linkage with clinical data.

3.2 Cross Sectional Study

For the cross sectional study (**Figure 1**), HIV-infected patients were conveniently sampled from two Cape Town ART clinics between 2007 and 2009.

Figure 1: Diagrammatic representation of the cross sectional study



Participants who were recruited included those who were ART-naïve, and those on either first-line ART treatment (consisting of stavudine or zidovudine, with lamivudine and either efavirenz or nevirapine) or second-line ART treatment (consisting of zidovudine with didanosine, and ritonavir-boosted lopinavir). Patients were excluded from recruitment based on a list of predefined conditions (**Table 2**).

Table 2. Cross Sectional Study - recruitment exclusion criteria

	Exclusion criteria
1	On ART for less than 6 months
2	Showing signs of acute opportunistic infections
3	Experiencing severe diarrhoea (>6 stools per day)
4	Diagnosed with tuberculosis within one month of starting treatment
5	History of diabetes mellitus
6	Received glucocorticoid therapy within the last six months
7	Pregnancy
8	Renal failure

After the initial screening and enrolment into the study, participants were provided with an appointment date and instructed to fast the night before the appointment. On the day of the appointment, participants underwent a 75g oral glucose tolerance test (OGTT). Venous blood samples were taken at 0, 30 and 120 minutes. The samples were kept on ice and centrifuged within 4 hours, aliquoted and frozen at -20°C, then stored at -70°C until analysis at the end of the recruitment in 2009.

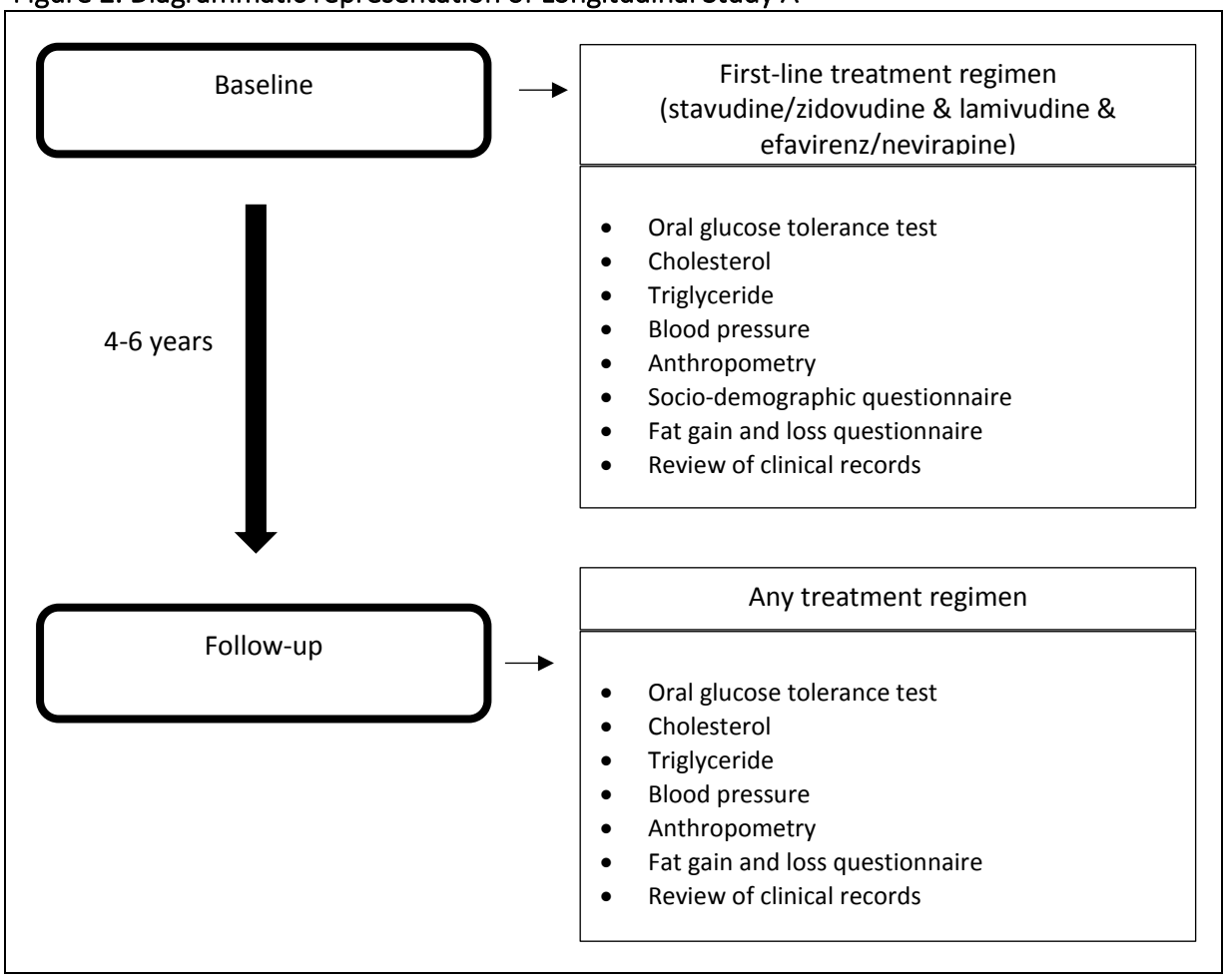
An interviewer administered questionnaire was used to collect socio-demographic information, while a second interviewer administered questionnaire collected information on fat gain and fat loss for all participants. Anthropometric measurements were taken by a single anthropometrist. Weight, height, circumferences (waist, hip, mid-upper arm, and mid-thigh), skinfold thickness (biceps, triceps, subscapular, abdomen, supra-iliac, thigh and calf) and sagittal abdominal diameter (SAD)] were measured. A sub-sample of this group had a dual-energy X-ray absorptiometry (DXA) scan to measure fat mass and fat free soft tissue mass.

Three blood pressure (BP) measurements were taken at 2-minute intervals using an Omron BP monitor with an appropriately sized cuff after the participant had been seated for 5 minutes. Patients' clinical records from the health facility were reviewed to obtain data on ART regimen, time on ART, viral load and CD count.

3.3 Longitudinal Study A

The first longitudinal study (**Figure 2**), followed participants on first-line treatment, who were enrolled in the Cross Sectional Study, for a period of 4-6 years.

Figure 2. Diagrammatic representation of Longitudinal Study A



Between 2011 and 2013, participants were contacted and procedures and risks explained before written, informed consent was obtained. A number of patients could not be located

as they had moved, changed contact details or had died. Patients who were able to be contacted, underwent an initial screening and were excluded from the follow-up study based on a set of predefined criteria (**Table 3**). The same measurements as described in the cross sectional study were used, and data collected using the same procedures and measurement instruments.

Table 3. Longitudinal Study A - recruitment exclusion criteria

	Exclusion criteria
1	Pregnancy
2	Non-adherence to medication
3	Showing signs of acute opportunistic infections
4	Experiencing severe diarrhoea (>6 stools per day)
5	Renal failure

3.4 Longitudinal Study B

For the second longitudinal study (**Figure 3**), newly diagnosed HIV-infected patients who were ART naïve when recruited, were followed for 24 months. Recruitment occurred between 2008 and 2010. Data were collected at baseline, 3 months, 6 months, 12 months, 18 months and 24 months. ART initiation occurred within one week of being diagnosed. In cases where initiation was delayed, participants were excluded from the study. Patients underwent an initial screening and were excluded from the study based on a set of predefined criteria (**Table 4**).

Figure 3: Diagrammatic representation of Longitudinal Study B

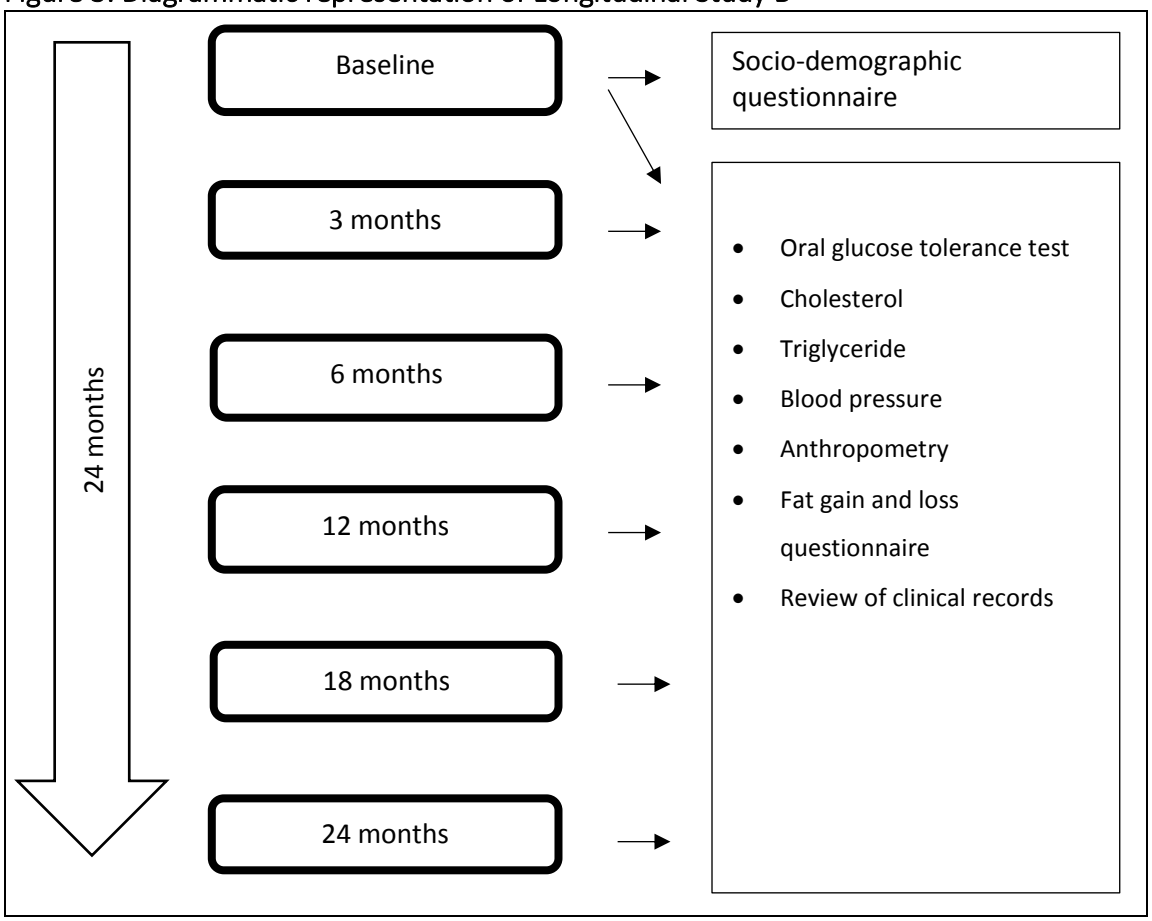


Table 4. Longitudinal Study B - recruitment exclusion criteria

	Exclusion criteria
1	Pregnancy
2	Showing signs of acute opportunistic infections
3	Experiencing severe diarrhoea (>6 stools per day)
4	Diagnosed with tuberculosis within one month of starting treatment
5	Renal failure

The same measurements as described in the cross sectional study were collected using the same procedures and measurement instruments at all time points.

Chapter 4

Changes in blood pressure, glucose levels, insulin secretion and anthropometry after long term exposure to antiretroviral therapy in South African women

Abrahams Z, Dave J, Maartens G, Levitt N. Changes in blood pressure, glucose levels, insulin secretion and anthropometry after long term exposure to antiretroviral therapy in South African women. AIDS Res Ther 2015;12:24. (**Appendix 1**)

4.1 Abstract

Background

A number of metabolic abnormalities, such as dysglycaemia, insulin resistance, lipodystrophy and dyslipidaemia, are associated with the use of antiretroviral drugs. We aimed to assess the effects of long-term antiretroviral exposure on blood pressure, glycaemia, insulin secretion and anthropometric measures in black South African women.

Methods

A convenience sample of HIV-infected women on first-line ART for a median of 16 months at baseline, had the following evaluations twice, at baseline and after approximately 5 years: anthropometry, including skin fold thicknesses, blood pressure, oral glucose test, and insulin. Insulin sensitivity and secretion (HOMA-IR, IGI and DI_o) were estimated.

Results

At baseline more than half the 103 women were using stavudine and efavirenz. The median interval between baseline and follow-up evaluation was 66 months. Weight, waist circumference, and waist-hip ratio increased over time, while limb skinfold thickness decreased over time. Systolic and diastolic blood pressure increased significantly and the proportion of participants with hypertension increased from 3.9% to 15.5% ($p<0.001$). There were increases from baseline in plasma glucose concentrations at 30 and 120 minutes; insulin concentrations at 0 and 30 minutes; and IGI and DI_o. The proportion of participants with diabetes increased from 1% to 7.5% ($p=0.070$).

Conclusion

In women with long-term exposure to ART, increases in hypertension and possibly diabetes were observed. Participants experienced an increase in central fat and a decrease in peripheral fat distribution. Early identification and management of these metabolic changes are important, especially in a region with the highest HIV-infected population in the world.

4.2 Introduction

Africa has made great strides in expanding access to antiretroviral therapy (ART), with an estimated 7.6 million people in sub-Saharan Africa receiving treatment by December 2012 [1]. The increase in access to ART has resulted in a dramatic decline in HIV-related deaths. However, several antiretroviral drugs are associated with a number of metabolic abnormalities [2] including dyslipidaemia, lipodystrophy, insulin resistance and dysglycaemia [3].

Several studies from Africa have shown an increased prevalence of dysglycaemia in HIV-infected patients, especially in patients on ART, but the duration of ART exposure was generally under 3 years [4-6]. There are conflicting data with regard to the impact of HIV and ART on hypertension, with some studies showing an increased risk of hypertension [7,8] and others showing no association [9-11]. A recent systematic review by Dillon *et al.* [12] found that HIV-infected patients in sub-Saharan Africa, irrespective of ART status, had lower systolic and diastolic blood pressure (BP) when compared to HIV-uninfected controls.

ART-related lipoatrophy is common in low- and middle-income countries [13-15], where stavudine has only recently been phased out and zidovudine is still widely being used. Studies using anthropometric measures show that fat loss is best detected by triceps skinfold and hip circumference measurements [13,16]. Central fat accumulation in patients on ART is thought to be a consequence of treating the HIV infection, as the gain in trunk and visceral fat is no different between HIV-infected participants on ART and HIV-uninfected controls, and does not differ by antiretroviral class [17].

Little is known about the long-term metabolic effects of ART in low- and middle-income countries. We aimed to assess the effects of long-term ART exposure on blood pressure, glycaemia, insulin secretion and anthropometric measures in black South African women.

4.3 Methods

Participants

In our initial cross sectional study, undertaken in 2007-2009 to examine the metabolic consequences of ART, a convenience sample of 345 HIV-infected black African women on first-line ART who were being followed up at ART clinics in Cape Town were selected. The recruitment procedure is described elsewhere [5]. At that stage the first-line ART regimen consisted of stavudine, lamivudine or zidovudine and efavirenz or nevirapine, and the second-line ART regimen consisted of zidovudine with didanosine and lopinavir/ritonavir [18]. Subsequently, the first-line regimen was changed to tenofovir and efavirenz or nevirapine, and lamivudine replaced didanosine in the second-line regimen. NRTI drug substitutions for toxicity or the convenience of a fixed dose combination are not considered switches to 2nd line ART. 103 of the initial 345 participants could be traced approximately 5 years later and underwent repeated assessments. The remainder could not be traced using their home address or telephone number and were no longer attending the health facility from which they were recruited; defaulted, were pregnant or had died. The baseline characteristics did not differ between those we traced and those not traced.

Testing procedures

We used the same procedures to collect information from participants at baseline and follow-up. Socio-demographic information was collected using an interviewer administered questionnaire. Clinical records were obtained from health facilities and reviewed to obtain data on ART regimen, time on ART and CD4 count. The Lipodystrophy Case Definition (LDCD) questionnaire [19] was used to collect self-reported information on fat gain or fat loss. Self-reported lipoatrophy was defined as in the HIV Outpatient Study (HOPS), as moderate or severe fat loss in 2 or more regions and self-reported lipohypertrophy defined as moderate or severe fat gain in two or more areas [20].

After an overnight fast, participants underwent a 75g oral glucose tolerance test (OGTT). Venous blood samples were taken prior to glucose ingestion, and after 30 and 120 minutes. The plasma was stored and analysed as previously described [5].

Anthropometric measurements: [weight, height, circumferences (waist, hip, mid-upper arm, and mid-thigh), skinfold thickness (biceps, triceps, subscapular, abdomen, supra-iliac, thigh and calf) and sagittal abdominal diameter (SAD)] were also done. Cut-point based lipoatrophy was defined as having a thigh skinfold thickness ≤28 mm or a triceps skinfold thickness of ≤14.5 mm [13]. Three BP measurements were taken at two-minute intervals using an Omron BP monitor with an appropriately sized cuff after the participant had been seated for five minutes. The average of the second and third BP measurements was used in the analyses. Hypertension was defined as BP ≥140/90 mmHg or using antihypertensive agents. Diabetes, IGT and IFG were defined using the American Diabetes Association criteria [21].

Ethical Approval

The study proposal was submitted and approved by the Research Ethics Committee of the Faculty of Health Sciences at the University of Cape Town. Written informed consent was obtained from all participants prior to participation in the study.

Data Analyses

Data analysis was carried out using the STATA/SE statistical software package version 12.0 (StataCorp., College Station, TX, USA). Baseline data were collected between February 2007 and June 2009 and follow-up data between July 2011 and July 2013. Because the data were not normally distributed, continuous variables were described as medians and inter-quartile ranges (IQR), and were compared using a non-parametric paired t-test. Binary variables were described using numbers and percentages, and compared using the McNemar chi-square test for paired data.

Markers of beta cell function and insulin resistance were estimated in the participants who did not have diabetes at follow-up. Beta-cell function was estimated using (1) IGI, calculated

as the ratio of the change in insulin to the change in glucose from 0 to 30 min ($_{\Delta Insulin0-30}/\Delta Glucose_{0-30}$), and (2) DI_o, calculated as a ($\Delta Insulin_{0-30}/\Delta Glucose_{0-30}$) x (1/fasting insulin). Insulin resistance was estimated using HOMA-IR, calculated as (fasting glucose x fasting insulin)/22.5.

4.4 Results

Participant characteristics are presented in **Table 1**. We enrolled 103 of the 345 women assessed at baseline for follow-up assessment. At baseline the participants had spent a median of 16 months on first-line ART and almost all were using stavudine (91%) and lamivudine (100%). At follow-up 84% were still on first-line ART but the percentage of those using stavudine had decreased to 39%, and those using zidovudine had increased from 10% to 38%. The median time on ART at follow-up was 82 months (6.8 years).

As shown in **Table 2**, waist circumferences increased significantly (p=0.038), while hip and mid-thigh circumferences decreased (p<0.001). All skinfold thicknesses changed significantly from baseline to follow-up. All 25% of the participants who reported having lipoatrophy at baseline, reported none at follow-up. However, when using objective measures [13] based on thigh and triceps cut-points, the percentage of participants with lipoatrophy increased from 44% to 64%; p=0.010. At baseline, 60% of those who self-reported moderate or severe fat loss in 2 or more regions, were correctly classified using thigh and triceps skinfold cut-points, while 61.5% of those who reported having lost none, or minimal amounts of fat were correctly classified (p=0.002).

Table 1. Comparison of baseline and follow-up characteristics of female participants

	Baseline Median [IQR] n=84	**Follow-up** Median [IQR] n=77
Age	33.5 [30.0-40.0]	40.1 [35.7-45.4]
Current CD4 count	3723 [261-471]	564 [427-774]
Time on ART (months)	16.0 [10.0-26.0]	82.4 [73.8-94.1]
	n[%]	**n[%]**
Education		
No schooling	5 [4.8]	
Primary School	14 [13.6]	
Secondary School	83 [80.6]	
Tertiary	1 [1.0]	
Drug Regimen		
1st line ART	84 [100]	65 [84.4]
2nd line ART	0 [0]	12 [15.6]
ART		
Stavudine	76 [90.5]	30 [39.0]
Lamivudine	84 [100]	77 [100]
Zidovudine	8 [9.5]	29 [37.7]
Tenofovir	0 [0.0]	18 [23.4]
Lopinavir	0 [0.0]	12 [15.6]
Efavirenz	41 [48.8]	36 [46.8]
Nevirapine	43 [51.2]	29 [37.7]

Both systolic and diastolic blood pressures increased ($p<0.001$) from baseline to follow-up (**Table 3**). Plasma glucose concentrations at 30 and 120 minutes, and insulin concentrations at 0 and 30 minutes also increased significantly from baseline to follow-up ($p<0.050$). Although the homeostatic assessment model (HOMA-IR) tended to increase ($p=0.089$) from baseline to follow-up, both the insulinogenic index (IGI) and the oral disposition index (DI_o) increased significantly ($p<0.001$).

Table 2. Comparison of anthropometric measures in females at baseline and follow-up

	Baseline Median [IQR] *n=103*	**Follow-up Median** Median [IQR] *n=94*	P-value*
Height (m)	1.6 [1.5-1.6]	1.6 [1.5-1.6]	0.401
Weight (kg)	69.2 [61.4-81.1]	70.1 [59.7-78.8]	0.402
BMI	27.9 [24.8-31.8]	27.8 [23.9-31.6]	0.443
Sagittal height (cm)	21 [19-24]	20.6 [18.5-23.5]	0.640
Circumferences			
Waist (cm)	89.8 [81.3-96.8]	90.8 [82.5-100.0]	**0.038**
Hip (cm)	103.0 [96.0-114.0]	100.0 [91.8-106.5]	**<0.001**
Waist-hip ratio	0.86 [0.81-0.92]	0.92 [0.85-0.98]	**<0.001**
Mid-upper arm (cm)	29.0 [27.0-32.0]	29.5 [26.8-32.5]	0.292
Mid-thigh (cm)	58.0 [53.0-63.0]	55.0 [49.5-59.5]	**<0.001**
Skinfold thickness			
Biceps (mm)	8.1 [5.8-10.5]	9.0 [6.4-12.6]	**0.011**
Triceps (mm)	19.0 [12.6-25.2]	16.3 [11.3-22.5]	**0.007**
Abdomen (mm)	25.1 [16.9-34.2]	32.2 [21.7-37.8]	**<0.001**
Thigh (mm)	32.8 [24.1-43.3]	24.3 [17.3-34.0]	**<0.001**
Sub-Scapular (mm)	21.5 [13.4-28.8]	29.0 [18.8-34.2]	**<0.001**
Supra-iliac (mm)	16.1 [9.9-22.4]	20.5 [13.3-28.9]	**<0.001**
Calf (mm)	17.8 [12.3-24.4]	13.1 [7.0-19.2]	**<0.001**
	n[%]	**n[%]**	**P-value****
Lipoatrophy			
Based on Patient Report	25 [24.5]	0	**<0.001**
Based on thigh (≤28 mm) and triceps (≤14.5 mm) skinfold cut-points	45 [43.7]	59 [64.1]	**0.010**

*non-parametric paired t-test
**McNemar Chi-square test for paired data

The proportion of participants with hypertension increased from baseline to follow-up, from 3.9% to 15.5%; p<0.001 (**Table 4**). While the proportion of participants with impaired glucose tolerance (IGT), impaired fasting glucose (IFG) and dysglycaemia did not change significantly from baseline to follow-up, there was a trend to an increase in the proportion of participants with diabetes (1% to 7.5%; p=0.070). At baseline and follow-up, diabetes, hypertension and dysglycaemia were significantly associated with lipoatrophy (p<0.001) based on thigh and triceps skinfold cut-points.

Table 3. Comparison of blood pressure, plasma glucose and insulin concentrations and markers of insulin sensitivity and beta cell function at baseline and follow-up (n=103)

	Baseline Median [IQR]	**Follow-up** Median [IQR]	P-value*
Blood Pressure			
Systolic	111 [101-121]	121 [112-133]	**<0.001**
Diastolic	72 [64-80]	80 [73-89]	**<0.001**
Glucose			
Fasting	5.1 [4.7-5.4]	4.9 [4.7-5.3]	0.365
30 mins	6.6 [5.8-7.4]	6.8 [5.9-8.0]	**0.040**
120 mins	5.4 [4.9-6.3]	5.6 [4.7-6.8]	**0.028**
Insulin			
Fasting	5.6 [3.3-9.5]	7.9 [4.1-12.9]	**0.009**
30 mins	35.1 [19.2-66.7]	177.7 [163.4-192.5]	**<0.001**
120 mins	24.0 [13.5-40.2]	23.7 [10.4-54.9]	0.993
Glycaemic parameters (without diabetics and outliers)			
HOMA-IR**	1.2 [0.7-2.2]	1.6 [0.9-2.7]	0.089
IGI***	23.7 [11.9-33.1]	80.0 [54.0-137.4]	**<0.001**
DI_o****	3.5 [2.3-7.9]	11.9 [4.9-23.3]	**<0.001**

*non-parametric paired t-test
**HOMA-IR= (fasting glucose x fasting insulin)/22.5
***IGI= $\Delta Insulin_{0\text{-}30}/\Delta Glucose_{0\text{-}30}$
****DI_o [Oral disposition index] = ($\Delta Insulin_{0\text{-}30}/\Delta Glucose_{0\text{-}30)}$ x (1/fasting insulin)

Table 4. Comparison of blood pressure and glucose abnormalities in females at baseline and follow-up (n=103)

	Baseline n[%]	**Follow-up** n[%]	P-value*
Hypertension	3 [3.9]	16 [15.5]	**<0.001**
Glucose abnormalities**			
Diabetes	1 [1.0]	7 [7.5]	0.070
Impaired Glucose Tolerance	6 [5.8]	9 [9.6]	0.344
Impaired fasting Glucose	17 [16.5]	10 [10.5]	0.308
Dysglycaemia	22 [21.4]	19 [20]	1.00

*McNemar Chi-square test for paired data
** n=94 at follow-up

Stavudine, efavirenz and nevirapine were significantly associated with diabetes, hypertension and dysglycaemia at follow-up (**Table 5**). Lipoatrophy was significantly associated (p<0.001) with zidovudine, tenofovir, lopinavir in addition to stavudine, efavirenz and nevirapine.

Table 5. P-values* representing associations between diabetes, hypertension, dysglycaemia and lipoatrophy, and different antiretroviral drugs at follow-up

	Diabetes	Hypertension	Dysglycaemia	Lipoatrophy**
Stavudine	**0.001**	**0.017**	**0.029**	**<0.001**
Zidovudine	**0.031**	1	0.845	**<0.001**
Tenofovir	**0.004**	0.690	0.856	**<0.001**
Lopinavir	0.077	0.664	0.690	**<0.001**
Efavirenz	**0.001**	**<0.001**	**<0.001**	**<0.001**
Nevirapine	**<0.001**	**0.029**	**0.029**	**<0.001**

*McNemar Chi-square test for paired data
**Defined by thigh and triceps skinfold cut-points

4.5 Discussion

Our results show that long term exposure to ART in South African women is associated with increases in blood pressure, glucose and insulin levels. These women also experienced changes in body composition with a significant increase in the waist-hip ratio, and in the prevalence of lipoatrophy when objective anthropometric measures (thigh and triceps skinfold cut-points [13]) were used instead of the subjective measure of patient report. These metabolic and body composition changes are all associated with an increased cardiovascular risk [22].

The prevalence of hypertension at baseline in our study was 3-fold lower than in women of a similar age-group from a similar area in Cape Town who participated in a community-based cardiovascular risk factor study (CRIBSA). At follow-up the prevalence of hypertension was lower when compared to similarly aged women from the CRIBSA Study. Although HIV testing

was not performed in the CRIBSA Study, participants were not known to be on ART and based on local data, the HIV-infected proportion was estimated to be about 10% [23]. Another study from rural KwaZulu Natal, South Africa [24] reported a 20% prevalence of hypertension in HIV-infected women compared to 40% in HIV-uninfected women 15 years and older. The lower BMI in people on ART compared to the HIV negative participants may be an explanation for the lower prevalence of hypertension in both of these South African studies. In contrast, studies from Tanzania [7] and Uganda [7,25] have reported a similar prevalence of hypertension in HIV-infected and HIV-uninfected participants, with those on ART in Tanzania having a higher BMI than those who were HIV-uninfected or HIV-infected and ART-naive.

Although the prevalence of new onset diabetes increased between baseline and follow-up in our study, this did not reach statistical significance, possibly due to the small sample size. Interestingly, the prevalence of new onset diabetes at follow-up, was 2-fold higher in our study than the prevalence of new onset diabetes in women of similar ages from the CRIBSA study [23]. The different methods used to assess dysglycaemia makes it difficult to compare studies from Africa. However, a study [26] that also used an OGTT to assess dysglycaemia found a similar prevalence of dysglycaemia, although the length of time on ART was longer in our study (81 months vs. 48 weeks). The rise in insulin secretion in relation to insulin resistance, as expressed by the DI_0, in the majority of the group at follow-up explains their lack of development of diabetes.

Although there was no increase in BMI at follow-up, the greater abdominal skinfold thickness and waist-hip ratio together with peripheral wasting suggests a marked difference in body composition with centralisation of body fat, in agreement with a number of other African studies [5,13,16,27]. HIV-associated central fat accumulation likely reflects the consequence of treating the HIV infection rather than a specific antiretroviral adverse drug reaction [17]. We found an increase in the percentage of participants with lipoatrophy when defined by thigh and triceps skinfold cut-points [13]. However, when using patient report to diagnose lipoatrophy, no women had lipoatrophy, including the 25% who reported lipoatrophy at baseline. The discrepancy we found in the proportion of women with lipoatrophy on anthropometry and patient report is likely due to the women having grown accustomed to

their new body shape and illustrates the limitations of diagnosing lipodystrophy using a subjective measure. Lipoatrophy is an antiretroviral adverse drug reaction, strongly associated with the use of thymidine analogue nucleoside reverse transcriptase inhibitors (NRTIs), stavudine and zidovudine [17]. In our study more than 30% of women were still on stavudine at follow-up and almost 20% were still taking zidovudine. The baseline prevalence of lipoatrophy we found is also similar to that of another South African study [16], which reported a 43% prevalence after two years of treatment, but they defined lipoatrophy only by subjective patient and healthcare worker reports.

Our study has some limitations. The lack of HIV-uninfected and ART-naïve control groups limits our ability to attribute the changes observed to the use of ART. The sample size was also relatively small, which limited our ability to assess whether the increased prevalence of diabetes over time was significant. Despite these limitations, ours is one of very few studies in Africa to use an OGTT to define dysglycaemia and to follow women on ART for over 5 years.

4.6 Conclusion

In this study from Africa we showed that long-term use of ART results in an increase in blood pressure (systolic and diastolic), the prevalence of hypertension and possibly diabetes. The greatest changes observed were in body composition, with an increase in central fat and a decrease in subcutaneous fat. The prevalence of lipoatrophy, when defined by skinfold cut-points, increased substantially. These findings have important implications for the management of HIV in Africa. The early identification and management of these cardiometabolic risks are crucial in the region with the highest HIV-infected population in the world.

4.7 Acknowledgements

Authors' Contributions

ZA captured and prepared the data, conducted all statistical analyses, interpreted the findings and drafted the manuscript; NL, GM and JD designed and conducted the study; NL, GM and JD edited the manuscript and drafted revisions. ZA reviewed co-author comments and suggestions and integrated them into the manuscript as appropriate. All authors read and approved the manuscript.

Financial competing interests

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Chapter 5

Changes in body fat distribution on dual-energy x-ray absorptiometry in black South Africans starting first-line antiretroviral therapy

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5.1 Abstract

Background

Long term use of antiretroviral therapy (ART) increases the risk of developing lipodystrophy. No studies from Africa have used longitudinal data to assess the development of lipoatrophy and lipohypertrophy. We use clinical anthropometry and dual-energy X-ray absorptiometry (DXA) to describe changes in body fat distribution over a 24 month period in individuals initiated on ART.

Methods

A convenience sample of black South African men and women were recruited from Community Healthcare Centres and followed for 24 months after commencing ART. Body fat distribution was assessed using anthropometric measurements and DXA scans at baseline and then at 3 months, 6 months, 12 months, 18 months, and 24 months after commencing ART. DXA was also used to estimate abdominal visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT). Clinical records were reviewed to obtain information on ART regimens, viral loads and CD4 counts.

Results

Women gained more overall weight and more regional fat in all areas analysed on DXA scans. Women, not men, experienced a significant increasing trend in trunk fat and a significant decreasing trend in limb fat, when expressed as a percentage of total body fat. In men, the risk of developing lipoatrophy was 2.85 times greater than women, after adjusting for age, baseline BMI and ART regimen. Lipohypertrophy occurred similarly in men and women. VAT and SAT increased significantly in men and women, with women gaining considerably more than men.

Conclusion

Using clinical anthropometry and DXA we describe distinct differences in body fat distribution in men and women after initiating ART. These findings are of great concern as an increased waist circumference is associated with increased mortality in HIV-infected populations. Further investigation is required to understand the mechanisms underlying the sex differences in changes in body fat distribution and its effects on cardiovascular risk.

5.2 Introduction

Since the introduction of antiretroviral therapy (ART), HIV-related deaths have dramatically decreased [1]. However, the long-term use of ART increases the risk of metabolic complications such as lipodystrophy, insulin resistance, dyslipidaemia and lactic acidosis. Lipodystrophy, in the form of lipoatrophy and/or lipohypertrophy, occurs with differing frequency and severity in patients using ART. The need to differentiate between lipoatrophy and lipohypertrophy has become imperative following a recent meta-analysis showing that lipoatrophy, but not lipohypertrophy, is an antiretroviral adverse drug reaction [2]. Lipodystrophy especially lipoatrophy, has been commonly reported in studies from Africa when using stavudine and zidovudine [3-6].

Subjective methods such as patient report [4,7,8] and objective methods, such as dual energy X-ray absorptiometry (DXA) [9,10], computerised tomography (CT) and magnetic resonance imaging (MRI), have been used to detect lipoatrophy and lipohypertrophy [2]. However, few studies from Africa have distinguished between lipoatrophy and lipohypertrophy [3,4,11], and none have used changes in DXA measures. Longitudinal studies using DXA measures to define lipoatrophy and lipohypertrophy have frequently been done in high-income countries [10,12] and have shown that lipoatrophy occurs more frequently in patients using a stavudine-based ART regimen. However, subjects in these studies were mostly middle-age white men, demographically different to the majority of patients receiving ART in sub-Saharan Africa.

The aim of our study was to use DXA scans to describe the changes in fat distribution over a 24 month period in a population of black South African men and women initiated on ART and, to investigate the factors associated with these changes.

5.3 Methods

Participants

A convenience sample of HIV-infected men and women presenting at Crossroads Community Health Centre in Cape Town were enrolled in a 24 month longitudinal study. A sample size calculation based on a population of 500, 5% margin for error, and 95% confidence interval, indicated that a sample size of 184 participants was needed. ART initiation occurred after enrolment into the study and completion of baseline evaluations. During the course of the study there was a change in the nucleoside reverse transcriptase inhibitors (NRTIs) used in first-line ART regimens in South Africa, from stavudine or zidovudine to tenofovir, together with lamivudine, and efavirenz or nevirapine. The second-line regimen consisted of zidovudine, didanosine and lopinavir/ritonavir, which was changed to zidovudine, lamivudine and lopinavir/ritonavir at the same time as the tenofovir change [13].

Testing procedures

An interviewer administered questionnaire was used to collect socio-demographic information from participants at the beginning of the study. Anthropometry (weight and height) and dual-energy X-ray absorptiometry (DXA) was performed at baseline, 3 months, 6 months, 12 months, 18 months, and 24 months. DXA (Discovery-W®, software version 12.7.3.7; Hologic, Bedford, MA) was used to measure fat mass according to standard procedures. DXA cut-off lines positioned at anatomical markers were used to obtain fat mass for the whole body as well as for the various regions of interest (arms, legs and trunk). The trunk comprised the region between the neck and waist, excluding the arms. Limb composition was determined by summing the arms and legs. The percentages for regions were calculated as a proportion of the whole body fat mass. A more detailed description has been previously described [14]. Abdominal visceral adipose tissue (VAT) and subcutaneous

adipose tissue (SAT) areas were estimated by a trained analyst using image analysis software (SliceOmatic V4.2, TomoVision, Montreal, Quebec, Canada). The DXA measure of VAT has been shown to perform as well as VAT measured using computerized tomography scans [15]. Clinical records obtained from health facilities were reviewed to obtain information on ART regimens, viral loads and CD4 counts.

Ethical approval

The study proposal was approved by the Research Ethics Committee of the Faculty of Health Sciences at the University of Cape Town. Written informed consent was obtained from all participants prior to participation in the study.

Data analysis

Data analysis was carried out using the STATA/SE statistical software package version 12.0 (StataCorp., College Station, TX, USA). Baseline measurements were collected between November 2008 and November 2010. Because the data were not normally distributed, continuous variables were described as medians and inter-quartile ranges (IQR), and were compared using Wilcoxon Rank Sum tests. Binary variables were described using frequency and percentages, and compared using chi-square tests. The Jonckheere-Terpstra test for ordered variables was used to measure trends over time.

Lipoatrophy was defined as (1) ≥ 20% loss of limb fat (all limbs combined) from baseline [10] and, (2) ≥ 10% loss of limb fat (all limbs combined) from baseline by DXA scan [16]. Lipohypertrophy was defined as ≥20% gain in trunk fat from baseline by DXA scan [17,18]. Differences in time to lipoatrophy and lipohypertrophy between men and women were calculated using the Kaplan-Meier method. The log rank test was used to test differences between groups. Cox proportional hazards regression modelling was used to analyse the contribution of the following variables, selected *a priori*: age, gender, BMI, and individual NRTIs and non-nucleoside reverse transcriptase inhibitors (NNRTIs), to the development of lipoatrophy. The Cox proportional hazards assumption was not met for lipohypertrophy, therefore logistic regression modelling was used to analyse the same variables as used in the

lipoatrophy model. Latent class mixed effects models are used to estimate mean longitudinal trajectories in potentially heterogeneous populations, by assigning a model of latent factors that determines class membership. The number of classes were set *a priori* and class membership was estimated by posterior probabilities. Latent class mixed effects models were fit to longitudinal data for limb fat and trunk fat change independently, with the same set of covariates as prior regression models. Two and three class models were fit and the final model chosen by best Aikaike Information Criterion (AIC). Posterior probabilities were estimated and latent classes characterised by summary statistics.

5.4 Results

Baseline characteristics of participants are shown in **Table 1**. The study sample consisted of 55 (29.4%) men and 132 (70.6%) women who were ART naïve. Men were significantly older than women (35 vs. 31 years; p=0.008). Significantly more women than men were obese (20% vs. 4%; p=0.005). After baseline measurements were collected, 25 participants (13.3%) were excluded from further participation in the study as they either did not meet the eligibility criteria for ART initiation or their ART initiation had been delayed. The number lost-to-follow-up increased from 25 to 57 (30.5%) participants at 12 months and 84 participants (44%) at 24 months on ART.

More men (71%) than women (56%) were initiated on a stavudine-based regimen. After 12 months on ART the proportion of participants on stavudine and tenofovir was no different than at baseline. However, after 24 months on ART, the proportion of participants on a stavudine-based regimen had decreased and those on a tenofovir-based regimen had increased. Only 1 participant was exposed to a drug regimen containing a protease inhibitor. After 6 months on antiretroviral therapy 73% of participants were virally suppressed (VL<50).

Change in weight, BMI and regional DXA fat measures in men and women over the 24 month follow-up period can be found in **Figure 1**. In women, the total limb and trunk fat increased (P<0.001), while limb fat as a percentage of whole body fat decreased (P<0.001), primarily as a result of the decrease in leg fat (45.2-42.1%; p<0.001). Trunk fat in women increased from

7.6 kg to 11.7 kg (p<0.001). In men, none of the regional changes in fat distribution were significant other than trunk fat, which increased from 3.65 kg to 4.16 kg (p=0.027). An increase in the derived variables of abdominal SAT and VAT were observed in both men and women (p<0.05).

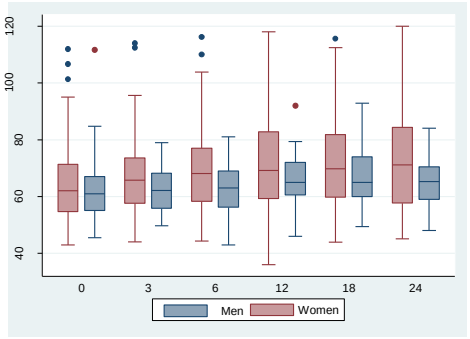
Table 1. Characteristics of participants at commencement of ART

	Total Median (IQR) n=187	**Women Median [IQR]** n=132	**Men Median [IQR]** n=55	**P-value**
Age (years)	33.0 [27.0-39.0]	31.0 [26.0-38.0]	35.0 [29.0-41.0]	**0.008**
CD4	156 [110-196]	161 [110-204]	137 [112-183]	0.126
	n [%]	**n [%]**	**n [%]**	
Smoker	60 [32.3]	24 [18.3]	36 [65.5]	**<0.001**
BMI status (kg/m^2)				
Underweight (BMI≤18.5)	10 [5.4]	4 [3.0]	6 [10.9]	**<0.001**
Normal weight (BMI 18.6-24.9)	108 [57.8]	66 [50.0]	42 [76.4]	
Overweight (BMI 25-29.9)	41 [21.9]	36 [27.3]	5 [9.1]	
Obese (BMI≥30)	28 [15.0]	26 [19.7]	2 [3.6]	
Antiretroviral drugs				
NRTI				
Stavudine	110 [60.8]	71 [56.4]	39 [70.9]	0.065
Zidovudine	20 [11.1]	19 [15.1]	1 [1.8]	**0.009**
Tenofovir	51 [28.2]	36 [28.6]	15 [27.3]	0.858
NNRTI				
Efavirenz	66 [36.5]	38 [30.2]	28 [50.9]	**0.008**
Nevirapine	115 [63.5]	88 [69.8]	27 [49.1]	**0.008**

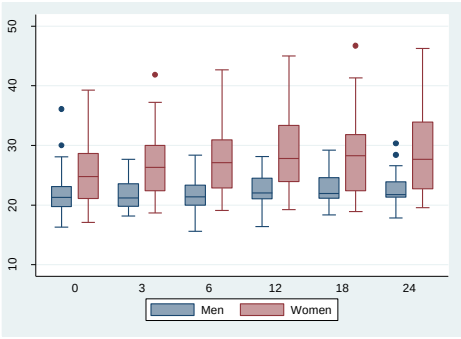
Kaplan-Meier plots of time to lipoatrophy and lipohypertrophy in men and women are shown in **Figure 2**. Lipoatrophy was observed in 20 of the 161 participants and occurred more frequently in men than in women (20% vs. 9.4%; p=0.066) but this difference was only significant when lipoatrophy was defined as ≥ 10% loss in limb fat (42.2% vs. 22.2%; p=0.011). Lipohypertrophy was observed in 90 of the 161 participants (55.9%), and was similar in men and women. Time to development of lipoatrophy and lipohypertrophy was not significantly different between men and women.

Figure 1. Body composition changes (weight, BMI, DXA measures) in men and women over 24 months

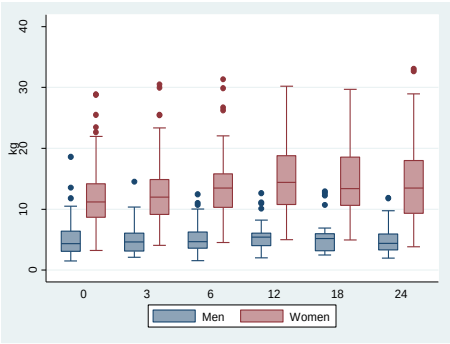
A: Weight (kg)
Men p=0.009; Women p<0.001



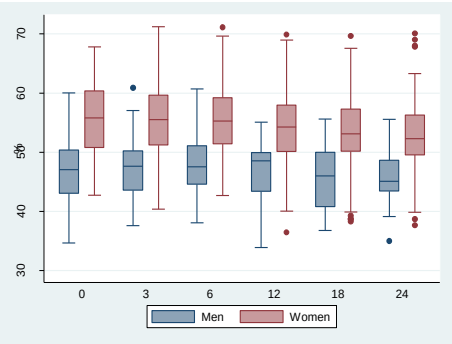
B: BMI
Men p=0.002; Women p<0.001



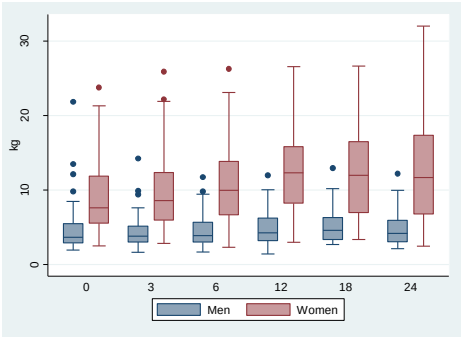
C: Limb fat (kg)
Men p=0.211; Women p<0.001



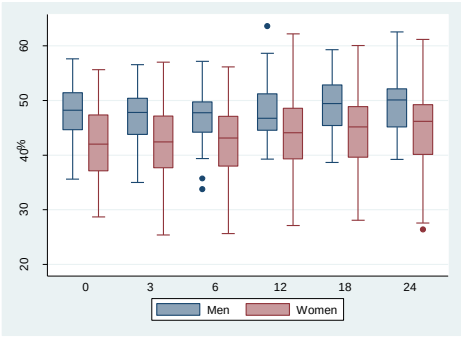
D: Limb fat (% of body fat)
Men p=0.248; Women p<0.001



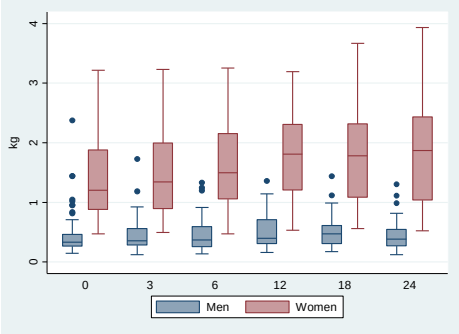
E: Trunk fat (kg)
Men p=0.027; Women p<0.001



F: Trunk fat (% of body fat)
Men p=0.085; Women p<0.001



G: Abdominal Subcutaneous Adipose Tissue (kg) Men p=O.049; Women p<0.001



H: Abdominal Visceral Adipose Tissue (kg)
Men p=0.032; Women p<0.001

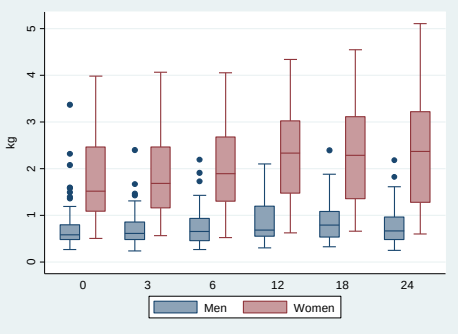
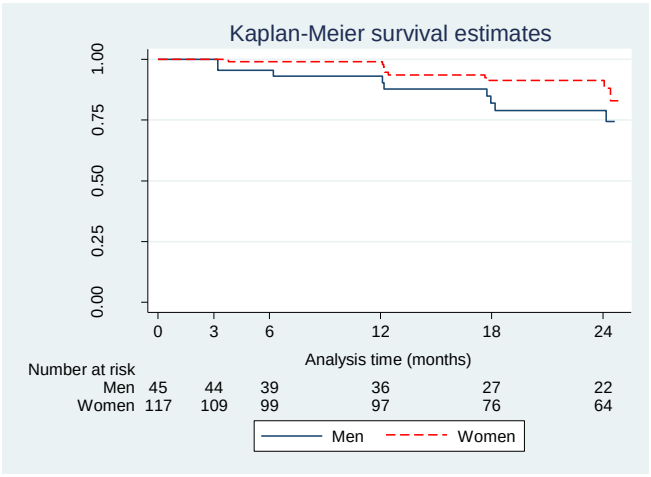
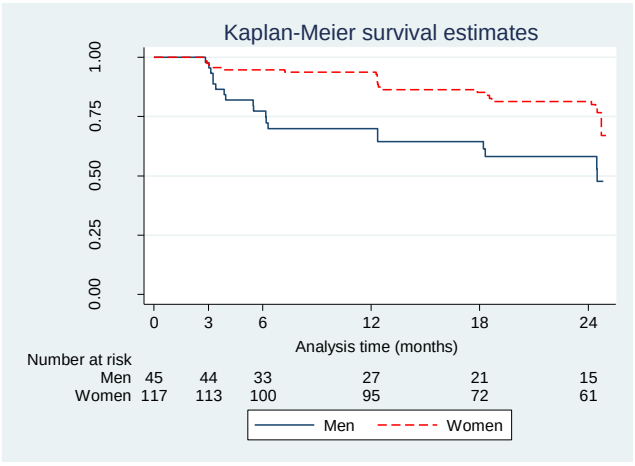


Figure 2. Kaplan Meier curves of time to lipoatrophy and lipohypertrophy in men and women

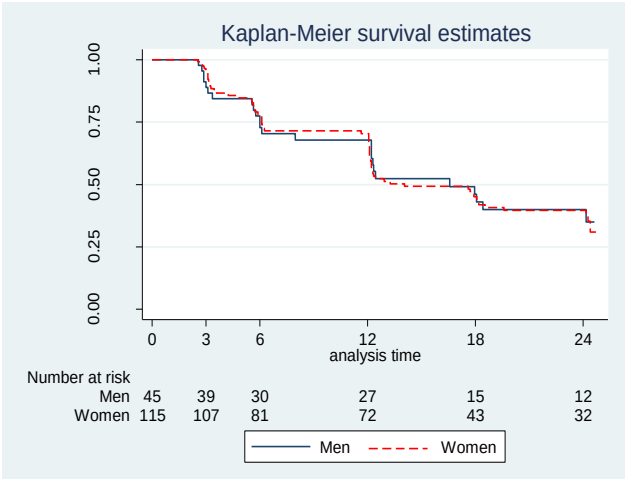
A: Lipoatrophy (limb fat loss ≥20%) P=0.089



B: Lipoatrophy (limb fat loss ≥10%) P=0.006



C: Lipohypertrophy (trunk fat gain ≥20%) P=0.992



Men have a greater than two times increased risk of developing lipoatrophy [HR=2.85 (0.96-8.47); p=0.060] than women (**Table 2**), after adjusting for age, baseline BMI and ART regimen. The risk of developing lipohypertrophy (**Table 3**) did not differ by gender but showed a large effect [OR=0.59 (0.23-1.52); p=0.271], though not statistically significant. None of the antiretroviral drugs were significantly associated with lipoatrophy or lipohypertrophy.

Table 2. Hazard Ratios for developing lipoatrophy using Cox regression modelling

Parameter	Univariate model		Full multivariate model	
	HR* (95% CI)	p-value	HR* (95% CI)	p-value
Age	0.98 (0.93-1.04)	0.505	0.97 (0.92-1.10)	0.352
Gender (ref. female)	2.11 (0.87-5.09)	0.097	2.85 (0.96-8.47)	0.060
BMI	0.97 (0.89-1.06)	0.462	1.03 (0.90-1.18)	0.665
NRTI1: AZT (ref. d4T)	0.81 (0.19-3.50)	0.774	0.67 (0.88-5.07)	0.697
NRTI2: TDF (ref. d4T)	0.55 (0.18-1.66)	0.287	0.50 (0.16-1.55)	0.227
NNRTI: EFV (ref. NVP)	0.87 (0.33-2.29)	0.778	0.81 (0.30-2.21)	0.686

*Hazard ratio; AZT-zidovudine; d4T-stavudine; TDF-tenofovir; EVF-efavirenz; NVP-nevirapine

Table 3. Odds ratios for developing lipohypertrophy using logistic regression modelling

Parameter	Univariate model		Full multivariate model	
	OR* (95% CI)	p-value	OR* (95% CI)	p-value
Age	0.99 (0.96-1.03)	0.842	1.02 (0.97-1.07)	0.335
Gender (ref. female)	0.98 (0.49-1.96)	0.956	0.59 (0.23-1.52)	0.271
BMI	0.91 (0.85-0.97)	0.004	0.92 (0.83-1.03)	0.156
NRTI1: AZT (ref. d4T)	0.57 (0.21-1.52)	0.259	1.50 (0.33-6.90)	0.605
NRTI2: TDF (ref. d4T)	0.63 (0.32-1.25)	0.186	0.72 (0.30-1.73)	0.467
NNRTI: EFV (ref. NVP)	1.17 (0.61-2.24)	0.644	0.76 (0.32-1.81)	0.537
Time on ART (months)	0.995 (0.994-0.997)	<0.001	0.99 (0.99-1.00)	**<0.001**

*Odds ratio; AZT-zidovudine; d4T-stavudine; TDF-tenofovir; EVF-efavirenz; NVP-nevirapine

Latent class modelling was used to show limb fat and trunk fat change over time for each individual (**Figure 3** and **Table 4**). The mean posterior of class membership in the limb fat model was class 1 = 0.91, class 2 = 0.89 and class 3 = 0.85. The mean posterior probability for class membership in the trunk fat model was class 1 = 0.91, class 2 = 0.94 and class 3 = 0.82. For both limb fat and trunk fat, class 2 showed an increase over time.

Figure 3. Limb fat (A) and trunk fat (B) longitudinal profiles for each individual where colour corresponds to estimate latent class membership based on posterior probabilities. Latent class labels (1, 2, 3) are arbitrary and merely reflect the group membership of each individual under a 3-group latent class model

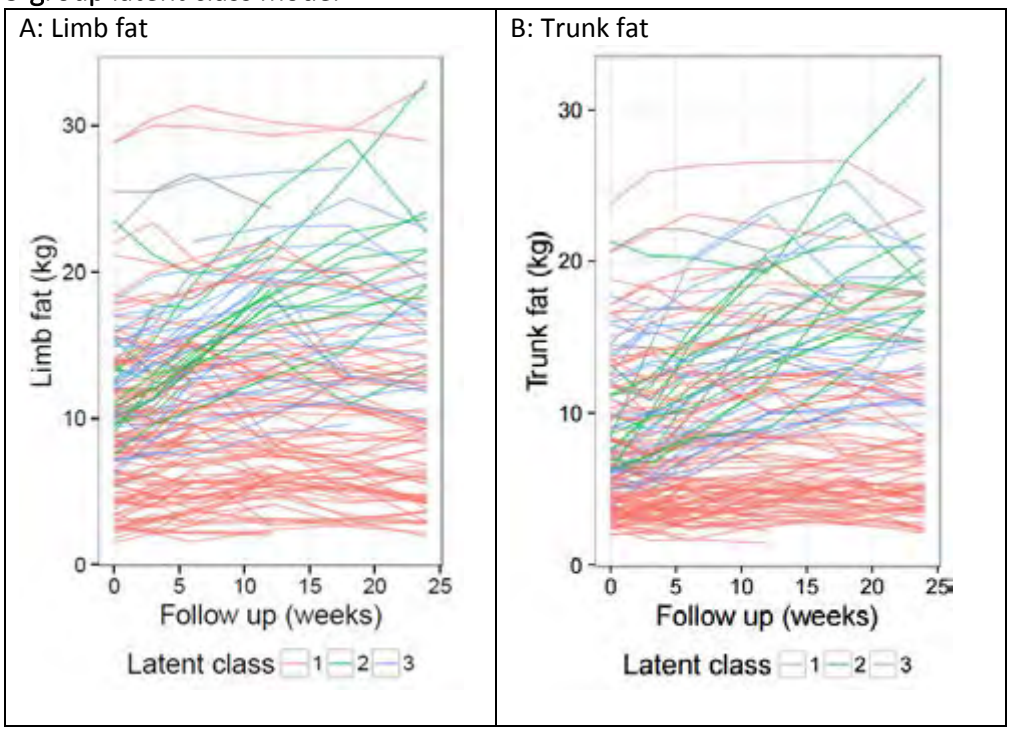


Table 4. Characteristics of classes as determined by latent class models for change in limb fat and trunk fat

Parameter	Class 1	Class 2	Class 3
Limb fat			
Number of individuals (n=174)	124	13	37
Baseline age (years)	33	30	31
Women (n [%])	76 [61.3]	13 [100]	34 [91.9]
Baseline weight (kg)	59.1	63.0	73.5
Baseline BMI (kg/m^2)	21.6	25.0	28.6
Trunk fat			
Number of individuals (n=174)	131	14	29
Baseline age (years)	33	31	34
Women (n [%])	82 [62.6]	14 [100]	27 [93.1]
Baseline weight (kg)	60.5	62.8	68.6
Baseline BMI	22.1	25.2	26.8

5.5 Discussion

This is the first longitudinal study from sub-Saharan Africa to document changes in body composition using DXA in patients starting ART. Following the initiation of ART we observed striking gender differences in weight gain, with women gaining more overall weight and more regional fat in all areas analysed on DXA scans. Women, but not men, had a significant decrease in limb fat expressed as a percentage of total body fat, and a significant increase in trunk fat percentage, which are markers of lipoatrophy and lipohypertrophy, respectively. The incidence of lipoatrophy expressed as a categorical variable was higher in men than women. The incidence of lipohypertrophy, when expressed as a categorical variable, occurred similarly in men and women. The derived variables, abdominal VAT and SAT, increased significantly from baseline in both men and women, with women gaining substantially more than men.

We found that men had a 2.85 times greater risk of developing lipoatrophy than women, and that lipoatrophy occurred more frequently in men. This data is supported by other studies [10,19,20], however, the data is conflicting as there are also studies showing the risk in women to be greater than in men [3,11], while others found the risk to be similar by gender [21]. The greater risk in men in our study may be due to their lower baseline BMI as well as having less body fat, especially in the limbs, compared to women. In addition, more men than women were using stavudine at baseline, even though the difference was not statistically significant. Epidemiological studies from South Africa show that in the general population, a smaller proportion of black men are overweight or obese compared to black women (38.5% vs. 64.8%) [22]. Our study showed that after 24 months on ART, 12% of all participants developed lipoatrophy when defined by ≥ 20% loss of limb fat, and this increased to 28% when defined as ≥ 10% limb fat loss. This is lower than the findings in a large multi-centre randomised control trial [10] that showed a 32% occurrence when defined by ≥ 20% limb fat loss and 40% when defined by ≥ 10% limb fat loss. The difference may be explained in part by the majority of male subjects in the multi-centre trial versus the majority of women in our study.

In 2003, the Lipodystrophy Italian Multicentre Study (LIMS) [21] reported different changes in fat distribution in men and women on ART, with women being more likely to develop fat accumulation around the abdomen. Similarly in 2009, McComsey *et al.* [23], found that women had a greater increase in trunk fat, SAT, VAT and TAT when compared to men.

A systematic review by De Waal *et al.* [2] found that lipohypertrophy was not an adverse drug reaction, but appeared rather to be a consequence of treating the HIV infection, which reduces the inflammatory response that causes wasting. Our finding that lipohypertrophy occurred with similar frequency in men and women irrespective of drug regimen is in keeping with the findings of the systematic review. By contrast, the systematic review found good evidence that lipoatrophy is an adverse drug reaction, due largely to thymidine analogue nucleoside reverse transcriptase inhibitors, especially stavudine. We showed that the incidence of lipoatrophy was 45% less with tenofovir than stavudine, in keeping with

numerous studies [3,24,25], but the difference was not statistically significant, likely due to our small sample size.

We also showed that over the 24 month period, weight and BMI increased significantly in both men and women. Interestingly, almost half of all women were either overweight or obese at the beginning of the study, when the criteria for ART eligibility was a CD4 count of <200 cells/mm^3. Furthermore, their median BMI increased from 24.8 kg/m^2 to 27.7 kg/m^2, and their median VAT increased by 190g during the two years. However, when using latent class analysis, we found a heterogeneous group within the sample, with the majority of individuals experiencing a longitudinal trajectory of limited, to no weight change over time. A minority of individuals, as supported by the other analyses, experienced longitudinal trajectories characterised by significant weight change, indicating that a minority of participants were responsible for the significant change in median weight and BMI. Our findings of increased weight and BMI mirror those of another South African study [26] showing that the BMI in HIV-infected women increased from 25 kg/m^2 to 27.4 kg/m^2 during the first year on ART. While the increase in weight and BMI may be due to a return to health in both men and women, a combination of mismatched weight perception and a cultural belief that underweight is associated with HIV infection may contribute to the increasing number of HIV-infected overweight and obese women. Studies on weight perception in HIV-infected women [26,27] reported a mismatch between actual weight and weight perception, with a tendency for women to judge themselves as less than their actual weight. In South Africa, HIV-infected women continue to experience discrimination, which may be the cause of intentional obesity as a mechanism of limiting the stigma [28]. These findings are of great concern as an increased waist circumference, and especially increase in VAT, is associated with increased mortality in HIV-infected populations [29].

Our study has some limitations. This was a non-randomised study, and there were no untreated or uninfected controls for comparison. The study sample was relatively small, with a high rate of loss to follow-up, resulting in a lack of power. There was minimal exposure to protease inhibitors, so we can only make inferences about first-line ART. Despite these limitations, this is the only study from Africa to use longitudinal DXA data.

5.6 Conclusion

In conclusion, we found that body fat changes differed in men and women on ART. Men, more frequently than women, developed lipoatrophy, while the risk of lipohypertrophy was no different in men and women. The percentage of trunk fat in women, but not men, increased significantly, with median BMI and VAT increasing substantially more in women than men. Further investigation is required to understand the mechanisms underlying the sex differences and its effects on cardiovascular risk.

5.7 Acknowledgements

Authors' Contributions

ZA conducted all statistical analyses, interpreted the findings and drafted the manuscript; JD, NL and GM designed and conducted the study; ML assisted with study design and data interpretation; NL, GM, JD and ML edited the manuscript and drafted revisions. All authors read and approved the manuscript.

Financial competing interests

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5.9 Supplementary Tables and Figures

Supplementary Figure 1. Trends for DXA measures in men and women

Supplementary Table 1. Comparison of anthropometric measures in men and women at baseline, 3, 6, 12, 18 and 24 months

Supplementary Table 2. Comparison of DXA measures in women at baseline, 3, 6, 12, 18 and 24 months

Supplementary Table 3. Comparison of DXA measures in men at baseline, 3, 6, 12, 18 and 24 months

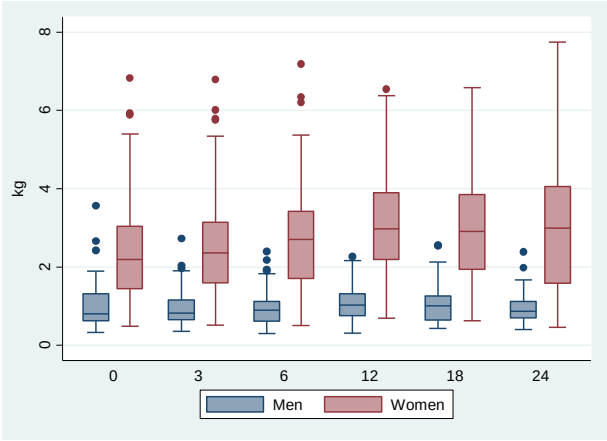
Supplementary Table 4. Comparison of ART in all participants with ≥ 20% fat loss in limbs

Supplementary Table 5. Comparison of ART in participants with ≥ 20% fat gain in trunk

Supplementary Figure 1. Trends for DXA measures in men and women

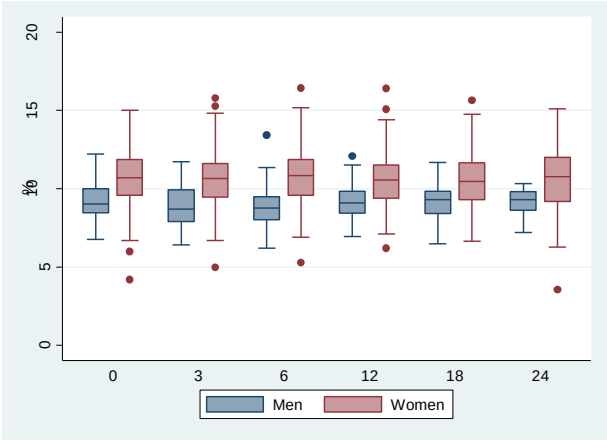
A: Arm fat (kg)

Men p=0.211; Women p<0.001



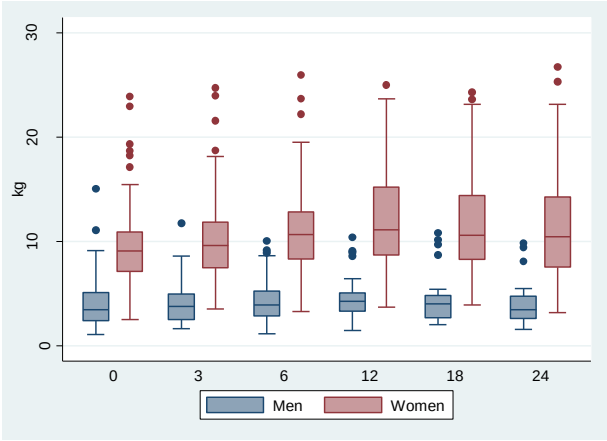
B: Arm fat (% of body fat)

Men p=0.737; Women p=0.673



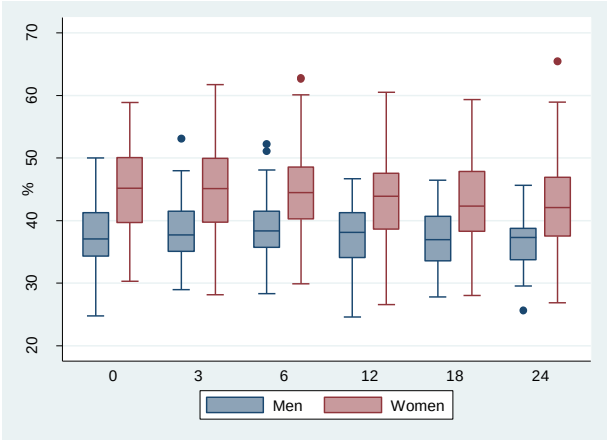
C: Leg fat (kg)

Men p=0.505; Women p<0.001



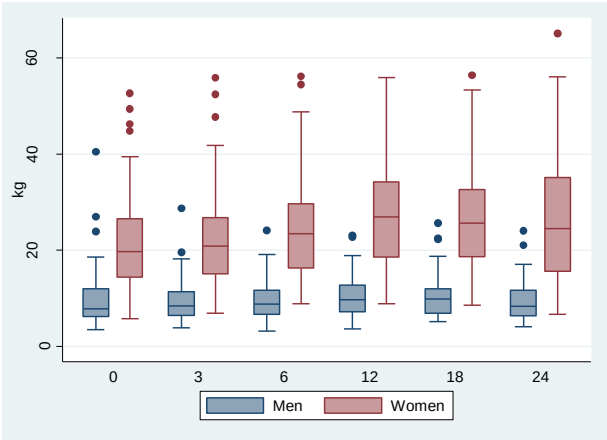
D: Leg fat (% of body fat)

Men p=0.340; Women p<0.001



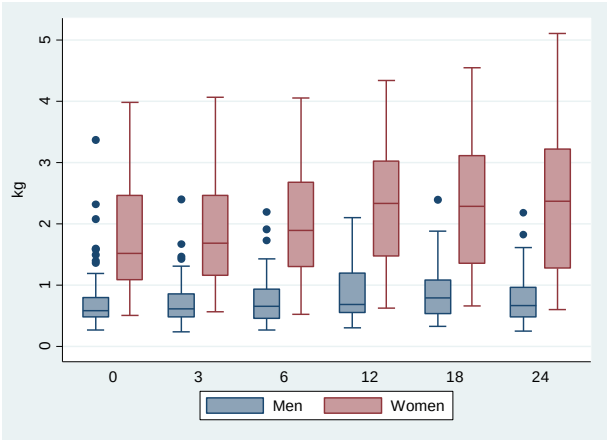
E: Body fat – excluding the head (kg)

Men p=0.085; Women p<0.001



F: Abdominal Total Adipose Tissue (kg)

Men p=0.039; Women p<0.001



Supplementary Table 1. Comparison of anthropometric measures in men and women at baseline, 3, 6, 12, 18 and 24 months

	Baseline Median [IQR]	**3 months Median [IQR]**	**6 months Median [IQR]**	**12 months Median [IQR]**	**18 months Median [IQR]**	**24 months Median [IQR]**	**P-value***
Women	*n=132*	*n=103*	*n=102*	*n=88*	*n=77*	*n=66*	
Height (m)	1.58 [1.55-1.62]	1.58 [1.54-1.62]	1.58 [1.55-1.62]	1.58 [1.55-1.62]	1.57 [1.54-1.62]	1.58 [1.55-1.63]	0.340
Weight (kg)	62.0 [54.7-71.3]	65.8 [57.5-73.6]	68.1 [58.2-77.0]	69.2 [59.3-82.8]	69.8 [59.8-81.8]	71.1 [57.6-84.4]	**<0.001**
BMI	24.8 [21.1-28.7]	26.3 [22.4-30.0]	27.1 [22.8-30.9]	27.8 [23.9-33.4]	28.3 [22.4-31.8]	27.7 [22.7-33.9]	**<0.001**
Men	*n=55*	*n=43*	*n=45*	*n=35*	*n=31*	*n=26*	
Height (m)	1.7 [1.7-1.8]	1.7 [1.7-1.7]	1.7 [1.7-1.8]	1.7 [1.7-1.8]	1.7 [1.7-1.7]	1.7 [1.7-1.7]	0.475
Weight (kg)	61.0 [55.0-67.0]	62.1 [55.8-68.2]	63 [56.2-69.0]	65.0 [60.6-72.0]	65.0 [60.0-74.0]	65.2 [59.0-70.5]	**0.009**
BMI	21.3 [19.7-23.1]	21.2 [19.7-23.6]	21.4 [20.0-23.3]	22.0 [21.0-24.5]	21.9 [21.2-24.6]	21.8 [21.3-23.9]	**0.003**

*Jonckheere-Terpstra test for ordered variables with an increasing trend

Supplementary Table 2. Comparison of DXA measures in women at baseline, 3, 6, 12, 18 and 24 months

	Baseline Median [IQR] *n=131*	**3 months Median [IQR]** *n=114*	**6 months Median [IQR]** *n=102*	**12 months Median [IQR]** *n=93*	**18 months Median [IQR]** *n=83*	**24 months Median [IQR]** *n=75*	**P-value***
Arms							
Fat (kg)	2.19 [1.44-3.04]	2.36 [1.60-3.14]	2.71 [1.71-3.42]	3.0 [2.19-3.90]	2.91 [1.93-3.85]	3.0 [1.58-4.06]	<0.001
Fat (%)	10.7 [9.6-11.9]	10.6 [9.5-11.6]	10.8 [9.5-11.9]	10.6 [9.4-11.5]	10.5 [9.3-11.6]	10.8 [9.2-12.0]	0.673
Legs							
Fat (kg)	9.08 [7.10-10.93]	9.60 [7.45-11.85]	10.66 [8.32-12.82]	11.12 [8.70-15.19]	10.602 [8.31-14.41]	10.47 [7.51-14.27]	<0.001
Fat (%)	45.2 [39.7-50.1]	45.1 [39.7-49.9]	44.5 [40.2-48.6]	43.9 [38.6-47.6]	42.3 [38.2-47.8]	42.1 [37.5-46.9]	<0.001**
Limbs							
Fat (kg)	11.20 [8.67-14.16]	11.98 [9.10-14.7]	13.47 [10.26-15.82]	14.40 [10.70-18.79]	13.39 [10.57-18.55]	13.49 [9.29-18.02]	<0.001
Fat (%)	55.8 [50.8-60.4]	55.5 [51.2-59.6]	55.3 [51.4-59.2]	54.3 [50.1-57.9]	53.1 [50.1-57.3]	52.3 [49.5-56.3]	<0.001**
Trunk							
Fat (kg)	7.61 [5.51-11.9]	8.57 [5.97-12.37]	9.95 [6.67-13.86]	12.32 [8.23-15.84]	11.99 [6.96-16.49]	11.69 [6.79-17.33]	<0.001
Fat (%)	42.0 [37.2-47.4]	42.4 [37.7-47.2]	43.1 [38.0-47.1]	44.1 [39.3-48.6]	45.2 [39.6-48.9]	46.2 [40.1-49.3]	<0.001
Whole body (excluding the head)							
Fat (kg)	19.70 [14.41-26.56]	20.84 [15.01-26.79]	23.42 [16.26-29.66]	26.92 [18.57-34.19]	25.64 [18.67-32.65]	24.52 [15.54-35.08]	<0.001
Abdominal Adipose Tissue							
Subcutaneous (SAT) (kg)	1.20 [0.88-1.88]	1.34 [0.90-1.99]	1.50 [1.05-2.15]	1.81 [1.21-2.31]	1.78 [1.09-2.32]	1.87 [1.03-2.43]	<0.001
Visceral (VAT) (kg)	0.32 [0.21-0.48]	0.34 [0.22-0.48]	0.38 [0.24-0.52]	0.46 [0.28-0.52]	0.50 [0.29-0.72]	0.51 [0.26-0.70]	<0.001
Total (TAT) (kg)	1.52 [1.09-2.46]	1.68 [1.15-1.47]	1.89 [1.30-2.68]	2.33 [1.48-3.03]	2.29 [1.35-3.11]	2.37 [1.27-3.22]	<0.001

*Jonckheere-Terpstra test for ordered variables with an increasing trend

** Jonckheere-Terpstra test for ordered variables with a decreasing trend

Supplementary Table 3. Comparison of DXA measures in men at baseline, 3, 6, 12, 18 and 24 months

	Baseline Median [IQR] *n=51*	**3 months Median [IQR]** *n=42*	**6 months Median [IQR]** *n=44*	**12 months Median [IQR]** *n=37*	**18 months Median [IQR]** *n=30*	**24 months Median [IQR]** *n=24*	**P-value***
Arms							
Fat (kg)	0.80 [0.61-1.31]	0.82 [0.64-1.15]	0.90 [0.61-1.11]	1.02 [0.75-1.32]	1.00 [0.63-1.26]	0.86 [0.69-1.12]	0.106
Fat (%)	9.0 [8.4-10.0]	8.7 [7.9-9.9]	8.8 [8.0-9.4]	9.1 [8.4-9.8]	9.3 [8.4-9.8]	9.3 [8.6-9.8]	0.368
Legs							
Fat (kg)	3.48 [2.37-5.09]	3.77 [2.5-4.95]	3.90 [2.88-5.24]	4.25 [3.32-5.08]	4.04 [2.67-4.84]	3.47 [2.59-4.74]	0.252
Fat (%)	37.0 [34.3-41.2]	37.7 [35.1-41.5]	38.3 [35.7-41.5]	38.1 [34.1-41.3]	37.0 [33.6-40.7]	37.3 [33.7-38.8]	0.830
Limbs							
Fat (kg)	4.32 [3.03-6.41]	4.60 [3.09-6.06]	4.65 [3.54-6.26]	5.41 [4.02-6.07]	5.18 [3.15-5.98]	4.37 [3.27-5.92]	0.211
Fat (%)	47.1 [43.0-50.4]	47.6 [43.6-50.2]	47.5 [44.6-51.1]	48.6 [43.4-49.9]	46.0 [40.8-50.0]	45.1 [43.4-48.7]	0.876
Trunk							
Fat (kg)	3.65 [2.87-5.49]	3.81 [3.01-5.15]	3.89 [2.99-5.66]	4.26 [3.23-6.25]	4.61 [3.37-6.32]	4.16 [3.07-5.94]	0.027
Fat (%)	48.2 [44.7-51.4]	47.8 [43.8-50.4]	47.8 [44.2-49.7]	46.8 [44.5-51.2]	49.4 [45.4-52.9]	50.1 [45.1-52.1]	0.085
Whole body (excluding the head)							
Fat (kg)	7.81 [6.21-12.01]	8.42 [6.46-11.37]	8.80 [6.66-11.72]	9.74 [7.24-12.72]	9.87 [6.92-11.99]	8.38 [6.34-11.69]	0.091
Adipose Tissue							
Subcutaneous (SAT) (kg)	0.33 [0.26-0.46]	0.36 [0.28-0.56]	0.37 [0.25-0.59]	0.40 [0.30-0.71]	0.47 [0.30-0.61]	0.38 [0.27-0.54]	0.049
Visceral (VAT) (kg)	0.25 [0.21-0.33]	0.25 [0.20-0.33]	0.27 [0.21-0.35]	0.27 [0.23-0.42]	0.31 [0.24-0.41]	0.29 [0.22-0.41]	0.032
Total (TAT) (kg)	0.58 [0.48-0.80]	0.61 [0.48-0.86]	0.65 [0.45-0.94]	0.68 [0.55-1.12]	0.79 [0.53-1.08]	0.67 [0.48-0.96]	0.039

*Jonckheere-Terpstra test for ordered variables with an increasing trend

Supplementary Table 4. Comparison of ART in all participants with ≥ 20% fat loss in limbs

	3 months n=2		6 months n=2		12 months n=6		18 months n=7		24 months n=6	
	n* [%]	**P-value**	**n* [%]**	**P-value**	**n* [%]**	**P-value**	**n* [%]**	**P-value**	**n* [%]**	**P-value**
D4T	2 [100]	**<0.001**	2 [100]	**<0.001**	4 [66.7]	**<0.001**	4 [57.1]	**<0.001**	4 [66.7]	**<0.001**
AZT	0	**0.001**	0	**0.002**	1 [16.7]	0.210	0	0.359	0	0.791
D4T + AZT	2 [100]	**<0.001**	2 [100]	**<0.001**	5 [83.3]	**<0.001**	4 [57.1]	**<0.001**	4 [66.7]	**<0.001**
TDF	0	**<0.001**	0	**<0.001**	1 [16.7]	**<0.001**	3 [42.8]	**<0.001**	2 [33.3]	**<0.001**
EFV	0	**<0.001**	1 [50.0]	**<0.001**	1 [16.7]	**<0.001**	4 [57.1]	**<0.001**	3 [50.0]	**<0.001**
NVP	2 [100]	**<0.001**	1 [50.0]	**<0.001**	5 [83.3]	**<0.001**	3 [42.8]	**<0.001**	3 [50.0]	**<0.001**

*****number of participants with ≥20% fat loss in the limbs; **chi-square test for paired data; d4T-stavudine; AZT-zidovudine; TDF-tenofovir; EFV-efavirenz; NVP-nevirapine

Supplementary Table 5. Comparison of ART in participants with ≥ 20% fat gain in trunk

	3 months n=22		6 months n=36		12 months n=49		18 months n=46		24 months n=27	
	n* [%]	**P-value****	**n* [%]**	**P-value****	**n* [%]**	**P-value****	**n* [%]**	**P-value****	**n* [%]**	**P-value****
D4T	16 [72.7]	**<0.001**	24 [66.7]	**<0.001**	30 [61.2]	0.119	27 [58.7]	1	15 [55.5]	0.215
AZT	1 [4.5]	0.405	1 [2.7]	**0.004**	5 [10.2]	**<0.001**	5 [10.9]	**<0.001**	3 [11.1]	**<0.001**
D4T + AZT	17 [77.3]	**<0.001**	25 [69.4]	**<0.001**	35 [71.4]	**0.001**	32 [69.6]	0.108	18 [66.7]	**0.009**
TDF	5 [22.7]	**0.006**	11 [39.6]	0.504	14 [28.6]	**0.040**	14 [30.4]	0.126	9 [33.3]	1
EFV	13 [59.1]	**<0.001**	18 [50.0]	0.085	17 [34.7]	0.126	17 [36.9]	**0.049**	10 [37.0]	0.860
NVP	9 [40.9]	**<0.001**	18 [50.0]	**<0.001**	32 [65.3]	**0.009**	29 [63.1]	0.079	17 [63.0]	**0.008**

*number of participants with ≥20% fat gain in the trunk; **chi-square test for paired data; d4T-stavudine; AZT-zidovudine; TDF-tenofovir; EFV-efavirenz; NVP-nevirapine

Chapter 6

The development of simple anthropometric measures to diagnose antiretroviral therapy-associated lipodystrophy in resource limited settings

Abrahams Z, Dave J, Maartens G, Lesosky M, Levitt N. The development of simple anthropometric measures to diagnose antiretroviral therapy-associated lipodystrophy in resource limited settings. AIDS Res Ther 2014;11:26. (Appendix 2)

6.1 Abstract

Background

Lipohypertrophy does not appear to be an adverse ART reaction while lipoatrophy is clearly associated with the use of stavudine and zidovudine. In low- and middle-income countries stavudine has only recently been phased out and zidovudine is still widely being used. Several case definitions have been developed to diagnose lipodystrophy, but none of them are generalizable to sub-Saharan Africa where black women have less visceral adipose tissue and more subcutaneous adipose tissue than white women. We aimed to develop a simple, objective measure to define lipoatrophy and lipohypertrophy by comparing patient report to anthropometric and dual-energy X-ray absorptiometry (DXA) -derived variables.

Methods

DXA and anthropometric measures were obtained in a cross sectional sample of black HIV-infected South African men (n=116) and women (n=434) on ART. Self-reported information on fat gain or fat loss was collected using a standard questionnaire. Receiver operating characteristic (ROC) curves were used to describe the performance of anthropometric and DXA-derived variables using patient reported lipoatrophy and lipohypertrophy as the reference standard.

Results

Lipoatrophy and lipohypertrophy were more common in women (25% and 33%, respectively) than in men (10% and 13%, respectively). There were insufficient numbers of men with DXA scans for meaningful analysis. The best predictors of lipoatrophy in women were the anthropometric variables triceps (AUC = 0.725) and thigh skinfold (AUC = 0.720) thickness; and the DXA-derived variables percentage lower limb fat (AUC = 0.705) and percentage lower limb fat/height (AUC = 0.713). The best predictors of lipohypertrophy in women were the anthropometric variable waist/hip ratio (AUC = 0.645) and the DXA-derived variable percentage trunk fat/percentage limb fat (AUC = 0.647).

Conclusions

We were able to develop simple, anthropometric measures for defining lipoatrophy and lipohypertrophy, using a sample of black HIV-infected South African women with DXA scans. This is of particular relevance in resource limited settings, where health professionals need simple and inexpensive methods of diagnosing patients with lipoatrophy and lipohypertrophy.

6.2 Introduction

Antiretroviral therapy (ART) has significantly reduced the morbidity and mortality of people infected with HIV [1], however, long-term use of ART has been associated with a number of metabolic complications such as dysglycaemia, insulin resistance, dyslipidaemia and lipodystrophy [2]. Lipodystrophy is characterized by either subcutaneous fat loss, which is most noticeable in the face, limbs, and buttocks (lipoatrophy), or fat accumulation (lipohypertrophy) seen in the abdomen, breast or posterior neck, or a combination of both [3,4].

Both subjective and objective methods have been used to diagnose lipodystrophy, resulting in a number of case definitions. The most widely used subjective methods of diagnosis are patient perception and report [5,6], physician examination and report [7], and physician confirmation of patient report [8-11]. Objective measures used are imaging by dual-energy X-ray absorptiometry (DXA) [6,12,13] and computed tomography (CT) scans [12,14]. These imaging measures are expensive and not widely available in resource limited settings. Anthropometric and DXA-derived variables have also been developed, in an attempt to provide standard measures of defining lipodystrophy [15-17]. Furthermore, criteria established to define lipodystrophy did not include data from any African country. These diagnostic criteria may not be generalizable to sub-Saharan Africans, as there are important ethnic differences in fat distribution, especially in black women who have less visceral adipose tissue and more subcutaneous adipose tissue than white women [18-20].

Lipohypertrophy does not appear to be an adverse ART reaction as participants on different ART drug regimens gained similar amounts of trunk fat over time [21]. Lipoatrophy, in contrast is clearly an adverse ART reaction. The use of stavudine and zidovudine is associated with subcutaneous fat loss and is partially reversed after changing to abacavir or tenofovir [21,22]. Lipoatrophy remains common in low- and middle-income countries where stavudine has only recently been phased out and zidovudine is still widely used [23]. However, even if lipohypertrophy is not associated with ART, lipodystrophy, and lipoatrophy in particular, is independently associated with an increased risk of vascular disease [24,25]. Therefore recognising lipodystrophy is important to identify patients at risk for vascular disease so that screening can be targeted for other vascular risk factors, while recognising lipoatrophy is important so that stavudine or zidovudine can be substituted.

The aim of our study was to develop a simple, objective measure to define lipoatrophy and lipohypertrophy by comparing patient report to anthropometric and DXA-derived variables in a sample of black South Africans on ART.

6.3 Methods

Participants

A convenience sample of HIV-infected black men and women presenting to ART clinics in Cape Town were selected. The recruitment procedure is described elsewhere [26]. The study sample comprised 116 men and 434 women on ART. At the time of the study two ART regimens were available to South Africans accessing primary health care facilities. The first-line ART regimen consisted of stavudine, lamivudine and efavirenz or nevirapine, and a second-line regimen consisting of zidovudine with lamivudine and lopinavir/ritonavir (LPV/r) [27].

Testing procedures

Questionnaires were used to collect socio-demographic information from participants. Their clinical records at the health facilities were reviewed to obtain data on ART regimen, time on ART, and CD4 count. Self-reported information on fat gain or fat loss was collected using a standard questionnaire [8]. Lipoatrophy was defined as moderate or severe fat loss in two or more regions and lipohypertrophy defined as moderate or severe fat gain in two or more areas [28].

Anthropometric measurements: [weight, height, circumferences (waist, hip, mid-upper arm, and mid-thigh), skinfold thickness (biceps, triceps, subscapular, abdomen, supra-iliac, thigh and calf) and sagittal abdominal diameter (SAD)] were taken and have previously been described [29]. DXA (Hologic Discovery-W, software version 12.7; scan region 195 x 65 cm2 and weight limit 160 kg) was used to measure fat mass and fat free soft tissue mass in a subsample of participants (women: n = 172; men: n = 53). DXA cut-off lines positioned at anatomical markers were used to obtain fat mass for the whole body as well as for the various regions of interest. A more detailed description has been previously described [26].

Ethical Approval

The study proposal was submitted and approved by the Research Ethics Committee of the Faculty of Health Sciences at the University of Cape Town. Written informed consent was obtained from all participants prior to participation in the study.

Data Analyses

Data analysis was carried out using the STATA/SE statistical software package version 12.0 (StataCorp., College Station, TX, USA). Data were collected between February 2007 and June 2009. Participants were categorised into those with and those without lipoatrophy. Continuous variables were described as medians and inter-quartile ranges (IQR), and were compared using Wilcoxon Rank Sum tests. Binary variables were described using chi-square tests.

Receiver operating characteristic (ROC) curves were used to describe the performance of a number of anthropometric and DXA-derived variables using patient reported lipoatrophy and lipohypertrophy as the reference standard. The area under the curve (AUC) was used to assess the diagnostic performance of each variable. In addition, sensitivity, specificity, likelihood ratios and predictive values were calculated for variables with the highest AUC at the optimum cut-points. Cut-point selection was based on positive likelihood ratios.

6.4 Results

Participant characteristics are presented in **Table 1**. The study sample consisted of 550 participants on ART. Based on patient report, 121 (22%) had lipoatrophy and 157 (29%) had lipohypertrophy. Both lipoatrophy and lipohypertrophy were significantly more common in women than in men ($p \leq 0.001$). Participants with lipoatrophy had spent a significantly longer period of time on ART (25 months vs. 17 months) and a longer time on stavudine (15.5 months vs. 13 months).

Anthropometric variables are shown separately for women and men (**Tables 2** and **3** respectively). In women, all median skinfold measurements, with the exception of sub-scapular skinfold thickness, were significantly lower in participants with lipoatrophy compared with those without lipoatrophy. Measurements for waist circumference, waist/hip ratio and supra-iliac skinfold thickness were significantly higher in women with lipohypertrophy compared with those without lipohypertrophy. There were no statistically significant differences in anthropometric variables in men with and without lipoatrophy (**Table 3**). Men with lipohypertrophy had a significantly ($P=0.008$) greater thigh circumference than those without (13.5 mm vs. 8.1 mm).

DXA-derived measures are shown for women only (**Table 4**), as there were insufficient numbers of men with DXA scans for meaningful analysis. Women with lipoatrophy as well as those with lipohypertrophy, had a significantly higher percentage trunk fat/lower limb fat and percentage trunk fat/total limb fat and significantly lower percentage lower limb fat/BMI.

Women with lipoatrophy had significantly less percentage limb fat while women with lipohypertrophy had significantly more percentage trunk fat.

Table 1. Characteristics of participants on ART

Variable	Lipoatrophy*		P-value**	Lipohypertrophy***		P-value**
	With Median [IQR] *n=121*	**Without** Median [IQR] *n=429*		**With** Median [IQR] *n=157*	**Without** Median [IQR] *n=393*	
Age	34 [30-42]	35 [30-41]	0.256	34 [29-41]	35 [30-41]	0.198
Current CD4 count	397 [249-539]	315 [218-481]	**0.023**	389 [248-548]	314 [220-481]	**0.015**
Time on ART (months)	25 [14-32]	17 [10-27]	**0.001**	20 [12-31]	17 [11-28]	0.080
Time on Stavudine (months)	15.5 [10-26]	13 [8-19]	**0.004**	13 [8-21]	13 [9-20]	0.961
	n [%]	**n [%]**		**n [%]**	**n [%]**	
Sex						
Men	12 [10.34]	104 [89.66]	**0.001**	15 [12.93]	101 [87.07]	**<0.001**
Women	109 [25.12]	325 [74.88]		142 [32.72]	292 [67.28]	
Highest standard passed						
No schooling	6 [18.8]	26 [81.3]	0.289	9 [28.13]	23 [71.88]	0.272
Primary school	14 [15.4]	77 [84.6]		20 [21.98]	71 [78.02]	
Secondary school	96 [23.4]	315 [76.6]		121 [29.44]	290 [70.56]	
Tertiary	5 [31.3]	11 [68.8]		7 [43.75]	9 [56.25]	
ART Regimen						
First-line	99 [22.30]	345 [77.70]	0.730	130 [29.28]	314 [70.72]	0.608
Second-line	22 [20.75]	84 [79.25]		27 [25.47]	79 [74.53]	

*based on patient report, those with moderate or severe fat loss in 2 or more regions

**Wilcoxon Rank-sum (Mann-Whitney) tests for continuous variables and chi-square tests for binary variables

***based on patient report, those with moderate or severe fat gain in 2 or more regions

Table 2. Anthropometric measurements of women on ART

Variable	Lipoatrophy*		P-value**	Lipohypertrophy***		P-value**
	With Median[IQR] n=106	Without Median [IQR] n=312		With Median [IQR] n=142	Without Median [IQR] n=292	
Height (m)	1.6 [1.5-1.6]	1.6 [1.5-1.6]	0.603	1.6 [1.6-1.6]	1.6 [1.5-1.6]	0.112
Weight (kg)	65.4 [56.0-74.5]	68.1 [58.9-80.9]	**0.019**	69.5 [60.7-80.4]	67.1 [57.6-79.3]	0.127
BMI	26.2 [23.8-29.3]	27.1 [24.1-31.6]	**0.036**	27.4[24.6-31.3]	26.65[23.9-31.2]	0.187
Sagittal Abdominal Diameter (cm)	20.5 [19.0-23.0]	20.0 [18.5-23.0]	0.552	21[19-24]	20[18-23]	**0.004**
Circumferences						
Waist (cm)	86.3 [79.5-96.3]	87.0 [78.5-97.0]	0.704	90.7[80.3-98.5]	86[78.4-95]	**0.003**
Hip (cm)	98.0 [92.0-104.0]	102.0 [95.0-	**<0.001**	100[95-110]	101[94-110]	0.824
Waist/hip ratio	0.90 [0.83-0.94]	0.85 [0.80-0.90]	**<0.001**	0.89[0.83-0.93]	0.84[0.79-0.90]	**<0.001**
Mid-upper arm (cm)	27.0 [25.0-29.0]	29.0 [26.5-32.0]	**<0.001**	28[26-32]	28[26-32]	0.789
Mid-thigh (cm)	54.5 [51.0-59.0]	57.0 [53.0-63.0]	**<0.001**	56[52-61]	57[52-63]	0.589
Skinfolds						
Bicep (mm)	6.0 [4.4-8.4]	8.1 [5.7-11.7]	**<0.001**	7.1[5.2-10.7]	8[5.4-11.45]	0.189
Triceps (mm)	13.3 [9.5-17.4]	18.4 [13.6-26.0]	**<0.001**	16.2[12-22]	18.3[13-25.15]	0.059
Abdomen (mm)	20.4 [11.9-30.0]	24.4 [16.8-35.3]	**0.001**	24.1[15.9-35.8]	23.6[15.0-31.2]	0.210
Thigh (mm)	23.8 [15.4-32.8]	34.7 [24.2-44.7]	**<0.001**	29.8[20.5-42.5]	32.0[22.5-43.5]	0.359
Sub-Scapular (mm)	16.9 [12.5-23.0]	19.2 [12.2-28.8]	0.058	20[14.8-29.4]	18.5[11.7-27.1]	0.050
Supra-iliac (mm)	13.2 [7.7-18.7]	15.1 [8.8-22.8]	**0.033**	16.6[9.9-24.0]	13.9[8.2-21.7]	**0.022**
Calf (mm)	10.8 [8.0-17.5]	17.5 [12.2-24.2]	**<0.001**	15.6[9.2-21.3]	17.3[11.4-23.6]	0.061

*based on patient report, those with moderate or severe fat loss in 2 or more regions

**Wilcoxon Rank-sum (Mann-Whitney) tests for continuous variables and chi-square tests for binary variables

***based on patient report, those with moderate or severe fat gain in 2 or more regions

Table 3. Anthropometric measurements of men on ART

Variable	Lipoatrophy*		P-value**	Lipohypertrophy***		P-value**
	With	Without		With	Without	
	Median [IQR]	Median [IQR]		Median [IQR]	Median [IQR]	
	n=12	n=104		n=15	n=101	
Height (m)	1.7 [1.6-1.7]	1.7 [1.6-1.7]	0.942	1.7 [1.6-1.8]	1.7 [1.7-1.7]	0.954
Weight (kg)	65.5 [59.7-68.6]	63.5 [57.0-74.4]	0.895	67.4 [57.8-78.3]	62.7 [57.3-73.4]	0.408
BMI	23.3 [20.2-23.9]	22.4 [20.6-25.2]	0.772	23.6 [21.1-25.5]	22.3 [20.5-24.8]	0.324
Sagittal Abdominal Diameter (cm)	17.0 [16.0-19.5]	18.0 [17.0-20.0]	0.384	17.0 [16.0-22.0]	18.0 [17.0-20.0]	0.967
Circumferences						
Waist (cm)	81.75 [77-84.9]	80.4 [75.0-90.2]	0.953	83.8 [75.0-97.0]	79.8 [75.8-89.5]	0.338
Hip (cm)	90 [87-93.5]	90.0 [85.0-97.0]	0.768	91.0 [85.0-97.0]	90.0 [85.0-96.0]	0.556
Waist/hip ratio	0.89 [0.85-0.95]	0.91 [0.87-0.94]	0.740	0.93 [0.86-0.97]	0.90 [0.86-0.94]	0.325
Mid-upper arm (cm)	27.0 [24-28.5]	26.0 [24.0-29.0]	0.877	27.0 [25.0-29.0]	26.0 [24.0-29.0]	0.817
Mid-thigh (cm)	50.5 [43.5-52.5]	49.0 [46.0-54.0]	0.401	49.0 [46.0-56.0]	49.0 [46.0-54.0]	0.567
Skinfolds						
Bicep (mm)	3.9 [3.4-4.2]	3.5 [3.0-4.5]	0.329	4.1 [3.2-4.8]	3.5 [3.0-4.3]	0.180
Triceps (mm)	6.8 [5.6-8.2]	6.4 [5.1-9.3]	0.921	8.4 [5.0-11.4]	6.4 [5.1-8.7]	0.444
Abdomen (mm)	13.5 [10.5-16.9]	12.3 [8.5-30.1]	1.00	15.8 [9.3-28.2]	12.3 [8.5 -18.1]	0.309
Thigh (mm)	8.4 [7.2-14.8]	8.5 [6.0-12.4]	0.490	13.5 [8.4-21.1]	8.1 [6.0-11.3]	**0.008**
Sub-Scapular (mm)	9.4 [6.5-13.2]	8.3 [6.3-13.4]	0.605	10.5 [5.7-18.0]	8.2 [6.5-11.1]	0.770
Supra-iliac (mm)	6.3 [4.4-6.8]	6.2 [4.9-9.4]	0.196	6.4 [4.7-12.1]	6.2 [4.9-8.5]	0.934
Calf (mm)	5.5 [4.8-9.0]	5.8 [4.4-8.2]	0.739	6.8 [5.4-10.5]	5.5 [4.4-7.7]	0.094

*based on patient report, those with moderate or severe fat loss in 2 or more regions

**Wilcoxon Rank-sum (Mann-Whitney) tests for continuous variables and chi-square tests for binary variables

***based on patient report, those with moderate or severe fat gain in 2 or more regions

Table 4. DXA-derived measurements of women on ART

Variable	Lipoatrophy*		P-value**	Lipohypertrophy***		P-value**
	With Median [IQR] n=29	Without Median [IQR] n=143		With Median [IQR] n=46	Without Median [IQR] n=126	
DXA-derived measures						
Arm fat (%)	37.4 [30.8-44.1]	38.3 [31.8-47.1]	0.380	39.8 [31.5-48.3]	38.1 [31.7-45.1]	0.319
Lower limb fat (%)	37.6 [31.5-40.6]	43.4 [36.6-49.7]	**0.001**	39.2 [33.3-49.7]	41.8 [36.3-48.8]	0.761
Trunk fat (%)	34.7 [29.9-38.0]	35.1 [27.9-43.1]	0.719	37.9 [30.7-45.3]	34.4 [26.6-40.4]	**0.019**
Lower limb fat/ht (%)	23.6 [19.7-25.4]	27.4 [23.4-31.5]	**0.001**	25.4 [21.9-31.1]	26.5 [23.2-29.7]	0.631
Total limb fat/ht (%)	22.7 [20.8-27.0]	25.4 [22.0-29.9]	**0.015**	25.4 [20.8-31.1]	25.2 [21.8-29.1]	0.825
Lower limb fat/BMI (%)	1.3 [1.2-1.7]	1.6 [1.3-1.8]	**0.049**	1.4 [1.2-1.6]	1.6 [1.3-1.8]	**0.019**
Total limb fat/BMI (%)	1.3 [1.3-1.6]	1.5 [1.3-1.6]	0.193	1.3 [1.2-1.5]	1.5 [1.3-1.7]	**0.006**
Trunk fat/lower limb fat (%)	0.94 [0.76-1.15]	0.83 [0.66-0.96]	**0.017**	0.93 [0.80-1.10]	0.81 [0.66-0.94]	**0.003**
Trunk fat/ total limb fat (%)	1.0 [0.8-1.0]	0.9 [0.7-1.0]	**0.045**	0.93 [0.85-1.03]	0.85 [0.72-0.97]	**0.003**

*based on patient report, those with moderate or severe fat loss in 2 or more regions
**Wilcoxon Rank-sum (Mann-Whitney) tests for continuous variables and chi-square tests for binary variables
***based on patient report, those with moderate or severe fat gain in 2 or more regions

ROC curves for lipoatrophy and lipohypertrophy were generated and reported in women for anthropometric and DXA-derived variables with the highest AUCs (**Figure 1**). For lipoatrophy, the two anthropometric variables with the highest AUCs were triceps skinfold thickness (AUC=0.725) and thigh skinfold thickness (AUC=0.720) and for lipohypertrophy they were waist/hip ratio (AUC=0.645) and waist circumference (AUC=0.589). For lipoatrophy, the two DXA-derived variables with the highest AUC were the percentage of lower limb fat standardised to height (AUC=0.713) and percentage lower limb fat (AUC=0.705); and for lipohypertrophy they were percentage trunk fat/percentage total limb fat (AUC=0.647) and percentage trunk fat/percentage lower limb fat (AUC=0.646). An illustration of anthropometric and DXA-derived variables in women is shown in **Figure 2**.

Optimum cut-points for lipoatrophy and lipohypertrophy variables, based on likelihood ratios, were selected. **Table 5** shows the sensitivity, specificity, likelihood ratios and predictive values for the two anthropometric and DXA-derived variables with the highest AUCs for lipoatrophy and lipohypertrophy at the optimum cut-points.

Figure 1. ROC curves for the 2 anthropometric and DXA-derived variables with the highest AUC for lipoatrophy (A) and lipohypertrophy (B) in women on ART

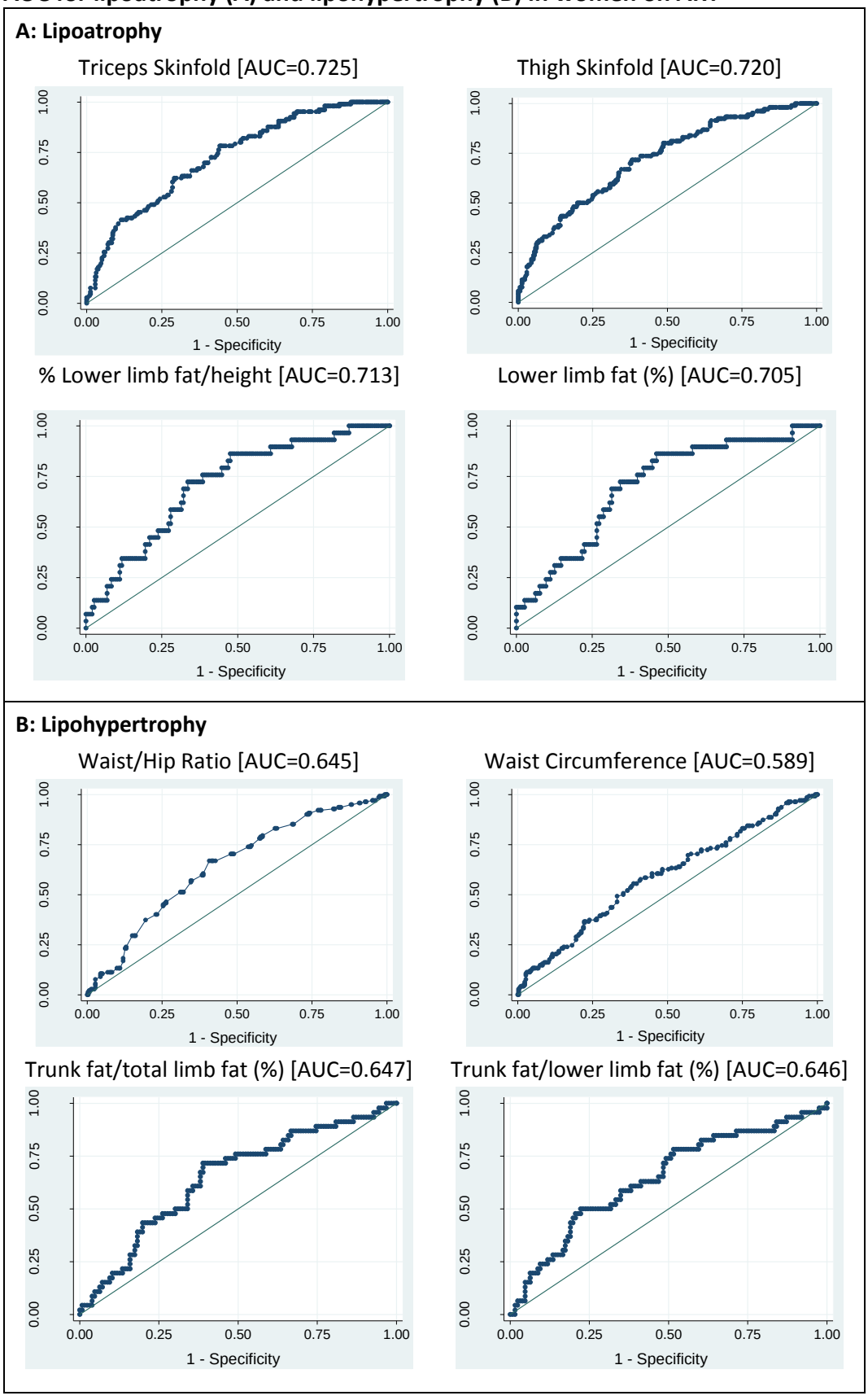


Figure 2. Lipoatrophy variables for women on ART with ROC AUC's of ≥0.6 and their 95% Confidence Intervals in descending order of AUC

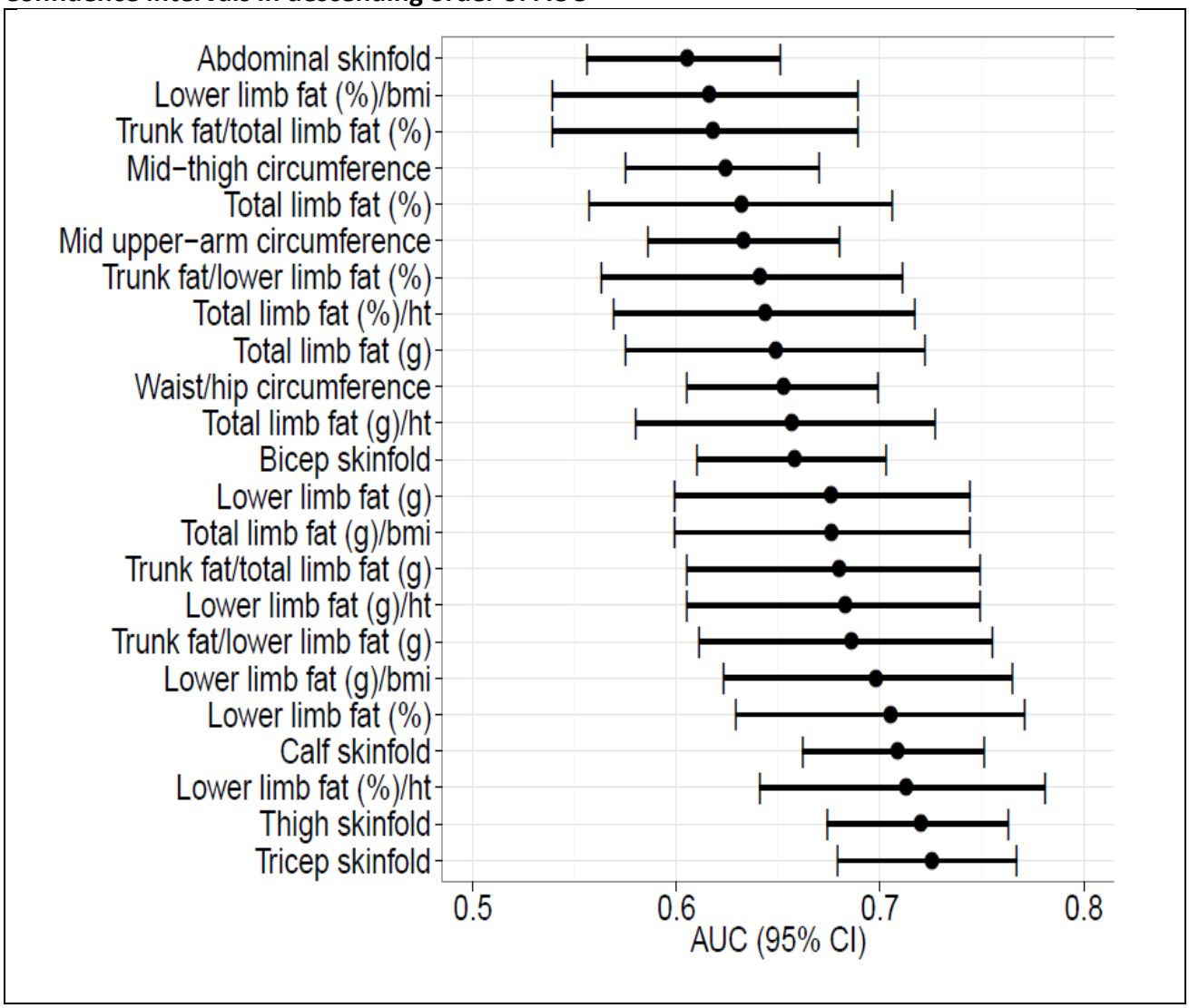


Table 5: Variables for prediction and classification used to identify lipoatrophy and lipohypertrophy cut-points in women on ART

	n	Sensitivity	Specificity	Likelihood Ratio Positive Test	Likelihood Ratio Negative Test	Positive Predictive Value	Negative Predictive Value	% correctly classified
Lipoatrophy variable cut-point								
Triceps skinfold ≤14.5 mm	418	0.62	0.71	2.13	0.53	0.42	0.85	68.66*
Thigh skinfold ≤28.0 mm	417	0.67	0.65	1.93	0.51	0.39	0.85	65.70*
% Lower limb fat/height ≤ 24.7	418	0.19	0.85	1.28	0.95	0.30	0.76	68.42*
Lower limb fat (%) ≤ 39.1	418	0.19	0.84	1.20	0.96	0.29	0.75	67.70*
Lipohypertrophy variable cut-point								
Waist/Hip Ratio ≥ 0.899	434	0.44	0.75	1.75	0.75	0.46	0.73	64.75**
Waist Circumference ≥ 88.5 cm	434	0.57	0.59	1.40	0.73	0.41	0.73	58.53**
Trunk fat/total limb fat (%) ≥0.8895	172	0.50	0.70	1.66	0.72	0.38	0.79	64.5**
Trunk fat/leg fat (%) ≥ 0.8816	172	0.72	0.61	1.84	0.46	0.40	0.86	64.0**

*percentage of those classified with and without lipoatrophy using the new cut-point compared to those defined by subject-report
**percentage of those classified with and without lipohypertrophy using the new cut-point compared to those defined by subject-report

6.5 Discussion

We showed that simple anthropometric measures were at least as good as DXA-derived measures to diagnose lipoatrophy and lipohypertrophy in African women on ART. The best predictors of lipoatrophy in women were the anthropometric variables triceps and thigh skinfold thicknesses; and the DXA-derived variables percentage lower limb fat and percentage lower limb fat/height. The best predictors of lipohypertrophy in women were the anthropometric variable waist/hip ratio and the DXA-derived variable percentage trunk fat/percentage limb fat. Women with lipoatrophy had considerably smaller limb circumferences, limb skinfold thicknesses and lower percentages of limb fat than women without lipoatrophy, despite similar BMIs. Lipoatrophy and lipohypertrophy were both more common in women than in the small sample of men.

Previous studies, conducted in high-income countries, developed objective measures for lipodystrophy, thus combining lipoatrophic and lipohypertrophic individuals [15-17]. They proposed the use of fat mass ratio (FMR), defined as the ratio between the percentage of trunk fat mass and the percentage of lower limb fat mass. We however sought to investigate lipoatrophy and lipohypertrophy as separate entities. Identification of lipoatrophy is important as it is an adverse antiretroviral drug reaction, which improves on switching antiretroviral drugs [21]. Although lipohypertrophy is thought to be a consequence of treating the HIV infection rather than an adverse antiretroviral drug reaction [21], like lipoatrophy, it is associated with an increased risk of vascular disease [30] therefore it is worth identifying so that appropriate screening and prevention interventions can be implemented.

Despite the subjective nature of assessing lipoatrophy and lipohypertrophy by using patient self-report, previous studies have shown a strong correlation between patient and physician reported lipodystrophy scores [31-33]. In South Africa, as well as in many other African countries, nurses, rather than physicians, prescribe antiretroviral therapy and follow-up patients. For these reasons we used patient self-report [5, 6] as the reference measure to define lipoatrophy and lipohypertrophy.

Our study, like several others [11, 21], showed a significant association between lipoatrophy and time on ART, and time on stavudine in particular. As South Africa has only recently phased out stavudine, and zidovudine is still being used, it is not unexpected that a quarter of the women and a tenth of the men, had lipoatrophy. The prevalence of lipoatrophy found in this study is not easy to compare with other studies, as studies from high-income countries focussed on men [12, 24], while most studies from Africa looked at the prevalence of lipodystrophy [34-36] rather than studying the two entities of lipoatrophy and lipodystrophy separately. Our finding that triceps skinfold thickness was a predictor of lipoatrophy is supported by other studies. George *et al.* [37], using a small sample of HIV-infected South Africans, found that after two years of exposure to ART, patients had significantly decreased triceps skinfold thickness. Similarly, a Ugandan study using a sample of HIV-infected men and women [36], found that decreased triceps skinfold thicknesses was associated with the use of zidovudine.

There were some limitations to our study. The cross sectional design, while allowing us to make associations, does not allow us to infer causality. With changes in fat distribution, repeated objective measures would have given us a better reference standard than patient report, even though patient report is commonly used [5, 6]. We did not have enough men with lipoatrophy or lipohypertrophy, to explore predictive anthropometric and DXA-derived variables. Finally, the likelihood ratios for the most predictive anthropometric and DXA-derived variables were only weakly diagnostic of self-reported lipoatrophy and lipohypertrophy. Future research of longitudinal studies in African cohorts, using changes in DXA-derived variables as the reference standard, is needed to confirm the value of anthropometric measures for the diagnosis of lipoatrophy and lipohypertrophy.

6.6 Conclusion

Using a large sample of black HIV-infected South African women who had DXA scans performed, we were able to develop anthropometric measures for defining lipoatrophy and lipohypertrophy. The development of anthropometric measures which admittedly needs training and well maintained skinfold callipers to ensure their accuracy, are of particular

relevance in resource limited settings, where health professionals need simple and inexpensive methods of diagnosing patients with lipoatrophy and lipohypertrophy.

6.7 Acknowledgements

Authors' Contributions

ZA conducted all statistical analyses, interpreted the findings and drafted the manuscript; JD, NL and GM designed and conducted the study; ML assisted with study design and data interpretation; NL, GM, JD and ML edited the manuscript and drafted revisions. All authors read and approved the manuscript.

Financial competing interests

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Chapter 7

Anthropometric definitions for antiretroviral associated lipodystrophy derived from a longitudinal South African cohort with dual-energy X-ray absorptiometry data

Abrahams Z, Maartens G, Levitt N, Dave JA. Anthropometric definitions for antiretroviral-associated lipodystrophy derived from a longitudinal South African cohort with serial dual-energy X-ray absorptiometry measurements. (In review – Int J Infect Dis)

7.1 Abstract

Background

The development of lipodystrophy is associated with the long-term use of antiretroviral therapy (ART). The diagnosis of lipodystrophy is difficult in resource-limited settings because of limited facilities for imaging, e.g. dual-energy X-ray absorptiometry (DXA), to show changes in body fat distribution. We assessed the agreement between patient reported lipodystrophy (using a standard instrument) and anthropometry with body fat changes measured using DXA to develop objective measures to define lipoatrophy and lipohypertrophy in a sample of black South Africans on ART.

Methods

ART-naïve HIV-infected adults were enrolled in a 24 month longitudinal study. Self-reported information on regional fat loss and fat gain, anthropometry, and DXA measures were collected at baseline, and at 3 months, 6 months, 12 months, 18 months, and 24 months after starting ART. Receiver operating characteristic (ROC) curves were used to describe the performance of anthropometric variables using change in limb and trunk fat measured by DXA as the reference standard.

Results

The proportion of men and women who developed lipoatrophy increased over the 24 month period, but it occurred more frequently in men (21.4% vs. 9.6%). Thigh skinfold and mid-arm circumference cut-points performed best at predicting lipoatrophy in women (correctly classifying 80% and 78%, respectively); and mid-arm circumference in men (correctly classifying 70%). Anthropometric measures performed poorly at defining DXA-defined lipohypertrophy. We showed very poor agreement between patient reported lipoatrophy and lipohypertrophy with changes in regional fat on DXA.

Conclusion

This is the first study from sub-Saharan Africa to describe longitudinal changes in body fat composition using DXA-derived measures. Anthropometric measures performed well for defining lipoatrophy, but not lipohypertrophy, in both men and women using DXA-derived measures as the reference standard.

7.2 Introduction

Long-term use of antiretroviral therapy (ART) is associated with a number of metabolic complications, including fat redistribution or lipodystrophy. Lipodystrophy is an all-encompassing term used to refer to lipoatrophy (loss of subcutaneous fat, particularly in the face and limbs), lipohypertrophy (increase in fat, particularly around the abdomen and breast), or a combination of both [1]. As both lipoatrophy and lipohypertrophy are associated with an increased risk of cardiovascular disease [2-4], it is important that simple, objective measures to diagnose lipodystrophy be developed for HIV care and treatment.

Lipodystrophy has been diagnosed using both subjective (self-report and examination by a clinician) [5-7] and objective methods (computed tomography, magnetic resonance imaging, and dual-energy X-ray absorptiometry (DXA)) [8-11]. Diagnosing lipodystrophy using costly objective methods is problematic, especially in resource limited settings [12] where self-report using standardised questionnaires, and clinical examination are the most commonly used assessment methods. While some studies found a high level of agreement between self-report and clinical examination [13,14], others reported poor agreement [15,16]. A cross-sectional study comparing patient and physician report to DXA-measured limb fat showed reasonable levels of agreement between lipoatrophy scores as measured by questionnaire and DXA-measured limb fat [17]. Studies on the diagnosis of lipodystrophy have mostly been done in men from high-income countries. There is minimal data on diagnosing lipodystrophy in sub-Saharan Africa, which bears the brunt of the HIV epidemic and where more women are infected than men. There is a high prevalence of obesity in black South African women and they have less visceral fat than white South African women [18]. We previously developed

anthropometric cut-points to diagnose lipodystrophy in a cross-sectional study, but the reference standard was patient report [11].

Therefore, the aim of our study was, firstly, to develop objective measures to define lipoatrophy and lipohypertrophy by comparing change in limb fat and trunk fat as measured by DXA to anthropometric variables; and secondly, to assess agreement between patient reported lipoatrophy and lipohypertrophy with objective measures derived by DXA in a sample of black South Africans starting ART.

7.3 Methods

Participants

A convenience sample of ART-naive HIV-infected men and women presenting at Crossroads Community Health Centre in Cape Town were enrolled in a 24 month longitudinal study. Patients were initiated on ART after enrolment into the study and completion of baseline evaluations. During the course of the study there was a change in the nucleoside reverse transcriptase inhibitors (NRTIs) used in first-line ART regimens in South Africa, from stavudine or zidovudine to tenofovir, together with lamivudine, and efavirenz or nevirapine. The second-line ART regimen consisted of zidovudine, lamivudine and lopinavir/ritonavir [19].

Testing procedures

Socio-demographic information was collected from participants at the beginning of the study using an interviewer administered questionnaire. The Lipodystrophy Case Definition questionnaire [6] was used to collect self-reported information on fat gain or fat loss. Self-reported lipoatrophy was defined as moderate or severe fat loss in two or more regions, and self-reported lipohypertrophy was defined as moderate or severe fat gain in two or more areas [20]. Anthropometry and DXA were performed at baseline, 3 months, 6 months, 12 months, 18 months, and 24 months. All anthropometric measurements were performed by the same anthropometrist and included weight, height, circumferences (waist, hip, mid-upper arm, and mid-thigh), sagittal abdominal diameter (SAD) and skinfold thickness (biceps,

triceps, subscapular, abdomen, supra-iliac, thigh and calf). DXA (Discovery-W®, software version 12.7.3.7; Hologic, Bedford, MA) was used to measure fat mass according to standard procedures by an independent observer. DXA cut-off lines positioned at anatomical markers were used to obtain fat mass for the whole body as well as for the various regions of interest (arms, legs and trunk). The trunk comprised the region between the neck and waist, excluding the arms. Limb composition was determined by summing the arms and legs. Clinical records obtained from health facilities were reviewed to obtain information on ART regimens, viral loads and CD4 counts.

Ethical approval

The study proposal was submitted and approved by the Research Ethics Committee of the Faculty of Health Sciences at the University of Cape Town. Written informed consent was obtained from all participants prior to participation in the study.

Data analysis

Data analysis was carried out using the STATA/SE statistical software package version 14.1 (StataCorp., College Station, TX, USA). Continuous variables were described as medians and inter-quartile ranges (IQR), and were compared using Wilcoxon Rank Sum tests. Binary variables were described using frequency and percentages, and compared using chi-square tests. The Jonckheere-Terpstra test for ordered variables was used to measure trends over time.

Two DXA-defined definitions of lipoatrophy were explored for the development of anthropometric measures: (1) ≥ 20% loss of limb fat from baseline [21] and, (2) ≥ 10% loss of limb fat (all limbs combined) from baseline by DXA scan [22]. Lipohypertrophy was defined as ≥ 20% gain in trunk fat from baseline by DXA scan [23,24]. Receiver operating characteristic (ROC) curves were used to describe the performance of a number of anthropometric variables in men and women using DXA-defined lipoatrophy and lipohypertrophy as the reference standard at 12, 18, and 24 months on ART. Anthropometric variable selection was based on associations with lipoatrophy or lipohypertrophy. The area under the ROC curve (AUC) was used to assess the diagnostic performance of each variable. In addition, sensitivity, specificity,

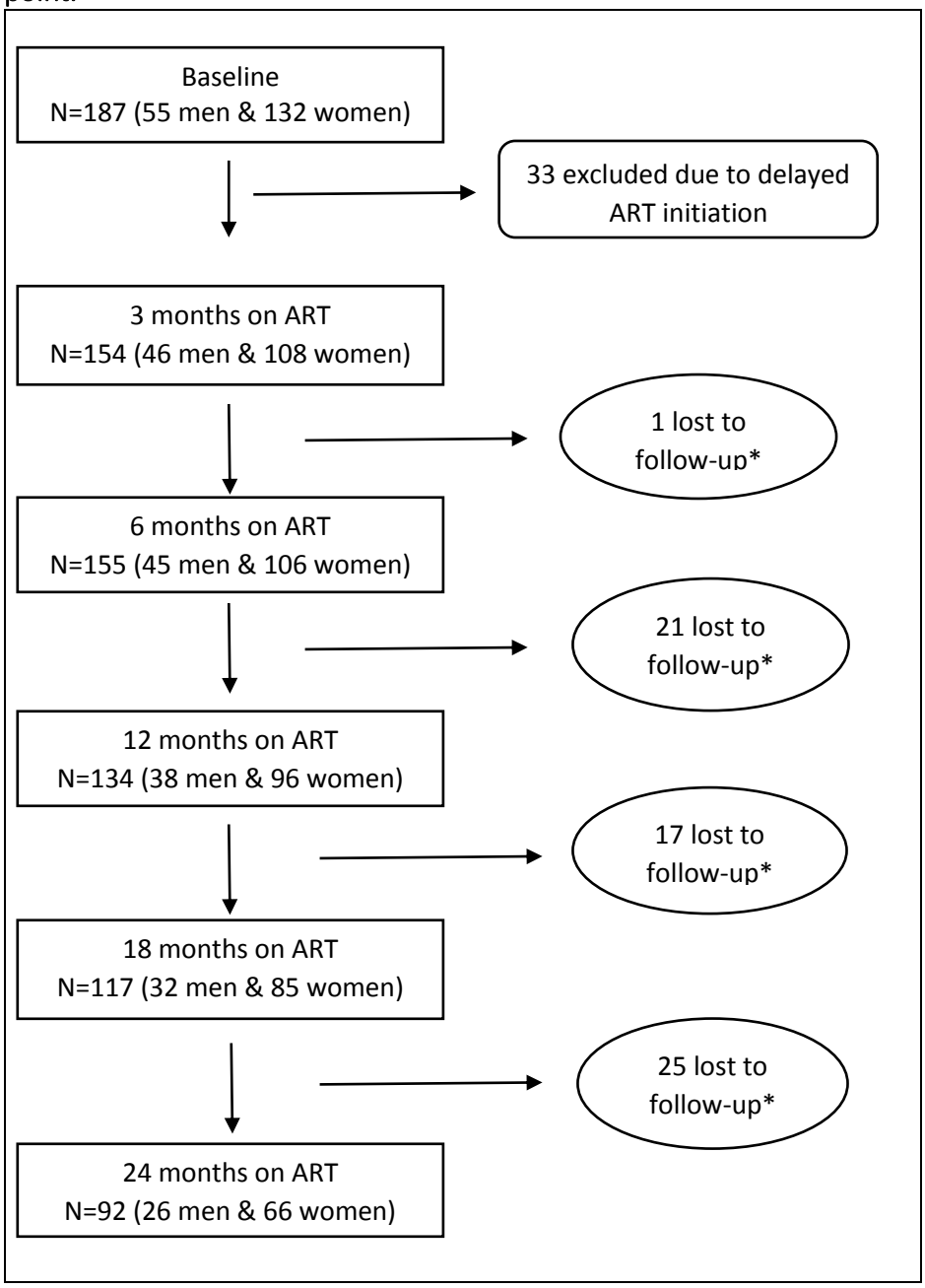
likelihood ratios and predictive values were calculated for variables with ROC AUCs of ≥ 0.65 at the optimum cut-points. Cut-point selection was based on positive likelihood ratios.

7.4 Results

ART-naïve participants (n=187; 29.4% men and 70.6% women) were enrolled into this study (**Figure 1**). After collection of baseline measurements, 33 participants (17.6%) were excluded from further participation as a result of delayed ART initiation. By 12 months on ART, 53 (28.3%) participants were lost to follow-up and this number increased to 95 (50.8%) participants at 24 months on ART. Patients were only classified as lost-to-follow-up when no further measurements were obtained from them. There was no difference in baseline characteristics between those lost to follow-up and those that remained in the study. Baseline characteristics are shown in **Table 1**. Men were significantly older than women (35 vs. 31 years; p=0.008), and more men (71%) than women (56%) were initiated on a stavudine-based regimen. The proportion of participants on stavudine and tenofovir did not differ between commencement of ART and 12 months later. However, after 24 months on ART, the proportion of participants on a stavudine-based regimen had decreased and those on a tenofovir-based regimen had increased. More women were initiated onto nevirapine (69.8%) than efavirenz (30.2%). Only 1 participant was exposed to a drug regimen containing a protease inhibitor. Viral suppression (viral load <50 copies/mL) was achieved in 73% of participants after 6 months of ART.

Change in anthropometric measures over the 24 month period is shown in **Table 2** for women and **Table 3** for men. In women, all anthropometric measures, except waist/hip ratio, showed a significantly increasing trend over time. In men, weight, BMI, mid-upper arm circumference, mid-thigh circumference, and skinfold thicknesses, showed a significantly increasing trend over time. Weight gain in women was two-fold higher than in men (9.1 kg vs. 4.2 kg).

Figure 1. Consort diagram showing the number of participants lost to follow-up at each time point.



*No further measurements were obtained from participants.

Table 1. Characteristics of participants at commencement of ART.

	Total Median [IQR] n=187	**Women** Median [IQR] n=132	**Men** Median [IQR] n=55	**P-value**
Age	33.0 [27.0-39.0]	31.0 [26.0-38.0]	35.0 [29.0-41.0]	**0.008**
CD4	156 [110-196]	161 [110-204]	137 [112-183]	0.126
	n [%]	**n [%]**	**n [%]**	
Smoker	60 [32.3]	24 [18.3]	36 [65.5]	**<0.001**
Antiretroviral drugs				
NRTI				
Stavudine	110 [60.8]	71 [56.4]	39 [70.9]	0.065
Zidovudine	20 [11.1]	19 [15.1]	1 [1.8]	**0.009**
Tenofovir	51 [28.2]	36 [28.6]	15 [27.3]	0.858
NNRTI				
Efavirenz	66 [36.5]	38 [30.2]	28 [50.9]	**0.008**
Nevirapine	115 [63.5]	88 [69.8]	27 [49.1]	**0.008**

Table 2. Comparison of anthropometric measures in women at baseline, 3, 6, 12, 18 and 24 months.

	Baseline Median [IQR] n=132	**3 months** Median [IQR] n=103	**6 months** Median [IQR] n=102	**12 months** Median [IQR] n=88	**18 months** Median [IQR] n=77	**24 months** Median [IQR] n=66	**P-value*** (increasing trend)
Height (m)	1.58 [1.55-1.62]	1.57 [1.54-1.62]	1.58 [1.55-1.62]	1.58 [1.55-1.62]	1.57 [1.54-1.62]	1.58 [1.55-1.63]	0.340
Weight (kg)	62.0 [54.8-71.3]	65.8 [57.5-73.6]	68.1 [58.2-77.0]	69.2 [59.3-82.8]	69.8 [59.8-81.8]	71.1 [57.6-84.4]	**<0.001**
BMI	24.8 [21.1-28.7]	26.3 [22.4-30.0]	27.1 [22.8-30.9]	27.8 [23.9-33.4]	28.3 [22.4-31.8]	27.7 [22.7-33.9]	**<0.001**
Sagittal abdominal diameter (cm)	18.5 [16.5-21.0]	19.0 [16.5-21.5]	20.3 [16.5-22.3]	20.0 [17.6-22.6]	21.0 [18.0-23.8]	21.0 [18.0-24.8]	**<0.001**
Circumferences							
Waist (cm)	80.8 [74.5-90.5]	84.5 [76.0-93.0]	84.0 [76.0-93.5]	86.9 [79.0-96.8]	85.5 [79.0-97.0]	89.5 [76.0-100.5]	**<0.001**
Hip (cm)	98.0 [91.8-104.0]	100.5 [93.0-108.0]	104.0 [94.0-110.0]	105.0 [94.0-113.5]	104.5 [94.0-113.0]	105.0 [93.0-112.5]	**<0.001**
Waist/hip ratio	0.84 [0.79-0.92]	0.84 [0.80-0.89]	0.83 [0.77-0.88]	0.83 [0.80-0.89]	0.84 [0.79-0.88]	0.86 [0.81-0.91]	0.143
Mid-upper arm (cm)	27.3 [24.0-27.0]	28.5 [25.5-31.5]	29.5 [26.0-32.5]	29.4 [26.0-33.0]	29.5 [25.8-33.0]	29.5 [25.5-33.5]	**<0.001**
Mid-thigh (cm)	55.0 [50.0-60.5]	56.0 [51.0-61.0]	59.0 [52.0-64.0]	59.0 [53.0-64.25]	59.0 [52.0-64.0]	60.0 [52.0-65.0]	**<0.001**
Skinfold thicknesses							
Biceps (mm)	7.0 [4.8-10.1]	7.4 [5.4-11.5]	8.4 [6.0-12.4]	8.7 [5.75-13.2]	8.6 [6.0-14.0]	9.0 [5.0-14.4]	**<0.001**
Triceps (mm)	17.2 [11.9-22.8]	19.2 [12.9-24.8]	21.4 [15.6-25.8]	22 [14.2-27.8]	21.2 [13.4-26.6]	21.1 [13.2-29.2]	**<0.001**
Abdomen (mm)	19.4 [13.9-31.4]	22.0 [15.8-32.0]	22.9 [17.0-34.0]	26.2 [18.65-38]	27.2 [19.0-37.0]	29.1 [19.1-39.8]	**<0.001**
Thigh (mm)	27.6 [20.4-36.0]	32.2 [23.2-39.0]	33.3 [23.2-41.4]	35.0 [24.1-44.2]	34.1 [25.0-42.3]	32.3 [22.0-41.2]	**<0.001**
Sub-Scapular (mm)	15.3 [10.0-24.4]	17.2 [11.3-27.3]	19.4 [11.9-29.2]	22.2 [12.6-32.0]	20.0 [13.0-30.3]	23.8 [13.6-31.0]	**<0.001**
Supra-iliac (mm)	11.1 [7.4-21.4]	13.4 [8.6-23.3]	15.5 [9.0-27.0]	18.5 [10.1-31.1]	17.6 [11.8-28.2]	21.2 [9.8-32.1]	**<0.001**
Calf (mm)	17.3 [13.4-21.4]	18.2 [14.6-23]	20.4 [15.0-25.5]	20.9 [16.2-27.3]	20.0 [15.0-26.2]	20.0 [14.4-26.2]	**<0.001**

*Jonckheere-Terpstra test for ordered variables

Table 3. Comparison of anthropometric measures in men at baseline, 3, 6, 12, 18 and 24 months.

	Baseline Median [IQR] n=55	**3 months** Median [IQR] n=43	**6 months** Median [IQR] n=45	**12 months** Median [IQR] n=35	**18 months** Median [IQR] n=31	**24 months** Median [IQR] n=26	**P-value*** (increasing trend)
Height (m)	1.7 [1.7-1.8]	1.7 [1.7-1.7]	1.7 [1.7-1.8]	1.7 [1.7-1.8]	1.7 [1.7-1.7]	1.7 [1.7-1.7]	0.475
Weight (kg)	61.0 [55.0-67.0]	62.1 [55.8-68.2]	63 [56.2-69.0]	65.0 [60.6-72.0]	65.0 [60.0-74.0]	65.2 [59.0-70.5]	**0.009**
BMI	21.3 [19.7-23.1]	21.2 [19.7-23.6]	21.4 [20.0-23.3]	22.0 [21.0-24.5]	21.9 [21.2-24.6]	21.8 [21.3-23.9]	**0.003**
Sagittal abdominal diameter (cm)	17.0 [15.8-18.5]	17.0 [15.5-18.0]	16.8 [15.0-18.0]	17.5 [16.3-20.0]	17.5 [16.5-19.5]	17.6 [16.3-19.0]	**0.017**
Circumferences							
Waist (cm)	78.3 [74.0-83.0]	78.8 [76.0-83.0]	78.0 [75.0-83.5]	80.5 [75.0-86.5]	80.0 [76.0-86.0]	79.6 [75.0-83.5]	0.092
Hip (cm)	88.4 [85.0-92.0]	88.0 [84.0-92.5]	90.0 [85.0-93.0]	90.0 [88.0-95.0]	91.0 [87.0-94.0]	89.0 [87.0-93.0]	0.071
Waist/hip ratio	0.89 [0.86-0.92]	0.89 [0.86-0.92]	0.87 [0.85-0.92]	0.89 [0.85-0.93]	0.89 [0.85-0.93]	0.89 [0.84-0.93]	0.593
Mid-upper arm (cm)	25.0 [24.0-27.0]	25.0 [24.0-27.0]	25.5 [24.0-27.0]	26.5 [25.0-28.5]	26.5 [25.0-28.0]	25.3 [24.5-27.0]	**0.011**
Mid-thigh (cm)	48.0 [45.0-52.0]	48.0 [45.5-51.5]	48.5 [45.5-52.0]	50.0 [48.0-52.5]	50.0 [48.0-54.5]	48.0 [46.0-51.0]	**0.017**
Skinfold thicknesses							
Biceps (mm)	3.2 [2.8-4.0]	3.4 [2.8-3.7]	3.3 [3.0-3.8]	3.4 [3.0-4.3]	3.8 [3.0-4.4]	3.7 [3.0-4.6]	**0.030**
Triceps (mm)	5.8 [4.9-8.6]	6.3 [4.8-8.2]	6.0 [5.0-8.8]	7.0 [5.1-10.9]	7.0 [5.0-10.0]	6.8 [5.7-8.9]	**0.035**
Abdomen (mm)	10.9 [7.8-15.6]	10.4 [8.6-14.4]	10.4 [7.7-17.8]	13.0 [8.4-20.4]	13.2 [8.4-19.4]	13.3 [9.3-18.5]	**0.033**
Thigh (mm)	7.7 [5.7-12.0]	8.0 [6.0-13.5]	8.6 [6.0-12.4]	8.0 [5.9-12.4]	8.8 [6.3-11.6]	9.0 [6.8-10.0]	0.141
Sub-Scapular (mm)	9.0 [6.5-9.7]	7.1 [6.4-9.3]	7.5 [6.6-10.2]	8.6 [6.6-11.4]	9.4 [7.2-12.3]	9.1 [6.8-11.9]	**0.011**
Supra-iliac (mm)	5.8 [4.8-7.6]	6.0 [4.8-7.5]	6.0 [4.8-8.2]	6.4 [5.0-10.4]	7.2 [4.8-9.4]	6.7 [4.6-9.0]	0.084
Calf (mm)	5.8 [4.6-8.2]	6.2 [5.0-8.4]	5.8 [5.0-9.1]	6.8 [4.4-8.3]	7.0 [5.2-10.0]	6.5 [5.2-8.2]	**0.043**

*Jonckheere-Terpstra test for ordered variables

Cumulative measures for lipoatrophy and lipohypertrophy in men and women are shown in **Table 4**. Few self-reported cases of lipoatrophy or lipohypertrophy were recorded. The incidence of lipoatrophy, defined by limb fat loss as measured by DXA, increased in both men and women over the 24 month period. A greater proportion of men (9 of 42; 21.4%) than women (11 of 114; 9.6%) developed lipoatrophy by 24 months on ART when defined as ≥ 20% limb fat loss. The proportion of participants who developed lipohypertrophy (defined as ≥ 20% trunk fat gain) increased in both men and women by 24 months on ART. A similar proportion of men (25 of 42; 60%) and women (65 of 114; 57%) developed lipohypertrophy.

Table 4. Cumulative number of participants with lipodystrophy assessed by patient report or DXA measures in women and men at 3, 6, 12, 18 and 24 months.

	3 months	6 months	12 months	18 months	24 months
Women					
Number at risk	114	101	94	83	75
Lost to observation	14	10	7	10	15
Lipoatrophy					
Patient Report	1	1	1	1	1
DXA ≥ 20% limb fat loss	1	1	6	8	11
DXA ≥ 10% limb fat loss	6	7	15	19	26
Lipohypertrophy					
Patient Report	6	15	16	17	17
DXA ≥ 20% trunk fat gain	17	31	53	63	65
Men					
Number at risk	42	44	37	30	28
Lost to observation	6	1	4	8	4
Lipoatrophy					
Patient Report	1	1	1	1	1
DXA ≥ 20 % limb fat loss	2	3	5	8	9
DXA ≥ 10 % limb fat loss	8	13	15	17	19
Lipohypertrophy					
Patient Report	1	3	3	3	3
DXA ≥ 20% trunk fat gain	7	13	21	24	25

The highest ROC AUC of anthropometric measures for lipoatrophy were observed in women at 18 months on ART, and in men at 24 months on ART (**Table 5**). Anthropometric measures for lipohypertrophy performed poorly in women with no ROC AUCs ≥ 0.65 (**Table 6**). In men, three anthropometric measures for lipohypertrophy at 18 months generated ROC AUCs ≥ 0.65 (sagittal abdominal diameter=0.732; waist circumference=0.661; waist/hip ratio=0.665) (**Table 6**). An illustration of ROC AUCs in descending order, for anthropometric measures associated with lipoatrophy (**Figure 2**) in women (A & B) and men (C & D), and for lipohypertrophy (**Figure 3**) in men, is shown. More anthropometric measures had a ROC AUC ≥ 0.65 when lipoatrophy was defined as limb fat loss ≥ 20% than when it was defined as ≥ 10%. Optimum cut-points for lipoatrophy variables with ROC AUCs of ≥ 0.65, based on likelihood ratios, were selected for men and women (**Table 7**). The thigh skinfold cut-point (≤24 mm) was able to correctly classify the greatest number of women (80.3%), both with lipoatrophy (sensitivity =62.5) and those without lipoatrophy (specificity=82.35). In men, the mid-arm circumference cut-point was able to correctly classify 69.6% of men, and was better at identifying those without lipoatrophy (specificity=83.33) than those with lipoatrophy (sensitivity=20.0).

Table 5. ROC AUC and 95% CI for anthropometric measures used to predict lipoatrophy defined as ≥20% limb fat loss in women and men at 12, 18 and 24 months on ART. ROC AUC values ≥0.65, which was our criterion for determining anthropometric cut-points, are in bold.

	12 months ROC AUC [95%CI]	18 months ROC AUC [95%CI]	24 months ROC AUC [95%CI]
Women	n=87	n=76	n=65
Waist/hip ratio	0.639 [0.534-0.744]	0.568 [0.447-0.679]	0.606 [0.471-0.720]
Mid-thigh circumference	**0.694 [0.581-0.785]**	**0.843 [0.740-0.916]**	**0.700 [0.566-0.801]**
Hip circumference	**0.748 [0.643-0.834]**	**0.859 [0.756-0.925]**	**0.748 [0.631-0.852]**
Mid-arm circumference	**0.695 [0.594-0.795]**	**0.740 [0.623-0.831]**	0.616 [0.486-0.733]
Biceps skinfold	**0.711 [0.606-0.805]**	0.605 [0.487-0.716]	0.625 [0.502-0.747]
Triceps skinfold	**0.714 [0.606-0.805]**	**0.764 [0.652-0.853]**	**0.660 [0.534-0.774]**
Calf skinfold	**0.677 [0.569-0.774]**	**0.791 [0.681-0.875]**	**0.680 [0.559-0.798]**
Thigh skinfold	**0.725 [0.618-0.815]**	**0.814 [0.710-0.895]**	**0.730 [0.598-0.827]**
Men	n=33	n=28	n=23
Waist/hip ratio	0.560 [0.364-0.719]	0.614 [0.406-0.785]	**0.667 [0.427-0.836]**
Mid-thigh circumference	0.565 [0.392-0.745]	0.621 [0.406-0.785]	**0.672 [0.427-0.836]**
Hip circumference	0.637 [0.451-0.796]	0.504 [0.306-0.694]	**0.650 [0.427-0.836]**
Mid-arm circumference	0.528 [0.335-0.692]	0.602 [0.406-0.785]	**0.706 [0.471-0.868]**
Biceps skinfold	0.607 [0.421-0.771]	0.546 [0.339-0.725]	**0.667 [0.427-0.836]**
Triceps skinfold	0.542 [0.364-0.719]	0.617 [0.406-0.785]	0.500 [0.306-0.732]
Calf skinfold	0.597 [0.421-0.771]	0.519 [0.339-0.725]	0.506 [0.306-0.732]
Thigh skinfold	0.583 [0.392-0.745]	0.553 [0.339-0.725]	0.511 [0.306-0.732]

Table 6. ROC AUC and 95% CI of anthropometric measures used to predict lipohypertrophy defined as ≥20% trunk fat gain in women and men at 12, 18 and 24 months on ART. ROC AUC values ≥0.65, which was our criterion for determining anthropometric cut-points, are in bold.

	12 months ROC AUC [95%CI]	18 months ROC AUC [95%CI]	24 months ROC AUC [95%CI]
Women	n=87	n=76	n=65
Sagittal abdominal diameter	0.518 [0.408-0.626]	0.559 [0.441-0.675]	0.540 [0.417-0.672]
Waist circumference	0.507 [0.396-0.615]	0.580 [0.460-0.691]	0.550 [0.425-0.677]
Hip circumference	0.543 [0.430-0.648]	0.594 [0.473-0.704]	0.592 [0.456-0.706]
Waist/hip ratio	0.520 [0.408-0.626]	0.521 [0.408-0.642]	0.531 [0.410-0.663]
Abdominal skinfold	0.519 [0.408-0.626]	0.577 [0.460-0.691]	0.502 [0.381-0.634]
Sub-scapular skinfold	0.540 [0.430-0.648]	0.530 [0.408-0.642]	0.527 [0.395-0.649]
Supra-iliac skinfold	0.526 [0.419-0.637]	0.606 [0.487-0.716]	0.510 [0.381-0.634]
Men	n=33	n=28	n=23
Sagittal abdominal diameter	**0.664 [0.482-0.820]**	**0.732 [0.537-0.889]**	**0.659 [0.427-0.836]**
Waist circumference	0.567 [0.392-0.745]	**0.661 [0.476-0.841]**	0.579 [0.345-0.768]
Hip circumference	0.584 [0.392-0.745]	0.631 [0.441-0.814]	**0.702 [0.471-0.868]**
Waist/hip ratio	0.592 [0.421-0.771]	**0.655 [0.441-0.814]**	0.501 [0.306-0.732]
Abdominal skinfold	0.546 [0.364-0.719]	0.608 [0.406-0.785]	0.643 [0.427-0.836]
Sub-scapular skinfold	0.523 [0.335-0.692]	0.576 [0.372-0.755]	0.532 [0.306-0.732]
Supra-iliac skinfold	0.564 [0.392-0.745]	0.544 [0.339-0.725]	0.575 [0.345-0.768]

Figure 2. Lipoatrophy measures in women (A & B) at 18 months on ART and men (C & D) at 24 months on ART with ROC AUCs and 95% confidence intervals in descending order of AUC.

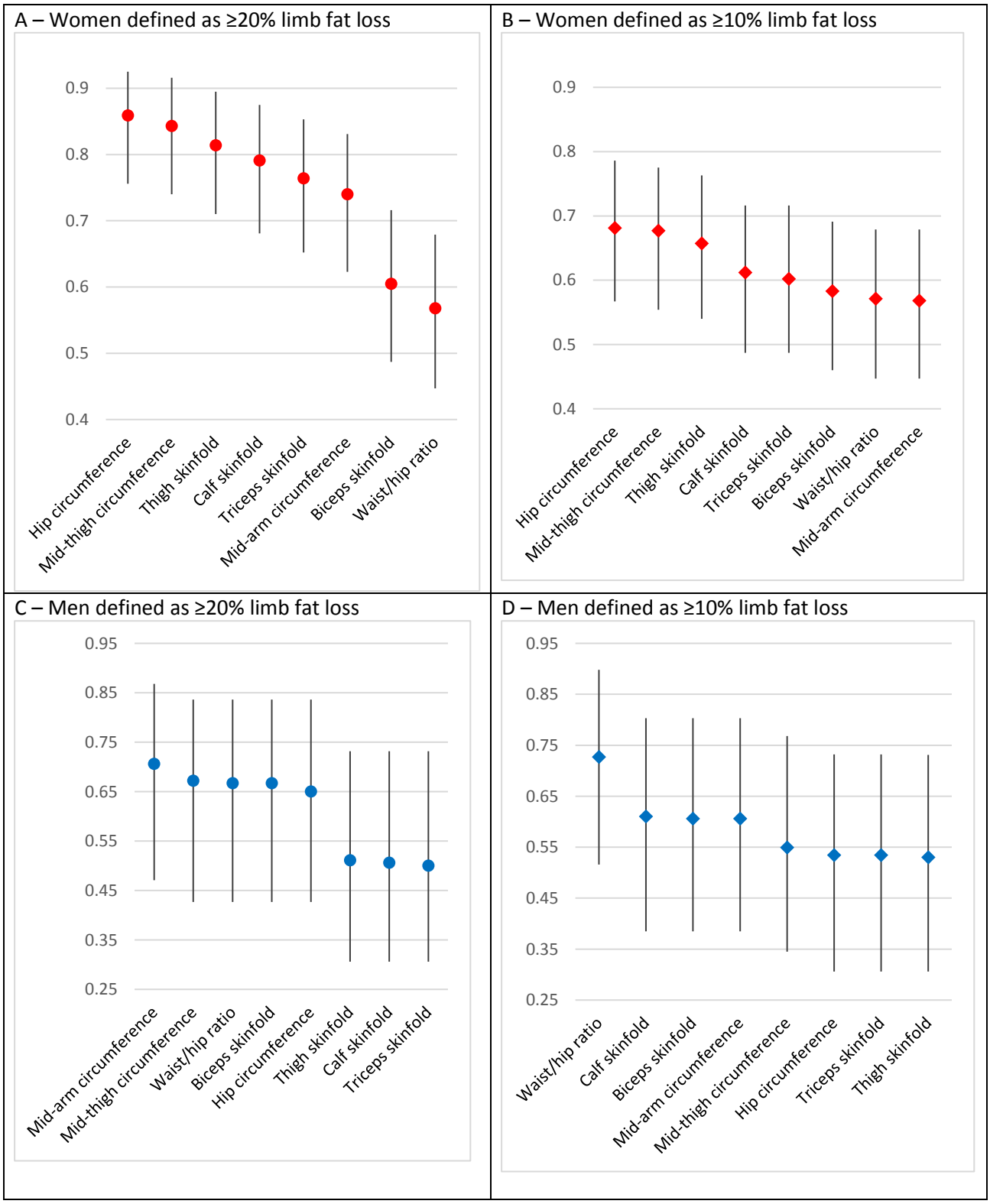


Fig 3. Lipohypertrophy measures (defined as ≥20% trunk fat gain) in (A) women and (B) men at 18 months on ART with ROC AUCs and 95% confidence intervals in descending order of AUC.

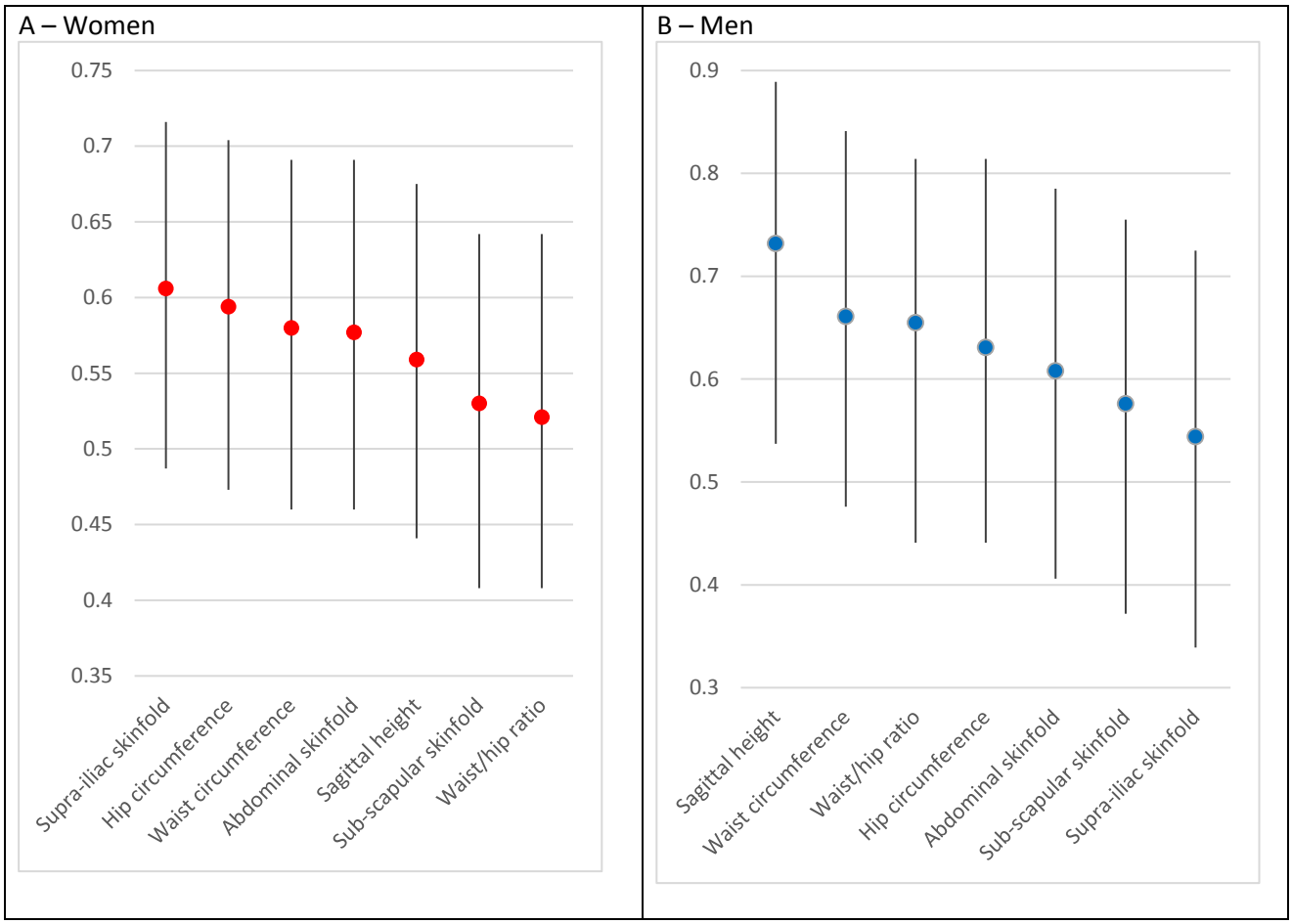


Table 7. Anthropometric cut-points to identify lipoatrophy in women at 18 months on ART and men at 24 months on ART. Anthropometric measures were selected for cut-point determination if their ROC AUC was ≥0.65.

	Cut-point	Sensitivity (%)	Specificity (%)	Likelihood ratio positive test	Likelihood ratio negative test	Positive predictive value (%)	Negative predictive value (%)	% correctly classified
Women (n=76)								
Hip circumference	≤96 cm	75.00	73.53	2.83	0.34	25.00	73.53	73.68
Mid-thigh circumference	≤55 cm	87.50	70.59	2.98	0.18	25.93	70.59	72.37
Mid-arm circumference	≤25 cm	23.53	93.22	3.47	0.82	50.00	93.22	77.63
Thigh skinfold	≤24 mm	62.50	82.35	3.54	0.46	29.41	82.35	80.26
Triceps skinfold	≤16 mm	25.00	96.15	6.50	0.78	75.00	96.15	73.68
Calf skinfold	≤16 mm	21.74	94.34	3.84	0.83	62.50	94.34	72.37
Men (n=23)								
Mid-arm circumference	≤24 cm	20.00	83.33	1.20	0.96	25.00	83.33	69.57
Mid-thigh circumference	≤48 cm	20.00	38.89	0.33	2.06	8.33	38.89	34.78
Hip circumference	≤88 cm	10.00	69.23	0.33	1.30	20.00	69.23	43.48
Waist/hip ratio	≤0.90	20.00	38.89	0.33	2.06	8.33	38.89	34.78
Biceps skinfold	≤3 mm	20.00	66.67	0.60	1.20	14.29	66.67	56.52

7.5 Discussion

This is the first study to develop simple diagnostic measures for lipodystrophy using longitudinal changes in DXA-derived measures of body fat composition as the reference standard. We found that simple anthropometric measures performed well for defining lipoatrophy in both men and women. The best predictors of lipoatrophy in women were hip and mid-thigh circumference, and thigh and calf skinfold thickness; and mid-arm circumference in men. The proportion of men and women who developed DXA-defined lipoatrophy increased over the 24 month period, but it occurred more frequently in men, possibly due to their lower baseline BMI as well as having less body fat, especially in the limbs. The proportion of men and women with DXA-defined lipohypertrophy increased until 18 months on ART, before decreasing. The best predictor of lipohypertrophy in men was sagittal abdominal diameter, and no anthropometric measure was useful for predicting lipohypertrophy in women. We found very poor agreement between DXA-defined lipodystrophy and patient report, which grossly under-estimated lipodystrophy.

Patient reports, using standardised questionnaires [6,20], are commonly used to assess lipodystrophy in low- and middle-income countries [25-27]. Results from studies comparing patient report to clinical examination have been contradictory [13-16]. One study reported a high level of agreement between patient and physician reported lipoatrophy and DXA-measured limb fat [17]. We on the other hand, found very poor agreement between self-report and DXA-defined lipodystrophy, with the proportion of participants with lipoatrophy and lipohypertrophy by self-report being far less than when defined by change in DXA measures in both men and women. These findings highlight the subjective nature of self-report and suggest that the incidence of lipodystrophy in African studies may have been underestimated when the diagnosis was based solely on self-report.

In low- and middle-income countries zidovudine continues to be used and stavudine was widely used until recently [28]. Lipoatrophy remains a common antiretroviral adverse drug reaction as both these drugs, especially stavudine, are associated with the development of lipoatrophy [26,29-32]. Accurate identification of lipoatrophy is important as it is

independently associated with increased vascular risk [2-4]. Objective measures based on DXA-derived variables have been developed to define lipodystrophy in France [33], Portugal [34], and India [34,35]. These cross sectional studies, which consisted mainly of men, proposed that fat mass ratio (FMR), defined as the ratio of the percentage of the trunk fat mass to the percentage of the lower limb fat mass measured by DXA, be used for the early diagnosis of lipodystrophy [33-35]. These measures may not be generalizable to sub-Saharan Africa, where black South African women have less visceral adipose tissue than white South African women, despite being more insulin resistant and showing a different metabolic phenotype in sub-Saharan Africa [18]. Using an earlier cross sectional study consisting of 550 participants on ART [11], our group developed objective measures for defining lipoatrophy and lipohypertrophy based on anthropometry and DXA-derived variables. We found the anthropometric variables triceps and thigh skinfold thickness to be the best predictors of lipoatrophy in women. However, all measures used patient report as the reference standard and may therefore be subject to the bias evident in self-report.

To our knowledge no prior studies have used change from baseline in DXA-derived variables as the reference standard to define lipoatrophy and lipohypertrophy in order to develop cut-points for anthropometric measures. We showed that several anthropometric measures could be used to diagnose lipoatrophy in women (hip, mid-thigh, and mid-arm circumferences; and thigh and calf skinfold), but mid-arm circumference was the only measure that had a ROC AUC of ≥ 0.65 in men. Given that skinfold measurement requires equipment and training to ensure accuracy, we propose the use of hip, mid-thigh, and mid-arm circumferences in women, and mid-arm circumference in men for defining lipoatrophy in African studies. Sagittal abdominal circumference was the only anthropometric measure that defined lipohypertrophy, and only achieved a ROC AUC of ≥ 0.65 in men.

We showed that in both men and women, weight, waist circumference, sagittal abdominal diameter (a predictor of visceral fat [36]) and abdominal skinfold thickness increased significantly over time. After 18 months on ART more than half the women, and almost half the men had developed lipohypertrophy as defined by ≥ 20% increase in trunk fat. Even though lipohypertrophy does not appear to be an antiretroviral adverse drug reaction, and is

instead thought to be a consequence of treating the HIV infection [32], detecting increased trunk fat is clinically relevant as it has been shown to be associated with an increased risk of cardiovascular disease [2-4] and death in HIV-infected populations [37].

There are some limitations to our study. There were no ART naive or HIV-uninfected control participants. The study sample was relatively small, with few participants being exposed to protease inhibitors, so only inferences about first-line ART can be made. Finally, rates of loss to follow up were high. Despite these limitations, ours is the only study to use longitudinal changes from baseline in fat distribution measured by DXA to develop cut-points of simple anthropometric measures for diagnosing lipoatrophy and lipohypertrophy.

7.6 Conclusion

We developed anthropometric cut-points for defining lipodystrophy in South African HIV-infected people on ART. These measures could be used in sub-Saharan Africa to identify lipoatrophy in both sexes and lipohypertrophy in men. Further research in other African countries is needed to validate these cut-points.

7.7 Acknowledgements

Authors' Contributions

ZA conducted all statistical analyses, interpreted the findings and drafted the manuscript; JD, NL and GM designed and conducted the study; NL, GM and JD edited the manuscript and drafted revisions. All authors read and approved the manuscript.

Financial competing interests

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that opinions, findings and conclusions or recommendations expressed in any publication generated by the NRF supported research are that of the author(s), and that the NRF accepts no liability whatsoever in this regard.

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7.9 Supplementary Tables and Figures

Supplementary Table 1. Anthropometric cut-points to identify lipohypertrophy in men at 18 months on ART. Anthropometric measures were selected for cut-point determination if their ROC AUC was ≥0.65.

Supplementary Figure 1. ROC curves for anthropometric measures of lipoatrophy in women and men at 12, 18 and 24 months on ART.

Supplementary Figure 2. ROC curves for anthropometric measures of lipohypertrophy in women and men at 12, 18 and 24 months on ART.

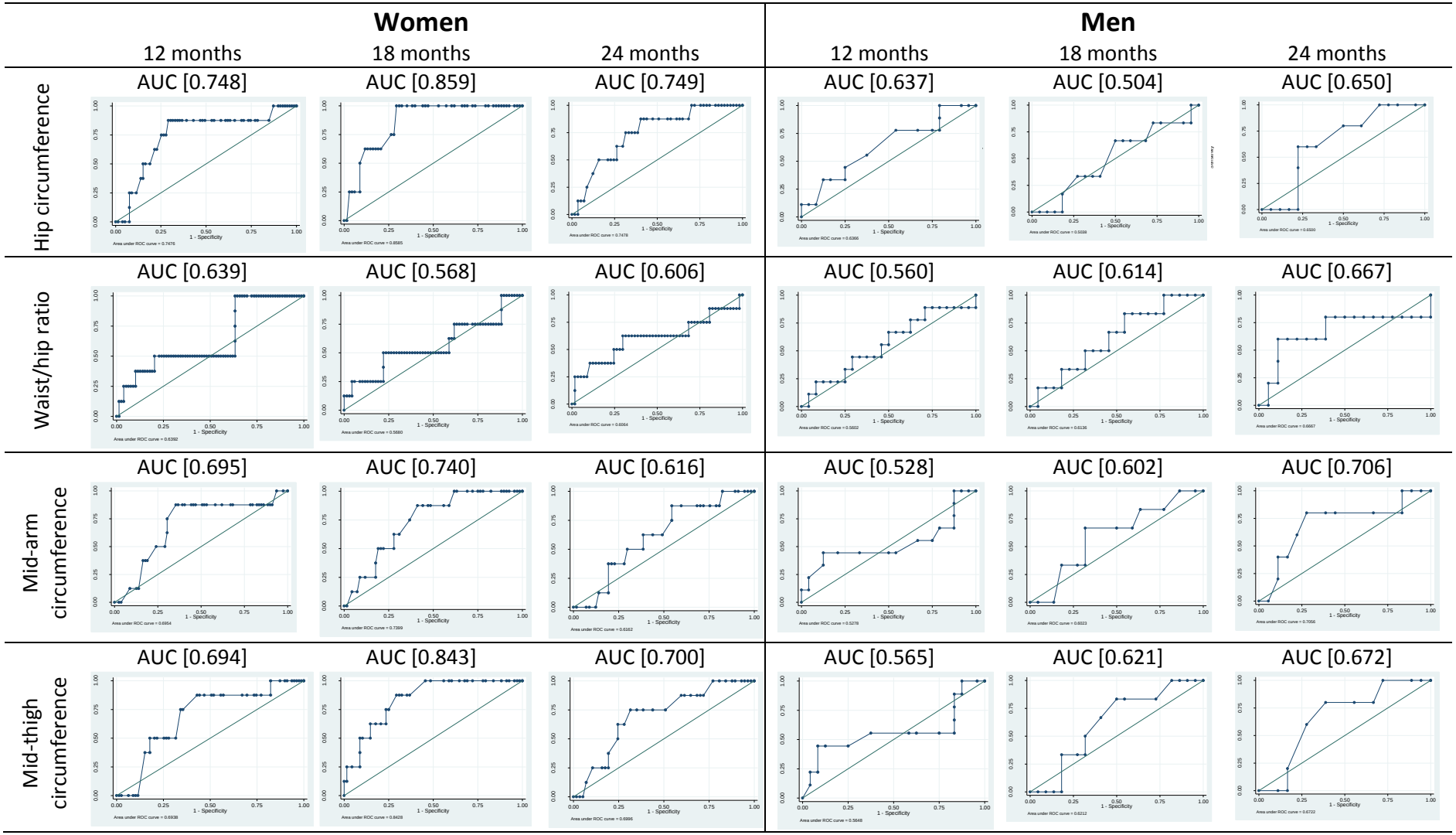
Supplementary Figure 3. ROC curves for anthropometric measures of lipoatrophy in the combined sample of women and men at 12, 18 and 24 months on ART.

Supplementary Figure 4. ROC curves for anthropometric measures of lipohypertrophy in the combined sample of women and men at 12, 18 and 24 months on ART.

Supplementary Table 1. Anthropometric cut-points to identify lipohypertrophy in men at 18 months on ART. Anthropometric measures were selected for cut-point determination if their ROC AUC was ≥0.65.

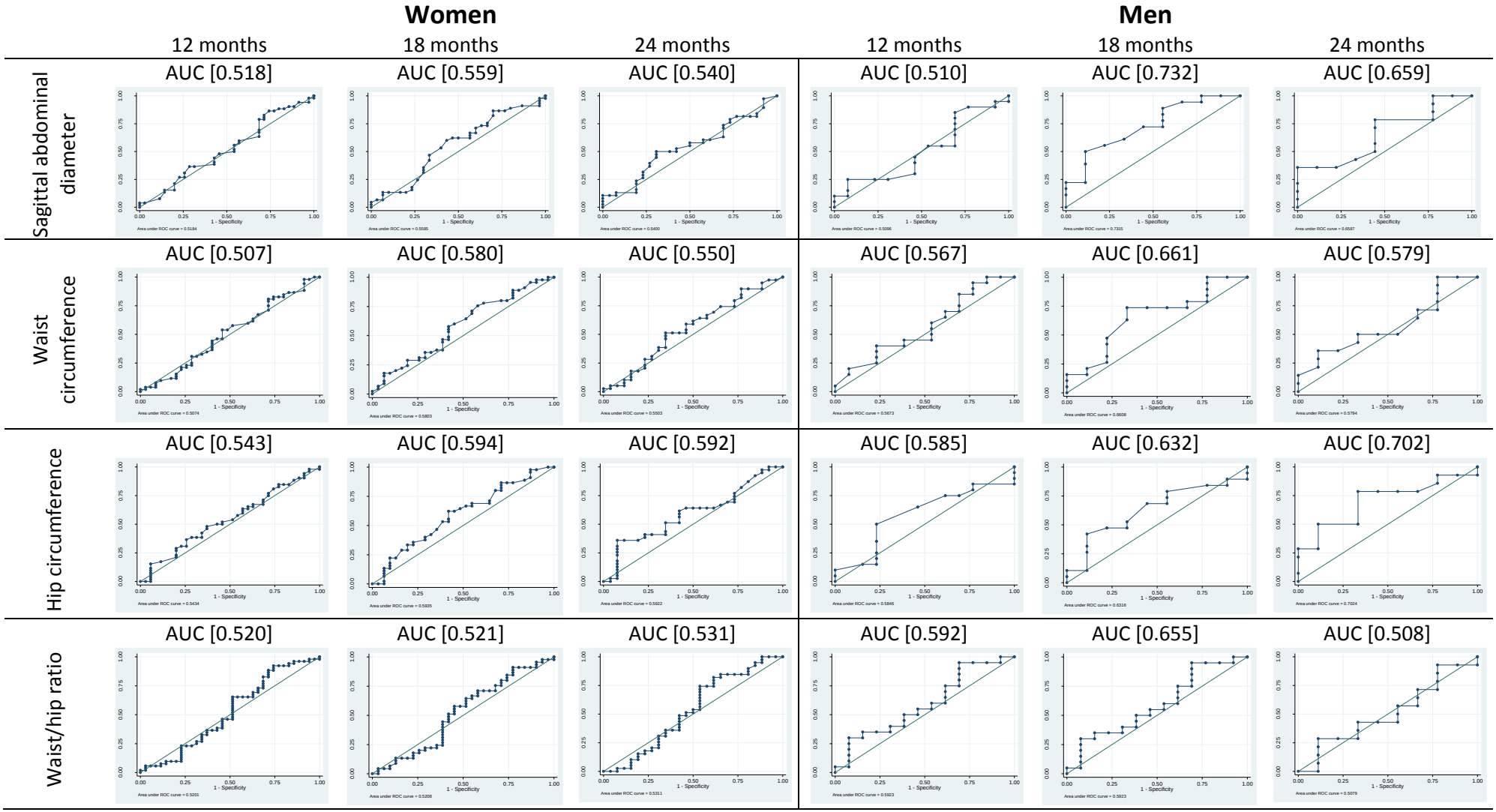
	Cut-point	**Sensitivity** (%)	**Specificity** (%)	**Likelihood ratio positive test**	**Likelihood ratio negative test**	**Positive predictive value** (%)	**Negative predictive value** (%)	**% correctly classified**
Men (n=23)								
Sagittal abdominal diameter	≥18cm	73.91	75.00	2.96	0.35	94.0	33.0	17.11
Waist circumference	≥80cm	80.00	46.15	1.49	0.43	63.0	67.0	12.22
Waist/hip ratio	≥0.84	73.68	44.44	1.33	0.59	74.0	44.0	14.14

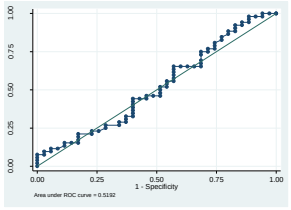
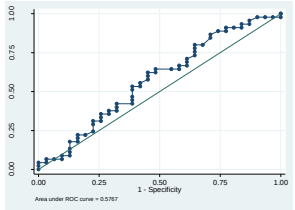
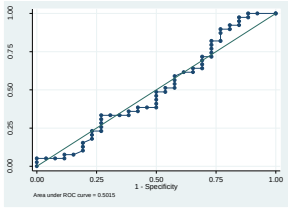
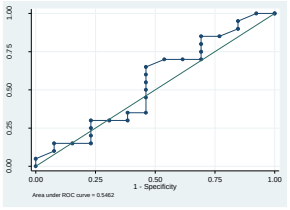
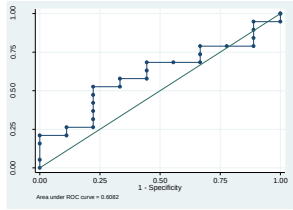
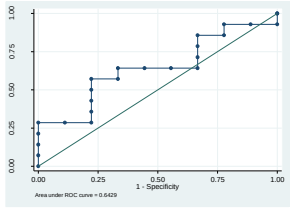
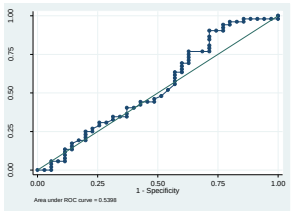
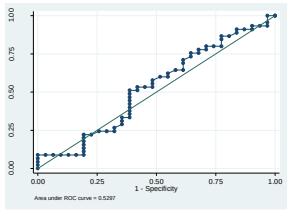
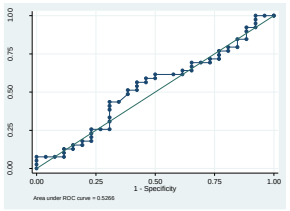
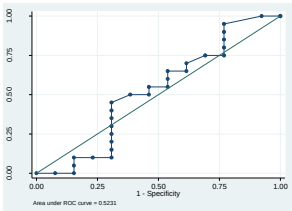
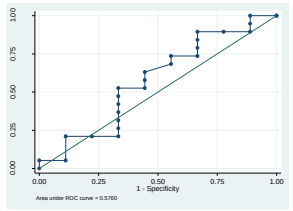
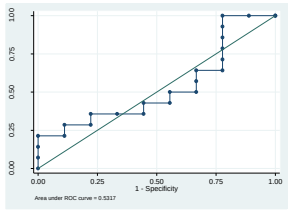
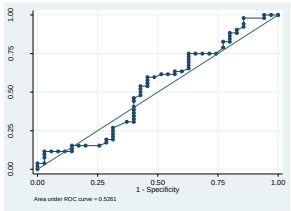
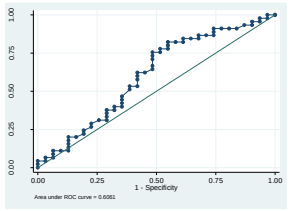
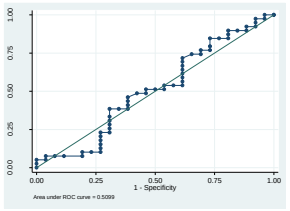
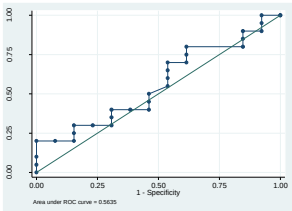
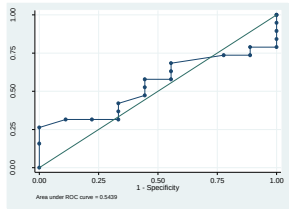
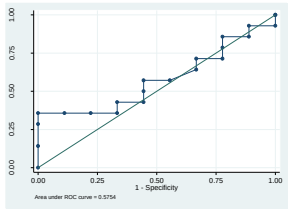
Supplementary Figure 1. ROC curves for anthropometric measures of lipoatrophy (defined as ≤20% limb fat loss) in women and men at 12, 18 and 24 months on ART.



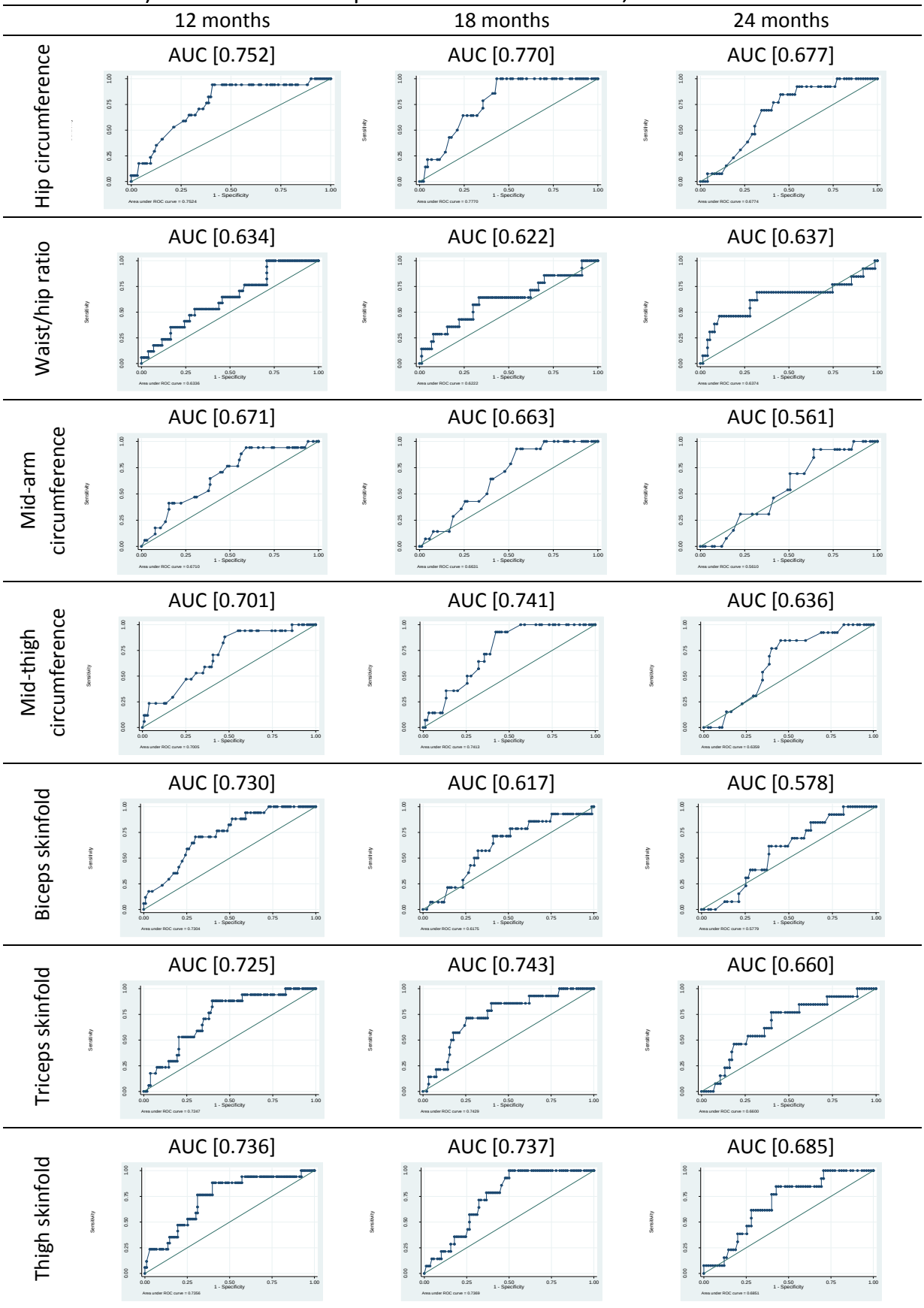
Biceps skinfold	AUC [0.711]	AUC [0.605]	AUC [0.625]	AUC [0.697]	AUC [0.546]	AUC [0.667]
Triceps skinfold	AUC [0.714]	AUC [0.764]	AUC [0.666]	AUC [0.542]	AUC [0.617]	AUC [0.500]
Thigh skinfold	AUC [0.725]	AUC [0.814]	AUC [0.730]	AUC [0.583]	AUC [0.553]	AUC [0.489]
Calf skinfold	AUC [0.677]	AUC [0.791]	AUC [0.680]	AUC [0.597]	AUC [0.519]	AUC [0.506]

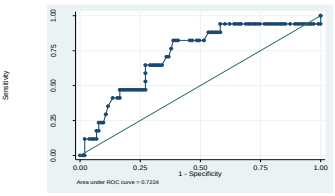
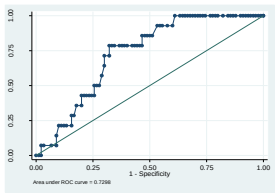
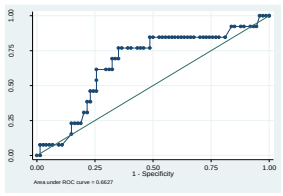
Supplementary Figure 2. ROC curves for anthropometric measures of lipohypertrophy (defined as ≤20% trunk fat gain) in women and men at 12, 18 and 24 months on ART.



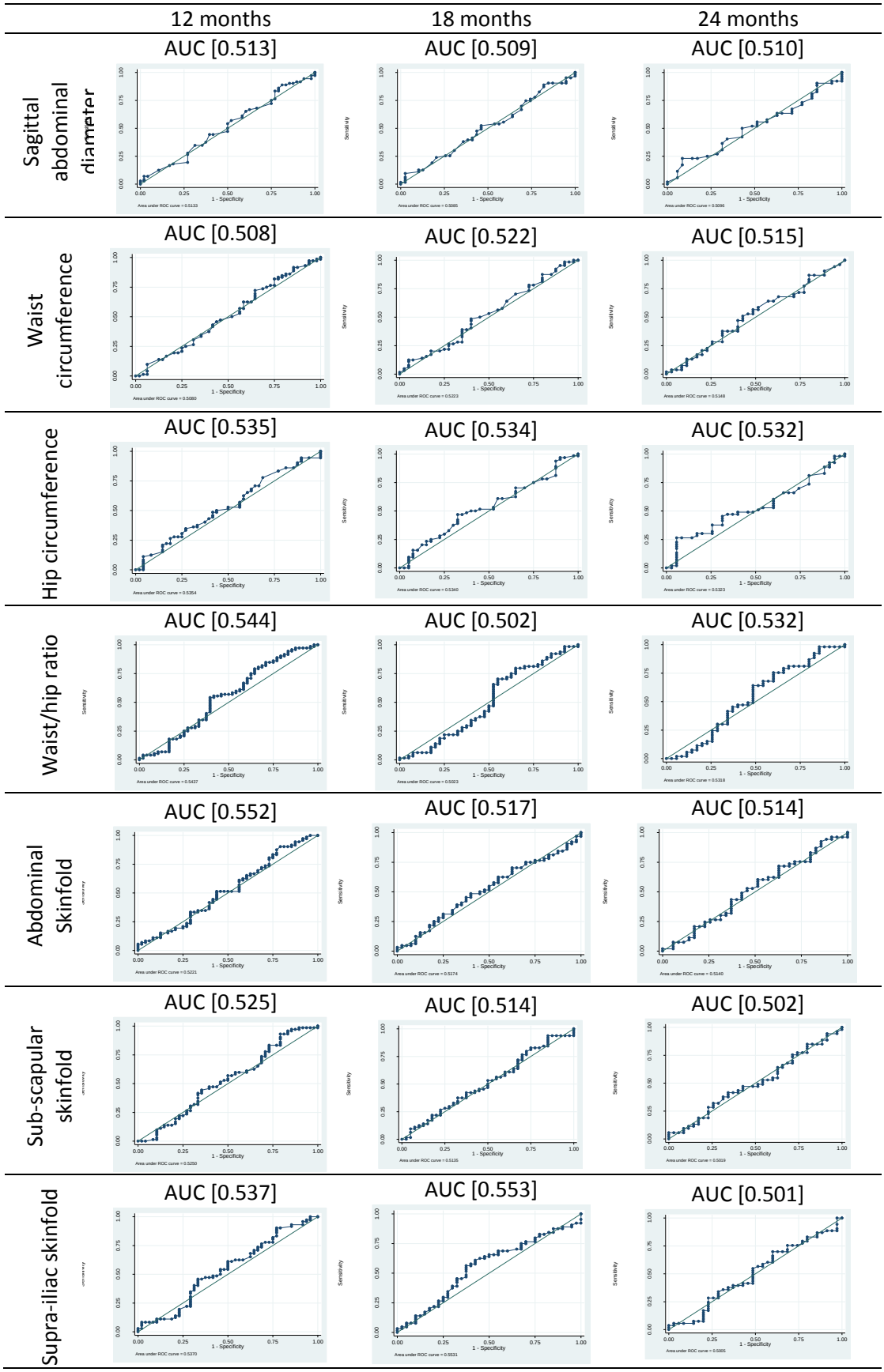
Abdominal Skinfold	AUC [0.519]	AUC [0.577]	AUC [0.502]	AUC [0.546]	AUC [0.608]	AUC [0.506]
						
Sub-scapular skinfold	AUC [0.540]	AUC [0.530]	AUC [0.527]	AUC [0.523]	AUC [0.576]	AUC [0.532]
						
Supra-iliac skinfold	AUC [0.526]	AUC [0.606]	AUC [0.510]	AUC [0.563]	AUC [0.544]	AUC [0.575]
						

Supplementary Figure 3. ROC curves for anthropometric measures of lipoatrophy (defined as ≤20% limb fat loss) in the combined sample of women and men at 12, 18 and 24 months on ART.



Calf skinfold	AUC [0.722]	AUC [0.730]	AUC [0.663]
			

Supplementary Figure 4. ROC curves for anthropometric measures of lipohypertrophy (defined as ≤ 20% trunk fat gain) in the combined sample of women and men at 12, 18 and 24 months on ART.



Chapter 8. Summary and Recommendations

This chapter presents a summary of the key findings in relation to the thesis aims and objectives, and its implications and recommendations for clinical practice, policy change and future research

8.1 Thesis aim and methods

This thesis investigated specific metabolic complications associated with the use of ART (hypertension, dysglycaemia and lipodystrophy), and thereafter developed simple anthropometric cut-points to assist in identifying those with lipodystrophy. Data for this thesis came from three datasets (one cross sectional and two longitudinal studies), collected between 2007 and 2013 of black HIV-infected men and women presenting to ART clinics in Cape Town.

To investigate the metabolic complications associated with the use of ART, a longitudinal dataset consisting of 103 women who had spent a median of 6.8 years on ART, was used. The complications assessed were: hypertension, defined as BP ≥140/90 mmHg or using antihypertensive agents; dysglycaemia using an oral glucose tolerance test (OGTT); insulin resistance estimated by HOMA-IR; β-cell function using the insulinogenic index (IGI) and the oral disposition index (DI_o); lipoatrophy defined as ≥ 20% loss of limb fat from baseline; and lipohypertrophy defined as ≥ 20% gain in trunk fat from baseline by DXA scan. Data were analysed using descriptive statistics which included non-parametric t-tests and McNemar chi-square t-tests for paired data. The second longitudinal dataset, consisting of 55 men and 132 women who were ART-naïve at baseline, was used to describe the changes in fat distribution over a 24 month period. Data analysis included (1) the Jonckheere-Terpstra test for ordered variables which was used to measure trends over time, (2) the Kaplan-Meier estimator which was used to assess differences in time to lipoatrophy and lipohypertrophy in men and women, (3) the Cox proportional hazards regression model and logistic regression model which was used to analyse the contribution of a number of *a priori* variables to the contribution of

lipoatrophy and lipohypertrophy, and (5) latent class mixed effects models which were used to estimate the mean longitudinal trajectories in potentially heterogeneous populations.

The cross sectional dataset, consisting of 116 men and 434 women on ART, was used to develop simple measures for defining lipoatrophy and lipohypertrophy using patient report as the reference standard. Data analysis consisted of descriptive statistics and receiver operating characteristic (ROC) curves, which were used to describe the performance of a number of anthropometric and DXA-derived variables. As patient report is a subjective method of assessment, a longitudinal dataset consisting of 187 participants, was used to validate the measures that were developed, this time using change in DXA measures as the reference standard. Data analysis consisted of descriptive statistics, the Jonckheere-Terpstra test for ordered variables which was used to measure trends over time, and ROC curves which were used to describe the performance of a number of anthropometric variables using change in DXA measures as the reference standard.

8.2 Summary findings

In summary, antiretroviral therapy was significantly associated with the development of metabolic complications, and anthropometric cut-points were developed that successfully predicted lipodystrophy.

The key findings are as follows:

1. There were significant changes in both systolic and diastolic blood pressure over time
2. Plasma glucose and insulin concentrations, as well as markers of insulin sensitivity and β-cell function changed significantly during the five year follow-up period
3. Change in body fat distribution following ART initiation occurred differently in men and women
4. When using patient report as the reference standard, the best predictors of lipoatrophy in women were triceps and thigh skinfold thickness, while lipohypertrophy was best defined by waist/hip ratio

5. When using change in DXA measures as the reference standard, thigh skinfold and mid-arm circumference correctly classified the greatest number of women with lipoatrophy, and mid-arm circumference correctly classified the greatest number of men with lipoatrophy
6. There was poor agreement between patient-reported lipodystrophy and DXA-derived lipodystrophy

Details and an in-depth discussion of these key findings can be found in the 3 published and 1 unpublished paper (Chapter 4-7). These findings and their interpretations are summarised as follows:

8.2.1 ART is associated with an increase in blood pressure levels

Median systolic and diastolic blood pressure increased significantly ($p<0.001$) during the five year follow-up period. While only 4% of participants had hypertension at baseline, the proportion significantly ($p<0.001$) increased to 15.5% after 5.5 years. The antiretroviral drugs stavudine, efavirenz and nevirapine were significantly ($p<0.001$) associated with the development of hypertension. These findings could be used to inform clinical practice in public health facilities, where efavirenz and nevirapine are still being used as part of the first-line ART treatment regimen.

8.2.2 ART is associated with an increase in diabetes

Plasma glucose concentrations showed a significant increase from baseline to follow-up at both 30 minutes ($p=0.040$) and 120 minutes ($p=0.028$), while plasma insulin concentration showed significant increases at 0 minutes ($p=0.009$) and 30 minutes ($p<0.001$). HOMA-IR increased from baseline to follow-up, indicating a decrease in insulin sensitivity. In addition, both IGI and DI_o increased significantly ($p<0.001$), indicating a rise in insulin secretion in relation to insulin resistance. While the proportion of participants with dysglycaemia did not change from baseline to follow-up, the proportion of participants with diabetes increased (1% to 7.5%), as did the proportion of participants with impaired glucose tolerance (5.8% to 9.6%). The development of diabetes and dysglycaemia were significantly associated with the antiretroviral drugs stavudine, efavirenz and nevirapine. As stavudine has been implicated in

the development of insulin resistance and diabetes in large studies from high-income countries [1,2] as well as a recent study from South Africa [3], and has only recently been phased out in South Africa, and efavirenz and nevirapine continue to be used as part of the first-line ART treatment regimen, these findings should be used as motivation for regular monitoring of glucose levels in patients on first-line ART treatment in South Africa.

8.2.3 ART is associated with changes in body fat distribution

Women who were on ART for a median of 16 months at baseline, experienced no change in weight or BMI during the 5.5 year follow-up period. However they had significant changes in fat distribution; with increases in abdominal, sub-scapular and supra-iliac skinfold thickness ($p<0.001$), as well as waist ($p=0.038$), hip ($p<0.001$) and mid-thigh ($p<0.001$) circumference. In addition, they experienced a significant decrease in their biceps ($p=0.011$), triceps ($p=0.007$), thigh and calf skinfold ($p<0.001$) thickness. However, when using a longitudinal study of men and women who were ART-naïve at baseline, both men and women experienced significant increases in weight ($p<0.009$) and BMI ($p<0.002$) following ART initiation. Women, experienced a significant increasing trend in trunk fat and a significant decreasing trend in limb fat, when expressed as a percentage of total body fat. These changes suggest centralisation of body fat, in addition to peripheral wasting, and is in agreement with numerous studies from sub-Saharan Africa [4-6]. In addition, these findings indicate that following ART initiation, patients experience a return to health as evidenced by an increase in weight and BMI. However, after several months on ART, weight and BMI stabilise, while limb fat continues to decrease and trunk fat continues to increase, thus indicating the continued development of lipoatrophy and lipohypertrophy. These findings highlight the need for a simple, inexpensive tool that can be used to assess lipoatrophy and lipohypertrophy in men and women.

8.2.4 Changes in body fat distribution occurs differently in men and women on ART

Women, compared to men gained significantly more overall weight (9kg vs. 4kg) and more regional fat in all areas analysed on DXA scans. Cox proportional hazards regression modelling showed an almost significant ($p=0.060$) association between gender and the development of

lipoatrophy. Men had a 2.9 times greater risk of developing lipoatrophy compared to women, after adjusting for age, baseline BMI and ART regimen. Women, but not men, experienced a significant decrease in limb fat when expressed as a percentage of total body fat ($p<0.001$ vs. $p=0.248$), and a significant increase in trunk fat percentage ($p<0.001$ vs. $p=0.085$). However, the incidence of lipoatrophy when expressed as a categorical variable was higher in men than women (20% vs. 9.4%; $p=0.066$), while the incidence of lipohypertrophy, when expressed as a categorical variable, occurred similarly in men (60%) and women (57%). Further investigation is required to understand the mechanisms underlying the sex differences in changes in body fat distribution and its effects on cardiovascular risk.

8.2.5 Development of objective measures to define lipoatrophy and lipohypertrophy

Using patient report as the reference standard, triceps and thigh skinfold thickness correctly identified the greatest percentage of women with lipoatrophy (68.7% and 65.7%, respectively), while waist/hip ratio correctly identified the greatest percentage with lipohypertrophy (64.8%). When using change in DXA measures as the reference standard, thigh skinfold thickness and mid-arm circumference correctly identified the greatest percentage of women with lipoatrophy (80.3% and 77.6%, respectively), and mid-arm circumference correctly identified the greatest percentage of men (69.6%) with lipoatrophy. These finding prove that simple measures of anthropometry can successfully be used to diagnose lipoatrophy and lipohypertrophy in men and women in South Africa.

8.2.6 Poor agreement between patient-reported lipodystrophy and DXA-defined lipodystrophy

The incidence of lipodystrophy by patient report was far lower than when defined by DXA measures. Based on patient report, only one man and one women developed lipoatrophy. However, when using change in DXA measures (defined as ≥ 20% limb fat loss), 11 women and 9 men were diagnosed with lipoatrophy. Similarly, fewer men and women (17 and 3, respectively) reported having lipohypertrophy compared to when lipohypertrophy was diagnosed using change in DXA measures (defined as ≥ 20% trunk fat gain) as the reference standard (25 men and 65 women). These findings highlight the subjective nature of self-report

and suggest that the incidence of lipodystrophy may be underestimated when the diagnosis is based solely on self-report.

8.3 New information (or contributions) from this thesis

Based on the summary findings of this thesis, the following new information emerged:

- Antiretroviral therapy may be associated with an increased risk of diabetes and hypertension in black South Africans
- Black men are at a greater risk of developing lipoatrophy when compared to black women
- There is poor agreement between patient reported lipodystrophy and DXA-defined lipodystrophy
- This is the first study in Africa to develop tools to define lipoatrophy and lipohypertrophy
- Anthropometric variables can be used to diagnose ART-related lipoatrophy and lipohypertrophy in South African men and women

8.4 Implications for clinical practice and policy change

The findings of this thesis showed that long-term use of ART results in an increase in both systolic and diastolic blood pressure, as well as an increase in the prevalence of hypertension and possibly diabetes. Furthermore, the findings showed that hypertension, dysglycaemia and diabetes were associated with the drugs stavudine, efavirenz and nevirapine, and diabetes was additionally associated with the drug zidovudine. During the coming years, the number of ART users are predicted to increase in low- and middle-income countries as they expand their ART programmes in following the World Health Organisation's recommendation to 'test and treat' [7] by initiating all HIV-infected patients, irrespective of CD4 count. However, as stavudine has only recently been phased out, and efavirenz and nevirapine

continues to be used in South Africa as part of their first-line ART treatment regimen [8], the incidence of antiretroviral-associated hypertension and diabetes may also increase. Even small increases in the prevalence of hypertension and diabetes may result in considerable public health impact on the prevalence of cardiovascular disease. These findings could be used to inform clinical practice in public health facilitiessuch asregular monitoring of blood pressure and blood sugar levels in patients on ART.

This thesis showed that more than 20% of men developed lipoatrophy; that the risk of development was far greater in men compared to women; and that the time to development did not differ by gender. On the other hand, lipohypertrophy occurred in more than half the participants, and occurred similarly in men and women. Both men and women, to varying degrees, experienced an increase in central fat and a decrease in subcutaneous fat, indicative of lipoatrophy and lipohypertrophy. The development of lipodystrophy is associated with an increase in cardiometabolic risk factors such as dyslipidaemia and dysglycaemia. Patients with lipodystrophy may develop impaired glucose tolerance and insulin resistance, as well as increased levels of triglycerides and decreased levels of HDL cholesterol. These findings have important implications for the management of HIV in Africa, as the early identification and management of these cardio-metabolic risks are crucial in the region with the highest HIV-infected population in the world.

This is the first study in Africa to develop tools to identify lipoatrophy and lipohypertrophy. This thesis showed that a number of anthropometric variables can be used to diagnose lipoatrophy and lipohypertrophy with varying degrees of accuracy. Those developed using change in DXA measures (i.e. hip, mid-thigh and mid-arm circumference) are likely to be more accurate than the measures developed using patient report as the reference standard (i.e. triceps and thigh skinfold thickness), due to the subjectivity of patient report. While training and well maintained skinfold callipers are necessary to accurately identify patients with lipoatrophy using triceps and thigh skinfold thickness, the circumferences are easily and quickly measured, and require minimal training and only an inexpensive tape measure. These measures are of particular relevance in resource limited settings such as South Africa, where

health professionals are overburdened and need simple, inexpensive and quick methods for diagnosing patients.

This thesis showed very poor agreement between self-report and DXA-defined lipodystrophy, with the proportion of participants with lipoatrophy and lipohypertrophy by self-report being far less than when defined by change in DXA measures in both men and women. Currently in South Africa, lipoatrophy and lipohypertrophy are diagnosed by patient report, and occasionally by a healthcare professional. These findings highlight the subjective nature of self-report and suggest that the incidence of lipodystrophy in African studies may have been underestimated when the diagnosis was based solely on self-report.

8.5 Clinical practice and policy recommendations

This section outlines recommendations for clinical practice and policy, based on the findings of the thesis. The overarching recommendation is to regularly monitor patients taking antiretrovirals, to ensure the early identification and treatment of the metabolic complications of ART.

1. Regular monitoring of blood pressure and glucose levels will be required to identify hypertensive and diabetic patients as early as possible. However, these recommendations are of particular concern in South Africa where healthcare facilities, based on national guidelines and in an attempt to decongest healthcare facilities [9], are now offering stable ART patients the option of collecting their medication from external service providers for a period of 6 months before returning to the healthcare facility for renewal of the prescription. These national guidelines only allow for annual assessment of patients. Hence, by following these guidelines, patients who develop elevated blood pressure or glucose levels as a result of their medication, risk going undetected for many months. To ensure the early identification and treatment of these metabolic complications, regular monitoring of blood pressure and glucose levels in patients on ART is recommended. While blood pressure is easy to monitor, providing ongoing screening for diabetes is more difficult. Fasting blood glucose levels

alone may miss patients with diabetes who have an abnormal 120 minute OGTT. However, an OGTT is cumbersome and takes 120 minutes to complete. An HbA1c test, while using a non-fasting blood sample, requires a laboratory test. In addition, the diagnostic cut-points used may not be applicable to patients on ART.

2. The findings of this thesis showed that patient-reported lipodystrophy had a tendency to underestimate the true incidence of lipoatrophy and lipohypertrophy. As lipodystrophy is subjectively measured by patient report in South Africa, and both lipoatrophy and lipohypertrophy are frequently found in patients on ART, objective tools for identifying those with lipoatrophy and lipohypertrophy are needed. Operational Managers at healthcare facilities in South Africa need to ensure that all ART patients undergo a clinical examination using objective measures to diagnose lipodystrophy before new prescriptions are dispensed.

3. In addition to regular monitoring of blood pressure and glucose levels, body fat changes in ART patients need to be identified. This will require the use of simple, inexpensive tools that require minimal training, and can be performed by an enrolled nurse, but can accurately diagnose both lipoatrophy and lipohypertrophy in men and women. The anthropometric cut-points developed in this thesis meets these requirements. The enrolled nurse in charge of the vitals station at healthcare facilities should be trained to measure circumferences of ART patients in addition to the normal weight and height measurements. This will help track changes in weight and BMI as well as limb fat loss and trunk fat gain.

4. After 24 months on ART, more than half the men and women enrolled in the study had developed lipohypertrophy. As central fat gain is a known cardiometabolic risk factor, patients who develop an enlarged waist circumference are potentially at risk of developing dyslipidaemia and dysglycaemia. It is thus essential to monitor glucose and cholesterol levels regularly. These measurement should be incorporated into the annual assessment patients attending public healthcare facilities need to undergo. In addition, patients should be provided with counselling and advice regarding

preventable risk factors that may impact their cardiovascular risk such as smoking cessation and dietary management to help decrease their risk of developing cardiovascular disease.

5. Before ART initiation, a large number of woman were either overweight or obese. After 24 months on ART, the proportion of overweight or obese women had increased substantially. Studies on weight perception in HIV-infected women reported a mismatch between actual weight and weight perception [10,11], with a tendency for women to judge themselves as less than their actual weight. In South Africa, HIV-infected women continue to experience discrimination, which may be the cause of intentional obesity as a mechanism of limiting the stigma [12]. While high levels of obesity have been reported in uninfected women as well, it is still of concern. These patients may benefit from regular weight assessments and counselling on the importance of exercise and a healthy diet when their weight increases. Adherence clubs could act as a platform for group counselling sessions so that individuals do not feel targeted.

8.6 Recommendations for future research

1. Further research using large prospective cohorts with adequate numbers on modern drug regimens as well as an HIV-naïve and an uninfected control is needed to confirm the increased risk of diabetes and hypertension found in this study; to identify specific drug interactions; and to confirm the greater risk of lipoatrophy in men compared to women.

2. Additional research is also required to understand the mechanisms underlying the sex differences in changes in body fat distribution and its effects on cardiovascular risk

3. As this is the first study in Africa to develop tools to define lipoatrophy and lipohypertrophy, these measures need to be validated in other cohorts in Africa using a longitudinal study design and an objective measure as the reference standard

8.7 Strengths and limitations of the studies

The study findings need to be interpreted within the context of its strengths and limitations. The cross sectional study design (Chapter 6), while allowing us to make associations, did not allow us to infer causality. With changes in fat distribution, repeated objective measures would have given us a better reference standard than patient report, even though patient report is commonly used. We did not have enough men with lipoatrophy or lipohypertrophy, to explore predictive anthropometric and DXA-derived variables. Finally, the likelihood ratios for the most predictive anthropometric and DXA-derived variables were only weakly diagnostic of self-report lipoatrophy and lipohypertrophy. In spite of these limitations, the sample size was relatively large, and unlike most studies from Africa, we used DXA measurements as well as anthropometry and patient self-report to define lipoatrophy and lipohypertrophy.

The lack of HIV-uninfected and ART-naïve control groups in Longitudinal Study A (Chapter 4) limited our ability to attribute the changes observed to the use of ART. The sample consisted exclusively of women, which prevented us from investigating whether hypertension, glycaemia and fat redistribution developed differently by gender. The sample size was also relatively small, which limited our ability to assess whether the increased prevalence of diabetes over time was significant. Despite its many limitations, this study has a few strengths. This is one of very few studies in Africa to follow women on ART for over 5 years, and to use an OGTT to define dysglycaemia.

Longitudinal study B (Chapter 5 and 7) was a non-randomised study, and there were no untreated or uninfected controls for comparison. Few participants were exposed to protease inhibitors, so only inferences about first-line ART could be made. The study sample was relatively small, with a high rate of loss to follow-up, resulting in a lack of power. Regardless of these limitations, this is the only study from Africa to use longitudinal data, and to collect DXA measurements at six time points during the 24 month period. The sample consisted of both men and women, which allowed us to assess how fat redistribution differed by gender. This is also the only study that used longitudinal changes from baseline in fat distribution

measured by DXA to develop cut-points of simple anthropometric measures for diagnosing lipoatrophy and lipohypertrophy.

As stavudine has already been discontinued in South Africa and the number of patients on zidovudine are minimal and may be replaced by a less toxic drug in the near future, the findings of this thesis are unfortunately not as important as they would have been 10 years ago. As the metabolic complications observed in this thesis were primarily related to the use of stavudine and zidovudine, we may see a decrease in their prevalence in the future.

8.8 References

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Appendices

Appendix 1

Abrahams Z, Dave J, Maartens G, Levitt N. Changes in blood pressure, glucose levels, insulin secretion and anthropometry after long term exposure to antiretroviral therapy in South African women. AIDS Res Ther 2015;12:24.

Appendix 2

Abrahams Z, Levitt N, Lesosky M, Maartens G, Dave J. Changes in body fat distribution on dual-energy x-ray absorptiometry in black South Africans starting first-line antiretroviral therapy. AIDS Patient Care STDs 2016;30(10);455-462.

Appendix 3

Abrahams Z, Dave J, Maartens G, Lesosky M, Levitt N. The development of simple anthropometric measures to diagnose antiretroviral therapy-associated lipodystrophy in resource limited settings. AIDS Res Ther 2014;11:26.