# MITOCHONDRIAL TOXICITIES, BODY-FAT ABNORMALITIES AND THE POSSIBLE ASSOCIATED CHANGE IN CARDIOVASCULAR RISK OF HIGHLY ACTIVE ANTI-RETROVIRAL THERAPY IN HIV-INFECTED INDIVIDUALS - A SOUTH AFRICAN PERSPECTIVE.

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A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa, for the degree of Doctor of Philosophy.

**DECLARATION**

I declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy to the University of the Witwatersrand, Johannesburg.

This thesis is submitted in the optional format, approved by the faculty, of published work with a supporting introduction, as a literature review, and discussion.

This work has not been submitted before for any degree or examination at any other University.

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**Colin Nigel Menezes**

## DEDICATION

This work is dedicated to the memory of my father, Jose Francisco da Piedade Menezes and to my mother, Maria Hazel de Quadros Menezes for her constant support and encouragement.

**ABSTRACT**

Despite the improved survival of human immunodeficiency virus (HIV) infected individuals with the introduction of highly active anti-retroviral therapy (HAART) in the South African public sector in 2004, new challenges have been brought to the fore. These include drug-related toxicities, particular those of stavudine, which remains in common use within developing countries.

A prospective analysis of 9040 HIV-1-infected adults initiated on HAART from 2004 to 2007 at the Themba Lethu Clinic, Helen Joseph Hospital in Johannesburg, confirmed the ability to roll out a successful HAART programme in a resource limited environment with a high retention rate of 70%. Nearly 30% of patients switched to non-stavudine based regimens due to side effects - predominantly peripheral neuropathy, symptomatic hyperlactataemia and lipoatrophy.

In an attempt to look for safer options, a prospective randomized controlled trial comparing standard and low dose stavudine with tenofovir was undertaken in 2009. Sixty patients were randomized 1:1:1 to either standard (30-40 mg), low (20-30 mg) dose stavudine or tenofovir (300 mg) each combined with lamivudine and efavirenz. Adipocyte mitochondrial DNA (mtDNA) levels, gene expression, anthropometry, markers of inflammation, lipid and glucose metabolism were assessed at various time intervals.

Results demonstrate early mitochondrial depletion among black South African patients receiving low and standard doses of stavudine, with preservation of gene expression levels, except for *NRF1* and *MTCYB*, when compared to patients on tenofovir. Mitochondrial toxicities occurred in both the stavudine arms. Immunological and virological outcomes were similar for all three arms. Both drugs caused lipid changes, but tenofovir had a more

favourable effect on anthropometry and adipokines. Both stavudine regimens increased fasting insulin and C-peptide levels, with the higher stavudine dose also causing increased fasting glucose and HOMA levels.

This study demonstrates an early association between mitochondrial depletion and stavudine therapy in the black South African population and shows that tenofovir has a minimal effect on mitochondrial numbers. Only two of eight adipocyte genes were significantly affected by stavudine therapy when compared with tenofovir, but this was only seen with the standard dose. This study highlights the occurrence of significant metabolic abnormalities with both drugs. Therefore, awareness of the potential increased cardiovascular risk should be of concern with tenofovir and stavudine, although toxicity is lower in the low dose compared to the standard dose stavudine regimen with no attenuation of anti-retroviral effectivity.

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A longitudinal study of stavudine-associated toxicities in a large cohort of South African HIV infected subjects. BMC Infect Dis. 2011.11:244

Paper 2:

The early effects of stavudine compared to tenofovir on adipocyte gene expression, mitochondrial DNA copy number and metabolic parameters in South African HIV-infected patients: A randomized trial. HIV Med. 2013; 14(4):217-25.

Paper 3:

A randomized clinical trial comparing metabolic parameters after 48 weeks of standard and low dose stavudine, and tenofovir therapy in HIV-infected South African patients. HIV Med. 2013 Aug 28. doi: 10.1111/hiv.12074.

### Poster presentation:

Wits Research Day, September 2012, Johannesburg, South Africa.

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The early effects of stavudine compared to tenofovir on adipocyte gene expression, mitochondrial DNA copy number and metabolic parameters in South African HIV-infected patients: A randomized trial. Presented at the Wits Research Day, September 2012, Johannesburg, South Africa.

**Conference presentation:**

Invited speaker - Conference of the Southern African HIV Clinicians' Society 2012, Cape Town, South Africa, November 2012.
Metabolic complications of HIV and HAART: The hyperlactataemia syndromes

## LIST OF ABBREVIATIONS

| | |
|---|---|
| $2^{\Delta}Cq$ | Relative quantities. |
| ABC | Abacavir. |
| AE | Adverse event. |
| AIDS | Acquired immunodeficiency syndrome. |
| ALT | Alanine aminotransferase. |
| ANOVA | Analysis of variance. |
| AST | Aspartate aminotransferase. |
| ATV | Atazanavir. |
| AZT | Zidovudine. |
| B2M | β2 microglobulin. |
| BMI | Body mass index. |
| bp | Base pair. |
| CCR5 | Chemokine (C-C motif) receptor 5. |
| CD4 count | Cluster of differentiation 4 count. |
| CI | Confidence interval. |
| COX 3 | Cytochrome c oxidase subunit III. |
| COX 4 | Cytochrome c oxidase subunit IV. |
| CPK | Creatinine kinase. |
| ΔCq | Quantification cycle. |
| CRABP1 | Cellular retinoic acid binding protein 1. |
| CT imaging | Computerised tomography imaging. |
| CVD | Cardiovascular disease. |
| CXCR4 | Chemokine (C-X-C motif) receptor 4. |

| | |
|---|---|
| DAD Study | Data collection on Adverse Effects of Anti-HIV Drugs Study. |
| DDI | Didanosine. |
| DEXA | Dual-energy radiographic absorptiometry. |
| DM | Diabetes mellitus. |
| dNTP | Deoxynucleoside triphosphate. |
| DRV | Darunavir. |
| DSMB | Data safety and monitoring board. |
| d4T | Stavudine. |
| ECG | Electrocardiogram**.** |
| EFV | Efavirenz. |
| ELISA | Enzyme-linked immunosorbent assay. |
| ETV | Etravirine. |
| FTC | Emtricitabine. |
| GFR | Cockcroft-Gault formula. |
| GGT | Gamma-glutamyltransferase. |
| GLUT 4 | Glucose transporter type 4. |
| HAART | Highly active anti-retroviral therapy. |
| HDL cholesterol | High-density lipoprotein cholesterol. |
| HGH | human growth hormone |
| HIV | Human immunodeficiency virus. |
| HOMA | Homeostasis model assessment. |
| HPRT | Hypoxanthine-guanine phosphoribosyltransferase. |
| hs-CRP | High sensitivity C-reactive protein. |
| ICAM-1 | Intercellular Adhesion Molecule -1. |

| | |
|---|---|
| IFG | Impaired fasting glucose. |
| IGT | Impaired glucose tolerance. |
| IL | Interleukin. |
| IQR | Interquartile range. |
| LA | Lactic acidosis. |
| LD | Lipodystrophy. |
| LDL cholesterol | Low-density lipoprotein cholesterol. |
| LEP | Leptin. |
| LPL | Lipoprotein lipase. |
| LPV | Lopinavir. |
| LRP1 | Low density lipoprotein receptor related protein 1. |
| MCC | Medicines Control Council. |
| MRI | Magnetic resonance imaging. |
| MTCYB | Mitochondrial cytochrome B. |
| mtDNA | Mitochondrial DNA. |
| MVC | Maraviroc. |
| ND | Not done. |
| NNRTI | Non-nucleoside reverse transcriptase inhibitor. |
| NRF1 | Nuclear respiratory factor-1. |
| NRTI | Nucleoside reverse transcriptase inhibitor. |
| Nts | Nucleotides. |
| NVP | Nevirapine. |
| PGC-1 α | PPAR-γ coactivator 1α. |
| PI | Protease inhibitor. |

| | |
|---|---|
| PN | Peripheral neuropathy. |
| PPARγ | Peroxisome-proleferator activated receptor γ. |
| qPCR | Quantitative polymerase chain reaction. |
| RAL | Raltegravir. |
| RNA RPL 13A | 60S ribosomal protein L 13A. |
| RTV | Ritonavir. |
| RXR-PPARγ | Retinoid X receptor-peroxisome proliferator-activated receptor γ. |
| SH | Symptomatic hyperlactatemia. |
| SMART | Strategies for management of antiretroviral therapy. |
| SQV | Saquinavir. |
| SREBP-1 | Sterol regulatory element binding protein-1. |
| TDF | Tenofovir. |
| TFAM | Mitochondrial transcription factor-A. |
| TNF | Tumour necrosis factor. |
| T20 | Enfuvirtide. |
| 3TC | Lamivudine. |
| VCAM-1 | Vascular cell adhesion molecule-1. |
| vWF | von Willebrand factor. |
| WHO | World Health Organization. |

## PREFACE

I formulated the study concept and design with Professor Ian Sanne, which was overseen by my supervisors, Professors Nigel Crowther, Frederick Raal and Ian Sanne. Assistance was received with the data collection and management from Mrs Desiree Van Amsterdam, Dr Mhairi Maskew, Ms Doreen Schulze and Mrs Avril Swarts Prinsloo. Dr Raquel Duarte, Dr Caroline Dickens and Ms Therese Dix-Peek assisted with the laboratory work. Professor Nigel Crowther, Dr Mhairi Maskew and Dr Denise Evans assisted with the data analysis. Mrs Marlene Naidoo assisted with the regulatory work. Dr Melanie-Anne John, Dr Francesca Condradie, Dr Mohammed Rassol, Dr Sharlaa Badal – Faesen, Dr Faizel Laher and Dr Pru Ive assisted with patient management.

This thesis is divided into five chapters:

**Chapter 1** provides a background on human immunodeficiency virus (HIV)-1 infection and the issues around the management of HIV-infected individuals, especially the complications associated with highly active anti-retroviral therapy (HAART), and introduces the aims of the thesis. **Chapter 2** contains the results of a published prospective analysis of a large cohort of HIV-1-infected adults initiated on HAART. It discusses the issues related to use of stavudine as first line therapy and forms the basis for the clinical trial using tenofovir as an option. The published version of this paper is included in Appendix 1. **Chapter 3** presents a published paper of the clinical trial and discusses the early effects of low and standard stavudine therapy when compared to tenofovir, on adipocyte gene expression, mitochondrial DNA copy number and metabolic parameters in South African HIV-infected patients after four weeks of therapy. The published version of this paper is included in Appendix 2.

**Chapter 4** contains the follow up paper (published after submission) on the longer term metabolic parameters after 48 weeks of therapy in this same cohort of patients. The published version of this paper is included in Appendix 3. **Chapter 5** summarises the results of the project and discusses new directions for research in this field. Each chapter has a list of its own references.

# CHAPTER 1

## 1. INTRODUCTION AND LITERATURE REVIEW

### 1.1. The human immunodeficiency virus (HIV)-1 infection and its natural history

HIV-1 infection is known to be transmitted through unprotected sexual intercourse, contaminated needles, blood products or through mother-to-child transmission. The HIV-1 binds to CD4+ T cells which include T-helper cells, monocytes/macrophages, eosinophils, microglial cells and dendritic cells (Fanales-Belasio *et al*, 2010).

The virus enters the cell by attaching to the CD4 receptor and its co-receptor, either chemokine (C-C motif) receptor 5 (CCR5) or chemokine (C-X-C motif) receptor 4 (CXCR4) on the surface of the cell, releasing its RNA and its various enzymes, which include the reverse transcriptase, the integrase enzyme, and the protease enzyme into the cell. Using the reverse transcriptase enzyme, a single-strand RNA genome is then transcribed into a double-strand DNA, and is integrated into a host chromosome. This viral DNA is then transported into the cell nucleus, where its integration into the cell's genome is carried out by the integrase enzyme. Using the host's nuclear transcription processes, viral proteins are produced which are cleaved into active forms by the protease enzyme and are packaged into new virions ready to infect more cells (Fanales-Belasio *et al*, 2010).

The natural history of HIV disease, which depends on the underlying host immune response and viral virulence factors, determines the outcome of the infection (Fanales-Belasio *et al*, 2010). If not treated, most patients will develop the acquired immunodeficiency syndrome (AIDS) which may result in an opportunistic infection or a malignancy.

### 1.2. The classes of antiretroviral drugs available

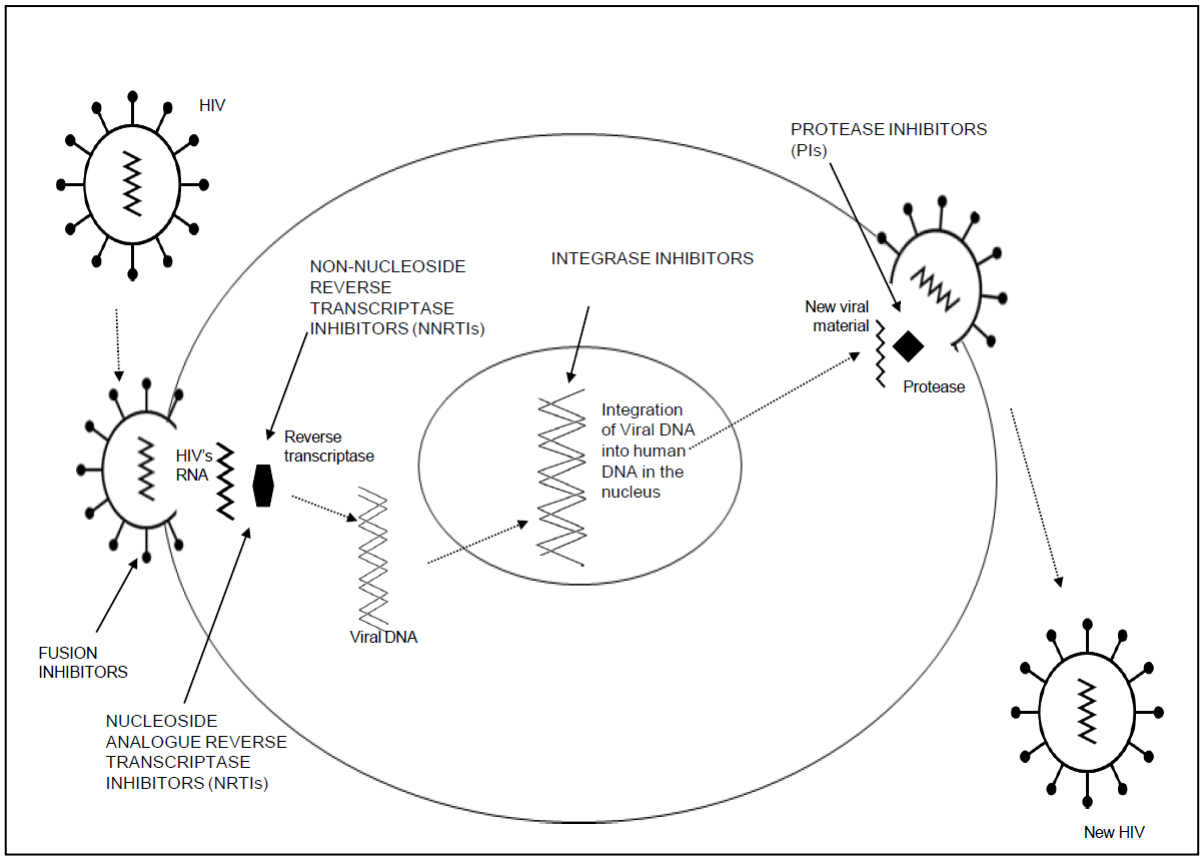
There are five classes of antiretroviral drugs available. These include the nucleoside reverse transcriptase inhibitors (NRTIs), which are analogues that mimic normal building blocks of DNA, preventing transcription of viral RNA to DNA; non-nucleoside viral reverse transcriptase inhibitor (NNRTIs), which fit into the genomic binding site of reverse transcriptase and directly inhibit its action; protease inhibitors (PI) which inhibit the viral protease enzyme, therefore inhibiting the final maturation stages of HIV replication resulting in non-infective viral particles; entry inhibitors that bind to the viral gp41, the host cell CD4 or chemokine (CCR5) receptors and block the entry of the HIV into the host cell; and integrase inhibitors that block the viral DNA integration into the host cell genome (Figure 1). Only some of these drug classes (Table 1) are currently available for use in the South African public sector, these include the NRTIs, NNRTIs, PIs and more recently the integrase inhibitors (Southern African HIV Clinicians Society Guidelines for Antiretroviral Therapy in Adults, 2012).

### 1.3. HIV variability and response to therapy

There are two forms of HIV, HIV-1 and HIV-2. In addition, the HIV-1 is further divided into three groups, M (Major), O (Outlier) and N (non-M/non-O). The group M includes nine subtypes or clades, designed A to K. There are at least 6 sub-subtypes within clades A and F (A1, A2, A3 and A4, and F1 and F2) (Fanales-Belasio *et al*, 2010).

It is possible that viruses of different subtypes can infect the same cell, sharing their genetic material, resulting in hybrids of new viruses. As a result, viruses within the same HIV-1 subtype may differ by up to 20%. In sub-Saharan Africa, there is a wide HIV-1 heterogeneity but subtypes A, C and D are the most prevalent, with subtype B viruses being rare but

**Figure 1: A schematic diagram of the mechanism of action of antiretroviral drugs within a host cell (Kindly drawn by Ms Jean Johnstone).**




prevalent in North and South America, Central and Western Europe, and Australia. HIV-2 is endemic in West Africa, but has spread to Europe and to India (Fanales-Belasio *et al*, 2010). Therefore, this might affect pathogenesis, infectivity and response to antiretroviral therapy with different HIV subtypes having different susceptibilities to antiretroviral drugs (Kaleebu *et al*, 2001. Velazquez-Campoy *et al*, 2001. Fanales-Belasio *et al*, 2010). Importantly, however, other reasons need to be considered, such as differences in resistance patterns to antiretroviral therapy at the time of initiation of therapy, differences in pharmacokinetics and adherence issues (van den Berg *et al*, 2005). A study in non-B HIV-1 subtypes noted poorer

**Table 1: List of classes of antiretroviral drugs available in Southern Africa (Adapted from the Southern African HIV Clinicians Society Guidelines for Antiretroviral Therapy in Adults, 2012).**

| Name of the class of antiretroviral drugs | Name of the available drugs |
|---|---|
| Nucleoside reverse transcriptase inhibitors (NRTIs) | Stavudine (d4T)<br>Lamivudine (3TC)<br>Zidovudine (AZT)<br>Didanosine (DDI)<br>Tenofovir disoproxil fumarate (TDF)<br>Abacavir (ABC)<br>Emtricitabine (FTC)* |
| Non-nucleoside viral reverse transcriptase inhibitor (NNRTIs) | Efavirenz (EFV)<br>Nevirapine (NVP)<br>Etravirine (ETV) |
| Protease inhibitor (PI) | Ritonavir (RTV)<br>Lopinavir (LPV)**<br>Atazanavir (ATV)<br>Saquinavir (SQV)<br>Darunavir (DRV) |
| Integrase inhibitors | Raltegravir (RAL) |

NB: Entry inhibitors - enfuvirtide (T20) and maraviroc (MVC) are not available.
* available only as a combination drug with tenofovir.
** available only as a combination drug with ritonavir.

virological outcome on PIs (Perno *et al*, 2001). Conversely, other studies have suggested that there are no differences in outcomes to tenofovir, adefovir and zidovudine for subtypes A–G (Palmer *et al*, 2001), and between subtypes F and A (Caride *et al*, 2000), or between B and C (Shafer *et al*, 1997) for NRTIs and NNRTIs. In two studies where African were compared to European HIV -1 infected patients, on various regimens of antiretroviral drugs, the one study (van den Berg *et al*, 2005) found an early poor virological response whilst the other study (Frater *et al*, 2002) found poorer longer term response in the African group but this was thought to be because of lack of adherence to therapy.

### 1.4. The burden of HIV-1 infection

According to the World Health Organization (WHO), by 2011, more than 60 million people have been infected with HIV since the beginning of the HIV-1 epidemic and nearly 30 million people have died of AIDS. In 2010, there were nearly 34 million people living with the HIV-1 infection, with an estimated 2.7 million new infections, and 1.8 million AIDS-related deaths. Sub-Saharan Africa continues to bear the global burden of HIV-1 with an estimated 68% of the HIV-1 infections worldwide. This region also accounted for 69% of the world's AIDS related deaths by that time (WHO, 2011).

More importantly, despite having the highest rates of this epidemic, there has been a noticeable drop in new HIV-1 infections in South Africa, this by at least 41%, between 2001 and 2011(UNAIDS, 2012). This may be because of the preventative programs, and the rapid scale up of highly active antiretroviral therapy (HAART) by the South African Government.

### 1.5. Access to antiretroviral therapy

A record 2.3 million people have been added to the anti-HIV treatment programs in the last two years in sub-Saharan Africa and this was an increase of 59%. South Africa has scaled up its treatment services to reach 1.7 million people which was an increase of nearly 75% in the preceding two years (UNAIDS, 2012).

The South African Government rolled out its HAART programme in the public health sector in 2004 (National Antiretroviral Treatment Guidelines, 2004), offering two regimens in the public sector. These contain a triple drug course, which consisted of 2 NRTIs, with either a NNRTI or PI. At diagnosis, unless contraindicated, patients were put on a first line regimen which consisted of stavudine, lamivudine and either efavirenz or nevirapine, and switched to second line therapy if they had virological failure to first line therapy. The second line

therapy consisted of zidovudine, didanosine and ritonavir/lopinavir. These drugs were cheap and easy to administer in the short term but were associated with more side effects in the long term. At that time, HAART was recommended when the CD4 count was 200 cells/µl or less, or when the patient presented at stage 4 of the WHO clinical staging system (National Antiretroviral Treatment Guidelines, 2004).

### 1.6. The issue of side effects of stavudine therapy: mitochondrial toxicities, body fat abnormities and cardiovascular risk

The success of controlling the HIV-1 infection with HAART has been affected by significant acute and long term complications. Acute complications are noted to occur frequently because of the use of stavudine. The hyperlactatemia syndromes (symptomatic hyperlactatemia and lactic acidosis) are the most serious side effects of stavudine use and are related to mitochondrial toxicity with high mortality rates (Bolhaar *et al*, 2007. Hernandez Perez *et al*, 2010).

Chronic complications include lipodystrophy, dyslipidaemias, insulin resistance, and the development of new onset diabetes mellitus (De Wit *et al*, 2008). These metabolic abnormalities, but not necessarily the lipodystrophy, result in an increased risk for cardiovascular disease (Fourie *et al*, 2011).

In 2010, the WHO changed its guidelines, advocating an earlier introduction of HAART at CD4 counts of 350 cells/µl or less and the use of less toxic drugs such as tenofovir and zidovudine as first line therapy, rather than stavudine (WHO Antiretroviral therapy guidelines, 2010 revision). In April that year, South Africa followed suit, and changed its guidelines to the use of tenofovir as first line therapy instead of stavudine (National Department of Health South Africa HAART guidelines, 2010). Tenofovir is a nucleotide

analogue, and was approved for use in 2001 in the United States. It is commonly used in initial therapy and has been shown to have a favourable lipid when compared to stavudine, with no difference in virological responses (Gallant *et al*, 2004). Despite the WHO advising countries to change their HAART guidelines, it is known that up to 56% of HIV-1-positive patients in other low and middle income countries are still receiving stavudine as first line therapy (WHO 2010) mainly because of its low cost and its availability in fixed drug combinations.

### 1.6.1. The hyperlactatemia syndromes (symptomatic hyperlactatemia and lactic acidosis)

#### 1.6.1.1. Epidemiology

The hyperlactataemia syndromes are associated with the use of NRTIs. Asymptomatic hyperlactataemia is common but does not predict for the symptomatic form of the disease. The symptomatic form of hyperlactataemia may have a good prognosis if recognised early and there is no associated liver dysfunction. Lactic acidosis is present if there is an associated metabolic acidosis and leads to multiple organ dysfunction.

The first case reports were published in the early 1990s (Freiman *et al*, 1993. Chattha *et al*, 1993) and described a rare but serious disease evidenced by a lactic acidosis, hepatic steatosis and occasionally liver failure (Chariot et *al*, 1999. Bissuel *et al*, 1994. Johri *et al*, 2000). Incidence rates of lactic acidosis from cohorts in Africa were much higher (Table 2) compared to studies in the West which have moved away from using stavudine and didanosine. Mortality rates were as high as 30% in one South African cohort (Bolhaar *et al*, 2007). In another South African study, substitutions of stavudine due to symptomatic hyperlactatemia occurred between six months and 18 months on treatment with a cumulative

incidence of 4.7% after three years on HAART, with women seven times more affected than men, after being on more than six months of therapy (Boulle *et al*, 2007).

#### 1.6.1.2. Pathogenesis

Mitochondria are found in all cells of the body except for erythrocytes (Maagaard *et al*, 2009) and because the mitochondria lack enzymes for DNA repair, they are particularly susceptible to mutagenic agents (Graziewicz *et al*, 2006). Mitochondrial DNA are responsible for the production of 13 mitochondrial polypeptides that are involved in the respiratory chain and essential for oxidative phosphorylation (Kakuda *et al*, 2000).

The mechanism by which NRTIs cause mitochondrial dysfunction is by inhibiting mtDNA polymerase γ with subsequent mtDNA depletion. In addition, NRTIs are also known to inhibit other specific enzymes involved in the tricarboxylic acid cycle and electron transport chain as well as inhibition of mitochondrial adenylate kinase and the ADP/ATP translocator (Moyle et *al*, 2000. Barile *et al*, 1994. Barile *et al*, 1997, 1998). Early effects of mitochondrial toxicity include decreased energy production and increased production of lactate, by affecting oxidative phosphorylation (Kakuda *et al*, 2000. Montaner *et al*, 2004). This would result in an increase in electron leakage from the electron-transport chain, which in turn increases the production of reactive oxygen species leading to a cascade of further oxidative damage and lipid peroxidation (Kakuda *et al*, 2000. Montaner *et al*, 2004).

In the presence of mitochondrial dysfunction, lactic acidosis (with or without an elevated anion gap and altered bicarbonate) may occur as aerobic respiration (oxidative phosphorylation) shifts to anaerobic respiration (glycolysis) (Kakuda *et al*, 2000 Montaner *et al*, 2004).

#### 1.6.1.3. Management

Early recognition and intervention is vital because lactic acidosis can progress to liver failure and death. It is a diagnosis of exclusion; other conditions need to be excluded such as diabetic ketoacidosis, renal failure, sepsis, heart failure, pancreatitis, severe anaemia, liver failure, bowel ischaemia and other drugs (e.g. metformin, INH overdose) (Southern African HIV Clinicians Society, 2012). The mainstay of management includes withdrawal of all NRTIs even before the diagnosis is confirmed if there is a high index of suspicion.

The Southern African HIV clinician society guidelines are based on other published guidelines (Schambelan *et al*, 2002. British HIV Association (BHIVA) guidelines, 2005.). Patients with mild asymptomatic hyperlactataemia (lactates of less than 5·0 mmol/L) would include cautious continuation of NRTIs which are less "mitochondrial toxic" while monitoring the patient closely for the development of symptoms and/or further increases in lactate (Southern African HIV Clinicians Society, 2012).

Patients who have serum lactate concentrations of /or above 5·0 mmol/L whether asymptomatic or symptomatic warrant discontinuation of therapy. Administration of intravenous fluids to pre-empt cardiovascular collapse and assist hepatic and renal clearance of lactate is important. The use of intravenous sodium bicarbonate is controversial as it may trigger respiratory acidosis. Ventilation may be required if respiratory fatigue occurs. Dialysis, inotropes and other supportive measures may be required as necessary. Other therapies such as thiamine, carnitine, riboflavin, co-enzyme Q and vitamin C which are either cofactors in oxidative phosphorylation or antioxidants may also be used (Brinkman *et al*, 2001. John *et al*, 2002. Falco *et al*, 2002. Southern African HIV Clinicians Society, 2012). The use of NRTIs in future regimens should be avoided. It can take several weeks to months for lactate concentrations to normalise. If a patient is on NNRTI regimen, a boosted PI

**Table 2: Studies of peripheral neuropathy and lactic acidosis in African HIV-infected populations**

| Toxicity | Reference | Year | Country | Study type | Sample size | Result |
|---|---|---|---|---|---|---|
| **Peripheral neuropathy** | Idoko *et al* | 2002 | Nigeria | Clinical trial | 1 patient | On zidovudine, zalcitabine, or a PI. Severe PN |
| | Wester *et al* | 2005 | Botswana | Longitudinal | 153 patients | 4.3 fold increase in moderate to severe PN if placed on a stavudine/didanosine regimen. |
| | Boulle *et al* | 2007 | South Africa | Cohort | 2679 patients; | PN related stavudine substitutions in 6.2% patients (95% CI 4.3-8.5) |
| | Hawkins *et al* | 2007 | Kenya | Longitudinal, observational | 1286 patients | Predominantly stavudine related regimens. 20.7% PN |
| | Forna *et al* | 2007 | Uganda | Cohort | 1029 patients | 96% on stavudine containing regimens. 36% PN, 9% were severe. 17.2% required drug change. |
| | Canestri *et al* | 2007 | Senegal | Clinical trial | 40 patients | 37.5% PN on a stavudine/didanosine regimen. |
| | Jamisse *et al* | 2007 | Mozambique | Prospective | 146 patients | 18% PN on either a stavudine or zidovudine based regimen. |
| | Sanne *et al* | 2009 | South Africa | Cohort | 7583 patients | Mainly stavudine. Incidence rates of 8.7/100 person years (95% CI 8.1-9.2) |
| | Minzi *et al* | 2009 | Tanzania | Retrospective review | 23 clinics | Increase 0.9% to 1% in the Dar-es-Salaam; 1.3% to 3.8% in the Mbeya in PN over a year if on stavudine. |
| | Sacktor *et al* | 2009 | Uganda | Prospective | 102 patients | 38% PN on stavudine based therapy. |
| | Beadles *et al* | 2009 | Malawi | Retrospective review | 4591 patients | On stavudine, 35% symptoms experienced but only 13% given diagnosis of PN. |
| | Shurie *et al* | 2010 | Ethiopia | Cross sectional | 203 patients | 43% on 30mg stavudine vs. 74% on 40mg stavudine; 20.7% on zidovudine |
| | van Griensven *et al* | 2010 | Rwanda | Cohort | 2190 patients | On stavudine based therapy. 8.0% PN. Incidence rates of 52/1000 patient years |

| Toxicity | Reference | Year | Country | Study type | Sample size | Result |
|---|---|---|---|---|---|---|
| **Lactic acidosis/ symptomatic hyperlactatemia** | Geddes *et al* | 2006 | South Africa | Observational case series | 891 patients | Stavudine related LA: Incidence rate of 19/1000 person years (95% CI 9-29). |
| | Wester *et al* | 2007 | Botswana | Randomized control trial | 650 patients | On stavudine or didanosine based therapy. 2% moderate to severe SH; 1% LA |
| | Boulle *et al* | 2007 | South Africa | Cohort | 2679 patients | LA/SH related stavudine substitutions in 4.7% (95% CI 3.0-6.8) |
| | Bolhaar *et al* | 2007 | South Africa | Retrospective cohort analysis | 1735 patients | On predominantly stavudine based therapy. Overall incidence rate 10.6 /1000 patient years; 16.1/1000 patient years (females); 1.2/1000 patient years (males); LA: 30.4% died. SH: None died. |
| | Sanne *et al* | 2009 | South Africa | Cohort | 7583 patients | Mainly stavudine. LA/SH: Incidence rates of 5.1 per 100 person years (95% CI 4.7-5.5) |
| | van Griensven *et al* | 2009 | Rwanda | Cohort | 2190 patients | On stavudine based therapy. LA/SH 3.1%; incidence rate 20/1000 patient years. |
| | Hernandez *et al* | 2010 | South Africa | Retrospective | 1719 patients | On stavudine based therapy. LA: Incidence rate 13.5 cases/1000 patient years (95% CI 9-29), 22.2% mortality. SH: Incidence rate 31.79 cases/1000 patient years (95% CI 14-40). |

*PN: Peripheral neuropathy; LA: Lactic acidosis; SH: Symptomatic hyperlactatemia; 95% CI = 95% confidence interval. Definitions for LA and SHL in different studies vary.

should be added. If the patient has already failed on NNRTI regimen and is on a boosted PI, other alternatives such raltegravir or etravirine should be added if available. Otherwise the patients should be continued on the boosted PI only (Southern African HIV Clinicians Society, 2012).

### 1.6.2. Peripheral neuropathy

#### 1.6.2.1. Epidemiology

A distal sensory peripheral neuropathy, characterized by the presence of symmetrical distal anesthesia and/or painful dysesthesia is another common side effect of antiretroviral therapy especially with the use of stavudine and didanosine, and is clinically indistinguishable from peripheral neuropathy associated with the HIV infection per se (Dalakas *et al*, 2001. Cornblath *et al*, 2006). Prevalence rates vary from country to country, with rates in the West between 10 and 35% (Keswani *et al*, 2002. McArthur *et al*, 2005. Cornblath *et al*, 2006). Data from neuropathy studies performed in Africa are shown in Table 2. One study from South Africa showed incidence rates of 8.7 per 100 person years (Sanne *et al*, 2009), with lower rates of 5.2 per100 person-years in Rwanda (van Griensven *et al*, 2010) and 2.8 per 100 person-years in another site in South Africa (Boulle *et al*, 2007). Prevalence rates were much higher in African studies compared to those in studies performed in Europe or the USA: 35% and above noted in African countries (Beadles *et al*, 2009. Canestri *et al*, 2007. Forna *et al*, 2007. Sacktor *et al*, 2009). Risk factors in most studies included male gender, a higher baseline BMIs, age and advanced clinical HIV disease (Boulle *et al*, 2007. van Griensven *et al*, 2009. Sanne *et al*, 2009). Most studies showed that toxicity was cumulative and resulted in a switch from stavudine to other alternative therapies, rather than being due to

treatment failure or contraindications and remained the most important trigger for treatment change (Boulle *et al*, 2007. van Griensven *et al*, 2009).

#### 1.6.2.2. Pathogenesis

Peripheral neuropathy is pathologically characterized by distal axonal degeneration of small myelinated and unmyelinated nerve fibers (Zhou *et al*, 2007), with evidence that abnormal mitochondria and mtDNA depletion are seen with dideoxycytidine (ddC)-associated peripheral neuropathy (Dalakas *et al*, 2001). Interestingly, there are also genetic predictors for the development of peripheral neuropathy as seen in white patients with a common European mitochondrial haplogroup T (Canter *et al*, 2008). This was also observed in black patients who belonged to mtDNA subhaplogroup L1c who were found to be at increased risk of developing peripheral neuropathy on antiretroviral therapy (Canter *et al*, 2010).

#### 1.6.2.3. Management

History and physical examination are important in the diagnosis of peripheral neuropathy. Patients who present with symptoms of numbness or dysesthesia after initiation of antiretroviral therapy especially with the use of stavudine and didanosine should be considered as having a peripheral neuropathy due to the antiretroviral therapy, once other causes are excluded. There are however no grading protocols for the severity of peripheral neuropathies. Whilst the ACTG grading system is available for the ACTG clinical trials, it is subjective and it is not a well known grading system to HIV clinicians especially those who are working in a resource limited environment and those who do not work in a research setting. This would explain variability in the rates observed in most studies.

Diagnostic tests would include nerve conduction studies which would confirm diminished or absent sensory action potential amplitudes with normal or slightly slower motor conduction velocities (Harrison *et al*, 1995). Other tests would include electromyography which could demonstrate denervation of distal muscles (Simpson *et al*, 1995), and biopsy of the sural or cutaneous nerve which would show axonal degeneration of myelinated and unmyelinated fibers (Simpson *et al*, 1995).

The goal of treatment is to control the pain and help maintain physical function for activities of daily living and quality of life. Some patients may need to switch antiretroviral therapy. Pharmacological treatment can be used to manage the patient's pain. For milder forms of pain, nonsteroidal anti-inflammatory drugs (NSAIDs) may be prescribed. Severe pain can be treated with stronger opioids, such as morphine and methadone. Antidepressants are commonly used in resource limited settings although a controlled study that compared amitriptyline and mexiletine showed equivalent pain relief to that of a placebo (Kieburtz *et al*, 1998). Anticonvulsants such as gabapentin were found to be effective in reducing pain (Hahn *et al*, 2004), and carbamazepine, and phenytoin are also commonly used in resource limited settings but there are little data to support their efficacy.

### 1.6.3. Lipodystrophy

#### 1.6.3.1. Epidemiology

When HAART was introduced in the 1990s, there was a dramatic improvement in patient morbidity and mortality (Brinkman *et al*, 1999). But soon afterwards, systemic metabolic alterations were observed, often in combination with body-fat distribution changes, and this was called the lipodystrophy syndrome (Brinkman *et al*, 1999). These metabolic alterations, which were characterized by insulin resistance and dyslipidaemia, were similar to that of the

metabolic syndrome that is seen with obesity. The body fat alterations were associated with wasting in subcutaneous adipose tissue in the face, arms, legs and buttocks i.e. lipoatrophy, with central fat accumulation, with some patients even presenting with breast enlargement as well as with fat accumulation in the dorso-cervical area, also known as "buffalo hump pads" (Lo *et al*, 1998. Carr *et al*, 1998, 2003). Some patients developed generalized or central fat accumulation i.e. lipohypertrophy, although most patients would present with a combination of both (mixed lipodystrophy) (Carr *et al*, 1998, 2003).

The prevalence rates of lipodystrophy vary widely ranging from 20% to 80% in HIV-1 patients receiving HAART (Carr *et al*, 2003, Jacobson *et al*, 2005) and this is due to an inconsistency in the diagnostic criteria that are used for identifying these patients. (Carr *et al*, 2003).

With regards to risk factors for the development of lipodystrophy; the recognition of lipodystrophy in patients on antiretroviral treatment coincided with the use of PIs, which caused visceral fat accumulation and the systemic metabolic changes. The use of NRTIs also contributed to fat wasting, but the concomitant use of PIs may have added to this effect. (Brinkman *et al*, 1999, Cossarizza A *et al*, 2001, Carr *et al*, 2003). Studies identified the use of stavudine and zidovudine, both NRTIs, as major risk factors for lipoatrophy (Mallal *et al*, 2000. John *et al*, 2001). Subsequent studies (Gallant *et al*, 2004. Podzamczer *et al*, 2008. Young *et al*, 2005) have confirmed the association between the use of stavudine and the risk of lipoatrophy. Other risk factors include increasing age, female gender, CD4 counts and viral loads (Carr *et al*, 2003).

The frequency of lipodystrophy in resource-limited countries where predominantly NRTI based regimens are being used is high, although it does vary from country to country (see Table 3). Studies from African countries have revealed rates as high as 34% (Mutimura *et al*,

2007. Sinxadi *et al*, 2010). Studies demonstrated that lipoatrophy tended to be common after one year of therapy, especially in patients who were on higher doses of stavudine (40mg) and in patients with a higher body weight (van Griensven *et al* 2010). Females were also more affected than males (Mutimura *et al*, 2007. van Griensven *et al* 2010).

#### 1.6.3.2. Pathogenesis

A proposed aetiology is presented in Figure 2. PIs were initially implicated because of their homology to lipid and adipocyte regulatory proteins (Carr *et al*, 1998). PIs can inhibit lipogenesis by reducing the differentiation of pre-adipocytes to mature adipocytes by reducing the levels of transcription factors involved in adipocyte differentiation - these include the sterol regulatory element binding protein-1 (*SREBP-1*), and peroxisome-proliferator activated receptor γ (*PPARγ*) (Zhang *et al*, 1999, Lenhard *et al*, 2000. Caron *et al*, 2001).

Mitochondria may be affected by the HIV infection itself and may contribute to the mitochondrial toxicity seen in patients on NRTIs. Some studies have confirmed significant lower levels of mtDNA in HIV-1 positive patients than in uninfected patients in peripheral blood mononuclear cells (Côté *et al*, 2002. Miró *et al*, 2004. Chiappini *et al*, 2004). In addition, studies have also confirmed decreased mtDNA content in skeletal muscle (Haugaard *et al*, 2005) and nerve tissue (Dalakas *et al*, 2005). Other tissues involved include adipose tissue (Villarroya *et al*, 2007).

There are several mechanisms by which NRTIs contribute to mitochondrial toxicity resulting in lipoatrophy. NRTIs impair mtDNA polymerase γ activity resulting in depletion of mtDNA. This has been confirmed by mtDNA depletion in subcutaneous adipose tissue seen in patients with lipoatrophy when compared to uninfected individuals or untreated HIV-1-

infected patients (Nolan *et al*, 2003. Pace *et al* 2003. Miro *et al*, 2005. Gerschenson *et al*, 2005).

However, other studies have also shown alterations in mitochondrial gene expression. Expression of mitochondrial and nuclear genes involved in mitochondrial biogenesis, adipogenesis, and metabolic and endocrine function are linked. *SREBP1* and *PPARγ*, together with *PPARα* regulate transcription of lipid metabolism genes and are linked to mitochondrial physiology through *PPARγ* coactivator 1 (*PGC1*). In turn, *PGC1* regulates transcription of mitochondrial genes through the master transcription factors - nuclear respiratory factor (*NRF*) 1 and 2, which regulate mitochondrial transcription factor A (*mtTFAM*) which in turn stimulates mitochondrial gene transcription, thus representing a link between mitochondrial biogenesis and adipose cell function (Enriquez *et al*, 1999.Spiegelman *et al*, 2000. Puigserver *et al*, 2003. Rosen *et al*, 2006).

Mitochondria produce cellular energy through the process of oxidative phosphorylation and the mitochondrial electron transport chain; it is thought that depletion of mtDNA may decrease cellular oxidative phosphorylation (Lewis *et al*, 2003). The mitochondrial electron transport chain which comprises 5 complexes (I—V) are encoded by both nuclear and mitochondrial genes. Mitochondrial DNA-encoded subunits including cytochrome *b* (Cyt *b*) of complex III and cytochrome c oxidase subunits I, II, and III (COX1, COX 2, and COX 3) of complex IV, play functional roles whereas nuclear-encoded components (COX4), play a more regulatory than a functional role (Enriquez *et al*, 1999. Barrientos et *al*, 2002). Therefore through a process of mitochondrial fine tuning, mitochondria can adapt transcription of mtRNA to meet altered oxidative requirements independent of regulation by nuclear factors or changes in mtDNA level (Martin *et al*, 1993. Enriquez *et al*, 1999).

**Table 3: Body fat abnormalities, dysglycaemia and dyslipidaemia in African HIV-1-infected populations**

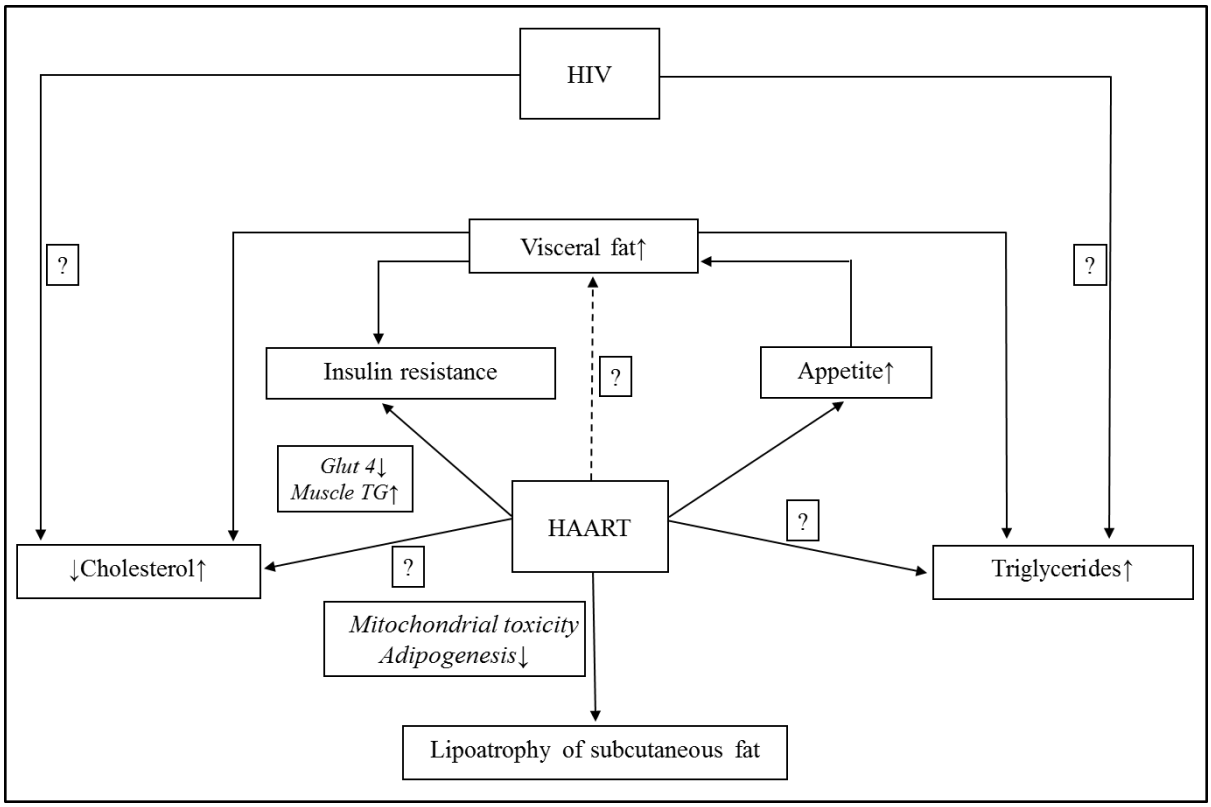
| Toxicity | Reference | Year | Country | Study type | Sample size | Result |
|---|---|---|---|---|---|---|
| **Lipodystrophy** | Boulle *et al* | 2007 | South Africa | Cohort | 2679 patients | LD related stavudine substitutions in 9.0% (95% CI 5.2-14.1) |
| | Mutimura *et al* | 2007 | Rwanda | Prospective cohort | 571 patients | On either a stavudine (predominantly) or zidovudine based regimen. LD observed in 34%. Peripheral LA with abdominal LH noted in 72% of LD patients. |
| | Sanne *et al* | 2009 | South Africa | Cohort | 7583 patients | Mainly stavudine. LD: Incidence rate 4.9/100 person years (95% CI 4.5-5.3). |
| | van Griensven *et al* | 2009 | Rwanda | Cohort | 2190 patients | On stavudine based therapy. LD: 7.2%; incidence rate of 47/1000 patient years. |
| | Mercier *et al* | 2009 | Senegal | Prospective cohort | 180 patients | On either a stavudine or zidovudine or a PI based regimen. Moderate-severe LD; 31.1% (95% CI 24.3-37.9); LA:13.3% ; LH:14.5%;mixed forms.3.3% |
| | George *et al* | 2009 | South Africa | Longitudinal | 42 patients | On stavudine based therapy. LD: 42.9%; Hypertriglyceridemia: 30.8%. Hypercholesterolemia: 35% in LD group.35% |
| | Sinxadi *et al* | 2010 | South Africa | Cross-sectional study | 47 patients | On stavudine. LD: 34%. |
| **Diabetes/ insulin resistance** | Mutimura *et al* | 2007 | Rwanda | Prospective cohort | 571 patients | On either a stavudine (predominantly) or zidovudine based regimen. IFG: 18% of LD (P=0.006 vs. HIV negative group); 16% in the non-LD group (P=0.01vs. HIV negative group); 2% for HIV negative group. |
| | Bakari *et al* | 2007 | Nigeria | Case report | 1 patient | Zidovudine, PIs. Developed diabetes |
| | Manuthu *et al* | 2008 | Kenya | Cross sectional | 295 patients | On stavudine. Dysglycemia: noted in 20.7%, with 22.9% on HAART and 17.9% HAART- naïve (p=0.248, OR 0.731, 95% CI 0.412-1.298) |
| | Mercier *et al* | 2009 | Senegal | Prospective cohort | 180 patients | On either a stavudine or zidovudine or a PI based regimen. Increase in fasting glucose in cases vs. |

| Toxicity | Reference | Year | Country | Study type | Sample size | Result |
|---|---|---|---|---|---|---|
| | | | | | | controls (4.86 vs. 3.60, P<0.0001). No difference in the fasting insulin levels. |
| | Sinxadi *et al* | 2010 | South Africa | Cross-sectional study | 47 patients | On stavudine. Dysglycaemia: 23%;IFG:19%; IGT: 4% |
| **Dyslipidaemia** | Mutimura *et al* | 2007 | Rwanda | Prospective cohort | 571 patients | On either a stavudine (predominantly) or zidovudine based regimen. Hypertriglyceridemia: 9% in LD group. Hypercholesterolemia: 14% in LD group. |
| | Manuthu *et al* | 2008 | Kenya | Cross sectional | 295 patients | On stavudine predominantly. Hypercholesterolemia: 39.2% in HAART group vs. HAART naïve group, prevalence of an elevated LDL in the HAART group was significantly higher than the HAART naïve group (40% vs. 11%; p value <0.0001) |
| | Buchacz *et al* | 2008 | Uganda | Prospective | 374 patients | On a stavudine regimen with a single drug substitution to zidovudine. Increase mean lipid levels from 0 to 24 months in total cholesterol 31 mg/dl (95% CI 26-36), LDL-cholesterol 26 mg/dl (95% CI 22-29), HDL-cholesterol 19 mg/dl (95% CI 17-21) |
| | Mercier *et al* | 2009 | Senegal | Prospective cohort | 180 patients | On either a stavudine or zidovudine or a PI based regimen. Triglyceride (1.03 mmol/L vs. 0.89 mmol/L, p=0.007) and HDL-C levels (1.33 mmol/L vs. 0.37 mmol/L, p=0.008) were increased in patients vs. controls |
| | Lesi *et al* | 2009 | Nigeria | Prospective cross sectional | 113 patients | On 'HAART'. 28.3% of patients with fasting cholesterol values >200mg/dl, 24% had an elevated LDL-cholesterol; 35% had elevated triglyceride levels. |
| | Salami *et al* | 2009 | Nigeria | Prospective cohort | 327 patients | Hypertriglyceridaemia was greater in the PI vs. NNRTI group (79% vs. 54%) with a higher risk RR 2.95 (95% CI 1.89-4.59) P= 0.0003; with hypercholesterolaemia (51% vs. 31%), also with a higher risk, RR 2.44(95% CI1.72-3.47) P =0.0001 |

| Toxicity | Reference | Year | Country | Study type | Sample size | Result |
|---|---|---|---|---|---|---|
| | Sinxadi *et al* | 2010 | South Africa | Cross-sectional study | 47 patients | On stavudine. Hypertriglyceridaemia: 23%. |

* LD: Lipodystrophy; IFG: Impaired fasting glucose; IGT: Impaired glucose tolerance; DM; Diabetes mellitus; HIV: Human immunodeficiency virus; HAART: Highly active antiretroviral therapy; PI: Protease inhibitor; NNRTI: Non-nucleoside reverse transcriptase inhibitor; 95% CI = 95% confidence interval.

**Figure 2: A schematic diagram of the aetiology of lipodystrophy and its metabolic consequences.**




In a study where HIV negative patients were exposed to dual NRTIs for two weeks, decreased transcription of mtRNA was observed with no significant depletion of mtDNA in adipose tissue, and this occurred with the up-regulation of *NRF1* and *TFAM* whereas *PGC1* was down-regulated. The investigators proposed that NRTIs caused mitochondrial dysfunction by means other than through inhibition of DNA polymerase-γ, and that disruption of expression of lipid metabolism genes played a role in NRTI-induced lipoatrophy (Mallon *et al*, 2005).

In another study when comparing stavudine versus zidovudine, patients who developed drug induced lipoatrophy after 18 months of therapy, were noted to develop significant mtDNA depletion and significant decrease in the ratio of *COX3* to *COX4* expression. In addition, a

significantly lower expression of *SREBP1* and the glucose transporter (*GLUT*)4 gene were also noted in the lipoatrophic patients. It was also noted that *PGC1B* was significantly higher in the lipoatrophic group than in those that had no lipoatrophy. In addition, in those patients with lipoatrophy, the stavudine group had a significantly lower expression of *POLG1*, *COX3/COX4* ratio and *SREBP1* than the zidovudine group (Sievers *et al*, 2009).

The role of NNRTIs in lipoatrophy has also been investigated. In one study, efavirenz was associated with a great risk of limb fat loss than PIs when combined with NRTIs (stavudine and zidovudine containing regimens) (Haubrich *et al*, 2009). In a recent systematic review, this causal relationship between NRTIs and lipoatrophy was confirmed, but it was suggested that the concomitant use of PIs had an ameliorating effect on the body fat changes. It was also suggested that efavirenz caused an additive toxicity. In addition, they noted that central fat gain was as a consequence of treating the HIV infection and not linked to any antiretroviral class (de Waal *et al*, 2013).

HIV-1 infection itself can also lead to alterations in gene expression for mitochondrial proteins. The expression of several nuclear and mtDNA-encoded genes for mitochondrial proteins has been shown to be reduced in adipose tissue from HIV-1-infected patients who were not on antiretroviral therapy (Giralt *et al*, 2006). This was consistent with previous studies of mtDNA depletion and mitochondrial respiratory dysfunction in peripheral blood mononuclear cells and subcutaneous fat from HIV-1-infected patients before antiretroviral therapy (Casula *et al*, 2005. Garrabou *et al*, 2011).

#### 1.6.3.3. Management

The diagnosis of lipodystrophy is generally clinically obvious; the patient may present with lipoatrophy or lipohypertrophy and this can be confirmed by anthropometric measurements

over time. Additionally, radiological imaging if available should also be used to confirm the diagnosis. This can be done by using dual energy x-ray absorptiometry (DEXA) scans, bioelectrical impedance analysis, computed tomography (CT), or magnetic resonance imaging (MRI) (Carr *et al*, 2003).

There are medical and surgical strategies for the management of lipoatrophy and there is a possibility that lipoatrophy changes can recover although it may be slow, or incomplete. This can include antiretroviral drug substitution. An improvement in lipoatrophy may occur when NRTIs are switched, although the dyslipidaemia maybe unaffected. Options include switching stavudine to abacavir or tenofovir (Carr *et al*, 2002. Moyle *et al*, 2006) which can result in a significant increase in limb fat mass for up to two years but may not result in complete resolution of clinical lipoatrophy. One could also reduce the stavudine dose (Milinkovic *et al*, 2007). Other drug interventions that can be used to reverse fat loss include the use of thiazolidinediones which improve adipocyte differentiation and increase limb fat mass. A meta-analysis has shown that pioglitazone and not rosiglitazone was more effective in increasing limb fat (Raboud *et al*, 2010). Statins and uridine have also been shown to improve lipoatrophy in small trials (Mallon *et al*, 2006. Sutinen *et al*, 2007).

Cosmetic surgery is an option for facial lipoatrophy. This includes surgically placed alloplastic, autologous, or synthetic implants, injection of temporary fillers such as poly-L-lactic acid, and injection of permanent fillers such as silicone (Guaraldi *et al*, 2011). Liposuction has been shown to be helpful in patients with lipohypertrophy although there was a potential for recurrence (Hultman *et al*, 2007). Exercise or diet may improve lipoatrophy (Leyes *et al*, 2008), but one should be careful in patients with low body mass indices (BMIs). Exercise training has been shown to improve outcomes in body composition and metabolic profiles in patients in resource limited settings (Mutimura *et al*, 2008).

On the other hand, in patients with lipohypertrophy, low caloric feeding and combined resistance and aerobic exercise has been shown to be beneficial in obese, HIV-infected women although there was no improvement in insulin resistance or lipids (Engelson *et al*, 2006).

### 1.6.4. Insulin resistance and diabetes mellitus

#### 1.6.4.1. Epidemiology

Prior to the advent of antiretroviral therapy, disorders of glucose metabolism were relatively uncommon in patients with HIV-1 infection (Samaras *et al*, 2009). Determination of the prevalence and incidence of glucose disorders in HIV-1-infected patients is complicated by multiple confounders, ranging from inherent differences in study populations to differences in the diagnostic methods used (Paik *et al*, 2011) .

Early cases of diabetes were described with PI use (Dubé *et al*, 1997. Walli *et al*, 1998), with other metabolic complications such as dyslipidaemia and lipodystrophy occurring at the same time, making it difficult to determine which factors were contributing to the changes in glucose metabolism (Samaras *et al*, 2009).

Evidence for increased prevalence of diabetes and insulin resistance was also derived from cohorts of patients on antiretroviral therapy with lipodystrophy where the rates of disorders of glucose metabolism such as diabetes mellitus, impaired fasting glucose, and impaired glucose tolerance were as high as 25% and were considered to be related to the specific PIs that were used (Carr *et al*, 1998. Carr *et al*, 1999). NRTIs also played a role, where cumulative exposure to nucleoside analogs was associated with insulin resistance in some studies (Brown *et al*, 2005. Tien *et al*, 2008). Rates from Africa vary (Table 3), with rates of dysglycaemia as high as 20% in two cross sectional studies (Manuthu *et al*, 2008. Sinxadi *et al*, 2010). Fat

redistribution may also contribute to insulin resistance (Meininger *et al*, 2002); one study suggested that the severity of lipoatrophy, among patients taking either stavudine or zidovudine, was associated with an increased risk of insulin resistance (De Wit *et al*, 2008). Other studies examining the prevalence and predictors of glucose intolerance and insulin resistance, and their association with antiretroviral therapy also found that obesity, gender, ethnicity, a family history of diabetes , and hepatitis C were all predictors (Mehta *et al*, 2003.Yoon *et al*, 2004. Amorosa *et al*, 2005. Salehian *et al*, 2005.Tien *et al*, 2008).

#### 1.6.4.2. Pathogenesis

Evidence generally suggests that nucleoside analogs exert their effects on glucose metabolism indirectly, through changes in body composition and mitochondrial toxicity (De Wit *et al*, 2008). This may be the case in Africa where predominantly NRTIs are used, and where rates of dysglycemia vary between 8-23% (Table 3).

On the other hand, PIs can inhibit the uptake of glucose into cells by interfering with the *GLUT* 4 glucose transporter (Murata *et al*, 2000. Hertel *et al*, 2004). Another proposed mechanism is the effects of PIs on adipokine metabolism. PIs reduce adipocyte differentiation by altering *SREBP*-1 maturation and *PPAR*-γ expression resulting in a decreased expression of adipocytokines (Zhang *et al*, 1999, Lenhard *et al*, 2000. Caron *et al*, 2001). Apart from direct PI-induced effects on insulin resistance, discrete and independent insults to glucose metabolism may arise from HAART-induced dyslipidaemia, lipotoxicity, and lipodystrophy (Gutierrez *et al*, 2012).

#### 1.6.4.3. Management

The diagnosis of diabetes mellitus should be confirmed using formal laboratory testing which would include a fasting blood glucose or an oral glucose tolerance test and more recently a glycosylated haemoglobin (WHO, 2011). Just like in HIV negative patients, diet and exercise are important in the management of insulin resistance and diabetes in overweight patients with HIV infection (Falutz *et al*, 2007). Similarly, if this is not achieved drug interventions may be required. Metformin has been shown to reduce insulin resistance and cardiovascular risk parameters in HIV-infected patients with lipodystrophy (Hadigan *et al*, 2000), but the use of metformin in patients on stavudine may increase the risk of lactic acidosis and may worsen lipoatrophy in HIV infected patients with lipoatrophy. Rosiglitazone has also been shown to improve insulin sensitivity and increased adiponectin levels (Hadigan *et al*, 2004).

### 1.6.5. Dyslipidaemia

#### 1.6.5.1. Epidemiology

Lipid abnormalities have been noted in HIV-1 infected patients even before the advent of antiretroviral therapy, and this was characterised by decreased levels of total, LDL and HDL cholesterol and elevated triglyceride levels (Constans *et al*, 1994. Grunfeld *et al*, 1998). HIV-1 associated dyslipidaemia is associated with increased levels of interferon α which has been correlated with elevated plasma triglycerides (Carpentier *et al*, 2005). Similarly, tumour necrosis factor (TNF) α has also been shown to be elevated in HIV-1-positive, antiretroviral therapy-naïve patients. Here it has been shown to interfere with free fatty acid metabolism, lipid oxidation and to attenuate insulin-mediated suppression of lipolysis (Johnson *et al*, 2004. Oh *et al*, 2007). The prevalence of lipid abnormalities varies depending on the type of antiretroviral therapy used. In patients receiving a PI-containing antiretroviral therapy, the

prevalence rates of dyslipidaemia were high (>70%) and it predominantly included hypertriglyceridaemia followed by hypercholesterolaemia (Dong *et al*, 1999. Carr *et al*, 1999. Savès *et al*, 2002). Although metabolic alterations are more common among patients with lipodystrophy, they are also present in those without body changes; in fact these metabolic abnormalities may precede the body fat changes (Vigouroux et *al*, 1999. Mulligan *et al*, 2000). Results from various studies are contradictory when comparing risk factors for the PI-related hyperlipidaemia. Although elevations in serum triglyceride and cholesterol levels have been associated with all the available PIs, hypertriglyceridaemia seems more frequent in patients receiving particular types of PI combination therapy (Carr *et al*, 1998. Carr *et al*, 1999). In a review (Calza *et al*, 2003), different PI-based therapies were associated with different lipid profiles, but hypertriglyceridaemia was noted to be more frequent in patients receiving a ritonavir, ritonavir/saquinavir or ritonavir/lopinavir combination therapy when compared with indinavir, nelfinavir and amprenavir based therapies. Atazanavir is the most lipid stable PI currently available (Cahn PE *et al*, 2004). In several studies, hypertriglyceridaemia has been found to be associated with male gender, advanced age, a higher body mass index, diagnosis of AIDS, higher mean CD4 count, and elevated cholesterol and triglyceride plasma levels prior to the PI therapy initiation while in other studies, gender, baseline lipid levels, stage of HIV-1 disease and body weight do not appear to be related to the occurrence of hypertriglyceridaemia (Mulligan *et al*, 2000. Savès *et al*, 2002. Thiébaut *et al*, 2003).

Lipid abnormalities also occur in patients on NRTIs such as stavudine and zidovudine. Data from countries in Africa confirm this (Table 3). A study from Senegal, in which the main drugs used were zidovudine and stavudine, showed both triglyceride and HDL-cholesterols were significantly raised in patients when compared to controls but the use of PIs may have

been a confounder (Mercier *et al*, 2009). In a smaller Ugandan study, where all patients were on stavudine based regimens, a comparison between two NNRTIs, efavirenz and nevirapine was also assessed. After two years of therapy, changes were thought to be related to the use of nevirapine (Buchacz *et al*, 2008). In a study from Rwanda, where no PIs were used and stavudine was the commonest drug used, high rates of elevated triglycerides and total cholesterol were noted in patients with lipodystrophy (Mutimura *et al*, 2007). Similarly, a longitudinal study from South Africa showed a significant increase in both triglyceride and cholesterol levels in patients on stavudine followed over a period of two years, but this was noted in patients with and without lipodystrophy (George *et al*, 2008).

#### 1.6.5.2. Pathogenesis

Concerning the proposed pathogenesis of PI-associated dyslipidaemias which are based partly on speculation in reviews rather than original research - PIs and cellular proteases acting in mitochondrial biogenesis underlie the metabolic alterations because of their homology. PIs bind to cellular retinoic acid binding protein 1 (*CRABP1*), which has a similar structural homology to the catalytic region of HIV-1 protease enzyme, thus inhibiting the formation of cis-9-retinoic acid from retinoic acid, leading to reduced retinoid X receptor-peroxisome proliferator-activated receptor γ (*RXR-PPARγ*) activity. This increases apoptosis and decreases proliferation of peripheral adipocytes causing peripheral lipoatrophy, and decreased adiponectin levels. As a result, there is a free fatty acid spill over from apoptotic peripheral adipocytes increasing free fatty acid flux to the liver and skeletal muscle. In the liver, the increased free fatty acid supply and the up-regulation of the triglyceride synthetic pathway through *SREBP1* increase hepatic triglyceride and ultimately secretion of

triglyceride- rich VLDL, decreased lipid storage and lipid release into the bloodstream. (Carr *et al*, 1998. Penzak *et al*, 2000. Calza *et al*, 2003. Oh *et al*, 2007).

PIs may also bind to low density lipoprotein receptor-related protein 1(*LRP1*) interfering with *LRP1*-lipoprotein lipase complex formation, reducing adipose storage capacity and increasing plasma triglyceride-rich lipoproteins (Oh *et al*, 2007). It is also possible that PIs may have direct effects on hepatic triglyceride synthesis by up regulating expression of key triglyceride biosynthetic enzymes (Carr *et al*, 1999).

As discussed in the previous sections, NRTIs can cause mitochondrial dysfunction through inhibition of DNA polymerase-γ, and that disruption of expression of lipid metabolism genes which may be responsible for abnormalities in several cell types such as adipocytes, leading to lipoatrophy, and in skeletal muscle leading to insulin resistance, with secondary dyslipidaemia (Cossarizza *et al*, 2003. Mallon *et al*, 2005. Pinti *et al*, 2006).

#### 1.6.5.3. Management

The diagnosis is made by an overnight fasting lipid profile, similar to that in a HIV negative patient. Exercise, weight reduction and diet are important components in the management of such patients (Lazzaretti *et al*, 2012. Mutimura *et al*, 2008. Stein *et al*, 2008). Generally, when initiating HAART in a patient with cardiovascular risk factors, drugs with a more favourable lipid profile should be given, such as atazanavir.

Other options would include lipid lowering drugs. Most statins are metabolized by the cytochrome P450 3A4 (CYP3A4) enzyme. With regards to NNRTIs, whilst nevirapine is a selective inducer of CYP3A4, efavirenz is both an inducer and inhibitor of CYP3A4 (Dubé e*t al*, 2003). There is no data on the combined use of nevirapine and statins. Efavirenz leads to significant induction of statin metabolism (Gerber *et al*, 2005) which may lead to decreased

efficacy of statin therapy, but on the other hand, efavirenz metabolism is not affected by statins. This would suggest that an increased dosing of statins may be needed in patients who are taking both agents, but closely monitor for drug toxicity.

All PIs down regulate the activity of CYP3A4, therefore leading to an increased serum concentration of the statin and the potential for adverse reactions such as rhabdomyolysis. Therefore statins such as lovastatin or simvastatin are contraindicated (Dubé e*t al*, 2003), but atorvastatin and pravastatin are other options. Atorvastatin levels are known to significantly increase with the use of PIs – especially with lopinavir/ritonavir, and therefore there is a concern for a rhabdomyolysis.

In contrast to the statins, fibrates are unlikely to have significant drug interactions with antiretroviral medications. Fibrates however have not been shown to reduce cardiovascular morbidity and mortality at the same degree as statin therapy. There is limited data on the use of ezetimibe in HIV positive patients although it was shown to be well tolerated with changes mainly in the LDL cholesterol (Bennett *et al*, 2007. Coll *et al*, 2006. Stebbing *et al*, 2009).

### 1.6.6. Cardiovascular risk

As summarised in Figure 3, the risk of cardiovascular disease (CVD) is due to a number of factors which include HIV associated inflammation, traditional cardiovascular risk factors and the metabolic side effects from antiretroviral therapy.

#### 1.6.6.1. The role of HIV associated inflammation

Through systemic inflammation, hypercoagulability and platelet activation, HIV infection via its viral proteins causes endothelial dysfunction and CVD (Dau *et al*, 2008). Independent of cardiovascular risk factors and antiretroviral therapy, HIV-1 replication and a low baseline

CD4 count are associated with an increased risk of myocardial infarctions in HIV-1-infected individuals (Lang *et al*, 2012). The HIV proteins gp120, Tat, and Nef induce expression of several adhesion molecules and inflammatory cytokines such as intercellular adhesion molecule (ICAM) -1, vascular cell adhesion molecule (VCAM)-1, E-selectin, tumour necrosis factor (TNF)-α, and interleukin (IL)-6 resulting in increased leukocyte adherence to the endothelium (Mu *et al*, 2007). Increased levels of vWF (von Willebrand factor), a glycoprotein facilitating platelet adhesion, are synthesized in endothelial cells and inflammatory cells, and correlate with circulating levels of inflammatory cytokines (Mu *et al*, 2007). A hypercoagulable state may also occur depending on plasma viral load. These HIV proteins can also induce endothelial apoptosis and increase endothelial permeability (Mu *et al*, 2007). Elevated levels of highly sensitivity C-reactive protein (hs-CRP), an inflammatory marker, are associated with an increased risk of cardiovascular events in the general population (Rutter *et al*, 2004. Pai *et al*, 2004) as well as in HIV-positive patients (Reingold *et al*, 2008. Triant *et al*, 2009).

#### 1.6.6.2. The role of traditional cardiovascular risk factors

The Women's Interagency HIV Study and the Multicenter AIDS Cohort Study evaluated the presence of major risk factors for cardiovascular disease and compared HIV-1 positive with HIV-1 negative controls where the 10-year risk of developing coronary heart disease was estimated using the Framingham risk score. It was noted that HIV-1-infected individuals had lower HDL-cholesterol and elevated triglyceride levels when compared with HIV-1 negative controls and the risk of coronary heart disease was significantly lower in patients who were treatment-naive compared with those patients taking PIs (Kaplan *et al*, 2007).

CVD is prevalent in many African countries, with studies from various countries demonstrating a rise in traditional risk factors (Dalal *et al*, 2011). In two studies from South Africa which discuss HIV and coronary artery disease specifically (Becker *et al*, 2010, 2011), it was noted that HIV infected patients who presented with acute coronary syndromes tended to be younger, were antiretroviral therapy naïve and had less traditional risk factors. They noted a lower atherosclerotic burden in the HIV group on angiography but a larger burden of thrombus, suggesting a prothrombotic state in the pathogenesis of acute coronary syndrome. They also noted that despite these HIV infected patients having fewer traditional risk factors, a thrombophilic state tended to be more common. In another study, lower HDL- cholesterol levels in the HIV infected patients, but significantly higher hs-CRP, ICAM-1 and VCAM-1 levels were noted (Fourie *et al*, 2011).

The extent of cardiovascular complications in HIV-1-infected patients on antiretroviral therapy in Africa will become more evident in future long term studies.

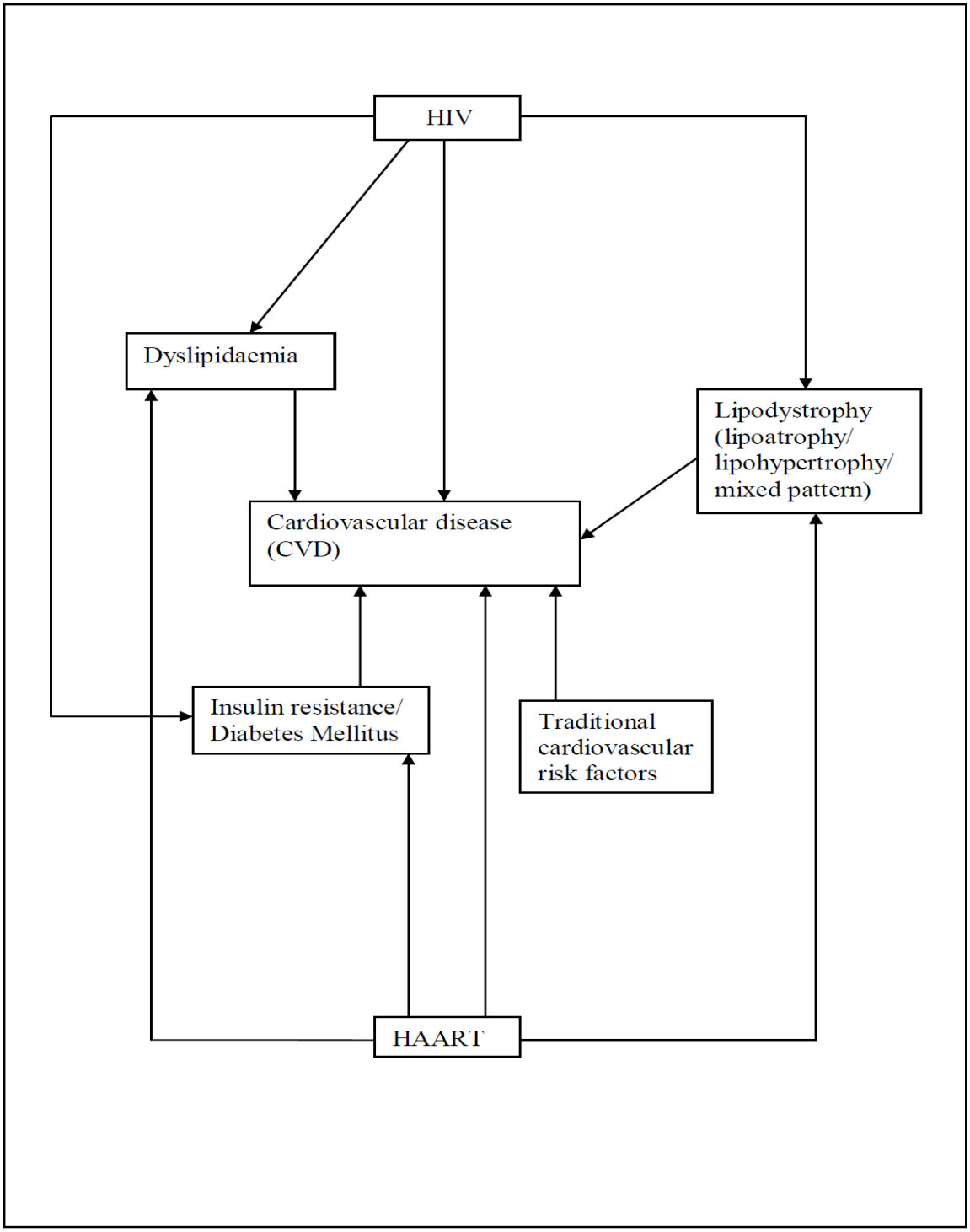
#### 1.6.6.3. The metabolic side effects of antiretroviral therapy.

Whilst antiretroviral therapy improves endothelial function (Arildsen *et al*, 2013); the metabolic side effects are a concern. Evidence from the Data collection on Adverse Effects of Anti-HIV Drugs Study (DAD) and other studies have established that exposure to certain antiretroviral drugs is associated with an increase in the rate of CVD events (Currier *et al*, 2003. Mary-Krause *et al*, 2003. DAD Study Group *et al*, 2007). In particular PI use, is significantly associated with cardiac events (DAD Study Group, 2007). This was mainly seen with the cumulative use of indinavir or lopinavir-ritonavir (Worm *et al*, 2010). Evidence also implicates NRTI use such as abacavir with an increased risk of myocardial infarction. The DAD cohort noted that there was an increased risk of myocardial infarction in those exposed

to abacavir and didanosine within six months of use prior to the myocardial infarction, but this was not seen with other NRTIs, including tenofovir (Worm *et al*, 2010). The Strategies for Management of Antiretroviral Therapy (SMART) study preliminary results demonstrated a fourfold risk of myocardial infarction and a significant increase in major cardiovascular events with abacavir use compared to other NRTIs. No association was found with didanosine (SMART study group, 2008). However, a meta-analysis of randomized controlled trials did not support this hypothesis (Cruciani *et al*, 2011).

Despite evidence that antiretroviral therapy may be associated with cardiovascular risk; its discontinuation may result in an even greater risk of disease. The virus itself may increase cardiovascular risk through endothelial dysfunction as discussed above and therefore suppression with antiretroviral therapy attenuates this risk. The SMART study group (2006) demonstrated that interruption of antiretroviral therapy was associated with an increased risk of major cardiovascular events compared to those on the continuous arm (SMART study group, 2006). There were more fatal and non-fatal cardiovascular events in the former compared to the later group suggesting that viral suppression may actually reduce cardiovascular risk in the short term. Patients with HIV-1 infection also appear to have a high rate of silent myocardial ischemia. In HIV-1-infected patients without known coronary artery disease from the SMART study, baseline electrocardiograms (ECGs) were assessed for the presence of asymptomatic Q-waves or ST segment depression. ECG evidence for asymptomatic ischemic heart disease was present in nearly 11 percent of the patients. (SMART study group, 2006).

**Figure 3: Summary of the HIV/HAART metabolic complications contributing to CVD.**



### 1.6.6.4. The role of adipokines and inflammatory markers

Adipokines may also have a role in the aetiology of CVD in HIV-1 patients. Leptin is expressed mainly in adipose tissue, its main function is to increase energy expenditure, and to inhibit both appetite and weight gain (Mallewa *et al*, 2008).The effect of antiretroviral therapy on leptin levels is controversial; where some studies have suggested that low levels of leptin are associated with lipodystrophy others have demonstrated no effect (Dzwonek *et al*, 2007. Calmy *et al*, 2009). One study from South Africa showed that leptin levels were associated with viral loads and contributed to disease progression (Azzoni *et al*, 2010). Some studies have suggested that because leptin has both proinflammatory and proatherogenic properties (Smith *et al*, 2011. Falasca *et al*, 2010), it may play a role in long-term cardiovascular events in HIV infected patients.

Adiponectin is a 30 kDa cytokine synthesized and secreted by adipose tissue, and is a potent insulin sensitizer (Mallewa *et al*, 2008).Its expression is reduced in obesity and type 2 diabetes, showing an inverse relationship with insulin resistance and visceral adiposity (Cui *et al*, 2011). Studies have shown adiponectin levels were suppressed in patients with HIV infection and fat redistribution, but the underlying mechanisms are not clear (Tong *et al*, 2003. Sankalé *et al*, 2006). Patients treated with antiretroviral therapy especially those with lipodystrophy, showed gradual down regulation in adiponectin serum levels (Luo *et al*, 2009), accompanied by accelerated cardiovascular impairment (Bezante *et al*, 2009). Therefore, the suppression of adiponectin levels in HIV-infected patients under antiretroviral therapy may negatively affect numerous metabolic parameters leading to cardiovascular events (Palios *et al*, 2012).

CRP has been shown to be a marker of increased cardiovascular events (Pai *et al*, 2004), and CRP levels have been shown to be elevated in patients with HIV compared with general

populations (Arinola *et al*, 2004. Noursadeghi et *al*, 2005. Dolan *et al*, 2005). Increased CRP and HIV infection were each associated with an approximately 2-fold increased risk for myocardial infarctions. Studies have shown high prevalence rates of elevated CRP in patients with HIV compared with control groups (Arinola *et al*, 2004. Noursadeghi et *al*, 2005. Dolan *et al*, 2005. Triant *et al*, 2009).

## 1.7. Management issues - implications for South Africa and other African countries

### 1.7.1. The issue of stavudine versus tenofovir.

Stavudine continues to be used as alternative first-line treatment in developing countries despite the change in the WHO antiretroviral therapy guidelines for treating patients with HIV infection (WHO, 2010). This is because of a lack of alternatives, shortages of tenofovir and abacavir, contraindications to the use of other NRTI drugs and its low cost and availability in fixed dose combinations. There is a dire need to increase the number of people receiving antiretroviral therapy worldwide, particularly in sub-Saharan Africa. According to the WHO, at least 15 million HIV-1 infected people are in need of therapy (WHO, 2012). In fact, shortages of tenofovir and abacavir continue to exist intermittently at health facilities across South Africa (Schowalter *et al*, 2012).

The standard dosage for stavudine is 40 mg given twice daily, as this regimen received regulatory approval in 1995 because of its efficacy, but data continues to suggest that stavudine related toxicities have been a major problem in South Africa and other African countries (Bolhaar *et al*, 2007. Boulle et al, 2007. Geddes *et al*, 2006. Hernandez Perez *et al*, 2010. Mutimura *et al*, 2007. van Griensven *et al* 2010) and is rarely used in North America and Europe because these side effects (Gallant *et al*, 20004).

In a landmark trial - the Gilead 903 trial, in 2004 - patients were randomized to receive lamivudine plus efavirenz, with either stavudine 30 or 40 mg twice daily, or tenofovir. This trial showed an equivalent efficacy when comparing the two drugs with a similar incidence of drug resistance rates at failure, but the proportion of patients with investigator-defined lipoatrophy was higher in the stavudine arm when compared to the tenofovir arm. However, the overall rate of serious adverse events between the arms over a period of three years was 27% in the tenofovir arm compared to 25% in the stavudine arm (Gallant *et al*, 2004).

In 2007, the WHO recommended a reduction in the dose of stavudine from 40mg to 30mg twice daily, after a review of dose-ranging studies that showed the same clinical and virological efficacy at the lower dose of 30mg twice daily but fewer complications, it was thought that the risk of peripheral neuropathy at this dose would be lower and patients were less likely to discontinue treatment (WHO, 2007). This advice did not suggest weight-adjusted doses, despite evidence from a review of clinical trials supporting a dose of 20 mg twice daily for body weights below 60kg (Hill, 2010). However, in 2010, the WHO and South African National Department of Health guidelines changed to a tenofovir based regimen as first line therapy. While the various toxicities associated with stavudine have been well documented, especially with the higher doses, it is less clear whether a lower dose of stavudine would have less toxic side effects.

The optimal dose for stavudine has been continually evaluated since its initial approval in 1995. A meta-analysis of nine clinical trials examining the dose of stavudine demonstrated that a dose of 30mg twice daily has equivalent efficacy to the 40mg standard-dose prescribed to adults weighing >60 kg, with some evidence of fewer side-effects, including neuropathy and to a lesser degree lipoatrophy (Hill *et al*, 2007). Interestingly, in this same meta-analysis (Hill *et al*, 2007), weight adjusted dosing and the mean body weight at baseline in three of

these studies (Ruxrungtham *et al*, 2000. Siangphoe *et al*, 2004. Ribera *et al*, 2005) was close to 60 kg, suggesting that 50% of the patients in these trials were receiving either stavudine 30mg twice daily in the control arm, or 20 mg twice daily in the low dose arm. The data in these studies suggested that there was strong evidence that lower doses of stavudine reduced the risk of peripheral neuropathy, and that there was some evidence for a correlation between stavudine dosing and the risk of lipid changes, lactic acidosis, and lipodystrophy (Hill *et al*, 2010).

Two studies where the "switch strategy" was used, patients were already on stavudine 40mg twice daily and this might have influenced the end results. In the first randomized controlled trial in the USA, the stavudine dose was cut by half, i.e. 40mg to 20mg twice daily in patients ≥ 60 kg or 30mg to 15mg twice daily in patients <60 kg, and this resulted in increased fat mtDNA and decreased lactate suggesting improvement in mitochondrial indices while preserving virologic suppression. The results suggested that for those patients who were more than 90% adherent, switching to low-dose stavudine improved mitochondrial indices while maintaining virologic suppression. (McComsey *et al*, 2008). Similarly, in the second study from Spain, patients on stavudine 40mg twice daily were either randomised to switch to 30mg twice daily, switch to tenofovir or remain on the same dose of stavudine. Although no differences were noted in mitochondrial indices, there were significant lipid improvements amongst those switched to tenofovir (Milinkovic *et al*, 2007).

The evidence from these stavudine dose ranging trials supports the use of stavudine at lower doses including doses as low as 20mg twice daily. The question remains on whether to use a lower dose of the cheaper stavudine-based therapy to treat more patients despite the risk of complications or treat fewer patients with better-tolerated but more expensive drugs with fewer side effects such as tenofovir. There have been no such studies in Africa which has the

biggest burden of HIV disease and limited resources. The only way to reliably answer this question would be to undertake a study comparing low dose stavudine with high dose stavudine and with tenofovir. Such a study is important because it is unlikely that stavudine will ever be completely phased out of use in countries with limited financial resources (Menezes *et al*, 2012).

### 1.7.2. Diagnostic challenges in resource-limited settings

The monitoring of side effects of HAART is difficult to achieve in resource-limited countries. The lack of laboratory and radiology services provides several challenges to the accurate diagnosis of lipodystrophy and the metabolic complications of HAART such as dyslipidaemia and diabetes. The availability and cost of routine measurements of cholesterol, glucose and glycosylated haemoglobin may be an issue in resource-limited areas. Not all medical facilities have access to sophisticated imaging such as dual-energy radiographic absorptiometry (DEXA) scans, computerised tomography (CT) imaging technology and magnetic resonance imaging (MRI) for assessment of lipodystrophy.

An alternative consensus definition for the diagnosis of lipodystrophy is required for resource –limited environments. An objective case definition of lipodystrophy has been developed (Carr *et al*, 2003), but it requires access to DEXA and CT imaging technology, which are not available in most resource-limited environments. A wide variation in lipoatrophy rates were noted in most studies from Africa (Boulle *et al*, 2007. van Griensven *et al*, 2010). It is possible that only cases that were severe enough to warrant a regimen change were noted. Similarly, a wide variability in the rates of peripheral neuropathies was observed in most studies. Therefore grading protocols for the diagnosis of the severity of peripheral

neuropathies are required as nerve conduction testing equipment, electromyography or the expertise and facilities for nerve biopsies are not available in most African centres.

### 1.7.3. Other treatment considerations

The cost of medications is a major issue for most resource limited countries. In 2002, six million people worldwide were eligible for antiretroviral therapy (using a CD4+ cell count threshold of 200/mm$^3$), but less than 5% of them received treatment. In 2011, in addition to the 8 million people on antiretroviral therapy, nearly 3 million people with CD4+ counts of less than 200/mm$^3$ were still in need therapy (WHO, 2012). The cost of antiretroviral therapy remains a major barrier to the scale-up of treatment. In 2010, approximately 56% of HAART regimens within resource-limited countries still contained stavudine because of its cheaper costs (WHO, 2010). A study from South Africa demonstrated that the price of tenofovir would need to decrease from USD$ 17 to USD$ 6.17 per month to have the same overall cost as stavudine based therapy (Rosen *et al*, 2008).

Drug–drug interactions are also an important concern, especially drug interactions between antiretroviral drugs and those drugs used for their treating metabolic consequences.

There are no contraindications or drug interactions related to the concurrent use of antiretroviral therapy and the antidiabetic drugs, although there may be an increased risk of lactic acidosis with the use of metformin and NRTIs, an increased risk of liver dysfunction with the use of PIs and thiazolidinediones has been described (Dau *et al*, 2008). Other agents such as the sulfonylureas and insulin can be used.

With regard to therapy of HAART associated dyslipidaemia, the use of lipid lowering agents may be limited especially because of the drug interactions. As discussed previously, simvastatin and atorvastatin, the commonly available cheaper options, are contraindicated

(National Antiretroviral Treatment Guidelines, 2004), whilst the availability of pravastatin which is generally safer, is limited.

The management of other risk factors such as hypertension include the use of calcium channel blockers, which are available but interactions with certain NNRTIs and PIs is a possibility, but other classes of antihypertensives are safe (Dau *et al*, 2008).

The need for plastic surgery with traditional cosmetic techniques such as facial fillers, liposuction, abdominolipectomy, dorsal hump lipectomy and breast reduction has increased dramatically (Wolfort *et al*, 1999. Strauch *et al*, 2004. Warren *et al*, 2008). However, there is a lack of this surgical facility in resource-limited settings. In a recent study from South Africa, nearly 6% of patients considered stopping antiretroviral therapy due the development of lipodystrophy, with 47% patients saying that they would consider surgery to correct unwanted physical changes (Zinn *et al*, 2013).

### 1.8. Aims of the thesis

Despite the major improvement in the survival of HIV-1 infected individuals since the South African Government introduced highly active anti-retroviral therapy in the public sector in 2004, there is an increasing recognition that adverse events remain an important source of morbidity and even mortality, especially with the effects of stavudine-related toxicities. Knowledge of the adverse effects of different therapeutic regimes and an understanding of the pathogenesis that modulate the risk of toxicity, are important in the management of HIV/AIDS patients as clinical practice is increasingly moving towards the use of regimes that combine high levels of tolerability and efficacy. In addition, HIV-1-infected individuals on HAART develop a pattern similar to that of the metabolic syndrome, ultimately putting them at risk for impending cardiovascular disease. Most studies on the metabolic effects of

HAART have been done in the West, but there is little data on patients from African countries including South Africa.

With this in mind, the first aim of this PhD was to make use of a large clinic-based survey of HIV-1-positive patients, newly initiated onto HAART over a period of three years (2004-2008), to compare and describe the effect of stavudine-based therapy on acute and chronic toxicities after initiation of HAART. This was the largest study of the side effects of stavudine therapy conducted to date in a South African HIV-1-positive population and formed the basis for the second aim of this PhD which was a clinical trial.

Whilst the various toxicities associated with stavudine have been well documented, what is less clear is whether the dose of stavudine plays a role in the development of these toxicities. Recent studies have suggested that reduced doses of stavudine (i.e. 20 or 30 mg twice daily) diminish its toxicity while maintaining efficacy. It is therefore possible that, because stavudine is cheaper than tenofovir, and its side effects can be minimized with dose reduction, it would allow more patients to be initiated on HAART in countries where the switch away from stavudine may not yet have occurred. In 2009, this open-label randomized controlled clinical trial was undertaken to assess whether there were any early differences (4 weeks) in the effect of tenofovir when compared with low-dose and standard dose stavudine on adipocyte-specific mtDNA copy number, gene expression and other metabolic parameters. In addition, data on anthropometry and body fat distribution, serological markers of inflammation, and lipid and glucose metabolism measured over 48 weeks of follow-up were also assessed. This was to be the first clinical trial to analyse early and long term effects of stavudine and tenofovir in a South African HIV-1-infected population and as a consequence indentify any possible associated change in cardiovascular risk factors associated with the use of these drugs in the South African setting.

## 1.9. References


Amorosa V, Synnestvedt M, Gross R, Friedman H, MacGregor RR, Gudonis D, *et al*. A tale of 2 epidemics: the intersection between obesity and HIV infection in Philadelphia. *J Acquir Immune Defic Syndr* 2005; **39(5)**:557-61.

Arildsen H, Sørensen KE, Ingerslev JM, Østergaard LJ, Laursen AL. Endothelial dysfunction, increased inflammation, and activated coagulation in HIV-infected patients improve after initiation of highly active antiretroviral therapy. *HIV Med* 2013; **14(1)**:1-9.

Arinola OG, Adedapo KS, Kehinde AO, Olaniyi JA, Akiibinu MO. Acute phase proteins, trace elements in asymptomatic human immunodeficiency virus infection in Nigerians. *Afr J Med Sci* 2004; **33(4)**:317-22.

Azzoni L, Crowther NJ, Firnhaber C, Foulkes AS, Yin X, Glencross D, *et al*. Association between HIV replication and serum leptin levels: an observational study of a cohort of HIV-1-infected South African women. *J Int AIDS Soc* 2010; **13**:33.

Bakari A, Sani-Bello F, Shehu M, Mai A, Aliyu I, Lawal II. Anti-retroviral therapy induced diabetes in a Nigerian. *Afr Health Sci* 2007; **7(3):**133-5.

Barile M, Valenti D, Hobbs GA, Abruzzese MF, Keilbaugh SA, Passarella S, *et al*. Mechanisms of toxicity of 3-azido-3-deoxythymidine. Its interaction with adenylate kinase. *Biochem Pharmacol* 1994; **4**: 1405–12.

Barile M, Valenti D, Passarella S, Quagliariello E. 3-Azido-3-deoxythmidine uptake into isolated rat liver mitochondria and impairment of ADP/ATP translocator. *Biochem Pharmacol* 1997; **53**: 913–20.

Barile M, Valenti D, Quagliariello E, Passarella S. Mitochondria as cell targets of zidovudine (zidovudine). *Gen Pharmacol* 1998; **31**: 531–38.

Barrientos A, Barros MH, Valnot I, Rotig A, Rustin P, Tzagoloff A. Cytochrome oxidase in health and disease. *Gene* 2002; **286**:53-63.

Beadles WI, Jahn A, Weigel R. Peripheral neuropathy in HIV-patients at an antiretroviral clinic in Lilongwe, Malawi. *Tropical Doctor* 2009; **29**:78-80.

Becker AC, Jacobson B, Singh S, Sliwa K, Stewart S, *et al*. The thrombotic profile of treatment-naïve HIV-positive black South Africans with acute coronary syndromes. *Clin Appl Thromb Hemost* 2011; **17(3)**:264

Becker AC, Sliwa K, Stewart S, Libhaber E, Essop AR, Zambakides CA, *et al*. Acute coronary syndromes in treatment naïve black South Africans with human immunodeficiency virus infection. *J Interv Cardiol* 2010; **23(1)**:70-7.

Bennett MT, Johns KW, Bondy GP. Ezetimibe is effective when added to maximally tolerated lipid lowering therapy in patients with HIV. *Lipids Health Dis* 2007; **6**:15.

Bezante GP, Briatore L, Rollando D, Maggi D, Setti M, Ghio M *et al*. Hypoadiponectinemia in lipodystrophic HIV individuals: a metabolic marker of subclinical cardiac damage. *Nutr Metab Cardiovasc Dis* 2009; **19(4)**: 277-282.

Bissuel F, Bruneel F, Habersetzer F, Chassard D, Cotte L, Chevallier M, *et al*. Fulminant hepatitis with severe lactate acidosis in HIV-infected patients on didanosine therapy. *J Intern Med* 1994: **235:** 367–71.

Bolhaar MG, Karstaedt AS. A high incidence lactic acidosis and symptomatic hyperlactemia in women receiving highly active antiretroviral therapy in Soweto, South Africa. *Clin Infect Dis* 2007; **45(2)**:254-60.

Boulle A, Orrell C, Kaplan R, van Cutsem G, McNally M, Hilderbrand K, *et al*. Substitution due to antiretroviral toxicity or contraindication in the first 3 years of antiretroviral treatment in a large South African cohort. *Antivir Ther* 2007; **12**:753-60.

Brinkman K. Management of hyperlactataemia: no need for routine lactate measurements. *AIDS* 2001; **15:** 795–97.

Brinkman K, Smeitink J, Romijn J, Reiss P. Mitochondrial toxicity induced by nucleoside-analogue reverse-transcriptase inhibitors is a key factor in the pathogenesis of antiretroviral-therapy-related lipodystrophy. *Lancet* 1999; **354**:1112-15.

British HIV Association (BHIVA) guidelines for the treatment of HIV-infected adults with antiretroviral therapy. 2005. http://www.bhiva.org/documents/Guidelines/Treatment%20Guidelines/Archive/2005/Treatment%20Guidelines%202005.pdf. Last accessed on the 22/4/2013.

Brown TT, Li X, Cole SR, Kingsley LA, Palella FJ, Riddler SA, *et al*. Cumulative exposure to nucleoside analogue reverse transcriptase inhibitors is associated with insulin resistance markers in the Multicenter AIDS Cohort Study. *AIDS* 2005; **19(13)**:1375-83.

Buchacz K, Weidle PJ, Moore D, Were W, Mermin J, Downing R, *et al*. Changes in lipid profile over 24 months among adults on first-line highly active antiretroviral therapy in the home-based AIDS care program in rural Uganda. *J Acquir Immune Defic Syndr* 2008; **47**:304–311.

Cahn PE, Gatell JM, Squires K, Percival LD, Piliero PJ, Sanne IA, et al. Atazanavir—a once-daily HIV protease inhibitor that does not cause dyslipidemia in newly treated patients: results from two randomized clinical trials. *J Int Assoc Physicians AIDS Care (Chic)* 2004; **3:** 92–98.

Calmy A, Gayet-Ageron A, Montecucco F, Nguyen A, Mach F, Burger F, *et al.* HIV increases markers of cardiovascular risk: results from a randomized, treatment interruption trial. *AIDS* 2009; **23(8)**: 929-939.

Calza L, Manfredi R, Chiodo F. Hyperlipidaemia in patients with HIV-1 infection receiving highly active antiretroviral therapy: epidemiology, pathogenesis, clinical course and management. *Int J Antimicrob Agents* 2003; **22(2)**:89-99.

Canestri A, Sow PS, Vray M, Ngom F, M'boup S, Kane CT, *et al*. Poor efficacy and tolerability of stavudine, didanosine and efavirenz-based regimen in treatment-naïve patients in Senegal. *MedGenMed* 2007; **9(4)**:7.

Canter JA, Haas DW, Kallianpur AR, Ritchie MD, Robbins GK, Shafer RW, *et al*. The mitochondrial pharmacogenomics of haplogroup T: MTND2*LHON4917G and antiretroviral therapy-associated peripheral neuropathy. *Pharmacogenomics J* 2008; **8:71**–77.

Canter JA, Robbins GK, Selph D, Clifford DB, Kallianpur AR, Shafer R, *et al*. African Mitochondrial DNA Subhaplogroups and Peripheral Neuropathy during Antiretroviral Therapy. *J Infect Dis*. 2010; **201(11)**: 1703–1707.

Casula M, Bosboom-Dobbelaer I, Smolders K, Otto S, Bakker M, de Baar MP, *et al*. Infection with HIV-1 induces a decrease in mtDNA. *J Infect Dis* 2005; **191**:1468–1471.

Caride E, Brindeiro R, Hertogs K, Larder B, Dehertogh P, Machado E, *et al*. Drug-resistant reverse transcriptase genotyping and phenotyping of B and non-B subtypes (F and A) of human immunodeficiency virus type I found in Brazilian patients failing HAART. *Virology* 2000; **275**:107-115.

Caron M, Auclair M, Vigouroux C, Glorian M, Forest C, Capeau J. The HIV protease inhibitor indinavir impairs sterol regulatory element-binding protein-1 intranuclear localization, inhibits preadipocyte differentiation, and induces insulin resistance. *Diabetes* 2001; **50(6)**:1378-88.

Carpentier A, Patterson BW, Uffelman KD, Salit I, Lewis GF. Mechanism of highly active anti-retroviral therapy-induced hyperlipidemia in HIV-infected individuals. *Atherosclerosis* 2005; **178(1)**:165-72.

Carr A. HIV lipodystrophy: risk factors, pathogenesis, diagnosis and management. *AIDS* 2003; **17**:S141-8.

Carr A, Grund B, Neuhaus J, El-Sadr WM, Grandits G, Gibert C, *et al*. Asymptomatic myocardial ischaemia in HIV-infected adults. *AIDS* 2008; **22(2)**:257-67.

Carr A, Emery S, Law M, Puls R, Lundgren JD, Powderly WG. An objective case definition of lipodystrophy in HIV-infected adults: a case-control study. *Lancet* 2003; **361**:726-735.

Carr A, Samaras K, Burton S, Law M, Freund J, Chisholm DJ, *et al*. A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. *AIDS* 1998; **12(7)**:F51-8.

Carr A, Samaras K, Chisholm DJ, Cooper DA. Pathogenesis of HIV-1-protease inhibitor-associated peripheral lipodystrophy, hyperlipidaemia, and insulin resistance. *Lancet* 1998; **351(9119)**:1881-3.

Carr A, Samaras K, Thorisdottir A, Kaufmann GR, Chisholm DJ, Cooper DA. Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor-associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study. *Lancet* 1999; **353**:2093-2099.

Carr A, Workman C, Smith DE, Hoy J, Hudson J, Doong N, *et al*. Abacavir substitution for nucleoside analogs in patients with HIV lipoatrophy: a randomized trial. *JAMA* 2002; **288(2)**:207-15.

Chariot P, Drogou I, de Lacroix-Szmania I, Eliezer-Vanerot MC, Chazaud B, Lombès A, *et al*. Zidovudine-induced mitochondrial disorder with massive liver steatosis, myopathy, lactic acidosis, and mitochondrial DNA depletion. *J Hepatol* 1999; **30:**156–60.

Chattha G, Arieff AI, Cummings C, Tierney LM. Lactic acidosis complicating acquired immunodeficiency syndrome. *Ann Intern Med* 1993; **118:** 37–39.

Chiappini F, Teicher E, Saffroy R, Pham P, Falissard B, Barrier A, *et al*. Prospective evaluation of blood concentration of mitochondrial DNA as a marker of toxicity in 157 consecutively recruited untreated or HAART-treated HIV-positive patients. *Lab Invest* 2004; **84(7)**:908-14.

Coll B, Aragonés G, Parra S, Alonso-Villaverde C, Masana L. Ezetimibe effectively decreases LDL-cholesterol in HIV-infected patients. *AIDS* 2006; **20(12)**:1675-7.

Constans J, Pellegrin JL, Peuchant E, Dumon MF, Pellegrin I, Sergeant C, *et al*. Plasma lipids in HIV-infected patients: a prospective study in 95 patients. *Eur J Clin Invest* 1994; **24**: 416–20.

Cornblath DR, Hoke A. Recent advances in HIV neuropathy. *Curr Opin Neurol* 2006; **19**: 446 - 450.

Cossarizza A, Riva A, Pinti M, Ammannato S, Fedeli P, Mussini C, *et al*. Increased mitochondrial DNA content in peripheral blood lymphocytes from HIV-infected patients with lipodystrophy. *Antivir Ther* 2003; **8(4)**:315-21.

Côté HC, Brumme ZL, Craib KJ, Alexander CS, Wynhoven B, Ting L, et al. Changes in mitochondrial DNA as a marker of nucleoside toxicity in HIV-infected patients. *N Engl J Med* 2002; **346(11)**:811-20.

Cruciani M, Zanichelli V, Serpelloni G, Bosco O, Malena M, Mazzi R, *et al*. Abacavir use and cardiovascular disease events: a meta-analysis of published and unpublished data. *AIDS* 2011; **25(16)**:1993-2004.

Cui J, Panse S, Falkner B. The role of adiponectin in metabolic and vascular disease: a review. *Clin Nephrol*. 2011; **75(1)**:26-33.

Currier JS, Taylor A, Boyd F, Dezii CM, Kawabata H, Burtcel B, *et al*. Coronary heart disease in HIV-infected individuals. *J Acquir Immune Defic Syndr* 2003; **33(4)**:506-12.

DAD Study Group, Friis-Møller N, Reiss P, Sabin CA, Weber R, Monforte Ad, *et al*. Class of antiretroviral drugs and the risk of myocardial infarction. *N Engl J Med* 2007; **356(17)**:1723-35.

Dalakas MC. Peripheral neuropathy and antiretroviral drugs. *J Peripher Nerv Syst* 2001; **6:**14–20.

Dalakas MC, Semino-Mora C, Leon-Monzon M. Mitochondrial alterations with mitochondrial DNA depletion in the nerves of AIDS patients with peripheral neuropathy induced by 2′3′-dideoxycytidine (ddC). *Lab Invest* 2001; **81**:1537–44.

Dalal S, Beunza JJ, Volmink J, Adebamowo C, Bajunirwe F, Njelekela M, *et al*. Non-communicable diseases in sub-Saharan Africa: what we know now. *Int J Epidemiol* 2011; **40(4)**:885-901.

Dau B, Holodniy M. The Relationship Between HIV Infection and Cardiovascular Disease. *Curr Cardiol Rev* 2008; **4:203**-218.

De Wit S, Sabin CA, Weber R, Worm SW, Reiss P, Cazanave C, *et al*. Incidence and risk factors for new-onset diabetes in HIV-infected patients: the Data Collection on Adverse Events of Anti-HIV Drugs (D: A: D) study. *Diabetes Care* 2008; **31(6)**:1224-9.

Dolan SE, Hadigan C, Killilea KM, Sullivan MP, Hemphill L, Lees RS, *et al*. Increased cardiovascular disease risk indices in HIV-infected women. *J Acquir Immune Defic Syndr* 2005; **39(1)**:44-54.

de Waal R, Cohen K, Maartens G. Systematic review of antiretroviral-associated lipodystrophy: lipoatrophy, but not central fat gain, is an antiretroviral adverse drug reaction. *PLoS One* 2013; **8(5)**:e63623.

Dong KL, Bausserman LL, Flynn MM, Dickinson BP, Flanigan TP, Mileno MD, *et al*. Changes in body habitus and serum lipid abnormalities in HIV-positive women on highly active antiretroviral therapy (HAART). *J Acquir Immune Defic Syndr* 1999; **21(2)**:107-13.

Dubé MP, Stein JH, Aberg JA, Fichtenbaum CJ, Gerber JG, Tashima KT, *et al*. Guidelines for the evaluation and management of dyslipidemia in human immunodeficiency virus (HIV)-infected adults receiving antiretroviral therapy: recommendations of the HIV Medical

Association of the Infectious Disease Society of America and the Adult AIDS Clinical Trials Group. *Clin Infect Dis* 2003; **37(5)**:613.

Dzwonek AB, Novelli V, Schwenk A. Serum leptin concentrations and fat distribution in HIV-1 infected children on highly active antiretroviral therapy. *HIV Medicine* 2007; **8(7)**:433-438.

Engelson ES, Agin D, Werber-Zion G, et al. Effects of a diet and exercise weight loss regimen on obese, HIV-infected women. *Metabolism* 2006; **55:1327**–1336.

Enriquez JA, Fernandez-Silva P, Montoya J. Autonomous regulation in mammalian mitochondrial DNA transcription. *Biol Chem* 1999; **380**:737-47.

Falasca K, Ucciferri C, Mancino P, Di Iorio A, Vignale F, Pizzigallo E, *et al*. Cystatin C, adipokines and cardiovascular risk in HIV infected patients. *Curr HIV Res* 2010; **8(5)**:405-10.

Fanales-Belasio E, Raimondo M, Suligoi B, Buttò S. HIV virology and pathogenetic mechanisms of infection: a brief overview. *Ann Ist Super Sanita* 2010; **46(1)**:5-14.

Falco V, Rodriguez D, Ribera E, et al. Severe nucleoside-associated lactic acidosis in human immunodeficiency virus-infected patients: report of 12 cases and review of the literature. *Clin Infect Dis* 2002; **34:** 838–46.

Falutz J. Therapy insight: shape changes and metabolic complications associated with HIV and highly active antiretroviral therapy. *Nat Clin Pract Endocrinol Metab* 2007; **3(9)**:651-61.

Forna F, Liechty CA, Solberg P, Asiimwe F, Were W, Mermin J, *et al.* Clinical toxicity of highly active antiretroviral therapy in a home-based AIDS care program in rural Uganda. *J Acquir Immune Defic Syndr* 2007; **44**:456–462.

Fourie C, Van Rooyen J, Pieters M, Conradie K, Hoekstra T, Schutte A. Is HIV-1 infection associated with endothelial dysfunction in a population of African ancestry in South Africa? *Cardiovasc J Afr* 2011; **22**:134-140.

Frater AJ, Dunn DT, Beardall AJ, Ariyoshi K, Clarke JR, McClure MO, *et al*. Comparative response of African HIV-1-infected individuals to highly active antiretroviral therapy. *AIDS* 2002; 16(8):1139-46.

Gallant JE, Staszewski S, Pozniak AL, DeJesus E, Suleiman JM, Miller MD, *et al*. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial. *JAMA* 2004; **292(2)**:191-201.

Galluzzi L, Pinti M, Guaraldi G, Mussini C, Troiano L, Roat E, *et al*. Altered mitochondrial RNA production in adipocytes from HIV-infected individuals with lipodystrophy. *Antivir Ther* 2005; **10**:M91-9.

Garrabou G, López S, Morén C, Martínez E, Fontdevila J, Cardellach F, *et al*. Mitochondrial damage in adipose tissue of untreated HIV-infected patients. *AIDS* 2011; **25(2)**:165-70.

Geddes R, Knight S, Moosa MY, Reddi A, Uebel K, Sunpath H. A high incidence of nucleoside reverse transcriptase inhibitor (NRTI)-induced lactic acidosis in HIV-infected patients in a South African context. *S Afr Med J* 2006; **96**:722-4.

George JA, Venter WD, Van Deventer HE, Crowther NJ. A longitudinal study of the changes in body fat and metabolic parameters in a South African population of HIV-positive patients receiving an antiretroviral therapeutic regimen containing stavudine. *AIDS Res Hum Retroviruses* 2009; **25(8)**:771-81.

Gerber JG, Rosenkranz SL, Fichtenbaum CJ, *et al*. Effect of efavirenz on the pharmacokinetics of simvastatin, atorvastatin, and pravastatin: results of AIDS Clinical Trials Group 5108 Study. *J Acquir Immune Defic Syndr* 2005; **39(3)**:307.

Gerschenson M, Shiramizu B, LiButti DE, Shikuma CM. Mitochondrial DNA levels of peripheral blood mononuclear cells and subcutaneous adipose tissue from thigh, fat and abdomen of HIV-1 seropositive and negative individuals. *Antivir Ther* 2005; **10**:M83-9.

Giralt M, Domingo P, Guallar JP, Rodriguez de la Concepción ML, Alegre M, Domingo JC, *et al*. HIV-1 infection alters gene expression in adipose tissue, which contributes to HIV-1/HAART-associated lipodystrophy. *Antivir Ther* 2006; **11(6)**:729-40.

Graziewicz MA, Longley MJ, Copeland WC. DNA polymerase gamma in mitochondrial DNA replication and repair. *Chem Rev* 2006; **106(2)**:383-405.

Grunfeld C, Kotler DP, Hamadeh R, Tierney A, Wang J, Pierson RN. Hypertriglyceridemia in the acquired immunodeficiency syndrome. *Am J Med* 1989; **86(1)**:27-31.

Guaraldi G, Fontdevila J, Christensen LH, Orlando G, Stentarelli C, Carli F *et al*. Surgical correction of HIV-associated facial lipoatrophy. *AIDS* 2011; **25(1)**:1-12.

Gutierrez AD, Balasubramanyam A. Dysregulation of glucose metabolism in HIV patients: epidemiology, mechanisms, and management. *Endocrine* 2012; **41(1)**:1-10.

Hahn K, Arendt G, Braun JS, von Giesen HJ, Husstedt IW, Maschke M, *et al*. A placebo-controlled trial of gabapentin for painful HIV-associated sensory neuropathies. *J Neurol* 2004; **251(10)**:1260-6.

Hadigan C, Corcoran C, Basgoz N, Davis B, Sax P, Grinspoon S. Metformin in the treatment of HIV lipodystrophy syndrome: A randomized controlled trial. *JAMA* 2000; **284(4)**:472-7.

Hadigan C, Yawetz S, Thomas A, Havers F, Sax PE, Grinspoon S. Metabolic effects of rosiglitazone in HIV lipodystrophy: a randomized, controlled trial. *Ann Intern Med* 2004; **140**:786–94.

Harrison MJG, McArthur JC. *AIDS and Neurology.* Edinburgh: Churchill Livingstone; 1995.

Haubrich RH, Riddler SA, DiRienzo AG, Komarow L, Powderly WG, Klingman K, et al. Metabolic outcomes in a randomized trial of nucleoside, nonnucleoside and protease inhibitor-sparing regimens for initial HIV treatment. *AIDS* 2009; **23(9)**:1109-18.

Haugaard SB, Andersen O, Pedersen SB, Dela F, Richelsen B, Nielsen JO, *et al*. Depleted skeletal muscle mitochondrial DNA, hyperlactatemia, and decreased oxidative capacity in HIV-infected patients on highly active antiretroviral therapy. *J Med Virol* 2005; **77(1)**:29-38.

Hawkins C, Achenbach C, Fryda W,Ngare D, Murphy R. Antiretroviral durability and tolerability in HIV-infected adults living in urban Kenya. *J Acquir Immune Defic Syndr* 2007; **45:**304–310.

Hernandez Perez E, Dawood H. Stavudine-induced hyperlactatemia/lactic acidosis at a tertiary communicable diseases clinic in South Africa. *J Int Assoc Physicians AIDS Care (Chic)* 2010; **9(2)**:109-12.

Hertel J, Struthers H, Horj CB, Hruz PW. A structural basis for the acute effects of HIV protease inhibitors on GLUT4 intrinsic activity. *J Biol Chem* 2004; **279:55147**–55152.

Hill A. d4T: keep it or abandon it? *Asian Biomedicine* 2010; **4(4)** 541-546.

Hill A, Ruxrungtham K, Hanvanich M, Katlama C, Wolf E, Soriano V, *et al*. Systematic review of clinical trials evaluating low doses of stavudine as part of antiretroviral treatment. *Expert Opin Pharmacother* 2007; **8(5)**:679-688.

Hultman CS, McPhail LE, Donaldson JH, Wohl DA. Surgical management of HIV-associated lipodystrophy: role of ultrasonic-assisted liposuction and suction-assisted lipectomy in the treatment of lipohypertrophy. *Ann Plast Surg* 2007; **58(3)**:255-63.

Idoko JA, Akinsete L, Abalaka AD, Keshinro LB, Dutse L, Onyenekwe B, *et al*. A multicentre study to determine the efficacy and tolerability of a combination of nelfinavir (Viracept), zalcitabine (Hivid) and zidovudine in the treatment of HIV infected Nigerian patients. *West Afr J med* 2002; 21(2):83-6.

Jamisse L, Balkus J, Hitti J, Gloyd S, Manuel R, Osman N, *et al*. Antiretroviral-associated toxicity among HIV-1-seropositive pregnant women in Mozambique receiving nevirapine based regimens. *J Acquir Immune Defic Syndr* 2007; **44(4)**:371-6.

John M, Mallal S. Hyperlactataemia syndromes in people with HIV infection. *Curr Opin Infect Dis* 2002; **15:** 23–29.

John M, Nolan D, Mallal S. Antiretroviral therapy and the lipodystrophy syndrome. *Antivir Ther* 2001; **6(1)**:9-20.

Johnson JA, Albu JB, Engelson ES, Fried SK, Inada Y, Ionescu G, *et al*. Increased systemic and adipose tissue cytokines in patients with HIV-associated lipodystrophy. *Am J Physiol Endocrinol Metab* 2004; **286**:E261-E271.

Johri S, Alkuja S, Siviglia G, Soni A. Steatosis lactic acidosis syndrome associated with stavudine and lamivudine therapy. *AIDS* 2000; **14:** 1286–87.

Jones SP, Qazi N, Morelese J, Lebrecht D, Sutinen J, Yki-Jărvinen H, *et al*. Assessment of adipokine expression and mitochondrial toxicity in HIV patients with lipoatrophy on stavudine- and zidovudine-containing regimens. *J Acquir Immune Defic Syndr* 2005; **40(5)**:565-72.

Kakuda TN.Pharmacology of nucleoside and nucleotide reverse transcriptase inhibitor-induced mitochondrial toxicity. *Clin Ther* 2000; **22(6)**:685-708.

Kaplan RC, Kingsley LA, Sharrett AR, Li X, Lazar J, Tien PC, *et al*. Ten-year predicted coronary heart disease risk in HIV-infected men and women. *Clin Infect Dis* 2007; **45(8)**:1074-81.

Kaleebu P, Ross A, Morgan D, Yirrell D, Oram J, Rutebemberwa A, *et al*. Relationship between HIV-1 Env subtypes A and D and disease progression in a rural Ugandan cohort. *AIDS* 2001; **15(3)**:293-9.

Keswani SC, Pardo CA, Cherry CL, Hoke A, McArthur JC. HIV-associated sensory neuropathies. *AIDS* 2002; **16**:2105-2117.

Kieburtz K, Simpson DM, Yiannoustsos C. A randomized trial of amitriptyline and mexiletene for painful neuropathy in HIV infection: the ACTG 242 study team. *Neurology* 1998; **51:1682**–1688.

Lang S, Mary-Krause M, Simon A, Partisani M, Gilquin J, Cotte L, *et al*. HIV replication and immune status are independent predictors of the risk of myocardial infarction in HIV-infected individuals. *Clin Infect Dis* 2012; **55(4)**:600-7.

Lazzaretti RK, Kuhmmer R, Sprinz E, *et al*. Dietary intervention prevents dyslipidemia associated with highly active antiretroviral therapy in human immunodeficiency virus type 1-infected individuals: a randomized trial. *J Am Coll Cardiol* 2012; **59**:979.

Lenhard JM, Furfine ES, Jain RG, Ittoop O, Orband-Miller LA, Blanchard SG, *et al*. HIV protease inhibitors block adipogenesis and increase lipolysis in vitro. *Antiviral Res* 2000; **47(2)**:121-9.

Lesi OA, Soyebi KS, Eboh CN. Fatty liver and hyperlipidemia in a cohort of HIV-positive Africans on highly active antiretroviral therapy. *J Natl Med Assoc* 2009; **101:**151-155.

Lewis W, Day BJ, Copeland WC. Mitochondrial toxicity of NRTI antiviral drugs: an integrated cellular perspective. *Nat Rev Drug Discov* 2003; **2**:812-22.

Leyes P, Martínez E, Forga Mde T. Use of diet, nutritional supplements and exercise in HIV-infected patients receiving combination antiretroviral therapies: a systematic review. *Antivir Ther* 2008; **13(2)**:149-59.

Lo JC, Mulligan K, Tai VW, Algren H, Schambelan M. "Buffalo hump" in men with HIV-1 infection. *Lancet* 1998; **351(9106)**:867-70.

Luo L, Zhang L, Tao M, Qiu Z, Xie J, Han Y, *et al*. Adiponectin and leptin levels in Chinese patients with HIV-related lipodystrophy: a 30-month prospective study. *AIDS Res Hum Retroviruses* 2009; **25(12)**:1265-72.

Maagaard A, Kvale D. Mitochondrial toxicity in HIV-infected patients both off and on antiretroviral treatment: a continuum or distinct underlying mechanisms? *J Antimicrob Chemother* 2009; **64(5)**:901-9.

Mallal SA, John M, Moore CB, James IR, McKinnon EJ. Contribution of nucleoside analogue reverse transcriptase inhibitors to subcutaneous fat wasting in patients with HIV infection. *AIDS* 2000; **14(10)**:1309-16.

Mallewa JE, Wilkins E, Vilar J, Mallewa M, Doran D, Back D, *et al*. HIV-associated lipodystrophy: a review of underlying mechanisms and therapeutic options. *J Antimicrob Chemother*. 2008; **62(4)**:648–660.

Mallon PW, Miller J, Kovacic JC, Kent-Hughes J, Norris R, Samaras K *et al*. Effect of pravastatin on body composition and markers of cardiovascular disease in HIV-infected men--a randomized, placebo-controlled study. *AIDS* 2006; **20(7)**:1003-10.

Mallon PW, Unemori P, Sedwell R, Morey A, Rafferty M, Williams K, *et al*. In vivo, nucleoside reverse-transcriptase inhibitors alter expression of both mitochondrial and lipid metabolism genes in the absence of depletion of mitochondrial DNA. *J Infect Dis* 2005; **191(10)**:1686-96.

Manuthu E, Joshi M, Lule G, Karari E. Prevalence of dyslipidemia and dysglycaemia in HIVinfected patients. *East Afr Med J* 2008; **85(1):**10-7.

Martin I, Vinas O, Mampel T, Iglesias R, Villarroya F. Effects of cold environment on mitochondrial genome expression in the rat: evidence for a tissue-specific increase in the liver, independent of changes in mitochondrial gene abundance. *Biochem J*1993; **296**:231-4.

Mary-Krause M, Cotte L, Simon A, Partisani M, Costagliola D; Clinical Epidemiology Group from the French Hospital Database. Increased risk of myocardial infarction with duration of protease inhibitor therapy in HIV-infected men. *AIDS* 2003; **17(17)**:2479-86.

McArthur JC, Brew BJ, Nath A. Neurological complications of HIV infection. *Lancet Neurol* 2005; **4**:543-555.

Mehta SH, Moore RD, Thomas DL, Chaisson RE, Sulkowski MS. The effect of HAART and HCV infection on the development of hyperglycemia among HIV-infected persons. *J Acquir Immune Defic Syndr* 2003; **33(5)**:577-84.

Mercier S, Gueye NF, Cournil A, Fontbonne A, Copin N, Ndiaye I, *et al*. Lipodystrophy and metabolic disorders in HIV-1–infected adults on 4- to 9-year antiretroviral therapy in Senegal: A Case–Control Study. *J Acquir Immune Defic Syndr* 2009; **51:**224–230.

McComsey GA, LoRe III V, O'Riordan M, Walker UA, Lebrecht D, Baron E, *et al*. Effect of Reducing the Dose of Stavudine on Body Composition, Bone Density and Markers of Mitochondrial Toxicity in HIV infected Subjects- a Randomized, Controlled study. *Clin Infect Dis*. 2008; **46(8)**: 1290–1296.

Meininger G, Hadigan C, Rietschel P, Grinspoon S. Body-composition measurements as predictors of glucose and insulin abnormalities in HIV-positive men. *Am J Clin Nutr* 2002; **76(2)**:460-5.

Menezes CN, Duarte R, Dickens C, Dix-Peek T, Van Amsterdam D, John M, *et al* . The early effects of stavudine compared to tenofovir on adipocyte gene expression, mitochondrial DNA copy number and metabolic parameters in South African HIV-infected patients: A randomized trial. *HIV Med* 2013;**14(4)**:217-25.

Mercier S, Gueye NF, Cournil A, Fontbonne A, Copin N, Ndiaye I, *et al*. Lipodystrophy and metabolic disorders in HIV-1–infected adults on 4- to 9-year antiretroviral therapy in Senegal: A Case–Control Study. *J Acquir Immune Defic Syndr* 2009; **51**:224–230.

Milinkovic A, Martinez E, López S, de Lazzari E, Miró O, Vidal S, *et al.* The impact of reducing stavudine dose versus switching to tenofovir on plasma lipids, body composition and mitochondrial function in HIV-infected patients. *Antivir Ther* 2007; **12(3)**:407-15.

Minzi, OMs, Irunde H, Moshiro C. HIV patients presenting common adverse drug events caused by highly active antiretroviral therapy in Tanzania. *Tanzan J Health Res* 2009; **11(10)**:5-10.

Miró O, López S, Cardellach F, Casademont J. Mitochondrial studies in HAART-related lipodystrophy: from experimental hypothesis to clinical findings. *Antivir Ther* 2005; **10**:M73-81.

Miró O, López S, Martínez E, Pedrol E, Milinkovic A, Deig E, et al. Mitochondrial effects of HIV infection on the peripheral blood mononuclear cells of HIV-infected patients who were never treated with antiretrovirals. *Clin Infect Dis* 2004; **39(5)**:710-6.

Montaner JS, Côté HC, Harris M, Hogg RS, Yip B, Harrigan PR, O'Shaughnessy MV. Nucleoside-related mitochondrial toxicity among HIV-infected patients receiving antiretroviral therapy: insights from the evaluation of venous lactic acid and peripheral blood mitochondrial DNA. *Clin Infect Dis* 2004; **38(2)**:S73-9.

Moyle G. Toxicity of antiretroviral nucleoside and nucleotide analogues: is mitochondrial toxicity the only mechanism? *Drug Saf* 2000; **23**: 467–81.

Moyle GJ, Sabin CA, Cartledge J, Johnson M, Wilkins E, Churchill D *et al*. A randomized comparative trial of tenofovir DF or abacavir as replacement for a thymidine analogue in persons with lipoatrophy. *AIDS* 2006; **20(16)**:2043-50.

Mu H, Chai H, Lin PH, Yao Q, Chen C. Current update on HIV-associated vascular disease and endothelial dysfunction. *World J Surg* 2007; **31(4)**:632-43.

Mulligan K, Grunfeld C, Tai VW, Algren H, Pang M, Chernoff DN, *et al* . Hyperlipidemia and insulin resistance are induced by protease inhibitors independent of changes in body composition in patients with HIV infection. *J Acquir Immune Defic Syndr* 2000; **23(1)**:35-43.

Murata H, Hruz PW, Mueckler M. The mechanism of insulin resistance caused by HIV protease inhibitor therapy. *J Biol Chem* 2000; **275:20251**–20254.

Mutimura E, Crowther NJ, Cade TW, Yarasheski KE, Stewart A. Exercise training reduces central adiposity and improves metabolic indices in HAART-treated HIV-positive subjects in Rwanda: a randomized controlled trial. *AIDS Res Hum Retroviruses* 2008; **24(1)**:15-23.

Mutimura E, Stewart A, Rheeder P, Crowther NJ. Metabolic function and the prevalence of lipodystrophy in a population of HIV-infected African subjects receiving highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* 2007; **46**:451-5.

National Department of Health. Clinical guidelines for the Management of HIV & AIDS in adults and adolescents, South Africa 2010: http://www.health-e.org.za/documents/0ba405d9042e557a9fb1987adb68cb97.pdf. Last accessed on the 31/03/2013.

National Department of Health. National Antiretroviral Treatment Guidelines, First Edition 2004: http://www.doh.gov.za/docs/factsheets/guidelines/artguide04-f.html. Last accessed on the 31/03/2013.

Nolan D, Hammond E, Martin A, Taylor L, Herrmann S, McKinnon E, *et al*. Mitochondrial DNA depletion and morphologic changes in adipocytes associated with nucleoside reverse transcriptase inhibitor therapy. *AIDS* 2003; **17(9)**:1329-38.

Noursadeghi M, Miller RF. Clinical value of C-reactive protein measurements in HIV-positive patients. *Int J STD AIDS*. 2005; **16(6)**:438-41.

Oh J, Hegele RA. HIV-associated dyslipidaemia: pathogenesis and treatment. *Lancet Infect Dis* 2007; **7(12)**:787-96.

Pace CS, Martin AM, Hammond EL, Mamotte CD, Nolan DA, Mallal SA. Mitochondrial proliferation, DNA depletion and adipocyte differentiation in subcutaneous adipose tissue of HIV-positive HAART recipients. *Antivir Ther* 2003; **8**:323-331.

Pai JK, Pischon T, Ma J, Manson JE, Hankinson SE, Joshipura K *et al*. Inflammatory markers and the risk of coronary heart disease in men and women. *N Engl J Med* 2004; **351**:2599–610.

Paik IJ, Kotler DP. The prevalence and pathogenesis of diabetes mellitus in treated HIV-infection. *Best Pract Res Clin Endocrinol Metab* 2011; **25**: 469-478.

Palios J, Kadoglou NP, Lampropoulos S. The pathophysiology of HIV-HAART-related metabolic syndrome leading to cardiovascular disorders: the emerging role of adipokines. *Exp Diabetes Res* 2012; 103063. doi: 10.1155/2012/103063.

Palmer S, Margot N, Gilbert H, Shaw N, Buckheit RJ, Miller M. Tenofovir, adefovir, and zidovudine susceptibilities of primary human immunodeficiency virus type 1 isolates with non-B subtypes or nucleoside resistance. *AIDS Res Hum Retroviruses* 2001; **17**:1167-1173.

Pan-Zhou XR, Cui L, Zhou XJ, Sommadossi JP, Darley-Usmar VM. Differential effects of antiretroviral nucleoside analogues on mitochondrial function in HepG2 cells. *Antimicrob Agents Chemother* 2000; **44:** 496–503.

Perno CF, Cozzi-Lepri A, Balotta C, Forbici F, Violin M, Bertoli A, *et al*. Secondary mutations in the protease region of human immunodeficiency virus and virologic failure in drug-naivepatients treated with protease inhibitor-based therapy. *J Infect Dis* 2001; **184(8)**:983-91.

Penzak SR, Chuck SK. Hyperlipidemia associated with HIV protease inhibitor use: pathophysiology, prevalence, risk factors and treatment. *Scand J Infect Dis* 2000; **32:** 111–23.

Pinti M, Salomoni P, Cossarizza A. Anti-HIV drugs and the mitochondria. *Biochim Biophys Acta* 2006; **1757(5-6)**:700-7.

Podzamczer D, Ferrer E, Sanchez P, Gatell JM, Crespo M, Fisac C, *et al*. Less lipoatrophy and better lipid profile with abacavir as compared to stavudine: 96-week results of a randomized study. *J Acquir Immune Defic Syndr* 2007; **44(2)**:139-47.

Puigserver P, Spiegelman BM. Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. *Endocr Rev* 2003; **24(4)**:78-90.

Raboud JM, Diong C, Carr A, Grinspoon S, Mulligan K, Sutinen J *et al*. A meta-analysis of six placebo-controlled trials of thiazolidinedione therapy for HIV lipoatrophy. *HIV Clin Trials* 2010; **11(1)**:39-50.

Reingold J, Wanke C, Kotler D, Lewis C, Tracy R, Heymsfield S, *et al*. Association of HIV infection and HIV/HCV coinfection with C-reactive protein levels: the fat redistribution and metabolic change in HIV infection (FRAM) study. *J Acquir Immune Defic Syndr* 2008; **48(2)**:142-8.

Ribera E, Paradinero J, Domingo P, Sauleda S, Luque S, Garcia-Arumi E, *et al*. A randomized study comparing the efficacy and tolerability of low-dose versus standard-dose stavudine in antiretroviral-naive patients (ETOX study). Presented at 3rd International Conference on AIDS Pathogenesis, Rio de Janiero, Brazil, July 2005 [abstr TuPe2.4c10].

Rosen ED, MacDougald OA. Adipocyte differentiation from the inside out. *Nat Rev Mol Cell Biol* 2006; **7**:885-96.

Rutter MK, Meigs JB, Sullivan LM, D'Agostino RB, Wilson PW. C-reactive protein, the metabolic syndrome, and prediction of cardiovascular events in the Framingham Offspring study. *Circulation* 2004; **110**:380–5.

Ruxrungtham K, Kroon E, Ungsedhapand C, Teeratakulpisarn S, Ubolyam S, Buranapraditkun S, *et al*. A randomised, dose-finding study with didanosine plus stavudine versus didanosine alone in antiviral-naive, HIV-infected Thai patients. *AIDS* 2000; **14**:1375 82.

Sacktor N, Nakasujja N, Skolasky RL, Robertson K, Musisi S, Ronald A, *et al*. Benefits and risks of stavudine therapy for HIV-associated neurologic complications in Uganda. *Neurology* 2009; **72**:165–170.

Salami A, Akande A, Olokoba A. Serum lipids and glucose abnormalities in HIV/AIDS patients on antiretroviral therapies. *West Afr J Med* 2009; **28(1):**10-5.

Salehian B, Bilas J, Bazargan M, Abbasian M. Prevalence and incidence of diabetes in HIV-infected minority patients on protease inhibitors. *J Natl Med Assoc* 2005; **97(8)**:1088-92.

Samaras K. Prevalence and pathogenesis of diabetes mellitus in HIV-1 infection treated with combined antiretroviral therapy. *J Acquir Immune Defic Syndr* 2009; **50(5)**:499–505.

Sankalé JL, Tong Q, Hadigan CM, Tan G, Grinspoon SK, Kanki PJ, *et al*. Regulation of adiponectin in adipocytes upon exposure to HIV-1. *HIV Med* 2006; **7(4)**:268–274.

Sanne I, Westreich D, Macphail AP, Rubel A, Majuba P, Van Rie A. Long term outcomes of antiretroviral therapy in a large HIV/AIDS care clinic in urban South Africa: a prospective cohort study. *J Acquir Immune Defic Syndr* 2009; **12**:753-760.

Savès M, Raffi F, Capeau J, Rozenbaum W, Ragnaud JM, Perronne C, *et al*. Factors related to lipodystrophy and metabolic alterations in patients with human immunodeficiency virus infection receiving highly active antiretroviral therapy. *Clin Infect Dis* 2002; **34(10)**:1396-405.

Schambelan M, Benson CA, Carr A, Currier JS, Dubé MP, Gerber JG, *et al*. Management of metabolic complications associated with antiretroviral therapy for HIV-1 infection: recommendations of an International AIDS Society-USA panel. *J Acquir Immune Defic Syndr*. 2002; **31**(3): 257-275.

Schowalter L, Conradie F. Approaches to tenofovir and abacavir drug shortages in South Africa: A guide for clinicians. *SAJHIVMED* 2012; **13(2)**:56-7

Shafer RW, Eisen JA, Merigan TC, Katzenstein DA. Sequence and drug susceptibility of subtype C reverse transcriptase from human immunodeficiency virus type 1 seroconverters in Zimbabwe. *J Virol* 1997; **71:5441**-5448.

Shurie JS, Deribew A. Assessment of the prevalence of distal symmetrical polyneuropathy and its risk factors among HAART-treated and untreated HIV infected individuals. *Ethiop Med J* 2010; **48(2)**:85-93.

Siangphoe U, Srikaew S, Waiwavaravuth C, Chariyasetphong T, Kamsaw O, Klinbua V, *et al*. Efficacy and safety of half dose compared to full dose stavudine (d4T) and zidovudine (AZT) in combination with didanosine (ddI) in Thai HIV-infected patients: 96-week results of ACTT002/ARV065 study. Presented at World AIDS Conference, Bangkok, July 2004 [abstr WePeB5952].

Sievers M, Walker UA, Sevastianova K, *et al*. Gene Expression and Immunohistochemistry in Adipose Tissue of HIV Type 1-Infected Patients with Nucleoside Analogues Reverse-Transcriptase Inhibitor-Associated Lipoatrophy. *JID* 2009; **200**:252-62.

Simpson DM, Tagliati N. Nucleoside analogue-associated peripheral neuropathy in human immunodeficiency virus infection. *J AcquirImmune Defic Syndr Hum Retroviral.*1995; **9**:153–161.

Simpson DM, Tagliati M, Ramcharitar S. Neurologic complications of AIDS: new concepts and treatments. *Mount Sinai J Med*. 1994; **61**:484–491.

Sinxadi PZ, van der Walt JS, McIlleron, Badri M, Smith PJ, Dave JA, *et al*. Lack of association between stavudine exposure and lipoatrophy, dysglycaemia, hyperlactataemia and hypertriglyceridaemia: a prospective cross sectional study. *AIDS Res Ther* 2010; **7**:23.

Smith CC, Yellon DM. Adipocytokines, cardiovascular pathophysiology and myocardial protection. *Pharmacol Ther* 2011; **129(2)**:206-19.

Southern African HIV Clinicians Society. Guidelines for Antiretroviral Therapy in Adults, September 2012: http://www.sahivsoc.org/upload/documents/Guidelines%20for%20antiretroviral%20treatment%20therapy%20in%20adults,%20September%202012.pdf. Last accessed on the 01/02/2013.

Spiegelman BM, Puigserver P, Wu Z. Regulation of adipogenesis and energy balance by PPARgamma and PGC-1. *Int J Obes Relat Metab Disord* 2000; **24**:S8-10.

Stebbing J, Asghar AK, Holmes P, Bower M, Isenman HL, Nelson M. Use of ezetimibe during HIV infection. *J Antimicrob Chemother* 2009; **63(1)**:218-20.

Stein JH, Hadigan CM, Brown TT *et al*. Prevention strategies for cardiovascular disease in HIV-infected patients. *Circulation* 2008; **118(2)**:e54.

Strauch B, Baum T, Robbins N. Treatment of human innunodeficiency virus-associated lipodystrophy with dermafat graft transfer to the malar area. *Plast Reconstr Surg* 2004; **113(1)**:363-70.

Strategies for Management of Antiretroviral Therapy (SMART) Study Group, El-Sadr WM, Lundgren JD, Neaton JD, Gordin F, Abrams D, *et al*. CD4+ count-guided interruption of antiretroviral treatment. *N Engl J Med* 2006; **355(22)**:2283-96.

Strategies for Management of Anti-Retroviral Therapy/INSIGHT; DAD Study Groups. Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients. *AIDS* 2008; **22(14)**:F17-24.

Sutinen J, Walker UA, Sevastianova K, Klinker H, Häkkinen AM, Ristola M, Yki-Järvinen H. Uridine supplementation for the treatment of antiretroviral therapy-associated lipoatrophy: a randomized, double-blind, placebo-controlled trial. *Antivir Ther*. 2007; **12(1)**:97-105.

The 2012 SEMDSA Guideline for the Management of Type 2 diabetes. *JEMDSA* 2012; **17(2)**: S7-S9.

Thiébaut R, Dabis F, Malvy D, Jacqmin-Gadda H, Mercié P, ValentinVD. Serum triglycerides, HIV infection, and highly active antiretroviral therapy, Aquitaine Cohort, France, 1996 to 1998. Groupe d'Epidémiologie Clinique du Sida en Aquitaine (GECSA). *J Acquir Immune Defic Syndr* 2000; **23(3)**:261-5.

Tien PC, Schneider MF, Cole SR, Levine AM, Cohen M, DeHovitz J, *et al*. Antiretroviral therapy exposure and insulin resistance in the Women's Interagency HIV study. *J Acquir Immune Defic Syndr* 2008; **49(4)**:369-76.

Tong Q, Sankalé JL, Hadigan CM, Tan G, Rosenberg ES, Kanki PJ, *et al*. Regulation of adiponectin in human immunodeficiency virus-infected patients: relationship to body composition and metabolic indices. *J Clin Endocrinol Metab* 2003; **88(4)**:1559–1564.

Triant VA, Meigs JB, Grinspoon SK. Association of C-reactive protein and HIV infection with acute myocardial infarction. *J Acquir Immune Defic Syndr* 2009; **51(3)**:268-73.

UNAIDS World AIDS Day Report – Results. 2012: http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2012/gr2012/JC2434_WorldAIDSday_results_en.pdf. Last accessed on the 05/03/2013.

van den Berg JB, Hak E, Vervoort SC, Hoepelman IM, Boucher CA, Schuurman R, *et al*. Increased risk of early virological failure in non-European HIV-1-infected patients in a Dutch cohort on highly active antiretroviral therapy. *HIV Med* 2005; **6(5)**:299-306.

van Griensven J, Zachariah R, Rasschaert F, Mugabo J, Atte EF, Reid T. Stavudine- and nevirapine-related drug toxicity while on generic fixed-dose antiretroviral treatment: incidence, timing and risk factors in a three-year cohort in Kigali, Rwanda. *Trans R Soc Trop Med Hyg* 2010; **104**:148-53.

Velazquez-Campoy A, Todd MJ, Vega S, Freire E. Catalytic efficiency and vitality of HIV-1 proteases from African viral subtypes. *Proc Natl Acad Sci USA* 2001; **98**: 6062–6067.

Vigouroux C, Gharakhanian S, Salhi Y, Nguyen TH, Chevenne D, Capeau J, *et al*. Diabetes, insulin resistance and dyslipidaemia in lipodystrophic HIV-infected patients on highly active antiretroviral therapy (HAART). *Diabetes Metab* 1999; **25(3)**:225-32.

Villarroya F, Domingo P, Giralt M. Mechanisms of antiretroviral-induced mitochondrial dysfunction in adipocytes and adipose tissue: in-vitro, animal and human adipose tissue studies. *Curr Opin HIV AIDS* 2007; **2**:261–267

Warren AG, Borud LJ. Excisional lipectomy for HIV-associated cervicodorsal lipodystropy. *Aesthet Surg J* 2008; **28(2)**: 147-52.

Wester CW, Kim S, Bussman H, Avalos A, Ndwapi N, Peter TF, *et al*. Initial response to highly active antiretroviral therapy in HIV-1C infected adults in a public sector treatment program in Botswana. *J Acquir Immune Defic Syndr* 2005; **40**:336-343.

WHO: Addendum to the 2006 WHO guidelines on antiretroviral therapy for HIV infection in adults and adolescents. http://www.who.int/hiv/art/ARTadultsaddendum.pdf. Last accessed on the 09/03/2013.

WHO: Antiretroviral therapy for HIV infection in adults and adolescents: Recommendations for a public health approach (2010 version).

http://whqlibdoc.who.int/publications/2010/9789241599764_eng.pdf. Last accessed on the 09/03/2013.

WHO. Forecasting antiretroviral demand. 2010. http://www.who.int/hiv/amds/forecasting/en/index4.html. Last accessed on the 28/10/2012.

WHO. Global HIV/AIDS response. Epidemic update and health sector progress towards Universal Access. Progress report 2011: http://whqlibdoc.who.int/publications/2011/9789241502986_eng.pdf. Last accessed on the 21/01/2013.

WHO. The strategic use of antiretrovirals to help end the HIV epidemic. (2012): Available from: http://apps.who.int/iris/bitstream/10665/75184/1/9789241503921_eng.pdf. Last accessed on the 17/1/2013.

WHO. Use of glycated haemoglobin (HbA1c) in the diagnosis of diabetes mellitus. (2011): Available from: http://www.who.int/diabetes/publications/report-hba1c_2011.pdf . Last accessed on the 16/4/2013.

Wolfort FG, Cetrulo CL Jr, Nevarre DR. Suction-assisted lipectomy for lipodystrophy syndromes attributed to HIV-protease inhibitor use. *Plast Reconstr Surg* 1999; **104(6)**:1814-20.

Worm SW, Sabin C, Weber R, Reiss P, El-Sadr W, Dabis F, *et al*. Risk of myocardial infarction in patients with HIV infection exposed to specific individual antiretroviral drugs from the 3 major drug classes: the data collection on adverse events of anti-HIV drugs (D: A: D) study. *J Infect Dis* 2010; **201(3)**:318-30.

Yoon C, Gulick RM, Hoover DR, Vaamonde CM, Glesby MJ. Case-control study of diabetes mellitus in HIV-infected patients. *J Acquir Immune Defic Syndr* 2004; **37(4)**:1464-9.

Young J, Rickenbach M, Weber R, Furrer H, Bernasconi E, Hirschel B, *et al*. Body fat changes among antiretroviral-naive patients on PI- and NNRTI-based HAART in the Swiss HIV cohort study. *Antivir Ther* 2005; **10(1)**:73-81.

Zhang B, MacNaul K, Szalkowski D, Li Z, Berger J, Moller DE. Inhibition of adipocyte differentiation by HIV protease inhibitors. *J Clin Endocrinol Metab* 1999; **84(11)**:4274-7.

Zinn RJ, Serrurier C, Takuva S, Sanne I, Menezes CN. HIV-associated lipodystrophy in South Africa: The impact on the patient and the impact on the plastic surgeon. *J Plast Reconstr Aesthet Surg* 2013 Mar 29. doi: pii: S1748-6815(13)00122-8. 10.1016/j.bjps.2013.02.032. [Epub ahead of print]

Zhou L, Kitch DW, Evans SR, Hauer P, Raman S, Ebenezer GJ *et al*. Correlates of epidermal nerve fiber densities in HIV-associated distal sensory polyneuropathy. Neurology 2007; **68**:2113-2119.

## CHAPTER 2

# 2. A LONGITUDINAL STUDY OF STAVUDINE-ASSOCIATED TOXICITIES IN A LARGE COHORT OF SOUTH AFRICAN HIV INFECTED SUBJECTS.

## 2.1. Abstract

**Background:**

There has been a major improvement in the survival of HIV-1 infected individuals since the South African Government introduced highly active anti-retroviral therapy (HAART) in the public sector in 2004. This has brought new challenges which include the effects of stavudine-related toxicities.

**Methods:**

Prospective analysis of a cohort of 9040 HIV-infected adults who were initiated on HAART at the Themba Lethu Clinic (TLC) in Johannesburg between April 1, 2004 to December 31, 2007, and followed up until June 30, 2008.

**Results:**

Amongst the 9040 study subjects, 8497(94%) were on stavudine based therapy and 5962 (66%) were women. The median baseline CD4 count was 81 cells/$mm^3$ (IQR 29-149). Median follow up on HAART was 19 months (IQR: 9.1-31.6). The proportion of HAART-related side effects for stavudine compared to non-stavudine containing regimens were, respectively: peripheral neuropathy,17.1% vs. 11.2% ($p< 0.001$); symptomatic hyperlactataemia, 5.7% vs. 2.2% ($p< 0.0005$); lactic acidosis, 2.5 vs. 1.3% ($p=0.072$); lipoatrophy, 7.3% vs. 4.6% ($p<0.05$). Among those on stavudine-based regimens, incidence rates for peripheral neuropathy were 12.1 cases/100 person-years (95%CI 7.0-19.5), symptomatic hyperlactataemia 3.6 cases/100 person-years (95%CI 1.2-7.5), lactic acidosis

1.6 cases/100 person-years (95%CI 0.4-5.2) and lipoatrophy 4.6 cases/100 person-years (95%CI 2.1-9.6). Females experienced more toxicity when compared to males in terms of symptomatic hyperlactataemia ($p< 0.0001$), lactic acidosis ($p< 0.0001$), lipoatrophy ($p< 0.0001$) and hypertension ($p< 0.05$).

**Conclusions:**

We demonstrate significant morbidity associated with stavudine. These data support the latest WHO guidelines, and provide additional evidence for other resource limited HAART rollout programs considering the implementation of non-stavudine based regimens as first line therapy.

## 2.2. Introduction

By the end of 2008, an estimated 33.4 million people worldwide were living with human immunodeficiency virus (HIV) infection (UNAIDS, 2009). Sub-Saharan Africa continues to bear a disproportionate share of the global burden of HIV with 67% of the HIV infections worldwide, of which 68% of them were amongst adults. This region also accounted for 72% of the world's AIDS related deaths (UNAIDS, 2009). South Africa's 2009 HIV prevalence rate in the adult population (aged 15-49 years) was estimated to be 17.8% (Department of Health, 2010).

There has been an increase in the provision of highly active antiretroviral therapy (HAART), with up to 44% of adults and children estimated to be receiving therapy with a profound reduction in mortality (Department of Health, 2010) and, with adherence to HAART it is possible to transform HIV from a fatal infection to a chronic and manageable illness (Hogg *et al*, 1997. Palella *et al*, 1998. Wit *et al*, 1999).

However, some of the anti-retroviral agents used in HAART regimens have severe side effects. Prominent amongst these drugs is stavudine, the use of which is associated with lactic acidosis /symptomatic hyperlactataemia, lipoatrophy, and peripheral neuropathy (Boulle *et al*, 2007). Other side effects of stavudine use include dyslipidaemia and insulin resistance, and it is an independent risk factor for the development of new onset diabetes mellitus (De Wit *et al*, 2008). Despite these serious side effects up to 60% of HIV-positive patients in low and middle income countries are receiving stavudine (Beck *et al*, 2006. Renaud-Théry *et al*, 2007).

The aim of this study was to make use of a large (N=9040) clinic-based survey of HIV-positive patients newly initiated onto HAART over a period of three years, to compare and describe the effect of stavudine based therapy on acute and chronic toxicities after initiation of HAART. We present baseline data gathered before the initiation of HAART and data collected for three years with each patient having a minimum of six months of follow-up. This is the largest study of the side effects of stavudine therapy conducted to date in a South African HIV-positive population.

## 2.3. Methods

### 2.3.1. Study population

The study population included HIV-1 infected individuals attending the Themba Lethu Clinic, a public sector HAART rollout facility based at the Helen Joseph Hospital, a teaching hospital attached to the University of the Witwatersrand, Johannesburg, South Africa. This clinic provides free antiretroviral therapy and other specialized services, and is one of the largest HAART rollout clinics in Africa. The program is funded by the South African

National and Gauteng Departments of Health, with support from Right to Care funded by USAID and PEPFAR.

### 2.3.2. Treatment

HAART was initiated in accordance with the 2004 South African National Antiretroviral Treatment Guidelines, which include initiation criteria of a CD4 count $\leq 200$ cells/mm$^3$ or WHO stage 4 AIDS defining illness irrespective of CD4 count (National Department of Health, 2004). The first line therapy consisted of stavudine, lamuvidine and efavirenz or nevirapine; however kaletra (ritonavir / lopinavir) was used as part of the first line therapy regimen if there were contra-indications to other first line drugs (National Department of Health, 2004). Until October 2007, stavudine was dosed according to patients' body weight: 30mg for those <60kg and 40mg for those $\geq$60kg. From October 2007, a universal 30mg dose was introduced and 40mg tablets of stavudine were withdrawn from the clinic. Single drug substitutions were permitted depending on the underlying clinical presentation of the patient.

### 2.3.3. Clinical and laboratory measurements

Patients who met the criteria for initiation of HAART received adherence counseling and screening for opportunistic infections prior to initiation of therapy. A history and physical examination was performed at every visit. All patients had a baseline chest X-ray. Laboratory monitoring was performed according to the clinic protocol, these included a CD4 count, a viral load, a full blood count (FBC), a creatinine and an alanine aminotransferase (ALT). Other serum biochemical tests were carried out as clinically indicated. Lipid levels and glucose where checked in any patient who presented with features suggestive of the lipodystrophy syndrome and diabetes. The results of these tests were not available for

analysis as the majority of patients were seen and diagnosed at other clinics where they would present for acute medical problems, and where their HAART regimen was modified. However, their diagnoses and new HAART regimens were captured for this study.

### 2.3.4. Diagnosis and definitions

Body mass index (BMI) was defined as body weight divided by the height, squared ($kg/m^2$). Patients who presented with symptoms of numbness or dysesthesia after initiation of HAART were defined as having peripheral neuropathy due to HAART, once other causes were excluded. Symptomatic hyperlactataemia was defined as the presence of suggestive symptoms with an uncuffed venous lactate level > 5 mmol/L with no evidence of a metabolic acidosis; and lactic acidosis was defined as an uncuffed lactate > 5 mmol/l and arterial pH < 7.35 or a total venous $CO_2$ < 20mmol/l, with other causes such as sepsis, renal failure, diabetic ketoacidosis and dehydration excluded. As a result, milder cases of hyperlactataemia were excluded by this definition. Pancreatitis was defined as the presence of abdominal symptoms with a serum amylase >125 U/L and lipase >60 U/L. The definition of lipoatrophy was based on the development of peripheral fat wasting (face, arms, buttocks or thighs) and/or central abdominal fat accumulation, and may include enlarged breasts. This was usually reported by the patient and confirmed by the doctor or, initially diagnosed by the doctor with patient confirmation. Hypertension was defined by the presence of three separate readings of systolic blood pressure > 140 mmHg and a diastolic blood pressure >90 mmHg. Diabetes was defined as the presence of symptoms with a fasting glucose of >= 7 mmol/L or a random blood glucose of > 11.1mmol/L. Dyslipidaemias were defined by the presence of abnormal lipid levels which included an elevated total cholesterol of > 5mmol/L, an elevated

triglyceride level of > 1.7mmol/L, and elevated LDL level of >3mmol/L. Only cases that were severe enough to warrant a regimen change were noted with certain toxicities.

### 2.3.5. Data collection and statistical analysis

We analyzed prospectively collected longitudinal cohort data from patients attending the clinic. Clinical data from patient records were captured onto an electronic database via a medical management software system, Therapy Edge-HIV$^{TM}$ (Associated Biological Systems, South Africa). Data was analyzed using the SAS® 9.1 statistical software package (SAS Institute, Inc., North Carolina, USA). Baseline characteristics of the study sample were summarized using simple proportions and medians with interquartile ranges. Differences in proportions of the toxicities were compared by initiating regimen with Chi-squared tests. Study subjects were followed from HAART initiation to the earliest of 1) death; 2) loss to follow up; 3) development of toxicity or 4) censor date 31 December 2008. Incidence rates with 95% confidence intervals for toxicity were calculated and compared by initiating regimen type (stavudine-based versus other). Crude and adjusted estimates of the effect of stavudine use on development of incident toxicity were estimated using Cox proportional hazard models. Models were controlled for confounding by baseline body mass index, CD4 count, age, and gender. Though the majority of subjects initiated HAART prior to the introduction of the universal 30mg stavudine dose, models were also adjusted for time period in which HAART was initiated (prior to or post October 2007). Kaplan Meier curves were used to estimate crude time to diagnosis of therapy-related complications stratified by initiated regimen. Subjects with existing toxicity at initiation of HAART were excluded from these analyses, including a peripheral neuropathy, of which the exact number is unavailable.

Use of the data for the study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand.

## 2.4. Results

Between 1 April 2004 and 1 July 2008, a total of 15,928 HIV-infected adults enrolled in care at the Themba Lethu Clinic. Of these, 5104 (32%) had early stage HIV infection and did not qualify for HAART, whilst the remaining 10,824 (68%) subjects were initiated on HAART. The study sample included the 9040 patients initiated on treatment between 1 April 2004 and 31 December 2007.The cohort profile is summarized in Figure 1.

### 2.4.1. Baseline characteristics of the study population

The baseline characteristics of the study cohort are summarized in Table 1. Two thirds of the study group was female. The majority of patients were in the age group 25-44 years. Even though 46.9% of the total study population were defined as being at only WHO Stage 1 for AIDS, they were already on HAART, indicating that their CD4 count rates were below 200 cells/$mm^3$ and this was confirmed by the median baseline CD4 count of 81 cells/$mm^3$ (IQR: 29-149). All patients in this study cohort were HAART naive at baseline. Ninety four percent of patients were on stavudine based regimens at baseline, with 79% of these initiated on stavudine, lamivudine and efavirenz as per the 2004 South African National guidelines (National Department of Health, 2004), while a smaller number were initiated on zidovudine (3.4%), this was because of contraindications to use of stavudine – particularly peripheral neuropathy. Less than one percent were initiated on a tenofovir-based regimen because of either anaemia or a peripheral neuropathy. Patients with a pre-existing peripheral neuropathy were not included in the data analysis.

### 2.4.2. Retention in care

The median time to follow up for this cohort on HAART was 19 months (IQR: 9.1-31.6). At the end of the study period, a total of 6415 patients (71%) were still alive and in care, 469 were confirmed deceased and a further 2156 were considered lost to follow up. Patients were considered lost to follow up if they missed their last scheduled appointment by more than 90 days or at least 180 days had lapsed since their last visit. It can be assumed that some of the patients who are lost to follow up may have died and so in total, there were 2,625 (16.5%) patients in this cohort who were either lost to follow up or dead by the end of the study period (see Figure 1).

### 2.4.3. Response to HAART

The patients had a median baseline CD4 count of 81 cells/ $mm^3$ (IQR 29-149). There was a significant ($p<0.0001$) increase in the CD4 count after initiation with a median of 205 cells/$mm^3$ (IQR 132-293) by 6 months on treatment. After six months of therapy, there was no significant change in BMI (means ± SD; 22.4 ± 5.0 and 23.7 ± 5.5 respectively).

## 2.5. Acute and chronic toxicities

Amongst the 9040 patients on therapy, 2488 patients (27.5%) had one or more incident toxicities recorded after treatment initiation and this lead to a drug switch. In terms of acute HAART-related toxicities, the proportion of patients diagnosed with peripheral neuropathy was significantly higher in the group receiving stavudine-based therapy (17.1% vs. 11.2%; $p<0.001$) compared to those on non-stavudine based therapy (Table 2).

**Figure 1. Profile of the Study Cohort**

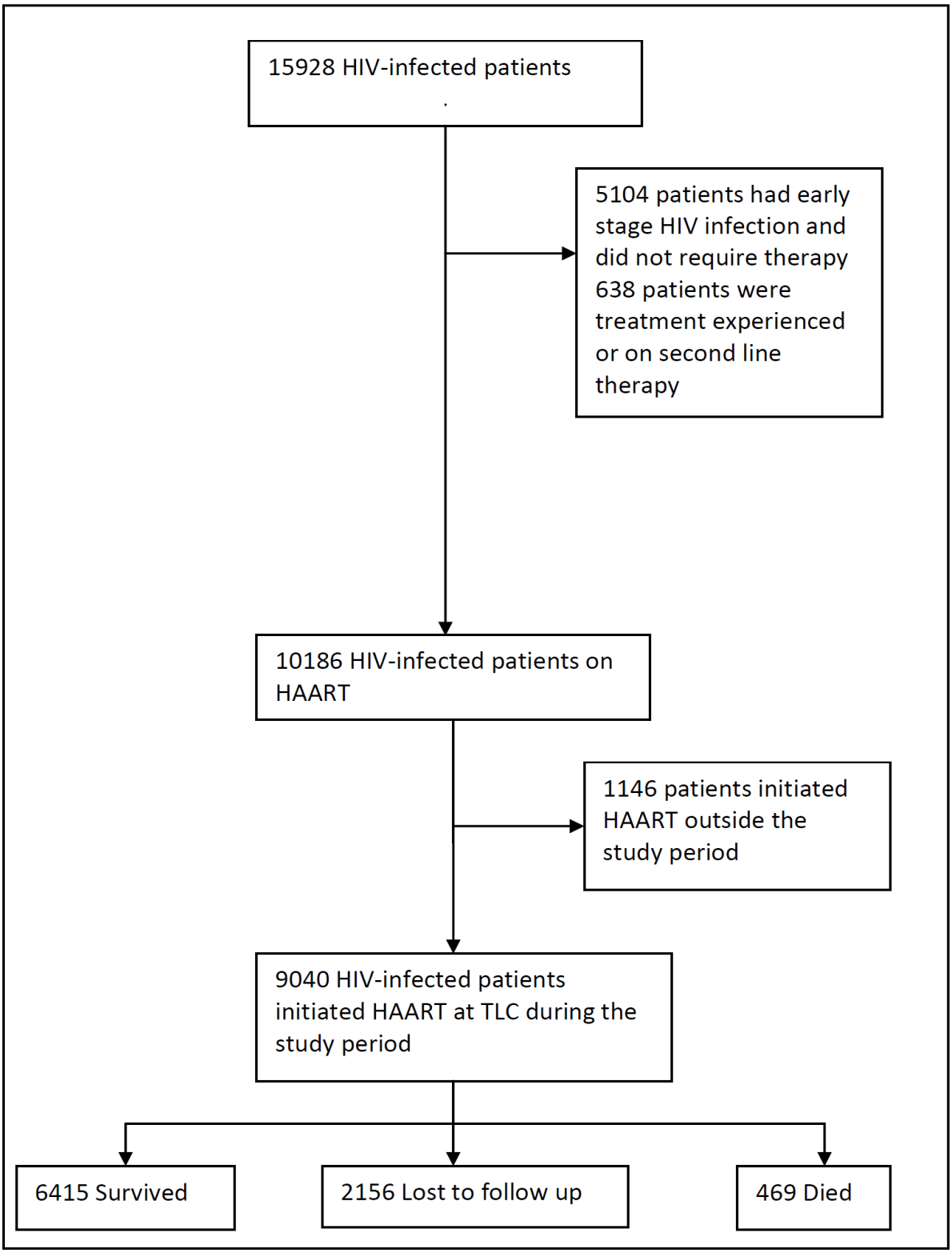


**Table 1. Cohort characteristics at baseline**

| Characteristic | Baseline value |
|---|---|
| **Gender** | **(N= 9040)** |
| Female | 5962 (66) |
| Male | 3078 (34) |
| **Age (years)** | **(N=9040)** |
| <25 years | 551 (6.1) |
| 25-34 years | 3822 (42.3) |
| 35-44 years | 3216 (35.5) |
| 45-54 years | 1173 (13.0) |
| >55 years | 277 (3.1) |
| **WHO AIDS classification** | **(N=8714)** |
| Stage 1 | 4086 (46.9) |
| Stage 2 | 1310 (15) |
| Stage 3 | 2507 (28.8) |
| Stage 4 | 811 (9.3) |
| **HAART** | **(N = 9040)** |
| d4T/3TC/EFV | 7138 (79) |
| d4T/3TC/NVP | 690 (7.6) |
| d4T/3TC/Kaletra | 669 (7.4) |
| AZT containing regimen | 308 (3.4) |
| TDF containing regimen | 59 (0.7) |
| other | 176 (1.9) |
| **BMI (kg/m$^2$)** | 22.4 ±5.0 (**N = 7010)** |
| **CD4 count (cells/mm$^3$)** | 81 (29-149) **(N = 7605)** |
| **Follow up time on HAART (months)** | 19.0 (9.1-31.6) **(N= 9040)** |

Data is expressed as N (%) except for BMI (mean±SD), CD4 count (median, IQR) and follow up time on HAART (median, IQR). WHO, World Health Organization, d4T,stavudine; 3TC, lamivudine; EFV, efavirenz; NVP, nevirapine; kaletra, ritonavir/lopinavir; AZT, zidovudine; TDF, tenofovir.

Peripheral neuropathy was reported equally in both genders (approximately 17%), with no difference in time to development between the drug groups.

There was also a significantly higher proportion of patients on stavudine based therapy presenting with symptomatic hyperlactataemia when compared to those on non-stavudine based therapy (5.75 vs. 2.2%, $p<0.0005$) (Table 2), with females more frequently affected than males (7.1% vs. 2.5 % ;$p<0.0001$). Although the rate of development of lactic acidosis was the same for both drug groups, it was experienced more frequently in females than males

(3.3% vs. 0.8%; p< 0.0001) and this was consistent with other studies (Boulle *et al*, 2007, Bolhaar *et al*, 2007, Sanne *et al*, 2009). The median time to onset was the same for both drug groups. Pancreatitis was equally rare in both treatment groups (see Table 2); both genders were equally affected, with the time to onset being the same in both drug groups. When compared to those on non-stavudine based regimens, those receiving stavudine had higher incidence rates of several toxicities including peripheral neuropathy [12.1/100 person-years (95%CI 7.0-19.5) vs. 7.9/100 person-years (95%CI 6.0-10.1)], symptomatic hyperlactataemia[3.6/100 person-years (95%CI 1.2-7.5) vs.1.4 /100 person-years (95%CI 0.7-2.5)], and lactic acidosis [1.6/100 person-years (95%CI 0.4-5.2) vs. 0.8/100 person-years (95%CI 0.3-1.7)] (Table 3).

In terms of the chronic HAART-related metabolic complications, 7.3% presented with lipoatrophy on stavudine based therapy, compared to 4.6% (p<0.05) patients on non-stavudine based therapy (Table 2). The median time to development of lipoatrophy was similar across the 2 treatment groups. Lipoatrophy was more predominantly seen in female than male patients (10.0% vs. 1.6%; p < 0.0001). Incidence rates for lipoatrophy were slightly higher in the stavudine based therapy group [3.0/100 person-years (95%CI 1.9-4.4) versus 4.6/100 person-years (95%CI 2.1-9.6)] compared to those on other regimens (Table 3). Only two percent of patients presented with hypertension, while only 0.3% presented with diabetes and 1.3% with dyslipidaemia on stavudine based therapy. Similar proportions developing these conditions were observed in both groups (Table 2). However, hypertension developed more quickly (p<0.05) in patients taking stavudine than in those not receiving this drug (Table 2). Hypertension was less common in females than males (1.8% vs. 2.4%; p<0.05) but females and males were equally affected in terms of diabetes and dyslipidaemia.

**Table 2. Frequency of and time (months) to HAART associated toxic complications by initiating regimen**

| Condition | Stavudine (N= 8497) | Other drugs (N=543) |
|---|---|---|
| **Peripheral neuropathy** | | |
| Frequency | 1454 (17.1)* | 61 (11.2)* |
| Time to diagnosis | 6.7 (3.8-111.8) | 5.1 (3.1-11.7) |
| **Symptomatic hyperlactataemia** | | |
| Frequency | 487 (5.7)* | 12 (2.2)* |
| Time to diagnosis | 14.5 (10.6-20.9) | 11.6 (8.7-19.4) |
| **Lactic Acidosis** | | |
| Frequency | 214 (2.5) | 7 (1.3) |
| Time to diagnosis | 10.8 (9.0-13.5) | 11.2 (9.0-13.5) |
| **Pancreatitis** | | |
| Frequency | 14 (0.2) | 1 (0.2) |
| Time to diagnosis | 10.4(4.0-13.0) | 8.3 (8.3-8.3) |
| **Lipoatrophy** | | |
| Frequency | 616 (7.3)** | 25 (4.6) ** |
| Time to diagnosis | 17.0 (11.4-23.1) | 15.0 (10.5-24.8) |
| **Hypertension** | | |
| Frequency | 167 (2.0) | 17 (3.1) |
| Time to diagnosis | 9.7 (4.6-18.4)** | 16.3 (9.3-30.5)** |
| **Diabetes** | | |
| Frequency | 28 (0.3) | 1 (0.2) |
| Time to diagnosis | 22.4 (12.5-28.1) | 9.0 (9.0-9.0) |
| **Dyslipidaemia** | | |
| Frequency | 109 (1.3) | 9(1.7) |
| Time to diagnosis | 24.6 (17.4-34.0) | 19.1 (15.3-26.1) |

Data is given as n (%) for prevalence and median (IQR) for time to diagnosis;
*p<0.005, **p<0.05 versus group receiving stavudine.

In multivariate analyses adjusted for age, gender, baseline BMI, CD4 counts and the stavudine dose at initiation of HAART, the use of stavudine continued to be associated with increased hazard of developing several toxicities: peripheral neuropathy (HR 2.02; 95% CI 1.35-3.03), symptomatic hyperlactataemia (HR 2.81; 95% CI 1.26-6.31), lactic acidosis (adjusted HR 2.55; 95% CI 0.81-8.00) and diabetes mellitus (adjusted HR 2.07; 95% CI 0.28-15.21) though some of these estimates lacked precision (Table 3).

Figures 2, 3, 4 and 5 present Kaplan Meier curves for the crude estimates of the time to development of HAART-related toxicities by initiating regimen. Those initiated on stavudine based regimens were more likely to develop peripheral neuropathy (log rank p< 0.001), hyperlactataemia (log rank p< 0.001), lactic acidosis (log rank p=0.098) or lipoatrophy (log rank p<0.05) than those on non-stavudine based regimens.

**Table 3: Crude and adjusted effects of stavudine use on toxicity initiated on HAART**

| Toxicity | No events | Person Time (years) | Rate/ 100 pys* (95% CI)‡ | Crude HR§ (95% CI)‡ | Adjusted† HR (95% CI)‡ |
|---|---|---|---|---|---|
| **Peripheral Neuropathy** | | | | | |
| *Other* | 61 | 775.9 | 7.9 (6.0-10.1) | 1.0 | 1.0 |
| *d4T-based* | 1454 | 12055.5 | 12.1 (7.0-19.5) | 1.53 (1.19-1.98) | 2.02 (1.35-3.03) |
| **Symptomatic hyperlactataemia** | | | | | |
| *Other* | 12 | 852.3 | 1.4 (0.7-2.5) | 1.0 | 1.0 |
| *d4T-based* | 487 | 13690.9 | 3.6 (1.2-7.5) | 2.70 (1.52-4.79) | 2.81 (1.26-6.31) |
| **Lactic acidosis** | | | | | |
| *Other* | 7 | 848.3 | 0.8 (0.3-1.7) | 1.0 | 1.0 |
| *d4T-based* | 214 | 13805.9 | 1.6 (0.4-5.2) | 2.09 (0.99-4.44) | 2.55 (0.81-8.00) |
| **Pancreatitis** | | | | | |
| *Other* | 1 | 858.9 | 0.1 (0.003-0.6) | 1.0 | 1.0 |
| *d4T-based* | 14 | 14126.2 | 0.1 (0.02-2.6) | 0.95 (0.13-7.21) | 0.40 (0.05-3.16) |
| **Lipoatrophy** | | | | | |
| *Other* | 25 | 834.0 | 3.0 (1.9-4.4) | 1.0 | 1.0 |
| *d4T-based* | 616 | 13534.3 | 4.6 (2.1-9.6) | 1.67 (1.12-2.50) | 1.60 (0.92-2.77) |
| **Hypertension** | | | | | |
| *Other* | 17 | 845.0 | 2.0 (1.2-3.2) | 1.0 | 1.0 |
| *d4T-based* | 167 | 13929.0 | 1.2 (0.2-4.0) | 0.66 (0.40-1.08) | 0.83 (0.39-1.78) |
| **Diabetes Mellitus** | | | | | |
| *Other* | 1 | 859.0 | 0.1 (0.003-0.6) | 1.0 | 1.0 |
| *d4T-based* | 28 | 14122.7 | 0.2 (0.02-2.6) | 1.90 (0.26-14.04) | 2.07 (0.28-15.21) |
| **Dyslipidaemia** | | | | | |
| *Other* | 9 | 856.5 | 1.1 (0.4-2.0) | 1.0 | 1.0 |
| *d4T-based* | 109 | 14097.2 | 0.8 (0.2-4.0) | 0.82 (0.41-1.61) | 0.61 (0.27-1.40) |

*pys = person years

§ HR = hazard ratio estimated from Cox proportional hazard models

‡ 95% CI = 95% confidence interval

† All models adjusted for age, gender, baseline CD4 count, baseline body mass index and time at which HAART was initiated (either prior to or post October 2007)

### 2.5.1. Other risk factors for development of toxicity

Age, CD4 counts and WHO staging at baseline did not appear to increase the risk of any adverse events after HAART initiation; however, BMIs at baseline did significantly increase the risk of both symptomatic hyperlactataemia and lactic acidosis. Relative to a baseline BMI of <25 kg/$m^2$, an increased risk of symptomatic hyperlactataemia was seen for those with a BMI of 25-30 kg/$m^2$ (RR 1.7; 95% CI 1.34-2.14, p< 0.0001) and those with a BMI of >30kg/$m^2$ (RR 1.82; 95% CI 1.35-2.44, p< 0.0001). The same was seen with lactic acidosis, where an increased risk was seen for those with a BMI of 25-30 kg/$m^2$ (RR 2.52; 95% CI 1.74-3.65, p< 0.0001) and those with a BMI of >30kg/$m^2$ (RR 3.10; 95% CI 2.00-4.79, p< 0.0001) when compared to subjects with a BMI of <25kg/$m^2$.

**Figure 2: Kaplan Meier crude estimates of time to development of peripheral neuropathy by initiating regimen (subjects with peripheral neuropathy at initiation of HAART were not included in the analysis).**

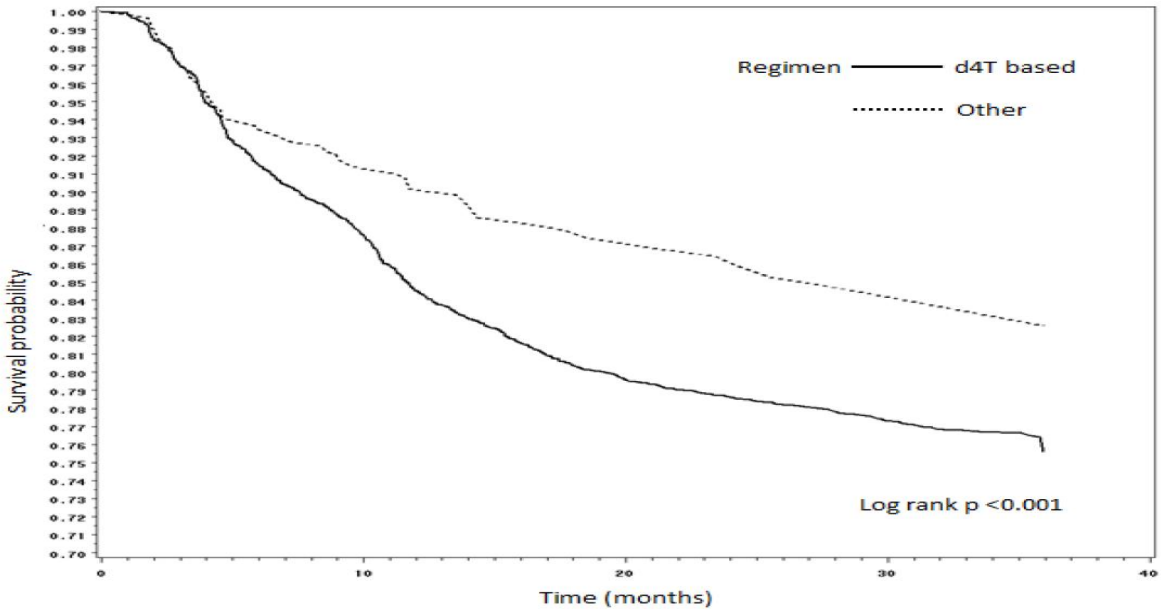


**Figure 3: Kaplan Meier crude estimates of time to development of hyperlactataemia by initiating regimen.**

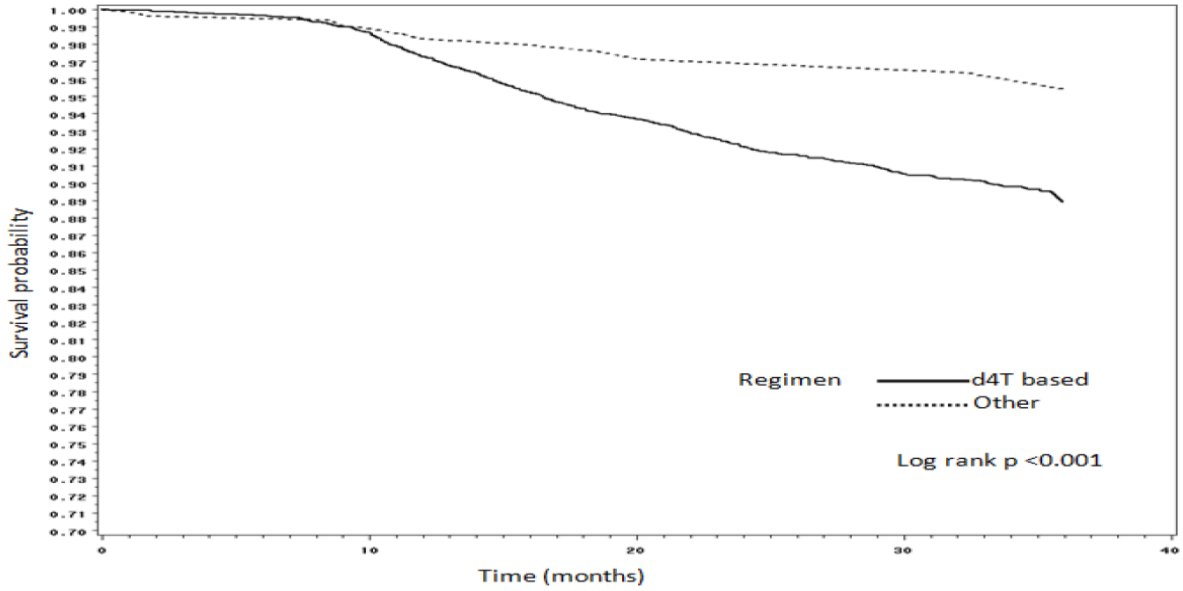



**Figure 4: Kaplan Meier crude estimates of time to development of lactic acidosis by initiating regimen.**

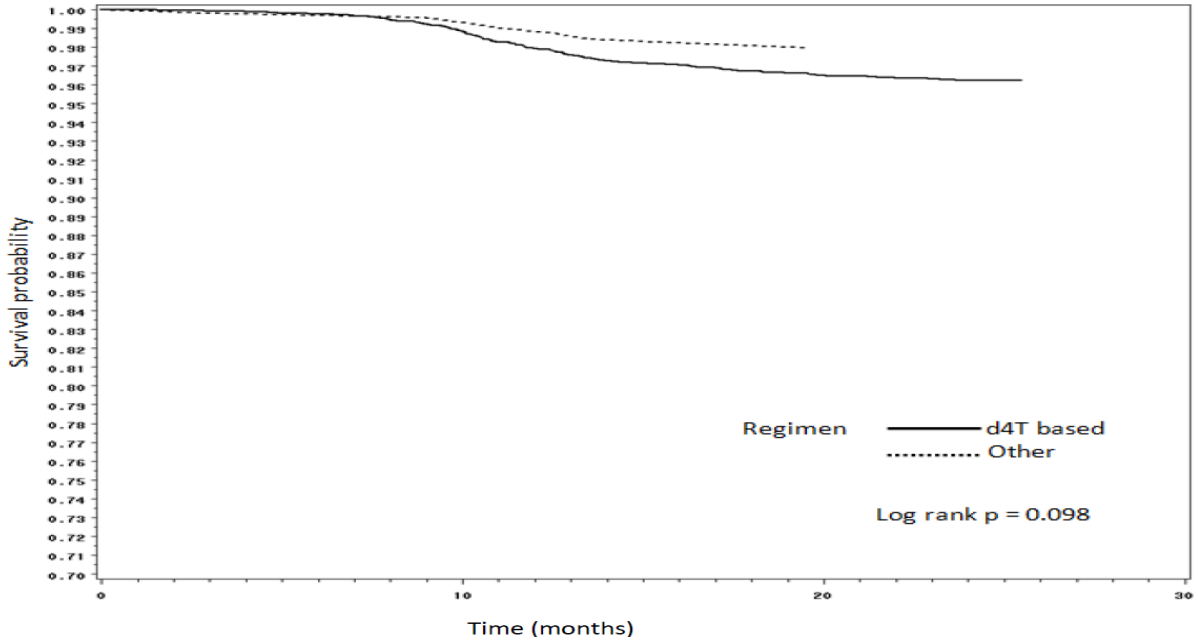
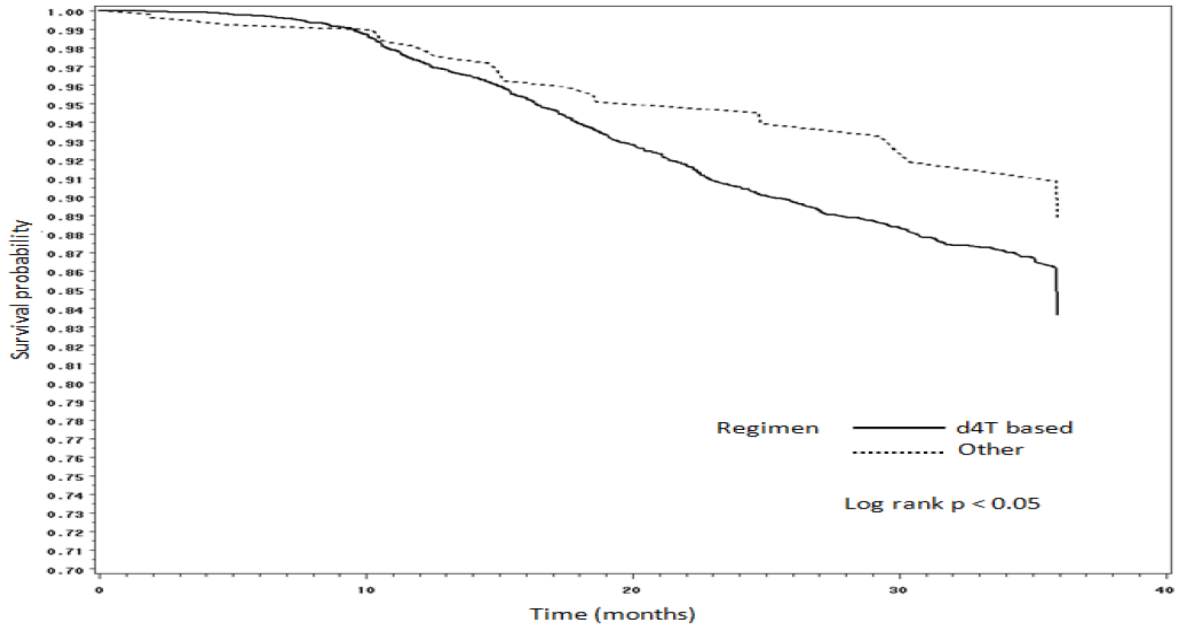


**Figure 5: Kaplan Meier crude estimates of time to development of lipoatrophy by initiating regimen.**




## 2.6. Discussion

This study describes data of three years of follow up of 9040 HIV-infected adults initiated on anti-retroviral treatment at the Themba Lethu Clinic, Johannesburg, South Africa. This high number of patients demonstrates the ability of rolling out a successful HAART programme despite being in a resource limited environment, and this, at a rapidity and scale that compares with reports from other African countries (Boulle *et al*, 2007. Coetzee *et al*, 2004 Stringer *et al*, 2006. Coetzee *et al*, 2004).

Despite the fact that stavudine is associated with significant complications, a large proportion of low and middle-income countries still use stavudine-based HAART as first line therapy (Beck *et al*, 2006. Renaud-Théry *et al*, 2007), mainly because of the cost implications of alternative drugs. In the present study nearly 30% of patients had to switch to non-stavudine based regimens, due to major side effects. These findings concur with those from another

large study in South Africa where 21% of patients switched regimens over a similar time period (Boulle *et al*, 2007).

This study provides estimates of the pattern of toxicities, the predominant ones being peripheral neuropathy, symptomatic hyperlactataemia, and lipoatrophy. Our incidence rates of peripheral neuropathy (12.1/100 person-years) were much higher compared to other African studies: 5.2/100 person-years in Rwanda (van Griensven *et al*, 2010) and 2.8/100 person-years in another site in South Africa (Boulle *et al*, 2007). The variability in these rates could be because there are no grading protocols for the severity of peripheral neuropathies. Our incidence rates of lactic acidosis were similar to a study from another province in South Africa, 1.6 versus 1.9 /100 person-years (Geddes *et al*, 2006). The proportion of lactic acidosis was slightly higher compared to another study from Botswana (Wester *et al*, 2007), 2.5% versus 1% in our cohort. When compared to this same study (Wester *et al*, 2007), the proportion developing symptomatic hyperlactataemia were much higher in our sample (5.7% versus 2%). These higher rates could be explained by the fact that clinicians were 'aware' of the higher proportion of females on stavudine in the clinic and were more 'sensitized' to the risk factors and serious side effects associated with stavudine. In addition, we had ready access to laboratory testing for lactic acid determination unlike other resource limited countries. The median time to the development of lactic acidosis was a little later in our study when compared to another study from South Africa (10.8 months versus 7.5 months) (Geddes *et al*, 2006) .

In terms of the chronic toxicities, incidence rates of lipoatrophy were 4.6 /100 person-years on stavudine based therapy and 3.0/100 person-years on the non-stavudine based therapy. Of note, the potential influence of zidovudine in the non-stavudine group may have affected the ability to determine the differences in rates of lipoatrophy although the numbers were

relatively smaller. One study from Rwanda (van Griensven *et al*, 2010) , where patients were also on stavudine based regimens showed a similar incidence rate of lipoatrophy to that reported in the present study at 4.7/100 person-years, while one other study from South Africa (Boulle *et al*, 2007) showed a lower incidence rate of 1.4/100 person-years . Another study from Rwanda showed a much higher proportion (34%) of patients developing lipoatrophy (Mutimura *et al*, 2007). The reason for this wide variation in lipoatrophy rates specifically, is possibly due to different diagnostic criteria used for identifying cases. Thus, in the present study and in the studies showing low but similar rates (Boulle *et al*, 2007. van Griensven *et al*, 2010), only cases that were severe enough to warrant a regimen change were noted. In the study showing a much higher proportion, milder cases of lipodystrophy that did not require a regimen change, were also recorded (Mutimura *et al*, 2007). An objective case definition of lipodystrophy has been developed (Carr et al, 2003); however, it requires access to DEXA and CT imaging technology, which is often not available in resource-limited settings. Therefore, an alternative consensus definition for the diagnosis of lipodystrophy is required for such environments.

Zidovudine may account for the relatively higher rates of mitochondrial toxicities noted in the non-stavudine group, but the degree to which it causes these side effects are not the same as that seen with stavudine. Zidovudine continues to be used as part of the second line therapy by the WHO and the South African HIV therapy guidelines – and is also a commonly used alternative drug because of the lack of other options, especially in the presence of contraindications to tenofovir.

A very small number of patients presented with diabetes (0.3%) and dyslipidaemias (1.3%). However, this could be because lipid or glucose levels were only tested when clinically

suspected due to cost implications, therefore probably underestimating the true prevalence of these metabolic disorders.

The major strength of this study is the large sample size. This cohort is similar to many other resource-limited settings where there is rapid scaling-up of comprehensive HIV care and HAART. It allowed for a relatively long duration of follow up of up to three years and a fairly high retention rate of 70%, with all clinicians working on common protocols for defining the various HAART-associated toxicities. However, despite this, these findings must be considered in the light of potential limitations. It is possible that only severe toxicities that warranted a change in regimen may have been reported and this may have led to an underestimation of the rates of some of the HAART-related toxicities, particularly lipoatrophy. Also, plasma glucose and serum lipid levels were not routinely measured and thus the true rates for glucose intolerance, diabetes and dyslipidaemia were not attained. Lower reported rates of the chronic toxicities could also be related to the rates of death (5%) and loss to follow up (24%), the majority of which occurred within the first six months on treatment as described in a previous report (Sanne *et al*, 2009 ).

## 2.7. Conclusion

These results support the move away from stavudine based regimens towards less toxic combination regimens as advocated by the World Health Organisation (WHO, 2006. WHO, 2010). South Africa has implemented these treatment guidelines, where tenofovir has now replaced stavudine as first line therapy. However in resource-limited countries, where staduvine is still being used because of cost implications, proper pharmacovigilance systems need to be established and alternative consensus definitions and grading protocols are required for identifying various HAART-related toxicities.

## 2.8. Acknowledgements

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## 2.9. References

Beck EJ, Vitoria M, Mandalia S, Crowley S, Gilks CF, Souteyrand Y. National adult antiretroviral therapy guidelines in resource-limited countries: concordance with 2003 WHO guidelines? *AIDS* 2006; **20**:1471-1479.

Boulle A, Orrell C, Kaplan R, van Cutsem G, McNally M, *et al*. Substitution due to antiretroviral toxicity or contraindication in the first 3 years of antiretroviral treatment in a large South African cohort. *Antivir Ther* 2007; **12**:753-60.

Carr A, Emery S, Law M, Puls R, Lundgren JD, Powderly WG. An objective case definition of lipodystrophy in HIV-infected adults: a case-control study. *Lancet* 2003; **361**:726-735.

Coetzee D, Hildebrand K, Boulle A, Maartens G, Louis F, Labatala V, *et al*. Outcomes after two years of providing antiretroviral treatment in Khayelitsha, South Africa. *AIDS* 2004; **18**:887-95.

De Wit S, Sabin CA, Weber R, Worm SW, Reiss P, Cazanave C, *et al*. Incidence and risk factors for new onset diabetes in HIV-infected patients. The data collection on adverse events of anti-HIV drugs (D: A: D) study. *Diabetes Care* 2008; **31**:1224-1229.

Department of Health, 2010. National Antenatal Sentinel HIV and Syphilis Prevalence Survey in South Africa, 2009: http://www.health-e.org.za/documents/85d3dad6136e8ca9d02cceb7f4a36145.pdf. Last accessed on the 31/08//2011.

Geddes R, Knight S, Moosa MY, Reddi A, Uebel K, Sunpath H. A high incidence of nucleoside reverse transcriptase inhibitor (NRTI)-induced lactic acidosis in HIV-infected patients in a South African context. *S Afr Med J* 2006; **96**:722-4.

Hogg RS, O'Shaughnessy MV, Gataric N, Yip B, Craib K, Schechter MT, *et al*. Decline in deaths from AIDS due to new antiretrovirals. *Lancet* 1997; **349**:1294.

Mutimura E, Stewart A, Rheeder P, Crowther NJ. Metabolic function and the prevalence of lipodystrophy in a population of HIV-infected African subjects receiving highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* 2007; **46**:451-5.

National Department of Health. National Antiretroviral Treatment Guidelines, First Edition 2004: http://www.doh.gov.za/docs/misc/2004/sec1.pdf. Last accessed on the 31/08/2011.

Palella FJ Jr, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, *et al*. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N Engl J Med* 1998; **338**:853-60.

Renaud-Théry F, Nguimfack BD, Vitoria M, Lee E, Graaff P, Samb B, *et al*. Use of antiretroviral therapy in resource-limited countries in 2006: distribution and uptake of first- and second-line regimens. *AIDS* 2007; **21**(Suppl 4):89-95.

Sanne I, Westreich D, Macphail AP, Rubel A, Majuba P, Van Rie A. Long term outcomes of antiretroviral therapy in a large HIV/AIDS care clinic in urban South Africa: a prospective cohort study. *J Int AIDS Soc* 2009; **12**:753-760.

Stringer JS, Zulu I, Levy J, Stringer EM, Mwango A, Chi BH, *et al*. Rapid Scale-up of Antiretroviral Therapy at Primary Care Sites in Zambia – Feasibility and Early Outcomes. *JAMA* 2006; **296**:782-793.

UNAIDS AIDS epidemic update. December 2009: http://data.unaids.org/pub/Report/2009/JC1700_Epi_Update_2009_en.pdf. Last accessed on the 07/12//2010.

van Griensven J, De Naeyer L, Mushi T, Ubarijoro S, Gashumba D, Gazille C, *et al*. High prevalence of lipoatrophy among patients on stavudine-containing first-line antiretroviral therapy regimens in Rwanda. *Trans R Soc Trop Med Hyg* 2007; **101(8)**:793-8.

van Griensven J, Zachariah R, Rasschaert F, Mugabo J, Atte EF, Reid T. Stavudine- and nevirapine-related drug toxicity while on generic fixed-dose antiretroviral treatment: incidence, timing and risk factors in a three-year cohort in Kigali, Rwanda. *Trans R Soc Trop Med Hyg* 2010; **104**:148-53.

Wester CW, Okezie OA, Thomas AM, Bussmann H, Moyo S, Muzenda T, *et al*. Higher-than-expected rates of lactic acidosis among highly active antiretroviral therapy-treated women in Botswana: preliminary results from a large randomized clinical trial. *J Acquir Immune Defic Syndr* 2007; **46**:318-22.

WHO: Addendum to the 2006 WHO guidelines on antiretroviral therapy for HIV infection in adults and adolescents. http://www.who.int/hiv/art/ARTadultsaddendum.pdf. Last accessed on the 09/12/2010.

WHO: Antiretroviral therapy for HIV infection in adults and adolescents: Recommendations for a public health approach (2010 version). http://whqlibdoc.who.int/publications/2010/9789241599764_eng.pdf . Last accessed on the 09/12/2010.

Wit FW, van Leeuwen R, Weverling GJ, Jurriaans S, Nauta K, Steingrover R, *et al*. Outcome and predictors of failure of highly active antiretroviral therapy: one-year follow-up of a cohort of human immunodeficiency virus type 1-infected persons. *J Infect Dis* 1999; **179**:790-798.

van Griensven J, De Naeyer L, Mushi T, Ubarijoro S, Gashumba D, Gazille C, *et al*. High prevalence of lipoatrophy among patients on stavudine-containing first-line antiretroviral therapy regimens in Rwanda. *Trans R Soc Trop Med Hyg* 2007; **101(8)**:793-8.

**CHAPTER 3**

# 3. THE EARLY EFFECTS OF STAVUDINE COMPARED TO TENOFOVIR ON ADIPOCYTE GENE EXPRESSION, MITOCHONDRIAL DNA COPY NUMBER AND METABOLIC PARAMETERS IN SOUTH AFRICAN HIV-INFECTED PATIENTS: A RANDOMIZED TRIAL.

## 3.1. Abstract

**Objective:**

Stavudine (d4T) is being phased out because of its mitochondrial toxicity and tenofovir (TDF) is recommended as part of first line HAART in South Africa. A prospective, open-label randomized controlled trial comparing standard and low dose d4T with TDF was performed to assess early differences in adipocyte mtDNA copy number, gene expression and metabolic parameters in black South African HIV-infected patients.

**Methods:**

Sixty patients were randomized 1:1:1 to either standard (30-40 mg) or low (20-30 mg) dose d4T or TDF (300 mg) each combined with lamivudine and efavirenz. Subcutaneous fat biopsies were obtained at weeks 0 and 4. Adipocyte mtDNA copies/cell and gene expression were measured using qPCR. Markers of inflammation, lipid and glucose metabolism were also assessed.

**Results:**

A 29% and 32% decrease in the mean mtDNA copies/cell was noted in the standard dose ($P<0.05$) and low dose d4T ($P<0.005$) arms respectively when compared to TDF at four weeks. *NRF1* and *MTCYB* gene expression levels were affected by d4T, with a significantly ($P<0.05$) greater fall in expression observed with the standard, but not the low dose compared

to TDF. No significant differences were observed in markers of inflammation, lipid and glucose metabolism.

**Conclusions:**

These results demonstrate early mitochondrial depletion among black South African patients receiving low and standard doses of d4T, with preservation of gene expression levels, except for *NRF1* and *MTCYB,* when compared to patients on TDF.

## 3.2. Introduction

Despite the benefits of the South African Government's highly active antiretroviral therapy (HAART) roll out programme which commenced in 2004, adverse events remain an important concern for patients on HAART in this country (Boulle *et al,* 2007, Sanne *et al,* 2009.Menezes *et al,* 2011). Understanding the adverse effects of different therapeutic regimens, as well as the underlying molecular and biochemical factors that modulate risk, are critical to improving the management of HIV/AIDS. This is particularly important now that clinical practice is moving towards regimens that combine high levels of both tolerability and efficacy.

Until 2010, South Africa's treatment guidelines for first-line public-sector HAART recommended stavudine (d4T) with lamivudine (3TC) and either efavirenz (EFV) or nevirapine (NVP) (National Department of Health, 2004). This d4T-based regimen, which is cheap and easy to administer in the short term, is associated with significant morbidity, particularly hyperlactataemia syndromes with long term risks of lipoatrophy, and peripheral neuropathy (Boulle *et al,* 2007.Sanne *et al,* 2009. Menezes *et al,* 2011). The main pathogenic mechanism thought to contribute to the metabolic changes and organ toxicities is mitochondrial toxicity, via the inhibition of mitochondrial DNA (mtDNA) polymerase γ

(POLG) and alterations in messenger RNA (mRNA) gene expression (Mallon *et al*, 2005. Kim *et al*, 2008. Sievers *et al*, 2009. Pace *et al*, 2003). As a result of the side effects of d4T, South Africa (SA) included tenofovir (TDF) in its recommended first-line public-sector HAART in 2010 (National Department of Health South Africa, 2010), despite its cost implications. A study from South Africa demonstrated that the price of TDF would need to fall from USD$ 17 to USD$ 6.17 per month to have the same overall cost as d4T based therapy (Rosen *et al*, 2008). TDF has been shown to have a more favourable effect on lipid and mtDNA profiles compared to d4T, with no difference in virologic response (Milinkovic et al, 2007).

While the various toxicities associated with d4T have been well documented, what is less clear is whether the dose of d4T plays a role in the development of these toxicities. The standard dosage for d4T is 40 mg given twice daily as this regimen received regulatory approval because of its efficacy. However, recent studies have suggested that reduced doses of d4T (i.e. 20 or 30 mg twice daily) diminishes its toxicity while maintaining efficacy (Milinkovic *et al*, 2007. Hill *et al*, 2007. Gallant *et al*, 2004).

It is therefore possible that because d4T is cheaper than TDF and its side effects can be minimized via reducing its dose, this would allow more patients to be initiated on HAART in countries where the switch away from d4T may have not yet occurred. Notwithstanding the recent changes in country-level HAART guidelines recommending the use of non-d4T based therapy (British HIV Association guidelines, 2012. Panel on Antiretroviral Guidelines for Adults and Adolescents, 2012), several resource-limited countries have yet to phase out d4T. According to the World Health Organization, in 2010 approximately 56% of HAART regimens within such countries still contained d4T (WHO, 2010).

Therefore, we have conducted an open-label, randomized controlled trial to identify early molecular and biochemical changes and clinical outcomes for patients on either standard or low dose d4T or TDF. We present here the results of four weeks of follow-up which set out to assess if there were any differences in the effect of TDF when compared to low dose and standard dose d4T on adipocyte specific mtDNA copy number, gene expression and other metabolic parameters. To our knowledge this is the first study to analyze the mitochondrial effects of d4T and TDF in a South African HIV- infected population

## 3.3. Methods

### 3.3.1. Study population and inclusion criteria

We enrolled HIV-1 infected, treatment naive individuals aged 18 years or older with a CD4+ cell count below 200 cells/$mm^3$ (consistent with the SA guidelines at the time (National Department of Health, 2004)). Patients were enrolled at the Themba Lethu HIV Clinic at the Helen Joseph Hospital, a public-sector hospital in Johannesburg. Eligible subjects had an absolute neutrophil count (ANC) ≥750/mm, hemoglobin ≥7.0 g/dL and platelet count ≥50,000/$mm^3$. Other inclusion criteria included an alanine and aspartate aminotransferase and an alkaline phosphatase ≤2.5 X upper limit of normal (ULN) and a total bilirubin ≤2.5 X ULN. Patients needed to have a creatinine clearance of ≥60 mL/min using the Cockcroft-Gault formula. In addition, participants of reproductive age had to have a negative serum or urine pregnancy test within 45 days prior to study entry. Those with reproductive potential had to be willing to use at least one reliable form of contraception.

Patients were excluded if they were breastfeeding or pregnant and if they had received any antiretroviral drugs (including occupational or sexual post exposure prophylaxis) other than single-dose nevirapine (NVP) within the past six months.

The study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand and the SA Medicines Control Council (MCC).

### 3.3.2. Randomization and masking

Patients were randomly assigned 1:1:1 to receive 3TC and EFV in combination with a) a standard dose of d4T (30 mg if weight <60 kg or 40 mg if weight >60 kg) according to the then SA guidelines (National Department of Health, 2004); b) a low-dose of d4T (20 mg if weight <60 kg or 30 mg if weight >60 kg); or c) TDF (300 mg). The definition of the standard and low dose of d4T is similar to several other reported studies (Hill *et al*, 2007). Coded identity numbers with the arm allocated were sealed in sequentially numbered envelopes. At enrolment, a member of the data collection team unsealed the next envelope to assign the drug arm allocated to the patient. Those analyzing patient samples were masked to the assignment.

### 3.3.3. Subcutaneous fat biopsies for the determination of mitochondrial DNA copy number and gene expression

At weeks 0 and 4, subcutaneous fat biopsies from the supra-iliac region were performed under local anesthetic on fasted subjects. Week 4 was chosen to avoid the confounding effects of diet and exercise arising with time. The adipose tissue biopsies were snap frozen in liquid nitrogen prior to storage at -70°C to avoid risk of mtDNA depletion.

Total DNA was extracted at the same time from adipose tissue collected at weeks 0 and 4 for each patient using the QIAmp DNA Mini Kit (QIAGEN, Inc., Hilden, Germany) and a modified protocol (Nolan *et al*, 2003). Mitochondrial and nuclear gene copy numbers were determined using a quantitative polymerase chain reaction (qPCR) approach and the number

of mitochondria per cell was calculated (Nolan *et al*, 2003) by comparing the mtDNA content to the nDNA content and by assuming that there are two copies of the nuclear-encoded human growth hormone HGH gene per cell, the number of cells per sample can be calculated and hence the number of mitochondria per cell. Thus, expressed in logarithmic form:

$$\log_{10}(\text{mtDNA copies/cell}) = \log_{10}(\text{mtDNA copies/ml}) - \log_{10}(\text{nDNA copies/ml}) - \log_{10}(0.5)$$

Total RNA was extracted using the RNeasy lipid tissue mini kit (QIAGEN), was quantified using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies Inc., Wilmington, Del.) and the integrity assessed using the 2100 Bioanalyser and RNA 6000 picochips (Agilent Technologies, Waldbronn, Germany). A 200 ng aliquot of total RNA was converted into cDNA using the Transcriptor high fidelity cDNA synthesis kit with random hexamers (Roche Diagnostics Ltd., Mannheim, Germany). Quantitative real time PCR was performed, in duplicate for each sample, using the SensiMix SYBR Low-ROX kit (Bioline Ltd, Luckenwalde, Germany) on a 7500 Real-Time PCR system (Applied Biosystems, Foster City, Calif.). Each 25 µl reaction mixture contained 1x SensiMix reaction mix, 0.25 µM of each primer and 6.25 ng cDNA. Thermocycling conditions for all primer pairs comprised an initial denaturation step of 95°C for 10 min; 45 amplification cycles of 95°C for 15 sec, 60°C for 30 sec and 72°C for 35 sec and a melt curve step of 95°C for 15 sec, 60°C for 20 sec ramping at 1% to 95°C for 20 sec. Genes assayed included the nuclear genes PPAR-γ coactivator 1α (*PGC-1*) which is important for mitochondrial biogenesis, nuclear respiratory factor-1 (*NRF-1*) which is a major respiratory gene transcription regulator and mitochondrial transcription factor-A (*TFAM*) which binds to and regulates mtDNA. Other genes measured included those involved in mitochondrial energy metabolism - cytochrome c oxidase subunit III (*COX 3),* cytochrome c oxidase subunit IV (*COX 4*) and mitochondrial cytochrome B

(*MTCYB*). Two nuclear genes involved in lipid metabolism, leptin (*LEP*) and lipoprotein lipase (*LPL*) were also assayed. Primer sequences for these genes were as previously published (Mallon *et al*, 2005). Gene expression was normalized against three reference genes: β2 microglobulin (*B2M*) (5'-TGCTGTCTCCATGTTTGATGTATCT-3' and 5'-TCTCTGCTCCCCACCTCTAAGT-3'), 60S ribosomal protein L 13A (RPL13A) (5'-CCTGGAGGAGAAGAGGAAAGAGA-3' and 5'-TTGAGGACCTCTGTGTATTTGTCAA-3') and hypoxanthine-guanine phosphoribosyltransferase (*HPRT*) (5'-TGACACTGGCAAAACAATGCA-3' and 5'-GGTCCTTTTCACCAGCAAGCT-3'). Primers for reference genes were designed using Primer Quest (http://eu.idtdna.com/Scitools/Applications/Primerquest). Comparison of gene expression between the week 0 and week 4 samples were calculated using the $2^{-\Delta\Delta Cq}$ method. In brief, ΔCq values for each patient were calculated for both the genes of interest and the reference genes by subtracting the week 4 sample's Cq value from the week 0 sample's Cq value. The relative quantities ($2^{\Delta Cq}$) of the genes of interest and the reference genes were calculated. To normalize to multiple reference genes, the geometric mean of the relative quantities of the reference genes was calculated (Hellemans *et al*, 2007). The normalized relative quantity of each gene of interest was determined by dividing the relative quantity of the gene of interest by this geometric mean. These normalized relative quantities were log transformed to allow for statistical analysis.

Of note, the patients' samples at baseline and one month were batched so that the RNA from both time points was extracted at the same time, in addition, the cDNA synthesis was done and the PCRs per gene were also done at the same time. Since samples were compared from the same patient run on the same assay, inter-run variation did not have to be accounted for (i.e. no inter-assay variation for 1 month and baseline samples from each patient). For

mtDNA extraction, 100 mg tissue for DNA extraction was used. The PCRs for F8R8 and HGH were run on the same day for the same samples. There was sufficient material to run this experiment on all 60 samples (1 month and baseline). For mRNA extraction, 1 ml lysis reagent per 100 mg tissue was used. Sufficient RNA was also obtained to measure gene expression changes on all 60 samples. The housekeeping genes were not measured per se, they were used to normalise the results. If there was a change in any of the procedures or reagents, it would have been corrected by the use of the housekeeping genes so that the results would represent the changes in gene of interest only.

These results were considered to be robust, as there were several levels of quality control that were employed. The determination of mtDNA copy number was based on a well established protocol (Nolan *et al*, 2003) that has been standardized and extensively critiqued (Cote *et al*, 2011). Another important strength of this study was the adoption of the minimum information for publication of quantitative real-time PCR experiments (MIQE) guidelines (Bustin *et al*, 2009) with the normalization of gene expression to multiple reference genes (Hellemans *et al*, 2007) which specifically addresses the issue of robustness of data.

### 3.3.4. Blood measurements

After an overnight fast, serum was taken for the measurement of total cholesterol, high density lipoprotein (HDL)- and low density lipoprotein (LDL) - cholesterol, triglycerides and glucose (Roche Integra analyzer 400, Roche Diagnostics), C-peptide and insulin (Immulite1000 analyzer Diagnostics Corp., Los Angeles, CA), and leptin, adiponectin and hs-CRP (*Fluorokine*®-Multi analytes profiling (MAP) kit, RnD Systems, Inc ). A full blood count was performed and urea, creatinine and electrolytes, liver function test, and CD4 counts (Beckman Coulter flow cytometry, Miami, FL) were also measured. Insulin resistance was

assessed using the homeostasis model assessment (HOMA) method (Matthews *et al*, 1985) calculated as: (fasting serum insulin (mU/L) X fasting plasma glucose (mmol/L))/22.5. A low HOMA-IR value indicates high insulin sensitivity, whereas, a high HOMA-IR value indicates low insulin sensitivity i.e., insulin resistance.

### 3.3.5. Statistical analysis

The primary endpoint was to determine if there are significant molecular and biochemical differences between all arms of treatment – with no threshold given to determine the presence of such depletion. Sample size calculations were based on the assumption that at least 70% of participants exposed to d4T would show features of mitochondrial depletion or features of lipodystrophy (Nolan *et al*, 2003), while the percentage of participants exposed to TDF experiencing these outcomes would be 30%. With the power of 80%, $\alpha$=0.05, and equal sample size per group, we required 28 subjects per group. Recruitment to the entire trial was stopped early after a data safety and monitoring board (DSMB) analysis of the first 60 patients showed that the group given d4T at both standard and low doses produced a greater fall in the mean mtDNA copies/cell than in the arm receiving TDF. Furthermore, the DSMB considered that there were compelling ethical grounds to stop the high dose d4T because the new South African guidelines were shortly to be implemented and TDF had become freely available. Accordingly, all patients on 40mg dose d4T were switched to TDF. Furthermore, all patients were still followed-up until the end of the study period, which was 48 weeks. As a result, because a "per-protocol" analysis was undertaken, the study may be underpowered to detect differences between the three arms, but this did not affect the differences that were observed.

Data were analyzed after creating an anonymized database and calculating differences between week 0 and 4 readings, using STATISTICA® version 10 (StatSoft, Inc.). Outliers, calculated using (lower quartile - 1.5 X interquartile range; upper quartile + 1.5 X interquartile range), were excluded from the analysis as they skewed the results. They were only excluded from the DNA analysis (2 samples) and not the RNA analysis. Comparisons were made both by randomization arm as well as by the dosage of drug given. The mtDNA copy number and gene expression data were analyzed, using an ANOVA test for multiple comparisons. Markers of inflammation, lipid and glucose metabolism analyzed with a Kruskall-Wallis test. Threshold of significance was set at $P$=0.05.

## 3.4. Results

### 3.4.1. Baseline characteristics of the study population

Ninety-one HIV-infected patients were screened. Sixty patients qualified and consented, and were randomized 1:1:1 to one of three treatment arms between September 2008 and December 2009. The baseline characteristics of the 60 enrolled patients are summarized in Table 1. There was a predominance of females (85%) in this study and 59/60 were black. A total of 78% were WHO stage 1 while 3% of patients were WHO stage 4, despite low CD4 counts. There was no statistically significant difference in the markers of inflammation, lipid and glucose metabolism between the arms at baseline.

### 3.4.2. Mitochondrial DNA copy numbers and gene expression levels in adipocytes

The effects of HAART on mtDNA copy number and gene expression were assessed in biopsy samples of subcutaneous adipose tissue (figure 1). The mean $\log_{10}$ mtDNA copies/cell at baseline and week 4 for the standard dose d4T arm (30-40 mg) was 2.74 ± 0.21 and 2.61 ±

0.31 respectively and for the low dose d4T arm (20-30 mg), it was 2.63 ± 0.23 and 2.46 ± 0.18 respectively. Whilst it was 2.70 ± 0.14 and 2.69 ± 0.19 respectively, for the TDF arm. There was a 29% decrease in the mean mtDNA copies/cell from baseline to four weeks in standard dose d4T arm (30-40 mg) ($P<0.05$), and a 32% decrease in the low dose d4T arm (20-30 mg) ($P<0.005$), when compared to the TDF (300 mg) arm which had only a 4% decrease in the mean mtDNA copies/cell.

With each individual dose of d4T (20 mg, 30 mg and 40 mg), there was also a drop in mean mtDNA copy numbers (22%, 35%, and 31% respectively) versus TDF (300 mg) (4%) at four weeks of HAART (figure 1). The decrease in mtDNA copy number for both the d4T 30 mg and 40 mg doses was significantly higher than TDF 300 mg ($P<0.005$ and $P<0.05$ respectively). The drop in the mtDNA copy number with the d4T 20 mg dose was not significant when compared to TDF ($P=0.40$).

The relative gene expression of *MTCYB* was significantly lower in the standard dose (30-40 mg) when compared to the TDF (300 mg) arm ($P<0.05$), but not in the low dose (20-30 mg) d4T arm (table 2). There were minimal changes in the expression of *COX3* and *COX4* when comparing the difference at four weeks for all three arms.

With respect to the expression of the nuclear genes involved in the regulation of mitochondrial transcription, there were minimal changes in *PGC1* and *TFAM* expression at week 4 when compared to baseline for all three arms. There was a statistically significant difference in *NRF1* expression in the standard dose d4T arm (30-40 mg) when compared to the TDF 300 mg arm ($P<0.05$). This was not observed in the low dose (20-30 mg) d4T arm. Expression of *LPL* and *LEP* were not statistically significant at week 4.

When the effects of the individual d4T doses (20, 30 and 40 mg) on gene expression were compared to those with TDF, no significant differences were noted for any of the 8 genes that were studied (data not shown).

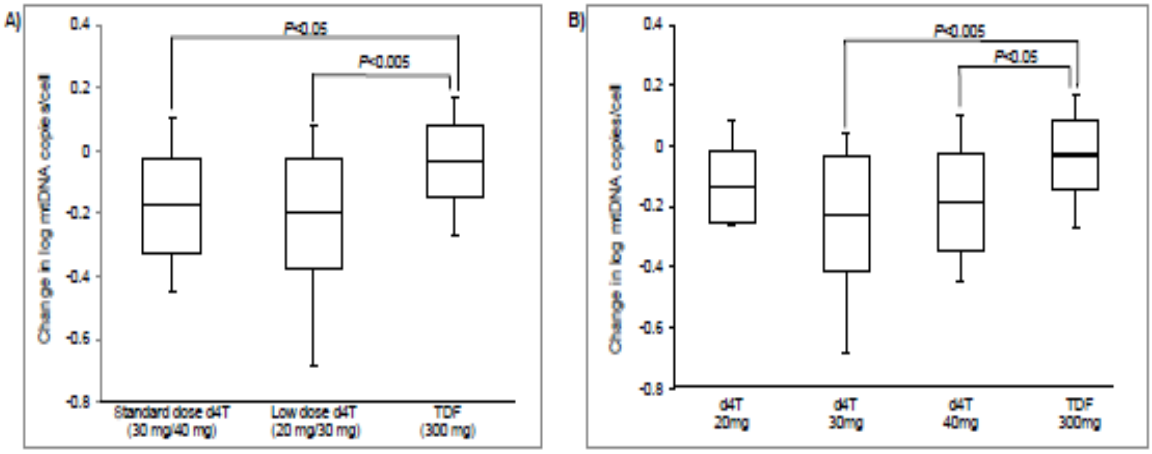
**Table 1. Patient characteristics at the commencement of HAART**

| Characteristic | Standard dose d4T (30 mg/40 mg) (N=20) | Low dose d4T (20 mg/30 mg) (N=20) | TDF (300mg) (N=20) |
|---|---|---|---|
| **Gender** | | | |
| **Female** | 16 (80%) | 18 (90%) | 17 (85%) |
| **Male** | 4 (20%) | 2 (10%) | 3 (15%) |
| **Age (years)** | 31(11) | 34(12) | 33(10) |
| **WHO AIDS classification** | | | |
| **Stage 1** | 14 (70%) | 17 (85%) | 16 (80%) |
| **Stage 2** | 3 (15%) | 1 (5%) | 3 (15%) |
| **Stage 3** | 1 (5%) | 2 (10%) | 1 (5%) |
| **Stage 4** | 2 (10%) | 0 (0%) | 0 (0%) |
| **BMI (kg/m$^2$)** | 25 (5) | 24 (7) | 24 (6) |
| **CD4 count (cells/mm$^3$)** | 155 (94) | 169 (78) | 135 (62) |
| **Inflammatory markers** | | | |
| **hs-CRP (pg/L)** | 0.80 (0.55) | 0.66 (0.94) | 0.75 (0.61) |
| **Leptin (pg/ml)** | 2.70 (15.02) | 3.17 (4.33) | 5.52 (8.49) |
| **Adiponectin (pg/ml)** | 11.78 (6.58) | 11.76 (8.43) | 13.78 (11.82) |
| **Markers of lipid and glucose metabolism** | | | |
| **C-peptide (nmol/ml)** | 0.40 (0.30) | 0.50 (0.20) | 0.40 (0.25) |
| **Insulin (mIU/L)** | 2.85(4.05) | 3.75 (4.65) | 3.60 (3.00) |
| **Total cholesterol (mmol/L)** | 3.20 (1.35) | 3.40 (1.10) | 3.80 (1.10) |
| **HDL cholesterol (mmol/L)** | 0.85 (0.40) | 0.80 (0.45) | 0.80 (0.40) |
| **Triglycerides (mmol/L)** | 0.70 (0.35) | 0.80 (0.70) | 0.80 (0.30) |
| **LDL cholesterol (mmol/L)** | 1.90 (1.05) | 2.15 (1.00) | 2.50 (0.70) |
| **Glucose (mmol/L)** | 3.95 (0.75) | 4.20 (0.80) | 4. 20 (0.60) |
| **HOMA** | 0.54 (0.96) | 0.70 (1.05) | 0.73 (0.60) |

Data is expressed as median (IQR) except for gender and WHO stage which are expressed as N (%).

**Figure 1: Comparison of log change in mtDNA copy number at week 4 by arm and dosage.**

A) Comparison of the three study arms, showing a significant difference in the standard (30 mg/ 40 mg) and low dose d4T (20 mg/ 30 mg) arms compared to TDF. B) A comparison of various dosages of d4T to TDF shows significant differences in the 30 mg and 40 mg d4T doses compared to TDF. The 20 mg dose was not significantly different ($P$=0.4). The line within the box-and-whisker plot marks the mean; the upper and lower boundaries of the box indicate one standard deviation to either side of the mean and the error bars above and below indicate the minimum and maximum values. There were 8 patients in the 20 mg dose stavudine group, 17 patients in the stavudine 30 mg dose group and 15 patients in the stavudine 40mg dose group.




### 3.4.3. Markers of inflammation, lipid and glucose metabolism

Following four weeks of HAART, there were no statistically significant changes in the lipid profile, glucose, insulin, C-peptide and HOMA indices when comparing the three arms and individual doses. No changes were noted in hs-CRP, leptin or adiponectin levels (table 2).

## 3.5. Discussion

This randomized controlled trial was designed to evaluate the *in vivo* effects of TDF against those of standard and low dose d4T regimens, with the reference regimen based on the Gilead

903 trial of Gallant *et al*, 2004. The Gilead 903 trial showed better outcomes and less adverse events attributed to mitochondrial toxicity in patients receiving TDF 300 mg when compared to d4T 40 mg given twice daily (Gallant *et al*, 2004) , but did not report mtDNA and gene expression evaluations. Our study is the first to compare mitochondrial effects of these drugs in a black population and at a time point in HAART therapy before the development of peripheral lipoatrophy.

The major finding in our study is the significant depletion, after only 4 weeks of therapy, of adipocyte mtDNA copy number in black South African HIV infected individuals receiving d4T. No effect of TDF on mtDNA copy number was observed. This study shows that the low dose d4T regimen produced a fall in mtDNA copy number similar to that observed with the standard d4T dose but greater than that seen with TDF. This finding is consistent with the results of other studies performed in non-black populations who had developed peripheral lipoatrophy at the time of sampling and after a longer duration of anti-retroviral therapy (Nolan *et al*, 2003. Schooley *et al*, 2002. Shikuma *et al*, 2001. Mallal *et al*, 2001. Buffet *et al*, 2005. Stankov *et al*, 2010. Boothby *et al*, 2009). Our results therefore suggest that mitochondrial pathology precedes changes in body fat distribution and occurs very early after the initiation of HAART.

This study also provides evidence of a dose response effect in that higher doses of d4T caused a greater loss of mtDNA after four weeks of HAART. Thus, d4T at a dose of 30 mg and 40 mg produced a greater fall in mtDNA than TDF, whereas the 20 mg dose of d4T was associated with a non-significant decrease in mtDNA, when compared to the TDF arm.

Despite the significant depletion in mtDNA, our study did not demonstrate a significant effect of d4T with regards to the expression of genes associated with mitochondrial energy

**Table 2: Comparison of the three arms with changes in relative gene expression in relation to housekeeping genes, markers of inflammation, lipid and glucose metabolism parameters from baseline at week 4:**

| Characteristic | Standard dose d4T (30 mg/40 mg) (N=20) | Low dose d4T (20 mg/30 mg) (N=20) | TDF (300 mg) (N=20) |
|---|---|---|---|
| **Genes involved in mitochondrial energy metabolism** | | | |
| **COX 4** | -0.01±0.34 | 0.04±0.49 | 0.07±0.54 |
| **COX 3** | -0.11±0.39 | 0.01±0.37 | 0.13±0.34 |
| **MTCYB** | -0.20±0.43* | -0.11±0.50 | 0.16±0.42 |
| **Nuclear genes involved in the regulation of mitochondrial transcription** | | | |
| **NRF-1** | -0.21±0.32* | 0.00±0.51 | 0.11±0.26 |
| **PGC-1α** | -0.23±0.75 | -0.03±0.36 | -0.02±0.57 |
| **TFAM** | -0.05±0.23 | 0.08±0.26 | 0.02±0.24 |
| **Genes involved in lipid metabolism** | | | |
| **LEP** | 0.09±0.34 | 0.09±0.40 | -0.06±0.36 |
| **LPL** | 0.06±0.22 | 0.08±0.29 | -0.01±0.20 |
| **Inflammatory markers** | | | |
| **hs-CRP (pg/L)** | 0.22 (0.39) | 0.15 (0.43) | 0.11 (0.33) |
| **Leptin (pg/ml)** | 0.18 (2.62) | 0.59 (2.20) | -0.13 (2.89) |
| **Adiponectin (pg/ml)** | 0.86 (5.29) | 2.12 (5.33) | 1.53(6.52) |
| **Markers of lipid and glucose metabolism** | | | |
| **C-peptide (nmol/ml)** | 0.00 (0.20) | -0.10 (0.10) | 0.00 (0.20) |
| **Insulin (mU/L)** | -0.25 (5.00) | 0.05 (4.70) | -0.60 (3.30) |
| **Total cholesterol (mmol/L)** | 0.60 (0.65) | 0.60 (1.00) | 0.60 (0.60) |
| **HDL cholesterol (mmol/L)** | 0.10 (0.25) | 0.20 (0.30) | 0.20 (0.30) |
| **Triglycerides (mmol/L)** | 0.20 (0.40) | 0.10 (0.30) | 0.20 (0.30) |
| **LDL cholesterol (mmol/L)** | 0.35 (0.75) | 0.40 (0.40) | 0.10 (0.70) |
| **Glucose (mmol/L)** | 0.30 (0.70) | 0.00 (1.10) | 0.20 (1.00) |
| **HOMA** | -0.05 (1.12) | -0.08 (0.88) | -0.17 (0.69) |

Data expressed as means ± SD except for markers of inflammation, lipid and glucose metabolism which are expressed as median (IQR); *p<0.05 versus TDF (300 mg).

metabolism and biogenesis, apart from *MTCYB* and *NRF-1*, although a step-wise decrease in some of the target genes – *COX 4, COX 3, MTCYB* and *NFF-1*, suggested toxicity related to a dose response, these trends did not reach statistical significance possibly as a consequence of the low sample size. However, these findings are in contrast to those of Mallon *et al.* who found significant reductions in gene expression without significant depletion of mtDNA after two weeks of HAART although notably, they compared d4T with zidovudine (AZT) and their study was in HIV negative patients (Mallon *et al*, 2005). Significant changes in the expression levels of *NRF1* and *MTCYB* were observed in the subjects given the standard d4T dose but not in those receiving the low dose therapy. *NRF1* is an important regulator of mitochondrial biogenesis and function. A master transcription factor, *NRF1* binds the regulatory regions of genes encoding subunits of the respiratory complex and various constituents of the mtDNA transcription and replication machinery. In addition, it serves to coordinate nucleo-mitochondrial activities by regulating mitochondrial and cytosolic enzymes (Scarpulla et *al*, 2008). These results suggest that downregulation of *NRF1* is an early event in NRTI toxicity. This change in *NRF1* expression is then cascaded to the factors which are under its control, with effects that are only observed much later in the time course of HAART (Pace *et al*, 2003. Sievers *et al*, 2009. Boothby *et al*, 2009).

Similar to our results, changes in *MTCYB* expression have been observed in other studies (Kim *et al*, 2008. Stankov *et al*, 2010). These changes may be associated with increased oxidative stress and apoptosis related to mitochondrial depletion (Kim *et al*, 2008. Komarov *et al*, 2008). The HIV-1 infection itself can also lead to alterations in gene expression for mitochondrial proteins and may have played a role here. The expression of several nuclear and mtDNA-encoded genes for mitochondrial proteins have been shown to be reduced in adipose tissue from HIV-1-infected patients who were not on antiretroviral therapy (Casula *et*

*al*, 2005. Giralt *et al*, 2006, Garrabou *et al*, 2011). In a recent study, an African mtDNA subhaplogroup L1c was implicated for the first time in susceptibility to NRTI associated toxicity. Studies comparing mitochondrial toxicity in African versus Caucasian subjects are limited. This is because of the complexities associated with mtDNA from African population. As a result, not much is known about functional differences in African mitochondria due to mtDNA variation (Canter *et al*, 2010).

Our findings must be considered in light of the study's limitations. Firstly, the study was stopped prematurely because of the introduction of the new South African National Treatment Guidelines which may have reduced the statistical power of the study. Our participant sample size however, was much larger than other studies where only 20 and 15 samples were analysed, respectively (Mallon *et al*, 2005. Kim *et al*, 2008), and was still sufficient for us to be able to detect significant effects of HAART on mtDNA copy number and gene expression. Another possible limitation is the short duration of therapy (4 weeks) at the time of sampling. Other studies have shown significant mtDNA and mtRNA depletion in HIV infected patients on a longer duration of anti-retroviral therapy of months to years (Pace *et al*, 2003. Sievers *et al*, 2009). In this study, the fourth week was chosen to avoid the potential confounding effects of changes in diet, exercise or bodyweight.

Despite these limitations, our findings are strengthened by the fact that the laboratory technique used to determine mtDNA copy number was based on a well established protocol (Nolan *et al*, 2003) that has been standardized and extensively critiqued (Cote *et al*, 2011). Another important strength of this study was the adoption of the minimum information for publication of quantitative real-time PCR experiments (MIQE) guidelines (Bustin *et al*, 2009) with the normalization of gene expression to multiple reference genes (Hellemans *et al*, 2007).

The findings of this study are important as previous trials in Europe, United States and Australia did not examine the effects of mitochondrial changes in an African population (Mallal *et al*, 2001. Shikuma *et al*, 2001. Schooley *et al*, 2002. Pace *et al*, 2003. Buffet *et al*, 2005. Mallon *et al*, 2005. Milinkovic *et al*, 2007. Kim *et al*, 2008. Boothby *et al*, 2009. Sievers *et al*, 2009. Stankov *et al*, 2010). Our study is the first randomized trial assessing the mitochondrial pathogenesis of NRTI-associated toxicities in an African population.

In conclusion, this study demonstrates an early association between mitochondrial depletion and d4T therapy in the black South African population and shows that TDF has a minimal effect on mitochondrial numbers. The expression levels of only 2 of 8 adipocyte genes were significantly affected by d4T therapy when compared with TDF, and this only with the standard dose (30 and 40 mg). These results support the new South African HAART guidelines which have evolved since 2004, with TDF 300 mg currently being recommended as first line therapy (National Department of Health South Africa, 2010). However, minimal effects on gene expression were noted with low dose (20-30 mg) d4T. Therefore, further larger studies using this regimen should be initiated, particularly in light of data showing that this dose is effective and safe (Hill *et al*, 2007. Milinkovic *et al*, 2007) and furthermore, it is unlikely that d4T will ever be completely phased out of use in countries with limited financial resources.

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## 3.7. References

Boothby M, McGee KC, Tomlinson JW, *et al.* Adipocyte differentiation, mitochondrial gene expression and fat distribution: differences between zidovudine and tenofovir after 6 months. *Antivir Ther.* 2009; **14(8)**:1089-100.

Boulle A, Orrell C, Kaplan R, *et al.* Substitution due to antiretroviral toxicity or contraindication in the first 3 years of antiretroviral treatment in a large South African cohort. *Antivir Ther* 2007; **12**:753-60.

Buffet M, Schwarzinger M, Amellal B, *et al.* Mitochondrial DNA depletion in adipose tissue of HIV-infected patients with peripheral lipoatrophy. *J Clin Virol.* 2005; **33(1)**:60-4.

Bustin SA, Benes V, Garson JA, *et al*. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem.* 2009; **55(4)**:611-622

Canter JA, Robbins GK, Selph D, Clifford DB, Kallianpur AR, Shafer R, *et al*. African mitochondrial DNA subhaplogroups and peripheral neuropathy during antiretroviral therapy. *J Infect Dis* 2010; **201(11)**:1703-7.

Cote HC, Gerschenson M, Walker UA, et al. Quality assessment of human mitochondrial DNA quantification: MITONAUTS, an international multicentre survey. *Mitochondrion* 2011; **11:520**-527

Gallant JE, Staszewski S, Pozniak AL, *et al*. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial. *JAMA* 2004; **292(2)**:191-201.

Hellemans J, Mortier G, De Paepe A, Speleman F, Vandesompele J. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome Biol.* 2007; **8(2)**:R19.

Hill A, Ruxrungtham K, Hanvanich M, *et al*. Systematic review of clinical trials evaluating low doses of stavudine as part of antiretroviral treatment. *Expert Opin. Pharmacother* 2007; **8(5)**:679-688.

Kim MJ, Jardel C, Barthélémy C, *et al*. Mitochondrial DNA content, an inaccurate biomarker of mitochondrial alteration in human immunodeficiency virus-related lipodystrophy. *Antimicrob Agents Chemother.* 2008; **52(5)**:1670-6.

Komarov AP, Rokhlin OW, Yu CA, Gudkov AV. Functional genetic screening reveals the role of mitochondrial cytochrome b as a mediator of FAS-induced apoptosis. *Proc Natl Acad Sci U S A.* 2008; **105(38)**:14453-8

Mallal SA, Hammond EL, Martin A, Taylor L, John M, Nolan DA. Mitochondrial DNA depletion, assessed by real-time PCR-based quantitative assay, in subcutaneous fat of HIV-infected patients: evidence for a role in the etiology of fat wasting. 4th International Conference on Nutrition and HIV Infection. Cannes, France, 19–21 April 2001, Abstract O7.

Mallon PW, Unemori P, Sedwell R, *et al*. In Vivo, Nucleoside Reverse –Transcriptase Inhibitors Alter Expression of Both Mitochondrial and Lipid Metabolism Genes in the Absence of Depletion of Mitochondrial DNA. *JID* 2005; **191**:1686-1696.

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28**: 412-419

Menezes CN, Maskew M, Sanne I, Crowther NJ, Raal FJ. A longitudinal study of stavudine-associated toxicities in a large cohort of South African HIV infected subjects. *BMC Infect Dis.* 2011; **11**:244.

Milinkovic A, Martinez E, López S, *et al.* The impact of reducing stavudine dose versus switching to tenofovir on plasma lipids, body composition and mitochondrial function in HIV-infected patients. *Antivir Ther* 2007; **12(3)**:407-15.

National Department of Health South Africa 2010. Clinical guidelines for the Management of HIV & AIDS in adults and adolescents: http://www.health-e.org.za/documents/0ba405d9042e557a9fb1987adb68cb97.pdf. Last accessed on the 31/05/2012.

National Department of Health. National Antiretroviral Treatment Guidelines, First Edition 2004: http://www.doh.gov.za/docs/misc/2004/sec1.pdf. Last accessed on the 31/05/2012.

Nolan D, Hammond E, Martin A, *et al.* Mitochondrial DNA depletion and morphologic changes in adipocytes associated with nucleoside reverse transcriptase inhibitor therapy. *AIDS* 2003; **17**:1329-1338.

Pace CS, Martin AM, Hammond EL, Mamotte CD, Nolan DA, Mallal SA. Mitochondrial proliferation, DNA depletion and adipocyte differentiation in subcutaneous adipose tissue of HIV-positive HAART recipients. *Antivir Ther* 2003; **8**:323-331.

Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services. http://aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf. Last accessed on the 06/08/2012.

Rosen S, Long L, Fox M, Sanne I. Cost and cost-effectiveness of switching from stavudine to tenofovir in first-line antiretroviral regimens in South Africa. *J Acquir Immune Defic Syndr* 2008; **48(3)**:334-44.

Sanne I, Westreich D, Macphail AP, Rubel A, Majuba P, Van Rie A. Long term outcomes of antiretroviral therapy in a large HIV/AIDS care clinic in urban South Africa: a prospective cohort study. *J Acquir Immune Defic Syndr*. 2009; **12**:753-760.

Scarpulla RC. Transcriptional paradigms in mammalian mitochondrial biogenesis and function. *Physiol Rev.* 2008; **88(2)**:611-38.

Schooley RT, Ruane P, Myers RA, *et al*. Tenofovir DF in antiretroviral-experienced patients: results from a 48-week, randomized, double-blind study. *AIDS* 2002; **16**:1257-63.

Shikuma CM, Hu N, Milne C, *et al.* Mitochondrial DNA decrease in subcutaneous adipose tissue of HIV-infected individuals with peripheral lipoatrophy. *AIDS* 2001; **15(14)**:1801-9.

Sievers M, Walker UA, Sevastianova K, *et al*. Gene Expression and Immunohistochemistry in Adipose Tissue of HIV Type 1-Infected Patients with Nucleoside Analogues Reverse-Transcriptase Inhibitor-Associated Lipoatrophy. *JID* 2009; **200**:252-62.

Stankov MV, Lücke T, Das AM, Schmidt RE, Behrens GM. Mitochondrial DNA depletion and respiratory chain activity in primary human subcutaneous adipocytes treated with

nucleoside analogue reverse transcriptase inhibitors. *Antimicrob Agents Chemother.* 2010; **54(1)**:280-7.

WHO: Antiretroviral therapy for HIV infection in adults and adolescents: Recommendations for a public health approach (2010 version). http://whqlibdoc.who.int/publications/2010/9789241599764_eng.pdf. Last accessed on the 31/05/2012.

Writing Group, Williams I, Churchill D, et al. British HIV Association guidelines for the treatment of HIV-1-positive adults with antiretroviral therapy 2012. *HIV Med.* 2012; **13**: 1-6.

**CHAPTER 4**

# 4. A RANDOMIZED CLINICAL TRIAL COMPARING METABOLIC PARAMETERS AFTER 48 WEEKS OF STANDARD AND LOW DOSE STAVUDINE, AND TENOFOVIR THERAPY IN HIV-INFECTED SOUTH AFRICAN PATIENTS.

## 4.1. Abstract

**Objective:**

Low dose stavudine therapy may have a lower toxicity profile compared to standard dose. A randomized controlled trial comparing these two doses with tenofovir was performed to assess the effects on anthropometry, markers of inflammation, lipid and glucose metabolism in black South African patients.

**Methods:**

Sixty patients were randomized 1:1:1 to either standard (30-40 mg) or low dose stavudine (20-30 mg) or tenofovir (300 mg), each combined with lamivudine and efavirenz for 48 weeks. Anthropometry, markers of inflammation, lipid and glucose metabolism were assessed using standard techniques.

**Results:**

In all three treatment arms, there was a significant increase in lipid levels over the study period. At 48 weeks, fasting glucose ($P<0.005$) and HOMA ($P<0.05$) increased significantly in the standard dose stavudine arm as did insulin and C-peptide levels in both the standard and low dose stavudine arms. At week 48, a significant decrease ($P<0.05$) in adiponectin was noted in the standard dose stavudine arm, but an increase ($P<0.005$) in the tenofovir arm. In both the stavudine arms significant increases in anthropometric measures occurred at 24

weeks but these decreased by week 48. Mitochondrial toxicities occurred in both the stavudine arms. Immunological and virological outcomes were similar for all three arms.

**Conclusions:**

This study highlights the occurrence of metabolic abnormalities with both stavudine and tenofovir treatment. Awareness of the potential increased cardiovascular risk should be of concern with the use of both these therapies.

## 4.2. Introduction

The introduction of highly active antiretroviral therapy (HAART) has significantly reduced morbidity and mortality in human immunodeficiency virus (HIV)-positive patients (Hogg *et al*, 1997. Palella *et al*, 1998). Attention has now focused on the emergence of metabolic complications in HIV-positive patients after long-term HAART (Carr *et al*, 1999. Carr *et al* 2000. Galli *et al*, 2002. Friis-Møller *et al*, 2003. Menezes *et al*, 2011).

Increased risk of myocardial infarction and rates of dyslipidaemia, insulin resistance and diabetes, and alterations in body fat distribution have been described with the use of HAART (Carr *et al*, 1999. Carr *et al* 2000. Galli *et al*, 2002. Friis-Møller *et al*, 2003. Menezes *et al*, 2011). Changes in leptin and adiponectin levels that are observed in HIV infection may play a major role in the pathogenesis of HIV/HAART-related metabolic syndrome (Lindegaard *et al*, 2004. Palios *et al*, 2012). Furthermore, elevated levels of high sensitivity C-reactive protein (hs-CRP), an inflammatory marker, which have been observed in HIV-infected male patients (Reingold *et al*, 2008), are associated with an increased risk of cardiovascular events in the general population (Rutter *et al*, 2004. Pai *et al*, 2004) as well as in HIV-positive patients (Triant *et al*, 2009).

Previously, the etiology of these metabolic abnormalities has been attributed to the use of protease inhibitors (PIs) (Carr *et al*, 1999). More recently, nucleoside reverse transcriptase inhibitors (NRTIs) have also been implicated, particularly stavudine (Boulle *et al*, 2007. Mutimura *et al*, 2007. van Griensven *et al*, 2010. Menezes *et al*, 2011). Stavudine continues to be used as alternative first-line treatment in developing countries despite the change in the WHO antiretroviral therapy guidelines for treating patients with HIV infection (WHO, 2010). Stavudine was used as first line HIV therapy in South Africa until 2010 and replaced with tenofovir (National Department of Health, South Africa, 2004 & 2010), but it continues to be used because of shortages of tenofovir and abacavir that occur intermittently at health facilities across South Africa (Schowalter *et al*, 2012). Compared to stavudine, tenofovir has fewer side effects and increases lipid levels to a lesser degree, with no difference in virological response (Gallant *et al*, 2004).

The recommended dose for stavudine is 40 mg twice daily, but studies have shown that lower doses (20 or 30 mg twice daily) diminishes toxicity while maintaining immunological efficacy (Hill *et al*, 2007. Milinkovic *et al*, 2007. McComsey *et al*, 2008). Therefore, in resource limited countries where the switch away from stavudine to the more expensive tenofovir has not yet occurred, low dose stavudine could still be used, allowing more patients to be initiated on to treatment.

To determine whether low dose stavudine therapy was as effective and safe as tenofovir in a resource-poor environment, we performed an open-label, randomized controlled trial. Biochemical changes and clinical outcomes for patients on either low or standard dose stavudine were compared to those patients receiving tenofovir.

We present here data on anthropometry and body fat distribution, serological markers of inflammation, and lipid and glucose metabolism measured over 48 weeks of follow-up in a cohort of black South African HIV-positive patients.

## 4.3. Methods

### 4.3.1. Study population

HIV-1 infected individuals aged 18 years or older with a CD4+ cell count below 200 cells/$mm^3$ were enrolled in to this study at the Themba Lethu HIV Clinic at the Helen Joseph Hospital, Johannesburg, South Africa. Inclusion criteria were: an absolute neutrophil count (ANC) ≥750/mm, a hemoglobin ≥7.0 g/dL, a platelet count ≥50,000/$mm^3$, a creatinine clearance of ≥60 mL/min calculated using the Cockcroft-Gault formula (Cockcroft *et al*, 1976), and an alanine and aspartate aminotransferase, an alkaline phosphatase and a total bilirubin of ≤ 2.5 x upper limit of normal. Patients of reproductive age had to have a negative serum or urine pregnancy test within 45 days prior to study entry, and had to be willing to use at least one reliable form of contraception. Exclusion criteria were: breastfeeding or pregnancy, receiving antiretroviral drugs including occupational or sexual post-exposure prophylaxis, other than single-dose nevirapine within the previous six months.

Patients were seen at screening and at week 0, 4, 24 and 48. Routine evaluations were taken at each time point, whilst markers of inflammation, lipid and glucose metabolism were measured at week 0, 4 and 48. These evaluations included a review of adverse events and concomitant medications, complete or symptom-directed physical examination, height and weight, hematology and chemistry profiles, calculated creatinine clearance, CD4+ cell count, plasma HIV-1 RNA and study drug accountability. Dual-energy radiographic absorptiometry (DEXA) scanning was performed at baseline and week 48.

Approval was received from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand and the SA Medicines Control Council (MCC). This study was compliant with the revised CONSORT statement guidelines (www.consort-statement.org).

### 4.3.2. Anti-retroviral therapy

As previously described (Menezes *et al*, 2012), patients were assigned in random 1:1:1 permuted blocks of five to receive lamivudine and efavirenz in combination with a) standard dose stavudine (30 mg if weight < 60 kg or 40 mg if weight > 60 kg) according to the then SA guidelines (National Department of Health, South Africa, 2004); b) low-dose stavudine (20 mg if weight < 60 kg or 30 mg if weight > 60 kg); or c) tenofovir 300 mg. The definition of the standard and low dose of stavudine is the same as that used in several other studies (Hill *et al*, 2007). Coded identity numbers with the arm allocated were sealed in sequentially numbered envelopes. At enrolment, a member of the data collection team unsealed the next envelope to assign the drug arm allocated to the patient. Staff analyzing patient samples were blinded to the treatment assigned to each patient.

### 4.3.3. Blood measurements

Fasting serum was taken for the measurement of total cholesterol, high density lipoprotein (HDL) -, and low density lipoprotein (LDL)-cholesterol, triglycerides and glucose (Roche Integra analyzer 400, Roche Diagnostics, Mannheim, Germany), C-peptide and insulin (Immulite1000 analyzer Diagnostics Corp., Los Angeles, CA, USA), and leptin, adiponectin and hs-CRP (Fluorokine®-Multianalyte profiling (MAP) kit, RnD Systems, Inc., Minneapolis, MN, USA). A full blood count was performed and creatinine, liver function

tests, viral load (Roche Cobas Amplicor, Indianapollis, IN) and CD4+ count (Beckman Coulter flow cytometry, Miami, FL, USA) were measured. Insulin resistance was assessed using the homeostasis model assessment (HOMA) method (Matthews *et al*, 1985).

### 4.3.4. Anthropometry and body composition measurements

Height and weight were measured using regularly calibrated instruments. Circumferences of the mid arm, chest, waist, hip and mid-thigh were obtained using a Gulick II measuring tape. Skinfolds of the triceps, biceps, subscapular, and suprailiac were measured using a Harpenden skin fold caliper. For all anthropometry measurements, two independent measurements were taken. If the first two measurements were outside of the acceptable range of variance for a particular body area, then a third measurement was taken. The mean of the two closest measures was then used in analysis. Total lean and fat mass were measured with a Hologic whole-body, dual-energy radiographic absorptiometry (DEXA) scanner (Hologic Inc., Waltham, MA, USA) and software, version 12.4. Staff analyzing performing the DEXA scans were blinded to the treatment assigned to each patient but those performing the anthropometry measurements were not.

### 4.3.5. Statistical analysis

Data that could be normalized was log transformed to normality and analyzed using parametric statistical analyses, whilst the remaining variables were analyzed using non-parametric statistical tests. With a power of 80%, $\alpha = 0.05$, and equal sample size per group, we required 28 patients per treatment arm, assuming that 70% of patients exposed to stavudine and 30% exposed to tenofovir would show features of mitochondrial toxicity (Nolan *et al*, 2003). However, the trial was stopped early after a data safety and monitoring

board (DSMB) analysis of the first 60 patients showed that the group given stavudine at both standard and low doses produced greater mitochondrial depletion than in the arm receiving tenofovir (Menezes *et al*, 2012). Furthermore, the DSMB considered that there were compelling ethical grounds to stop the high dose stavudine because the new SA guidelines were shortly to be implemented (National Department of Health, South Africa 2010), and tenofovir had become freely available. At that point, the four patients who were still on 40 mg of stavudine were switched to tenofovir. Those patients who switched therapy or who, for any other reason, had to prematurely leave the study were excluded from the final analysis. Therefore, the trial was not adequately powered to demonstrate non-inferiority in terms of efficacy. Data were analyzed using STATISTICA® version 10 (StatSoft, Inc., Tulsa, OK, USA). Results were summarized using proportions and medians with interquartile ranges. Comparisons were made at different time intervals within each arm using the Wilcoxon matched paired test or Students paired t test, whilst comparisons across arms were performed using ANOVA for baseline levels. The week 4 and week 48 data were analysed using ANOVA and ANCOVA, with adjustment for baseline levels. This adjustment had no effect on the outcomes.

## 4.4. Results

### 4.4.1. Baseline characteristics of the study population

Figure 1 shows the cohort profile: 60 eligible patients were randomized 1:1:1 to the three treatment arms between September 2008 and December 2009.

There was a predominance of females (85%) in this study and 59 of the 60 patients were black (Table 1). All patients were clinically asymptomatic with 78% of the patients being WHO stage 1.

### 4.4.2. Lipid metabolism

There were significant increases in both total and HDL-cholesterol levels from baseline to weeks 4 and 48 for all three arms (Table 2). Triglyceride levels increased significantly at week 4 in the standard stavudine and tenofovir arms only. The LDL- cholesterol increased significantly in the low dose stavudine arm at weeks 4 and 48, but increased only at week 4 for the standard dose stavudine arm and at week 48 for the tenofovir arm (Table 2). The percentage of patients with hypercholesterolemia, hypertriglyceridemia, elevated LDL and reduced HDL in each arm, as defined by NCEP-ATP III guidelines (2002), were calculated at week 48 and no significant differences were found.

### 4.4.3. Glucose metabolism

A decrease in C-peptide levels was noted at week 4 and a significant increase by week 48 for the low dose stavudine arm, with a significant increase at week 48 also noted in the standard stavudine arm.

When comparing the fasting insulin levels in all patients, an increase was noted at week 48 in both stavudine arms but not in the tenofovir arm. Significant differences were noted when comparing the change in insulin levels at week 48 from baseline between the standard dose stavudine and tenofovir arm (Table 3).

When comparing the HOMA scores, a significant increase was noted at week 48 for the standard stavudine arm only.

### 4.4.4. Inflammatory markers

There was a significant increase in the levels of hs-CRP at week 4 in the standard dose stavudine arm and in the leptin levels at week 4 for the low dose stavudine arm, and at week

**Figure 1: Profile of the Trial Cohort**

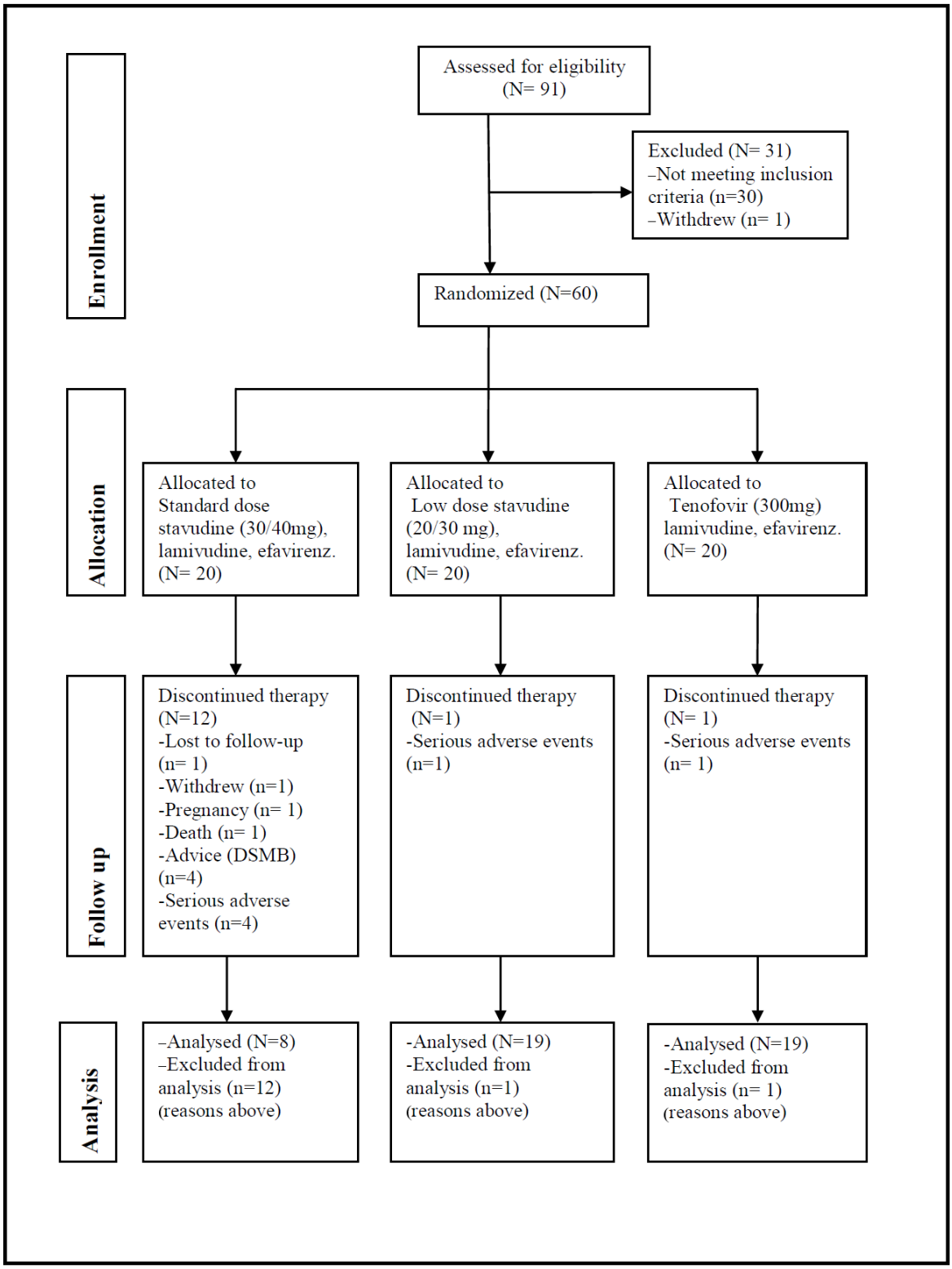

**Table 1. Patient characteristics at the commencement of HAART**

| Characteristic | Standard dose stavudine (30 mg/40 mg) (N=20) | Low dose stavudine (20 mg/30 mg) (N=20) | Tenofovir (300 mg) (N=20) |
|---|---|---|---|
| **African race** | 20 (100%) | 19 (95%) | 19 (95%) |
| **Gender, Female** | 16 (80%) | 18 (90%) | 17 (85%) |
| **Age (years)** | 31.0 (11.0) | 34.0 (12.0) | 33.0 (10.0) |
| **BMI ($kg/m^2$)** | 25 (5) | 24 (7) | 24 (6) |
| **Hemoglobin (g/d)** | 12.3 (2.7) | 12.3 (2.2) | 12.0(2.0) |
| **GGT( IU/L )** | 24 (24) | 30 (11) | 22 (11) |
| **ALT( IU/L)** | 21 (21) | 20 (15) | 18 (12) |
| **Creatinine clearance (ml/min)** | 108 (17) | 108 (25) | 99 (40) |

Data are expressed as median (IQR) except for race and gender which are expressed as N (%). BMI: Body Mass Index, GGT: gamma-glutamyltransferase, ALT: alanine aminotransferase

48 for the tenofovir arm (Table 2). A significant decrease in the adiponectin levels was noted in the standard dose stavudine arm at week 48, and an increase at weeks 4 and 48 in the tenofovir arm. The week 48 adiponectin level was significantly higher in the tenofovir than the standard dose stavudine arm, and this was similar when comparing the change in adiponectin levels over the course of the study between the standard dose stavudine and tenofovir arms (Table 3).

### 4.4.5. Immunological and virological efficacy

No differences were noted at week 24 in CD4+ cell counts and viral loads. There was a significant increase in the CD4+ cell counts in all three arms at week 48 when compared to baseline (Table 2).

**Table 2: Comparison of the markers of inflammation, glucose and lipid metabolism at different time intervals:**

| Characteristic | Standard dose stavudine (30 mg/40 mg) | | | Low dose stavudine (20 mg/30 mg) | | | Tenofovir (300 mg) | | |
|---|---|---|---|---|---|---|---|---|---|
| **Time (week)** | **0** | **4** | **48** | **0** | **4** | **48** | **0** | **4** | **48** |
| **N** | 20/20 | 20/20 | 8/20 | 20/20 | 20/20 | 19/20 | 20/20 | 20/20 | 19/20 |
| **Total Cholesterol (mmol/L)** | 3.20 (1.35) | 4.10*** (1.35) | 4.35* (1.20) | 3.40 (1.10) | 4.10** (1.70) | 4.50*** (1.50) | 3.80 (1.10) | 4.20** (1.10) | 4.60*** (0.7) |
| **HDL-Cholesterol (mmol/L)** | 0.85 (0.40) | 1.00* (0.50) | 1.35* (0.55) | 0.80 (0.45) | 1.00** (0.50) | 1.25*** (0.60) | 0.80 (0.40) | 1.10** (0.60) | 1.30*** (0.40) |
| **Triglycerides (mmol/L]** | 0.70 (0.35) | 0.90** (0.45) | 0.95 (0.20) | 0.80 (0.70) | 0.80 (0.60) | 1.10 (0.70) | 0.80 (0.30) | 0.90* (0.40) | 0.80 (0.30) |
| **LDL-Cholesterol (mmol/L)** | 1.90+ (1.05) | 2.60*** (1.15) | 2.35 (1.20) | 2.15 (1.00) | 2.40** (1.20) | 2.70*** (1.70) | 2.50 (0.70) | 2.80 (1.25) | 2.85* (0.70) |
| **C-peptide (nmol/ml)** | 0.40 (0.30) | 0.50 (0.20) | 0.50* (0.20) | 0.50 (0.20) | 0.40* (0.25) | 0.60* (0.30) | 0.40 (0.25) | 0.45 (0.25) | 0.50 (0.10) |
| **Insulin (mU/L)** | 2.85 (4.05) | 3.65 (3.00) | 3.95* (2.60) | 3.75 (4.65) | 3.10 (2.80) | 6.25* (5.50) | 3.60 (3.00) | 3.00 (2.65) | 4.80 (3.90) |
| **Glucose (mmol/L)** | 3.95 (0.75) | 4.40 (0.40) | 4.40* (0.70) | 4.20 (0.80) | 4.30 (0.70) | 4.40 (0.70) | 4.20 (0.60) | 4.35 (0.70) | 4.60 (0.40) |
| **HOMA** | 0.54 (0.96) | 0.69 (0.81) | 0.83* (0.59) | 0.7 (1.05) | 0.51 0.595 | 1.25 (1.30) | 0.73 (0.60) | 0.59 (0.56) | 0.91 (0.88) |
| **hs-CRP (pg/L)** | 0.80 (0.55) | 0.84* (0.75) | 0.79 (0.35) | 0.66 (0.94) | 0.86 (0.82) | 0.94 (0.53) | 0.75 (0.61) | 0.83 (0.73) | 0.93 (0.56) |
| **Leptin (pg/ml)** | 2.69+ (15.02) | 4.09 (10.08) | 1.84 (4.43) | 3.17 (4.33) | 4.07* (6.07) | 3.77 (7.25) | 5.52 (8.49) | 4.79 (12.92) | 11.82* (27.45) |
| **Adiponectin (pg/ml)** | 11.78 (6.58) | 13.53 (4.08) | 7.21* + (3.86) | 11.75 (8.43) | 15.90 (11.14) | 12.18 (14.73) | 13.78 (11.82) | 16.07* (12.47) | 17.42** (10.75) |
| **CD4 count (cells/mm$^3$)** | 155 (94) | ND | 285 * (52) | 169 (78) | ND | 286 ** (162) | 135 (62) | ND | 293*** (160) |
| **Viral load, (copies/ml )** | 15000 (88650) | ND | 102* (2) | 42500 (97500) | ND | 101 *** (1) | 18000 (76000) | ND | 101*** (1) |
| **Viral load, <400copies/ml, N (%)** | - | - | 7/8 (88%) | - | - | 18/18(100%) | - | - | 18/19(95%) |
| **Viral load, <50copies/ml, N (%)** | - | - | 7/8(88%) | - | - | 18/18(100%) | - | - | 16/19(84%) |

Data is expressed as median (IQR); *p<0.05; **p<0.005; *** p<0.0005 versus week 0; + p<0.05 versus same time point in tenofovir arm; ND-Not done.

**Table 3: Changes from baseline to week 48 in markers of glucose and lipid metabolism, inflammatory markers, and anthropometric measurements in each of the 3 study arms:**

| Characteristic | Standard dose stavudine (30 mg/40 mg) (N=20) | Low dose stavudine (20 mg/30 mg) (N=20) | Tenofovir (300 mg) (N=20) |
|---|---|---|---|
| **N** | 8/20 | 19/20 | 18/20 |
| **C-peptide (nmol/ml)** | 0.1 (0.3) | 0.1 (0.3) | 0.1 (0.3) |
| **Insulin (mU/L)** | 2.3 (4.4)* | 1.4 (5.3) | 0.8 (5.1) |
| **Glucose (mmol/L)** | 0.6 (1.3) | 0.3 (0.7) | 0.5 (0.8) |
| **HOMA** | 0.5 (1.0) | 0.3 (1.2) | 0.2 (1.2) |
| **Total Cholesterol (mmol/L)** | 0.7 (1.7) | 1.4 (2.5) | 0.7 (1.6) |
| **HDL-Cholesterol (mmol/L)** | 0.3 (0.8) | 0.4 (0.8) | 0.5 (0.9) |
| **Triglycerides (mmol/L)** | 0.2 (0.6) | 0.1 (0.6) | 0.1 (0.3) |
| **LDL-Cholesterol (mmol/L)** | 0.2 (0.9) | 0.7 (1.5) | 0.2 (0.8) |
| **hs-CRP (pg/L)** | 0.1 (0.4) | 0.1 (0.9) | 0.1 (0.5) |
| **Leptin (pg/ml)** | 0.8 (4.7) | 1.4 (11.2) | 1.9 (13) |
| **Adiponectin (pg/ml)** | -0.6 (6.8)* | 0.1 (12.4) | 3.6 (3.5) |
| **BMI ($kg/m^2$)** | 0.5 (1.8) | 1.3 (2.6) | 0.5 (4.0) |
| **Mid-arm (cm)** | 1.5 (2.5) | 0.0 (3.0) | 0.5 (5.1) |
| **Waist (cm)** | 2.0 (7.0) | 4.0 (9.0) | 2.8 (8.6) |
| **Hip (cm)** | 3.0 (11) | 1.0 (6.8) | 1.4 (10.8) |
| **Mid-thigh (cm)** | 2.0 (6.0) | 2.0 (5.3) | 2.0 (6.0) |
| **Triceps (mm)** | 4.0 (17.6) | 0.1 (11.4) | 6.0 (24.3) |
| **Subscapular (mm)** | 3.0 (17.6) | 3.6 (6.9) | -0.9 (16.4) |
| **Suprailiac (mm)** | 3.6 (18.6) | 5.8 (11.5) | 2.1 (7.1) |
| **Trunk Fat (g)** | 1565.2 (3939.9) | 1467.1 (4172) | 470.2 (2935.9) |
| **Trunk % Fat** | 4.10 (11.8) | 3.20 (7.1) | 1.20 (7.3) |
| **Tot Limb Fat (g)** | 0.0 (5700.9) | -68.4 (2754.6) | 0.0 (3126.5) |
| **Tot Limb % Fat** | -1.5 (7.9) | -0.9 (3.9) | 0.0 (3.6) |

Data expressed as median (IQR); p value by Kruskal Wallis; *$p<0.05$ versus Tenofovir.

Similarly, there was a significant decrease in viral loads (≤ 400 copies/ml) noted in all three arms at week 48 when compared to baseline but number of patients who had virological suppression at week 48 did not differ significantly between treatment arms (Table 2).

An ITT analysis was performed for CD4 counts and viral loads at week 48 and no significant differences were noted.

### 4.4.6. Anthropometric measurements and body composition

There was a significant increase in the BMI of patients in the standard dose stavudine arm by weeks 4 and 24 and in the low dose stavudine arm by weeks 24 and 48; although no significant changes were observed for patients in the tenofovir arm (Table 4).

A significant increase was noted in the waist measurements at weeks 24 and 48 in both the standard and low dose stavudine arms but only at week 48 in the tenofovir arm. A significant increase in hip measurements was noted by week 24 in both stavudine arms but this was not observed with tenofovir. A similar trend was noted in the mid thigh measurements, but with an increase at week 48 for the tenofovir arm.

With regards to the skinfold measurements, a similar increasing trend was noted at week 24 for the two stavudine arms with a tendancy for measures to fall by week 48 to levels close to, or below those at baseline. For the tenofovir arm, there was an increase in skinfold measurements across the study period but changes were not statistically significant. (Table 4)

Exactly 47% of the patients in the standard stavudine dose arm, 40 % in the low dose stavudine arm, and 55% in the tenofovir arm ($P$=0.637) developed a BMI $\geq$ 25kg/m$^2$ at week 48.

### 4.4.7. Safety and therapy switching

There were 14 adverse events (AEs) in the standard stavudine dose arm, 17 AEs in the low dose stavudine arm and 12 AEs in the tenofovir arm. There was one case of renal dysfunction, in the tenofovir arm. There was one death on the standard stavudine dose arm due to a high grade B cell lymphoproliferative disorder. One patient was lost to follow up, one patient withdrew from the study and one patient fell pregnant and was withdrawn from the study.

With regards to mitochondrial AEs, there were three patients in the standard stavudine dose arm who developed a lactic acidosis while one patient developed a hyperlactatemia. In the low dose stavudine arm, three patients developed a peripheral neuropathy, of which one of these patients also had an associated hyperlactatemia and clinical features of lipoatrophy warranting switch in therapy.

Within the standard dose stavudine arm, 12 patients had to switch therapy for various reasons. At week 0, these subjects differed from those who did not change therapy in terms of higher measures of body fat, hsCRP and leptin levels. Notably, at baseline 66.7% of patients who switched therapy were overweight/obese compared to 12.5% of patients who did not switch therapy ($P<0.05$). A significant difference was noted when comparing the median (IQR) suprailiac skinfold thickness (43.3 mm (25.3) vs. 16 mm (12.9); $P< 0.005$), mean truncal % fat (32.98 ± 10.23 vs. 21.44 ± 9.93; $P <0.05$), hs-CRP (0.86 pg/L (0.53) vs. 0.54 pg/L (0.64); $P< 0.05$), and leptin levels ( 8.74 pg/ml (26.57) vs. 1.37 pg/ml (1.62); $P< 0.005$) for those who switched therapy in standard dose stavudine arm to those who did not.

### 4.5. Discussion

Despite the move away from stavudine to towards less toxic regimens, more than 1.55 million people were still receiving stavudine therapy by the end of 2012 (WHO, 2010). It is known that even though stavudine is cheaper and effective as initial therapy, the standard dose (30 or 40 mg) is associated with long term complications. However, studies have demonstrated excellent antiviral efficacy with a lower dose of 20 mg (Hill *et al*, 2007. Milinkovic *et al*, 2007. McComsey *et al*, 2008). Generic tenofovir is used as a non-stavudine first line therapy but is more expensive (Rosen *et al*, 2008). This study was a prospective, open-label randomized controlled trial designed to evaluate the *in vivo* effects of tenofovir

**Table 4: Comparison of the anthropometric measurements and body fat composition at different time intervals:**

| Characteristic | Standard dose stavudine (30 mg/40 mg) | | | | Low dose stavudine (20 mg/30 mg) | | | | Tenofovir (300 mg) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 weeks | 4 weeks | 24 weeks | 48 weeks | 0 weeks | 4 weeks | 24 weeks | 48 weeks | 0 weeks | 4 weeks | 24 weeks | 48 weeks |
| N | 20/20 | 20/20 | 19/20 | 8/20 | 20/20 | 20/20 | 20/20 | 19/20 | 20/20 | 20/20 | 20/20 | 19/20 |
| BMI (kg/m$^2$) | 24.82 (4.92) | 24.87* (4.62) | 25.84** (5.65) | 24.69 (4.64) | 23.45 (6.50) | 23.92 (6.20) | 25.48*** (5.50) | 23.79* (5.61) | 24.25 (5.68) | 24.08 (4.44) | 24.88 (5.32) | 25.06 (6.36) |
| Mid-arm (cm) | 28.75 (4.50) | 29.00 (3.75) | 30.5** (4.50) | 31.00* (2.50) | 28.75 (6.50) | 28.00 (7.00) | 30.00* (7.00) | 29.00 (5.50) | 29.00 (4.75) | 29.50 (4.75) | 30.00 (4.50) | 31.50 (8.00) |
| Chest (cm) | 81.75 (8.00) | 81.50 (6.50) | 83.00 (11.00) | 82.00 (12.00) | 79.00 (6.25) | 79.00 (11.50) | 79.50 (8.20) | 80.50* (7.00) | 79.75 (7.50) | 80.75 (10.50) | 81.00 (8.75) | 81.00 (10.00) |
| Waist (cm) | 82.00 (8.00) | 81.00 (9.00) | 83.00* (11.00) | 83.00* (14.50) | 75.75 (12.50) | 81.00 (13.00) | 80.50** (14.75) | 82.00** (13.00) | 78.75 (13.50) | 78.00 (12.50) | 83.75 (12.25) | 81.50* (15.00) |
| Hip (cm) | 100.50 (13.00) | 102.50 (12.00) | 105.00* (10.00) | 102.00 (11.50) | 99.50 (16.25) | 99.00 (21.00) | 101.50** (14.00) | 98.00 (9.00) | 100.00 (14.50) | 101.00 (16.00) | 103.50 (12.00) | 109.00 (16.00) |
| Mid-thigh (cm) | 57.00 (8.75) | 58.00 (6.75) | 59.50** (7.00) | 57.00 (7.00) | 55.25 (8.75) | 54.00 (13.00) | 56.00* (8.50) | 56.50 (5.00) | 59.50 (10.00) | 60.00 (13.50) | 61.25 (9.50) | 63.00* (12.00) |
| Triceps skinfold (mm) | 43.70 (37.80) | 49.80 (22.50) | 63.00* (33.00) | 43.40 (33.20) | 43.50 (29.70) | 51.00 (30.40) | 50.70** (27.10) | 39.80 (20.40) | 46.50 (29.90) | 46.10* (33.30) | 52.50 (27.00) | 54.00 (50.50) |
| Biceps skinfold (mm) | 16.00 (10.10) | 16.80 (8.20) | 18.60 (14.40) | 12.40 (9.00) | 18.00 (13.20) | 19.40 (15.00) | 18.00 (13.40) | 13.2* (10.80) | 15.10 (11.40) | 16.50 (9.40) | 20.10 (10.90) | 18.00 (10.80) |
| Subscapular skinfold (mm) | 32.80 (23.90) | 30.50 (22.00) | 36.00 (25.40) | 34.70 (17.70) | 25.80 (16.30) | 29.60 (18.00) | 31.80*** (18.60) | 29.00 (21.00) | 30.40 (16.90) | 32.90 (18.70) | 35.70 (20.00) | 36.60 (23.40) |
| Suprailiac skinfold (mm) | 27.00 (28.60) | 27.40 (26.90) | 27.60* (31.40) | 25.30 (14.80) | 25.80 (23.10) | 26.00 (26.10) | 30.30* (19.20) | 27.6* (18.00) | 28.60 (24.70) | 27.60 (25.30) | 30.00 (22.50) | 33.00 (28.40) |
| Trunk fat (g) | 17687.70 (11903.50) | ND | ND | 19759.00 (14212.70) | 17538.70 (13110.20) | ND | ND | 19490.45 (12170.20) | 23000.10 (10254.40) | ND | ND | 24924.90 (17053.00) |
| Trunk % fat | 28.10 (19.50) | ND | ND | 30.80 (19.60) | 29.40 (10.80) | ND | ND | 33.90 (12.80) | 34.60 (11.70) | ND | ND | 37.10 (18.00) |
| Tot al limb fat (g) | 16878.60 (12021.10) | ND | ND | 21512.15 (13510.75) | 16138.95 (16609.90) | ND | ND | 17378.30 (12214.90) | 21961.00 (10268.60) | ND | ND | 24105.20 (17061.50) |
| Total limb % fat | 28.90 (21.00) | ND | ND | 37.60 (23.30) | 30.40 (15.10) | ND | ND | 35.00 (13.70) | 35.70 (12.40) | ND | ND | 40.45 (17.60) |

Data is expressed as median (IQR); * p<0.05; **p<0.005; *** p<0.0005 versus week 0; ND-Not done.

against those of standard and low dose stavudine regimens, with the reference regimen based on the Gilead 903 trial by Gallant *et al* (Gallant *et al*, 2004). The Gilead 903 trial showed better outcomes and less adverse events attributed to mitochondrial toxicity in patients receiving tenofovir 300 mg when compared to patients receiving 40 mg stavudine given twice daily (Gallant *et al*, 2004). To our knowledge, this work presents the first trial to compare the use of these drugs in a black population.

Studies in treatment naïve HIV infected patients have shown that peripheral fat initially increases during the first few months of HAART, but decreases linearly thereafter and truncal fat increases around six months of therapy (Mallon *et al*, 2003. Magkos *et al*, 2011). Similarly, in the current study significant increases in the majority of anthropometric measurements were noted in the stavudine arms by six months with a drop towards one year of therapy, whilst such changes were seen in a minority of the anthropometric variables with tenofovir therapy.

This study demonstrated that insulin and C-peptide levels increased significantly in both stavudine arms, but not with tenofovir therapy. However, significant rises in glucose and HOMA levels were only observed with the standard stavudine dose. Previous research showed that the severity of lipoatrophy, among patients taking either stavudine or zidovudine, was associated with an increased risk of insulin resistance (De Wit *et al*, 2008).

Consistent with other studies (Savès *et al*, 2002. Gallant *et al*, 2004. Shlay *et al*, 2005), we demonstrated a significant increase in fasting lipids after one year of HAART, and this was noted within both stavudine arms and with tenofovir. Lipid abnormalities have been noted in HIV-1 infected patients even before the advent of antiretroviral therapy, with cholesterol levels being lower and triglyceride levels higher in HIV-infected compared to non-infected subjects (Constans *et al*, 1994). In the present study, the early increase of cholesterol levels

after only 4 weeks of therapy may be explained by the 'return to baseline' effect of commencing antiretroviral therapy, but this cannot explain the rise in triglyceride levels. Furthermore, dyslipidaemia has been widely reported with exposure to NRTIs, particularly with stavudine or zidovudine, and less so with tenofovir (Savès *et al*, 2002. Mallon *et al*, 2003. Gallant *et al*, 2004. Shlay *et al*, 2005. De Wit *et al*, 2008. Magkos *et al*, 2011). The observed effects might also be associated with the use of efavirenz. However, its effects on lipids remain largely conflicting (van Leth *et al*, 2004. Jones *et al*, 2005).

Inflammatory markers appear to have a role in the pathogenesis of HIV/HAART related metabolic syndrome. The effect of HAART on leptin levels is controversial; a few studies have demonstrated that low levels of leptin are associated with lipodystrophy while other studies have demonstrated no effect of HAART on leptin (Dzwonek *et al*, 2007. Calmy *et al*, 2009). We noted an increase in leptin levels at week 4 for patients in the low dose stavudine arm and at week 48 for the tenofovir arm. Patients on HAART, especially those with lipodystrophy, show a gradual reduction in serum adiponectin levels, which is thought to be associated with increased cardiovascular risk (Bezante *et al*, 2009). In our study, there was a significant decrease at week 48 in the standard dose stavudine arm but a significant increase in the tenofovir arm at 4 and 48 weeks. These changes in serum adiponectin levels in the stavudine arm may be significant in terms of the ongoing risk of lipoatrophy as well as metabolic complications. hs-CRP has been shown to be a marker of increased cardiovascular events (Pai *et al*, 2004). There was a tendency for hs-CRP levels to increase in all 3 treatment arms, but this reached statistical significance only in the standard dose stavudine group at 4 weeks and then fell to baseline levels at 48 weeks.

The AEs associated with mitochondrial toxicities were noted only with the stavudine regimens. However, none of these were seen in individuals treated with the 20 mg dose.

Our findings must be considered in light of the study's limitations. Our sample size was small as the study had to be stopped prematurely because of the mitochondrial results (Menezes *et al*, 2012) and due to the introduction of the new South African National HAART guidelines. Secondly, despite this being a randomized controlled trial, it was open labeled, which may have been a confounding factor. The metabolic abnormalities may be more prevalent with increasing duration of therapy and therefore not seen after only 48 weeks of therapy. However, these data are important as our study is the first randomized trial assessing the frequency of NRTI- associated metabolic toxicities related to the two most relevant anti-retroviral agents currently used in a routine clinical setting in black African populations.

In conclusion, the current study demonstrates that in South African HIV-positive patients, tenofovir has more favorable effects on anthropometry and adipokine levels whilst both stavudine regimens increase fasting insulin and C-peptide levels, with the higher stavudine dose also causing increased fasting glucose and higher HOMA levels. However, both stavudine and tenofovir cause raised lipid levels. Therefore, awareness of the potential increased cardiovascular risk should be of concern with the use of both these therapies. More AEs were noted in the standard compared to the low dose stavudine arm. Therefore, where options are limited and there is an absolute need for stavudine to be used, low dose stavudine may be a cautious option as it is equally efficient in viral suppression and has lower rates of complications than the standard dose.

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**References**

Bezante GP, Briatore L, Rollando D, *et al*. Hypoadiponectinemia in lipodystrophic HIV individuals: a metabolic marker of subclinical cardiac damage. *Nutr Metab Cardiovasc Dis* 2009; **19(4)**: 277-282.

Boulle A, Orrell C, Kaplan R, et *al*: Substitution due to antiretroviral toxicity or contraindication in the first 3 years of antiretroviral treatment in a large South African cohort. *Antivir Ther* 2007; **12**:753-60.

Calmy A, Gayet-Ageron A, Montecucco F, *et al.* HIV increases markers of cardiovascular risk: results from a randomized, treatment interruption trial. *AIDS* 2009; **23(8)**: 929-939.

Carr A, Cooper DA. Adverse effects of antiretroviral therapy. *Lancet* 2000; **356:1423**-1430.

Carr A, Samaras K, Thorisdottir A, Kaufmann GR, Chisholm DJ, Cooper DA. Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor-associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study. *Lancet* 1999; **353**:2093-2099.

Cockcroft DW, Gault MH. "Prediction of creatinine clearance from serum creatinine". *Nephron* 1976; **16** (1): 31–41.

Constans J, Pellegrin JL, Peuchant E, Dumon MF, Pellegrin I, Sergeant C, *et al*. Plasma lipids in HIV-infected patients: a prospective study in 95 patients. *Eur J Clin Invest* 1994; **24**: 416–20.

De Wit S, Sabin CA, Weber R, *et al*. Incidence and risk factors for new-onset diabetes in HIV-infected patients: the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study. *Diabetes Care* 2008; **31(6)**:1224-9.

Dzwonek AB, Novelli V, Schwenk A. Serum leptin concentrations and fat distribution in HIV-1 infected children on highly active antiretroviral therapy. *HIV Med* 2007; **8(7)**:433-438.

Friis-Møller N, Sabin CA, Weber R, *et al.* Combination antiretroviral therapy and the risk of myocardial infarction. *N Engl J Med* 2003; **349(21)**:1993-2003.

Gallant JE, DeJesus E, Arribas JR, *et al*. Tenofovir DF, emtricitabine, and efavirenz vs. zidovudine, lamivudine, and efavirenz for HIV. *N Engl J Med* 2006; **354(3)**:251-60.

Gallant JE, Staszewski S, Pozniak AL, *et al*. Efficacy and safety of tenofovir DF vs. stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial. *JAMA* 2004; **292(2)**:191-201.

Galli M, Ridolfo AL, Adorni F, *et al*. Body habitus changes and metabolic alterations in protease inhibitor-naive HIV-1-infected patients treated with two nucleoside reverse transcriptase inhibitors. *J Acquir Immune Defic Syndr* 2002; **29:21**-31

Hill A, Ruxrungtham K, Hanvanich M, *et al*. Systematic review of clinical trials evaluating low doses of stavudine as part of antiretroviral treatment. *Expert Opin Pharmacother* 2007; **8(5)**:679-688.

Hogg RS, O'Shaughnessy MV, Gataric N, *et al*. Decline in deaths from AIDS due to new antiretrovirals. *Lancet* 1997; **349**:1294

Jones R, Sawleshwarkar S, Michailidis C, Jackson A, Mandalia S, Stebbing J, *et al*. Impact of antiretroviral choice on hypercholesterolaemia events: the role of the nucleoside reverse transcriptase inhibitor backbone. *HIV Med* 2005; **6(6)**:396-402.

Lindegaard B, Keller P, Bruunsgaard H, Gerstoft J, Pedersen BK. Low plasma level of adiponectin is associated with stavudine treatment and lipodystrophy in HIV-infected patients. *Clin Exp Immunol* 2004; **135(2)**:273-9.

Magkos F, Mantzoros CS. Body fat redistribution and metabolic abnormalities in HIV-infected patients on highly active antiretroviral therapy: novel insights into pathophysiology and emerging opportunities for treatment. *Metabolism* 2011; **60(6)**:749-53.

Mallon PW, Miller J, Cooper DA, Carr A. Prospective evaluation of the effects of antiretroviral therapy on body composition in HIV-1-infected men starting therapy. *AIDS* 2003; **17(7):**971–979.

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28**: 412-419.

McComsey GA, LoRe III V, O'Riordan M, *et al*. Effect of Reducing the Dose of Stavudine on Body Composition, Bone Density and Markers of Mitochondrial Toxicity in HIV infected Subjects- a Randomized, Controlled study. *Clin Infect Dis*. 2008; **46(8)**: 1290–1296

Menezes CN, Duarte R, Dickens C, *et al*. The early effects of stavudine compared to tenofovir on adipocyte gene expression, mitochondrial DNA copy number and metabolic parameters in South African HIV-infected patients: A randomized trial. *HIV Med* 2013; **14(4)**:217-25.

Menezes CN, Maskew M, Sanne I, Crowther NJ, Raal FJ. A longitudinal study of stavudine-associated toxicities in a large cohort of South African HIV infected subjects. *BMC Infect Dis* 2011; **11**:244.

Milinkovic A, Martinez E, López S, *et al*. The impact of reducing stavudine dose versus switching to tenofovir on plasma lipids, body composition and mitochondrial function in HIV-infected patients. *Antivir Ther* 2007; **12(3)**:407-15.

Mutimura E, Stewart A, Rheeder P, Crowther NJ. Metabolic function and the prevalence of lipodystrophy in a population of HIV-infected African subjects receiving highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* 2007; **46**:451-5.

National Department of Health. National Antiretroviral Treatment Guidelines, First Edition 2004: http://www.doh.gov.za/docs/misc/2004/sec1.pdf. Last accessed on the 20/03/2013.

National Department of Health South Africa 2010. Clinical guidelines for the management of HIV & AIDS in adults and adolescents: http://www.health-e.org.za/documents/0ba405d9042e557a9fb1987adb68cb97.pdf. Last accessed on the 20/03/2013.

Nolan D, Hammond E, Martin A, *et al.* Mitochondrial DNA depletion and morphologic changes in adipocytes associated with nucleoside reverse transcriptase inhibitor therapy. *AIDS* 2003; **17:**1329-1338.

Pai JK, Pischon T, Ma J, *et al*. Inflammatory markers and the risk of coronary heart disease in men and women. *N Engl J Med* 2004; **351**:2599–610.

Palella FJ Jr, Delaney KM, Moorman AC, *et al*. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N Engl J Med* 1998; **338**:853-60.

Palios J, Kadoglou NP, Lampropoulos S. The pathophysiology of HIV-HAART-related metabolic syndrome leading to cardiovascular disorders: the emerging role of adipokines. *Exp Diabetes Res* 2012; **2012**:103063.

Reingold J, Wanke C, Kotler D, *et al*. Association of HIV infection and HIV/HCV coinfection with C-reactive protein levels: the fat redistribution and metabolic change in HIV infection (FRAM) study. *J Acquir Immune Defic Syndr* 2008; **48(2)**:142-8.

Rosen S, Long L, Fox M, Sanne I. Cost and cost-effectiveness of switching from stavudine to tenofovir in first-line antiretroviral regimens in South Africa. *J Acquir Immune Defic Syndr* 2008; **48(3)**:334-44.

Rutter MK, Meigs JB, Sullivan LM, D'Agostino RB, Wilson PW. C-reactive protein, the metabolic syndrome, and prediction of cardiovascular events in the Framingham Offspring study. *Circulation* 2004; **110**:380–5.

Savès M, Raffi F, Capeau J, *et al*. Factors related to lipodystrophy and metabolic alterations in patients with human immunodeficiency virus infection receiving highly active antiretroviral therapy. *Clin Infect Dis* 2002; **34**:1396-405.

Schowalter L, Conradie F. Approaches to tenofovir and abacavir drug shortages in South Africa: A guide for clinicians. *SAJHIVMED* 2012; **13(2)**:56-7

Shlay JC, Visnegarwala F, Bartsch G, *et al.* Body composition and metabolic changes in antiretroviral-naive patients randomized to didanosine and stavudine vs. abacavir and lamivudine. *J Acquir Immune Defic Syndr* 2005; 38(2):147-55.

Third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. *Circulation* 2002, **106**:3143–3421.

Triant VA, Meigs JB, Grinspoon SK. Association of C-reactive protein and HIV infection with acute myocardial infarction. *J Acquir Immune Defic Syndr* 2009; **51(3)**:268-73.

van Griensven J, Zachariah R, Rasschaert F, Mugabo J, Atte EF, Reid T. Stavudine- and nevirapine-related drug toxicity while on generic fixed-dose antiretroviral treatment: incidence, timing and risk factors in a three-year cohort in Kigali, Rwanda. *Trans R Soc Trop Med Hyg* 2010; **104**:148-53.

van Leth F, Phanuphak P, Stroes E, Gazzard B, Cahn P, Raffi F, *et al*. Nevirapine and efavirenz elicit different changes in lipid profiles in antiretroviral therapy naive patients infected with HIV-1. *PLoS Med* 2004; 1(1):e19.

World Health Organization. Forecasting antiretroviral demand. 2010. http://www.who.int/hiv/amds/forecasting/en/index4.html (last accessed on 20/03/2013).

# CHAPTER 5

# 5. DISCUSSION AND CONCLUSION

## 5.1. Summary of the results

There is growing concern regarding the rise in the rates of non-communicable diseases, especially cardiovascular disease, that has occurred in South Africa over the last 15 years. This is likely to increase further in the coming years, with HIV-1infection and its associated complications, compounding this problem (Mayosi *et al*, 2009).

Whilst HIV-1 infection has been turned into a chronic, manageable disease, the serious acute mitochondrial and long term metabolic side effects caused by HAART are a major concern especially with the use of stavudine. This drug was used as first line HIV therapy in South Africa until 2010 (National Antiretroviral Treatment Guidelines, 2004). Our clinic-based longitudinal study (Menezes *et al*, 2011) of 9040 HIV-infected adults, demonstrated the concerns associated with stavudine based therapy. Although this study demonstrated a fairly high retention rate of 70%, nearly 30% of the patients had to switch to non-stavudine based regimens, due to major side effects. Those patients receiving stavudine had higher incidence rates of acute toxicities which included peripheral neuropathy [12.1/100 person-years (95% CI 7.0-19.5) vs. 7.9/100 person-years (95% CI 6.0-10.1)], symptomatic hyperlactataemia [3.6/100 person-years (95% CI 1.2-7.5) vs.1.4 /100 person-years (95% CI 0.7-2.5)], and lactic acidosis [1.6/100 person-years (95% CI 0.4-5.2) vs. 0.8/100 person-years (95% CI 0.3-1.7)] when compared to those on non-stavudine based regimens.

In terms of the metabolic complications, lipoatrophy was seen in 7.3% of patients on stavudine based therapy compared to 4.6% ($p<0.05$) patients on non-stavudine based therapy with a very small proportion of patients presented with diabetes and dyslipidaemia.

According to the WHO, more than 1.55 million people will still be receiving stavudine therapy by the end of 2012 (WHO, 2010). Because stavudine is cheaper than tenofovir and its side effects can be minimized by a dose reduction, it is therefore possible that more patients could be initiated on antiretroviral therapy in countries where the switch has still not occurred. In addition, zidovudine which is used as part of second line therapy or as an alternative drug in the presence of contraindications to tenofovir, also causes mitochondrial side effects but to a lesser extent than stavudine (van Griensven *et al*, 2007). Our clinical trial (Menezes *et al*, 2012) was the first randomized trial that assessed the mitochondrial pathogenesis of NRTI-associated toxicities in an African population when comparing the use of various doses of stavudine and tenofovir therapy.

A major finding of this study is the significant depletion of adipocyte mtDNA in black South African HIV-1 infected individuals on both high and low dose stavudine therapy after only four weeks of therapy. We also showed that tenofovir had a minimal effect on mitochondrial numbers. There was a 29% and 32% decrease in the mean mtDNA copies/cell in the standard-dose and low-dose stavudine arms, respectively, when compared with tenofovir at four weeks. We also noted that despite the significant depletion in mtDNA, the expression levels of only two of eight adipocyte genes (*MTCYB and NRF-1*) associated with mitochondrial energy metabolism and biogenesis were significantly affected by standard dose (30 and 40 mg) stavudine therapy when compared with tenofovir, with minimal effects on gene expression with low dose (20-30 mg) stavudine .

Data after one year of therapy demonstrated that both stavudine and tenofovir caused raised lipid levels but surprisingly this was also noted as early as four weeks of therapy. Early insulin resistance was noted with both stavudine regimens with data suggesting that both

stavudine regimens increased fasting insulin and C-peptide levels, with the higher stavudine dose also causing an increased fasting glucose and higher HOMA levels.

Tenofovir was noted to have a more favourable effect on anthropometry by one year of therapy. Notably, in both the stavudine arms significant increases in anthropometric measures occurred at 24 weeks but these decreased by week 48. This fat redistribution was not objectively confirmed on DEXA scanning in this study possibly because only total truncal and total limb fat was measured .

We noted an increase in leptin levels after four weeks of therapy for patients in the low dose stavudine arm and after 48 weeks of therapy for the tenofovir arm. In our study, there was a significant decrease in serum adiponectin levels at week 48 in the standard dose stavudine arm but a significant increase in the tenofovir arm at 4 and 48 weeks. The hs-CRP serum levels demonstrated a tendency to increase in all three treatment arms, but this reached statistical significance only in the standard dose stavudine group at 4 weeks and then fell to baseline levels at 48 weeks.

This trial demonstrated that there was a significant increase in the CD4+ cell counts in all three arms after one year of therapy when compared to baseline, and similar levels of virological suppression ($\leq$ 400 copies/ml).

The adverse events associated with mitochondrial toxicities were noted only with the stavudine regimens but none were seen in individuals treated with the 20 mg dose.

### 5.2. Discussion of the results

The first study (Chapter 2) demonstrated the ability of a resource limited clinic to roll out a successful HAART programme at a pace and scale that compared with other African

countries (Boulle *et al*, 2007. Coetzee *et al*, 2004. Stringer *et al*, 2006). This was the largest such study conducted in a South African HIV-1-positive population.

When compared to other studies from Africa (Boulle *et al*, 2007. van Griensven *et al*, 2010. Mutimura *et al*, 2007), there was a wide variation in the rates of lipoatrophy; the reason for this is possibly due to the use of different diagnostic criteria. It would appear that only cases that were presenting with severe enough body dysmorphic changes to warrant a regimen change were noted in our study. The study from Rwanda, showed a significantly higher proportion of lipodystrophy because milder cases of lipodystrophy that did not warrant a regimen change, were also recorded (Mutimura *et al*, 2007). The low rates of diabetes and dyslipidaemias may be because lipid or glucose levels were only tested when clinically suspected due to cost implications, probably underestimating the true prevalence of dyslipidaemia and diabetes in our clinic. Other reasons could be that the patients may have died (death rates of 5%) or lost to follow up (24%), the majority of which occurred within the first six months on therapy.

In addition to the disparity in the rates of toxicities, this study highlighted other issues associated with the management of HIV-1 infected patients in resource limited environments, such as the importance of close monitoring for adverse events and when to anticipate such problems. There is a need for grading protocols for assessing the severity of peripheral neuropathies, as well as an alternative definition for the diagnosis of lipodystrophy considering the lack of access to routine DEXA and MRI imaging technology. Routine metabolic monitoring such as glucose and lipid profiles must also be instituted.

With regards to the clinical trial, these findings were consistent with studies undertaken in non-African populations who developed peripheral lipoatrophy at the time of sampling and after a longer duration of anti-retroviral therapy (Boothby *et al*, 2009. Buffet *et al*, 2005.

Mallal *et al*, 2001. Nolan *et al*, 2003. Schooley *et al*, 2002. Shikuma *et al*, 2001. Stankov *et al*, 2010). Our results suggest that mitochondrial pathology precedes changes in body fat distribution and occurs very early after the initiation of antiretroviral therapy. In addition, *NRF1* is known to be an important regulator of mitochondrial biogenesis and function, where it binds to the regulatory regions of genes encoding subunits of the respiratory complex and various constituents of the mtDNA transcription and replication machinery. It serves to coordinate nucleo-mitochondrial activities by regulating mitochondrial and cytosolic enzymes (Scarpulla *et al*, 2008). These results suggest that downregulation of *NRF1* is an early event in NRTI toxicity. This change in *NRF1* expression triggers a cascade of events under its control, with effects that are only observed much later in the time course of antiretroviral therapy (Boothby *et al*, 2009. Pace *et al*, 2003. Sievers *et al*, 2009). Similarly, changes in *MTCYB* expression have been observed in other studies (Kim *et al*, 2008. Stankov *et al*, 2010). These changes may be associated with increased oxidative stress and apoptosis related to mitochondrial depletion (Kim *et al*, 2008. Komarov *et al*, 2008). The findings of this study are important as previous trials in Europe, the USA and Australia did not examine the effects of mitochondrial changes in a black population (Boothy *et al*, 2009. Buffet *et al*, 2005. Kim *et al*, 2008. Mallal *et al*, 2001. Mallon *et al*, 2005. Milinkovic *et al*, 2007. Nolan *et al*, 2003. Pace *et al*, 2003. Schooley *et al*, 2002. Shikuma *et al*, 2001. Sievers *et al*, 2009. Stankov *et al*, 2010).

These findings are strengthened by the fact that the laboratory technique used in this study to determine mtDNA copy number was based on a well-established protocol (Nolan *et al*, 2003) that has been standardized and extensively critiqued (Cote *et al*, 2011), and which followed the minimum information for publication of quantitative real-time PCR experiments (MIQE)

guidelines (Bustin *et al*, 2009) with the normalization of gene expression to multiple reference genes (Hellemans *et al*, 2007).

In this era where the benefits of HAART have clearly improved the morbidity and mortality of patients with HIV-1 infection, CVD has emerged as a cause for concern in these patients. In our study, data at 48 weeks of therapy demonstrated that in South African, HIV-1 positive patients both stavudine and tenofovir caused raised lipid levels. While dyslipidaemias have been known to be associated with exposure to NRTIs, this has been mainly associated with stavudine and zidovudine, and less so with tenofovir (Gallant *et al*, 2004. Gallant *et al*, 2006). Consistent with other studies (Gallant *et al*, 2004. Savès *et al*, 2002. Shlay *et al*, 2005), including studies from Africa (Buchacz *et al*, 2010. Mutimura *et al*, 2007. Sinxadi *et al*, 2010. Zannou *et al*, 2009), we demonstrated a significant increase in fasting lipids on HAART, but surprisingly this was noted after only 4 weeks of therapy with both stavudine and tenofovir. The observed effects might also be explained by the use of efavirenz, but its effects on lipids remain largely conflicting (van Leth *et al*, 2004. Jones *et al*, 2005).

Early insulin resistance was noted with both stavudine regimens, and other studies have shown that cumulative exposure to NRTIs such as stavudine and zidovudine is associated with abnormal glucose tolerance (Brown *et al*, 2005. De Wit *et al*, 2008. Tien *et al*, 2008).

Tenofovir was noted to have a more favorable effect on anthropometry that stavudine. Studies in treatment naïve HIV infected patients have shown that peripheral fat initially increases during the first few months of HAART, but decreases linearly thereafter and truncal fat increases around six months of therapy (Makgos *et al*, 2011. Mallon *et al*, 2003). In fact, studies demonstrated that lipoatrophy tended to be common after one year of therapy and especially in patients who were on higher doses of stavudine (40mg) (Mutimura *et al*, 2007. van Griensven *et al*, 2010).

The effect of antiretroviral therapy on leptin levels is controversial; some studies have suggested that low levels of leptin are associated with lipodystrophy while others have demonstrated no effect (Dzwonek *et al*, 2007. Calmy *et al*, 2009). We noted early changes in leptin levels in patients on the low dose stavudine arm and late changes in the tenofovir arm. Patients on HAART, especially those with lipodystrophy, show a gradual reduction in serum adiponectin levels, which is thought to be associated with increased cardiovascular risk (Bezante *et al*, 2009). We noted a significant decrease after a year of therapy in the standard dose stavudine arm but a significant increase in the tenofovir arm at 4 and 48 weeks. This is an important finding because in non-HIV infected patients low adiponectin levels have been shown to be associated with an increased risk of diabetes and associated metabolic disorders (Koenig *et al*, 2006).

Our data would suggest that low dose stavudine is more patient friendly than the standard dose, and is equally efficacious in viral suppression and immunological outcomes, and may therefore still be a treatment option in resource limited settings.

These results support the new South African HAART guidelines which have evolved since 2004, with tenofovir 300 mg currently being recommended as first line therapy. This study also demonstrates that in South African, HIV-positive patients both stavudine and tenofovir cause raised lipid levels, although tenofovir has more favourable effects on anthropometry and adipokine levels. Both stavudine regimens increase fasting insulin and C-peptide levels, with the higher stavudine dose also causing increased fasting glucose and higher HOMA levels. This data also suggests that a potential for increased cardiovascular risk exists with the use of both tenofovir and stavudine, and that cardiovascular risk factor modification strategies would be important in the management of patients receiving these therapies.

### 5.3. Limitations and directions for future studies

1. Even though our sample size was much larger than other studies, and was still sufficient for us to be able to detect significant effects of HAART on mtDNA copy number and gene expression, the trial had to be stopped prematurely on the advice of the DSMB and this may have reduced the statistical power of the study. The DSMB analysis of the first 60 patients showed that the group given stavudine at both standard and low doses produced greater mitochondrial depletion than in the arm receiving tenofovir. Furthermore, the DSMB considered that there were compelling ethical grounds to stop the high dose stavudine because the new SA guidelines were shortly to be implemented (National Department of Health, South Africa 2010), and tenofovir had become freely available. At that point, the four patients who were still on 40 mg of stavudine were switched to tenofovir. The other patients were left on the 20 mg and 30mg stavudine doses, because it took some time before tenofovir was eventually available. Therefore there were 20 patients in the standard dose stavudine arm at baseline and 4 weeks; and only 8 patients at 48 weeks.

2. Adipose biopsies should be performed after a longer duration of antiretroviral therapy as other studies found significant mtDNA and mtRNA depletion on a longer duration of therapy of months to years.

3. Metabolic abnormalities may be more prevalent with increasing duration of therapy and may not be seen after only 48 weeks of therapy; therefore, further studies using the low dose stavudine (20mg stavudine vs. tenofovir) should be initiated to confirm our findings, particularly in light of data showing that this dose is effective and safe and, furthermore, it is unlikely that stavudine will ever be completely phased out of use in countries with limited financial resources.

4. The impact of HIV/AIDS on CVD needs to be studied further. Whilst a lot is known about the aetiology and complications of the epidemic of CVD in the HIV negative population, there is a paucity of data to describe the emergence and impact of CVD in HIV-1-infected patients on antiretroviral therapy in Africa. The emergence of lipodystrophy with its associated complications such as insulin resistance/diabetes, hypertension and dyslipidaemia will have major public health implications in the future and will put further strain on the already limited financial resources available within the public health sector not only in South Africa, but also across antiretroviral therapy programmes in sub-Saharan Africa.

## 5.4. References

Bezante GP, Briatore L, Rollando D, Maggi D, Setti M, Ghio M *et al*. Hypoadiponectinemia in lipodystrophic HIV individuals: a metabolic marker of subclinical cardiac damage. *Nutr Metab Cardiovasc Dis* 2009; **19(4)**: 277-282.

Boothby M, McGee KC, Tomlinson JW, Gathercole LL, McTernan PG, Shojaee-Moradie F, *et al*. Adipocyte differentiation, mitochondrial gene expression and fat distribution: differences between zidovudine and tenofovir after 6 months. *Antivir Ther* 2009; **14(8)**:1089-100.

Boulle A, Orrell C, Kaplan R, van Cutsem G, McNally M, Hilderbrand K, *et al*. Substitution due to antiretroviral toxicity or contraindication in the first 3 years of antiretroviral treatment in a large South African cohort. *Antivir Ther* 2007; **12**:753-60.

Brown TT, Li X, Cole SR, Kingsley LA, Palella FJ, Riddler SA, *et al*. Cumulative exposure to nucleoside analogue reverse transcriptase inhibitors is associated with insulin resistance markers in the Multicenter AIDS Cohort Study. *AIDS* 2005; **19(13)**:1375-83.

Buchacz K, Weidle PJ, Moore D, Were W, Mermin J, Downing R, *et al*. Changes in lipid profile over 24 months among adults on first-line highly active antiretroviral therapy in the home-based AIDS care program in rural Uganda. *J Acquir Immune Defic Syndr* 2008; **47**:304–311.

Buffet M, Schwarzinger M, Amellal B, Gourlain K, Bui P, Prévot M, *et al*. Mitochondrial DNA depletion in adipose tissue of HIV-infected patients with peripheral lipoatrophy. *J Clin Virol* 2005; **33(1)**:60-4.

Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, *et al*. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem* 2009; **55(4)**:611-622

Calmy A, Gayet-Ageron A, Montecucco F, Nguyen A, Mach F, Burger F *et al.* HIV increases markers of cardiovascular risk: results from a randomized, treatment interruption trial. *AIDS* 2009; **23(8)**: 929-939.

Coetzee D, Hildebrand K, Boulle A, Maartens G, Louis F, Labatala V, *et al*. Outcomes after two years of providing antiretroviral treatment in Khayelitsha, South Africa. *AIDS* 2004; **18**:887-95.

Côté HC, Gerschenson M, Walker UA, Miro O, Garrabou G, Hammond E, *et al*. Quality assessment of human mitochondrial DNA quantification: MITONAUTS, an international multicentre survey. *Mitochondrion* 2011; **11**:520-527.

De Wit S, Sabin CA, Weber R, Worm SW, Reiss P, Cazanave C, *et al*. Incidence and risk factors for new-onset diabetes in HIV-infected patients: the Data Collection on Adverse Events of Anti-HIV Drugs (D: A: D) study. *Diabetes Care*. 2008; **31(6)**:1224-9.

Dzwonek AB, Novelli V, Schwenk A. Serum leptin concentrations and fat distribution in HIV-1 infected children on highly active antiretroviral therapy. *HIV Medicine* 2007; **8(7)**:433-438.

Gallant JE, DeJesus E, Arribas JR, Pozniak AL, Gazzard B, Campo RE, *et al*. Tenofovir DF, emtricitabine, and efavirenz vs. zidovudine, lamivudine, and efavirenz for HIV. *N Engl J Med* 2006; **354(3)**:251-60.

Gallant JE, Staszewski S, Pozniak AL, DeJesus E, Suleiman JM, Miller MD, *et al*. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial. *JAMA* 2004; **292(2)**:191-201.

Hellemans J, Mortier G, De Paepe A, Speleman F, Vandesompele J. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome Biol* 2007; **8(2)**:R19.

Kim MJ, Jardel C, Barthélémy C, Jan V, Bastard JP, Fillaut-Chapin S, *et al*. Mitochondrial DNA content, an inaccurate biomarker of mitochondrial alteration in human immunodeficiency virus-related lipodystrophy. *Antimicrob Agents Chemother* 2008; **52(5)**:1670-6.

Koenig W, Khuseyinova N, Baumert J, Meisinger C, Löwel H. Serum concentrations of adiponectin and risk of type 2 diabetes mellitus and coronary heart disease in apparently healthy middle-aged men: results from the 18-year follow-up of a large cohort from southern Germany. *J Am Coll Cardiol* 2006; **48(7)**:1369-77.

Komarov AP, Rokhlin OW, Yu CA, Gudkov AV. Functional genetic screening reveals the role of mitochondrial cytochrome b as a mediator of FAS-induced apoptosis. *Proc Natl Acad Sci U S A* 2008; **105(38)**:14453-8.

Magkos F, Mantzoros CS. Body fat redistribution and metabolic abnormalities in HIV-infected patients on highly active antiretroviral therapy: novel insights into pathophysiology and emerging opportunities for treatment. *Metabolism* 2011; **60(6)**:749-53.

Mallal SA, Hammond EL, Martin A, Taylor L, John M, Nolan DA. Mitochondrial DNA depletion, assessed by real-time PCR-based quantitative assay, in subcutaneous fat of HIV-infected patients: evidence for a role in the etiology of fat wasting. 4th International Conference on Nutrition and HIV Infection. Cannes, France, 19–21 April 2001, Abstract O7.

Mallon PW, Miller J, Cooper DA, Carr A. Prospective evaluation of the effects of antiretroviral therapy on body composition in HIV-1-infected men starting therapy. *AIDS* 2003; **17(7)**:971–979.

Mallon PW, Unemori P, Sedwell R, Morey A, Rafferty M, Williams K, *et al*. In vivo, nucleoside reverse-transcriptase inhibitors alter expression of both mitochondrial and lipid metabolism genes in the absence of depletion of mitochondrial DNA. *J Infect Dis* 2005; **191(10)**:1686-96.

Mayosi BM, Flisher AJ, Lalloo UG, Sitas F, Tollman SM, Bradshaw D. The burden of non-communicable diseases in South Africa. *Lancet* 2009; 374: 934–47

Menezes CN, Duarte R, Dickens C, Dix-Peek T, Van Amsterdam D, John M, *et al* . The early effects of stavudine compared to tenofovir on adipocyte gene expression, mitochondrial DNA copy number and metabolic parameters in South African HIV-infected patients: A randomized trial. *HIV Med* 2013;14(4):217-25.

Menezes CN, Maskew M, Sanne I, Crowther NJ, Raal FJ. A longitudinal study of stavudine-associated toxicities in a large cohort of South African HIV infected subjects. *BMC Infect Dis* 2011; **11**:244.

Milinkovic A, Martinez E, López S, de Lazzari E, Miró O, Vidal S, *et al.* The impact of reducing stavudine dose versus switching to tenofovir on plasma lipids, body composition and mitochondrial function in HIV-infected patients. *Antivir Ther* 2007; **12(3)**:407-15.

Mutimura E, Stewart A, Rheeder P, Crowther NJ. Metabolic function and the prevalence of lipodystrophy in a population of HIV-infected African subjects receiving highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* 2007; **46**:451-5.

National Department of Health. National Antiretroviral Treatment Guidelines, First Edition 2004: http://www.doh.gov.za/docs/factsheets/guidelines/artguide04-f.html. Last accessed on the 09/07/2010.

Nolan D, Hammond E, Martin A, Taylor L, Herrmann S, McKinnon E, *et al*. Mitochondrial DNA depletion and morphologic changes in adipocytes associated with nucleoside reverse transcriptase inhibitor therapy. *AIDS* 2003; **17(9)**:1329-38.

Pace CS, Martin AM, Hammond EL, Mamotte CD, Nolan DA, Mallal SA. Mitochondrial proliferation, DNA depletion and adipocyte differentiation in subcutaneous adipose tissue of HIV-positive HAART recipients. *Antivir Ther* 2003; **8**:323-331.

Savès M, Raffi F, Capeau J, Rozenbaum W, Ragnaud JM, Perronne C, *et al*. Factors related to lipodystrophy and metabolic alterations in patients with human immunodeficiency virus infection receiving highly active antiretroviral therapy. *Clin Infect Dis* 2002; **34(10)**:1396-405.

Scarpulla RC. Transcriptional paradigms in mammalian mitochondrial biogenesis and function. *Physiol Rev* 2008; **88(2)**:611-38.

Schooley RT, Ruane P, Myers RA, Beall G, Lampiris H, Berger D, *et al*. Tenofovir DF in antiretroviral-experienced patients: results from a 48-week, randomized, double-blind study. *AIDS* 2002; **16**:1257-63.

Shikuma CM, Hu N, Milne C, Yost F, Waslien C, Shimizu S, *et al*. Mitochondrial DNA decrease in subcutaneous adipose tissue of HIV-infected individuals with peripheral lipoatrophy. *AIDS* 2001; **15(14)**:1801-9.

Shlay JC, Visnegarwala F, Bartsch G, Wang J, Peng G, El-Sadr WM, *et al.* Body composition and metabolic changes in antiretroviral-naive patients randomized to didanosine and stavudine vs. abacavir and lamivudine. *J Acquir Immune Defic Syndr* 2005; **38(2)**:147-55.

Sievers M, Walker UA, Sevastianova K, Setzer B, Wågsäter D, Eriksson P, *et al*. Gene Expression and Immunohistochemistry in Adipose Tissue of HIV Type 1-Infected Patients with Nucleoside Analogues Reverse-Transcriptase Inhibitor-Associated Lipoatrophy. *J Infect Dis* 2009; **200**:252-62.

Sinxadi PZ, van der Walt JS, McIlleron, Badri M, Smith PJ, Dave JA, *et al*. Lack of association between stavudine exposure and lipoatrophy, dysglycaemia, hyperlactataemia and hypertriglyceridaemia: a prospective cross sectional study. *AIDS Res Ther* 2010; **7**:23.

Stankov MV, Lücke T, Das AM, Schmidt RE, Behrens GM. Mitochondrial DNA depletion and respiratory chain activity in primary human subcutaneous adipocytes treated with

nucleoside analogue reverse transcriptase inhibitors. *Antimicrob Agents Chemother* 2010; **54(1)**:280-7.

Stringer JS, Zulu I, Levy J, Stringer EM, Mwango A, Chi BH, *et al*. Rapid Scale-up of Antiretroviral Therapy at Primary Care Sites in Zambia -Feasibility and Early Outcomes. *JAMA* 2006; **296**:782-793.

Tien PC, Schneider MF, Cole SR, Levine AM, Cohen M, DeHovitz J, *et al*. Antiretroviral therapy exposure and insulin resistance in the Women's Interagency HIV study. *J Acquir Immune Defic Syndr* 2008; **49(4)**:369-76.

van Griensven J, De Naeyer L, Mushi T, Ubarijoro S, Gashumba D, Gazille C, *et al*. High prevalence of lipoatrophy among patients on stavudine-containing first-line antiretroviral therapy regimens in Rwanda. *Trans R Soc Trop Med Hyg* 2007; **101(8)**:793-8.

van Griensven J, Zachariah R, Rasschaert F, Mugabo J, Atte EF, Reid T. Stavudine- and nevirapine-related drug toxicity while on generic fixed-dose antiretroviral treatment: incidence, timing and risk factors in a three-year cohort in Kigali, Rwanda. *Trans R Soc Trop Med Hyg* 2010; **104**:148-53.

WHO. Forecasting antiretroviral demand. 2010. http://www.who.int/hiv/amds/forecasting/en/index4.html. Last accessed on the 28/10/2012.

Zannou DM, Denoeud L, Lacombe K, Amoussou-Guenou D, Bashi J, Akakpo J, *et al*. Incidence of lipodystrophy and metabolic disorders inn patients starting non-nucleoside reverse transcriptase inhibitors in Benin. *Antivir Ther* 2009; **14(3)**:371-80.

# CHAPTER 6

# 6. APPENDICES

## 6.1. Appendix 1 – Paper 1

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BMC Infectious Diseases

RESEARCH ARTICLE Open Access

# A longitudinal study of stavudine-associated toxicities in a large cohort of South African HIV infected subjects

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**Abstract**

**Background:** There has been major improvement in the survival of HIV-1 infected individuals since the South African Government introduced highly active anti-retroviral therapy (HAART) in the public sector in 2004. This has brought new challenges which include the effects of stavudine-related toxicities.

**Methods:** Prospective analysis of a cohort of 9040 HIV-infected adults who were initiated on HAART at the Themba Lethu Clinic (TLC) in Johannesburg between April 1, 2004 to December 31, 2007, and followed up until June 30, 2008.

**Results:** Amongst the 9040 study subjects, 8497(94%) were on stavudine based therapy and 5962 (66%) were women. The median baseline CD4 count was 81 cells/mm$^3$ (IQR 29-149). Median follow up on HAART was 19 months (IQR: 9.1-31.6). The proportion of HAART-related side effects for stavudine compared to non-stavudine containing regimens were, respectively: peripheral neuropathy,17.1% vs. 11.2% ($p < 0.001$); symptomatic hyperlactataemia, 5.7% vs. 2.2% ($p < 0.0005$); lactic acidosis, 2.5 vs. 1.3% ($p = 0.072$); lipoatrophy, 7.3% vs. 4.6% ($p < 0.05$). Among those on stavudine-based regimens, incidence rates for peripheral neuropathy were 12.1 cases/100 person-years (95%CI 7.0-19.5), symptomatic hyperlactataemia 3.6 cases/100 person-years (95%CI 1.2-7.5), lactic acidosis 1.6 cases/100 person-years (95%CI 0.4-5.2) and lipoatrophy 4.6 cases/100 person-years (95%CI 2.1-9.6). Females experienced more toxicity when compared to males in terms of symptomatic hyperlactataemia ($p < 0.0001$), lactic acidosis ($p < 0.0001$), lipoatrophy ($p < 0.0001$) and hypertension ($p < 0.05$).

**Conclusions:** We demonstrate significant morbidity associated with stavudine. These data support the latest WHO guidelines, and provide additional evidence for other resource limited HAART rollout programs considering the implementation of non-stavudine based regimens as first line therapy.

### Background

By the end of 2008, an estimated 33.4 million people worldwide were living with human immunodeficiency virus (HIV) infection [1]. Southern Africa continues to bear a disproportionate share of the global burden of HIV with 67% of the HIV infections worldwide, of which 68% of them were amongst adults. This region also accounted for 72% of the world's AIDS related deaths [1]. South Africa's 2009 HIV prevalence rate in the adult population (aged 15-49 years) was estimated to be 17.8% [2].

There has been an increase in the provision of highly active antiretroviral therapy (HAART), with up to 44% of adults and children estimated to be receiving therapy with a profound reduction in mortality [2] and, with adherence to HAART it is possible to transform HIV from a fatal infection to a chronic and manageable illness [3-5].

However, some of the anti-retroviral agents used in HAART regimens have severe side effects. Prominent amongst these drugs is stavudine, the use of which is associated with lactic acidosis/symptomatic hyperlactataemia, lipoatrophy, and peripheral neuropathy [6]. Other side effects of stavudine use include dyslipidaemia and insulin resistance, and it is an independent risk factor for the development of new onset diabetes mellitus [7]. Despite these serious side effects up to 60% of HIV-positive

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patients in low and middle income countries are receiving stavudine [8,9].

The aim of this study was to make use of a large (N = 9040) clinic-based survey of HIV-positive patients newly initiated onto HAART over a period of three years, to compare and describe the effect of stavudine based therapy on acute and chronic toxicities after initiation of HAART. We present baseline data gathered before the initiation of HAART and data collected for three years with each patient having a minimum of six months of follow-up. This is the largest study of the side effects of stavudine therapy conducted to date in a South African HIV-positive population.

## Methods

### Study population

The study population included HIV-1 infected individuals attending the Themba Lethu Clinic, a public sector HAART rollout facility based at the Helen Joseph Hospital, a teaching hospital attached to the University of the Witwatersrand, Johannesburg, South Africa. This clinic provides free antiretroviral therapy and other specialized services, and is one of the largest HAART rollout clinics in Africa. The program is funded by the South African National and Gauteng Departments of Health, with support from Right to Care funded by USAID and PEPFAR.

### Treatment

HAART was initiated in accordance with the 2004 South African National Antiretroviral Treatment Guidelines, which include initiation criteria of a CD4 count ≤ 200 cells/$mm^3$ or WHO stage 4 AIDS defining illness irrespective of CD4 count [10]. The first line therapy consisted of stavudine, lamuvidine and efavirenz or nevirapine; however kaletra (ritonavir/lopinavir) was used as part of the first line therapy regimen if there were contra-indications to other first line drugs [10]. Until October 2007, stavudine was dosed according to patients' body weight: 30 mg for those < 60 kg and 40 mg for those ≥ 60 kg. From October 2007, a universal 30 mg dose was introduced and 40 mg tablets of stavudine were withdrawn from the clinic. Single drug substitutions were permitted depending on the underlying clinical presentation of the patient.

### Clinical and laboratory measurements

Patients who met the criteria for initiating of HAART received adherence counseling and screening for opportunistic infections prior to initiation of therapy. A history and physical examination was performed at every visit. All patients had a baseline chest X-ray. Laboratory monitoring was performed according to the clinic protocol. Other serum biochemical tests were carried out as clinically indicated. The results of these tests were not available for analysis as the majority of patients were seen and diagnosed at other clinics where they would present for acute medical problems, and where their HAART regimen was modified. However, their diagnoses and new HAART regimens were captured for this study.

### Diagnosis and definitions

Body mass index (BMI) was defined as body weight divided by the height, squared ($kg/m^2$). Patients who presented with symptoms of numbness or dysesthesia after initiation of HAART were defined as having peripheral neuropathy due to HAART, once other causes were excluded. Symptomatic hyperlactataemia was defined as the presence of suggestive symptoms with an uncuffed venous lactate level > 5 mmol/L with no evidence of a metabolic acidosis; and lactic acidosis was defined as an uncuffed lactate > 5 mmol/l and arterial pH < 7.35 or a total venous $CO_2$ < 20 mmol/l, with other causes such as sepsis, renal failure, diabetic ketoacidosis and dehydration excluded. Pancreatitis was defined as the presence of abdominal symptoms with a serum amylase > 125 U/L and lipase > 60 U/L. The definition of lipoatrophy was based on the development of peripheral fat wasting (face, arms, buttocks or thighs) and/or central abdominal fat accumulation, and may include enlarged breasts. This was usually reported by the patient and confirmed by the doctor or, initially diagnosed by the doctor with patient confirmation. Hypertension was defined by the presence of three separate readings of systolic blood pressure > 140 mmHg and a diastolic blood pressure > 90 mmHg. Diabetes was defined as the presence of symptoms with a fasting glucose of > = 7 mmol/L or a random blood glucose of 11.1 mmol/L. Dyslipidaemias were defined by the presence of abnormal lipid levels which included an elevated total cholesterol of > 5 mmol/L, an elevated triglyceride level of > 1.7 mmol/L, and elevated LDL level of > 3 mmol/L.

### Data collection and statistical analysis

We analyzed prospectively collected longitudinal cohort data from patients attending the clinic. Clinical data from patient records were captured onto an electronic database via a medical management software system, Therapy Edge-HIV™ (Associated Biological Systems, South Africa). Data was analyzed using the SAS® 9.1 statistical software package (SAS Institute, Inc., North Carolina, USA). Baseline characteristics of the study sample were summarized using simple proportions and medians with interquartile ranges. Differences in proportions of the toxicities were compared by initiating regimen with Chi-squared tests. Study subjects were followed from HAART initiation to the earliest of 1) death; 2) loss to follow up; 3) development of toxicity or 4) censor date 31 December 2008. Incidence rates with 95% confidence intervals for toxicity were calculated and compared by initiating regimen type (stavudine-based versus other). Crude and adjusted estimates of

the effect of stavudine use on development of incident toxicity were estimated using Cox proportional hazard models. Models were controlled for confounding by baseline body mass index, CD4 count, age, and gender. Though the majority of subjects initiated HAART prior to the introduction of the universal 30 mg stavudine dose, models were also adjusted for time period in which HAART was initiated (prior to or post October 2007). Kaplan Meier curves were used to estimate crude time to diagnosis of therapy-related complications stratified by initiated regimen. Subjects with existing toxicity at initiation of HAART were excluded from these analyses.

Use of the data for the study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand.

## Results

Between 1 April 2004 and 1 July 2008, a total of 15,928 HIV-infected adults enrolled in care at the Themba Lethu Clinic. Of these, 5104 (32%) had early stage HIV infection and did not qualify for HAART, whilst the remaining 10,824 (68%) subjects were initiated on HAART. The study sample included the 9040 patients initiated on treatment between 1 April 2004 and 31 December 2007. The cohort profile is summarized in Figure 1.

### Baseline characteristics of the study population

The baseline characteristics of the study cohort are summarized in Table 1. Two thirds of the study group was female. The majority of patients were in the age group 25-44 years. Even though 46.9% of the total study population were defined as being at only WHO Stage 1 for AIDS, they were already on HAART, indicating that their CD4 count rates were below 200 cells/mm$^3$ and this was confirmed by the median baseline CD4 count of 81 cells/mm$^3$ (IQR: 29-149).

Ninety four percent of patients were on stavudine based regimens at baseline, with 79% of these initiated on stavudine, lamivudine and efavirenz as per the 2004 South African National guidelines [10], while a smaller number were initiated on zidovudine- (3.4%) and tenofovir-based regimens (0.7%).

### Retention in care

The median time to follow up for this cohort on HAART was 19 months (IQR: 9.1-31.6). At the end of the study period, a total of 6415 patients (71%) were still alive and in care, 469 were confirmed deceased and a further 2156 were considered lost to follow up. Patients were considered lost to follow up if they missed their last scheduled appointment by more than 90 days or at least 180 days had lapsed since their last visit. It can be assumed that some of the patients who are lost to follow up may have died and so in total, there were 2,625 (16.5%) patients in this cohort who were either lost to follow up or dead by the end of the study period (see Figure 1).

### Response to HAART

The patients had a median baseline CD4 count of 81 cells/mm$^3$ (IQR 29-149). There was a significant ($p < 0.0001$) increase in the CD4 count after initiation with a median of 205 cells/mm$^3$ (IQR 132-293) by 6 months on treatment. After six months of therapy, there was no significant change in BMI (means ± SD; 22.4 ± 5.0 and 23.7 ± 5.5 respectively).

### Acute and chronic toxicities

Amongst the 9040 patients on therapy, 2488 patients (27.5%) had one or more incident toxicities recorded after treatment initiation. In terms of acute HAART-related toxicities, the proportion of patients diagnosed with peripheral neuropathy was significantly higher in the group receiving stavudine-based therapy (17.1% vs. 11.2%; $p < 0.001$) compared to those on non-stavudine based therapy (Table 2). Peripheral neuropathy was reported equally in both genders (approximately 17%), with no difference in time to development between the drug groups. There was also a significantly higher proportion of patients on stavudine based therapy presenting with symptomatic hyperlactataemia when compared to those on non-stavudine based therapy (5.75 vs. 2.2%, $p < 0.0005$) (Table 2), with females more frequently affected than males (7.1% vs. 2.5%; $p < 0.0001$). Although the rate of development of lactic acidosis was the same for both drug groups, it was experienced more frequently in females than males (3.3% vs. 0.8%; $p < 0.0001$). The median time to onset was the same for both drug groups. Pancreatitis was equally rare in both treatment groups (see Table 2); both genders were equally affected, with the time to onset being the same in both drug groups. When compared to those on non-stavudine based regimens, those receiving stavudine had higher incidence rates of several toxicities including peripheral neuropathy [12.1/100 person-years (95%CI 7.0-19.5) vs. 7.9/100 person-years (95%CI 6.0-10.1)], symptomatic hyperlactataemia [3.6/100 person-years (95%CI 1.2-7.5) vs.1.4/100 person-years (95%CI 0.7-2.5)], and lactic acidosis [1.6/100 person-years (95%CI 0.4-5.2) vs. 0.8/100 person-years (95%CI 0.3-1.7)] (Table 3).

In terms of the chronic HAART-related metabolic complications, 7.3% presented with lipoatrophy on stavudine based therapy, compared to 4.6% ($p < 0.05$) patients on non-stavudine based therapy. The median time to development of lipoatrophy was similar across the 2 treatment groups. Lipoatrophy was more predominantly seen in female than male patients (10.0% vs. 1.6%; $p < 0.0001$). Incidence rates for lipoatrophy were slightly higher in the stavudine based therapy group [3.0/100 person-years (95% CI 1.9-4.4) versus 4.6/100 person-years (95%CI 2.1-9.6)]

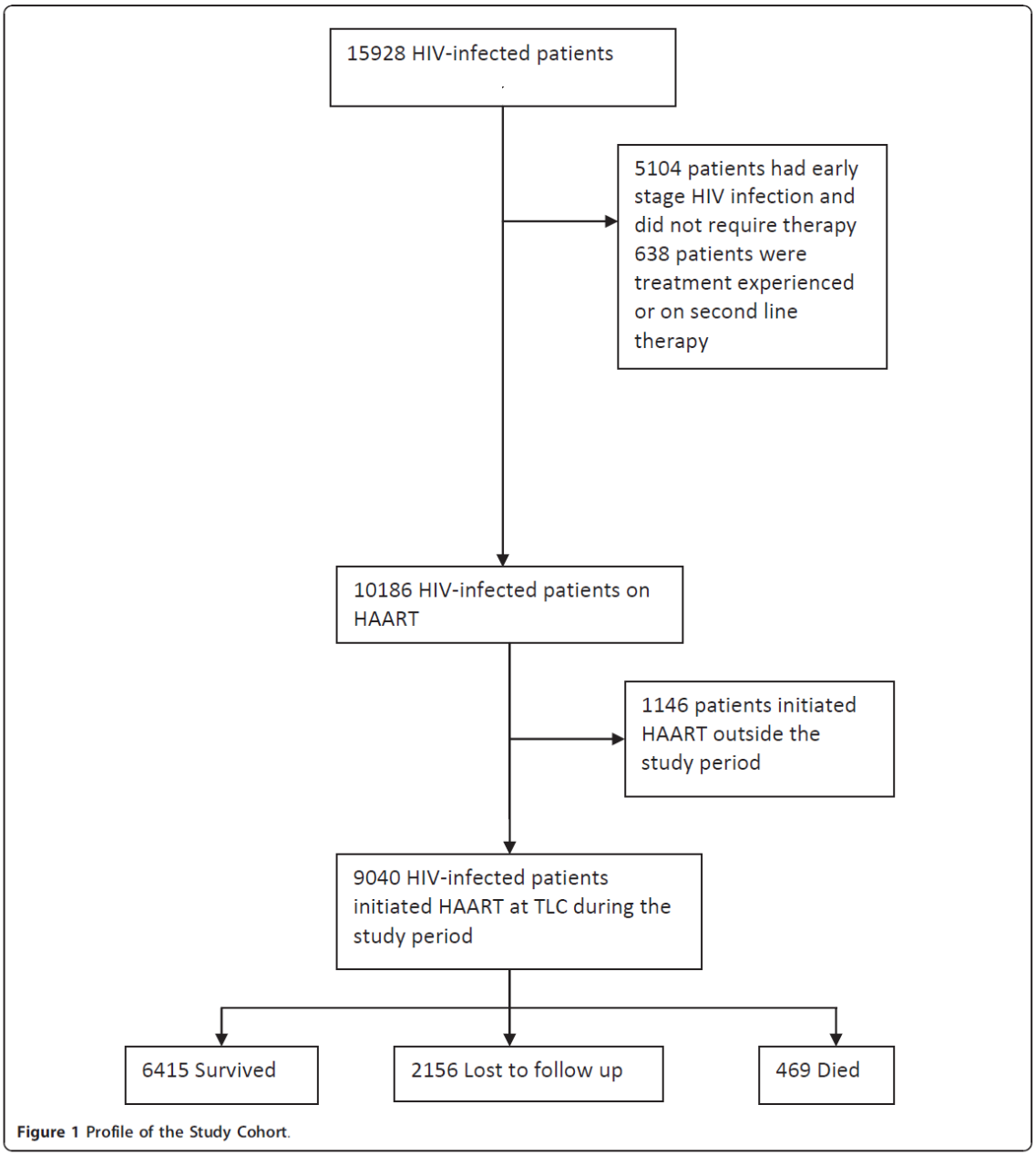


**Figure 1** Profile of the Study Cohort.

compared to those on other regimens (Table 3). Only two percent of patients presented with hypertension, while only 0.3% presented with diabetes and 1.3% with dyslipidaemia on stavudine based therapy. Similar proportions developing these conditions were observed in both groups (Table 2). However, hypertension developed more quickly ($p < 0.05$) in patients taking stavudine than in those not receiving this drug (Table 2). Hypertension was less common in females than males (1.8% vs. 2.4%; $p < 0.05$) but females and males were equally affected in terms of diabetes and dyslipidaemia.

In multivariate analyses adjusted for age, gender, baseline BMI, CD4 counts and the time period initiating HAART, the use of stavudine continued to be associated

**Table 1 Cohort characteristics at baseline**

| Characteristic | Baseline value |
|---|---|
| Gender | (N = 9040) |
| Female | 5962 (66) |
| Male | 3078 (34) |
| Age (years) | (N = 9040) |
| < 25 years | 551 (6.1) |
| 25-34 years | 3822 (42.3) |
| 35-44 years | 3216 (35.5) |
| 45-54 years | 1173 (13.0) |
| > 55 years | 277 (3.1) |
| WHO AIDS classification | (N = 8714) |
| Stage 1 | 4086 (46.9) |
| Stage 2 | 1310 (15) |
| Stage 3 | 2507 (28.8) |
| Stage 4 | 811 (9.3) |
| HAART | (N = 9040) |
| d4T/3TC/EFV | 7138 (79) |
| d4T/3TC/NVP | 690 (7.6) |
| d4T/3TC/Kaletra | 669 (7.4) |
| AZT containing regimen | 308 (3.4) |
| TDF containing regimen | 59 (0.7) |
| other | 176 (1.9) |
| BMI (kg/m$^2$) | 22.4 ± 5.0 (N = 7010) |
| CD4 count (cells/mm$^3$) | 81 (29-149) (N = 7605) |
| Follow up time on HAART (months) | 19.0 (9.1-31.6) (N = 9040) |

Data is expressed as N (%) except for BMI (mean ± SD), CD4 count (median, IQR) and follow up time on HAART (median, IQR). WHO, World Health Organization, d4T, stavudine; 3TC, lamivudine; EFV, efavirenz; NVP, nevirapine; kaletra, ritonavir/lopinavir; AZT, zidovudine; TDF, tenofovir.

**Table 2 Frequency of and time to HAART associated toxic complications by initiating regimen**

| Condition | Stavudine (N = 8497) | Other drugs (N = 543) |
|---|---|---|
| Peripheral neuropathy | 1454 (17.1)* | 61 (11.2)* |
| Frequency | 6.7 (3.8-111.8) | 5.1 (3.1-11.7) |
| Time to diagnosis | | |
| Symptomatic hyperlactataemia | 487 (5.7)* | 12 (2.2)* |
| Frequency | 14.5 (10.6-20.9) | 11.6 (8.7-19.4) |
| Time to diagnosis | | |
| Lactic Acidosis | 214 (2.5) | 7 (1.3) |
| Frequency | 10.8 (9.0-13.5) | 11.2 (9.0-13.5) |
| Time to diagnosis | | |
| Pancreatitis | 14 (0.2) | 1 (0.2) |
| Frequency | 10.4(4.0-13.0) | 8.3 (8.3-8.3) |
| Time to diagnosis | | |
| Lipoatrophy | 616 (7.3)** | 25 (4.6) ** |
| Frequency | 17.0 (11.4-23.1) | 15.0 (10.5-24.8) |
| Time to diagnosis | | |
| Hypertension | 167 (2.0) | 17 (3.1) |
| Frequency | 9.7 (4.6-18.4)** | 16.3 (9.3-30.5)** |
| Time to diagnosis | | |
| Diabetes | 28 (0.3) | 1 (0.2) |
| Frequency | 22.4 (12.5-28.1) | 9.0 (9.0-9.0) |
| Time to diagnosis | | |
| Dyslipidaemia | 109 (1.3) | 9 (1.7) |
| Frequency | 24.6 (17.4-34.0) | 19.1 (15.3-26.1) |
| Time to diagnosis | | |

Data is given as n (%) for prevalence and median (IQR) for time to diagnosis; *p < 0.005, **p < 0.05 versus group receiving stavudine.

with increased hazard of developing several toxicities: peripheral neuropathy (HR 2.02; 95% CI 1.35-3.03), symptomatic hyperlactataemia (HR 2.81; 95% CI 1.26-6.31), lactic acidosis (adjusted HR 2.55; 95% CI 0.81-8.00) and diabetes mellitus (adjusted HR 2.07; 95% CI 0.28-15.21) though some of these estimates lacked precision (Table 3).

Figures 2, 3, 4 and 5 present Kaplan Meier curves for the crude estimates of the time to development of HAART-related toxicities by initiating regimen. Those initiated on stavudine based regimens were more likely to develop peripheral neuropathy (log rank $p < 0.001$), hyperlactataemia (log rank $p < 0.001$), lactic acidosis (log rank $p = 0.098$) or lipoatrophy (log rank $p < 0.05$) than those on non-stavudine based regimens.

### Other risk factors for development of toxicity

Age, CD4 counts and WHO staging at baseline did not appear to increase the risk of any adverse events after HAART initiation; however, BMIs at baseline did significantly increase the risk of both symptomatic hyperlactataemia and lactic acidosis. Relative to a baseline BMI of < 25 kg/m$^2$, an increased risk of symptomatic hyperlactataemia was seen for those with a BMI of 25-30 kg/m$^2$ (RR 1.7; 95% CI 1.34-2.14, $p < 0.0001$) and those with a BMI of > 30 kg/m$^2$ (RR 1.82; 95% CI 1.35-2.44, $p < 0.0001$). The same was seen with lactic acidosis, where an increased risk was seen for those with a BMI of 25-30 kg/m$^2$ (RR 2.52; 95% CI 1.74-3.65, $p < 0.0001$) and those with a BMI of > 30 kg/m$^2$ (RR 3.10; 95% CI 2.00-4.79, $p < 0.0001$) when compared to subjects with a BMI of < 25 kg/m$^2$.

## Discussion

This study describes data of three years of follow up of 9040 HIV-infected adults initiated on anti-retroviral treatment at the Themba Lethu Clinic, Johannesburg, South Africa. This high number of patients demonstrates the ability of rolling out a successful HAART programme despite being in a resource limited environment, and this, at a rapidity and scale that compares with reports from other African countries [6,11,12].

Despite the fact that stavudine is associated with significant complications, a large proportion of low and middle-income countries still use stavudine based HAART as first line therapy [8,9], mainly because of the cost implications of alternative drugs. In the present study nearly 30% of patients had to switch to non-stavudine based regimens, due to major side effects. These findings concur with those from another large study in South Africa where 21% of patients switched regimens over a similar time period [6].

This study provides estimates of the pattern of toxicities, the predominant ones being peripheral neuropathy, symptomatic hyperlactataemia, and lipoatrophy. Our incidence

**Table 3 Crude and adjusted effects of stavudine use on toxicity initiated on HAART**

| Toxicity | No events | Person Time (years) | Rate/100 pys* (95% CI)‡ | Crude HR§ (95% CI)‡ | Adjusted† HR (95% CI)‡ |
|---|---|---|---|---|---|
| **Peripheral Neuropathy** | 61 | 775.9 | 7.9 (6.0-10.1) | 1.0 | 1.0 |
| *Other* | 1454 | 12055.5 | 12.1 (7.0-19.5) | 1.53 (1.19-1.98) | 2.02 (1.35-3.03) |
| *d4T-based* | | | | | |
| **Symptomatic hyperlactataemia** | 12 | 852.3 | 1.4 (0.7-2.5) | 1.0 | 1.0 |
| *Other* | 487 | 13690.9 | 3.6 (1.2-7.5) | 2.70 (1.52-4.79) | 2.81 (1.26-6.31) |
| *d4T-based* | | | | | |
| **Lactic acidosis** | 7 | 848.3 | 0.8 (0.3-1.7) | 1.0 | 1.0 |
| ***Other*** | 214 | 13805.9 | 1.6 (0.4-5.2) | 2.09 (0.99-4.44) | 2.55 (0.81-8.00) |
| ***d4T-based*** | | | | | |
| **Pancreatitis** | 1 | 858.9 | 0.1 (0.003-0.6) | 1.0 | 1.0 |
| *Other* | 14 | 14126.2 | 0.1 (0.02-2.6) | 0.95 (0.13-7.21) | 0.40 (0.05-3.16) |
| *d4T-based* | | | | | |
| **Lipoatrophy** | 25 | 834.0 | 3.0 (1.9-4.4) | 1.0 | 1.0 |
| *Other* | 616 | 13534.3 | 4.6 (2.1-9.6) | 1.67 (1.12-2.50) | 1.60 (0.92-2.77) |
| *d4T-based* | | | | | |
| **Hypertension** | 17 | 845.0 | 2.0 (1.2-3.2) | 1.0 | 1.0 |
| *Other* | 167 | 13929.0 | 1.2 (0.2-4.0) | 0.66 (0.40-1.08) | 0.83 (0.39-1.78) |
| *d4T-based* | | | | | |
| **Diabetes Mellitus** | 1 | 859.0 | 0.1 (0.003-0.6) | 1.0 | 1.0 |
| *Other* | 28 | 14122.7 | 0.2 (0.02-2.6) | 1.90 (0.26-14.04) | 2.07 (0.28-15.21) |
| *d4T-based* | | | | | |
| **Dyslipidaemia** | 9 | 856.5 | 1.1 (0.4-2.0) | 1.0 | 1.0 |
| *Other* | 109 | 14097.2 | 0.8 (0.2-4.0) | 0.82 (0.41-1.61) | 0.61 (0.27-1.40) |
| *d4T-based* | | | | | |

*pys = person years.

§ HR = hazard ratio estimated from Cox proportional hazard models.

‡ 95% CI = 95% confidence interval.

† All models adjusted for age, gender, baseline CD4 count, baseline body mass index and time at which HAART was initiated (either prior to or post October 2007).

rates of peripheral neuropathy (12.1/100 person-years) were much higher compared to other African studies: 5.2/100 person-years in Rwanda [13] and 2.8/100 person-years in another site in South Africa [6]. The variability in these rates could be because there are no grading protocols for the severity of peripheral neuropathies. Our incidence rates of lactic acidosis were similar to a study from another province in South Africa, 1.6 versus 1.9/100 person-years [14]. The proportion of lactic acidosis was slightly higher compared to another study from Botswana [15], 2.5% versus 1% in our cohort. When compared to this same study [15], the proportion developing symptomatic hyperlactataemia were much higher in our sample (5.7% versus 2%). The median time to the development of lactic acidosis was a little later in our study when compared to another study from South Africa (10.8 months versus 7.5 months) [14].

In terms of the chronic toxicities, incidence rates of lipoatrophy were 4.6/100 person-years on stavudine based therapy and 3.0/100 person-years on the non-stavudine based therapy. One study from Rwanda [13], where patients were also on stavudine based regimens showed a similar incidence rate of lipoatrophy to that reported in the present study at 4.7/100 person-years, while one other study from South Africa [6] showed a lower incidence rate of 1.4/100 person-years. Another study from Rwanda showed a much higher proportion (34%) of patients developing lipoatrophy [16]. The reason for this wide variation in lipoatrophy rates specifically, is possibly due to different diagnostic criteria used for identifying cases. Thus, in the present study and in the studies showing low but similar rates [6,13], only cases that were severe enough to warrant a regimen change were noted. In the study showing a much higher proportion, milder cases of lipodystrophy that did not require a regimen change, were also recorded [16]. An objective case definition of lipodystrophy has been developed [17]; however, it requires access to DEXA and CT imaging technology, which is often not available in resource-limited settings. Therefore, an alternative consensus definition for the diagnosis of lipodystrophy is required for such environments.

A very small number of patients presented with diabetes (0.3%) and dyslipidaemias (1.3%). However, this could be because lipid or glucose levels were only tested when clinically suspected due to cost implications, therefore probably underestimating the true prevalence of dyslipidaemia and diabetes.

The major strength of this study is the large sample size. This cohort is similar to many other resource-limited settings where there is rapid scaling-up of

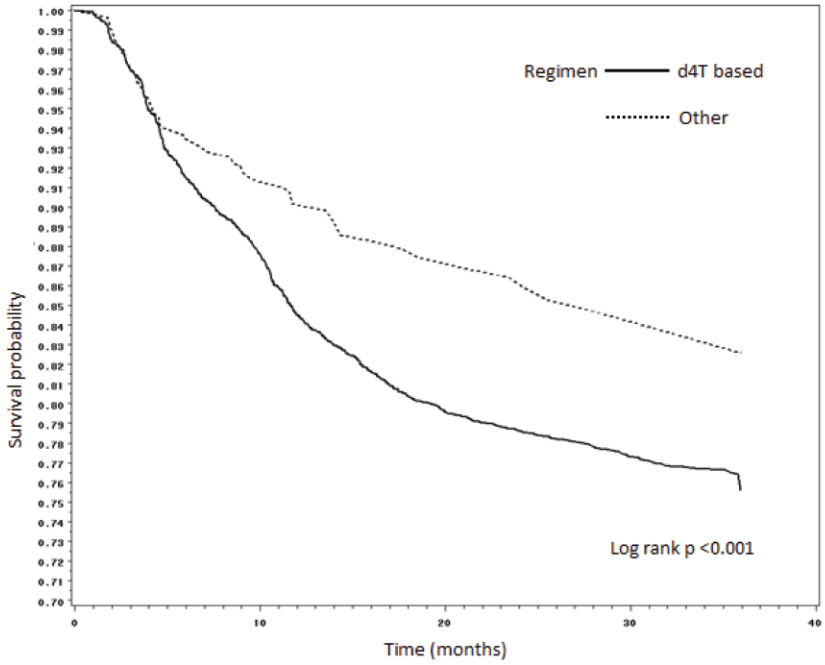


**Figure 2** Kaplan Meier crude estimates of time to development of peripheral neuropathy by initiating regimen (subjects with peripheral neuropathy at initiation of HAART were not included in the analysis).

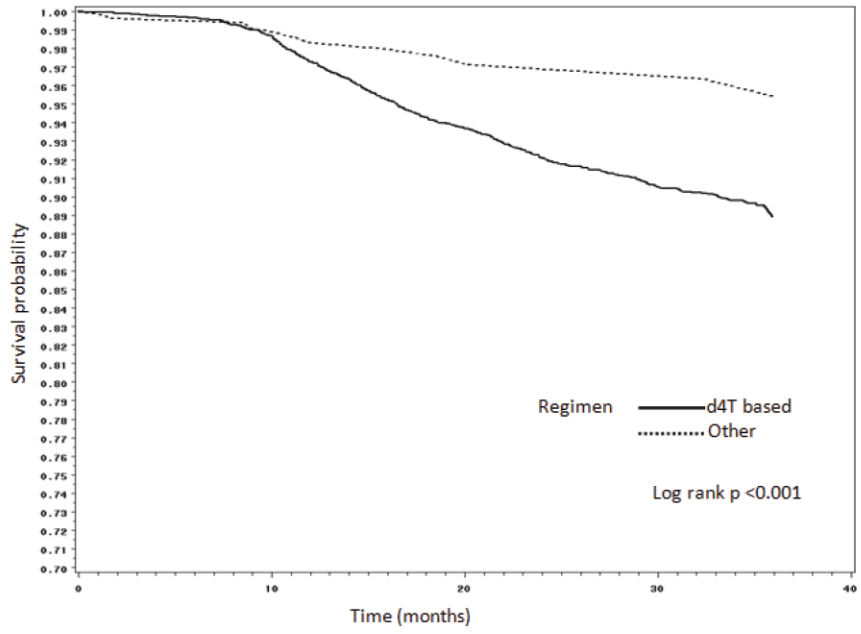


**Figure 3** Kaplan Meier crude estimates of time to development of hyperlactataemia by initiating regimen.

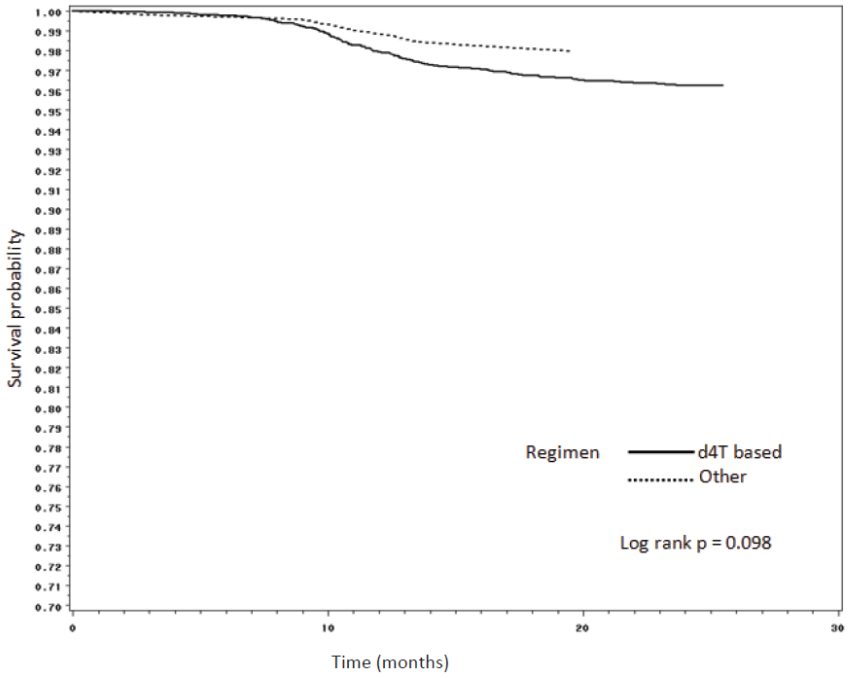


**Figure 4** Kaplan Meier crude estimates of time to development of lactic acidosis by initiating regimen.

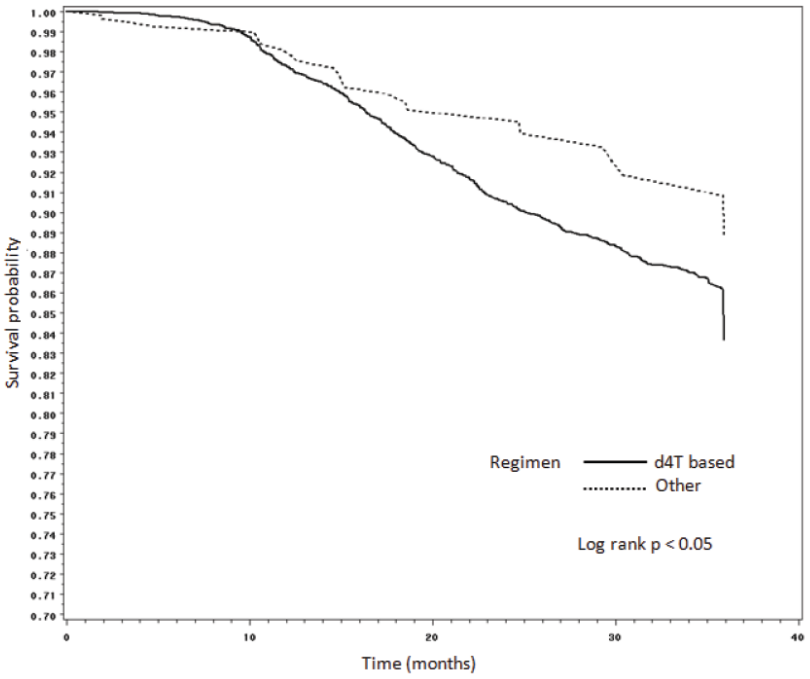


**Figure 5** Kaplan Meier crude estimates of time to development of lipoatrophy by initiating regimen.

comprehensive HIV care and HAART. It allowed for a relatively long duration of follow up of up to three years and a fairly high retention rate of 70%, with all clinicians working on common protocols for defining the various HAART-associated toxicities. However, despite this, these findings must be considered in the light of potential limitations. It is possible that only severe toxicities that warranted a change in regimen may have been reported and this may have led to an underestimation of the rates of some of the HAART-related toxicities, particularly lipoatrophy. Also, plasma glucose and serum lipid levels were not routinely measured and thus the true rates for glucose intolerance, diabetes and dyslipidemia were not attained. Lower reported rates of the chronic toxicities could also be related to the rates of death (5%) and loss to follow up (24%), the majority of which occurred within the first six months on treatment as described in a previous report [18].

## Conclusion

These results support the move away from stavudine based regimens towards less toxic combination regimens as advocated by the World Health Organisation [19,20]. South Africa has implemented these treatment guidelines, where tenofovir has now replaced stavudine as first line therapy. However in resource-limited countries, where staduvine is still being used because of cost implications, proper pharmacovigilance systems need to be established and alternative consensus definitions and grading protocols are required for identifying various HAART-related toxicities.

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### Authors' contributions

Study concept and design: CNM, MM, IS. Acquisition and analysis of data: MM. Interpretation of data: CNM, MM. Drafting of manuscript: CNM. Critical revisions for important intellectual content: CNM, MM, NJC, IS, FJR. All authors read and approved the final manuscript.

### Competing interests

The authors declare that they have no competing interests.

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### References

1. UNAIDS AIDS epidemic update. 2009 [http://data.unaids.org/pub/Report/2009/JC1700_Epi_Update_2009_en.pdf], Last accessed on the 07/12//2010.
2. Department of Health: National Antenatal Sentinel HIV and Syphilis Prevalence Survey in South Africa, 2009. 2010 [http://www.health-e.org.za/documents/85d3dad6136e8ca9d02cceb7f4a36145.pdf], Last accessed on the 31/08//2011.
3. Palella FJ Jr, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, Aschman DJ, Holmberg SD: Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N Engl J Med* 1998, **338**:853-60.
4. Hogg RS, O'Shaughnessy MV, Gataric N, Yip B, Craib K, Schechter MT, Montaner JS: Decline in deaths from AIDS due to new antiretrovirals. *Lancet* 1997, **349**:1294.
5. Wit FW, van Leeuwen R, Weverling GJ, Jurriaans S, Nauta K, Steingrover R, Schuijtemaker J, Eyssen X, Fortuin D, Weeda M, de Wolf F, Reiss P, Danner SA, Lange JM: Outcome and predictors of failure of highly active antiretroviral therapy: one-year follow-up of a cohort of human immunodeficiency virus type 1-infected persons. *J Infect Dis* 1999, **179**:790-798.
6. Boulle A, Orrell C, Kaplan R, van Cutsem G, McNally M, Hilderbrand K, Myer L, Egger M, Coetzee D, Maartens G, Wood R, International Epidemiological Database to Evaluate Aids in Southern Africa Collaboration: Substitution due to antiretroviral toxicity or contraindication in the first 3 years of antiretroviral treatment in a large South African cohort. *Antivir Ther* 2007, **12**:753-60.
7. De Wit S, Sabin CA, Weber R, Worm SW, Reiss P, Cazanave C, El-Sadr W, Monforte A, Fontas E, Law MG, Friis-Møller N, Phillips A, Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study: Incidence and risk factors for new onset diabetes in HIV-infected patients. The data collection on adverse events of anti-HIV drugs (D: A: D) study. *Diabetes Care* 2008, **31**:1224-1229.
8. Beck EJ, Vitoria M, Mandalia S, Crowley S, Gilks CF, Souteyrand Y: National adult antiretroviral therapy guidelines in resource-limited countries: concordance with 2003 WHO guidelines? *AIDS* 2006, **20**:1471-1479.
9. Renaud-Théry F, Nguimfack BD, Vitoria M, Lee E, Graaff P, Samb B, Perriëns J: Use of antiretroviral therapy in resource-limited countries in 2006: distribution and uptake of first- and second-line regimens. *AIDS* 2007, **21(Suppl 4)**:89-95.
10. National Department of Health: National Antiretroviral Treatment Guidelines, First Edition 2004. [http://www.doh.gov.za/docs/misc/2004/sec1.pdf], Last accessed on the 31/08/2011.
11. Stringer JS, Zulu I, Levy J, Stringer EM, Mwango A, Chi BH, Mtonga V, Reid S, Cantrell RA, Bulterys M, Saaq MS, Marlink RG, Mwinqa A, Ellerbrock TV, Sinkala M: Rapid Scale-up of Antiretroviral Therapy at Primary Care Sites in Zambia - Feasibility and Early Outcomes. *JAMA* 2006, **296**:782-793.
12. Coetzee D, Hildebrand K, Boulle A, Maartens G, Louis F, Labatala V, Reuter H, Ntwana N, Goemaere E: Outcomes after two years of providing antiretroviral treatment in Khayelitsha, South Africa. *AIDS* 2004, **18**:887-95.
13. van Griensven J, Zachariah R, Rasschaert F, Mugabo J, Atte EF, Reid T: Stavudine- and nevirapine-related drug toxicity while on generic fixed-dose antiretroviral treatment: incidence, timing and risk factors in a three-year cohort in Kigali, Rwanda. *Trans R Soc Trop Med Hyg* 2010, **104**:148-53.
14. Geddes R, Knight S, Moosa MY, Reddi A, Uebel K, Sunpath H: A high incidence of nucleoside reverse transcriptase inhibitor (NRTI)-induced lactic acidosis in HIV-infected patients in a South African context. *S Afr Med J* 2006, **96**:722-4.
15. Wester CW, Okezie OA, Thomas AM, Bussmann H, Moyo S, Muzenda T, Makhema J, van Widenfelt E, Musonda R, Novitsky V, Gaolathe T, Ndwapi N, Essex M, Kuritzkes DR, DeGruttola V, Marlink RG: Higher-than-expected rates of lactic acidosis among highly active antiretroviral therapy-treated women in Botswana: preliminary results from a large randomized clinical trial. *J Acquir Immune Defic Syndr* 2007, **46**:318-22.
16. Mutimura E, Stewart A, Rheeder P, Crowther NJ: Metabolic function and the prevalence of lipodystrophy in a population of HIV-infected African

subjects receiving highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* 2007, **46**:451-5.
17. Carr A, Emery S, Law M, Puls R, Lundgren JD, Powderly WG: **An objective case definition of lipodystrophy in HIV-infected adults: a case-control study.** *Lancet* 2003, **361**:726-735.
18. Sanne I, Westreich D, Macphail AP, Rubel A, Majuba P, Van Rie A: **Long term outcomes of antiretroviral therapy in a large HIV/AIDS care clinic in urban South Africa: a prospective cohort study.** *J Int AIDS Soc* 2009, **12**:753-760.
19. WHO: **Addendum to the 2006 WHO guidelines on antiretroviral therapy for HIV infection in adults and adolescents.** [http://www.who.int/hiv/art/ARTadultsaddendum.pdf], Last accessed on the 09/12/2010.
20. WHO: **Antiretroviral therapy for HIV infection in adults and adolescents: Recommendations for a public health approach (2010 version).** [http://whqlibdoc.who.int/publications/2010/9789241599764_eng.pdf], Last accessed on the 09/12/2010.

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## 6.2. Appendix 2 – Paper 2

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*HIV Medicine* (2012)

ORIGINAL RESEARCH

# The early effects of stavudine compared with tenofovir on adipocyte gene expression, mitochondrial DNA copy number and metabolic parameters in South African HIV-infected patients: a randomized trial

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**Objectives**

Stavudine is being phased out because of its mitochondrial toxicity and tenofovir (TDF) is recommended as part of first-line highly active antiretroviral therapy (HAART) in South Africa. A prospective, open-label, randomized controlled trial comparing standard- and low-dose stavudine with TDF was performed to assess early differences in adipocyte mtDNA copy number, gene expression and metabolic parameters in Black South African HIV-infected patients.

**Methods**

Sixty patients were randomized 1:1:1 to either standard-dose (30–40 mg) or low-dose (20–30 mg) stavudine or TDF (300 mg) each combined with lamivudine and efavirenz. Subcutaneous fat biopsies were obtained at weeks 0 and 4. Adipocyte mtDNA copies/cell and gene expression were measured using quantitative polymerase chain reaction (qPCR). Markers of inflammation and lipid and glucose metabolism were also assessed.

**Results**

A 29% and 32% decrease in the mean mtDNA copies/cell was noted in the standard-dose ($P < 0.05$) and low-dose stavudine ($P < 0.005$) arms, respectively, when compared with TDF at 4 weeks. Nuclear respiratory factor-1 (*NRF1*) and mitochondrial cytochrome B (*MTCYB*) gene expression levels were affected by stavudine, with a significantly ($P < 0.05$) greater fall in expression observed with the standard, but not the low dose compared with TDF. No significant differences were observed in markers of inflammation and lipid and glucose metabolism.

**Conclusions**

These results demonstrate early mitochondrial depletion among Black South African patients receiving low and standard doses of stavudine, with preservation of gene expression levels, except for *NRF1* and *MTCYB*, when compared with patients on TDF.

**Keywords:** gene expression, insulin resistance, lipids, mitochondria, stavudine, tenofovir

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## Introduction

Despite the benefits of the South African Government's highly active antiretroviral therapy (HAART) roll-out programme which commenced in 2004, adverse events remain an important concern for patients on HAART in this country [1–3]. Understanding the adverse effects of different therapeutic regimens, as well as the underlying molecular and biochemical factors that modulate risk, is critical to improving the management of HIV/AIDS. This is particularly important now that clinical practice is moving towards regimens that combine high levels of both tolerability and efficacy.

Until 2010, South Africa's treatment guidelines for first-line public-sector HAART recommended stavudine with lamivudine (3TC) and either efavirenz (EFV) or nevirapine (NVP) [4]. This stavudine-based regimen, which is cheap and easy to administer in the short term, is associated with significant morbidity, particularly hyperlactataemia syndromes with long-term risks of lipoatrophy, and peripheral neuropathy [1–3]. The main pathogenic mechanism thought to contribute to the metabolic changes and organ toxicities is mitochondrial toxicity, via the inhibition of mitochondrial DNA (mtDNA) polymerase γ (POLG) and alterations in messenger RNA (mRNA) gene expression [5–8]. As a result of the side effects of stavudine, South Africa included tenofovir (TDF) in its recommended first-line public-sector HAART in 2010 [9], despite its cost implications. A study from South Africa demonstrated that the price of TDF would need to fall from USD$17 to USD$6.17 per month to have the same overall cost as stavudine-based therapy [10]. TDF has been shown to have a more favourable effect on lipid and mtDNA profiles compared with stavudine, with no difference in virological response [11].

While the various toxicities associated with stavudine have been well documented, what is less clear is whether the dose of stavudine plays a role in the development of these toxicities. The standard dosage for stavudine is 40 mg given twice daily, as this regimen received regulatory approval because of its efficacy. However, recent studies have suggested that reduced doses of stavudine (i.e. 20 or 30 mg twice daily) diminish its toxicity while maintaining efficacy [11–13].

It is therefore possible that, because stavudine is cheaper than TDF and its side effects can be minimized via reducing its dose, this would allow more patients to be initiated on HAART in countries where the switch away from stavudine may not yet have occurred. Notwithstanding the recent changes in country-level HAART guidelines recommending the use of non-stavudine-based therapy [14,15], several resource-limited countries have yet to phase out stavudine. According to the World Health Organization, in 2010 approximately 56% of HAART regimens within such countries still contained stavudine [16].

Therefore, we have conducted an open-label, randomized controlled trial to identify early molecular and biochemical changes and clinical outcomes for patients on either standard- or low-dose stavudine or TDF. We present here the results of 4 weeks of follow-up through which we set out to assess whether there were any differences in the effect of TDF when compared with low-dose and standard-dose stavudine on adipocyte-specific mitochondrial DNA copy number, gene expression and other metabolic parameters. To our knowledge this is the first study to analyse the mitochondrial effects of stavudine and TDF in a South African HIV-infected population

## Methods

### Study population and inclusion criteria

We enrolled HIV-1-infected, treatment-naïve individuals aged 18 years or older with a CD4 cell count below 200 cells/μL (consistent with the South African guidelines at the time [4]). Patients were enrolled at the Themba Lethu HIV Clinic at the Helen Joseph Hospital, a public-sector hospital in Johannesburg. Eligible subjects had an absolute neutrophil count (ANC) $\geq 750$ cells/μL, haemoglobin $\geq 7.0$ g/dL and a platelet count $\geq 50\,000$ platelets/μL. Other inclusion criteria included an alanine and aspartate aminotransferase measurement and an alkaline phosphatase measurement $\leq 2.5 \times$ the upper limit of normal (ULN) and a total bilirubin measurement $\leq 2.5 \times$ ULN. Patients needed to have a creatinine clearance of $\geq 60$ mL/min using the Cockcroft–Gault formula. In addition, female participants of reproductive age had to have a negative serum or urine pregnancy test within 45 days prior to study entry. Those with reproductive potential had to be willing to use at least one reliable form of contraception.

Patients were excluded if they were breastfeeding or pregnant and if they had received any antiretroviral drugs (including occupational or sexual post exposure prophylaxis) other than single-dose nevirapine (NVP) within the past 6 months.

The study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand and the South African Medicines Control Council (MCC).

### Randomization and masking

Patients were randomly assigned 1:1:1 to receive 3TC and EFV in combination with (i) a standard dose of stavudine (30 mg if weight $< 60$ kg or 40 mg if weight $> 60$ kg) according to the then South African guidelines [4]; (ii) a

low dose of stavudine (20 mg if weight <60 kg or 30 mg if weight > 60 kg); or (iii) TDF (300 mg). The definitions of the standard and low doses of stavudine are similar to those used in several other studies [12]. Coded identity numbers with the arm allocated were sealed in sequentially numbered envelopes. At enrolment, a member of the data collection team unsealed the next envelope to assign the drug arm allocated to the patient. Those analysing patient samples were masked to the assignment.

### Subcutaneous fat biopsies for the determination of mitochondrial DNA copy number and gene expression

At weeks 0 and 4, subcutaneous fat biopsies from the supra-iliac region were performed under local anaesthetic on fasted subjects. Week 4 was chosen to avoid the confounding effects of diet and exercise arising with time. The adipose tissue biopsies were snap-frozen in liquid nitrogen prior to storage at −70°C to avoid risk of mtDNA depletion.

Total DNA was extracted at the same time from adipose tissue collected at weeks 0 and 4 for each patient using the QIAmp DNA Mini Kit (Qiagen, Inc., Hilden, Germany) and a modified protocol [17]. Mitochondrial and nuclear gene copy numbers were determined using a quantitative polymerase chain reaction (qPCR) approach and the number of mitochondria per cell calculated as previously described [17]. Total RNA was extracted using the RNeasy Lipid Tissue Mini Kit (Qiagen) and quantified using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies Inc., Wilmington, Delaware) and the integrity was assessed using the 2100 Bioanalyser and RNA 6000 picochips (Agilent Technologies, Waldbronn, Germany). A 200 ng aliquot of total RNA was converted into cDNA using the Transcriptor high-fidelity cDNA synthesis kit with random hexamers (Roche Diagnostics Ltd, Mannheim, Germany). Quantitative real-time PCR was performed, in duplicate for each sample, using the SensiMix SYBR Low-ROX kit (Bioline Ltd, Luckenwalde, Germany) on a 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA). Each 25 μl reaction mixture contained 1 × SensiMix reaction mix, 0.25 μM of each primer and 6.25 ng of cDNA. Thermocycling conditions for all primer pairs comprised an initial denaturation step of 95°C for 10 min; 45 amplification cycles of 95°C for 15 s, 60°C for 30 s and 72°C for 35 s; and a melt curve step of 95°C for 15 s and 60°C for 20 s ramping at 1% to 95°C for 20 s. Genes assayed included the nuclear genes peroxisome proliferator-activated receptor (PPAR)-γ coactivator 1α (*PGC1*), which is important for mitochondrial biogenesis, nuclear respiratory factor-1 (*NRF1*), which is a major respiratory gene transcription regulator, and mitochondrial transcription factor-A (*TFAM*), which binds to and regulates mtDNA. Other genes measured included those involved in mitochondrial energy metabolism: cytochrome *c* oxidase subunit III (*COX3*), cytochrome *c* oxidase subunit IV (*COX4*) and mitochondrial cytochrome B (*MTCYB*). Two nuclear genes involved in lipid metabolism, leptin (*LEP*) and lipoprotein lipase (*LPL*), were also assayed. Primer sequences for these genes were as previously published [5]. Gene expression was normalized against three reference genes: β2 microglobulin (*B2M*) (5′-TGCTGTCTCCATGTTTGATGTATCT-3′ and 5′-TCTCTGCTCCCCACCTCTAAGT-3′), 60S ribosomal protein L 13A (RPL13A) (5′-CCTGGAGGAGAAGAGGAAAGAGA-3′ and 5′-TTGAGGACCTCTGTGTATTTGTCAA-3′) and hypoxanthine-guanine phosphoribosyltransferase (*HPRT*) (5′-TGACACTGGCAAAACAATGCA-3′ and 5′-GGTCCTTTTCACCAGCAAGCT-3′). Primers for reference genes were designed using Primer Quest (http://eu.idtdna.com/Scitools/Applications/Primerquest). Comparison of gene expression between the week 0 and week 4 samples was performed using the $2^{-\Delta\Delta Cq}$ method. In brief, change in quantification cycle (ΔCq) values for each patient were calculated for both the genes of interest and the reference genes by subtracting the Cq value of the week 4 sample from the Cq value of the week 0 sample. The relative quantities ($2^{\Delta Cq}$) of the genes of interest and the reference genes were calculated. To normalize to multiple reference genes, the geometric mean of the relative quantities of the reference genes was calculated [18]. The normalized relative quantity of each gene of interest was determined by dividing the relative quantity of the gene of interest by this geometric mean. These normalized relative quantities were log-transformed to allow for statistical analysis.

### Blood measurements

After an overnight fast, serum was taken for the measurement of total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides and glucose (Roche Integra Analyzer 400; Roche Diagnostics), C-peptide and insulin (Immulite1000 Analyzer; Diagnostics Corp., Los Angeles, CA), and leptin, adiponectin and high sensitivity C-reactive protein (hs-CRP) [Fluorokine®-Multianalyte profiling (MAP) kit; RnD Systems, Inc., Minneapolis, MN]. A full blood count and a liver function test were performed, urea, creatinine and electrolytes were measured, and CD4 counts were determined (XL-MCL flow cytometer, Beckman Coulter, Miami, FL). Insulin resistance was assessed using the homeostasis model assessment (HOMA) method [19], and was calculated as: [fasting serum insulin (mU/L) × fasting plasma glucose (mmol/L)]/22.5.

### Statistical analysis

Sample size calculations were based on the assumption that at least 70% of participants exposed to stavudine would show features of mitochondrial depletion or features of lipodystrophy [17], while the percentage of participants exposed to TDF experiencing these outcomes would be 30%. With the power of 80%, $\alpha = 0.05$, and equal sample size per group, we required 28 subjects per group. Recruitment to the entire trial was stopped early after a data safety and monitoring board (DSMB) analysis of the first 60 patients demonstrated that the group given stavudine at both standard and low doses showed a greater fall in the mean mtDNA copies/cell than those in the arm receiving TDF. Furthermore, the DSMB considered that there were compelling ethical grounds to stop the high-dose (40 mg) stavudine because the new South African guidelines were shortly to be implemented and TDF had become freely available. Accordingly, all patients on the 40 mg dose of stavudine were switched to TDF. Furthermore, all patients were still followed up until the end of the study period, which was 48 weeks.

As a result, the study may be underpowered to detect differences between the three arms, but this did not affect the differences that were observed.

Data were analysed after creating an anonymized database and calculating differences between week 0 and 4 readings, using STATISTICA® version 10 (StatSoft, Inc., Tulsa, OK). Outliers, calculated using (lower quartile – 1.5 × interquartile range; upper quartile + 1.5 × interquartile range), were excluded from the analysis. Comparisons were made both by randomization arm and by the dosage of drug given. The mitochondrial DNA copy number and gene expression data were analysed using an analysis of variance (ANOVA) for multiple comparisons. Markers of inflammation and lipid and glucose metabolism analysed with a Kruskall–Wallis test. The threshold of significance was set at $P = 0.05$.

## Results

### Baseline characteristics of the study population

Ninety-one HIV-infected patients were screened. Sixty patients qualified for inclusion and consented to participate in the study, and were randomized 1:1:1 to one of three treatment arms between September 2008 and December 2009. The baseline characteristics of the 60 enrolled patients are summarized in Table 1. There was a predominance of female patients (85%) in this study and 59 of 60

Table 1 Patient characteristics at the commencement of highly active antiretroviral therapy (HAART)

| Characteristic | Standard-dose stavudine (30 mg/40 mg) ($n = 20$) | Low-dose stavudine (20 mg/30 mg) ($n = 20$) | TDF (300 mg) ($n = 20$) |
|---|---|---|---|
| Gender | | | |
| Female | 16 (80%) | 18 (90%) | 17 (85%) |
| Male | 4 (20%) | 2 (10%) | 3 (15%) |
| Age (years) | 31 (11) | 34 (12) | 33 (10) |
| WHO AIDS classification | | | |
| Stage 1 | 14 (70%) | 17 (85%) | 16 (80%) |
| Stage 2 | 3 (15%) | 1 (5%) | 3 (15%) |
| Stage 3 | 1 (5%) | 2 (10%) | 1 (5%) |
| Stage 4 | 2 (10%) | 0 (0%) | 0 (0%) |
| BMI (kg/m$^2$) | 25 (5) | 24 (7) | 24 (6) |
| CD4 count (cells/μL) | 155 (94) | 169 (78) | 135 (62) |
| Inflammatory markers | | | |
| hs-CRP (pg/L) | 0.80 (0.55) | 0.66 (0.94) | 0.75 (0.61) |
| Leptin (pg/ml) | 2.70 (15.02) | 3.17 (4.33) | 5.52 (8.49) |
| Adiponectin (pg/ml) | 11.78 (6.58) | 11.76 (8.43) | 13.78 (11.82) |
| Markers of lipid and glucose metabolism | | | |
| C-peptide (nmol/ml) | 0.40 (0.30) | 0.50 (0.20) | 0.40 (0.25) |
| Insulin (mIU/L) | 2.85(4.05) | 3.75 (4.65) | 3.60 (3.00) |
| Total cholesterol (mmol/L) | 3.20 (1.35) | 3.40 (1.10) | 3.80 (1.10) |
| HDL cholesterol (mmol/L) | 0.85 (0.40) | 0.80 (0.45) | 0.80 (0.40) |
| Triglycerides (mmol/L) | 0.70 (0.35) | 0.80 (0.70) | 0.80 (0.30) |
| LDL cholesterol (mmol/L) | 1.90 (1.05) | 2.15 (1.00) | 2.50 (0.70) |
| Glucose (mmol/L) | 3.95 (0.75) | 4.20 (0.80) | 4.20 (0.60) |
| HOMA | 0.54 (0.96) | 0.70 (1.05) | 0.73 (0.60) |

Data are expressed as median (interquartile range) except for gender and World Health Organization (WHO) stage, which are expressed as *n* (%).
BMI, body mass index; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; hs-CRP, high sensitivity C-reactive protein; LDL, low-density lipoprotein; TDF, tenofovir.

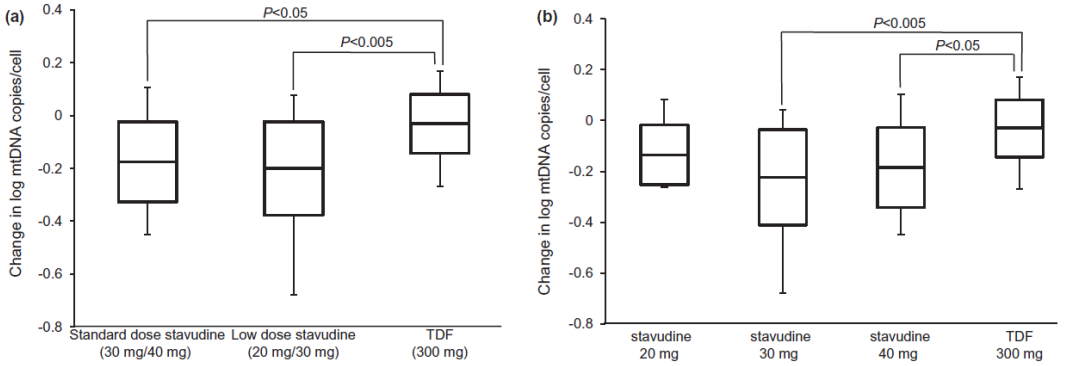


**Fig. 1** Comparison of log change in mtDNA copy number at week 4 by arm and dosage. (a) Comparison of the three study arms, showing a significant difference in the standard-dose (30 mg/40 mg) and low-dose (20 mg/30 mg) stavudine arms compared with tenofovir (TDF). (b) A comparison of various dosages of stavudine with TDF shows significant differences in the 30 and 40 mg stavudine doses compared with TDF. The 20 mg dose was not significantly different ($P = 0.4$). The line within the box-and-whisker plot marks the mean; the upper and lower boundaries of the box indicate 1 standard deviation to either side of the mean and the error bars above and below indicate the minimum and maximum values.

were Black. A total of 78% were at WHO stage 1 while 3% of patients were at WHO stage 4, despite low CD4 counts. There was no statistically significant difference in the markers of inflammation and lipid and glucose metabolism between the arms at baseline.

### Mitochondrial DNA copy numbers and gene expression levels in adipocytes

The effects of HAART on mtDNA copy number and gene expression were assessed in biopsy samples of subcutaneous adipose tissue (Fig. 1). There was a 29% decrease in the mean mtDNA copies/cell from baseline to 4 weeks in the standard-dose stavudine arm (30–40 mg) ($P < 0.05$), and a 32% decrease in the low-dose stavudine arm (20–30 mg) ($P < 0.005$), when compared with the TDF (300 mg) arm, which had only a 4% decrease in the mean mtDNA copies/cell.

For each individual dose of stavudine (20, 30 and 40 mg), there was also a drop in mean mtDNA copy number (22, 35 and 31%, respectively) *vs.* TDF (300 mg) (4%) at 4 weeks of HAART (Fig. 1). The decrease in mtDNA copy number for both the stavudine 30 and 40 mg doses was significantly higher than for TDF 300 mg ($P < 0.005$ and $P < 0.05$, respectively). The drop in mtDNA copy number with the stavudine 20 mg dose was not significant when compared with TDF ($P = 0.40$).

The relative gene expression of *MTCYB* was significantly lower in the standard-dose (30–40 mg) arm when compared with the TDF (300 mg) arm ($P < 0.05$), but not in the low-dose (20–30 mg) stavudine arm (Table 2). There were minimal changes in the expression of *COX3* and *COX4* at 4 weeks when compared to baseline for all three arms.

With respect to the expression of the nuclear genes involved in the regulation of mitochondrial transcription, there were minimal changes in *PGC1* and *TFAM* expression at week 4 when compared with baseline for all three arms. There was a statistically significant difference in *NRF1* expression in the standard-dose stavudine arm (30–40 mg) when compared with the TDF 300 mg arm ($P < 0.05$). Such a difference was not observed in the low-dose (20–30 mg) stavudine arm. Changes in the expression of *LPL* and *LEP* from baseline were not statistically significant at week 4 in any of the arms.

When the effects of the individual stavudine doses (20, 30 and 40 mg) on gene expression were compared with those of TDF, no significant differences were noted for any of the eight genes that were studied (data not shown).

### Markers of inflammation and lipid and glucose metabolism

Following 4 weeks of HAART, there were no statistically significant changes in the lipid profile, glucose, insulin, C-peptide and HOMA indices when comparing the three arms and individual doses. No changes were noted in hs-CRP, leptin or adiponectin levels (Table 2).

## Discussion

This randomized controlled trial was designed to evaluate the *in vivo* effects of TDF against those of standard- and low-dose stavudine regimens, with the reference regimen based on the Gilead 903 trial of Gallant *et al.* [13]. The Gilead 903 trial showed better outcomes and fewer adverse events attributed to mitochondrial toxicity in patients

Table 2 Comparison of the three arms in terms of changes in relative gene expression in relation to housekeeping genes, and markers of inflammation and lipid and glucose metabolism parameters from baseline at week 4

| Characteristic | Standard-dose stavudine (30 mg/40 mg) ($n = 20$) | Low-dose stavudine (20 mg/30 mg) ($n = 20$) | TDF (300 mg) ($n = 20$) |
|---|---|---|---|
| Genes involved in mitochondrial energy metabolism | | | |
| *COX4* | −0.01 ± 0.34 | 0.04 ± 0.49 | 0.07 ± 0.54 |
| *COX3* | −0.11 ± 0.39 | 0.01 ± 0.37 | 0.13 ± 0.34 |
| *MTCYB* | −0.20 ± 0.43* | −0.11 ± 0.50 | 0.16 ± 0.42 |
| Nuclear genes involved in the regulation of mitochondrial transcription | | | |
| *NRF1* | −0.21 ± 0.32* | 0.00 ± 0.51 | 0.11 ± 0.26 |
| *PGC1α* | −0.23 ± 0.75 | −0.03 ± 0.36 | −0.02 ± 0.57 |
| *TFAM* | −0.05 ± 0.23 | 0.08 ± 0.26 | 0.02 ± 0.24 |
| Genes involved in lipid metabolism | | | |
| *LEP* | 0.09 ± 0.34 | 0.09 ± 0.40 | −0.06 ± 0.36 |
| *LPL* | 0.06 ± 0.22 | 0.08 ± 0.29 | −0.01 ± 0.20 |
| Inflammatory markers | | | |
| hs-CRP (pg/L) | 0.22 (0.39) | 0.15 (0.43) | 0.11 (0.33) |
| Leptin (pg/ml) | 0.18 (2.62) | 0.59 (2.20) | −0.13 (2.89) |
| Adiponectin (pg/ml) | 0.86 (5.29) | 2.12 (5.33) | 1.53 (6.52) |
| Markers of lipid and glucose metabolism | | | |
| C-peptide (nmol/ml) | 0.00 (0.20) | −0.10 (0.10) | 0.00 (0.20) |
| Insulin (mU/L) | −0.25 (5.00) | 0.05 (4.70) | −0.60 (3.30) |
| Total cholesterol (mmol/L) | 0.60 (0.65) | 0.60 (1.00) | 0.60 (0.60) |
| HDL cholesterol (mmol/L) | 0.10 (0.25) | 0.20 (0.30) | 0.20 (0.30) |
| Triglycerides (mmol/L) | 0.20 (0.40) | 0.10 (0.30) | 0.20 (0.30) |
| LDL cholesterol (mmol/L) | 0.35 (0.75) | 0.40 (0.40) | 0.10 (0.70) |
| Glucose (mmol/L) | 0.30 (0.70) | 0.00 (1.10) | 0.20 (1.00) |
| HOMA | −0.05 (1.12) | −0.08 (0.88) | −0.17 (0.69) |

Data are expressed as means ± standard deviation except for markers of inflammation and lipid and glucose metabolism, which are expressed as median (interquartile range); *$P < 0.05$ *vs.* TDF (300 mg).
BMI, body mass index; *COX*, cytochrome *c* oxidase; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; hs-CRP, high sensitivity C-reactive protein; LDL, low-density lipoprotein; *LEP*, leptin; *LPL*, lipoprotein lipase; *MTCYB*, mitochondrial cytochrome B; *NRF1*, nuclear respiratory factor-1; *PGC1α*, peroxisome proliferator-activated receptor-γ coactivator 1α; TDF, tenofovir; *TFAM*, mitochondrial transcription factor-A.

receiving TDF 300 mg when compared with stavudine 40 mg given twice daily [13], but did not report mtDNA and gene expression evaluations. Our study is the first to compare mitochondrial effects of these drugs in a Black population and at a time-point in HAART therapy before the development of peripheral lipoatrophy.

The major finding in our study is the significant depletion, after only 4 weeks of therapy, of adipocyte mtDNA copy number in Black South African HIV-infected individuals receiving stavudine. No effect of TDF on mtDNA copy number was observed. This study shows that the low-dose stavudine regimen produced a fall in mitochondrial DNA copy number similar to that observed with the standard stavudine dose but greater than that seen with TDF. This finding is consistent with the results of other studies performed in non-Black populations who had developed peripheral lipoatrophy at the time of sampling and after a longer duration of antiretroviral therapy [17,20–25]. Our results therefore suggest that mitochondrial pathology precedes changes in body fat distribution and occurs very early after the initiation of HAART.

This study also provides evidence of a dose–response effect in that higher doses of stavudine caused a greater loss of mtDNA after 4 weeks of HAART. Thus, stavudine at doses of 30 and 40 mg produced a greater fall in mtDNA than TDF, whereas the 20 mg dose of stavudine was associated with a nonsignificant decrease in mtDNA, when compared with the TDF arm. Despite the significant depletion in mtDNA, our study did not demonstrate a significant effect of stavudine with regard to the expression of genes associated with mitochondrial energy metabolism and biogenesis, apart from *MTCYB* and *NRF1*. This is similar to the findings of other studies [6–8,25], where only minimal changes were reported at the gene expression level. However, these findings are in contrast to those of Mallon *et al.*, who found significant reductions in gene expression without significant depletion of mtDNA after 2 weeks of HAART, although, notably, they compared stavudine with zidovudine (ZDV) and their study was in HIV-negative patients [5]. Significant changes in the expression levels of *NRF1* and *MTCYB* were observed in the subjects given the standard stavudine dose but not in those receiving the low-dose therapy. *NRF1* is an important regulator of mitochondrial biogenesis and function. A master transcription factor, NRF1 binds the regulatory regions of genes encoding subunits of the respiratory complex and various

constituents of the mtDNA transcription and replication machinery. In addition, it serves to coordinate nucleo-mitochondrial activities by regulating mitochondrial and cytosolic enzymes [26]. These results suggest that down-regulation of *NRF1* is an early event in nucleoside reverse transcriptase inhibitor (NRTI) toxicity. This change in *NRF1* expression is then cascaded to the factors that are under its control, with effects that are only observed much later in the time-course of HAART [7,8,25].

Similar to our results, changes in *MTCYB* expression have been observed in other studies [6,24]. These changes may be associated with increased oxidative stress and apoptosis related to mitochondrial depletion [6,27].

Our findings must be considered in light of the study's limitations. Firstly, the study was stopped prematurely because of the introduction of the new South African National Treatment Guidelines, which may have reduced the statistical power of the study. Our participant sample size, however, was much larger than those of other studies [3,5,6], and was still sufficient for us to be able to detect significant effects of HAART on mtDNA copy number and gene expression. Another possible limitation is the short duration of therapy (4 weeks) at the time of sampling. Other studies have shown significant mtDNA and mtRNA depletion in HIV-infected patients on a longer duration of antiretroviral therapy of months to years [7,8]. In this study, the 4th week was chosen to avoid the potential confounding effects of changes in diet, exercise or body weight.

Despite these limitations, our findings are strengthened by the fact that the laboratory technique used to determine mtDNA copy number was based on a well-established protocol [17] that has been standardized and extensively critiqued [28]. Another important strength of this study was the adoption of the minimum information for publication of quantitative real-time PCR experiments (MIQE) guidelines [29] with the normalization of gene expression to multiple reference genes [18].

The findings of this study are important as previous trials in Europe, the USA and Australia did not examine the effects of mitochondrial changes in an African population [5–8,11,17,20–25]. Our study is the first randomized trial assessing the mitochondrial pathogenesis of NRTI-associated toxicities in an African population.

In conclusion, this study demonstrates an early association between mitochondrial depletion and stavudine therapy in the Black South African population and shows that TDF has a minimal effect on mitochondrial numbers. The expression levels of only two of eight adipocyte genes were significantly affected by stavudine therapy when compared with TDF, and this only with the standard dose (30 and 40 mg). These results support the new South African HAART guidelines which have evolved since 2004, with TDF 300 mg currently being recommended as first-line therapy [9]. However, minimal effects on gene expression were noted with low-dose (20–30 mg) stavudine. Therefore, further larger studies using this regimen should be initiated, particularly in light of data showing that this dose is effective and safe [11–13] and, furthermore, it is unlikely that stavudine will ever be completely phased out of use in countries with limited financial resources.

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*Authors' contributions*: Study concept and design: CNM and IS. Acquisition of data: DVA and CNM. Laboratory analysis: CD, TDP and RD. Analysis of data: CNM, RD, CD, TDP, MM, MPF, PM and NJC. Interpretation of data: CNM, RD, CD, TDP and NJC. Drafting of manuscript: CNM. Critical revisions for important intellectual content: all authors. Read and approved the final manuscript: all authors.

## References

1 Boulle A, Orrell C, Kaplan R *et al.* Substitution due to antiretroviral toxicity or contraindication in the first 3 years of antiretroviral treatment in a large South African cohort. *Antivir Ther* 2007; 12: 753–760.

2 Sanne I, Westreich D, Macphail AP, Rubel A, Majuba P, Van Rie A. Long term outcomes of antiretroviral therapy in a large HIV/AIDS care clinic in urban South Africa: a prospective cohort study. *J Acquir Immune Defic Syndr* 2009; 12: 753–760.

3 Menezes CN, Maskew M, Sanne I, Crowther NJ, Raal FJ. A longitudinal study of stavudine-associated toxicities in a large cohort of South African HIV infected subjects. *BMC Infect Dis* 2011; 11: 244–253.

4 National Department of Health. National Antiretroviral Treatment Guidelines, First Edition. 2004. Available at www.doh.gov.za/docs/misc/2004/sec1.pdf (accessed 31 May 2012).

5 Mallon PW, Unemori P, Sedwell R *et al.* In Vivo, Nucleoside Reverse –transcriptase inhibitors alter expression of both mitochondrial and lipid metabolism genes in the absence of depletion of mitochondrial DNA. *JID* 2005; 191: 1686–1696.

6 Kim MJ, Jardel C, Barthélémy C *et al.* Mitochondrial DNA content, an inaccurate biomarker of mitochondrial alteration in human immunodeficiency virus-related lipodystrophy. *Antimicrob Agents Chemother* 2008; 52: 1670–1676.

7 Sievers M, Walker UA, Sevastianova K *et al.* Gene expression and immunohistochemistry in adipose tissue of HIV Type 1-infected patients with nucleoside analogues reverse-transcriptase inhibitor-associated lipoatrophy. *JID* 2009; 200: 252–262.

8 Pace CS, Martin AM, Hammond EL, Mamotte CD, Nolan DA, Mallal SA. Mitochondrial proliferation, DNA depletion and adipocyte differentiation in subcutaneous adipose tissue of HIV-positive HAART recipients. *Antivir Ther* 2003; 8: 323–331.

9 National Department of Health South Africa. 2010. Clinical guidelines for the Management of HIV & AIDS in adults and adolescents. Available at www.health-e.org.za/documents/0ba405d9042e557a9fb1987adb68cb97.pdf (accessed 31 May 2012).

10 Rosen S, Long L, Fox M, Sanne I. Cost and cost-effectiveness of switching from stavudine to tenofovir in first-line antiretroviral regimens in South Africa. *J Acquir Immune Defic Syndr* 2008; 48: 334–344.

11 Milinkovic A, Martinez E, López S *et al.* The impact of reducing stavudine dose versus switching to tenofovir on plasma lipids, body composition and mitochondrial function in HIV-infected patients. *Antivir Ther* 2007; 12: 407–415.

12 Hill A, Ruxrungtham K, Hanvanich M *et al.* Systematic review of clinical trials evaluating low doses of stavudine as part of antiretroviral treatment. *Expert Opin Pharmacother* 2007; 8: 679–688.

13 Gallant JE, Staszewski S, Pozniak AL *et al.* Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial. *JAMA* 2004; 292: 191–201.

14 Writing Group, Williams I, Churchill D *et al.* British HIV Association guidelines for the treatment of HIV-1-positive adults with antiretroviral therapy 2012. *HIV Med* 2012; 13: 1–6.

15 Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services. Available at http://aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf (accessed 6 August 2012).

16 WHO. Antiretroviral therapy for HIV infection in adults and adolescents: recommendations for a public health approach. (2010 version). Available at http://whqlibdoc.who.int/publications/2010/9789241599764_eng.pdf (accessed 31 May 2012).

17 Nolan D, Hammond E, Martin A *et al.* Mitochondrial DNA depletion and morphologic changes in adipocytes associated with nucleoside reverse transcriptase inhibitor therapy. *AIDS* 2003; 17: 1329–1338.

18 Hellemans J, Mortier G, De Paepe A, Speleman F, Vandesompele J. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome Biol* 2007; 8: R19.

19 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; 28: 412–419.

20 Schooley RT, Ruane P, Myers RA *et al.* Tenofovir DF in antiretroviral-experienced patients: results from a 48-week, randomized, double-blind study. *AIDS* 2002; 16: 1257–1263.

21 Shikuma CM, Hu N, Milne C *et al.* Mitochondrial DNA decrease in subcutaneous adipose tissue of HIV-infected individuals with peripheral lipoatrophy. *AIDS* 2001; 15: 1801–1809.

22 Mallal SA, Hammond EL, Martin A, Taylor L, John M, Nolan DA. Mitochondrial DNA depletion, assessed by real-time PCR-based quantitative assay, in subcutaneous fat of HIV-infected patients: evidence for a role in the etiology of fat wasting. *4th International Conference on Nutrition and HIV Infection*. Cannes, France, April 2001 [Abstract O7].

23 Buffet M, Schwarzinger M, Amellal B *et al.* Mitochondrial DNA depletion in adipose tissue of HIV-infected patients with peripheral lipoatrophy. *J Clin Virol* 2005; 33: 60–64.

24 Stankov MV, Lücke T, Das AM, Schmidt RE, Behrens GM. Mitochondrial DNA depletion and respiratory chain activity in primary human subcutaneous adipocytes treated with nucleoside analogue reverse transcriptase inhibitors. *Antimicrob Agents Chemother* 2010; 54: 280–287.

25 Boothby M, McGee KC, Tomlinson JW *et al.* Adipocyte differentiation, mitochondrial gene expression and fat distribution: differences between zidovudine and tenofovir after 6 months. *Antivir Ther* 2009; 14: 1089–1100.

26 Scarpulla RC. Transcriptional paradigms in mammalian mitochondrial biogenesis and function. *Physiol Rev* 2008; 88: 611–638.

27 Komarov AP, Rokhlin OW, Yu CA, Gudkov AV. Functional genetic screening reveals the role of mitochondrial cytochrome b as a mediator of FAS-induced apoptosis. *Proc Natl Acad Sci USA* 2008; 105: 14453–14458.

28 Cote HC, Gerschenson M, Walker UA *et al.* Quality assessment of human mitochondrial DNA quantification: MITONAUTS, an international multicentre survey. *Mitochondrion* 2011; 11: 520–527.

29 Bustin SA, Benes V, Garson JA *et al.* The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem* 2009; 55: 611–622.

## 6.3. Appendix 3 – Paper 3

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ORIGINAL RESEARCH

# A randomized clinical trial comparing metabolic parameters after 48 weeks of standard- and low-dose stavudine therapy and tenofovir disoproxil fumarate therapy in HIV-infected South African patients

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Objectives

Low-dose stavudine therapy may have a lower toxicity profile compared with standard dose. A randomized controlled trial comparing these two doses of stavudine with tenofovir disoproxil fumarate (tenofovir DF) was performed to assess the effects on anthropometry, markers of inflammation, and lipid and glucose metabolism in Black South African patients.

Methods

Sixty patients were randomized 1:1:1 to either standard-dose (30–40 mg) or low-dose (20–30 mg) stavudine or tenofovir DF (300 mg), each combined with lamivudine and efavirenz, for 48 weeks. Anthropometry, markers of inflammation, and lipid and glucose metabolism were assessed using standard techniques.

Results

In all three treatment arms, there was a significant increase in lipid levels over the study period. At 48 weeks, fasting glucose level ($P < 0.005$) and homeostasis model assessment (HOMA) score ($P < 0.05$) increased significantly in the standard-dose stavudine arm, as did insulin and C-peptide levels in both the standard- and low-dose stavudine arms. At week 48, a significant decrease ($P < 0.05$) in adiponectin was noted in the standard-dose stavudine arm, but there was an increase ($P < 0.005$) in the tenofovir DF arm. In both the stavudine arms, significant increases in anthropometric measures occurred at 24 weeks but these decreased by week 48. Mitochondrial toxicities occurred in both the stavudine arms. Immunological and virological outcomes were similar for all three arms.

Conclusions

This study highlights the occurrence of metabolic abnormalities with both stavudine and tenofovir DF treatment. Awareness of the potential increased cardiovascular risk should be of concern with the use of both these therapies.

Keywords: glucose, lipids, randomized trial, stavudine, tenofovir disoproxil fumarate

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## Introduction

The success in controlling HIV-1 infections with highly active antiretroviral therapy (HAART) [1,2] has been marred by the emergence of metabolic complications in HIV- positive patients after long-term therapy [3–7].

An increased risk of myocardial infarction, increased rates of dyslipidaemia, insulin resistance and diabetes, and alterations in body fat distribution have been described with the use of HAART [3–7]. Changes in leptin and adiponectin levels that are observed in HIV infection may play a major role in the pathogenesis of HIV/HAART-related metabolic syndrome [8,9]. Furthermore, elevated levels of high-sensitivity C-reactive protein (hs-CRP), an inflammatory marker, which have been observed in HIV-infected male patients [10], are associated with an increased risk of cardiovascular events in the general population [11,12] and in HIV-positive patients [13].

Previously, the aetiology of these metabolic abnormalities has been attributed to the use of protease inhibitors (PIs) [4]. More recently, nucleoside reverse transcriptase inhibitors (NRTIs) have been implicated, particularly stavudine [3,14–16]. Stavudine continues to be used as an alternative first-line treatment in developing countries despite the change in the World Health Organization (WHO) antiretroviral therapy guidelines for treating patients with HIV infection [17]. Stavudine was used as first-line HIV therapy in South Africa until 2010, when it was replaced with tenofovir disoproxil fumarate (tenofovir DF) [18,19], but it continues to be used because of shortages of tenofovir DF and abacavir that occur intermittently at health facilities across South Africa [20].

In the Gilead 903 trial [21], no major differences were noted between stavudine and tenofovir DF with regard to serious adverse events (AEs). Other studies have shown modest rates of nephrotoxicity with tenofovir DF [22–25], with one study finding significant nephrotoxicity at a yearly incidence of 1% [26].

The recommended dose for stavudine is 40 mg twice daily, but studies have shown that lower doses (20 or 30 mg twice daily) diminish toxicity while maintaining immunological efficacy [27–29]. Therefore, in resource-limited countries where the switch away from stavudine to the more expensive tenofovir DF has not yet occurred, low-dose stavudine could still be used, allowing more patients to be initiated on treatment.

To determine whether low-dose stavudine therapy was as effective and safe as tenofovir DF in a resource-poor environment, we performed an open-label, randomized controlled trial. Biochemical changes and clinical outcomes for patients on either low- or standard-dose stavudine were compared with those for patients receiving tenofovir DF.

The effects of these two drugs on adipocyte gene expression, mitochondrial DNA copy number and metabolic parameters after only 4 weeks of therapy in a cohort of Black South African HIV-positive patients were discussed in an earlier paper [30]. We present here follow-up data on anthropometry, serological markers of inflammation, and lipid and glucose metabolism measured after 48 weeks of therapy.

## Methods

### Study population

HIV-1-infected individuals aged 18 years or older with a CD4 cell count < 200 cells/μL were enrolled in this study at the Themba Lethu HIV Clinic at the Helen Joseph Hospital, Johannesburg, South Africa. Inclusion criteria were: an absolute neutrophil count (ANC) ≥ 750 cells/μL, a haemoglobin concentration ≥ 7.0 g/dL, a platelet count ≥ 50 000 cells/μL, a creatinine clearance of ≥ 60 mL/min calculated using the Cockcroft–Gault formula [31], and alanine and aspartate aminotransferase, alkaline phosphatase and total bilirubin concentrations of ≤ 2.5 times the upper limit of normal. Patients of reproductive age had to have a negative serum or urine pregnancy test within 45 days prior to study entry, and had to be willing to use at least one reliable form of contraception. Exclusion criteria were: breastfeeding or pregnancy, and receiving antiretroviral drugs including occupational or sexual post-exposure prophylaxis, other than single-dose nevirapine, within the previous 6 months.

Patients were seen at screening and at weeks 0, 4, 24 and 48. Routine evaluations were performed at each time-point, while markers of inflammation, and lipid and glucose metabolism were measured at weeks 0, 4 and 48. These evaluations included a review of AEs and concomitant medications, a complete or symptom-directed physical examination, height and weight measurements, and evaluations of haematology and chemistry profiles, calculated creatinine clearance, CD4 cell count, plasma HIV-1 RNA and study drug accountability. Dual-energy radiographic absorptiometry (DEXA) scanning was performed at baseline and week 48.

Approval was received from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand and the SA Medicines Control Council (MCC). This study was compliant with the revised CONSORT statement guidelines (http://www.consort-statement.org).

### Antiretroviral therapy

As previously described [31], patients were assigned in random 1:1:1 permuted blocks of five to receive lamivudine and efavirenz in combination with (a) standard-dose stavudine (30 mg if weight < 60 kg or 40 mg if weight > 60 kg) according to the then South African guidelines [18]; (b) low-dose stavudine (20 mg if weight < 60 kg or 30 mg if weight > 60 kg); or (c) tenofovir DF 300 mg. The definitions of the standard and low doses of stavudine are the same as those used in other studies [29]. Staff analysing patient samples were blinded to the treatment assigned to each patient.

### Blood measurements

Fasting serum was taken for the measurement of total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides and glucose (Roche Integra Analyzer 400; Roche Diagnostics, Mannheim, Germany), C-peptide and insulin (Immulite1000 Analyzer; Diagnostics Corp., Los Angeles, CA), and leptin, adiponectin and hs-CRP (Fluorokine®-Multianalyte profiling (MAP) kit; RnD Systems, Inc., Minneapolis, MN). A full blood count was performed and creatinine, liver function, viral load (Cobas Amplicor Analyzer, Roche Diagnostics, Indianapolis, IN) and CD4 count (XL-MCL flow cytometer, Beckman Coulter, Miami, FL) were measured. Insulin resistance was assessed using the homeostasis model assessment (HOMA) method [32].

### Anthropometry and body composition measurements

Height and weight were measured using regularly calibrated instruments. Circumferences of the mid-arm, chest, waist, hip and mid-thigh were obtained using a Gulick II measuring tape (FitnessMart®, Gays Mills, WI). Skinfolds of the triceps, biceps, subscapular and suprailiac were measured using a Harpenden skin fold caliper (British Indicators, West Sussex, UK). For all anthropometry measurements, two independent measurements were taken. If the first two measurements were outside of the acceptable range of variance for a particular body area, then a third measurement was taken. The mean of the two closest measures was then used in analysis. Total lean and fat masses were measured with a Hologic whole-body, dual-energy radiographic absorptiometry (DEXA) scanner (Hologic Inc., Waltham, MA) and software, version 12.4.

### Statistical analysis

Data that could be normalized were log-transformed to normality and analysed using parametric statistical analyses, while the remaining variables were analysed using nonparametric statistical tests. With a power of 80%, $\alpha$ = 0.05, and equal sample size per group, we required 28 patients per treatment arm, assuming that 70% of patients exposed to stavudine and 30% exposed to tenofovir DF would show features of mitochondrial toxicity [33]. However, the trial was stopped early after a data safety and monitoring board (DSMB) analysis of the first 60 patients in March 2010 showed that the group given stavudine at both standard and low doses had greater mitochondrial depletion than those in the arm receiving tenofovir [30]. Furthermore, the DSMB considered that there were compelling ethical grounds to stop treatment with high-dose stavudine because the new South African guidelines were shortly to be implemented [19], and tenofovir DF had become freely available. At that point, the four patients who were still on 40 mg of stavudine were switched to tenofovir. Those patients who switched therapy or who, for any other reason, had to prematurely leave the study were excluded from the final analysis.

Data were analysed using STATISTICA® version 10 (StatSoft, Inc., Tulsa, OK). Results were summarized using proportions and medians with interquartile ranges (IQRs). Comparisons were made at different time intervals within each arm using the Wilcoxon matched pairs test or Student's paired *t*-test, while comparisons across arms were performed using analysis of variance (ANOVA) for baseline levels or analysis of covariance (ANCOVA), with adjustment for baseline levels.

## Results

### Baseline characteristics of the study population

Figure 1 shows the cohort profile: 60 eligible patients were randomized 1:1:1 to the three treatment arms between September 2008 and December 2009.

There was a predominance of female patients (85%) in this study and 58 of the 60 patients were Black (Table 1). All patients were clinically asymptomatic, with 78% of the patients being WHO stage 1.

### Mitochondrial toxicity

As previously described [30], the tenofovir DF arm had a 4% decrease in the mean mtDNA copies/cell from baseline to 4 weeks, while there was a 29% decrease in the standard-dose stavudine arm ($P < 0.05$), and a 32% decrease in the low-dose stavudine arm ($P < 0.005$).

Relative gene expression was also affected, with mitochondrial cytochrome B (*MTCYB*) and nuclear respiratory factor-1 (*NRF1*) expression being significantly lower in the standard-dose stavudine arm compared with the tenofovir DF arm ($P < 0.05$).

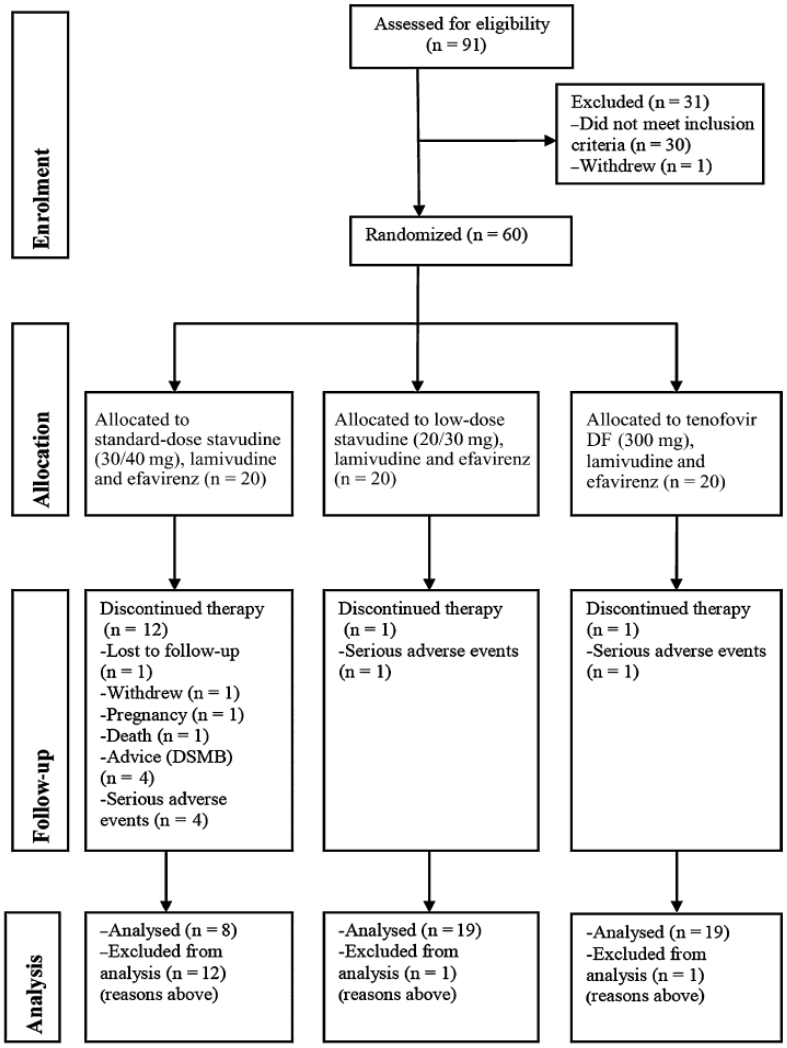


**Fig. 1** Profile of the trial cohort. DSMB, data safety and monitoring board.

**Table 1** Patient characteristics at the commencement of highly active antiretroviral therapy (HAART)

| Characteristic | Standard-dose stavudine (30/40 mg) (*n* = 20) | Low-dose stavudine (20/30 mg) (*n* = 20) | Tenofovir DF (300 mg) (*n* = 20) |
|---|---|---|---|
| African race | 20 (100) | 19 (95) | 19 (95) |
| Female gender | 16 (80) | 18 (90) | 17 (85) |
| Age (years) | 31 (11) | 34 (12) | 33 (10) |
| Weight (kg) | 64 (17) | 62 (17) | 66 (19) |
| BMI (kg/m$^2$) | 25 (5) | 24 (7) | 24 (6) |
| Haemoglobin (g/dL) | 12 (3) | 12 (2) | 12 (2) |
| GGT (IU/L) | 24 (24) | 30 (11) | 22 (11) |
| ALT (IU/L) | 21 (21) | 20 (15) | 18 (12) |
| Creatinine clearance (mL/min) | 108 (17) | 108 (25) | 99 (40) |

Data are expressed as median (interquartile range), except for race and gender which are expressed as n (%).
Patients in the standard-dose stavudine arm were given 30 mg if weight < 60 kg or 40 mg if weight > 60 kg; patients in the low-dose stavudine arm were given 20 mg if weight < 60 kg or 30 mg if weight > 60 kg.
BMI, body mass index; GGT, gamma-glutamyltransferase; ALT, alanine aminotransferase.

Table 2 Comparison of the markers of inflammation, glucose and lipid metabolism at different time intervals

| Characteristic | Standard-dose stavudine (30/40 mg) | | | Low-dose stavudine (20/30 mg) | | | Tenofovir DF (300 mg) | | |
|---|---|---|---|---|---|---|---|---|---|
| Time (weeks) | 0 | 4 | 48 | 0 | 4 | 48 | 0 | 4 | 48 |
| n | 20/20 | 20/20 | 8/20 | 20/20 | 20/20 | 19/20 | 20/20 | 20/20 | 19/20 |
| Total cholesterol (mmol/L) | 3.20 (1.35) | 4.10*** (1.35) | 4.35* (1.20) | 3.40 (1.10) | 4.10** (1.70) | 4.50*** (1.50) | 3.80 (1.10) | 4.20** (1.10) | 4.60*** (0.7) |
| HDL cholesterol (mmol/L) | 0.85 (0.40) | 1.00* (0.50) | 1.35* (0.55) | 0.80 (0.45) | 1.00** (0.50) | 1.25*** (0.60) | 0.80 (0.40) | 1.10** (0.60) | 1.30*** (0.40) |
| Triglycerides (mmol/L) | 0.70 (0.35) | 0.90** (0.45) | 0.95 (0.20) | 0.80 (0.70) | 0.80 (0.60) | 1.10 (0.70) | 0.80 (0.30) | 0.90* (0.40) | 0.80 (0.30) |
| LDL cholesterol (mmol/L) | 1.90+ (1.05) | 2.60*** (1.15) | 2.35 (1.20) | 2.15 (1.00) | 2.40** (1.20) | 2.70*** (1.70) | 2.50 (0.70) | 2.80 (1.25) | 2.85* (0.70) |
| C-peptide (nmol/mL) | 0.40 (0.30) | 0.50 (0.20) | 0.50* (0.20) | 0.50 (0.20) | 0.40* (0.25) | 0.60* (0.30) | 0.40 (0.25) | 0.45 (0.25) | 0.50 (0.10) |
| Insulin (mU/L) | 2.85 (4.05) | 3.65 (3.00) | 3.95* (2.60) | 3.75 (4.65) | 3.10 (2.80) | 6.25* (5.50) | 3.60 (3.00) | 3.00 (2.65) | 4.80 (3.90) |
| Glucose (mmol/L) | 3.95 (0.75) | 4.40 (0.40) | 4.40* (0.70) | 4.20 (0.80) | 4.30 (0.70) | 4.40 (0.70) | 4.20 (0.60) | 4.35 (0.70) | 4.60 (0.40) |
| HOMA | 0.54 (0.96) | 0.69 (0.81) | 0.83* (0.59) | 0.7 (1.05) | 0.51 (0.59) | 1.25 (1.30) | 0.73 (0.60) | 0.59 (0.56) | 0.91 (0.88) |
| hs-CRP (pg/L) | 0.80 (0.55) | 0.84* (0.75) | 0.79 (0.35) | 0.66 (0.94) | 0.86 (0.82) | 0.94 (0.53) | 0.75 (0.61) | 0.83 (0.73) | 0.93 (0.56) |
| Leptin (pg/mL) | 2.69+ (15.02) | 4.09 (10.08) | 1.84 (4.43) | 3.17 (4.33) | 4.07* (6.07) | 3.77 (7.25) | 5.52 (8.49) | 4.79 (12.92) | 11.82* (27.45) |
| Adiponectin (pg/mL) | 11.78 (6.58) | 13.53 (4.08) | 7.21*+ (3.86) | 11.75 (8.43) | 15.90 (11.14) | 12.18 (14.73) | 13.78 (11.82) | 16.07* (12.47) | 17.42** (10.75) |
| CD4 count (cells/μL) | 155 (94) | ND | 285* (52) | 169 (78) | ND | 286** (162) | 135 (62) | ND | 293*** (160) |
| Viral load (copies/mL ) | 15000 (88650) | ND | 12*** (5) | 42500 (97500) | ND | 12*** (0) | 18000 (76000) | ND | 12*** (11) |
| Viral load < 400 copies/mL [*n* (%)] | – | – | 7/8 (88) | – | – | 18/18 (100) | – | – | 18/19 (95) |
| Viral load < 50 copies/mL [*n* (%)] | – | – | 7/8 (88) | – | – | 18/18 (100) | – | – | 16/19 (84) |

HDL, high-density lipoprotein; HOMA, homeostasis model assessment; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; ND, not done.
Data are expressed as median (interquartile range), unless otherwise stated. *$P < 0.05$; **$P < 0.005$; *** $P < 0.0005$ *vs.* week 0; +$P < 0.05$ *vs.* same time-point in the tenofovir arm.

## Lipid metabolism

There were significant increases in both total and HDL cholesterol levels from baseline to weeks 4 and 48 for all three arms (Table 2). Triglyceride levels increased significantly at week 4 in the standard-dose stavudine and tenofovir DF arms only. LDL cholesterol increased significantly in the low-dose stavudine arm at weeks 4 and 48, but increased only at week 4 in the standard-dose stavudine arm and at week 48 in the tenofovir arm (Table 2). The percentages of patients with hypercholesterolaemia, hypertriglyceridaemia, elevated LDL cholesterol and reduced HDL cholesterol in each arm, as defined by NCEP-ATP (National Cholesterol Education Program – Adult Treatment Panel) III guidelines [34], were calculated at week 48 and no significant differences were found.

## Glucose metabolism

A decrease in C-peptide levels was noted at week 4 and a significant increase by week 48 in the low-dose stavudine arm, with a significant increase at week 48 also noted in the standard-dose stavudine arm (Table 2).

When fasting insulin levels were compared in all patients, an increase was noted at week 48 in both stavudine arms but not in the tenofovir DF arm. The change in insulin level from baseline to week 48 was significantly higher in the standard-dose stavudine arm [median (IQR) 2.3 (4.4) nmol/L] than in the tenofovir DF arm [median (IQR) 0.8 (5.1) nmol/L; $P < 0.05$].

When the HOMA scores were compared, a significant increase was noted at week 48 for the standard-dose stavudine arm only (Table 2).

## Inflammatory markers

There was a significant increase in the level of hs-CRP at week 4 in the standard-dose stavudine arm and in leptin levels at week 4 in the low-dose stavudine arm, and at week 48 in the tenofovir DF arm (Table 2).

A significant decrease in the adiponectin level was noted in the standard-dose stavudine arm at week 48, and an

increase at weeks 4 and 48 in the tenofovir DF arm. The week 48 adiponectin level was significantly higher in the tenofovir DF arm than in the standard-dose stavudine arm, and the change in adiponectin level from baseline to week 48 in the tenofovir DF arm [3.6 (3.5) pg/mL] was significantly greater ($P < 0.05$) than that in the standard-dose stavudine arm [–0.6 (6.8) pg/mL].

### Immunological and virological efficacy

No differences were noted among treatment arms at week 24 in CD4 cell count or viral load. There was a significant increase in the CD4 cell counts in all three arms at week 48 when compared with baseline (Table 2). No significant differences were noted when the median CD4 count changes from baseline to week 48 were compared; the median (IQR) change was 111 (54) cells/μL for standard-dose stavudine, 122 (161) cells/μL for low-dose stavudine, and 131 (126) cells/μL for tenofovir DF.

Similarly, a significant decrease in viral load (< 50 copies/mL) was noted in all three arms at week 48 when compared with baseline, but the number of patients who had virological suppression at week 48 did not differ significantly among treatment arms (Table 2).

An intent-to-treat (ITT) analysis was performed for CD4 count and viral load at week 48 and no significant differences were noted.

### Anthropometric measurements and body composition

There was a significant increase in the body mass index (BMI) of patients in the standard-dose stavudine arm by weeks 4 and 24 and in the low-dose stavudine arm by weeks 24 and 48, although no significant changes were observed for patients in the tenofovir DF arm (Table 3).

A significant increase was noted in waist circumference at weeks 24 and 48 in both the standard- and low-dose stavudine arms but only at week 48 in the tenofovir DF arm. A significant increase in hip circumference was noted by week 24 in both stavudine arms but this was not observed with tenofovir DF. A similar trend was noted for mid-thigh, but with an increase at week 48 in the tenofovir DF arm.

With regard to skinfolds, a similar increasing trend was noted at week 24 for the two stavudine arms, with a tendency for skinfold thicknesses to fall by week 48 to levels close to or below those at baseline. In the tenofovir DF arm, there was an increase in skinfold thicknesses across the study period but changes were not statistically significant (Table 3).

Forty-seven per cent of patients in the standard-dose stavudine arm, 40% in the low-dose stavudine arm, and 55% in the tenofovir DF arm ($P = 0.637$) developed a BMI ≥ 25 kg/m$^2$ at week 48.

### Safety and therapy switching

There were 14 AEs in the standard-dose stavudine arm, 17 AEs in the low-dose stavudine arm and 12 AEs in the tenofovir DF arm. There were no statistically significant changes in creatinine clearance in any of the three arms. There was one case of renal dysfunction, in the tenofovir DF arm. There was one death in the standard-dose stavudine arm caused by a high-grade B-cell lymphoproliferative disorder. One patient was lost to follow-up, one patient withdrew from the study and one patient became pregnant and was withdrawn from the study.

With regard to mitochondrial AEs, there were three patients in the standard-dose stavudine arm who developed lactic acidosis, while one patient developed hyperlactataemia. In the low-dose stavudine arm, three patients developed peripheral neuropathy, of whom one also had associated hyperlactataemia and clinical features of lipoatrophy warranting a switch of therapy.

Within the standard-dose stavudine arm, 12 patients had to switch therapy for various reasons. At week 0, these patients differed from those who did not change therapy in having higher body fat measurements and hs-CRP and leptin levels. Notably, at baseline 66.7% of patients who switched therapy were overweight/obese compared with 12.5% of patients who did not switch therapy ($P < 0.05$). Significant differences were noted between those who switched and did not switch therapy in the standard-dose stavudine arm in median suprailiac skinfold thickness [median (IQR) 43.3 (25.3) *vs.* 16 (12.9) mm, respectively; $P < 0.005$], mean per cent truncal fat (mean 32.98 ± SD 10.23 *vs.* 21.44 ± 9.93, respectively; $P < 0.05$), median hs-CRP [median (IQR) 0.86 (0.53) *vs.* 0.54 (0.64) pg/L, respectively; $P < 0.05$], and median leptin level [median (IQR) 8.74 (26.57) *vs.* 1.37 (1.62) pg/mL, respectively; $P < 0.005$].

## Discussion

Despite the move away from stavudine towards less toxic regimens, more than 1.55 million people were still receiving stavudine therapy by the end of 2012 [17]. It is known that, although stavudine is cheaper than other antiretroviral drugs and is effective as initial therapy, the standard dose (30 or 40 mg) is associated with short- and long-term complications. However, studies have demonstrated excellent antiviral efficacy with a lower dose of 20 mg [27–29]. The Gilead 903 trial showed no major

Table 3 Comparison of anthropometric measurements and body fat composition at different time intervals

| | Standard-dose stavudine (30/40 mg) | | | | Low-dose stavudine (20/30 mg) | | | | Tenofovir (300 mg) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Characteristic | 0 weeks | 4 weeks | 24 weeks | 48 weeks | 0 weeks | 4 weeks | 24 weeks | 48 weeks | 0 weeks | 4 weeks | 24 weeks | 48 weeks |
| n | 20/20 | 20/20 | 19/20 | 8/20 | 20/20 | 20/20 | 20/20 | 19/20 | 20/20 | 20/20 | 20/20 | 19/20 |
| BMI (kg/m$^2$) | 24.82 | 24.87* | 25.84** | 24.69 | 23.45 | 23.92 | 25.48*** | 23.79* | 24.25 | 24.08 | 24.88 | 25.06 |
| | (4.92) | (4.62) | (5.65) | (4.64) | (6.50) | (6.20) | (5.50) | (5.61) | (5.68) | (4.44) | (5.32) | (6.36) |
| Mid-arm circumference (cm) | 28.75 | 29.00 | 30.5** | 31.00* | 28.75 | 28.00 | 30.00* | 29.00 | 29.00 | 29.50 | 30.00 | 31.50 |
| | (4.50) | (3.75) | (4.50) | (2.50) | (6.50) | (7.00) | (7.00) | (5.50) | (4.75) | (4.75) | (4.50) | (8.00) |
| Chest circumference (cm) | 81.75 | 81.50 | 83.00 | 82.00 | 79.00 | 79.00 | 79.50 | 80.50* | 79.75 | 80.75 | 81.00 | 81.00 |
| | (8.00) | (6.50) | (11.00) | (12.00) | (6.25) | (11.50) | (8.20) | (7.00) | (7.50) | (10.50) | (8.75) | (10.00) |
| Waist circumference (cm) | 82.00 | 81.00 | 83.00* | 83.00* | 75.75 | 81.00 | 80.50** | 82.00** | 78.75 | 78.00 | 83.75 | 81.50* |
| | (8.00) | (9.00) | (11.00) | (14.50) | (12.50) | (13.00) | (14.75) | (13.00) | (13.50) | (12.50) | (12.25) | (15.00) |
| Hip circumference (cm) | 100.50 | 102.50 | 105.00* | 102.00 | 99.50 | 99.00 | 101.50** | 98.00 | 100.00 | 101.00 | 103.50 | 109.00 |
| | (13.00) | (12.00) | (10.00) | (11.50) | (16.25) | (21.00) | (14.00) | (9.00) | (14.50) | (16.00) | (12.00) | (16.00) |
| Mid-thigh circumference (cm) | 57.00 | 58.00 | 59.50** | 57.00 | 55.25 | 54.00 | 56.00* | 56.50 | 59.50 | 60.00 | 61.25 | 63.00* |
| | (8.75) | (6.75) | (7.00) | (7.00) | (8.75) | (13.00) | (8.50) | (5.00) | (10.00) | (13.50) | (9.50) | (12.00) |
| Triceps skinfold thickness (mm) | 43.70 | 49.80 | 63.00* | 43.40 | 43.50 | 51.00 | 50.70** | 39.80 | 46.50 | 46.10* | 52.50 | 54.00 |
| | (37.80) | (22.50) | (33.00) | (33.20) | (29.70) | (30.40) | (27.10) | (20.40) | (29.90) | (33.30) | (27.00) | (50.50) |
| Biceps skinfold thickness (mm) | 16.00 | 16.80 | 18.60 | 12.40 | 18.00 | 19.40 | 18.00 | 13.2* | 15.10 | 16.50 | 20.10 | 18.00 |
| | (10.10) | (8.20) | (14.40) | (9.00) | (13.20) | (15.00) | (13.40) | (10.80) | (11.40) | (9.40) | (10.90) | (10.80) |
| Subscapular skinfold thickness (mm) | 32.80 | 30.50 | 36.00 | 34.70 | 25.80 | 29.60 | 31.80*** | 29.00 | 30.40 | 32.90 | 35.70 | 36.60 |
| | (23.90) | (22.00) | (25.40) | (17.70) | (16.30) | (18.00) | (18.60) | (21.00) | (16.90) | (18.70) | (20.00) | (23.40) |
| Suprailiac skinfold thickness (mm) | 27.00 | 27.40 | 27.60* | 25.30 | 25.80 | 26.00 | 30.30* | 27.6* | 28.60 | 27.60 | 30.00 | 33.00 |
| | (28.60) | (26.90) | (31.40) | (14.80) | (23.10) | (26.10) | (19.20) | (18.00) | (24.70) | (25.30) | (22.50) | (28.40) |
| Trunk fat (g) | 17687.70 | ND | ND | 19759.00 | 17538.70 | ND | ND | 19490.45 | 23000.10 | ND | ND | 24924.90 |
| | (11903.50) | | | (14212.70) | (13110.20) | | | (12170.20) | (10254.40) | | | (17053.00) |
| Trunk % fat | 28.10 | ND | ND | 30.80 | 29.40 | ND | ND | 33.90 | 34.60 | ND | ND | 37.10 |
| | (19.50) | | | (19.60) | (10.80) | | | (12.80) | (11.70) | | | (18.00) |
| Tot al limb fat (g) | 16878.60 | ND | ND | 21512.15 | 16138.95 | ND | ND | 17378.30 | 21961.00 | ND | ND | 24105.20 |
| | (12021.10) | | | (13510.75) | (16609.90) | | | (12214.90) | (10268.60) | | | (17061.50) |
| Total limb % fat | 28.90 | ND | ND | 37.60 | 30.40 | ND | ND | 35.00 | 35.70 | ND | ND | 40.45 |
| | (21.00) | | | (23.30) | (15.10) | | | (13.70) | (12.40) | | | (17.60) |

BMI, body mass index; ND, not done.
Data are expressed as median (IQR), unless otherwise stated. *$P < 0.05$; **$P < 0.005$; *** $P < 0.0005$ *vs.* week 0.

differences in the rate of clinical AEs between the stavudine and tenofovir DF arms, apart from lipid changes and investigator-reported lipodystrophy [21]. Generic tenofovir DF is used as nonstavudine first-line therapy but is more expensive [35].

This was a prospective, open-label randomized controlled trial designed to evaluate the *in vivo* effects of tenofovir DF against those of standard- and low-dose stavudine regimens, with the reference regimen based on the Gilead 903 trial [21]. To our knowledge, this work presents the first trial to compare the use of these drugs in a Black South African population.

Studies in treatment-naïve HIV-infected patients have shown that peripheral fat initially increases during the first few months of HAART, but decreases linearly thereafter and truncal fat increases at around 6 months of therapy [36,37]. Similarly, in the current study significant increases in the majority of anthropometric measurements were noted in the stavudine arms by 6 months, with a drop towards 1 year of therapy, while such changes were seen in a minority of the anthropometric variables with tenofovir DF therapy.

This study demonstrated that insulin and C-peptide levels increased significantly in both stavudine arms, but not with tenofovir DF therapy. However, significant rises in glucose level and HOMA score were only observed with the standard stavudine dose. Previous research showed that the severity of lipoatrophy in patients taking either stavudine or zidovudine was associated with an increased risk of insulin resistance [38].

Consistent with other studies [21,39,40], we found a significant increase in fasting lipids after 1 year of HAART, and this was noted within both stavudine arms and with tenofovir DF. Lipid abnormalities were noted in HIV-1-infected patients even before the advent of antiretroviral therapy, with cholesterol levels being lower and triglyceride levels higher in HIV-infected compared with uninfected subjects [41]. In the present study, the early increase of cholesterol levels after only 4 weeks of therapy may be explained by the 'return to baseline' effect of commencing antiretroviral therapy, but this cannot explain the rise in triglyceride levels. Furthermore, dyslipidaemia has been widely reported with exposure to NRTIs, particularly stavudine or zidovudine, and less so with tenofovir DF [21,36–40,42].

Inflammatory markers appear to have a role in the pathogenesis of HIV/HAART-related metabolic syndrome. The effect of HAART on leptin levels is controversial; a few studies have demonstrated that low levels of leptin are associated with lipodystrophy, while other studies have demonstrated no effect of HAART on leptin [43,44]. We noted an increase in leptin levels at week 4 for patients in the low-dose stavudine arm and at week 48 for the tenofovir DF arm. Patients on HAART, especially those with lipodystrophy, show a gradual reduction in serum adiponectin levels, which is thought to be associated with increased cardiovascular risk [45]. In our study, there was a significant decrease at week 48 in the standard-dose stavudine arm but a significant increase in the tenofovir DF arm at 4 and 48 weeks. These changes in serum adiponectin levels in the stavudine arm may be significant in terms of the ongoing risk of lipoatrophy as well as metabolic complications. hs-CRP has been shown to be a marker of an increased risk of cardiovascular events [12]. There was a tendency for hs-CRP levels to increase in all three treatment arms, but this reached statistical significance only in the standard-dose stavudine group at 4 weeks and then fell to baseline levels at 48 weeks.

The AEs associated with mitochondrial toxicities were noted only with the stavudine regimens.

Our findings must be considered in the light of the study's limitations. First, our sample size was small, as the study had to be stopped prematurely because of the mitochondrial results [30] and following the introduction of the new South African National HAART guidelines. Secondly, despite this being a randomized controlled trial, it was open- label, which may have been a confounding factor. The metabolic abnormalities may be more prevalent with increasing duration of therapy and therefore not seen after only 48 weeks of therapy. However, these data are important as our study is the first randomized trial assessing the frequency of NRTI-associated metabolic toxicities related to the two most relevant antiretroviral agents currently used in a routine clinical setting in Black African populations.

In conclusion, the current study demonstrates that, in South African HIV-positive patients, tenofovir DF has more favourable effects on anthropometry and adipokine levels while both stavudine regimens increase fasting insulin and C-peptide levels, with the higher stavudine dose also causing increased fasting glucose levels and higher HOMA scores. However, both stavudine and tenofovir DF caused raised lipid levels. Therefore, awareness of the potential increased cardiovascular risk should be of concern with the use of both these therapies. More AEs were noted in the standard-dose compared with the low-dose stavudine arm. Therefore, where options are limited and there is an absolute need for stavudine to be used, low-dose stavudine may be a carefully considered option as it is equally efficient in viral suppression and has lower rates of complications than the standard dose.

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*Conflicts of interest:* The authors have no conflicts of interest to declare.

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*Authors' contributions:* CNM and IS were responsible for the study concept and design, CNM and DVA for data acquisition, RD, CD and TDP for laboratory analysis, CNM, NJC and DE for data analysis, CNM and NJC for data interpretation, and CNM for drafting of the manuscript. All authors read and approved the final version of the manuscript.

## References

1 Palella FJ Jr, Delaney KM, Moorman AC *et al.* Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N Engl J Med* 1998; 338: 853–860.
2 Hogg RS, O'Shaughnessy MV, Gataric N *et al.* Decline in deaths from AIDS due to new antiretrovirals. *Lancet* 1997; 349: 1294.
3 Menezes CN, Maskew M, Sanne I, Crowther NJ, Raal FJ. A longitudinal study of stavudine-associated toxicities in a large cohort of South African HIV infected subjects. *BMC Infect Dis* 2011; 11: 244.
4 Carr A, Samaras K, Thorisdottir A, Kaufmann GR, Chisholm DJ, Cooper DA. Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor-associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study. *Lancet* 1999; 353: 2093–2099.
5 Carr A, Cooper DA. Adverse effects of antiretroviral therapy. *Lancet* 2000; 356: 1423–1430.
6 Galli M, Ridolfo AL, Adorni F *et al.* Body habitus changes and metabolic alterations in protease inhibitor-naive HIV-1-infected patients treated with two nucleoside reverse transcriptase inhibitors. *J Acquir Immune Defic Syndr* 2002; 29: 21–31.
7 Friis-Møller N, Sabin CA, Weber R *et al.* Combination antiretroviral therapy and the risk of myocardial infarction. *N Engl J Med* 2003; 349: 1993–2003.
8 Lindegaard B, Keller P, Bruunsgaard H, Gerstoft J, Pedersen BK. Low plasma level of adiponectin is associated with stavudine treatment and lipodystrophy in HIV-infected patients. *Clin Exp Immunol* 2004; 135: 273–279.
9 Palios J, Kadoglou NP, Lampropoulos S. The pathophysiology of HIV-HAART-related metabolic syndrome leading to cardiovascular disorders: the emerging role of adipokines. *Exp Diabetes Res* 2012; 2012: 103063.
10 Reingold J, Wanke C, Kotler D *et al.* Association of HIV infection and HIV/HCV coinfection with C-reactive protein levels: the fat redistribution and metabolic change in HIV infection (FRAM) study. *J Acquir Immune Defic Syndr* 2008; 48: 142–148.
11 Rutter MK, Meigs JB, Sullivan LM, D'Agostino RB, Wilson PW. C-reactive protein, the metabolic syndrome, and prediction of cardiovascular events in the Framingham Offspring study. *Circulation* 2004; 110: 380–385.
12 Pai JK, Pischon T, Ma J *et al.* Inflammatory markers and the risk of coronary heart disease in men and women. *N Engl J Med* 2004; 351: 2599–2610.
13 Triant VA, Meigs JB, Grinspoon SK. Association of C-reactive protein and HIV infection with acute myocardial infarction. *J Acquir Immune Defic Syndr* 2009; 51: 268–273.
14 Boulle A, Orrell C, Kaplan R *et al.* Substitution due to antiretroviral toxicity or contraindication in the first 3 years of antiretroviral treatment in a large South African cohort. *Antivir Ther* 2007; 12: 753–760.
15 Mutimura E, Stewart A, Rheeder P, Crowther NJ. Metabolic function and the prevalence of lipodystrophy in a population of HIV-infected African subjects receiving highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* 2007; 46: 451–455.
16 van Griensven J, Zachariah R, Rasschaert F, Mugabo J, Atte EF, Reid T. Stavudine- and nevirapine-related drug toxicity while on generic fixed-dose antiretroviral treatment: incidence, timing and risk factors in a three-year cohort in Kigali, Rwanda. *Trans R Soc Trop Med Hyg* 2010; 104: 148–153.
17 World Health Organization. Forecasting antiretroviral demand. 2010. Available at www.who.int/hiv/amds/forecasting/en/index4.html (accessed 20 April 2013).
18 National Department of Health. National Antiretroviral Treatment Guidelines, First Edition 2004: Available at www.doh.gov.za/docs/misc/2004/sec1.pdf (accessed 20 April 2013).
19 National Department of Health South Africa. 2010. Clinical guidelines for the management of HIV & AIDS in adults and adolescents: Available at www.health-e.org.za/documents/0ba405d9042e557a9fb1987adb68cb97.pdf (accessed 20 April 2013).
20 Schowalter L, Conradie F. Approaches to tenofovir and abacavir drug shortages in South Africa: a guide for clinicians. *South Afr J HIV Med* 2012; 13: 56–57.
21 Gallant JE, Staszewski S, Pozniak AL *et al.* Efficacy and safety of tenofovir DF vs. stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial. *JAMA* 2004; 292: 191–201.
22 Overton ET, Nurutdinova D, Freeman J, Seyfried W, Mondy KE. Factors associated with renal dysfunction within an

urban HIV-infected cohort in the era of highly active antiretroviral therapy. *HIV Med* 2009; 10: 343–350.
23 Gallant JE, Parish MA, Keruly JC, Moore RD. Changes in renal function associated with tenofovir disoproxil fumarate treatment, compared with nucleoside reverse-transcriptase inhibitor treatment. *Clin Infect Dis* 2005; 40: 1194–1198.
24 Young B, Buchacz K, Baker RK *et al.* Renal function in tenofovir exposed and tenofovir-unexposed patients receiving highly active antiretroviral therapy in the HIV Outpatient Study. *J Int Assoc Physicians AIDS Care (Chic Ill)* 2007; 6: 178–187.
25 Ryom L, Mocroft A, Kirk O *et al.* Association between antiretroviral exposure and renal impairment among HIV-positive persons with normal baseline renal function: the D:A:D study. *J Infect Dis* 2013; 207: 1359–1369.
26 Fux CA, Simcock M, Wolbers M *et al.* Tenofovir use is associated with a reduction in calculated glomerular filtration rates in the Swiss HIV Cohort Study. *Antivir Ther* 2007; 12: 1165–1173.
27 Milinkovic A, Martinez E, López S *et al.* The impact of reducing stavudine dose versus switching to tenofovir on plasma lipids, body composition and mitochondrial function in HIV-infected patients. *Antivir Ther* 2007; 12: 407–415.
28 McComsey GA, LoRe III V, O'Riordan M *et al.* Effect of Reducing the Dose of Stavudine on Body Composition, Bone Density and Markers of Mitochondrial Toxicity in HIV infected Subjects- a Randomized, Controlled study. *Clin Infect Dis* 2008; 46: 1290–1296.
29 Hill A, Ruxrungtham K, Hanvanich M *et al.* Systematic review of clinical trials evaluating low doses of stavudine as part of antiretroviral treatment. *Expert Opin Pharmacother* 2007; 8: 679–688.
30 Menezes CN, Duarte R, Dickens C *et al.* The early effects of stavudine compared to tenofovir on adipocyte gene expression, mitochondrial DNA copy number and metabolic parameters in South African HIV-infected patients: a randomized trial. *HIV Med* 2013; 14: 217–225.
31 Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976; 16: 31–41.
32 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; 28: 412–419.
33 Nolan D, Hammond E, Martin A *et al.* Mitochondrial DNA depletion and morphologic changes in adipocytes associated with nucleoside reverse transcriptase inhibitor therapy. *AIDS* 2003; 17: 1329–1338.
34 Third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. *Circulation* 2002; 106: 3143–3421.
35 Rosen S, Long L, Fox M, Sanne I. Cost and cost-effectiveness of switching from stavudine to tenofovir in first-line antiretroviral regimens in South Africa. *J Acquir Immune Defic Syndr* 2008; 48: 334–344.
36 Mallon PW, Miller J, Cooper DA, Carr A. Prospective evaluation of the effects of antiretroviral therapy on body composition in HIV-1-infected men starting therapy. *AIDS* 2003; 17: 971–979.
37 Magkos F, Mantzoros CS. Body fat redistribution and metabolic abnormalities in HIV-infected patients on highly active antiretroviral therapy: novel insights into pathophysiology and emerging opportunities for treatment. *Metabolism* 2011; 60: 749–753.
38 De Wit S, Sabin CA, Weber R *et al.* Incidence and risk factors for new-onset diabetes in HIV-infected patients: the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study. *Diabetes Care* 2008; 31: 1224–1229.
39 Savès M, Raffi F, Capeau J *et al.* Factors related to lipodystrophy and metabolic alterations in patients with human immunodeficiency virus infection receiving highly active antiretroviral therapy. *Clin Infect Dis* 2002; 34: 1396–1405.
40 Shlay JC, Visnegarwala F, Bartsch G *et al.* Body composition and metabolic changes in antiretroviral-naive patients randomized to didanosine and stavudine vs. abacavir and lamivudine. *J Acquir Immune Defic Syndr* 2005; 38: 147–155.
41 Constans J, Pellegrin JL, Peuchant E *et al.* Plasma lipids in HIV-infected patients: a prospective study in 95 patients. *Eur J Clin Invest* 1994; 24: 416–420.
42 Gallant JE, DeJesus E, Arribas JR *et al.* Tenofovir DF, emtricitabine, and efavirenz vs. zidovudine, lamivudine, and efavirenz for HIV. *N Engl J Med* 2006; 354: 251–260.
43 Calmy A, Gayet-Ageron A, Montecucco F *et al.* HIV increases markers of cardiovascular risk: results from a randomized, treatment interruption trial. *AIDS* 2009; 23: 929–939.
44 Dzwonek AB, Novelli V, Schwenk A. Serum leptin concentrations and fat distribution in HIV-1 infected children on highly active antiretroviral therapy. *HIV Med* 2007; 8: 433–438.
45 Bezante GP, Briatore L, Rollando D *et al.* Hypoadiponectinemia in lipodystrophic HIV individuals: a metabolic marker of subclinical cardiac damage. *Nutr Metab Cardiovasc Dis* 2009; 19: 277–282.

## 6.4. Appendix 4 – Ethics

**UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG**

Division of the Deputy Registrar (Research)

**HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)**
R14/49 Menezes

| | |
|---|---|
| **CLEARANCE CERTIFICATE** | **PROTOCOL NUMBER M070614** |
| **PROJECT** | A Cohort Study: Review of the Mtochondrial Toxicities of HIV-Infected Individuals on Highly Anti-Retroviral Therapy-A SA Perspective |
| **INVESTIGATORS** | Dr CN Menezes |
| **DEPARTMENT** | Infectious Disease Unit |
| **DATE CONSIDERED** | 07.06.29 |
| **DECISION OF THE COMMITTEE*** | APPROVED UNCONDITIONALLY |

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

**DATE** 07.07.26 **CHAIRPERSON** .....
(Professors PE Cleaton-Jones, A Dhai, M Vorster, C Feldman, A Woodiwiss)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Prof FJ Raal

---

**DECLARATION OF INVESTIGATOR(S)**

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

**Wits Clinical Research**

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Postnet Suite 189, Private Bag x2600, Houghton, 2041

**COURIERED**

Dr CN Menezes,

Clinical HIV Research Unit
Posnet Suite 176
Private Bag x2600
Houghton
2041

Fax: 011 482 2130

Dear Dr Menezes,

**PROTOCOL NO: CHRU 01**

**PROTOCOL TITLE: Molecular, Biochemical and Clinical Differences Between Stavudine and Tenofovir, each Combined with Lamivudine and Efavirenz, in HIV-Infected Patients - A South African Perspective**

**PRC REFERENCE NUMBER: 070603**

**********

Please be advised that your trial application was:

**APPROVED**

**The Expert Reviewer /(s):** Prof PA Cooper

Also reviewed by: Ms R Wills: Acting Chairperson Protocol Review Committee
Dr S Khan: Gauteng Department of Health
Dr ML Likibi: Gauteng Department of Health

Yours sincerely

MS RENÉ WILLS
**Chairperson: Protocol Review Committee**
**29 August 2007**

**cc.**
Clinical HIV Research Unit - C Mrs Marlene Naidoo

**Tel** 011 276 8809 **Cell** 084 403 2619 **Fax** 011 482 2130

**University of the Witwatersrand, Johannesburg**

**Human Research Ethics Committee: (Medical)**
**FWA Registered No IRB 00001223**

**SECRETARIAT:** Suite 189, Private Bag x2600, Houghton 2041, South Africa • Tel: +27-11-274-9200 • Fax: +27-11-274-9281

29 August 2007

**FAXED & COURIED**

Mrs Marlene Naidoo

Regulatory Manager
Wits Clinical HIV Research Unit
Helen Joseph Hospital
Perth Road, Westdene

Fax: 011 482 2130

Dear Mrs Naidoo,

**PROTOCOL: CHRU 01 - MOLECULAR, BIOCHEMICAL AND CLINICAL DIFFERENCES BETWEEN STAVUDINE AND TENOFOVIR, EACH COMBINED WITH LAMIVUDINE AND EFAVIRENZ, IN HIV-INFECTED PATIENTS - A SOUTH AFRICAN PERSPECTIVE**

**ETHICS REFERENCE NO: 070603**

**RE : FINAL ETHICS APPROVAL**

This is to certify that the above-mentioned trial was reviewed by the University of the Witwatersrand, Human Research Ethics Committee (HREC), and the Protocol Review Committee (PRC) on: 29 June 2007.

The University of the Witwatersrand, Human Research Ethics Committee Approval Granted for the above mentioned study is valid for five years. Where required by Sponsor to have approval on a more frequent basis it remains the responsibility of the Sponsor and Investigator to apply for continuing review and approval, or for the duration of the Trial.

1. THIS APPROVAL IS SUBJECT TO THE FOLLOWING PROVISOS:

* A copy of the MCC Approval and/or MCC Notification letter must be submitted to the Ethics Regulatory Office Secretariat before the study commences.

* The study is conducted according to the protocol submitted to the University of the Witwatersrand, Human Research Ethics Committee. Any amendments to the protocol must first be submitted to the Human Research Ethics Committee for approval.

* During the study, the University of the Witwatersrand, Human Research Ethics Committee is informed immediately of :
  - Any Unexpected Serious Adverse Events or Unexpected Adverse Drug Reactions, which, in the Investigator and/or the Sponsor's opinion are suspected to be related to the study drug. (International and Local Reports).
  - Any data received during the trial which, may cast doubt on the validity of the continuation of the study .

* The University of the Witwatersrand, Human Research Ethics Committee is notified of any decision to discontinue the study and the reason stated.

* The Investigators authorised by this approval participate in this study. Additional Investigators shall be submitted to the University of the Witwatersrand, Human Research Ethics Committee for approval prior to their participation in the study.

* In the event of an authorised Investigator ceasing to participate in the study, the University of the Witwatersrand, Human Research Ethics Committee must be informed and the reason for such cessation given.

2. PRINCIPLES OF INFORMED CONSENT:

The University of the Witwatersrand, Human Research Ethics Committee requires that in all studies, the Principles of Informed Consent are adhered to. This applies to volunteers as well as patients.

3. PROGRESS REPORTS:

* The University of the Witwatersrand, Human Research Ethics Committee requests that the MCC Progress Reports be submitted twice a year either in March and September or six monthly from start of study to the HREC Secretariat Office - 011 274 9281 and a report of the final results, at the conclusion of the study.

4. TRANSPORT AND STORAGE OF BLOOD AND TISSUE SAMPLES IN SOUTH AFRICA:

* If blood specimens are to be stored for future analysis and it is planned that such analysis will be done outside Wits then the blood must be stored at Wits with release of sub-samples ONLY once projects have been approved by the local Research Ethics Committee applicable to where the research will be done, as well as by the Wits Human Research Ethics Committee: (Medical).

5. REIMBURSEMENT TO PATIENTS FOR TRANSPORT:

* The Human Research Ethics Committee: (Medical) does not agree with the R150 reimbursement per visit as stipulated by the Medicines Control Council of SA but that reimbursement should be appropriate according to the situation.

6. GENETIC TESTING

* The Human Research Ethics Committee: Medical; will not approve open-ended genetic testing as this does not fit the Human Research Ethics Committee criteria.

7. THE SUPPORTING APPROVAL DOCUMENTS ARE ATTACHED:

7.1 Ethics Approval Form signed by the Chairperson of the HREC - Kindly return the copy of the Approval Form signed by the Principal Investigator /(s) per fax: 011 274 9281 for our records.

7.2 Protocol Review Committee Approval Signature page signed by the Chairperson of the PRC.

7.3 List of members present at the HREC meeting held as per INDEPENDENT ETHICS COMMITTEE APPROVAL FORM 2003

8. WE AWAIT YOUR RESPONSES AS REQUESTED:

* MCC Approval and/or Notification before the above study may commence.
* Copy of Approval Form signed by the Principal Investigator.
* Kindly forward the above to the undersigned at fax: 011 274 9281 at your earliest convenience.

The above has been noted for the Ethics Committee information and records.

***KINDLY FORWARD TO THE RELEVANT INVESTIGATORS / CRA / SPONSOR / STUDY CO-ORDINATORS - WHERE APPLICABLE***

Regards,

**PROF PETER CLEATON-JONES**

For and on behalf of the Human Research Ethics Committee: (Medical)

health

Department:
Health
REPUBLIC OF SOUTH AFRICA

MEDICINES CONTROL COUNCIL
The Registrar of Medicines, Private Bag X828, PRETORIA, 0001
Tel (012) 312 0000 Fax (012) 312 3105
*Tel:* *Inquiries:*
*Fax:* *Reference:*

**FAX AND MAIL TO**

**MRS NOLUNTU FUNANI**

**N2/19/8/2 (2271)**

**Dr C Menezes**

**Datum * Date** **07 September 2007**

**Helen Joseph Hospital**
**Perth Road**
**Westdene**
**Johannesburg**
**2092**

Tel: 012 312 0276
Fax: 012 312 3106

Fax: 0114822130

Dear Dr Menezes,

AUTHORISATION FOR THE IMPORTATION OF UNREGISTERED MEDICINE IN TERMS OF SECTION 21 OF THE MEDICINES AND RELATED SUBSTANCES CONTROL ACT, 1965 (ACT 101 OF 1965)

PRODUCT: TENOFOVIR DISOPROXIL FUMARATE, LOPINAVIR, ZIDOVUDINE, EFAVIRENZ, LAMIVUDINE, STAVUDINE.

Your application letter dated **31 Aug 2006** refers

1. **RESOLUTION AND APPROVAL**

It was recently resolved by the Medicines Control Council that; the clinical trial application according to the following Protocol be approved :-

**CHRU 01 protocol version 1.0 dated 30-Aug-06**

**Molecular, Biochemical and clinical differences between Stavudine and Tenofovir, each combined with Lamivudine and Efavirenz, in HIV-infected patients-A South African Perspective.**

1.1 **BEFORE COMMENCEMENT OF TRIAL**

Please Note: Copies of written Ethics Committee approval/(s) to be submitted to MCC before the study commences

2. **AUTHORISATION**

Authorisation is hereby granted for the importation and administration of a sufficient quantity, for the duration of the trial, of the unregistered medicine:

TENOFOVIR DISOPROXIL FUMARATE, LOPINAVIR, ZIDOVUDINE, EFAVIRENZ, LAMIVUDINE, STAVUDINE.

solely for the purpose of a clinical trial to be conducted by:

| | | |
|---|---|---|
| Dr CN Menezes | Clinical HIV Research Unit, Helen Joseph Hospital | Principal |
| Dr M John | Clinical HIV Research Unit, Helen Joseph Hospital | |
| Prof FJ Raal | Clinical HIV Research Unit, Helen Joseph Hospital | |
| Dr IM Sanne | Clinical HIV Research Unit, Helen Joseph Hospital | |

3. **PLEASE FORWARD**

It is a requirement that a copy of this letter be forwarded to all the relevant Trialist/(s), including the approving Ethics Committee(s).

4. **THIS AUTHORISATION IS SUBJECT TO THE FOLLOWING PROVISOS:**

(a) The Council shall be informed immediately of any toxic effects or death, which may occur during the Clinical Trial and of any data received which, might cast doubt on the validity of the continuation of the Clinical Trial.

(b) The Council shall be notified of any decision to discontinue the Clinical Trial. The reason for such cancellation shal be stated.

(c) The Clinical Trial shall be conducted in accordance with the Protocol submitted to the Council. Any Amendment/(s) to the Protocol shall first be submitted to the Council for approval. All Clinical Trials be conducted in accordance with ICH GCP Guidelines, and the South African Clinical Trials Guidelines.

(d) The medicine shall be administered by or under the direction of the authorised Trialist. In the case where the Trialist permits another Medical Practitioner to administer a medicine, which is exempted from the registration for the purpose of the Trial, the Trialist shall remain responsible for any eventuality arising from such usage.

(e) Where a Trialist who is not authorised in the initial Authorisation, is requested to participate in the Clinical Trial, the Council requests that the relevant MCC Curriculum Vitae Format be completed detailing their Full Names, Address and Qualifications of the proposed Trialist (Practitioner) concerned, and be submitted to the Council for Approval.

(f) In the event of the authorised Trialist ceasing to participate in the Clinical Trial, the Council shall be informed and the reason for such cessation shall be given.

5. **PROGRESS REPORTS**

The Council must be furnished with signed six-monthly Progress Report from each Trialist including a report of the Final Results.

6. **INFORMED CONSENT**

It is a Council requirement that in all Clinical Trials the 'Principles of Informed Consent' should be adhered to. This applies to Trial Volunteers, as well as Participants (Patients). (Reference: Section 4.8 of ICH GCP Guidelines and Section 3.5 of SACT Guidelines).

***PLEASE NOTE: : Dispenser at site: Mrs C Barker. Ethics approval dated 29/08/2007 is noted.***

Yours faithfully,

MRS NOLUNTU FUNANI
**FOR AND ON BEHALF OF REGISTRAR OF MEDICINE**

MCC TRIAL REFERENCE NO: 20061022