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ASSESSING HIV LIPODYSTROPHY SYNDROME: A COMPARISON OF DIFFERENT METHODS TO AN OBJECTIVE CASE DEFINITION

by

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'Africa carries 25% of the world's disease burden yet has only 3% of the world's health workers and 1% of the world's economic resources to meet that challenge' [1]

I would like to acknowledge the following people for the role they played in the successful completion of this research project:

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ABSTRACT

Background: Morphological changes of Human Immunodeficiency Virus lipodystrophy syndrome (HIV LDS) are said to be the new face of HIV / Acquired Immune Deficiency Syndrome (AIDS) which may negatively influence the adherence to Highly Active Antiretroviral Therapy (HAART). Methods of assessing HIV LDS vary among researchers and practitioners who make it difficult to compare incidence and prevalence figures among clinics in South Africa. Instruments available in other countries to assess HIV LDS are costly and time consuming for the clinical setup. A standard, objective and practical instrument is needed for the South African clinical setup to address this problem.

Objectives: The primary objective of the study was to assess the agreement between methods routinely used to classify HIV LDS in the clinical setup (i.e. National Cholesterol Education Programme [NCEP] criteria, subjective self-reporting and routine anthropometry) to a published, objective case definition using diagnostic testing. The secondary objective was to develop and cross-validate a classification instrument for HIV LDS utilising parameters from the studied test methods.

Design and sample: The study was a cross-sectional, analytical and non-experimental analysis of 1421 HIV positive adult patients (69% female) enrolled on HAART. Of the 283 subjects who met the inclusion criteria, 253 consented to participate. Purposeful sampling was performed dividing the study population into a case (n=79) - and control (n=73) group according to a screening process. The dichotomous outcome (HIV LDS **(+)** or – **Θ**) of the tests – and reference method was statistically analysed by means of diagnostic testing. The new classification instrument was developed using logistic regression on all the variables and validated with a cross-validation technique.

Setting: Outpatient clinic at Kalafong Hospital, Gauteng Province, South Africa.

Results: *Primary objective*: The diagnostic properties (sensitivity, specificity, Kappa coefficient and p-value for McNemar's test respectively) of the test methods were as follows: NCEP criteria: (45%, 83%, 0.29 and 0.54); subjective self-reporting (74%, 59%, 0.26 and 0.00); Kotler anthropometry (71%, 52%, 0.18 and 0.00); routine anthropometry (62%, 54%, 0.12 and 0.00); and Dong&Hendricks anthropometry: (10%, 88%, 0.00 and 0.00). *Secondary objective*: A new, simple classification instrument (with limited blood samples) had the following diagnostic properties: (sensitivity 81% [71% validated], specificity 79% [75% validated], Kappa 0.54 [0.41 validated], McNemar's test of symmetry p=0.00, and the area under the receiver operating curve [AUC] was 0.88). A classification

instrument without blood samples had a sensitivity of 69%, specificity of 67%, Kappa = 0.310, McNemar's p= 0.00 and AUC= 0.75).

Conclusion: In a resource limited setting the NCEP criteria appears to be the "best" among the methods tested for identifying HIV LDS. Two newly developed instruments showed even better diagnostic properties. Use of these might lead to an accurate, consistent detection of HIV LDS in the typical South African setting. However, practical implications to the individual and the health care system still need to be investigated further. The results can also be used in longitudinal studies.

ABSTRAK

Agtergrond: Morfologiese veranderinge in die Menslike Immuniteitsgebreks Virus (MIV) lipodistrofie sindroom (MIV LDS) word beskryf as die nuwe beeld van MIV / Verworwe Immuniteits Gebrek Sindroom (VIGS) wat die korrekte gebruik van hoogs aktiewe anti-retrovirale behandeling (HAART) negatief kan beïnvloed. Die assessering van MIV LDS verskil tussen navorsers en gesondheidsorgwerkers wat dit moeilik maak om die insidensie en prevalensie tussen klinieke in Suid Afrika te vergelyk. Instrumente beskikbaar in ander lande om MIV LDS te bepaal is duur en tydsaam om te gebruik in die kliniese opset. ‘n Standaard, objektiewe en praktiese instrument is nodig om die problem aan te spreek in die Suid-Afrikaanse opset.

Doelwitte: Die primêre doelwit van die studie was om deur middel van diagnostiese toetsing die ooreenstemming tussen metodes wat algemeen gebruik word om MIV LDS in die kliniese opset (Nationale Cholesterol Opvoedings Program [NCEP] kriteria, subjektiewe self-rapportering en roetine-antropometrie) teenoor ‘n gepubliseerde objektiewe gevaldefinisie, te bepaal. Die sekondêre doelwit was om aan die hand van parameters uit die toetsmetodes ‘n klassifikasie-instrument vir MIV LDS te ontwikkel en te kruis-valideer.

Ontwerp en steekproef: Die studie was ‘n dwarsnit, analitiese, nie-eksperimentele analise van 1421 MIV positiewe volwasse pasïente (69% vrouens) op HAART. Van die 283 pasïente wat aan die insluitingskriteria voldoen het, het 253 toestemming gegee om deel te neem. Doelbewuste steekproefverdeling was gedoen om die studiepopulasie in ‘n geval (n=79) - en kontrole (n=73) groep te verdeel volgens ‘n siftingsprosedure. Die digitome uitkoms (MIV LDS **(+)** of – **Θ**) van die toets- en verwysingsmetodes was statisties geanaliseer d.m.v diagnostiese toetsing. Die nuwe klassifikasie-instrument was ontwikkel met behulp van logistiese regressie-analise van al die veranderlikes asook gevalideer m.b.v ‘n kruis-validasie tegniek.

Setting: Buite-pasïentkliniek Kalafong Hospital, Gauteng Provinsie, Suid Afrika.

Resultate: Primêre doelwit**:** Die diagnostiese eienskappe (sensitiwiteit, spesifisiteit, Kappa koeffisïent en p-waarde vir McNemar se toets respektiewelik) van die toetsmetodes was soos volg: NCEP kriteria: (45%, 83%, 0.29 en 0.54); subjektiewe self-rapportering (74%, 59%, 0.26 en 0.00); Kotler antropometrie (71%, 52%, 0.18 en 0.00); roetiene antropometrie (62%, 54%, 0.12 en 0.00) en Dong&Hendricks antropometrie:

(10%, 88%, 0.00 en 0.00). Sekondêre doelwit**:** ‘n Nuwe, eenvoudige klassifikasie-instrument (met beperkte bloedmonsters) het die volgende diagnostiese eienskappe getoon: (sensitiwiteit 81% [71% gevalideer], spesifisiteit 79% [75% gevalideer], Kappa 0.54 [0.41 gevalideer], McNemar se toets p=0.00 en die area onder die kurwe [AUC] was 0.88). ‘n Klassifikasie-instrument sonder bloedmonsters het ‘n sensitiwieit van 69% gehad, ‘n spesifisiteit van 67%, Kappa = 0.310, McNemar se p = 0.00 en AUC= 0.75).

Samevatting: In ‘n hulpbron-beperkte opset is NCEP kriteria die ‘beste’ toetsmetode om MIV LDS te identifiseer. Die twee nuwe klassifikasie intrumente het nog beter diagnostiese eienskappe getoon. Die gebruik van hierdie resulate kan lei tot meer akkurate, konsekwente deteksie van MIV LDS in die tipiese Suid Afrikaanse kliniese opset. Die impak op die individu asook die gesondheidsorg stelsel moet egter in ag geneem word voordat daar besluit kan word om die resulate te implementeer in die praktyk. Die resultate kan ook gebruik word vir verdere longitudinale studies.

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LIST OF ABBREVIATIONS

AIDS : ACQUIRED IMMUNE DEFICIENCY SYNDROME
AMA : ARM MUSCLE AREA
AFA : ARM FAT AREA
ART : ANTIRETROVIRAL THERAPY
BIA : BIOELECTRICAL IMPEDANCE ANALYSIS
BMI : BODY MASS INDEX
BP : BLOOD PRESSURE
CDC : CENTRE OF DISEASE CONTROL
CVD : CARDIOVASCULAR DISEASE
CT : COMPUTED TOMOGRAPHY
DEXA : DUAL ENERGY X-RAY ABSORPTIOMETRY
DM : DIABETES MELLITUS
DNA : DEOXYRIBONUCLEIC ACID
FFM : FAT FREE MASS
FM : FAT MASS
HAART : HIGHLY ACTIVE ANTIRETROVIRAL THERAPY
HDL : HIGH-DENSITY LIPOPROTEIN
HIV : HUMAN IMMUNODEFICIENCY VIRUS
HIV LDS : HIV LIPODYSTROPHY SYNDROME
IOPD : IMMUNOLOGY OUTPATIENT DEPARTMENT
LBM : LEAN BODY MASS
LDL : LOW-DENSITY LIPOPROTEIN
LDS : LIPODYSTROPHY SYNDROME
MAC : MID ARM CIRCUMFERENCE
MRI : MAGNETIC RESONANCE IMAGING
MT : MITOCHONDRIAL
NCEP : NATIONAL CHOLESTEROL EDUCATION PROGRAM
NHLS : NATIONAL HEALTH LABORATORY SERVICES
NNRTI : NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS
NPV : NEGATIVE PREDICTIVE VALUE
NRTI : NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS
PI : PROTEASE INHIBITORS

PPV : POSITIVE PREDICTIVE VALUE
RNA : RIBONUCLEIC ACID
ROC : RECEIVER OPERATING CHARACTERISTIC OR CURVE
TG : TRIGLYCERIDES
TSF : TRICEPS SKINFOLD
VLDL : VERY-LOW DENSITY LIPOPROTEIN
VL : VIRAL LOAD
WC : WAIST CIRCUMFERENCE
WHO : WORLD HEALTH ORGANIZATION
WHR : WAIST HIP RATIO

ASSESSING HIV LIPODYSTROPHY SYNDROME: A COMPARISON OF DIFFERENT METHODS TO AN OBJECTIVE CASE DEFINITION

Keywords

Acquired Immune Deficiency Syndrome (AIDS), anthropometry, highly active antiretroviral therapy (HAART), Human Immunodeficiency Virus (HIV), lipodystrophy syndrome (LDS), National Cholesterol Education Programme (NCEP) criteria, subjective self-reporting.

1 RESEARCH PROBLEM IN CONTEXT

1.1 THEORETICAL JUSTIFICATION FOR STUDY

In an environment characterised by a high prevalence of HIV/AIDS [2] and an increased use of HAART, incidences of long-term adverse side effects of HAART can be on the increase. [3,4,5,6,7,8] More research needs to focus on the long-term side effects of HAART for a better understanding of the management and consequences thereof. [3,4,5,6,7,8] HIV LDS is one of the long-term adverse side effects of HAART and is well documented as the new face of HIV/AIDS which influences adherence to HAART. [4,9,10] With more research and funding, standardised assessment of HIV LDS may lead to a better quality of life, because the management of HIV LDS may improve self-esteem, body image perception and thus adherence to HAART for the HIV infected individual. [10]

1.2 PRACTICAL JUSTIFICATION FOR STUDY

Having the opportunity to work at an immunology clinic as a member of a multi disciplinary team presented me with the opportunity to develop an electronic database to assist me in understanding the nutritional profile of the patients at the clinic. The electronic database contains all HIV/AIDS patients' nutritional information. One area that stood out after analysis of the database was the anthropometry. It showed that over 40% of the clinic's HIV positive patients were overweight or obese and only 10% were undernourished (unpublished data, July 2006). These results are contrary to the popular belief that the typical HIV patient is undernourished, and the following questions arose:

- Is this the result of the side effects of the antiretroviral treatment?
- Is it beneficial for a HIV positive person on HAART to be overweight or obese?

These questions lead me to be more sensitive to the overweight/obese patient's nutritional profile. With more overweight and obese HIV positive patients presenting with lactic acidosis and complaining of body shape changes, I started to focus on the adverse side effects of HAART. These findings motivated me to start my research study.

The importance of this study is that it objectively evaluates the diagnostic value of various available methods for assessing HIV LDS in women. It furthermore contributes towards determining the best routine method to identify HIV LDS in clinical setups similar to the Immunology Outpatient Clinic (IOPD) at Kalafong Hospital, District of Tshwane, South Africa. A uniform way of assessing HIV LDS might lead to earlier detection and treatment of HIV LDS. Prevalence of HIV LDS could then be more consistently reported to identify trends. The results of the study can be used for future longitudinal studies. The results can be made public in peer-reviewed literature for everyone's use.

1.3 RESEARCH AIM AND OBJECTIVES

1.3.1 Research aim

To determine, in a South African IOPD, the agreement of generally used methods to an internationally acknowledged objective case definition of HIV LDS.

1.3.2 Objectives

1.3.2.1. The primary objective

The primary objective of the study was to investigate the agreement between each of the following test methods on the one hand, and an objective case definition of HIV LDS on the other hand:

- National Cholesterol Education Program (NCEP) criteria
- Subjective self-reporting
- Anthropometry.

1.3.2.2. Secondary objective

The secondary objective of the study was to develop a classification instrument for HIV LDS utilising parameters from the studied test/reference method(s).

1.3.2.3. Tertiary objective

The tertiary objective of the study was to cross-validate the new classification instrument for HIV LDS.

1.3.3 Conceptual framework

The conceptual framework for the study is shown in Figure 1.1.

1.4 CLARIFICATION OF TERMINOLOGY

The following terms were conceptualized and/or operationally defined for the purpose of this study:

HIV Lipodystrophy (HIV LDS)
HIV LDS is the combination of *metabolic*- and *morphological changes* associated with the long-term use of HAART. The *metabolic changes* of HIV LDS include dyslipidemia, insulin resistance and lactic acidosis. The *morphological changes* include fat wasting (lipoatrophy) and – accumulation (lipohypertrophy). [10]

Antiretroviral Therapy (ART)
ART is a combination of drugs to treat HIV infected individuals. [11]

Highly active antiretroviral therapy (HAART)
HAART is defined as the combination of three classes of ART, namely nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI) and protease inhibitors (PI). [11]

Assessment

To make a judgement of value, quality, outcomes, result or size. To judge the presence or absence of HIV LDS using different methods including an objective case definition, NCEP criteria, subjective self-reporting and anthropometry. The presence of HIV LDS reflects a positive state, while the absence of HIV LDS a negative state. The symbols **(+)** and Θ were used throughout the study to indicate the presence or absence of HIV LDS respectively.

Comparison

Comparison is the evaluation of similarities and differences of qualities. For the purpose of this study, the agreement between the reference method and the test methods was determined with diagnostic testing (sensitivity, specificity, positive and negative predictive value).

Non-assignees

Non-assignees are the individuals excluded from the study according to the defined inclusion and exclusion criteria.

Test methods

Test methods are methods that need to be tested for accuracy to measure a specific quality against a reference method. The test methods in this study are the NCEP criteria, subjective self-reporting and anthropometric methods. These methods were tested against the reference method to determine if these methods could accurately identify HIV LDS. [12]

Diagnostic testing

Diagnostic testing is the statistical method where the results under investigation (test methods) are used to classify individuals into two groups according to the presence or absence of an outcome e.g. HIV LDS. Sensitivity, specificity, positive and negative predictive value are used to quantify the diagnostic ability of the given test. [12]

Sensitivity

Sensitivity is defined as a statistical measure of the true positives that are correctly identified by the test method. [12]

Specificity

Specificity is defined as a statistical measure of the true negatives that are correctly identified by the test method. [12]

Positive predictive value (PPV)

A positive predictive value (PPV) is the proportion of subjects with positive results who are correctly diagnosed. [12]

Negative predictive values (NPV)

A negative predictive value (NPV) is the proportion of subjects with negative results who are correctly identified. [12]

Receiver operating characteristic or curve (ROC)

A receiver operating characteristic or curve is a graphical presentation of the sensitivity (true positive rate) versus the false positive rate (1- specificity). The best prediction method would present a graph in the upper left corner of the ROC space, which shows that all true positives and no false positives were found. [13]

Table 1.1 summarizes the conceptualization and operationalization for the purpose of this study. See Figure 1.2 for a schematic representation of how the study was conducted.

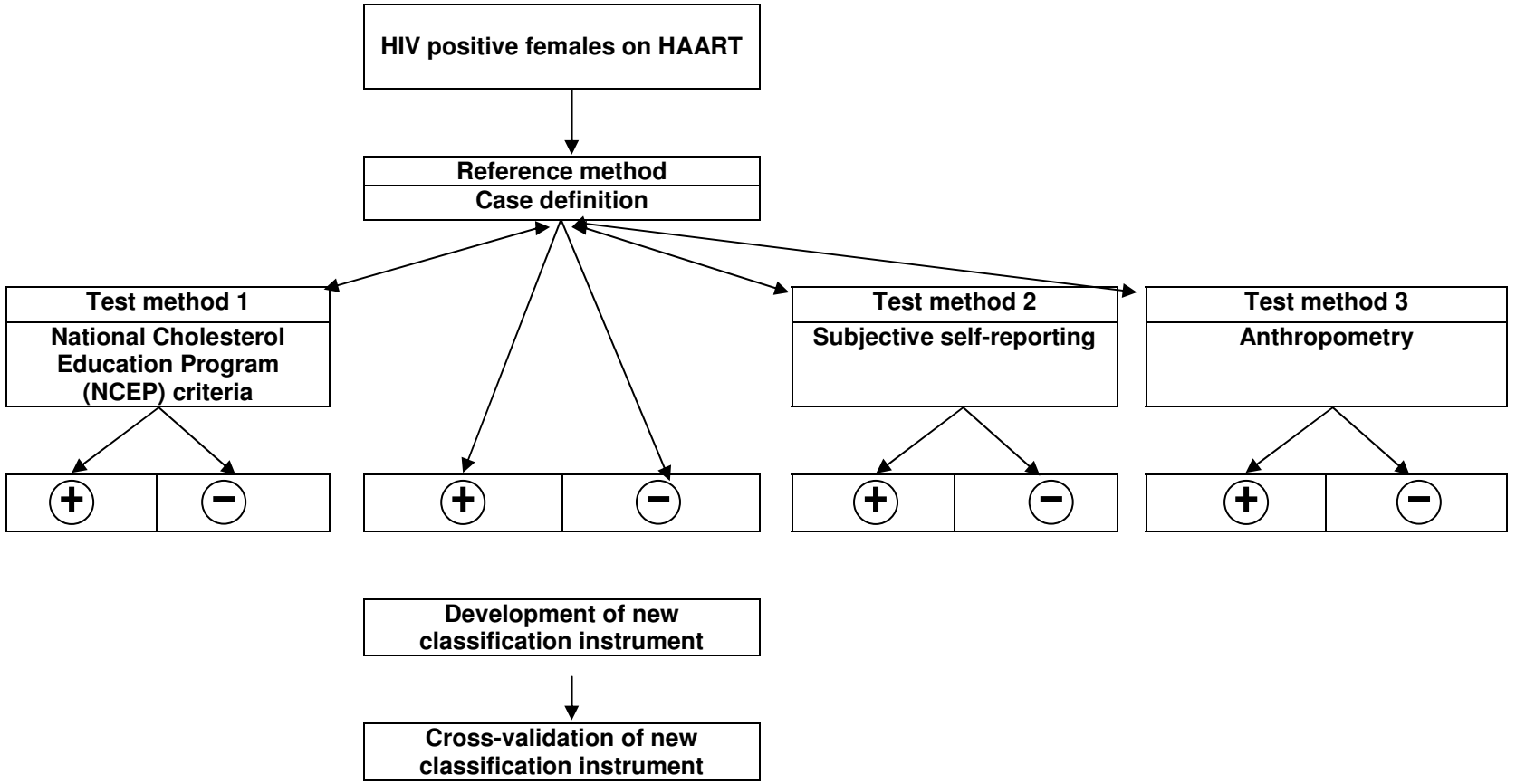


FIGURE 1.1: CONCEPTUAL FRAMEWORK

TABLE 1.1: CLARIFICATION OF TERMINOLOGY

TERMINOLOGY	CONCEPTUALIZATION	OPERATIONALIZATION (also see chapter 3)
Screening	A detection manoeuvre aimed at identifying characteristics associated with a specific problem. By identifying these characteristics a person who is at risk for developing a specific problem is identified to be assessed more comprehensively. [14] In this study: The process where subjects who visited the IOPD were identified as cases, controls or non-assignees according to the presence or absence of morphological changes.	The screening form consisted of two parts namely lipoatrophy and -hypertrophy. Each referred to specific areas of the body and was scored for the severity of morphological changes as mild, moderate and severe. [9,15] (see Appendix A).
Cases	Cases were the individuals with the outcome under investigation, namely HIV LDS.	In this study, cases were subjects with one or more moderate and/or severe feature of lipoatrophy and/or -hypertrophy according to the screening process (see Appendix A).
Controls	Controls were the individuals without the outcome under investigation.	In this study controls were subjects without any feature of lipoatrophy and/or -hypertrophy according to the screening process (see Appendix A).
Reference method	Also known as the 'golden standard'; refers to a thoroughly investigated method. In this study: The simpler objective case definition of HIV LDS. [15]	Refer to Table 3.1 for all the variables and the formula for scoring. A final score of at least zero classified a subject as HIV LDS **(+)**.[15]
Test method 1 National Cholesterol Education Programme (NCEP) criteria	NCEP criteria consists of the following elements: ▪ Abdominal obesity (waist circumference) ▪ Fasting triglycerides (TG) ▪ High-density lipoprotein (HDL) ▪ Blood pressure (BP) ▪ Fasting glucose. [3,16,17,18]	If a subject exceeded the specified individual cut-off of 3 or more of the elements (see Figure 1.2), she was classified as HIV LDS **(+).**

Test method 2 **Subjective self-reporting**	The subjective self-identification of morphological changes by the subjects. [19,20]	Every subject was asked to self-identify any morphological changes since starting on HAART. A confirmation classified a person as HIV LDS **(+)**.
Test method 3 **Anthropometry**	The science of measuring the size, weight and proportions of the human body. [21] In this study it referred to the measurement of HIV LDS (lipoatrophy and lipohypertrophy) by means of skinfolds, circumferences, weight and height.	**Kotler circumferences** If the waist circumference (WC) was more than 75.3 cm ***and*** waist hip ratio (WHR) more than 0.9, a subject was classified as HIV LDS **(+)**. [10,22] **Routine anthropometry:** **Lipoatrophy (+):** Arm muscle area (AMA) ***and*** arm fat area (AFA) below the 10th percentile of the National Centre for Health Statistics (NCHS), 1976-1980. [21] **Lipohypertrophy (+):** WC more than 88 cm. If lipohypertrophy ***and/or*** lipoatrophy were present, then a subject was classified as HIV LDS **(+).** **Dong&Hendricks** **Lipoatrophy (+):** Body mass index (BMI) =18.5–24.9 kg/m^2, ***and*** WHR <0.9 ***and*** triceps skinfold (TSF) less than the 10th percentile per age group from the NHCS, 1976-1980. [23] **Lipohypertrophy (+):** WC more than 88 cm. If lipohypertrophy ***and/or*** lipoatrophy were present, then a subject was classified as HIV LDS **(+).**

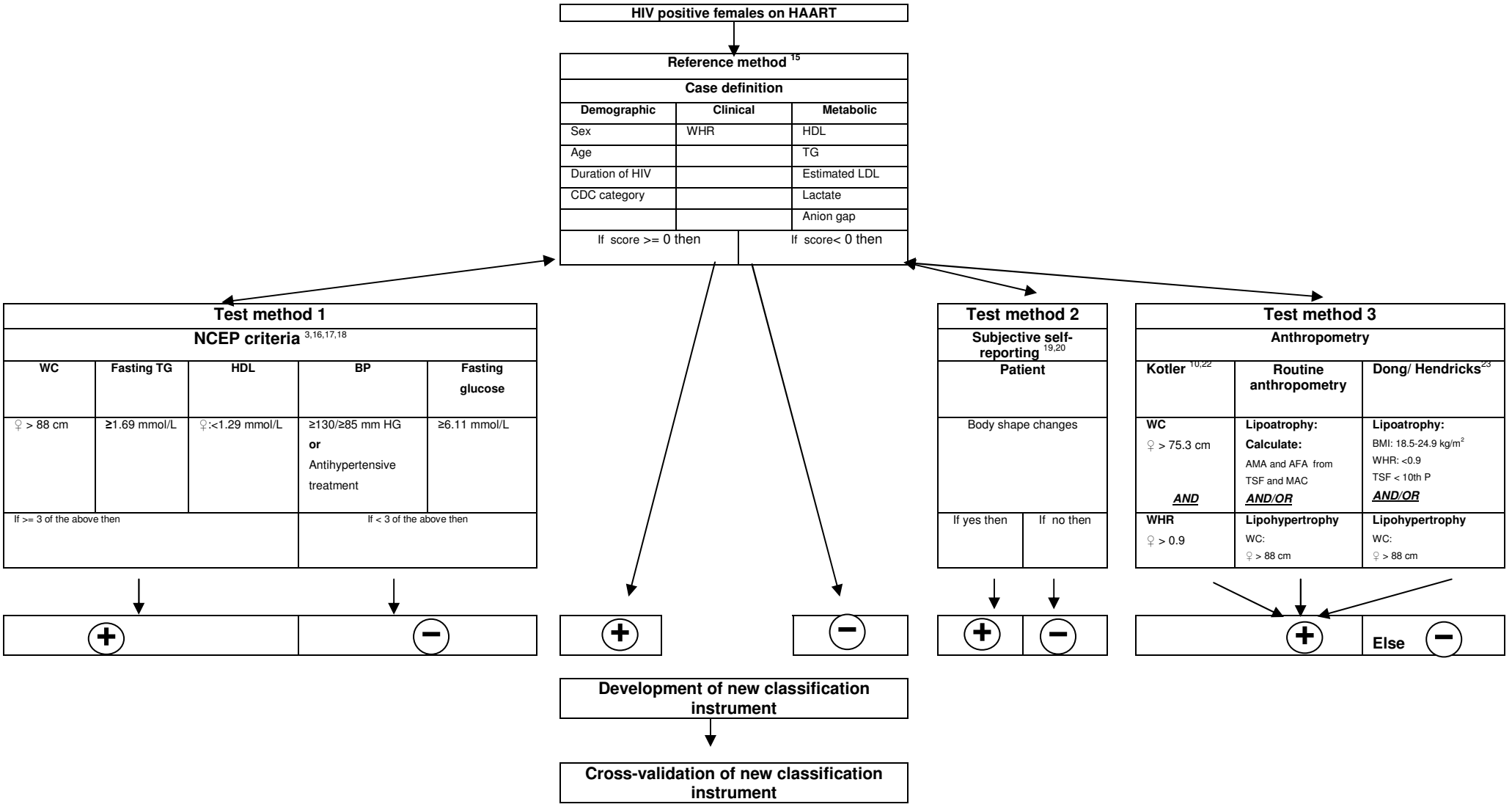


FIGURE 1.2: OPERATIONALIZATION OF CONCEPTUAL FRAMEWORK

2 LITERATURE REVIEW

2.1 INTRODUCTION

Although the introduction of HAART has drastically reduced the mortality and morbidity in HIV/AIDS patients, it can not eradicate HIV. [4,5,7,9,24,25] For successful treatment, individuals must be committed to take HAART for the rest of their lives. [5,6,7,8] With the long-term use of HAART, adverse side effects can develop. One of the most prevalent secondary adverse side effects of HAART is HIV LDS. [3,4,5,6,7,8,24,26,27,28,29]

2.2 HIV ASSOCIATED LIPODYSTROPHY

2.2.1 What is HIV LDS?

HIV LDS is described as a syndrome which includes a combination of metabolic- and morphological changes. [4,5,10,23,29,30,31] These changes are consistent with the metabolic syndrome also known as Syndrome X. [4,5,8,32]

The metabolic changes of HIV LDS include the following:

- Dyslipidemia
- Insulin resistance
- Lactic acidosis. [3,4,5,7,10,15,16,24,30,31,32,33,34,35,36]

The morphological changes of HIV LDS include the following:

- Fat wasting (atrophy)
- Fat accumulation (hypertrophy) [3,4,5,7,10,15,16,24,30,31,32,33,34,35,36]

These changes put an individual at risk for early onset hypercholesterolemia, cardiovascular disease (CVD) and diabetes mellitus (DM). [3,6,10,15,16,27,36,37,38,39]

There is still some controversy whether the metabolic- and morphological changes are separate conditions or various stages of the same pathophysiologic phenomenon. [4,40] The development of HIV LDS is not drug class specific and can occur with all the classes of ART. [5,15,20,24] The effects of these complications can also vary within the same drug

classes and may be influenced by infection, genetics and environmental factors. [5]

HIV LDS can occur in individuals not on HAART, although the majority of cases are associated with HAART. [3,24] It is also not clear if HIV LDS is directly caused by the HAART or indirectly caused by fat that was lost in the periphery and deposited in alternative visceral sites. [24]

The incidence of HIV LDS varies dramatically due to the inconsistency of the diagnosis of HIV LDS, but is said to be as high as 71%. [10] A prospective population based study found that an estimate of 50% of individuals will develop adverse changes of HIV LDS within the first year of treatment, 20% of individuals on HAART will experience fat redistribution, 60% experience dyslipidemia and 35% will develop glucose intolerance in the first two years of treatment. [4,9]

Except for the adverse physical health impact of HIV LDS, it also has psychological effects on the individuals which can lead to poor body image, anxiety, depression and low self-esteem. This disfiguring of the body has become the new face of HIV and can unintentionally disclose the HIV-status of individuals. [4,9,10] This can lead to stigma which can result in decreased compliance with treatment which lowers the effectiveness of the treatment. [6,10,15,37] Healthcare professionals should incorporate the adverse effects of HIV LDS as part of routine assessment to manage these changes. [8,36]

With increased life expectancy of a HIV infected person due to HAART, the epidemic of obesity is increasing. It is documented that overweight and obesity are common problems in HIV infected persons especially women. Overweight and obesity itself increase the risk for developing HIV LDS, dyslipidemia and insulin resistance associated in HIV infected persons on HAART. [41,42,43] Assessment, monitoring and risk factor modifications need to be in place to prevent long-term side effects of HAART e.g. coronary artery disease and HIV LDS, because younger adults become infected with HIV and treatment of HAART starts earlier. [41,42]

2.2.2 Etiology of HIV LDS

The mechanism for developing HIV LDS is not exactly clear, but two hypotheses that have widespread popularity are mitochondrial toxicity by NRTIs and PI- inhibiting lipid metabolism. [4,5,7,24,41]

2.2.2.1. Mitochondrial toxicity

NRTIs inhibit the polymerase gamma enzyme which is responsible for the replication of mitochondrial (mt) DNA. This leads to altered mitochondrial function. The effects of the altered mt function are:

- Increase in catalytic pathways and apoptosis in peripheral adipose tissue
- Changes in the differentiation of brown – and white adipose tissue
- Systemic metabolism changes due to the release of hormones
- Decreased oxidative phosphorylation that leads to disordered lipid metabolism. [4,5,7,24,31,39]

The effect is reversible upon switching to other classes of ART and is not a secondary effect of HIV infection. [4, 5, 7, 24, 41]

2.2.2.2. PI- inhibiting lipid metabolism

There is a high degree of homology between HIV protease and two proteins that regulate lipid metabolism. [4] These two proteins, cytoplasmic retinoic acid-binding protein-1 and low-density lipoprotein receptor-related protein can be inhibited by PI. This leads to stimulation of lipolysis and inhibition of lipogenesis resulting in hyperlipidemia, HIV LDS and insulin resistance. [4, 5,24,26,41]

2.2.3 Risk factors for developing HIV Lipodystrophy Syndrome (LDS)

From the literature it becomes clear that one has to assess individual cardiovascular risk before starting HAART as well as continue monitoring of risk throughout treatment. The Framingham calculator is widely used to calculate an individual's 10 year risk estimate of myocardial infarction or cardiac death. [16, 17]

Common risk factors associated with the development of HIV LDS include the following:

- Positive family history
- Current history of severe immune suppression
- Age
- Gender, especially female
- Presence of opportunistic illnesses
- AIDS diagnosis (Stage four disease or CD_4^+ less than 200 cells/mm^3)
- Duration of HAART
- Type of HAART, especially Stavudine (NRTI), ritonavir and indinavir (PI's)
- Duration of disease. [4,5,6,10,36]

Thus, the cause of HIV LDS is multifactorial and the ART side effects depend on each individual's history and underlying conditions. [4, 5,8,23]

2.2.4 Treatment strategies for HIV Lipodystrophy Syndrome (LDS)

Depending on the risk for developing CVD three main treatment strategies are available for HIV LDS namely therapeutic lifestyle behaviour changes, drug therapy and modifying ART. [25, 40]

2.2.4.1. Therapeutic lifestyle behaviour changes

Lifestyle modifications have proven benefits in the morbidity and mortality in chronic disease populations. It is suggested that these changes can also be beneficial to HIV infected individuals taking HAART. These modifications include dietary changes, physical activity and smoking abstinence. [10, 16,17,18,25,39,40,44]

This is the recommended first-line intervention to decrease excessive pill burden associated with HAART, which can influence adherence to treatment. [4] Once diagnosed with HIV LDS, individuals might be more receptive towards making lifestyle behaviour changes. [25]

Therapeutic dietary changes include a low to moderate glycemic index (GI)-, low fat -, low cholesterol diet. [23, 28] Endurance – and resistance exercises are the most successful way to decrease overall body adiposity and building lean body mass. [4]

2.2.4.2. Drug therapy

If lifestyle behaviour changes are not sufficient in treating HIV LDS or the risk for developing CVD is extremely high, lipid lowering drugs (including statins, fibrates and niacin) and insulin-sensitising agents (metformin) can be added. One has to keep in mind the possible drug interaction between some of the lipid lowering drugs and ART (especially PI and Nevirapine). [3,4,5,16,17,18,28,37,39,40,41]

2.2.4.3. Modifying ART

The third option for treating HIV LDS is switching ART from one class to another e.g. switching Stavudine for Abacavir. The new ART that is introduced needs to be monitored carefully for developing toxicity as well as virology failure. [4,5,10,16,37,41]

2.2.4.4. Alternative treatment options

Other treatment options for HIV LDS are hormonal therapy and plastic surgery. These strategies are limited in South Africa and very expensive. [4, 10, 28,37,39,41]

2.2.5 Metabolic Changes in HIV Lipodystrophy Syndrome (LDS)

As previously mentioned the metabolic changes associated with HIV LDS are dyslipidemia, insulin resistance and lactic acidosis. [3,4,5,7,8,10,15,16,24,31,33,34,36,39]

2.2.5.1. Dyslipidemia

Dyslipidemia is an abnormal lipid profile and is the most common manifestation of HIV LDS. This abnormal lipid profile includes:

- Increase in fasting total plasma cholesterol
- Increase in fasting total plasma LDL
- Increase in fasting total plasma TG
- Decrease in fasting total plasma HDL. [5,7,10,15,30,38,39,44]

Dyslipidemia may occur within the first 12 months of HAART in individuals and may worsen with time. [10] Dyslipidemia and insulin resistance may be precursors of morphological changes in HIV LDS. [10]

2.2.5.2. Insulin resistance

Insulin resistance is a state where a higher level of insulin is needed to regulate blood glucose and to obtain a biological response. [3, 5,7,10,15,31,45] Increased free fatty acids, increased TG and morphological changes are directly associated with the development of insulin resistance. [4,10,29,31,35,40,46]

The most severe insulin abnormalities occur in individuals with a combination of central lipohypertrophy and peripheral lipoatrophy. [4,8,45] Some studies showed that insulin resistance often precedes HIV LDS saying insulin resistance is a primary feature of the metabolic syndrome. [3, 10]

The incidence of developing non-insulin dependent DM is not well documented with HIV LDS, but can occur. [5, 15, 39]

2.2.5.3. Lactic acidosis

Lactic acidosis is a fatal condition which is accompanied by fatigue, loss of appetite, nausea and significant weight loss. [5,7,15] It can be caused by inadequate function of mt which leads to an excess production of lactate from pyruvate or decreased hepatic clearance.

Insulin resistance is also associated with increased plasma lactate and release from muscle tissue. [34] Hyperlactatemia is usually unnoticed if there are efficient compensatory mechanisms. [7]

2.2.6 Morphological changes in HIV Lipodystrophy Syndrome (LDS)

Morphological changes in HIV LDS consist mainly of two dimensions namely lipoatrophy and lipohypertrophy. The two dimensions can occur independently from each other or simultaneously. They worsen with prolonged treatment of HAART. [4, 5, 7,10,19,27]

In the literature, gender-related differences in morphological changes have been reported. On the one hand, women are five times more likely than men to develop HIV LDS when treated with NRTI alone. [5] They are also more likely to develop metabolic- and/or

morphological changes. One stu[illegible]e women are more aware of body changes, it might be misjudged with actual increased risk for developing HIV LDS. [10] On the other hand, when morphological differences were compared between HIV positive and - negative men, age independent morphological differences (especially lipoatrophy of the face, arms, legs and buttocks) where found in the HIV positive HAART group upon physical examination. [9]

2.2.6.1. Lipoatrophy

Lipoatrophy is fat wasting of the extremities and includes subcutaneous fat wasting of the arms, legs, buttocks and face, resulting in sunken cheeks and prominent bones, arteries and veins. [3,4,5,6,7,8,10,15,25,27,31,37,39,40,45,47] Peripheral lipoatrophy is becoming a more distinguishing feature in HIV LDS than central lipohypertrophy. [9, 20] and can be defined as normal BMI and WHR with a TSF below the 10th percentile of the reference value. [23] The risk of developing lipoatrophy is positively associated with use and duration of NRTIs. [4,5,6,10,24] Patients who take Stavudine have a significantly higher risk for developing lipoatrophy than patients with alternative NRTIs. [6, 47] Men are especially at risk for development lipoatrophy. [4, 36]

2.2.6.2. Lipohypertrophy

Lipohypertrophy is fat accumulation in visceral areas and includes breast enlargement or gyneacomastia, buffalo hump, increase in neck circumference, dorsocervical fat accumulation and non specific lipomatous growth. [3,4,5,6,7,8,10,15,25,27,31,37,39,40,45,47] The increase in visceral circumferences is due to an increase in intra-abdominal fat. [5]

Lipohypertrophy occurs earlier than lipoatrophy regardless of the site. [4] Lipohypertrophy is known to promote insulin resistance. [40, 45] Women are more likely to develop lipohypertrophy. [4, 36] There is an increased risk for developing lipohypertrophy with prolonged use of PI [6, 10, 24] but other researchers found no increase risk with any specific ART. [47] High fibre diets, but not low Glycemic Index (GI) diets, are associated with a decreased risk for fat deposits. [23]

When comparing differences in morphological changes in HIV negative and – positive men, no significant difference exist in the incidence of breast – and abdominal

hypertrophy. [9] Jain et al. found t[illegible]d a lower fat mass compared to HIV negative patients except for fat in the abdominal region. [5]

2.3 ASSESSMENT OF HIV LIPODYSTROPHY SYNDROME (LDS)

The assessment of HIV LDS varies widely among researchers due to a lack of standardisation. [10]

2.3.1. Current methods for assessing HIV Lipodystrophy Syndrome (LDS)

Methods that are used in HIV LDS research studies include subjective self-identification, assessment by physician, anthropometric measurements, bioelectrical impedance analysis (BIA), computed tomography (CT) scan, magnetic resonance imaging (MRI) and dual energy X-ray absorptiometry (DEXA). These methods of assessing HIV LDS vary widely in objectivity, methodology, accuracy, cost and standardisation of classification. A standard, universal acceptable case definition is needed to address the above problems. A case definition for HIV LDS should include all clinical components seen in HIV LDS, link abnormalities or pathophysiologic mechanisms and strengthen the association between abnormalities and specific HAART used. [9, 15]

2.3.1.1. Objective case definition of HIV Lipodystrophy Syndrome (LDS)

The HIV Lipodystrophy Case Definition Study Group identified the need for an objective case definition for HIV LDS. A case-control study design was used with patients completing a lipodystrophy specific questionnaire and physical examination. Cases were defined as persons with at least one moderate and/or severe feature of LDS. Data collection for both cases and controls included background information, clinical-, biochemical-, dietary – and body composition data. A training dataset was developed through universal analysis. A stepwise logistic regression was applied to the training dataset with variables that were significant at $p<0.05$. Diagnostic testing was used on the validation dataset. [15]

The result of the study was the development of three models for assessing HIV LDS differing in sensitivity and specificity. The model with the best sensitivity (79%) and specificity (80%) is the most comprehensive model and includes metabolic, clinical, body

imaging data (thus both, metabo[illegible]ponents of HIV LDS). The second model is simpler and is intended where no body imaging data are available. It has a sensitivity of 73% and specificity of 71% (this also includes both metabolic and morphological components of HIV LDS). The third model is intended if no biochemical and body imaging data are available with a sensitivity of 75% and specificity of 60%. See Table 2.3 for a comparison between the comprehensive – and simpler model. [15]

The comprehensive model is ideal to be used for research purposes, but it is expensive, requiring equipment that is not readily available and time consuming. The simpler objective case definition of HIV LDS is more cost effective, less time consuming and more practical for the clinical setup, because the model does not use DEXA and computed tomography (CT) scan components. [15]

All variables in the models have a score. These are added and a constant is subtracted. If the answer is equal to or larger than zero, then a person is likely to have HIV LDS. The higher the score of the model, the more severe is the HIV LDS. The score can also be used to monitor the severity of HIV LDS in individuals over time. [10, 15]

TABLE 2.1: COMPREHENSIVE – vs. SIMPLER OBJECTIVE CASE DEFINITION OF HIV LDS

Comprehensive objective case definition			**Simpler objective case definition**	
Demographic			**Demographic**	
	Age (years)			Age (years)
	Gender			Gender
	HIV duration (years)			HIV duration (years)
	Centre of disease control (CDC) category			CDC category
Clinical	WHR		**Clinical**	WHR
Laboratory	HDL (mmol/L)		**Laboratory**	HDL (mmol/L)
	Lactate (mmol/L)			Lactate (mmol/L)
	Triglycerides (mmol/L)			Triglycerides (mmol/L)
	LDL (mmol/L)			LDL (mmol/L)
	Anion gap (mmol/L)			Anion gap (mmol/L)
Body Composition	VAT:SAT ratio [a]			
	Trunk: Limb fat ratio			
	Leg fat percent			
Constant	-43		**Constant**	-5.104
Total				
Sensitivity	79%			73%
Specificity	80%			71%

[a] VAT (intra-abdominal adipose tissue[visceral fat]) :SAT (subcutaneous adipose tissue [subcutaneous fat])[15]

2.3.1.2. National Cholesterol ...NCEP)

The CVD- and DM risk associated with HIV LDS [3,6,10,15,16] and it's similarity to the metabolic syndrome also known as Syndrome X, [4,5,8,33] allow health practitioners to assess HIV LDS using a simple working definition published by the NCEP. [3,16,17,18] The model includes components of metabolic changes (e.g. dyslipidemia and insulin resistance) and morphological changes like lipohypertrophy measured by WC, but does not accommodate any peripheral atrophy of arms, legs, hips and buttocks. It is also not clear at what site the waist- and hip circumferences were taken.

The model defines metabolic syndrome as the presence of 3 or more of the following risk factors:

- Abdominal obesity measured by WC (men > 102 cm and women > 88 cm)
- Elevated fasting TG (≥ 1.7 mmol/L)
- Low HDL levels (< 1.04 mmol/L for men and 1.3 mmol/L for women)
- Elevated blood pressure (≥ 130/85 mm Hg)
- Increased fasting glucose value (≥ 6.11 mmol/L). [3,16,17,18]

2.3.1.3. Self-identification of morphological changes

Several studies have suggested that self-identification of morphological changes may be accurate. [19, 20] Patients who self-reported truncal enlargement compared to patients without, had significant excess visceral adipose tissue as confirmed by MRI scans. [20] Other researchers are sceptical. Peripheral lipoatrophy makes the abdomen look bigger and the increase in visceral fat make the periphery look smaller. The study found that patients that self-identified increased truncal fat have a decrease in peripheral fat without an absolute increase in truncal fat measured by DEXA compared to healthy controls. The data did not support increase in visceral obesity with peripheral lipoatrophy. This phenomenon is called pseudo truncal obesity rather than actual increase in truncal fat. Safrin and Grunfeld argue that during loss of weight, visceral fat is spared giving the impression of increased regional body fat. [8]

2.3.1.4. Body composition methods

Body composition methods can be useful ways of assessing morphological changes in HIV LDS. Although no metabolic variables are included in body composition methods, the

risk of developing non-communi ositively associated with body composition e.g. central obesity (increase in WC) is positively associated with the development of hypertension (HT), CVD, DM and increased mortality. [48]

Body composition methods need to be accurate to ensure effective diagnosis and nutrition support of HIV LDS. [49, 50] The ideal body composition instrument or method needs to be cost-effective, non invasive, easy to operate by non skilled persons, and is highly reproducible and accurate in producing results. [49] The most accurate models of body composition are multi compartment models which include fat-mass, total body water, body mineral mass and protein. These models should be used as reference method to test simpler methods of determining body composition. [50]

2.3.1.4.1. Dual energy X-ray absorptiometry (DEXA), computed tomography (CT) scanning and magnetic resonance imaging (MRI)

DEXA is a two dimensional multi compartment model which does not make assumptions about fat mass, lean tissue and bone densities. It is a safe method with low radiation exposure, fast and easy to use. [5, 21, 50] It can measure regional fat accurately but does not allow separate quantification of visceral fat or fat accumulation, and is costly. [5, 8,10,19,20]

CT is an imaging technique which measures differences in transmission of x-ray beams through different body tissue densities. Magnetic resonance imaging (MRI) is a body imaging technique. [21] CT scans and MRI's are three dimensional multi compartment models which can quantify visceral and regional fat. MRI and CT scanning can give a better understanding of regional body composition but is not routinely available in clinical practice. [19] The analysis of results is not well standardised between groups and the methods are more expensive and invasive than DEXA. [5, 8,10,19,51] CT scans and MRI's are good reference methods to use for research purposes. [51]

2.3.1.4.2. Bioelectrical impedance analysis (BIA)

Bioelectrical impedance analysis (BIA) is a method where a small current is passed through the body and the impedance is measured. Depending on the equation used to predict body composition it can be reasonably accurate, although it cannot be used to define regional body fat. [19] It is relatively inexpensive and easy to perform. [5,8,10,19,21,49,52,53,54]

2.3.1.4.3. Comparative studies

When comparing percentage body fat derived from skinfold measurements to BIA, the percentage body fat from two-site skinfolds was much lower than when measured using BIA. [19] This could be due to only two skinfolds that were taken. The two sites for skinfolds (triceps and subscapular) that were chosen are often influenced by peripheral atrophy and visceral hypertrophy. The researchers of the studies concluded that percentage body fat as measured by 2-site anthropometry and BIA is not an adequate method to measure morphological changes in a HIV LDS population because it cannot identify regional fat depots. [19, 53]

Breast or chest- and thigh circumference are useful for monitoring HIV LDS, but no reference values are available to evaluate or draw conclusions from once-off assessments. TSF, mid arm- and mid thigh circumference have been suggested as surrogates for peripheral lipoatrophy. [19]

Schwenk et al. compared three bedside methods, segmental BIA, anthropometric measurements (waist-, hip- and thigh circumferences) and linear analogue scale assessments (LASA), for the assessment of HIV LDS. The main aim of the study was firstly to see if there was a difference in BIA results in patients with HIV LDS compared to no HIV LDS, secondly if segmental BIA of the leg was better in identifying patients with HIV LDS compared to whole body BIA and thirdly if there is any best bedside method for the clinical setup. The results of the study showed that BIA did not discriminate between patients with- and without HIV LDS. The discriminative power of BIA between patients with- and without HIV LDS did not improve with the use of leg segmental BIA. BIA may be biased estimating body composition due to the distorted representation of limbs and trunk due to lipoatrophy and – hypertrophy. The three bedside methods did not fulfil the requirements for an assessment tool for HIV LDS because BIA is insensitive to redistribution of body compartments and WHR was found to have limited sensitivity and specificity for regional obesity. [51]

In a study comparing different methods of measuring body composition (BIA and skinfolds) compared to a reference method (DEXA), fat-free-mass and fat-mass measured by BIA did not differ significantly from DEXA values if the “right” prediction equations were used. [49, 55] The conclusion of the study was that BIA can accurately measure fat-free-mass and fat mass if Kotler et al and Heitmann’s equations are used in HIV patients. [49] Skinfolds

prediction equations differ signif Percentage body fat was calculated from different prediction equations from nine skinfolds sites and compared with DEXA. The Durnin-Womersley-equation had the smallest bias of all the equations, but still overestimated fat-free-mass and underestimated fat-mass. [49, 54]

Differences in body composition between races were documented in several studies e.g. black people had a greater bone mineral density and body protein contents than white people. This leads to a greater fat free body density and underestimate of percentage body fat in black people. The distribution of fat seems to differ between races e.g. blacks had less subcutaneous fat in the limbs than in the trunk, and had more fat on the back and lateral portions than white persons. Race and ethnicity are not always accounted for in prediction equations e.g. the Durnin-Womersley equation. This can lead to systematic errors if the reference for body composition did not take differences of ethnicity and race into account. New formulae are warranted for converting body density to percentage body fat in black men instead of using the Brozek and Siri formulas which were derived from white male cadaver studies. Cross-validation of new conversion formulas still needs to be done. [54, 56]

In a study done by Papa et al body composition was assessed with isotope dilution and compared with bioimpedance spectroscopy and skinfolds. Stable isotope dilution is a reference technique for measuring total body water (TBW). Bioimpedance spectroscopy significantly correlated with isotope dilution but overestimated TBW in HIV positive mothers. Fat free mass (FFM) was overestimated by 1.4 kg and FM underestimated with 1.4 kg. The difference is considered small and acceptable for the clinic setup. [54]

2.3.1.5. Anthropometry

Inexpensive ways of assessing morphological changes are anthropometric measurements e.g. skinfolds and circumferences. [5, 10] Anthropometric measurements are easy to perform, non-invasive, readily available and practical to use in a clinical environment. [45, 54] Accurate measurements are dependent on proper training and standardisation of techniques [54] as well as the prediction equation that is used. [19]

2.3.1.5.1. Weight

One of the simplest anthropometric methods in HIV positive persons is monitoring weight. Many HIV infected individuals experience substantial weight loss in a short period of time before initiation of treatment e.g. due to AIDS-induced cachexia, opportunistic illnesses and progression of HIV disease. Earlier studies supported the idea that weight loss in untreated AIDS patients is mostly loss of body cell mass, but later studies showed an equal loss of both fat and lean body mass. Malnourished women, in contrast with men, tend to loose more fat than lean body mass. [36, 57]

Once initiated on HAART individuals appear to regain their weight although it is mostly from the fat compartment. [5] Even though body weight can be maintained throughout treatment on HAART, the loss of lean body mass (LBM) can still continue while visceral fat is accumulating. [5, 36] The manifestation of HIV wasting has changed in the era of HAART to body composition changes: LBM is wasted (lipoatrophy) and fat is centrally accumulated (lipohypertrophy), resulting in unchanged weight. [19, 36] The monitoring and interpretation of weight in the face of HIV LDS can thus be misleading and unreliable as a measure of fat-free –mass and changes in HIV infection. [57]

2.3.1.5.2. Waist- and hip circumferences

Abdominal obesity is highly correlated with the increase in visceral adipose tissue mass. WC has been shown to be more associated with visceral adipose tissue mass than WHR and BMI. WC measurement is a practical way of evaluating the presence of regional fat depots. A WC of more than 102 cm for men and 88 cm for women is positively associated with fat depots. [23]

The most common sites for measuring WC are as follows:

- Immediately below the lowest area of the ribs
- At the narrowest waist (according to the Anthropometric Standardization Report
- Manual)
- Midpoint between lowest rib and iliac crest (World Health Organization [WHO] guidelines)
- Immediately above iliac crest (NIH guidelines). [48]

One study compared the above ing WC against a reference method (DEXA). They found that the absolute values differed significantly amongst women (and less with men) per site. The WC, body fat mass and percentage fat in the trunk region as measured by DEXA correlated significantly in both sexes and significantly with percentage body fat in women. The association of WC with trunk fat is higher than that of total fat. [8, 48]

The fat redistribution and metabolic changes in HIV infection (FRAM) study compared simple anthropometric measurements with MRI. The association of anthropometric measurements with metabolic risk indicators in HIV LDS was found to be similar to those for full regional adipose tissue volumes from MRI in HIV infected subjects. The study found that the best anthropometric correlation with a metabolic risk in HIV infected subjects was WC which correlated the strongest to the homeostatic model assessment (quantifying insulin resistance and β-cell function) and HDL; and WHR to TG. WC was found to be the best measurement for visceral obesity and WHR with hyperinsulinemia. [58]

As stated above, the technique of measuring WC can vary among studies, which necessitates caution in the interpretation of results. Gerrior et al, for example, used the site at the narrowest waist [19], Schwenk et al. used the WHO guidelines [51] and Meiniger et al used immediately above the iliac crest. [45] Some of the studies did not specify the site, yet recommended the use thereof. Absolute value cut-off points for WC and WHR are typically used for classifying risk. If the same site as suggested by a particular study is not used, inappropriate conclusions and comparisons among studies may be made.

A model that was developed to differentiate between simply obesity and visceral fat accumulation as verified by CT and MRI scans demonstrated that an HIV infected individual will have lipohypertrophy when the WC is larger than 88.2 cm and 75.3 cm and WHR larger than 0.95 and 0.9 for men and women respectively. [10,22]

2.3.2. Conclusion

The best methods to assess body composition appear to be those that that rely on or include MRI, CT scans and DEXA, but these are not readily available in the clinical practice and they are very costly. While BIA and skinfolds have been shown to be relatively accurate in predicting body composition, neither can quantify regional body fat and peripheral atrophy, which is important in HIV LDS. The value of skinfolds lies in the

assessment of fat accumulation g (lipoatrophy) at specific sites e.g. triceps - and thigh skinfolds. [49,54]

Because WC represents visceral fat accumulation (lipohypertrophy) and hip circumference subcutaneous fat loss (lipoatrophy), WC and WHR are said to be good indices of body composition changes in HIV LDS. [45] WC and WHR may be useful for routine follow ups for patients with HIV LDS. [19] WHR was also found to significantly predict fasting hyperinsulinemia and insulin under the curve independent of BMI and PI use which is important to assess in HIV LDS. [45] As a result indices of circumferences are worth investigating further for their assessment value of HIV LDS.

From the literature there is currently no consensus and/or standardization regarding practical, clinic-based methods for assessing HIV LDS.

3 METHODOLOGY

3.1 DESIGN

The study was conducted in the quantitative domain. The research design was cross-sectional, analytical and non-experimental.

3.2 ETHICAL CONSIDERATIONS

Permission to access patients' Immunology clinic records was granted by the Head of Department: Human Nutrition, Head of IOPD and the Chief Executive Officer of Kalafong Hospital.

The study was registered at the Harmonized Ethics Committee, Gauteng Department of Health. The application ID provided by the NHREC was 838. The study was also submitted and approved by the University of Pretoria's Ethics Committee (number: S97/2007) as well as the School of Health Care Sciences' Postgraduate Committee.

All HIV positive female adult individuals (who met the inclusion criteria of the study; see 3.3) enrolled on HAART were approached for written informed consent (see Appendix B) on Immunology clinic days (Tuesday, Wednesday, and Friday) when visiting the IOPD during the first month after approval by the ethics committee (i.e. 4 July 2007). Every individual that consented and met the inclusion criteria of the study was included in the study population. The consent form was in triplicate with one copy kept with the individual, one in the patient file and one kept with the researcher. If an individual was illiterate and/or could not read English an interpreter was used to get verbal consent.

Each subject in the study was assigned a unique study number. The study number was randomly linked to the data. Subjects' clinic file number and - name had no link to the study number. The unique study number was used throughout the study instead of the subjects' file number. Once all the information was collected, the table containing the study number linked to the clinic file number was destroyed. In this way anonymity was ensured throughout and after the study.

Photographic images were taken from cases after written consent was given on a

voluntary basis (during the data collection) and used for academic purposes.

3.3 SAMPLING

The population was HIV positive persons enrolled on HAART at the IOPD of Kalafong Hospital, Tshwane District, Gauteng Province, South Africa. See Figure 3.3 for a flow diagram of how sampling took place.

Inclusion criteria

- HIV positive female, adult (older than 18 years) patients at IOPD, Kalafong Hospital
- 12/13/14-, 24/25/26-, 36/37/38- and 48/49/50 months duration on HAART

(Only the above mentioned months of duration on HAART were included to ensure that routine blood analyses could be used for the study as blood is only drawn at specific months of treatment as part of the IOPD procedures.)

Exclusion criteria

- HIV positive male persons
- HIV negative persons
- Children (younger than 18 years)
- Pregnant women
- Active AIDS defining illness (prior AIDS defining illnesses may significantly influence body composition. [55]

Since HIV LDS was thought to be a relatively uncommon event, thus limiting the number of cases, purposeful sampling was performed. Cases were defined as a person with at least one moderate and/or severe LDS feature according to the screening process and controls as persons without any LDS features according to the screening process (See Appendix A: Screening of morphological changes).

The ideal ratio of controls to cases of one to one was targeted, but if fewer cases were available, two controls for every case would have been assigned. The proposed number of cases and controls were 75 each. The sample was randomly selected with a table of random numbers.

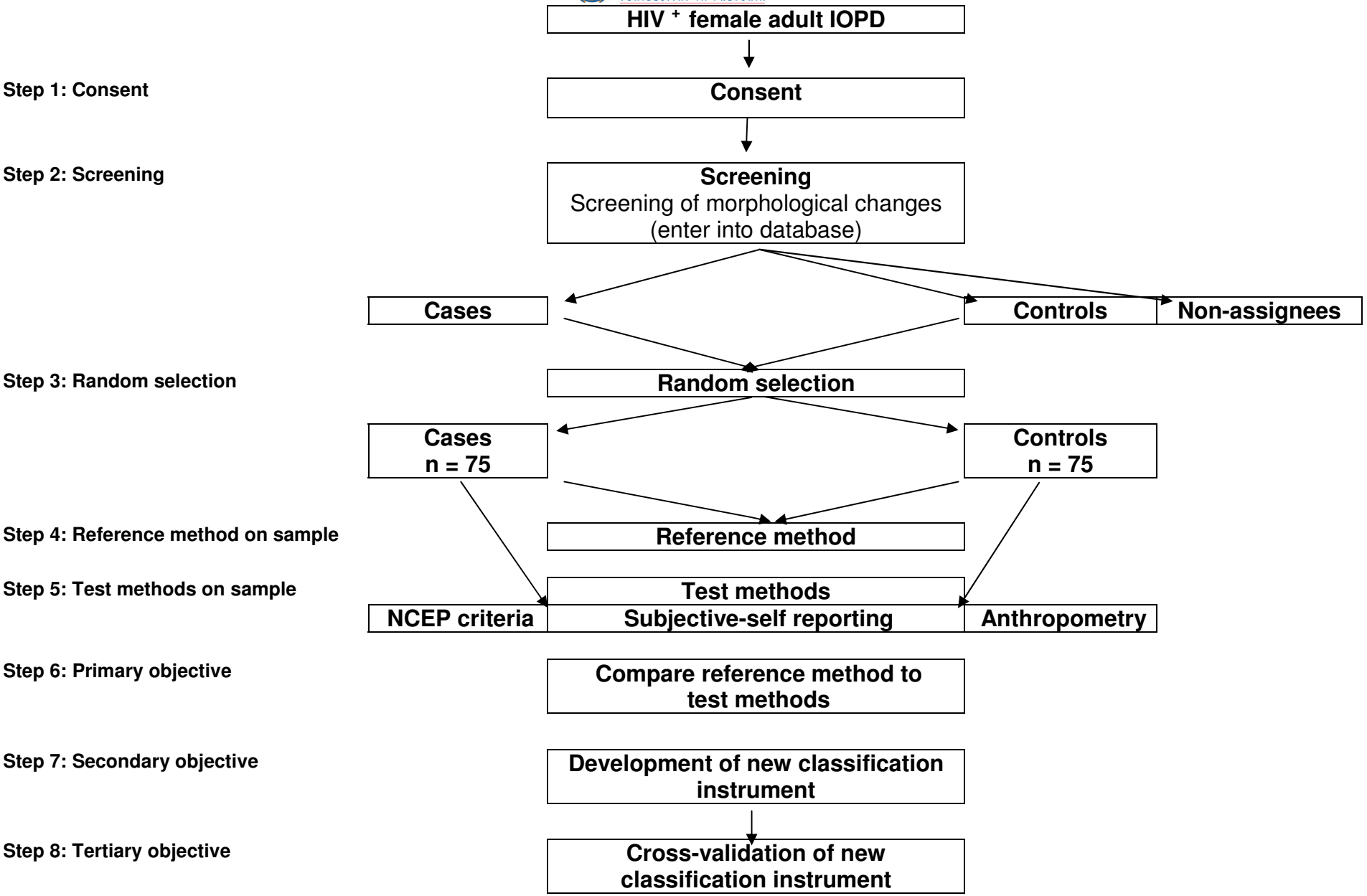


FIGURE 3.1: FLOW DIAGRAM OF SAMPLING PROCESS AND STUDY PROFILE

3.4 DATA COLLECTION

All background information (study number, age, gender, details of HIV infection and HAART) and measurements were recorded per subject on a data collection form (see Appendix C) and captured into a study-specific electronic database. The information in the database was automatically converted by Microsoft Excel into the reference – and test methods.

Data were collected over a period of three months by the researcher. The consent- and screening process took place during the first month. The measurements contained in the reference - and test methods were taken during the second - and third month of the study. This ensured that the screening process did not influence the outcome of the reference – or test methods.

The validity of the screening form cannot be fully known. Furthermore, it is possible for observers taking measurements to employ techniques which obtain different readings for the same subject. To address measurement error which could lead to information bias , distorting the study conclusions, only the researcher measured and collected variables and information. Measurements were also repeated to assess the validity of the measurement.

3.4.1 Screening

On the same day that consent was given by individuals, the screening process took place to identify cases, controls and non-assignees. The screening form was completed by the researcher while busy with the patient consultation. Subjects were unaware of the assessment, to ensure that the researcher was not influenced by the subjects' opinion.
All screening forms were captured on a study-specific electronic database. This screening tool has previously been used in other studies, but in a different format (See Appendix A for the screening form). [9,15]

To address selection bias (to ensure that the sample is representative of the target population) cases and controls underwent an identical detection manoeuvre i.e. standardized screening of morphological changes by the researcher.

A pilot study was done on a similar population to test and standardize the screening process. Changes were made to the screening form to ensure a more logical flow of the screening process e.g. lipohypertrophy at the different sites were investigated before lipoatrophy were examined.

A pilot study was done to test the data collection form and study-specific electronic database. Computer generated classifications were compared to classifications done manually. Necessary changes were made to the instrument as needed.

3.4.2 Demographic data

The gender of the subjects was recorded from the IOPD clinic file. If no gender information was available, it was concluded from the subject's national identification number (ID). Gender was recorded as either 'male' or 'female'. (Males were excluded from the study)

The age of every subject was calculated from the date of birth or ID recorded in the IOPD clinic file. The first two numbers of the ID represented the year in which the subject was born, the second two numbers the month and the next two numbers the day of the month. The date of birth was subtracted from the date of collecting the data. Age was reported to the nearest year and then categorized as 'younger and/or equal' or 'older' than 40 years.

For the question regarding the duration of HIV infection, the assumption was made that the subject knew more or less how long she had been infected with HIV.

CDC category staging as well as WHO classification were done by two experienced HIV specialist medical doctors working in the IOPD clinic at Kalafong Hospital. They were briefed before the study was conducted. The staging was then recorded from the IOPD clinic file of the subject. The CDC category was recorded as either stage A (asymptomatic), stage B (symptomatic conditions occur) or stage C (AIDS). The WHO classification was recorded as stage 1 (asymptomatic), stage 2, stage 3 or stage 4 (AIDS).

3.4.3 Anthropometry

All anthropometric measurements were done by the researcher, who is a qualified dietitian experienced in taking anthropometric measurements. Measurements were taken twice and captured into the study-specific database. All equipment was calibrated before the study was conducted. Focus was put on consistency in the technique to help eliminate potential (random) sources of errors. All measurements were done in the researcher's consultation room behind an examination screen. Only the researcher and the subject were allowed in the consultation room while anthropometric measurements took place to ensure that the privacy of the subject was attended to.

Skinfolds were taken with a calibrated Harpenden (John Bull) caliper, with a jaw tip pressure of 10 g/mm^2. All skinfolds were measured at the right hand side of the body. The measurement was read after four seconds to the nearest 0.2 mm. The two measurements were taken at least 15 seconds apart.

The location of each specific skinfold site was marked with a non-permanent marker. A non-elastic measuring tape (1 cm width) was used to measure circumferences and to locate midpoints where necessary. Once the specific site was located, the thumb and index finger of the left hand grasped the skinfold 1 cm proximal from the skinfold site away from the body so that the pressure from the fingers holding the skinfold did not influence the reading. The caliper was held in the right hand, perpendicular to the long axis of the skinfold. The caliper tips were placed with parallel folds at each side. [21]

3.4.3.1 Weight

Weight was measured to the nearest 0.1 kg on a Seca 766 electronic scale bare foot and dressed in light underclothing. Subjects had to stand in the middle of the scale's platform, with body weight equally distributed on both feet without touching anything.

3.4.3.2 Height

Height (stature) was measured to the nearest 0.1 cm with a Seca 766 stadiometer. The subject was measured bare foot, hair ornaments and hats were removed with minimal underclothing on to ensure correct positioning of the body. The subject's head was in the

Frankfort horizontal plane, standing erected, heels together, arms hanging loosely on the side, legs straight, shoulders relaxed. The subject's back of head, scapulae, buttocks and heels were against the vertical surface of the stadiometer. The headboard of the stadiometer was lowered to the highest point of the head. Measurement was taken after exhalation of breath with the observer's eye level to the headboard to avoid parallax error. [21]

3.4.3.3 Waist circumference

The WC was measured with a non-elastic tape (1 cm width) measure to the nearest 0.1 cm. Subjects were asked to remove any outer clothing that could restrict the accurate measurement of the WC or influenced the accuracy of the measurement (e.g. tight fitting underclothing). The measurement was taken on bare skin.

The highest point of the hipbone at the iliac crest on the right side of the subject was located and a small mark was made with a non-permanent marker just above the lateral border of the iliac crest. The tape was positioned in a plane that is perpendicular to the long axis of the body. The subjects stood in an erect position, with abdominal muscle relaxed, arms at the side and feet together. The measurement was taken at the end of normal expiration. [21,48]

3.4.3.4 Hip circumference

Hip circumference was measured with the same condition as with the WC. The location of the hip circumference was at the largest extension of the buttocks (gluteal) or hip. [21]

3.4.3.5 Mid arm circumference (MAC)

The mid arm circumference (MAC) was measured at the exact midpoint of the lateral projection of the acromiom process of the scapula and the inferior margin of the olecranon process of the ulna of the right arm to the nearest 0.1 cm. [21]

3.4.3.6 Triceps skinfold (TSF)

The TSF was measured between the midpoint of the lateral projection of the acromiom process of the scapula, and the inferior margin of the olecranon process of the ulna on the posterior aspect of the right arm over the triceps muscle. The site was marked at the midpoint of the acromiom and olecranon process on the lateral side of the arm with the elbow flexed to 90 degrees. After the site was marked, the subjects arm hung loosely at the side with the

palm facing anteriorly. A mark was made to the posterior midline at the same level as the previous mark. The TSF was taken standing behind the subject in a vertical direction with the skin grasp with the left hand of the researcher 1 cm proximal to the skinfold site. [21]

3.4.3.7 Subscapular skinfold

The subscapular skinfold was measured 1 cm below the lowest angle of the scapula of the long axis in a 45-degree angle direction down and to the right side of the body. Subjects were standing relaxed with arms hanging loosely on the side. The skinfold was taken while standing behind the subject with the left hand of the researcher grasping the skin 1 cm above and medial to the site along the axis. [21]

3.4.3.8 Biceps skinfold

The biceps skinfold was measured between the midpoint of the lateral projection of the acromiom process of the scapula, and the inferior margin of the olecranon process of the ulna on the anterior aspect of the right arm over the belly of the biceps muscle. The site was marked at the midpoint of the acromiom and olecranon process on the lateral side of the arm with the elbow flexed to 90-degrees. After the site was marked, the subjects arm hanged loosely at the side with the palm facing anteriorly. A mark was made to the anterior midline at the same level as the previous mark. The biceps skinfold was taken in a vertical direction with the skin grasp with the left hand one cm proximal to the skinfold site. [21]

3.4.3.9 Supra iliac skinfold

The supra iliac skinfold was measured at the midaxillary line above the iliac crest in a diagonal direction. The subjects were in a standing position, feet together and arms hanging loosely at the sides. The skinfold was measured at the midaxillary line with the left hand of the researcher grasping the skinfold 1 cm posterior to the midaxillary line. [21]

3.4.3.10 Thigh skinfold

The thigh skinfold was measured at the anterior midline of the midpoint of the inguinal crease and the proximal border of the patella. The subject had to shift his weight to the left foot while flexing the knee slightly with the right foot flat on the floor. The skinfold was measured in a vertical direction with the skin grasped with the left hand one cm proximal to the skinfold site. [21]

3.4.3.11 Thigh circumference

Thigh circumference was measured at the exact position as describe with thigh skinfold at the midpoint of the inguinal crease and the proximal border of the patella. [21]

3.4.4 Biochemical data

Routine blood tests were used. The under mentioned procedure describes how blood was taken at the IOPD.

For this study, blood was taken by one experienced phlebotomist under the following circumstances:

- Overnight fast.
- Well hydrated.
- Tourniquet use was minimised to less than one minute if searched for a vein and was released before the blood withdrawal began.
- Seated for at least 15 minutes.
- Injection site was sterilised with alcohol swab.
- Tubes for serum were filled first, and then plasma samples.

Metabolic variables were only taken specifically for this study if the tests were not routinely performed in the IOPD clinic within eight weeks of the rest of the assessment. National Health Laboratory Services (NHLS) analysed all blood tests.

3.4.4.1. High-density lipoprotein

A direct method for HDL assay was used to avoid isolation and variation in results due to chemical reagents (NHLS procedures).

3.4.4.2. Triglycerides

Enzymatic method for measuring TG was used (NHLS procedures).

3.4.4.3. Lactate

After blood was taken for lactate levels, blood was shaken well and placed on wet ice until analyses. Ion selective electrodes method was used as methodology (NHLS procedures).

3.4.4.4. Anion gap

Anion gap was calculated from the sum of sodium-, potassium-, chloride- and bicarbonate values (NHLS procedures).

3.4.4.5. Fasting glucose

Enzymatic auto analyzer method i.e. Glucose oxidase mediated peroxidise/4-aminiantipyrine, hexokinase G-6DH was used for plasma glucose (NHLS procedures).

3.4.5 Clinical information

Blood pressure

BP was taken by one trained nurse with a serviced simple mercury sphygmomanometer and a stethoscope. Subjects were in a sitting position with the right arm resting on a desk so that the arm was level with the heart with the palm facing up. A standard cuff width of 12 cm wide was used. The cuff of the sphygmomanometer was placed so that the bottom part was about 2.5 cm above the elbow. The cuff was wrapped snugly around the arm. With the middle and index finger the subject's pulse was found at the brachial artery. The diaphragm of the stethoscope was placed at the brachial artery pulse. The cuff was inflated rapidly to the peak inflation level, with the bulb, with the valve on the bulb fastened. The cuff was deflated at a rate of 2 mmHG per second until a clear repetitive tapping sound was heard (systolic pressure). Deflating was continued until the repetitive sound had stopped (diastolic pressure). The cuff was deflated completely. Measurements were recorded to nearest 2 mmHG and repeated twice. [59]

3.4.6 Subjective self-reporting

Each subject was asked to self-identify any morphological changes since starting on HAART. The specific wording was as follows "*Have you noticed any changes in your body shape since*

taking the HAART e.g. breast - and/or stomach enlargement, or wasting of the arms, legs or buttocks". A simple 'yes' or 'no' answer was recorded. There was no need to translated the subjective self-reporting question in any another language.

3.5 DATA ANALYSIS

All statistical analysis was done in consultation with a biostatistician.

Each entry in the database was checked (by the researcher) against the collection form to eliminate any finger errors. Descriptive and inferential statistics were used to analyse the data.

3.5.1 Descriptive statistics

Descriptive statistics were used to describe central tendency e.g. mean and median and spread of the population e.g. variance, standard deviation (SD) and standard error of mean (SEM) of continuous variables. Statistical software that was used by the biostatistician: Stata Corp. 2003. Stata Statistical Software: Release 8.0. College Station, TX: Stata Corporation.

Where multiple readings were taken for the variables, the average of the readings was used in the statistical calculations to make use of the additional information available. A one way ANOVA was performed for each of these variables.

3.5.1.1. Waist hip ratio (WHR)

WHR was calculated as follows:
WHR = waist circumference divided by hip circumference. [21]

3.5.1.2. Body mass index (BMI)

BMI was calculated as follows:
BMI= weight in kilograms divided by height in metres squared (i.e. BMI = kg/m^2). [21]

3.5.1.3. Muscle - and fat mass

AMA was calculated from TSF and MAC by using Heymsfield and co-workers revised equation for corrected AMA.

AMA and – fat area were calculated as follows:

- Arm muscle circumference (cm) = MAC(cm) - π*TSF(cm)
- Upper arm area (AA) = (π/4)* MAC(cm)
- AMA (cm^2) = $[(MAC(cm) – \pi*TSF(cm))^2 /4\pi] – 6.5$
- AFA = AA – AMA. [21]

3.5.1.4. Low-density lipoprotein

Low-density lipoprotein (LDL) was estimated using the Friedewald equation:
LDL = (total cholesterol level) - (HDL level) - (triglyceride level/5). [15] (NHLS procedures)

Each of the test methods (NCEP criteria, subjective self-reporting and anthropometry) as well as the reference method i.e. the objective case definition, had to have a dichotomous outcome, either HIV LDS **(+)** or HIV LDS Θ. Each method's outcome was defined as follows (see also Figure 1.2 for a summary):

3.5.1.5. Reference method: The simpler objective case definition

Each of the variables (demographic, clinical and laboratory) in the reference method was scored. Refer to Table 3.1 for all the variables and the formula for scoring. A final score of at least zero classified a subject as HIV LDS **(+)**. [15]

3.5.1.6. Test method 1: NCEP criteria

The NCEP criteria defined the following variables and cut-off points to get to a final outcome:

- WC: a cut-off point of > 88 cm
- Fasting TG: a cut-off point of ≥1.69 mmol/L
- HDL: a cut-off point of <1.29 mmol/L
- BP : a cut-off point of:
    - ≥ 130 mmHg for systolic BP and/or
    - ≥ 85 mmHg for diastolic BP and/or

- If the subject was on Antihypertensive treatment

- Fasting glucose: a cut-off point of ≥ 6.11 mmol/L

If a subject exceeded the specified individual cut-off of 3 or more of the variables, she was classified as HIV LDS **(+)**.[3, 16, 17, 18]

TABLE 3.1: REFERENCE METHOD (i.e. the simpler objective case definition of HIV LDS) [15]

Variable		Unit	Score [a]	Total
Demographic				
	Age (years)	<= 40	0	
		> 40	1.139	
	Gender	Male	0	
		Female	1.279	
	HIV duration (years)	<= 4	0	
		> 4	1.373	
	CDC category	A	0	
		B	0.181	
		C	0.731	
Clinical	**WHR**		* 2.114	
Laboratory	**HDL (mmol/L)**		*-1.593	
	Lactate (mmol/L)		* 0.291	
	Triglycerides (mmol/L)		* 0.204	
	LDL (mmol/L)	<= 3.0	0	
		> 3.0	0.625	
	Anion gap (mmol/L)		* 0.0701	
Constant			-5.104	
Total				

[a] Add score totals for each variable. Where multiply sign *, first multiply value before adding.

3.5.1.7. Test method 2: Subjective self-reporting

Each subject was asked to self-identify any morphological changes since starting on HAART. A confirmation classified a person as HIV LDS **(+)**.

3.5.1.8. Test method 3.1: Anthropometry: Kotler

A subject was classified as HIV LDS **(+)** if the following was true:

- If the WC was >75.3 cm ***and***
- If the WHR was >0.9. [10,22]

3.5.1.9. Test method 3.2: Anthropometry: Routine anthropometry

A subject was classified as HIV LDS **(+)** if lipohypertrophy ***and/or*** lipoatrophy were present:

Lipoatrophy (+) was defined as follows:

- AMA: a cut-off point of <10th percentile of NCHS 1976-1980 ***and***
- AFA: a cut-off point of <10th percentile of NCHS 1976-1980. [21]

Lipohypertrophy (+) was defined as follows:

- If the WC was > 88 cm

3.5.1.10. Test method 3.3: Anthropometry: Dong&Hendricks

A subject was classified as HIV LDS **(+)** if lipohypertrophy ***and/or*** lipoatrophy were present:

Lipoatrophy (+) was defined as follows:

- BMI=18.5–24.9 kg/m^2, ***and***
- WHR<0.9 ***and***
- TSF<10th percentile per age group from the NHCS, 1976-1980. [23]

Lipohypertrophy (+) was defined as follows:

- If the WC was > 88 cm

3.5.2 Inferential statistics

Statistical Quality Control

Anthropometric measurements were taken twice and the intraclass correlation between measurements calculated to ensure consistency and reduce measurement bias in the data used in the statistical analysis. In all cases the intraclass correlation between measurements were greater than 0.99.

3.5.2.1. Agreement between the test methods and the reference method

The agreement between the reference method and each of the test methods was assessed using the Kappa-statistic (κ) where:

$\kappa \leq 0.4$ denotes poor agreement;

$0.4 < \kappa \leq 0.75$ moderate agreement and

κ > 0.75 excellent agreement. [60]

Furthermore along with diagnostic statistics (sensitivity, specificity, negative predictive values and positive predictive values, refer to Table 3.2), McNemar's test for symmetry was used to determine the direction of deviation from the reference method.

Sensitivity

Sensitivity was calculated as the number of true positives (a) divided by the sum of the true positives (a) and false negatives (c) (Sensitivity = a/a+c). [12]

Specificity

Specificity was calculated as the number of true negatives (d) divided by the sum of the true negatives (b) and false positives (d) (Specificity= d/b+d). [12]

TABLE 3.2: EXAMPLE OF DIAGNOSTIC STATISTICS CALCULATIONS

			HIV LDS as determined by the reference method		
			Positive	**Negative**	
Test methods	e.g. **NCEP criteria**	**Positive**	**a** (True positive)	**b** (False positive)	**PPV**
		Negative	**c** (False negative)	**d** (True negative)	**NPV**
			↓ **Sensitivity**	↓ **Specificity**	

Positive predictive value

PPV as calculated as the number of true positives (a) divided by the sum of the true positives (a) and false positives (b) (PPV = a/a+b). [12]

Negative predictive values

NPV was calculated as the number of true negatives (d) divided by the sum of the true negatives (c) and false negatives (d) (NPV = d/c+d). [12]

3.5.2.2. Development of a new classification instrument for HIV LDS

A logistic regression was done on the following variables: screening, age, duration HIV, WHO

stage of disease, duration HAART, morphological changes, systolic BP, diastolic BP, CD_4^+, HDL, LDL, TG, lactate, anion gap, glucose, weight, height, WC, hip circumference, MAC, thigh circumference, TSF, biceps skinfold, subscapular skinfold, supra iliac skinfold, thigh skinfold, on the reference method, the objective case definition.

3.5.2.2.1. Development of an IDEAL classification instrument for HIV LDS

A stepwise logistic regression was done on the full data-set, thus including the following variables: screening, age, duration HIV , WHO stage of disease, duration HAART, morphological changes, systolic BP, diastolic BP, CD_4^+, HDL, LDL, TG, lactate, anion gap, glucose, weight, height, WC, hip circumference, MAC, thigh circumference, TSF, biceps skinfold, subscapular skinfold, supra iliac skinfold, thigh skinfold, on the reference method, the objective case definition (pe of 0.15 and pr of 0.2) to develop an ideal classification instrument for HIV LDS**.**

3.5.2.2.2. Development of a SIMPLER INSTRUMENT with blood samples for HIV LDS

The following variables were removed before the stepwise logistic regression due to cost and accuracy implications: duration of HIV, lactate and anion gap.

A stepwise logistic regression was done on the following variables: screening, age, WHO stage of disease, duration HAART, morphological changes, systolic BP, diastolic BP, CD_4^+, weight, height, WC, hip circumference, MAC, thigh circumference, TSF, biceps skinfold, subscapular skinfold, supra iliac skinfold, thigh skinfold, HDL, LDL and TG on the reference method, the objective case definition (pe of 0.15 and pr of 0.2) to develop a SIMPLER INSTRUMENT with blood samples for HIV LDS**.**

The SIMPLER classification instruments (with limited blood samples) for HIV LDS followed from using stepwise logistic regression.
The fitted logistic regression in general notation was:
$y = \beta_0 + \beta_1 * \text{thigh skinfold} + \beta_2 * \text{age} + \beta_3 * \text{HDL} + \beta_4 * \text{duration of HAART} + \beta_5 * \text{morphological changes} + \beta_6 * \text{thigh circumference} + \beta_7 * \text{diastolic BP} + \beta_8 * \text{MAC} + \beta_9 * \text{weight} + \beta_{10} * \text{LDL}$

The probability of being LDS **(+)** followed from:
$p = e^Y/1+e^Y$

= exp(y)/1 +exp(y) where

a given subject was classified as LDS **(+)** if p > 0.3 (.i.e. cut point that was found to optimize diagnostic statistics). Equivalently this subject is LDS **(+)** when y > -0.85.

The agreement between the SIMPLER INSTRUMENT with blood samples for HIV LDS and the reference method were assessed using the Kappa-statistic (κ), diagnostic statistics (sensitivity, specificity, NPV and PPV) and McNemar's test for symmetry.

3.5.2.2.3. Development of a SIMPLER INSTRUMENT (NO BLOOD SAMPLES) for HIV LDS

The need for an even simpler classification instrument (with no biochemical variables) was acknowledged as it is not always possible to analyse blood samples. The following variables were thus removed before it was put into the stepwise logistic regression: HDL, LDL, TG, lactate, anion gap and glucose.

A stepwise logistic regression was done on these variables: screening, age, WHO stage of disease, duration HAART, morphological changes, systolic BP, diastolic BP, CD_4^+, weight, height, WC, hip circumference, MAC, thigh circumference, TSF, biceps skinfold, subscapular skinfold, supra iliac skinfold, thigh skinfold, on the reference method, the objective case definition. (pe of 0.15 and pr of 0.2)**.**

The SIMPLER classification instruments (without blood samples) for HIV LDS followed from using stepwise logistic regression.
The fitted logistic regression in general notation was:
$y = \beta_0 + \beta_1 * MAC + \beta_2 * age + \beta_3 *$morphological changes $+ \beta_4 *$ subscapular skinfold $+ \beta_5 *$ diastolic BP.

The probability of being LDS **(+)** follows from:
$p = e^Y/1+e^Y$
= exp(y)/1 +exp(y) where
a given subject will be classified as LDS **(+)** if p > 0.28 (.i.e. cut point that was found to optimize diagnostic statistics). Equivalently this subject is LDS **(+)** when y > -0.94

The agreement between the SIMPLER classification instrument (which excluded blood

samples) for HIV LDS and the reference method were assessed using the Kappa-statistic (κ), diagnostic statistics (sensitivity, specificity, NPV and PPV) and McNemar's test for symmetry.

3.5.2.3. Cross-validation of the new classification instrument

The classification instrument was tested making use of cross-validation. In the cross-validation, for each subject separately, the model used as new classification instrument was fitted to the remaining 151 subjects' (152-1) data, after which the omitted subject was classified with the latter model. Subsequently diagnostic statistics were again calculated.

4 RESULTS

4.1 DESCRIPTION OF THE STUDY POPULATION AND SAMPLE

4.1.1 Study population and sample selection

A total of 1421 patients (69% female) visited the IOPD between July and August 2007. Of these 283 met the inclusion criteria. Two hundred and fifty three patients (89%) consented to participate in the study, of which six subjects were non-assigned due to falling pregnant during the study period. See Table 4.1 for a summary of the study population and sample, and Figure 4.1 for a flow diagram of the study profile.

Based on the screening of these 247 consenting and assigned patients, 174 subjects were identified as cases and 73 subjects as controls. Table 4.2 is a summary of the screening for morphological changes and appendix D is a complete summary of cases and controls. Overall lipohypertrophy was more common than lipoatrophy in the subjects. Four cases had lipohypertrophy at all three sites (i.e. neck, breast and waist) and 19 had lipoatrophy at all four sites (face, arms, buttocks and legs). Two subjects presented with lipohypertrophy and –atrophy at all sites. The most common moderate to severe form of lipohypertrophy was to the breast and waist, and the most common sites for lipoatrophy were the buttock and legs. The least common morphological change was the buffalo hump.

From the 174 screened cases 79 were randomly selected as final cases. All 73 controls, as identified by the screening process, were retained as such in the final sample. Figure 4.2 shows images and descriptions of some of the cases.

4.1.2 Description of sample

Table 4.3 describes the sample. In total, 99% of the subjects were black as the research site was located in a historically black township. The mean age of the sample was 38 years (±8). The mean duration of known HIV status was 3 years (±2) although some subjects reported as high as 12 years. The cases had a mean duration of HAART of 27 months (±9) compared to

the controls of 21 months (±9). The BP measurements of the two groups were similar with a systolic BP range of 80–150 mmHg (±13) and diastolic BP range of 60-110 mmHg (±11). The CD_4^+ count of cases was higher than the controls although the viral load (VL) for the majority was lower than detectable limits i.e. <50 copies/mL. HDL levels were similar between the two groups with a mean of 1.08 mmol/L(±0.2) (normal: >1.29mmol/L). Cases had a slightly higher mean LDL level of 2.83 (±0.6) (normal: <2.59 mmol/L) and TG level of 1.63 mmol/L (±0.95) (normal: <1.69 mmol/L). Lactate and anion gap levels were higher than the normal level for both groups with outliers showing as high as 8 mmol/L due to recent lactic acidosis. Glucose levels were also similar in both groups although one outlier showed a level of 26.4 mmol/L(This subject was later identified with DM). Weight, BMI, percentage body fat, WC, hip circumference and thus WHR were found to be higher in the cases than the controls. Mean sub-scapular – and supra iliac skinfolds were also higher.

TABLE 4.1: STUDY POPULATION AND SAMPLE

Number of patients visiting IOPD				**Eligible for study**		
Date	**Total**	**Male**	**Female**	**Total eligible**	**Refused consent/ Non-assigned**	**Consent given**
2007/07/24	163	35	128	27	5	22
2007/07/25	106	27	79	20	2	18
2007/07/27	98	20	78	34	7	27
2007/07/31	101	38	63	17	3	14
2007/08/01	80	30	50	18	4	14
2007/08/03	114	33	81	27	3	24
2007/08/07	139	46	93	21	0	21
2007/08/08	104	38	66	17	2	15
2007/08/10	106	47	59	30	2	28
2007/08/14	134	33	101	32	3	29
2007/08/15	129	42	87	18	2	16
2007/08/17	147	53	94	22	4	18
Total	**1421**	**442**	**979**	**283**	**36**	**247**

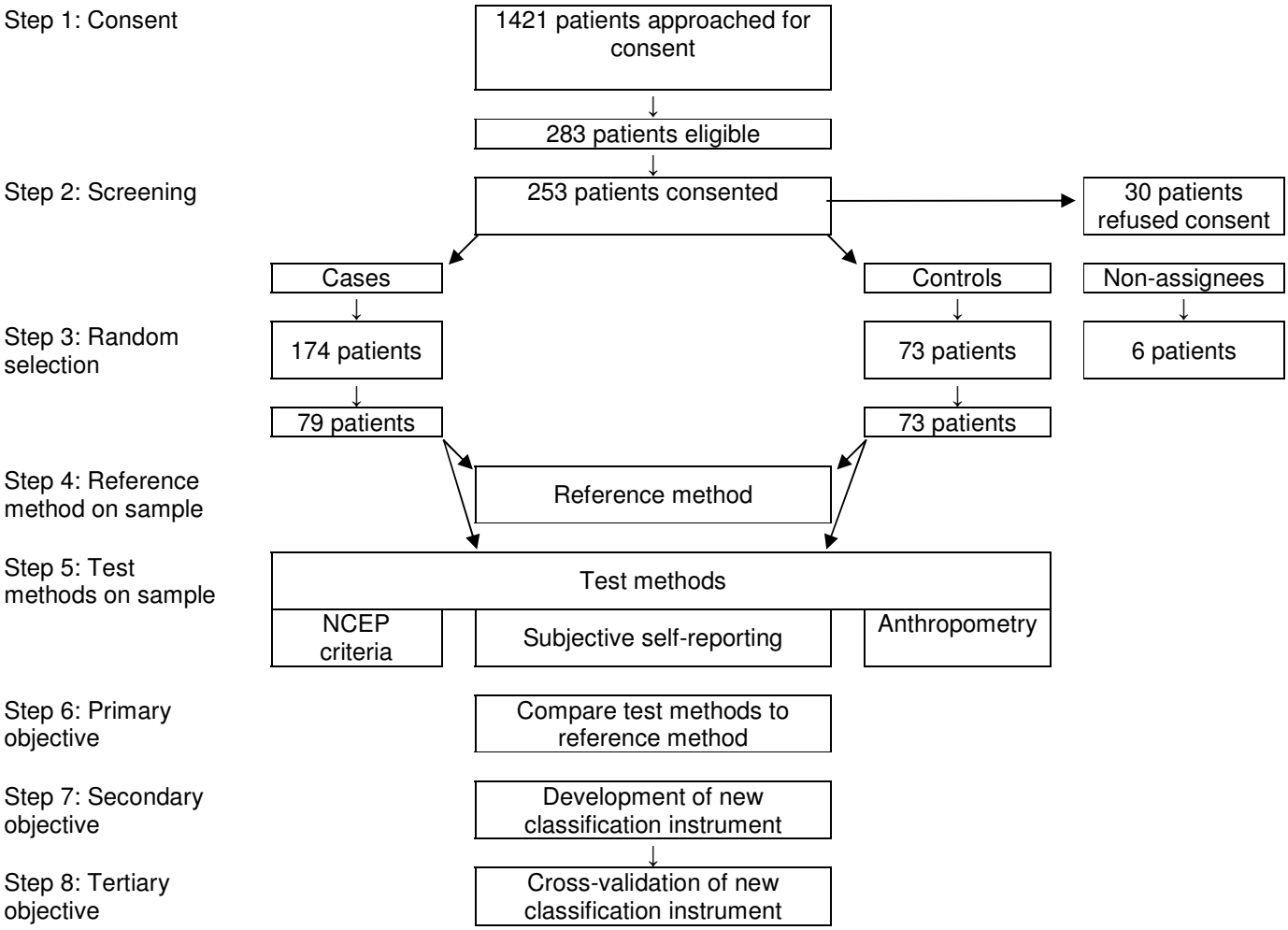


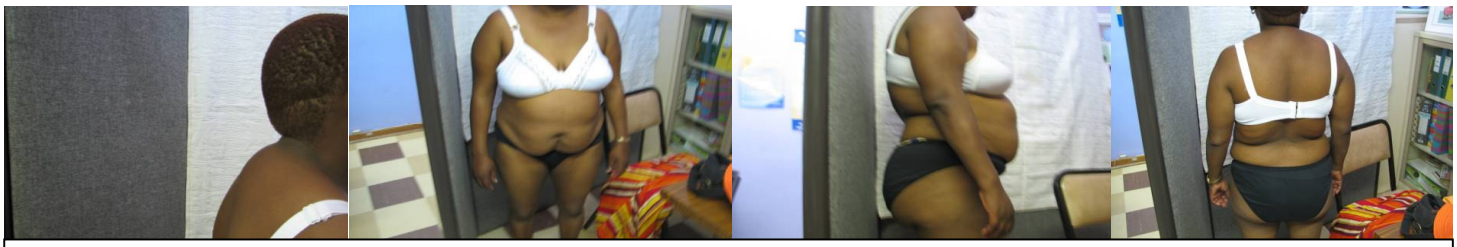
FIGURE 4.1: STUDY PROFILE

TABLE 4.2: SUMMARY OF THE SCREENING FOR MORPHOLOGICAL CHANGES (N=174[a])

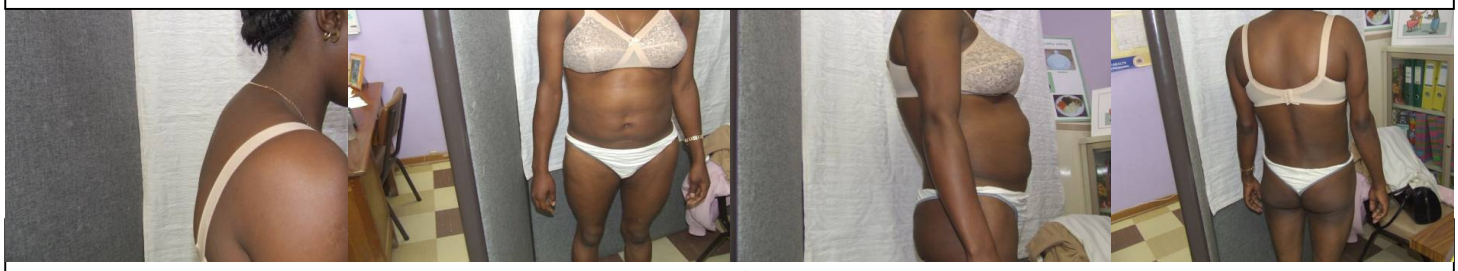
	LDS feature of cases	Mild	Moderate	Severe	Total (%)[b]
Lipohypertrophy (n=173; 99%)	Neck	10	1	0	11 (6)
	Breast	26	62	33	121 (70)
	Waist	17	121	29	167 (96)
Lipoatrophy (n=134; 77%)	Face	76	5	0	81 (47)
	Arms	42	6	1	49 (28)
	Buttocks	52	46	4	102 (59)
	Legs	41	44	3	88 (51)

[a] More than one LDS feature was possible per case

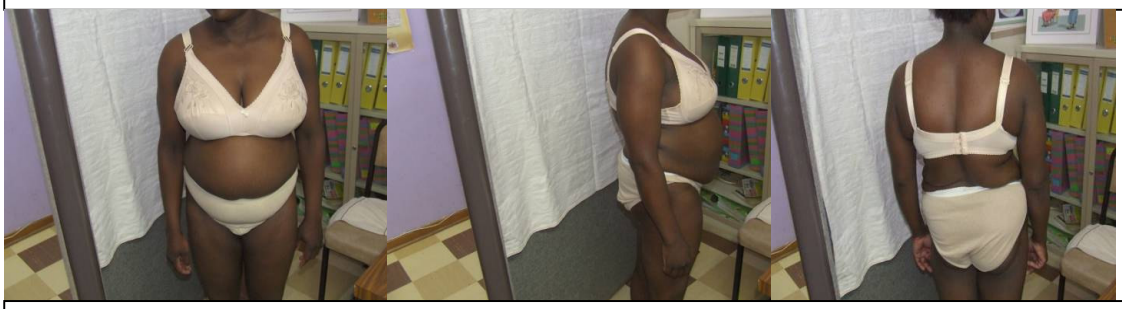
[b] % expression of total cases (n=174)



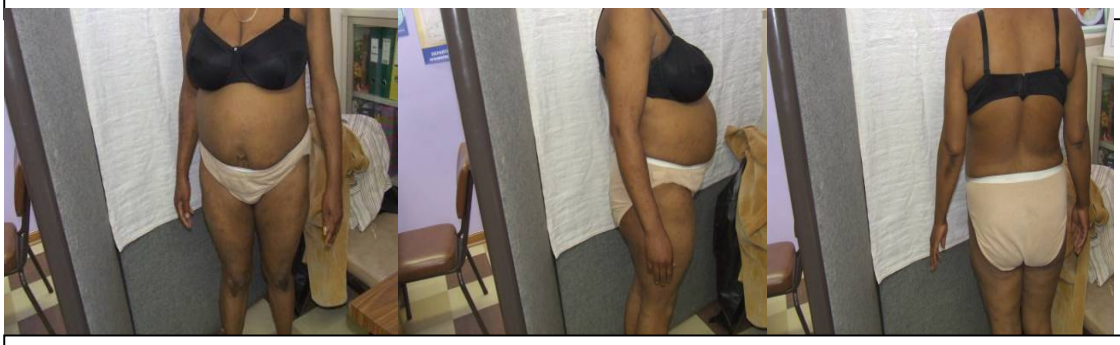
39 year old female, 35 months on HAART (AZT, 3TC and Efavirenz) presenting with moderate to severe lipohypertrophy of the neck (buffalo hump), breast, stomach and back.



31 year old female, 25 months on HAART (AZT, 3TC and Efavirenz) presenting with lipohypertrophy of the neck (buffalo hump) (mild), and breast (severe). Lipoatrophy can be seen at the arms (moderate), buttocks and legs (severe).



34 year old female, 24 months on HAART (AZT, 3TC and Efavirenz) presenting with lipohypertrophy of the breast and stomach (severe). Lipoatrophy can be seen at the arms (moderate), buttocks and legs (severe).



46 year old female, 35 months on HAART (AZT, 3TC and Efavirenz) presenting with lipohypertrophy of the breast (severe) and stomach (moderate). Severe lipoatrophy can be seen at the arms, buttocks and legs.

FIGURE 4.2: IMAGES OF SOME CASES' MORPHOLOGICAL CHANGES

TABLE 4.3: DESCRIPTION OF CONTROLS AND CASES IN THE SAMPLE (N=152)

	Controls (n=73)						Cases (n=79)					
Variable	**Min**	**Max**	**Mean**	**Median**	**SD**	**Skew**	**Min**	**Max**	**Mean**	**Median**	**SD**	**Sl**
Age (years)	22.00	63.00	38.55	38.00	8.85	0.61	25.00	66.00	37.77	36.00	8.40	
Duration HIV (years)	1.00	12.00	3.40	3.00	2.15	1.64	1.00	11.00	3.99	3.00	1.92	
WHO classification (Stage 1-4)	1.00	3.00		3.00			1.00	3.00		3.00		
Duration HAART (months)	12.00	43.00	20.95	15.00	9.43	0.73	12.00	39.00	27.18	26.00	8.86	
Systolic blood pressure (mmHg)	90.00	150.00	113.73	110.00	13.89	0.07	80.00	150.00	116.52	120.00	13.44	
Diastolic blood pressure (mmHg)	60.00	110.00	76.47	80.00	10.77	0.20	50.00	110.00	77.39	80.00	11.70	
CD_4^+ (absolute cell count/mL)	88.00	1,039.00	364.01	339.00	178.81	1.18	125.00	1 061.00	425.87	384.00	172.57	
Viral load (copies/mL)	88.00	9 500.00	2 205.60	350.00	3 091.96	1.66	36.00	1 400 000.00	105 840.00	325.00	372 779.00	
High density lipoprotein (mmol/L)	0.90	1.80	1.08	1.10	0.15	1.90	0.80	2.10	1.08	1.00	0.27	
Low density lipoprotein (mmol/L)	1.50	3.50	2.44	2.40	0.39	0.55	1.40	4.90	2.83	2.90	0.62	
Triglycerides (mmol/L)	0.60	3.70	1.24	1.20	0.49	3.10	0.40	8.00	1.63	1.50	0.95	
Lactate (mmol/L)	0.70	8.00	1.91	1.60	1.24	2.62	0.70	7.10	2.24	2.00	1.18	
Anion gap (mmol/L)	7.00	23.00	12.48	12.00	2.88	0.84	8.00	18.00	13.44	14.00	2.34	
Glucose (mmol/L)	3.00	7.10	4.49	4.30	0.74	1.59	3.70	26.40	5.29	4.90	2.71	
Weight (kg)	42.90	89.13	60.42	59.70	8.90	0.54	42.78	98.60	70.35	69.00	12.94	
Height (m)	1.43	1.71	1.59	1.59	0.06	-0.28	1.40	1.74	1.59	1.59	0.07	
Waist circumference (cm)	67.10	102.00	82.95	82.90	7.48	0.39	73.00	127.50	95.08	93.05	10.79	
Hip circumference (cm)	80.00	118.05	95.23	93.50	8.15	0.88	80.00	143.00	100.21	99.00	11.10	
Mid arm circumference (cm)	22.00	36.60	28.24	28.40	3.08	0.36	21.50	43.50	30.62	30.00	4.03	
Thigh circumference (cm)	40.55	69.00	51.44	51.00	5.31	0.93	40.00	73.00	53.97	54.70	6.75	
Triceps skinfold (mm)	6.40	44.70	18.99	18.50	8.06	0.79	8.00	45.60	21.67	20.60	8.72	
Biceps skinfold (mm)	3.45	37.10	13.12	12.00	7.75	1.59	4.00	42.10	15.61	14.35	7.60	
Subscapular skinfold (mm)	5.85	40.50	17.35	16.00	7.56	0.89	12.05	62.45	28.04	25.70	11.50	
Supra iliac skinfold (mm)	4.50	39.35	19.02	18.40	8.84	0.41	4.00	52.60	25.02	24.65	9.33	
Thigh skinfold (mm)	9.55	76.70	39.74	39.95	16.98	0.33	7.60	75.65	36.56	34.60	16.63	
Waist hip ratio	0.70	1.03	0.87	0.87	0.06	0.12	0.82	1.14	0.95	0.95	0.05	
Arm muscle circumference (cm)	17.38	26.90	22.28	22.44	2.08	-0.16	18.66	29.61	23.81	23.74	2.43	
Arm area (cm^2)	38.52	106.60	64.21	64.18	14.16	0.66	36.78	150.58	75.88	71.62	20.50	
Arm muscle area (cm^2)	17.53	51.14	33.32	33.55	7.33	0.08	21.21	63.27	39.08	38.37	9.39	
Arm fat area (cm^2)	13.70	63.89	30.89	29.56	11.53	0.83	14.60	89.35	36.79	33.83	14.8	
Body mass index (kg/m^2)	17.79	34.38	24.00	23.71	3.39	0.67	16.53	41.85	27.74	27.25	5.07	

Table 4.4 shows the number of cases and controls with their other illnesses, CDC category (as well as WHO classification) and HAART regimens. Of the sample population, 84% had no other illnesses or co-morbidities during the data collection period. The most common illness was peripheral neuropathy and HT. The majority of subjects had symptomatic HIV, although none of them had an AIDS-defining illness (as per classification of WHO). The majority of subjects were treated with first-line regimen of HAART (3TC, D4T and Efavirenz) with less than 5% on second-line regimen of HAART. The rest of the subjects were on a combination of first- and second line treatment.

TABLE 4.4: DISEASE- AND TREATMENT PROFILES OF SAMPLE

	Cases (n=79)	Controls (n=73)	Total (n=152)
Other illnesses			
	n (%)	**n (%)**	**n (%)**
None	63 (42)	64 (42)	127 (84)
Diabetes mellitus	1 (<1)	0	1 (<1)
Hypertension on treatment	5 (3)	1 (<1)	6 (4)
Hypertension on treatment + Tuberculosis mycobacterium	1 (<1)	0	1 (<1)
Hypertension on treatment + Diabetes mellitus treatment	1 (<1)	0	1 (<1)
Lactic acidosis	2 (1)	1 (<1)	3 (2)
Peripheral neuropathy	5 (3)	7 (5)	12 (8)
Peripheral neuropathy + lactic acidosis	1 (<1)	0	1 (<1)
CDC classification			
	n (%)	**n (%)**	**n (%)**
A	7 (5)	5 (3)	12 (8)
B	70 (46)	60 (39)	130 (85)
C	2 (1)	8 (5)	10 (6)
WHO classification			
	n (%)	**n (%)**	**n (%)**
1	8 (5)	5 (3)	13 (8)
2	27 (18)	27 (18)	54 (36)
3	44 (29)	41 (27)	85 (56)
4	0	0	0
HAART regimen			
	n (%)	**n (%)**	**n (%)**
3TC, D4T and Stocrin	19 (13)	44 29)	63 (42)
3TC, AZT and Stocrin	33 (22)	10 (6)	43 (28)
3TC, D4T and Nevirapine	9 (6)	16 (11)	25 (17)
3TC, AZT and Nevirapine	10 (6)	1 (<1)	11 (7)
AZT, DDI and Kaletra	4 (3)	1 (<1)	5 (3)
3TC, AZT, Kaletra	1 (<1)	1 (<1)	2 (1)
3TC, D4T, Kaletra	2 (1)	0	2 (1)
Kaletra, 3TC	1 (<1)	0	1 (<1)

4.2 RELIABILITY OF MEASUREMENTS

Table 4.5 shows the intraclass correlation where multiple readings were taken for the variables. All readings had an intraclass correlation of >0.99.

TABLE 4.5: INTRACLASS CORRELATION OF MULTIPLE READINGS OF VARIABLES

Variable	Intraclass correlation
Systolic blood pressure	0.99
Diastolic blood pressure	0.99
Weight	0.99
Height	0.99
Waist circumference	0.99
Hip circumference	0.99
Mid arm circumference	0.99
Thigh circumference	0.99
Triceps skinfold	0.99
Biceps skinfold	0.99
Subscapular skinfold	0.99
Supra iliac skinfold	0.99
Thigh skinfold	0.99

4.3 AGREEMENT BETWEEN THE TEST METHODS AND THE REFERENCE METHOD

The primary objective of the study was to investigate the agreement between the test methods (i.e. NCEP criteria, subjective self-reporting and anthropometry [Kotler, routine anthropometry and Dong&Hendricks), and the reference method, i.e. the objective case definition. Table 4.6 is a cross tabulation between, on the one hand, the reference method, and, on the other hand, the various test methods and the screening process. This was used to calculate the sensitivity, specificity and PPV and NPV for each test method (Table 4.7).

TABLE 4.6: CROSS TABULATION OF THE AGREEMENT BETWEEN TEST – AND REFERENCE METHODS

			Reference method		
			Positive (n=42)	Negative (n=110)	Total (n=152)
Screening		Positive	30	49	79
		Negative	12	61	73
Test methods	**NCEP criteria**	Positive	19	19	38
		Negative	23	91	114
	Subjective self-reporting	Positive	31	45	76
		Negative	11	65	76
	Anthropometry: Kotler	Positive	30	53	83
		Negative	12	57	69
	Routine anthropometry	Positive	26	51	77
		Negative	16	59	75
	Anthropometry: Dong&Hendricks	Positive	4	13	17
		Negative	38	97	135

TABLE 4.7 : DIAGNOSTIC STATISTICS OF THE TEST METHODS

Method	Sensitivity (%)	Specificity (%)	PPV [a] (%)	NPV [b] (%)	Kappa [c]	McNemar's [d]
Screening	71	55	38	84	0.21	0.00
NCEP criteria	45	83	50	80	0.29	0.54
Subjective self-reporting	74	59	41	86	0.26	0.00
Anthropometry: Kotler	71	52	36	83	0.18	0.00
Routine anthropometry	62	54	34	79	0.12	0.00
Anthropometry: Dong&Hendricks	10	88	24	72	0.00	0.00

[a] Positive predictive value

[b] Negative predictive value

[c] Kappa coefficient

[d] McNemar's test of symmetry (two-tailed p-value)

Subjective self-reporting, Kotler anthropometry and the screening process identified the most subjects correctly as HIV LDS **(+)**, with respective sensitivities (true positives) of 74%, 71% and 71%. The lowest sensitivity was achieved by the Dong&Hendricks anthropometry (10%).

The NCEP criteria had the best PPV (the proportion of subjects with a positive test result who are correctly classified as HIV LDS **(+)**), with 50%, followed by subjective self-reporting of 41%. Anthropometric measurement: Dong&Hendricks had the lowest PPV with 24%. The Dong&Hendricks anthropometry and NCEP criteria correctly identified the most HIV LDS Θ, i.e. 88% and 83% respectively, compared to the reference method (specificity or true negatives). Subjective self-reporting, Kotler anthropometry and

screening for morphological changes had the best NPV (the proportion of subjects who are correctly identified as HIV LDS Θ).

The NCEP criteria had the best kappa coefficient of all the test methods and screening with a κ-value of 0.29 although all were less than 0.4. Only for NCEP criteria the two-tailed p-value as measured by McNemar's test of symmetry was 0.54 which is not significantly different from the reference method.

4.4 NEW CLASSIFICATION INSTRUMENTS FOR HIV LDS

The secondary objective of the study was to develop a classification instrument for HIV LDS utilizing parameters from the testing procedures. The ideal would be that such an instrument would be based on all the available parameters (i.e. screening, age, duration HIV, WHO, duration HAART, morphological changes, systolic blood pressure, diastolic blood pressure, CD_4^+, HDL, LDL, TG, lactate, anion gap, glucose, weight and height). However, since this resulted in a model with too many variables, including relatively costly biochemical analyses (e.g. anion gap and lactate), a more feasible model, which included a lipogram, was developed. Using stepwise logistic regression this classification model follows from the output in Table 4.8.

TABLE 4.8: A SIMPLER INSTRUMENT WITH BLOOD SAMPLES FOR HIV LDS

Variable	Coefficient	p value
Thigh skinfold (mm)	0.04	0.09
Age (years)	0.08	0.01
High density lipoprotein (mmol/L)	-8.95	0.00
Duration HAART (months)	0.04	0.14
Morphological changes	1.28	0.02
Thigh circumference (cm)	-0.15	0.11
Diastolic blood pressure (mmHg)	0.04	0.07
Mid arm circumference (cm)	-0.20	0.11
Weight (kg)	0.07	0.13
Low density lipoprotein (mmol/L)	0.79	0.09
Constant	5.36	0.25

The information from Table 4.8 can be presented as follows (Figure 4.3):

y = 5.36 + 0.04 thigh skinfold (mm) – 0.15 thigh circumference (cm) - 0.20 MAC (cm) + 0.07 weight (kg) + 0.79 LDL (mmol/L) – 8.95 HDL (mmol/L) + 0.08 age (years) + 0.04 duration HAART (months) + 1.28 morphological changes (yes=1; no =0) + 0.04 diastolic BP (mmHg).

FIGURE 4.3: A SIMPLER INSTRUMENT WITH BLOOD SAMPLES FOR HIV LDS

From Figure 4.3 the probability of being LDS **(+)** is determined from the expression:

p = prob (LDS **(+)**) = exp (y) / (1+exp(y))

and a given subject will be classified as LDS **(+)** if p > 0.3 or, alternatively, if y > -0.85. The diagnostic statistics associated with the cut-off p > 0.3 are given in Table 4.9. Figure 4.4 is an illustration the cut-point (p > 0.3) that was found to optimize diagnostic statistics (sensitivity and specificity) for the SIMPLER INSTRUMENT with blood samples for HIV LDS.

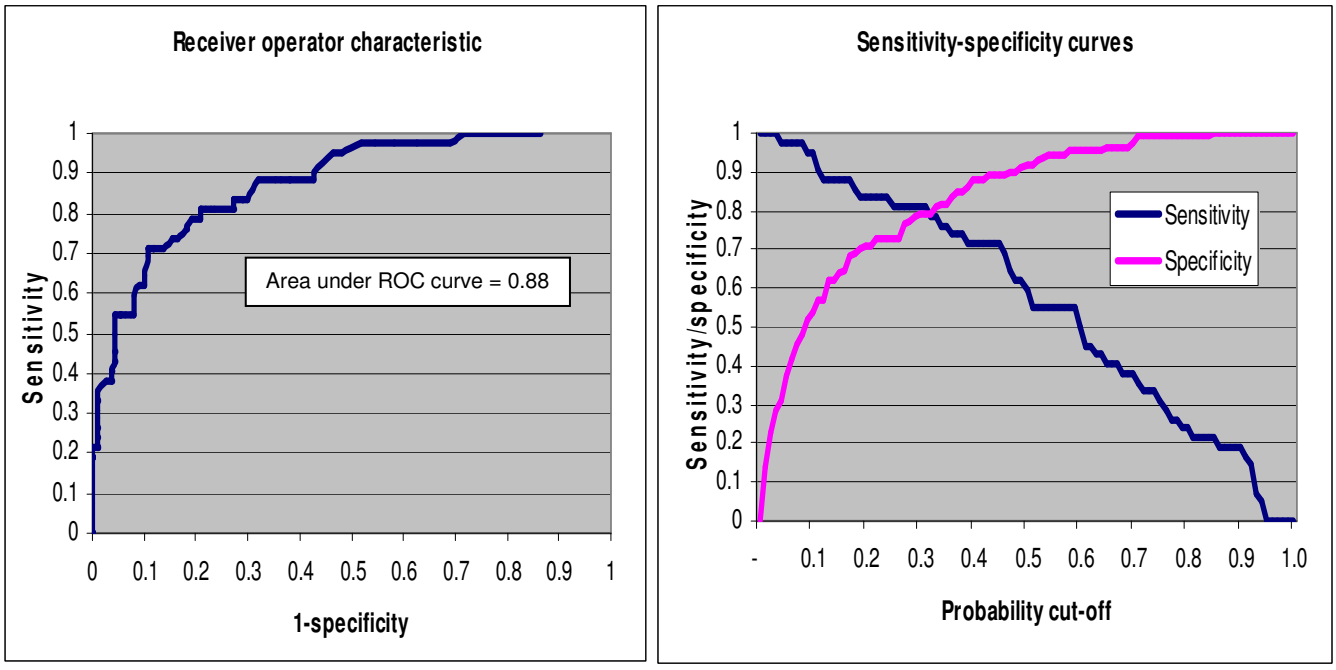


FIGURE 4.4: RECEIVER OPERATOR CHARACTERISTIC AND SENSITIVITY-SPECIFICITY CURVES FOR THE SIMPLER INSTRUMENT WITH BLOOD SAMPLES

TABLE 4.9: DIAGNOSTIC STATISTICS OF A SIMPLER INSTRUMENT WITH BLOOD SAMPLES

		Reference method		
		Positive (n=42)	**Negative (n=110)**	**Total (n=152)**
SIMPLER INSTRUMENT with blood samples	**Positive (p>0.3)**	34	23	57
	Negative (p=<0.3)	8	87	95
Sensitivity: 81% Specificity: 79% Positive predictive value: 60% Negative predictive value: 92% Kappa statistic: 0.54 McNemar's test of symmetry (two-tailed p-value): 0.00 Area under Receiver Operating Curve (ROC): 0.88				

In the absence of a lipogram (i.e. excluding all blood tests from the analyses) stepwise logistic regression suggests the classification instrument of Table 4.10.

TABLE 4.10: A SIMPLER INSTRUMENT (NO BLOOD SAMPLES)

Variable	**Coefficient**	**p Value**
Mid arm circumference (cm)	-0.097	0.18
Age (years)	0.04	0.06
Subscapular skinfold (mm)	0.04	0.08
Diastolic blood pressure (mmHg)	0.04	0.045
Morphological changes	1.18	0.01
Constant	-4.53	0.04

The information from Table 4.10 can be reduced to the following formula (Figure 4.5):

y = -4.53 – 0.097 MAC (cm) + 0.04 subscapular skinfold (mm) + 0.04 age (years) + 1.18 morphological changes (yes=1; no =0) + 0.04 diastolic blood pressure (mmHg).

FIGURE 4.5: A SIMPLER INSTRUMENT (NO BLOOD SAMPLES) FOR HIV LDS

From Figure 4.5 the probability of being LDS **(+)** is determined from the expression:

p = prob (LDS **(+)**) = exp (y) / (1+exp(y)).

Here a subject will be classified as HIV LDS **(+)** if p>0.28 or y= -0.94.

The diagnostic statistics associated with cut-off p>0.28 are given in Table 4.11. Figure 4.6 is an illustration the cut-point (p > 0.28) that was found to optimize diagnostic statistics

(sensitivity and specificity) for the SIMPLER INSTRUMENT (NO BLOOD SAMPLES) for HIV LDS.

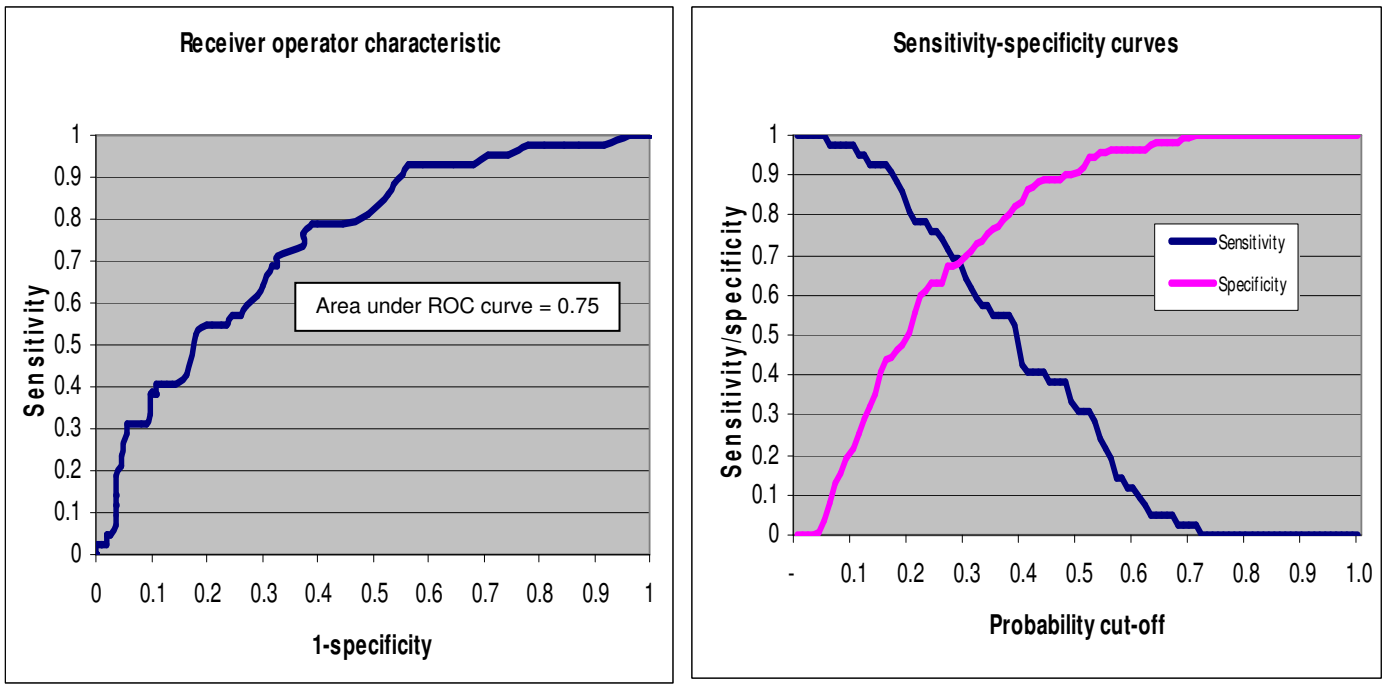


FIGURE 4.6: RECEIVER OPERATOR CHARACTERISTIC AND SENSITIVITY-SPECIFICITY CURVES FOR THE SIMPLER INSTRUMENT (NO BLOOD SAMPLES)

TABLE 4.11: DIAGNOSTIC STATISTICS OF A SIMPLER INSTRUMENT (NO BLOOD SAMPLES) FOR HIV LDS

		Reference method		
		Positive (n=42)	**Negative (n=110)**	**Total (n=152)**
SIMPLER INSTRUMENT (NO BLOOD SAMPLES)	**Positive (p>0.28)**	29	36	65
	Negative (p=<0.28)	13	74	87
Sensitivity: 69.05% Specificity: 67.27% Positive predictive value: 44.62% Negative predictive value: 85.06% Kappa statistic: 0.31 McNemar's test of symmetry (two-tailed p-value): 0.00 Area under ROC: 0.75				

4.5 CROSS-VALIDATION OF SIMPLER INSTRUMENT WITH BLOOD SAMPLES

The SIMPLER INSTRUMENT with blood samples was subjected to cross-validation. As expected, the diagnostic statistics were found to be lower than above (Table 4.12).

TABLE 4.12: DIAGNOSTIC STATISTICS OF THE SIMPLER INSTRUMENT WITH BLOOD SAMPLES AFTER CROSS-VALIDATION

		Reference method		
		Positive (n=42)	**Negative (n=110)**	**Total (n=152)**
SIMPLER INSTRUMENT with blood samples	**Positive**	30	28	58
	Negative	12	82	94
Sensitivity: 71.4% Specificity: 74.5% Positive predictive value: 51.7% Negative predictive value: 87.1% Kappa statistic: 0.41				

Table 4.13 summarised the comparison of all the test methods i.e. NCEP criteria, subjective self-reporting and anthropometry to the new classification instruments.

TABLE 4.13: COMPARISON OF THE DIAGNOSTIC STATISTICS BETWEEN THE TEST METHODS AND THE SIMPLER CLASSIFICATION INSTRUMENTS FOR HIV LDS

Method	Sensitivity (%)	Specificity (%)	PPV [a] (%)	NPV [b] (%)	Kappa [c]	McNemar's [d]
NCEP criteria	45	83	50	80	0.29	0.54
Subjective self-reporting	74	59	41	86	0.26	0.00
Anthropometry: Kotler	71	52	36	83	0.18	0.00
Routine anthropometry	62	54	34	79	0.12	0.00
Anthropometry: Dong&Hendricks	10	88	24	72	0.00	0.00
SIMPLER INSTRUMENT with blood samples	81	79	60	92	0.54	0.00
SIMPLER INSTRUMENT with blood samples after validation	71	75	52	87	0.41	0.00
SIMPLER INSTRUMENT (NO BLOOD SAMPLES)	69	67	45	85	0.31	0.00

[a] Positive predictive value

[b] Negative predictive value

[c] Kappa coefficient

[d] McNemar's test of symmetry (two-tailed p-value)

5 DISCUSSION

With the long-term use of HAART, adverse side effects may develop in individuals. HIV LDS is one of the most prevalent secondary side effects of HAART. [3,4,5,6,7,24] Several studies have been done in the field of HIV LDS, but the lack of standardisation in the assessment thereof makes it difficult to compare and generalize results due to the inconsistency and variation of methods use (i.e. anthropometry and subjective self-reporting) to assess this adverse effect in HIV treatment. [10] This study's objective was to first compare existing methods of HIV LDS assessment (NCEP criteria, subjective self-reporting and anthropometric prediction [Kotler, routine and Dong&Hendricks]) to a reference method i.e. the objective case definition by means of diagnostic testing. Furthermore a new classification instrument was developed and validated from the existing variables of the different test methods used in the clinical setup. These objectives were set to fulfil the need for an objective classification- and standard reporting of HIV LDS, as well as for simpler and less expensive diagnosis of LDS since the reference method is too expensive and time consuming for use in the clinical setup [10] in South Africa.

The study was carried out in Kalafong Hospital, South Africa in 2007. The typical number of patients attending the IOPD during the period of August 2005 to December 2007 (which included the data collection period of this study) had a gender ratio of 7:3 for females and males respectively. [Unpublished data] The study population's gender distribution during the data collection period was similar to that of the IOPD population of 69% females and 31% males. Informal methods used to assess HIV LDS in the IOPD clinic before the study period showed an estimated prevalence of 20% of HIV LDS and that less than 1% of these were male. [Unpublished data during October 2005 – June 2007] Furthermore, women are five times more likely to develop metabolic- and/or morphological changes associated with HIV. [10] These considerations led to the decision to focus on females only in this study and consequently the sample population only consisted of females. The majority of the subjects in the study were black, which was similar to the IOPD clinic race-ethnicity distribution due to the research site location. [Unpublished data]

The high consent rate (90%) was due the trusting relationship that existed between the dietetic department and the IOPD-patients. Among non-participants disclosure of their HIV status and the social implication thereof may defer them from participation in spite of the assurance of confidentiality. [4,9,10]

As outlined in chapter 3 (Methodology), cases and controls were used to purposefully sample participants since HIV LDS was thought to be a relatively uncommon event, thus limiting cases. We anticipated to identify more controls than cases using the screening process and for this reason a target was set to have at least one case (HIV LDS **(+)**) for every two controls (HIV LDS Θ). Surprisingly the screening process identified more cases than controls (2:1). This could be due to the objective, standardised screening tool that was used for the first time to identify morphological changes in the population in stead of the wide range of lack of standardisation methods that were used in the past (see addendum A for screening tool). This screening tool is an objective way to test for the presence of morphological changes, without any subjective opinions from the observer or the patient. This screening tool was also used in the objective case definition study to screen for cases and controls. [15] Lipohypertrophy was more common than lipoatrophy in the cases with 99% and 77% respectively as identified by the screening process. Lipohypertrophy at the waist area (96%) was the most common and neck (buffalo hump) the least (6%) prevalent feature. Lipoatrophy was more common at the buttocks (59%) and least seen in the arm area (28%). The overall morphological change least common was in the neck area followed by the arms. Due to the inconsistency in reporting of HIV LDS and thus morphological changes these results are difficult to compare with previous studies. [10,15] If the results are compared to the 'Objective case definition' of the lipodystrophy case definition group, lipoatrophy was found to be more common than lipohypertrophy. This difference could be due to the gender composition of their study population (where more than 80% were men) [15] as it is known that lipoatrophy is more common amongst men and lipohypertrophy amongst women. [4, 36] Another explanation for the this difference could be due to the relative short duration on HAART for the cases (less than three years) as it is known that the development of lipoatrophy is positively associated with the duration of HAART. [4,5,6,10]

Distinctive differences in certain variables between controls and cases were expected because the screening process was based on the morphological changes in the subjects. Anthropometric measurements that showed clear differences between the controls and the cases were weight (kg) 60 (± 9) vs. 70 (± 13) and BMI (kg/m^2) 24 (± 3) vs. 28 (± 5); WC (cm) 83 (± 7.5) vs. 95 (± 10.8); hip circumference (cm) 95 (± 8.2) vs. 100 (± 11.1) and WHR 0.87 (±0.06) vs. 0.95 (± 0.05); respectively. The manifestation of HIV wasting has

changed in the era of HAART to body composition changes due to LBM wasting (lipoatrophy) even though it may not be reflected by weight changes due to fat accumulation at central parts (lipohypertrophy).The interpretation of weight and thus BMI in the face of HIV LDS can be misleading and unreliable as a measure of fat free mass and the changes thereof. [57] It was not known how many of the participants already had a WC of greater than 88cm when they started to use HAART and did not develop a large WC after initiation of HAART but WC has been shown to be more associated with visceral adipose tissue mass than WHR and BMI. WC measurement is thus a practical means of evaluating the presence of regional fat depots. In this study the mean WC for the cases was 95 cm (±11) compared to the cut-off of 88 cm which is positively associated with fat depots. [23] Because WC would indicate visceral fat deposits (lipohypertrophy) and hip circumference would reflect subcutaneous fat loss (lipoatrophy), WHR is said to be a good index of body composition changes in HIV LDS. [45] In the sample the mean WHR for the cases was 0.95 (±0.05) compare to the cut-off suggested by authors of larger than 0.9 cm which represents lipohypertrophy. [10,22] In terms of skinfolds, the only distinctive difference between the controls and cases was for subscapular skinfolds 17 mm (± 8) vs. 28 mm (± 12) respectively) and supra iliac skinfolds 19 mm (± 9) vs. 25 mm (± 9) respectively). Because skinfolds value lies in that it can measure sub-cutaneous fat at site-specific areas, this study results can be interpreted as representative of lipohypertrophy. [49,54]

It was expected to also see more distinctive differences in the peripheral measurements (MAC and thigh circumference, triceps-, biceps and thigh skinfolds) between cases and controls to demonstrate the presence of lipoatrophy in the cases. The absence of lipoatrophy can be related back to the screening result where more lipohypertrophy (99%) was seen in cases compared to lipoatrophy (77%) as well as that the sample consisted of females only with lipoatrophy more common in men. [4,36]

It was expected to see some differences in the rest of the variables between cases and controls because of their association to HIV LDS as described in the literature. The mean age in the sample was 38 (± 8) years, which was similar for cases and controls. The duration of HIV was longer in the cases (4 years ± 3) than controls (3.4 ± 3) as expected although the duration of HIV should be interpreted with caution as the assumption was made that subjects knew when they were infected and could in most subjects rather provide the duration since testing HIV positive. [15] The mean duration of HAART was less

than three years for the cases and for the controls less than two years which was expected because of the association with developing HIV LDS. [15]

The absolute CD_4^+ lymphocyte count was higher in the cases than the controls and the VL was undetectable for the majority of the sample which corresponds with other studies showing higher CD_4^+ and lower VL for patients who were diagnosed with HIV LDS compared to patients without HIV LDS. [10, 20] Baseline measurements for VL and CD_4^+ were not recorded which could have been valuable as it is known that patients who are initiated on HAART with a higher VL, are at a higher risk to develop HIV LDS. [10,20] Some of the subjects had detectable VL which could reflect resistance to the HAART or non-adherence because it is expected to have an undetectable VL at six months after HAART initiation. The objective case definition study population showed similar subjects characteristics. [15] (Refer to Table 4.3).

Cases' lipograms were slightly higher than those of controls (with the exception of mean HDL were similar 1.08 mol/L (± 0.27 cases and 0.15 controls). Lactate, anion gap as well as glucose levels were higher in cases than controls which can all be interpreted as metabolic changes associated with HIV LDS. [5,10,15,38]

Overall the sample was "healthy". Only 16% reported other illness like DM, HT or peripheral neuropathy. It is of interest to note that 59% of the sample was on HAART regimen 1 (D4T, 3TC and Nevirapine/Efavirenz) and 35% on HAART regimen 1 with D4T replaced with AZT. It is a common practice in resource limited countries to switch ART-drug D4T with AZT in the presence of metabolic and/or morphological side effects of HAART. [11] It's is also worth mentioning that 95% of the subjects were on a PI-sparing regimen. It was previously thought that only PI attribute to morphological- and metabolically side effects, but this population's HAART regimen and presence of these side effects agrees with findings that the development of HIV LDS is not drug class specific and can occur with all the classes of ART. [5,15,24]

5.1 RELIABILITY OF MEASUREMENTS

In order to address quality control issues for the 13 parameters, two readings were taken. For all of these parameters intraclass correlation was calculated and was found to be close to one indicating very good intra-rater reliability of measurements.

5.2 AGREEMENT BETWEEN THE TEST METHODS AND THE REFERENCE METHOD

A kappa coefficient of at least 0.4 was taken to indicate a good agreement. [60] None of the test methods i.e. NCEP criteria (κ=0.29), subjective self-reporting (κ= 0.26) and anthropometry measurements had good agreement with the reference method i.e. objective case definition to assess if a subject was HIV LDS **(+)** or HIV LDS Θ.

The two-tailed p value for McNemar's test of symmetry was 0.00 for all the test methods except for NCEP criteria with a value of 0.5371. By conventional criteria, this difference is considered to be extremely statistically significant. This means that test method NCEP criteria is the only method that classifies subjects in the same direction as the reference method e.g. the non-agreeing cells (false positives and false negatives) were symmetrical. All the other test methods show a bias where either the false positives or the false negatives dominate (refer to Table 4.6). [61]

With diagnostic testing a trade-off usually exists between measurements depending on the risk that one is willing to take to over- or under classify subjects. If the end goal is to identify as many people as possible as HIV LDS **(+)** while taking the risk to classifying healthy people as HIV LDS **(+)**, a test method with the highest sensitivity (and highest PPV) would be chosen. If the goal is to implement a screening tool for HIV LDS Θ, or if the test method will be used in a resource-limited country or -setup, test methods with higher specificity and NPV would then be best to choose. A HIV LDS Θ result will reassure the observer/health professional that the patient does not have HIV LDS.

The suitability of tests which over- or under classify subjects as HIV LDS **(+),** is debatable. On the one hand over classifying subjects as HIV LDS **(+)** might address, support or manage future adherence issues and metabolic complications if present, but the burden

on the individual (to deal with another 'label' on top of his/her HIV status) as well as on the health system (cost of treatment) must also be taken into account.

Subjective self-reporting, Kotler anthropometry and the screening process identified the most subjects correctly (sensitivity or true positives) as HIV LDS **(+)**, with 74%, 71% and 71% respectively, compared to the reference method. Routine anthropometry had a sensitivity of 62% and NCEP criteria's sensitivity was 45%. The lowest sensitivity was by the anthropometric test method of Dong&Hendricks with 10%. However, this could be due to the fact that the reference cut-off points are based on data derived from a USA database of mainly white persons.[23] NCEP criteria had the best positive predictive value (PPV) (the proportion of subjects with a positive test result who are correctly classified as HIV LDS **(+)**), with 50%, followed by subjective self-reporting of 41%. Anthropometry: Dong&Hendricks had the lowest PPV with 24%.

The Dong&Hendricks anthropometry and NCEP criteria identified the most subjects correctly as HIV LDS Θ (specificity or true negatives) with 88% and 82% respectively. Subjective self-reporting as well as routine and Kotler anthropometry all had much lower specificities with 59%, 54% and 52% respectively. Subjective self-reporting and anthropometric prediction by Kotler as well as the screening method had the best NPV (the proportion of subjects who are correctly identified as HIV LDS Θ).

To conclude, although test method Dong&Hendricks had the highest specificity and lowest false positive rate (1 – specificity), it did not perform well with the Kappa coefficient and McNemar's test for symmetry. The test method with the best overall agreement to the reference method was NCEP criteria. NCEP criteria had a specificity of 83% (false positive rate of 17%), PPV of 50% and NPV of 80% (false negative rate 20%). NCEP criteria also had the best agreement with the reference method as calculated with Kappa coefficient and McNemar's test for symmetry. It must thus be concluded that from the methods tested NCEP criteria was the best method to assess HIV LDS for use in a resource limited country like SA.

5.3 DEVELOPMENT AND VALIDATION OF A NEW CLASSIFICATION INSTRUMENT FOR HIV LDS

Three classification instruments were developed to fulfil the secondary objective of the study.

The first instrument that was developed was the IDEAL classification instrument for HIV LDS. This was the 'ideal model' and as close to the truth to classify a subject as HIV LDS **(+)** or - Θ compared to the reference method i.e. the objective case definition with an area under the curve (AUC) of 0.97. This model was not very practical to be used in the clinical setup because its length was 13 variables (compared to the reference method's variables of ten), would be time consuming to perform in the clinical setup and costly e.g. because of the anion gap and lactate blood values. One can argue that in that case, the reference method might as well be used for the purpose of identifying HIV LDS. As the goal of developing a new classification instrument was to develop a simple instrument to classify a subject as HIV LDS **(+)** or - Θ in the clinical setup, a few variables were removed that would have implications on cost and/or time in the clinical setup.

This led to the development of a SIMPLER classification instrument (which included blood samples) with an AUC of 0.88. The instrument has 10 variables (of which four are anthropometric, two are biochemical and four are observations or subjective information) which are all easy to do in the clinical setup. Three of these variables i.e. age, HDL and LDL overlap with the reference method. If these variables were to be included in an electronic Excel type application it could be very easy to use, implement and interpreted in the clinical setup (refer to Appendix E).

The four anthropometric variables included in the SIMPLER classification instrument (which included blood samples) were weight (kg), MAC (cm), thigh circumference (cm) and thigh skinfold (mm). MAC, thigh circumference and thigh skinfold are said to be surrogates for peripheral lipoatrophy. [19] The fat redistribution and metabolic changes in HIV Infection (FRAM) -study highlighted that lipoatrophy is the hallmark of body fat changes [58] and that lipoatrophy is a more distinguishing feature in HIV LDS is than central hypertrophy. [19,20,58] However, none of the suggested anthropometric measurement i.e. WC or WHR that was found by the FRAM-study to be associated with HIV LDS were included

in this instrument. [58] It was unexpected to see weight as a variable in the SIMPLER instrument as it is said that it can be misleading and unreliable as a measure of fat free mass and the changes thereof because a specific weight can be maintained over time even though fat redistribution can take place as demonstrated with body imaging. [57]

The two biochemical variables included in the SIMPLER classification instrument (which included blood samples) were HDL and LDL, both associated with the metabolic changes associated with HIV LDS. [5, 7,10,15,30,38,39,44]

The four observations or subjective information included in the SIMPLER classification instrument (which included blood samples) were age, duration of HAART, morphological changes and diastolic BP. Age and duration of HAART are known to be risk factors associated with the development of HIV LDS. [4,5,7,10,47] Elevated diastolic BP (≥90 mmHg) was found to be associated with the metabolic changes associated with HIV LDS if compared with controls [29] although other researchers only referred to the association of elevated BP in HIV LDS patients, and not specifically to diastolic BP. [62,63] Subjective self-reporting, is very easy to perform and can be accurate [19,20], but it is criticized as nothing more than pseudo truncal obesity where peripheral lipoatrophy makes the abdomen look bigger and the increase in visceral fat makes the periphery look smaller. [8]

The question arose how the SIMPLER classification instrument (which included blood samples) compared with NCEP criteria (highlighted earlier as the best of the test methods). The agreement as measured by diagnostic statistics, kappa coefficient and McNemar's symmetry test was as follows (refer to Table 4.13):

- Diagnostic statistics: sensitivity of 45% vs. 71% (validated), specificity of 83% vs. 75% (validated), PPV of 50% vs. 52% (validated) and NPV of 80% vs. 87 (validated) respectively for NCEP criteria and SIMPLER classification instrument (which included blood samples).
- Kappa coefficient equals 0.54 before validation, and 0.41 after validation compared to NCEP criteria's 0.29. This indicated good agreement.
- McNemar's test for symmetry's p-value equalled 0.00 compared to NCEP criteria's 0.5371. By conventional criteria, this difference is considered to be extremely statistically significant. This means that the SIMPLER classification instrument

(which included blood samples) did not classify the subjects in the same direction as the reference method (refer to Table 4.9).

Although specificity is more desirable than sensitivity in a resource limited clinical setup, the new instrument also demonstrated a more favourable sensitivity than NCEP criteria. The instrument thus classifies more subjects correctly as HIV LDS Θ and/or - **(+)**. Furthermore a good agreement was shown with kappa's coefficient although it did not classify subjects in the same direction from the reference method as calculated by McNemar's test for symmetry. It is my opinion that this instrument would be an appropriate model to implement in the clinical setup and is worth further investigation and to assess the implementation in the clinical setup.

Finally, a SIMPLER classification instrument (which excluded blood samples) was developed for the clinical setup where access to biochemical information was limited. This instrument had an AUC of 0.75. The instrument has five variables (of which two contain anthropometric data and three observations or subjective information) which are all easy to do in the clinical setup. Only one of these variables (age) overlaps with the reference method. The two anthropometric variables included in the SIMPLER INSTRUMENT (NO BLOOD SAMPLES) were MAC (cm), and subscapular skinfold (mm). MAC may represent peripheral lipoatrophy. [19] The measurement at the subscapular skinfold site might indicate the presence of lipohypertrophy due to the site-area. [49, 54]

The three observations or subjective information included in the SIMPLER INSTRUMENT (NO BLOOD SAMPLES) were age, morphological changes and diastolic BP. These variables were similar to the SIMPLER classification instrument (which included blood samples) of HIV LDS.

The SIMPLER INSTRUMENT (NO BLOOD SAMPLES) of HIV LDS was compared with test method NCEP criteria (which were highlighted as the best method to be used of all the routine methods to assess HIV LDS compared to the reference method). The agreement as measured by diagnostic statistics, kappa coefficient and McNemar's symmetry test was as follows (refer to Table 4.13):

- Diagnostic statistics: sensitivity of 45% vs. 69.05%, specificity of 83% vs. 67.27%, PPV of 50% vs. 44.62% and NPV of 80% vs. 85.07 respectively for NCEP criteria and SIMPLER INSTRUMENT (NO BLOOD SAMPLES).

- Kappa coefficient equals 0.31 compared to NCEP criteria's 0.29 indicating poor agreement.
- McNemar's test for symmetry's p-value equalled 0.00 compared to NCEP criteria's 0.5371. By conventional criteria, this difference is considered to be extremely statistically significant. This means that the SIMPLER INSTRUMENT (NO BLOOD SAMPLES) did not classify the subjects in the same direction as the reference method (refer to Table 4.11).

To conclude: the SIMPLER classification instrument (which excluded blood samples) did not show better agreement than test method NCEP criteria as well as the SIMPLER classification instrument (which included blood samples) as measured by kappa coefficient, McNemar's symmetry test and diagnostic testing. Although this instrument would be ideal in the clinical setup where access to laboratory analysis is limited, the diagnostic accuracy is compromised by removing all the biochemical variables. More subjects would be classified falsely as HIV LDS positive which could negatively impact on the subjects burden of the disease as well as impact on the health system which need to respond with treatment options for the subjects who are classified as HIV LDS **(+)** with this instrument. The question needs to be asked is: if this instrument is to be implemented, will it actually improve patient outcome? For this reason it is my opinion that the SIMPLER classification instrument (which excluded blood samples) should not be used for the clinical setup.

5.4 STEPWISE EVALUATION OF THE DIAGNOSTIC TEST: THE SIMPLER CLASSIFICATION INSTRUMENT FOR HIV LDS

To conclude the study a stepwise evaluation process as suggested by Van den Bruel et al was used as guidance for potential implementation of the SIMPLER INSTRUMENT with blood samples in the clinical setup. [64]

The five criteria recommended by Van den Bruel et al are technical accuracy, place in the clinical pathway, diagnostic accuracy, impact on patient outcome and cost-effectiveness. Every criterion needs to be considered in turn to assess the diagnostic ability of the test. Evidence should be provided that the test meets that criterion before progressing to the next step of the evaluation process. A decision can then be made to implement the new

diagnostic test or not depending on the quality of the evidence that was produced at each step. Furthermore the risk-benefit balance to all stakeholders should determine if the diagnostic test would be implemented. [64]

In the following discussion the SIMPLER classification instrument (which included blood samples) for HIV LDS is assessed against these criteria.

Step 1: Technical accuracy

The ability of the SIMPLER classification instrument for HIV LDS to produce useful information under research conditions was assessed as part of the primary objective of this study by comparing it to the reference standard i.e. objective case definition for sensitivity and specificity and then validating the instrument.

Further research is needed to field-test the new instrument in the IOPD population before it can be widely implemented.

The new instrument has several variables which involve standardised methods and equipment to measure biochemical values, anthropometry and BP which influence the reproducibility of the results. Intra-rater reliability was measured in the study however inter-rater reliability was not tested as only the researcher did the measurements. The reproducibility of measurements in a non-research setup is unknown as the accuracy of anthropometric measurements, blood tests and BP readings depends on the skill and experience of the individual taking the measurements. [21]

Step 2: Place in the clinical pathway

Consideration needs to be given to where in the clinical pathway the SIMPLER classification instrument for HIV LDS will be placed: before the pathway as a triage, replacing an existing test, or placed after the pathway as an add-on?

The new instrument's intended goal is to replace existing test methods in the clinical setup although it must be mentioned that currently in some South African IOPD settings no HIV LDS testing is done. Where no HIV LDS testing is done, the SIMPLER classification instrument for HIV LDS would be an add-on. Although the new instrument proved to be

more accurate than the test methods for the sample population as assessed in objective one in the research study, this will not necessarily apply in the general clinical setup.

The impact or burden on the individual as well as on the health system also needs to be assessed. The simpler instrument is likely to be more expensive than the current approaches.

Future studies could compare the instrument to existing methods against several criteria including accuracy, invasiveness for patient, cost and difficulty of interpretation of results. The outcome of these studies could assist in deciding whether it will be practical to replace existing methods with the new instrument.

Step 3: Diagnostic accuracy

An important factor to consider is whether the SIMPLER classification instrument for HIV LDS could correctly detect or exclude HIV LDS. In order to assess diagnostic accuracy, the test was compared to a reference standard i.e. objective classification instrument in a clinically relevant population, assessing sensitivity, specificity, positive predictive- and negative predictive values. Head-to-head comparisons of the new- and existing test methods were done to determine its diagnostic accuracy relative to these methods.
The SIMPLER classification instrument (which included blood results) for HIV LDS was also validated. Diagnostic accuracy is as follows after validation: sensitivity 71%, specificity of 75%, PPV 52% and NPV 87%.

Step 4: Impact on patient outcome

Positive diagnosis of HIV infection and related conditions has a physical, financial and psychological impact on the affected individual. A key requirement of the diagnostic instrument is that it should improve patient outcome. Expected benefits need to be balanced against the expected harm to the patient as well as to the health system.

The expected harm includes the burden, pain, risk as well as cost to the individual. The patient already carries the burden of a HIV positive diagnosis, having to take lifelong HAART. Now the patient will be labeled with another diagnosis as a result of his/her status and treatment thereof. The false positive rate of the instrument might complicate the burden on the patient and health system further.

The health system needs to respond to the diagnosis of HIV LDS in terms of treating patients as such as well as to change current clinical procedures to accommodate the new diagnosis. Treatment options that need to be considered include medication (switching HAART and adding new medication), availability of surgical options and lifestyle programmes. Does the health system have the capacity or resources (money, people, equipment and time) to deal with this as they already struggle to get HAART available and accessible to all? Further studies are needed to answer the above.

On the other hand consistence, standardized method of diagnosing HIV LDS will lead to better management and treatment of patients experiencing HIV LDS as HIV LDS remains largely untreated in the SA clinical setup due to the underestimation of the condition. Some patients might benefit from the new instrument and even improve their quality of life (QOL) as it will give patients affirmative diagnosis and initiate symptomatic treatment. This will improve reporting on the prevalence thereof which would help with motivation for better treatment options for these patients e.g. drug switching, surgical options etc. which is not normally available in routine practices due to underestimation of the problem. If the test were to be implemented in practice it might impact on life expectancy (as metabolic complications would be detected and treatment earlier e.g. avoidance of cardiovascular complications), QOL (including body imaging) and avoidance of other test procedures for the patient.

Step 5: Cost-effectiveness

Consideration also needs to be given to the cost of the SIMPLER classification instrument for HIV LDS as well as the acceptability to society and the health system.

The cost to the individual as well as the expected risk and benefits to the health system need to be calculated with cost-effectiveness analysis studies or economic models. A cost effectiveness study computing cost per unit of effective measure using the new test was not done, however the majority of variables included in the test are part of routine clinical practices in the IOPD and should not have further cost implications to the clinic.

An Excel based computer calculation could improve the acceptability and implementation of the new instrument (see Appendix E). All patients attending the IOPD would have

equitable access to the test, and it can be made available within the broader health care structure [64]

With this stepwise approach, a decision to implement the SIMPLER classification instrument for HIV LDS would seem to be a complicated matter as the benefit to the individual and health system needs to outweigh the possible harm or burden that might be experienced. More studies need to be undertaken before a decision to implement the new instrument in the clinical setup is taken, as one needs to know more about the instrument than its testing accuracy.

5.5 LIMITATIONS AND RECOMMENDATIONS

A limitation of the study is that the majority of the subjects in the sample population were black females while the appropriateness of the reference data to black women has not been established. The fact that this was a cross-sectional study and no baseline data were available also meant that changes in anthropometric measurements since starting HAART could not be noted.

Access to body imaging in South Africa is very limited and expensive, but would have been ideal to use in the reference method. It also has to be noted that only HIV infected subjects were included in the study as the study objective was not to distinguish between HIV uninfected LDS and – infected LDS but how to identify subjects with HIV LDS.

It is also unfortunate that the SIMPLER INSTRUMENT (NO BLOOD SAMPLES) was not validated with cross-validation. This can however be done with further consultation with a biostatistician.

An Excel based computer HIV LDS calculator can easily be developed to calculate if HIV LDS is present in patients in the clinical setup (see Appendix E).

The study successfully assessed current test methods to diagnose HIV LDS. It is concluded that from the methods tested NCEP criteria is the best method. A new classification instrument could achieve even better test accuracy than the current methods of assessing HIV LDS in the clinical setup. Implementation of the study results may lead to

an accurate, consistent, standardized way of detecting and reporting of HIV LDS. The results of the study need to be used in further follow-up studies as well as cost effectiveness studies to address issues such as external validity and (inter-rater) reliability before it can be finally implemented in South African IOPD clinics.

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APPENDIX A

APPENDIX A: SCREENING OF MORPHOLOGICAL CHANGES

Study number		**Date**	/ /
Initiation date		**Dietitian**	

Notes:

For each body region, indicate the severity of fat accumulation or – wasting.
Lipohypertrophy refers to generalized fat gain in the specific area.
Lipoatrophy refers to generalized fat wasting in a specific area.
Severity to be scored as **mild** (slightly noticeable), **moderate** (readily obvious) and **severe** (obvious).
Cases are subjects with one or more moderate and/or severe feature of lipoatrophy and/or hypertrophy

LIPOHYPERTROPHY

Is there any lipohypertrophy in any of the following areas?

1. Neck (buffalo hump)	
☐ **NO** or ☐ **YES** →	Mild ☐ Moderate ☐ Severe ☐
2. Breast	
☐ **NO** or ☐ **YES** →	Mild ☐ Moderate ☐ Severe ☐
3. Waist / abdomen	
☐ **NO** or ☐ **YES** →	Mild ☐ Moderate ☐ Severe ☐

LIPOATROPHY

Is there any lipoatrophy in any of the following areas?

1. Face	
☐ **NO** or ☐ **YES** →	Mild ☐ Moderate ☐ Severe ☐
2. Arms	
☐ **NO** or ☐ **YES** →	Mild ☐ Moderate ☐ Severe ☐
3. Buttocks	
☐ **NO** or ☐ **YES** →	Mild ☐ Moderate ☐ Severe ☐
4. Legs	
☐ **NO** or ☐ **YES** →	Mild ☐ Moderate ☐ Severe ☐

Case / Control / non-assignee

APPENDIX B

APPENDIX B: PATIENT / PARTICIPANT INFORMATION LEAFLET & INFORMED CONSENT FORM

TITLE OF STUDY:

ASSESSING HIV LIPODYSTROPHY SYNDROME: A COMPARISON OF DIFFERENT METHODS TO AN OBJECTIVE CASE DEFINITION

Dear Mr. / Mrs. ..... Date ...../...../....

1) INTRODUCTION

We invite you to participate in a research study. This information leaflet will help you to decide if you want to participate. Before you agree to take part you should fully understand what is involved. If you have any questions that this leaflet does not fully explain, please do not hesitate to ask the investigator.

2) THE NATURE AND PURPOSE OF THIS STUDY

I understand that I am being asked to take part in a research study.

What is lipodystrophy?

Lipodystrophy is a side effect that can occur after long-term use of anti-HIV medicine. It consists out of two parts: The first part has to do with your body shape. The breasts and/or stomach appear bigger while the face, arms, legs and buttocks appear smaller. The second part is seen in the blood e.g. high cholesterol (fat) and/or -glucose (blood sugar).

Aim of the study

The aim of this study is to research body shape changes linked to anti-HIV medicines. By studying this one can see which the most common features in lipodystrophy are. This will help us understand lipodystrophy better. If we understand lipodystrophy better, a tool can be developed to identify it uniformly and manage it sooner.

Purpose of the study

The purpose of this study is to check if the existing methods we use in the immunology clinic accurately identify body shape changes. Secondly we want to develop a new method to identify body shape changes with the results collected from the study.

What are the consequences of lipodystrophy?

Lipodystrophy may lead to diseases like heart disease and Diabetes Mellitus (sugar disease). It may also influence the way you see yourself (poor body image) because the body can look out of proportion.

Can lipodystrophy be treated?

Lipodystrophy can be treated. Although this study's purpose is not to treat lipodystrophy, the next step will be to identify the appropriate management options. Management of lipodystrophy usually consists of lifestyle changes (changing diet, daily exercise and stop smoking) as well as changing or adding medication.

3) EXPLANATION OF PROCEDURES TO BE FOLLOWED.

What will be expected from me?

- Permission to access and use information in my Immunology Clinic file e.g. date of birth, stage of HIV/AIDS, duration on anti-HIV medicine etc.
- Permission to access and use my blood results that were taken previously at the Immunology Clinic.
- Measuring your blood pressure.
- Answering some questions with regard to duration of HIV infection and possible body shape changes etc.
- Measurements including weight, height, waist-, arm-, hip-, thigh size (using a measuring tape) and skinfolds. Skinfolds are measured using a special tool (caliper). It feels like a small pinch that will be made on my arm, back, waist and leg.

When will all of the above be performed?

All of the activities will be performed on your monthly Immunology Clinic day visit.

4) RISK AND DISCOMFORT INVOLVED.

The only possible discomfort involved is the measurement of your blood pressure, waist- hip-, arm- and leg size with a measuring tape and skinfolds of your arm, leg, back and waist with a caliper.

These measurements will take approximately 10 additional minutes of your time, which may be an inconvenience. Your position in the queue to see other health workers e.g. doctor, sister, counsellor, and pharmacist will not be affected by this delay.

5) POSSIBLE BENEFITS OF THIS STUDY.

The benefit of the study will be that it will enable us to identify and manage people with lipodystrophy.

6) WHAT ARE YOUR RIGHTS AS A PARTICIPANT?

Your participation in this study is entirely voluntary. You can refuse to participate or stop at any time during the study without giving any reason. Your withdrawal will not affect you or your treatment in any way.

7) HAS THE STUDY RECEIVED ETHICAL APPROVAL?

This clinical study Protocol was submitted to the Faculty of Health Sciences Research Ethics Committee, University of Pretoria and written approval has been granted by that committee. The study has been structured in accordance with the Declaration of Helsinki (last update: October 2000), which deals with the recommendations guiding doctors in biomedical research involving human/subjects.

A copy of the Declaration may be obtained from the investigator should you wish to review it.

8) INFORMATION AND CONTACT PERSON

If I have any questions concerning this study, I should contact:
Mrs. Elmarie van Wyk (012- 318 6642)

9) COMPENSATION

Your participation is voluntary. No compensation will be given for your participation.

10) CONFIDENTIALITY.

All information that you give will be kept strictly confidential.

Steps to be taken by the investigator to ensure confidentiality and anonymity:

- Once you agree to participate in the study, a unique study number will be assigned to your file number according to a table created by the investigator (Mrs E van Wyk).
- The unique study number will be used throughout the study instead of your file number.
- Once all the information is collected, the table containing the study number linked to your file number will be destroyed. This will ensure that nobody can identify you.
- Once we have analysed the information no one will be able to identify you. Research reports and articles in scientific journals will not include any information that may identify you. Results will be published or presented in such a fashion that you remain unidentifiable.

11) CONSENT TO PARTICIPATE IN THIS STUDY.

I confirm that the person asking my consent to take part in this study has told me about nature, process, risks, discomforts and benefits of the study. I have also received, read and understood the above written information (Information Leaflet and Informed Consent) regarding the study. I am aware that the results of the study, including personal details, will be anonymously processed into research reports. I am participating willingly. I have had time to ask questions and have no objection to participate in the study. I understand that there is no penalty should I wish to discontinue with the study and my withdrawal will not affect any treatment in any way.

I have received a signed copy of this informed consent agreement.

.....	.....
Patient / Proxy signature	Date
Mrs. E van Wyk	.....
Person obtaining informed consent	Date
.....	.....
Witness	Date

VERBAL PATIENT INFORMED CONSENT (applicable when patients cannot read or write)

I, the undersigned, Mrs. E van Wyk, have read and have explained fully to the patient named ……and/or is/her relative, the patient information leaflet, which has indicated the nature and purpose of the study in which I have asked the patient to participate. The explanation I have given has mentioned both the possible risks and benefits of the study. The patient indicated that he/she understands that he/she will be free to withdraw from the study at any time for any reason and without jeopardizing his/her subsequent comprehensive management at the Immunology clinic, to which he/she agrees.

I hereby certify that the patient has agreed to participate in this study.

……..	…….	……..
Patient's Name	***Patient's Signature***	***Date***
Mrs. E van Wyk	…….	……..
Investigator's Name	***Investigator's Signature***	***Date***
……..	…….	……..
Witness's Name	***Witness's Signature***	***Date***

(Witness - sign that he/she has witnessed the process of informed consent)

APPENDIX C

APPENDIX C: DATA COLLECTION FORM

Study #: _____ **Initiation date: _____/___/___**

A	Background information		Date	Value 1	Value 2	Comments
	Gender					
	Duration of HIV	<= 4 years				
		> 4 years				
	WHO staging	1				
		2				
		3				
		4				
	CDC staging	A				
		B				
		C				
	HAART regimen					
	Duration on HAART					
	Other illnesses e.g.					
B	**Subjective reporting**					
	Any morphological changes from staring on Rx (patient answer, no influence form observer)	Yes				
		No				
	Blood pressure					
C	**Biochemistry**					
	CD_4^+					
	VL					
	HDL					
	Lactate					
	Triglycerides					
	LDL	<= 3.0				
		> 3.0				
	Anion gap					
D	**Anthropometry**					
	Weight (kg)					
	Height (m)					
	WC (cm)					
	Hip circumference (cm)					
	MAC (cm)					
	Thigh circumference (cm)					
	Triceps skinfold (mm)					
	Biceps skinfold (mm)					
	Sub scapular skinfold (mm)					
	Supra iliac skinfold (mm)					
	Thigh skinfold (mm)					

APPENDIX D

APPENDIX D: SCREENING OF MORPHOLOGICAL CHANGES

Study #	Lipohypertrophy						Lipoatrophy								Screening
	Neck		Breast		Waist		Face		Arms		Buttocks		Legs		
	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	Case/control/non-assignee
1	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
2	no	none	no	none	yes	moderate	no	none	no	none	yes	moderate	yes	moderate	Case
3	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
4															non-assignee
5															non-assignee
6	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
7	no	none	yes	severe	yes	moderate	yes	mild	no	none	no	none	no	none	case
8	no	none	yes	mild	yes	moderate	no	none	no	none	yes	moderate	yes	moderate	case
9	no	none	yes	moderate	yes	moderate	no	none	no	none	yes	mild	yes	moderate	case
10	no	none	yes	mild	yes	moderate	no	none	no	none	yes	moderate	yes	mild	case
11	no	none	no	none	yes	moderate	no	none	yes	mild	yes	mild	no	none	case
12	no	none	yes	moderate	yes	moderate	no	none	yes	mild	no	none	yes	mild	case
13	no	none	yes	moderate	yes	mild	yes	mild	no	none	yes	mild	yes	moderate	case
14	no	none	yes	moderate	yes	moderate	no	none	no	none	no	none	no	none	case
15	no	none	yes	moderate	yes	severe	no	none	no	none	yes	mild	yes	mild	case
16	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
17	no	none	yes	moderate	yes	moderate	no	none	yes	mild	yes	mild	no	none	case
18	no	none	no	none	no	none	no	none	no	none	yes	moderate	yes	moderate	case
19	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
20	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
21	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
22	no	none	yes	moderate	yes	moderate	no	none	no	none	yes	mild	no	none	case
23	no	none	yes	severe	yes	moderate	no	none	yes	mild	yes	mild	yes	moderate	case
24	no	none	no	none	yes	moderate	yes	mild	no	none	no	none	no	none	case
25	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
26	no	none	yes	moderate	yes	moderate	no	none	yes	mild	yes	mild	yes	moderate	case
27	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
28	no	none	no	none	yes	severe	no	none	no	none	yes	moderate	yes	moderate	case
29	no	none	yes	severe	yes	moderate	no	none	yes	mild	no	none	no	none	case
30	no	none	yes	moderate	yes	moderate	no	none	no	none	no	none	no	none	case
31	no	none	no	none	yes	moderate	no	none	no	none	yes	moderate	no	none	case
32	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
33	no	none	no	none	yes	moderate	no	none	no	none	no	none	no	none	case
34	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
35	no	none	yes	moderate	yes	mild	no	none	yes	mild	no	none	no	none	case
36	no	none	no	none	yes	moderate	no	none	yes	mild	yes	mild	no	none	case
37	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
38	no	none	yes	severe	yes	severe	no	none	no	none	no	none	yes	mild	case
39	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
40	yes	mild	no	none	yes	moderate	no	none	no	none	yes	moderate	yes	mild	case

Study #	Lipohypertrophy						Lipoatrophy								Screening
	Neck		Breast		Waist		Face		Arms		Buttocks		Legs		
	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	Case/control/non-assignee
41	no	none	yes	mild	yes	moderate	no	none	no	none	no	none	no	none	case
42	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
43	no	none	yes	moderate	yes	mild	no	none	yes	mild	yes	mild	yes	mild	case
44	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
45	no	none	no	none	yes	moderate	no	none	no	none	yes	mild	no	none	case
46	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
47	no	none	no	none	yes	moderate	no	none	no	none	yes	mild	yes	mild	case
48	no	none	yes	mild	yes	moderate	no	none	no	none	yes	mild	no	none	case
49	no	none	yes	severe	yes	severe	no	none	yes	mild	yes	moderate	yes	mild	case
50	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
51	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
52	no	none	yes	mild	yes	severe	no	none	yes	mild	yes	mild	yes	mild	case
53	no	none	no	none	yes	severe	no	none	no	none	yes	mild	yes	mild	case
54	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
55	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
56	no	none	yes	moderate	yes	moderate	yes	mild	yes	mild	yes	mild	yes	mild	case
57	no	none	no	none	yes	moderate	no	none	no	none	yes	mild	yes	mild	case
58	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
59	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
60	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
61	no	none	yes	moderate	yes	moderate	no	none	no	none	no	none	no	none	case
62	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
63	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
64	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
65	no	none	yes	moderate	yes	moderate	no	none	no	none	no	none	no	none	case
66															non-assignee
67	no	none	yes	severe	yes	moderate	no	none	no	none	no	none	no	none	case
68	no	none	no	none	yes	moderate	no	none	yes	mild	no	none	yes	mild	case
69	no	none	yes	severe	yes	moderate	no	none	yes	mild	yes	mild	no	none	case
70	no	none	yes	moderate	yes	severe	yes	mild	no	none	no	none	no	none	case
71	no	none	yes	moderate	no	none	yes	mild	no	none	no	none	no	none	case
72	no	none	yes	severe	yes	moderate	yes	mild	no	none	yes	mild	no	none	case
73	no	none	yes	severe	yes	moderate	no	none	no	none	no	none	no	none	case
74	no	none	yes	mild	yes	moderate	no	none	no	none	yes	moderate	yes	moderate	case
75	no	none	no	none	yes	moderate	no	none	no	none	no	none	no	none	case
76	no	none	yes	moderate	yes	moderate	yes	mild	no	none	yes	mild	yes	moderate	case
77	no	none	yes	moderate	yes	moderate	yes	mild	no	none	no	none	no	none	case
78	no	none	yes	moderate	yes	moderate	yes	mild	no	none	yes	mild	yes	mild	case
79	no	none	yes	moderate	yes	mild	yes	mild	yes	mild	yes	mild	no	none	case
80	no	none	no	none	yes	moderate	no	none	no	none	yes	mild	no	none	case

Study #	Lipohypertrophy						Lipoatrophy								Screening
	Neck		Breast		Waist		Face		Arms		Buttocks		Legs		
	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	Case/control/non-assignee
81	no	none	yes	moderate	yes	mild	yes	mild	no	none	no	none	no	none	case
82	no	none	yes	mild	yes	moderate	yes	mild	yes	mild	no	none	no	none	case
83	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
84	no	none	no	none	yes	mild	no	none	no	none	yes	mild	no	none	case
85	no	none	no	none	yes	moderate	no	none	no	none	no	none	no	none	case
86	no	none	yes	mild	yes	moderate	yes	mild	yes	mild	yes	mild	yes	mild	case
87	no	none	no	none	yes	moderate	no	none	no	none	no	none	no	none	case
88	yes	mild	yes	severe	yes	severe	yes	mild	yes	mild	yes	mild	yes	mild	case
89	no	none	yes	moderate	yes	moderate	no	none	no	none	yes	mild	no	none	case
90	no	none	yes	mild	yes	moderate	yes	mild	no	none	no	none	yes	mild	case
91	no	none	yes	moderate	yes	moderate	no	none	no	none	yes	moderate	yes	moderate	case
92	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
93															non-assignee
94	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
95	no	none	yes	moderate	yes	moderate	no	none	yes	mild	yes	moderate	yes	mild	case
96	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
97	no	none	no	none	yes	moderate	no	none	no	none	no	none	no	none	case
98	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
99	no	none	yes	moderate	yes	moderate	yes	mild	yes	mild	no	none	yes	moderate	case
100															non-assignee
101	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
102	no	none	no	none	no	none	no	none	no	none	yes	none	no	none	Control
103	yes	mild	yes	moderate	yes	mild	yes	mild	no	none	yes	moderate	yes	moderate	case
104	no	none	yes	severe	yes	severe	no	none	no	none	no	none	no	none	case
105	no	none	yes	moderate	yes	severe	no	none	no	none	yes	mild	no	none	case
106	no	none	yes	moderate	yes	mild	yes	mild	no	none	yes	moderate	yes	moderate	case
107	no	none	yes	severe	yes	severe	yes	mild	no	none	yes	mild	yes	mild	case
108	no	none	yes	mild	yes	moderate	yes	moderate	no	none	yes	moderate	yes	moderate	case
109	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
110	no	none	yes	moderate	yes	mild	yes	mild	yes	moderate	yes	mild	yes	moderate	case
111	no	none	yes	mild	yes	moderate	no	none	no	none	no	none	no	none	case
112	no	none	yes	severe	yes	moderate	yes	moderate	no	mild	yes	mild	yes	moderate	case
113	no	none	yes	moderate	yes	severe	yes	mild	no	none	no	none	no	none	case
114	no	none	yes	mild	yes	moderate	no	none	no	none	yes	mild	yes	mild	case
115	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
116	no	none	yes	severe	yes	severe	yes	mild	yes	mild	yes	moderate	yes	mild	case
117	no	none	yes	moderate	yes	moderate	yes	mild	yes	moderate	yes	moderate	yes	moderate	case
118	no	none	no	none	yes	moderate	no	none	yes	mild	yes	severe	yes	severe	case
119	no	none	no	none	yes	moderate	no	none	no	none	no	none	no	none	case
120	no	none	no	none	yes	moderate	no	none	no	none	no	none	no	none	case

Study #	Lipohypertrophy						Lipoatrophy								Screening
	Neck		Breast		Waist		Face		Arms		Buttocks		Legs		
	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	Case/control/non-assignee
121	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
122	no	none	yes	mild	yes	moderate	yes	mild	no	none	no	none	no	none	case
123	no	none	yes	moderate	yes	moderate	no	none	yes	mild	no	none	no	none	case
124	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
125	yes	mild	yes	moderate	yes	moderate	no	none	no	none	yes	mild	yes	moderate	case
126	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
127	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
128	no	none	yes	moderate	yes	moderate	no	none	no	none	yes	mild	yes	mild	case
129	no	none	no	none	yes	moderate	yes	mild	no	none	yes	mild	no	none	case
130	no	none	no	none	yes	moderate	yes	mild	no	none	yes	moderate	yes	mild	case
131	no	none	yes	severe	yes	severe	no	none	no	none	yes	mild	no	none	case
132	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
133	no	none	no	none	yes	moderate	yes	moderate	no	none	yes	moderate	yes	mild	case
134	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
135	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
136	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
137	no	none	yes	moderate	yes	moderate	yes	mild	yes	mild	no	none	no	none	case
138	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
139	no	none	yes	moderate	yes	severe	no	none	no	none	no	none	no	none	case
140	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
141	no	none	yes	severe	yes	severe	no	none	no	none	no	none	no	none	case
142	no	none	yes	severe	yes	severe	no	none	yes	severe	yes	mild	yes	mild	case
143	no	none	yes	mild	yes	moderate	yes	mild	no	none	yes	severe	yes	mild	case
144	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
145	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
146	no	none	yes	moderate	yes	moderate	no	none	no	none	yes	moderate	yes	moderate	case
147	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
148	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
149	no	none	yes	moderate	no	none	yes	mild	no	none	no	none	no	none	case
150	no	none	no	none	yes	moderate	no	none	no	none	no	none	no	none	case
151	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
152	no	none	no	none	yes	severe	no	none	no	none	no	none	no	none	case
153	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
154	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
155	no	none	no	none	yes	moderate	no	none	no	none	no	none	no	none	case
156	no	none	no	none	yes	mild	yes	mild	yes	moderate	yes	moderate	yes	moderate	case
157	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
158	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
159	no	none	no	none	yes	mild	no	none	no	none	yes	moderate	yes	moderate	case
160	no	none	no	none	yes	moderate	no	none	no	none	yes	mild	no	none	case

Study #	Lipohypertrophy						Lipoatrophy								Screening
	Neck		Breast		Waist		Face		Arms		Buttocks		Legs		
	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	Case/control/non-assignee
161	no	none	yes	moderate	yes	moderate	no	none	no	none	no	none	no	none	case
162	no	none	yes	moderate	yes	moderate	no	none	yes	moderate	yes	moderate	yes	moderate	case
163	no	none	yes	moderate	yes	severe	no	none	no	none	yes	moderate	yes	moderate	case
164	no	none	no	none	yes	moderate	no	none	no	none	no	none	no	none	case
165	no	none	yes	moderate	yes	moderate	yes	mild	no	none	yes	severe	yes	severe	case
166	no	none	yes	severe	yes	severe	yes	mild	yes	mild	yes	moderate	yes	moderate	case
167	no	none	yes	moderate	yes	mild	no	none	no	none	no	none	no	none	case
168	no	none	yes	moderate	no	none	yes	mild	no	none	yes	mild	yes	mild	case
169	no	none	no	none	yes	moderate	no	none	no	none	no	none	no	none	case
170	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
171	yes	mild	yes	moderate	yes	severe	no	none	no	none	yes	moderate	yes	moderate	case
172	no	none	no	none	yes	moderate	yes	mild	no	none	yes	mild	yes	mild	case
173	no	none	no	none	yes	moderate	yes	mild	no	none	yes	moderate	yes	moderate	case
174	no	none	yes	moderate	yes	moderate	yes	mild	no	none	no	none	no	none	case
175	no	none	yes	moderate	yes	moderate	no	none	no	none	no	none	no	none	case
176	no	none	no	none	yes	moderate	no	none	no	none	yes	mild	yes	mild	case
177	no	none	yes	mild	yes	moderate	no	none	no	none	no	none	no	none	case
178	no	none	no	none	no	none	no	none	no	none	no	none	no	none	non-assignee
179	yes	mild	no	none	yes	moderate	no	none	no	none	yes	moderate	yes	moderate	case
180	no	none	no	none	no	none	no	none	no	none	yes	none	no	none	Control
181	no	none	yes	severe	yes	severe	no	none	no	none	no	none	no	none	case
182	no	none	no	none	yes	moderate	no	none	no	none	no	none	no	none	case
183	no	none	no	none	no	none	no	none	no	none	yes	moderate	yes	moderate	case
184	no	none	yes	moderate	yes	moderate	yes	mild	no	none	no	none	no	none	case
185	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
186	no	none	yes	severe	yes	severe	yes	mild	no	none	yes	mild	yes	mild	case
187	no	none	yes	mild	yes	moderate	no	none	no	none	yes	mild	yes	mild	case
188	no	none	yes	severe	yes	mild	yes	mild	no	none	yes	moderate	yes	moderate	case
189	yes	moderate	no	none	yes	moderate	yes	moderate	yes	mild	yes	moderate	yes	mild	case
190	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
191	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
192	no	none	yes	mild	yes	moderate	no	none	no	none	yes	mild	yes	moderate	case
193	no	none	no	none	yes	moderate	no	none	no	none	no	none	no	none	case
194	no	none	yes	severe	yes	moderate	yes	mild	yes	mild	yes	moderate	yes	moderate	case
195	no	none	yes	moderate	yes	moderate	no	none	no	none	no	none	no	none	case
196	no	none	yes	severe	yes	moderate	yes	mild	yes	mild	yes	moderate	yes	mild	case
197	no	none	yes	moderate	yes	severe	no	none	no	none	no	none	no	none	case
198	no	none	yes	moderate	yes	moderate	yes	mild	no	none	yes	mild	yes	mild	case
199	no	none	yes	mild	yes	moderate	yes	mild	no	none	no	none	no	none	case
200	no	none	yes	severe	yes	moderate	no	none	yes	mild	yes	moderate	yes	mild	case

Study #	Lipohypertrophy						Lipoatrophy								Screening
	Neck		Breast		Waist		Face		Arms		Buttocks		Legs		
	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	Case/control/non-assignee
201	no	none	yes	moderate	yes	moderate	no	none	no	none	no	none	no	none	case
202	no	none	yes	moderate	yes	severe	yes	mild	no	none	yes	mild	no	none	case
203	no	none	yes	moderate	yes	moderate	no	none	no	none	no	none	no	none	case
204	no	none	yes	mild	yes	moderate	yes	mild	no	none	yes	moderate	no	none	case
205	no	none	no	none	yes	moderate	yes	mild	no	none	no	none	no	none	case
206	no	none	yes	moderate	yes	moderate	yes	mild	yes	moderate	yes	moderate	yes	mild	case
207	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
208	no	none	yes	mild	yes	moderate	yes	mild	yes	mild	yes	mild	yes	moderate	case
209	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
210	no	none	yes	moderate	yes	mild	no	none	no	none	no	none	no	none	case
211	no	none	yes	moderate	yes	moderate	no	none	yes	moderate	no	none	no	none	case
212	no	none	yes	moderate	yes	moderate	yes	mild	yes	mild	yes	mild	yes	mild	case
213	no	none	no	none	yes	severe	no	none	no	none	yes	moderate	yes	severe	case
214	no	none	yes	mild	yes	mild	yes	mild	no	none	no	none	no	none	case
215	yes	mild	yes	severe	yes	moderate	yes	mild	yes	mild	yes	severe	yes	moderate	case
216	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
217	no	none	yes	moderate	yes	moderate	yes	mild	no	none	no	none	no	none	case
218	no	none	no	none	yes	moderate	yes	mild	no	none	yes	moderate	no	none	case
219	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
220	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
221	no	none	yes	severe	yes	moderate	no	none	no	none	no	none	no	none	case
222	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
223	no	none	no	none	no	none	yes	mild	no	none	yes	moderate	yes	moderate	case
224	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
225	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
226	no	none	no	none	yes	moderate	yes	mild	yes	mild	yes	mild	yes	moderate	case
227	no	none	yes	mild	yes	moderate	yes	moderate	yes	mild	no	none	no	none	case
228	no	none	no	none	yes	severe	yes	mild	yes	mild	yes	moderate	yes	mild	case
229	yes	mild	yes	severe	yes	severe	yes	mild	no	none	no	none	no	none	case
230	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
231	no	none	no	none	yes	moderate	yes	mild	yes	mild	no	none	no	none	case
232	no	none	yes	severe	yes	moderate	yes	mild	yes	mild	yes	moderate	yes	moderate	case
233	no	none	yes	moderate	yes	moderate	yes	mild	no	none	yes	moderate	yes	moderate	case
234	no	none	yes	mild	yes	moderate	no	none	yes	mild	yes	moderate	yes	mild	case
235	no	none	yes	severe	yes	severe	yes	mild	yes	mild	yes	mild	yes	mild	case
236	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
237	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
238	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
239	no	none	yes	severe	yes	mild	no	none	no	none	yes	moderate	yes	moderate	case
240	no	none	no	none	yes	moderate	no	none	no	none	no	none	no	none	case

Study #	Lipohypertrophy						Lipoatrophy								Screening
	Neck		Breast		Waist		Face		Arms		Buttocks		Legs		
	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	yes/no	Mild/Moderate/Severe/None	Case/control/non-assignee
241	no	none	yes	mild	yes	moderate	no	none	no	none	no	none	no	none	case
242	no	none	yes	mild	yes	moderate	yes	mild	no	none	yes	moderate	yes	moderate	case
243	no	none	no	none	no	none	no	none	no	none	no	none	no	none	Control
244	no	none	no	none	no	none	yes	mild	no	none	yes	moderate	yes	moderate	case
245	no	none	yes	moderate	yes	moderate	no	none	no	none	no	none	yes	moderate	case
246	no	none	yes	severe	yes	moderate	no	none	yes	mild	yes	mild	no	none	case
247	no	none	no	none	yes	moderate	no	none	yes	mild	yes	mild	yes	mild	case
248	no	none	yes	moderate	yes	moderate	yes	mild	no	none	no	none	no	none	case
249	no	none	yes	severe	yes	moderate	no	none	no	none	no	none	no	none	case
250	no	none	no	none	yes	moderate	no	none	no	none	no	none	no	none	case
251	no	none	no	none	no	none	no	none	no	none	yes	none	no	none	Control
252	yes	mild	yes	mild	yes	mild	no	none	yes	mild	no	none	yes	moderate	case
253	yes	mild	yes	severe	yes	moderate	yes	mild	no	none	yes	moderate	yes	moderate	case

APPENDIX E

APPENDIX E: ELECTRONIC CALCULATOR FOR NEW HIV LDS CLASSIFICATION INSTRUMENT

To use the Excel based calculator, security settings in Excel need to be set to 'Medium' as follows:

- Open a blank Excel document
- Choose 'Tools', then 'Macros', then 'Security'
- Switch security setting to 'Medium'
- Open calculator
- Choose 'Enable macros'

This only has to be done once if you use the same computer