# THE EFFECT OF HIGHLY ACTIVE ANTI-RETROVIRAL TREATMENT ON GLUCOSE AND LIPID METABOLISM IN HUMAN IMMUNODEFICIENCY VIRUS POSITIVE PATIENTS AT CLINICS IN THE POLOKWANE LOCAL MUNICIPALITY, LIMPOPO PROVINCE, SOUTH AFRICA

**by**

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**A dissertation submitted in fulfilment of the requirements for the degree of**

**MASTER OF SCIENCE**

**in**

**PHYSIOLOGY**

**in the**

**FACULTY OF SCIENCE AND AGRICULTURE**

**SCHOOL OF MOLECULAR AND LIFE SCIENCES**

**at the**

**UNIVERSITY OF LIMPOPO**

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2016

## DECLARATION

I, Mapula Mercy Mashao, declare that ‘’The effect of highly active anti-retroviral treatment on glucose and lipid metabolism in human immunodeficiency virus positive patients at clinics in the Polokwane Local Municipality, Limpopo Province, South Africa” hereby submitted to the University of Limpopo, for the degree Master of Science in Physiology, has not previously been submitted by me for a degree at this or any other University; that it is my work in design and execution, and that all material contained herein has been duly acknowledged.

_____ 08 January 2016

**Ms M.M. Mashao** **Date**

## DEDICATION

I would like to thank God of Israel for taking me through this long awaited journey and giving me the strength and courage to make this dissertation a living document. A special dedication to my family and my daughter Kgaogelo.

## ACKNOWLEDGEMENTS

I would like to thank the following people for their respective contributions to this research:

- A special thank you to all the patients that gave consent to participate in the study.
- A special thank you to my supervisor, Dr M. van Staden, for her guidance, support and encouragement.
- A special thank you to my Co-supervisor, Dr L.J.C. Erasmus for his support and guidance.
- The staff members at Tirisano, Thekganang and Phela o Phediše clinic who assisted in the study.
- My research collaborators, Mr J.S. Mahlangu, Ms S.K. Mpherwane, Ms E. Phalane, Ms W. Mabitsela, Ms N.M. Mokabane, Ms M. Maanogo, Ms L. Mathebula and Ms M. Matshipi.
- The University of Limpopo for funding my studies
- The Department of Medical Sciences (UL), for the use of their laboratories and equipment.
- The Department of Health and Social Development for granting permission to conduct the study at these three clinics.
- The statisticians: Dr S. Ntuli, Mr Netshidzivhani and Mr T. Darikwa.
- The physician, Dr C.C. Clark, for overseeing any emergencies.
- Nurses S.K. Mpherwane, L.C. Senona, M.G. Machaka who assisted with drawing blood.

## ABSTRACT

**Relevance:** An increase in the number of HIV positive patients receiving HAART raises important concerns about the metabolic impact of these regimens. The treatment effectively reduces viral load and increase $CD4^+$ count; unfortunately it seems to disrupt carbohydrate and lipid metabolic pathways thereby increasing the risk for CDL by placing an already chronically ill HIV population at risk of more chronic diseases. As a developing country, accessibility to safer regimens of HAART is limited thus patients exposed to toxicities from long term exposure to sub-optimal regimens are even at greater risk. The aim of this study was to assess the long term effects of HAART on biochemical parameters and body composition as an indication of carbohydrate and lipid metabolism.

**Methods:** A prospective cohort of 87 patients receiving HAART for 12 months or more was conducted at baseline and follow-up. Venous blood was collected after an overnight fast. An automated enzymatic colorimetric test was used to analyse plasma glucose and serum TC, HDL-C and TG. The LDL-C levels were calculated from TC and HDL-C. Leptin levels were analysed using human leptin radioimmunoassay kit. Insulin was analysed using an automated access ultrasensitive insulin assay. Anthropometric measurements were taken for the determination of body fat distribution and BMI. All statistical analyses were performed using SPSS version 23.

**Results**: Total cholesterol, LDL-C, and waist circumference significantly decreased from baseline to follow-up ($p<0.05$). Triglycerides and LDL-C levels were significantly affected by durations between 24–47 and 49–72 months respectively. There were no significant changes in the mean levels of leptin observed within the two lines of regime. Mean leptin levels were 11.36±8.52 ng/ml and 9.67±6.42 ng/ml at baseline and follow-up respectively. Furthermore, the duration of HAART significantly affected BMI and WC at 49–72 months. Patients that met the criteria for diagnosis of DM were only found in PI containing regimens at 6.3% and 5.9% baseline and follow-up respectively. In the first line regimen, the prevalence of DM was only found at follow-up.

**Conclusion:** The present study demonstrated that longer duration between months 49–72 has significant negative effects on the glucose and lipid metabolism of HIV positive patients. The study also highlighted that patients on combinations

containing PIs and NRTIs such as stavudine and zidovudine are at higher risk of developing metabolic diseases.

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## LIST OF ABBREVIATIONS

| ABBREVIATION | DESCRIPTION |
|---|---|
| 3TC | Lamivudine |
| Apo | Apolipoprotein |
| ARV | Antiretroviral |
| ART | Antiretroviral therapy |
| ATV | Atazanavir |
| AZT | Zidovudine |
| BMI | Body mass index |
| CDL | Chronic disease of lifestyle |
| d4T | Stavudine |
| ddl | Didanosine |
| DM2 | Type 2 diabetes mellitus |
| EFV | Efavirenz |
| ETC | Electron transport chain |
| FDC | Fixed dose combination |
| FTC | Emitricitabine |
| GLUT-4 | Glucose transporter-4 |
| HALS | HIV/HAART associated lipodystrophy syndrome |
| HAART | Highly active antiretroviral treatment |
| HDL | High density lipoprotein |
| IDL | Intermediate density lipoprotein cholesterol |
| IFG | Impaired fasting glucose |
| IGT | Impaired glucose tolerance |
| IR | Insulin resistance |
| IRS | Insulin receptor substrate |
| JAK-2 | Janus Kinase-2 |
| LDL | Low density lipoprotein cholesterol |
| LPL | Lipoprotein lipase |
| LPV/r | Lopinavir ritonavir boosted |
| mtDNA | Mitochondrial deoxyribonucleic acid |

| | |
|---|---|
| NRTI | Nucleoside reverse transcriptase inhibitor |
| NtRTI | Nucleotide reverse transcriptase inhibitor |
| NNRTI | Non-nucleoside reverse transcriptase inhibitor |
| NSB | Non-specific binding |
| NVP | Nevirapine |
| PDK1 | Phosphoinositide-dependent kinase |
| PI | Protease inhibitor |
| PIP2 | Phosphatidylinositol biphosphate |
| PIP3 | Phosphatidylinositol triphosphate |
| PPAR-$\gamma$ | Peroxisome proliferator activator receptor gamma |
| RIA | Radioimmuno-assay |
| ROS | Reactive oxygen species |
| SH | Src homology |
| SH2 | Src homology 2 |
| SREBP-1 | Sterol regulatory element binding protein-1 |
| TDF | Tenofovir |
| TG | Triglycerides |
| VLDL | Very low density lipoprotein cholesterol |
| WC | Waist circumference |
| W:H | Waist to hip ratio |
| WHO | World health organization |

# CHAPTER 1

# INTRODUCTION

## 1.1 GENERAL LITERATURE REVIEW

### 1.1.1 Introduction

Sub-Saharan Africa, has the highest prevalence of human immune deficiency virus (HIV) infections in the world (Dillon et al., 2013). An estimated 5.51 million people in South Africa (SA) is living with HIV (STATSSA, 2014). Access to antiretroviral (ARV) treatment since 2004 (Connolly et al., 2004), resulted in 1.069 million individuals 15 years and older and 93 000 children younger than 15 years receiving ARVs in 2005. These numbers increased to 1.555 million and 183 000 respectively (STATSSA, 2010) by 2010.

In SA a limited number of studies explored the metabolic diseases found in HIV positive patients receiving highly active anti-retroviral treatment (HAART). Julius (2010), investigated the burden of metabolic diseases amongst HIV positive patients who were on HAART for more than 1 year in a Johannesburg hospital. The study found that the prevalence of type 2 diabetes mellitus (DM2) was one percent, hypertension 16.8%, hypercholesterolemia 35.5% and metabolic syndrome 20.4% in the age group 18–45 years. Similarly Awotedu et al. (2010), investigated the prevalence of metabolic syndrome among HIV HAART patients in the Eastern Cape Province. They found that the prevalence of hypertriglyceridaemia was 28.4%; elevated LDL 64.2%; insulin resistance 12.8% and metabolic syndrome 26.6%.

Metabolic syndrome is a cluster of metabolic disturbances phenotypically associated with a state of insulin resistance (IR), visceral obesity, metabolic dysregulation and increased risk for DM2 and cardiovascular disease (CVD) (Samaras, 2008). These diseases contribute to the chronic diseases of lifestyle as they are diseases that share similar risk factors. Chronic diseases of lifestyle (CDLs) and HIV/AIDS contribute to the quadruple burden of disease in South Africa (Steyn, 2006). The quadruple burden is due to the co-existence of degenerative diseases and diseases associated with poverty, the growing HIV epidemic and the high prevalence of injuries in SA and other developing countries.

The risk factors for CDL include unhealthy lifestyle habits such as smoking tobacco; excessive alcohol intake; consuming a diet that is high in saturated fat and low in fibre and a lack of physical activity (Bradshaw et al., 2006). These are probably as prevalent amongst the HIV population as it is for the rest of the non-infected SA population, because they share similar environmental and lifestyle conditions. The fact that HAART effectively reduces viral load and increase $CD4^+$ count (Barbaro and Lacobellis, 2009) result in an increased survival of HIV patients, subsequently increasing the probability to develop CDLs. This will predispose an already chronically ill HIV population to an increased risk of acquiring other chronic diseases. Currently the HIV treatment regimen does not provide for special screening of CDL or its related risk factors. However, the co-existence of CDL and HIV in a single patient or in a specific cohort of patients will confound an already complicated disease profile.

Highly active antiretroviral treatment is an effective intervention which consists of a combination of three or more anti-retroviral drugs (Busari et al., 2009). Highly active anti-retroviral treatment is initiated in response to the $CD4^+$ T-lymphocyte count which is a major indicator of immunodeficiency (Richman et al., 2010). The long-term use of HAART is strongly associated with disruption of the carbohydrate and lipid metabolism (Samaras, 2009), subsequently resulting in the development of metabolic diseases, such as DM2 and dyslipidaemia (George, 2006).

The National Health Insurance Policy Report, observed a disparity in the prevalence of CDLs among $HIV^+$ and non-infected individuals (McLeod, 2009). In females and males the highest prevalence of CDLs, in individuals receiving ARV treatment, were observed in the age ranges 25–44 and 30–49 respectively. This is in contrast to the cohort not receiving ARV, where the highest prevalence for CDLs were observed in the 50–85 and 55–$85^+$ age groups for females and males respectively (McLeod, 2009). Therefore, $HIV^+$ patients on ARVs and with pre-existing CDL, are prone to develop an intensified metabolic disorder profile.

## 1.2 LITERATURE REVIEW

### 1.2.1 Introduction

Currently in SA, HAART is the standard method of treatment in $HIV^+$ patients. It reduces the viral load and increase $CD4^+$ counts; thereby, improving the survival rate of those infected (Barbaro and Lacobellis, 2009). There is a remarkable transition in

the development of drug combinations that work synergistically towards effectively suppressing the viral load. The notion to use ARV-cocktails was reported in 1996 (Hester, 2012); and with novel drugs being developed, there is the capacity to improve the quality of life for those living with HIV.

With increasing morbidity and mortality rates in the HIV population, the criteria for selecting patients eligible for ART was revised in 2010. These criteria included patients with multi-drug resistant tuberculosis or patients with non-multi-drug resistant tuberculosis with a $CD4^+$ T-cell count of <350 cells/ µL; thereby, resulting in approximately 2.6 million HIV positive patients in SA eligible to receive ART by 2012 (April et al., 2014). Currently treatment guidelines favour a $CD4^+$ T-cell count of <500 cells/ µL when selecting patients that are eligible for initiation of anti-retroviral therapy (ART) (Hester, 2012).

The South African antiretroviral roll out programme was introduced in 2002, based on the WHO, 2002 recommendations (Table 1.1) (Wood, 2006). The majority of people infected with HIV receive treatment in public sector health care facilities, where the recommended first line regime was a triple-drug combination consisting of stavudine (d4T), lamivudine (3TC) and efavirenz (EFV) or d4T, 3TC and NVP for females of reproductive age. The second line regime was a combination of zidovudine (AZT), didanosine (ddI) and lopinavir boosted with ritonavir (LPV/r) for patients who failed first line regime (Awotedu, 2010).

Currently the First line regime is a triple drug combination consisting of tenofovir (TDF), 3TC combined with either EFV or nevirapine (NVP) (Department of Health, 2013). In 2013, a new triple fixed dose combination (FDC) pill was introduced. It contains TDF, emitricitabine (FTC) and EFV in one pill (Davies, 2013). The second line regime remained unchanged and consist of ritonavir-boosted protease inhibitor consisting of AZT, TDF, ddI, and lopinavir/ritonavir (LPV/r) (Wallis et al., 2011). The third line regime is seldom used in this SA setting, it involves a special referral for patients failing on second line regime. The most likely combination is raltegravir/duranavir/etravirine (South African Antiretroviral Treatment Guidelines, 2013).

**Table 1.1:** The three lines of regimen that are used in South Africa (South African Antiretroviral Treatment Guidelines, 2013).

| Regimen line | Combination of drugs |
|---|---|
| **first line regime: NRTIs+NNRTIs containing regimens** | |
| 1A | TDF+3TC+EFV |
| 1B | TDF+FTC+EFV |
| 1C | d4T +3TC+NVP |
| | AZT+3TC+NVP |
| | TDF+EFV+NVP |
| | TDF+3TC+NVP |
| | AZT+3TC+EFV |
| | d4T+3TC+EFV |
| **Second line regime: PI containing regimen** | |
| 2A | TDF+3TC+LPV/r |
| | AZT+3TC+LPV/r |
| | d4T+3TC+LPV/r |
| | AZT+3TC+ATV |
| **Third line regime** | |
| Special referral, most likely regimen prescribed is Raltegravir. | |

Wood, (2006) conducted an analysis comparing toxicities associated with d4T and costs of d4T and other drugs. The findings indicated that d4T had more toxicities hence its affordable price at R42.59/month compared to R126.79 and R123.08 for AZT and TDF+FTC (one pill) respectively with fewer toxicities. Therefore, the need for access to safer regimens received major attention and resulted in the replacement of d4T in combination with other regimens by FDC. Airoldi et al. (2010), conducted a qualitative study on the FDC pill. Perceptions of patients indicated a better adherence and compliance with FDC compared to the old regimen which consists of three individual drugs. These findings suggest that access to new HAART regimens with a good efficacy is slowly expanding in South Africa.

### 1.2.2 HAART alters carbohydrate and lipid metabolism

The benefits of HAART have been greatly overshadowed by metabolic diseases such as insulin resistance, DM2 and dyslipidaemia (Tsiodras et al., 2010). Highly active anti-retroviral treatment has been implicated as one of the more significant risk factors associated with exacerbated metabolic effects. In the general population, risk factors such as a positive family history, increased BMI, smoking and mature age result in the development of metabolic diseases (Van Zyl et al., 2012). The same risk factors affect the HIV population. However, risk factors associated with HAART induced metabolic abnormalities including treatment duration, more advanced stages of the disease and exposure to drugs such as PIs pose a significant threat to the HIV population as opposed to the general population who are not on HAART (Ali et al., 2014).

Reports on PI-induced metabolic disorders have been widely exhausted, with older PIs such as indinavir, ritonavir and saquinavir being implicated. Although nuclease reverse transcriptase inhibitors (NRTIs) have been associated with mitochondrial toxicity. However mechanisms associated with these regimens have not been clearly explained in the pathophysiology of carbohydrate and lipid metabolism. Estimates on the prevalence of patients who develop IR due to the use of PIs are as high as 80%, compared to approximately 2% prior to the advent of HAART; thus indicating the exacerbated metabolic disorders associated with HAART (Ryan et al., 2010). Similarly, Duro et al. (2013) reported a higher prevalence of lipid abnormalities in HAART treated patients compared to ART naïve patients. It was further observed that dyslipidaemia was prevalent in 70–80% of patients receiving a PI boosted regimen (Souza et al., 2013).

### 1.2.3 HAART is associated with increased blood glucose levels

#### 1.2.3.1 Overview of the carbohydrate metabolism

Carbohydrates form part of the macronutrients required by the body as a source of energy. Food sources for carbohydrates include starch, glucose, cane sugar (sucrose), sugars in fruit (fructose), honey (fructose and glucose), milk sugar (lactose), maple syrup, and molasses (Singh et al., 1999). Carbohydrates are food sources that are usually not ingested in one form but rather in a complex form. The glycaemic response of food intake is affected by interactions between different macronutrients

including proteins and fats (Ang et al., 2012). Glucose can enter the blood postprandial or from the liver and other tissues (as a result of metabolism).

Glucose homeostasis is tightly regulated by the balance between glucose entering and exiting the circulation (Wei et al., 2000). Glucose is a source of energy that is used for the normal functioning of the body and thus should be maintained within acceptable limits (Wei et al., 2000). Normal blood glucose levels should be maintained at <5.6 mmol/L (Samaras, 2009). If these values are not maintained glucose abnormalities such as impaired fasting glucose may develop.

Insulin is a hormone produced by pancreatic beta cells. It lowers blood glucose levels significantly by supressing hepatic glucose production and by enhancing glucose uptake primarily into the skeletal muscles, adipose tissue and the liver (Kruszynska, 2003). It also promotes storage of glucose as glycogen through pathways involving glycogenesis in the liver by inhibiting overproduction of hepatic glucose (Wysokiński, 2014). Thus any problem with the action of insulin will result in serious disruption of glucose homeostasis leading to dysglycaemia.

Negative feedback mechanisms regulate the secretion of insulin whenever blood glucose levels are elevated. These feedback loops also involve the secretion of glucagon, a hormone produced and secreted by pancreatic alpha cells, which is responsible for increasing blood glucose levels whenever they are too low for the body's energy requirements (Sherwood, 2012). Even though glucagon is not part of the focus of this study, its effect in regulating blood glucose levels cannot be discounted (Figure 1.1). Insulin and glucagon act as antagonists in order to effectively regulate blood glucose. Furthermore, other hormones such as growth hormone and catecholamine increase blood glucose whenever it is too low.

The skeletal muscle is the largest organ that has the highest affinity for insulin hence it accounts for up to 85% of glucose uptake in the body. The skeletal muscle plays an important role in interactions with hepatic and adipose tissue in mediating insulin sensitivity in these organs (Carnagarin et al., 2015). Facilitation of glucose uptake by the muscle is mediated by recruitment of glucose transporter-4 (GLUT-4) (Wysokiński, 2014). It is one of 13 sugar transporters. Glucose transporters 1, 4, 5 and 12 are the isoforms involved in mediating glucose transport in the skeletal muscle (Carnagarin et al., 2015).

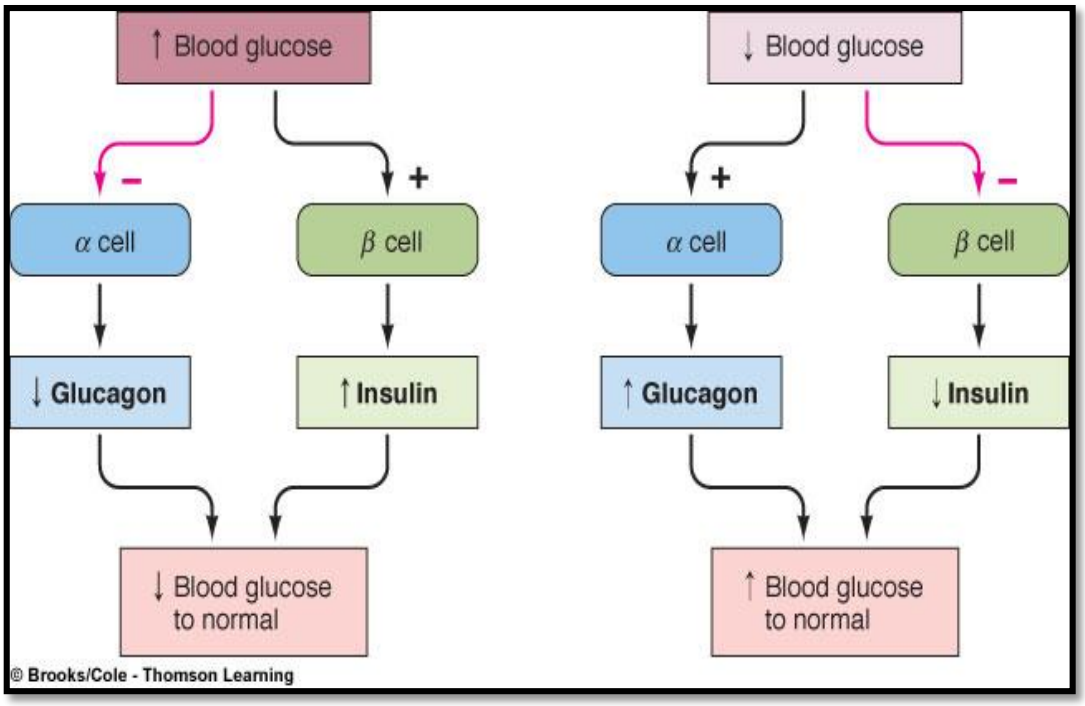



**Figure 1.1:** Negative feedback loops regulating secretion of insulin and glucagon in maintaining blood glucose levels (Sherwood, 2012).

Insulin signalling pathways involves signal transduction that occurs at cellular level. The three mechanisms include hormone receptor interaction resulting in signal receptor activation; transformation of signal transduction into intracellular message which results in formation of modified proteins and transport systems (Carnagarin et al., 2015). Mitochondria are involved in glucose stimulated insulin secretion by establishing signals during oxidative glucose catabolism that trigger and intensify insulin release from the pancreas (Jensen and Affourtit, 2015).

Figure 1.2 shows the mechanism of insulin-mediated molecular events regulating glucose homeostasis. Insulin binds to the insulin receptor substrate (IRS) on the membrane surface and activation of tyrosine kinase insulin receptor results in phosphorylation where the substrate proteins bind to the SRC homology 2 (SH2) domains of the effectors. This pathway moves into a series of reactions where the phosphorylated IRS employs P13K to hydrolyse the membrane bound phosphatidylinositol biphosphate (PIP2) to phosphatidylinositol-3, 4, 5- triphosphate (PIP3) which then employs phosphoinositide-dependant kinase 1 (PDK1) into a series of other reactions that ultimately result in release of GLUT-4 from storage vesicles to the membrane surface for glucose to bind (Carnagarin et al., 2015).

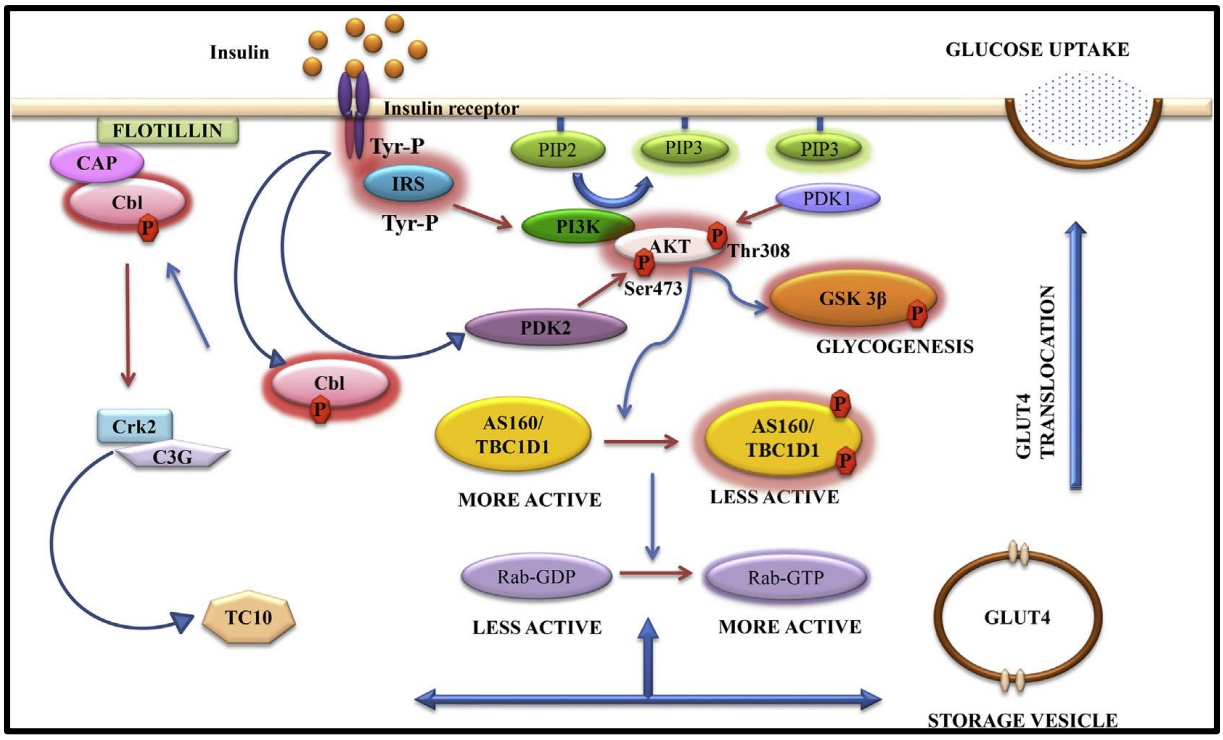



**Figure 1.2:** Insulin mediated molecular pathways regulating glucose homeostasis (Carnagarin et al., 2015).

Disruptions in the homeostatic control of the glucose metabolism can result in the progression from normo-glycaemia to impaired glucose tolerance (IGT). This transition stems from a defect in insulin-mediated glucose uptake in muscle, a dysfunction of the pancreatic cells, a disruption of secretory function of adipocytes, and an impaired insulin action in liver (Szablewski, 2011). Insulin resistance is a state where target cells fail to respond to normal levels of circulating insulin, which results in the inability of insulin to regulate normal glucose and lipid homeostasis (Wang et al., 2004). This occurs due to abnormalities in insulin-signalling pathways where there are defects in the receptors involving down-regulation and reduced affinity of insulin to bind to the receptors (Carnagarin et al., 2015). This in turn results defective GLUT-4 transport systems where glucose becomes elevated in the circulation instead of entering the cells.

Chronic elevated blood glucose levels can result in the development of DM2, which is further associated with an increased risk for CVDs (Wei et al., 1998a).

Increased blood glucose levels reflects on impaired carbohydrate metabolism, defects in insulin secretion and/or its functions as well as defects in glucose regulatory mechanisms (Wei et al., 1998b).

#### 1.2.3.2 Mechanism of insulin resistance and diabetes in HAART patients

Insulin sensitivity is primarily affected by PIs through the direct inhibition of GLUT-4 transporters. In addition, nucleoside reverse transcriptase inhibitorss can also influence insulin sensitivity via induced mitochondrial toxicity (Feeney and Mallon, 2011) (Figure 1.3). In general HAART-associated IR resembles the pathogenesis of DM2, where it mimics abnormalities in glucose homeostasis found in lipodystrophic cases. Lipodystrophy is a syndrome that is associated with a mixed pattern of lipoatrophy (fat loss) and lipohypertrophy (fat accumulation) (George, 2006). Insulin resistance is a biological marker indicating a significant metabolic side effect associated with HIV patients receiving HAART (Tsiodras et al., 2010). These mechanisms form a hierarchy chain leading to decreased insulin sensitivity and a hyperinsulinaemic state resulting in hyperglycaemia and hyperlipidaemia (Calza et al., 2008).

Although lipodystrophy is not the main focus of this study, its involvement in the mechanisms associated with HAART induced IR and DM make it an important aspect to cover in this discussion. It is crucial to note that risk factors such as genetic predisposition, increased free fatty acid concentrations, visceral fat accumulation, increased muscle and fat (Figure 1.3), hormonal alteration, chronic inflammation and co-morbid diseases are prevalent in the general population as risk factors for DM, furthermore including exposure to HAART increases the susceptibility of the HIV population to developing IR and DM (Paik and Kotler, 2011). It has been reported that the onset of DM is accelerated by the co-existence of lipodystrophy in HIV positive patients (Reid et al., 2012).

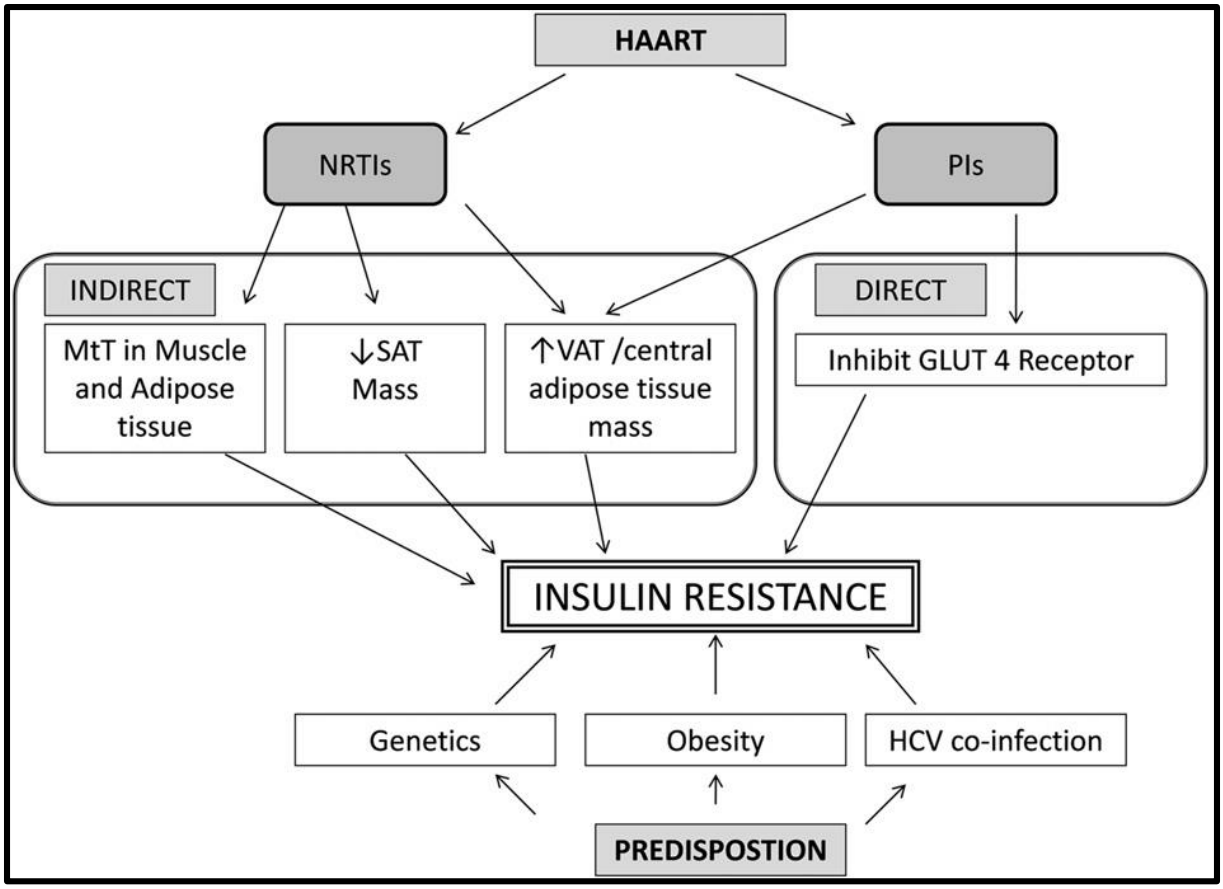



**Figure 1.3**: Mechanism associated with HAART induced insulin resistance; Mitochondrial toxicity (MtT), SAT (subcutaneous adipose tissue), VAT (visceral adipose tissue), GLUT 4 (glucose transporter 4) and HCV (Hepatitis C virus) (Feeney and Mallon, 2011).

#### 1.2.3.3 Prevalence of diabetes mellitus and glucose intolerance in HAART treated patients

An increase in glucose disorders has been observed in HIV patients receiving HAART (Julius, 2010). In 2003, the prevalence of IGT was estimated at approximately 46%, DM2 at 7% and pathologic insulin sensitivity at 61% (Tanwani and Mokshagundam, 2003). Savès et al. (2002) found that the prevalence of DM2 was 6% higher in patients who received HAART for 20 months as compared to those who received HAART for 12 months; however, of the 28 patients with DM2, 9 (32%) were diagnosed only after the 2 hour oral glucose tolerance test. These findings suggest that the duration of HAART plays a significant role in the development of some metabolic diseases, especially dyslipidaemia.

Initiation of HAART in ART-naïve patients requires screening of such individuals for fasting blood glucose, serum lipid parameters, full blood count and chemistry profiles for pre-selection of HAART regimen. However, it is important that the glucose and lipid profiles are monitored on a continuous 3 months basis to reduce the prevalence of metabolic diseases associated with glucose and lipid metabolism (Hester, 2012). The South African guidelines have suggested screening HAART patients showing high risk factors every 6 months, however criteria that is used to categorize patients as high risk may differ in different clinical settings and may make it complex to monitor these variables (Reid et al., 2012). However, it would be cost effective to monitor the effects of these regimens as opposed to treating them whenever they start to prevail.

### 1.2.4 The effect of PIs on glucose metabolism

Protease inhibitors emerged in the mid-1990s with indinavir and ritonavir being the first PIs marketed. Indinavir has not been used since the advent of new PIs such as LPV/r and atazanavir (ATV). In 2000, the co-formulation of PIs began when ritonavir was boosted with lopinavir to increase the PI potency (Hester, 2012). Currently, Kaletra™ and Aluvia™ are the two PIs available, where ritonavir is co-formulated with lopinavir. Ritonavir is metabolized by the hepatic cytochrome P450 enzymes CYP3A4 and CYP3A5. These enzymes may reduce the antiviral activity of ritonavir and make it insufficient to suppress viral replication (Reyskens and Essop, 2014); therefore, when it is boosted with lopinavir, it will require multiple mutations to induce high level resistance (Hester, 2012). Co-formulating with lopinavir is based on its capability to block CYP3A4 and CYP3A5; thereby increasing the potency of ritonavir to suppress viral replication (Reyskens and Essop, 2014). Seemingly these PIs were synthesized with the focus on pharmacokinetics of the drug in suppressing viral replication; however, the metabolic effects of these drugs in the human body were underestimated.

The impact of PIs on glucose metabolism ranges from impaired glucose tolerance to DM (Tanwani and Mokshagundam, 2003). Protease inhibitors have been found to increase IR and reduce insulin secretion by inhibiting GLUT-4 mediated glucose transport (Kalra et al., 2011). The GLUT-4 transporter, mainly expressed in tissues such as skeletal muscle, cardiac muscle and fat, is responsible for most of the body's glucose disposal. It is known to be the main transporter isoform mediating

insulin-stimulated glucose uptake (Hruz et al., 2002). A number of PIs, such as indinavir and ritonavir, have been strongly associated with insulin resistance (Noor et al., 2001; Feeney and Mallon, 2011).

Mechanisms associated with PI-induced IR have been identified. Firstly, metabolic pathways involving insulin receptor substrate-1 phosphorylation, subsequently affecting insulin sensitivity (Calza et al., 2008); secondly, down-regulation of GLUT-4 has been identified as a mechanism for diabetes in patients taking ritonavir however findings did not show effects when ritonavir is combined with ATV (Paik and Kotler, 2011); thirdly, inhibition of peroxisome proliferator-activated gamma receptor activity subsequently resulting in reduced adipocyte differentiation and lastly, PIs have been found to impair beta cell function by up to 50% (Reid et al., 2012). In this regard it seems that the mechanisms reported are PI-regimen specific and that not all PIs are negatively associated with abnormal metabolic outcomes.

The pathophysiology of PI-induced metabolic disorders involves a multifaceted response (Figure 1.4); and oxidative stress induced by reactive oxygen species (ROS) in endothelial cells, adipocytes and macrophages have been implicated (Chandra et al., 2009). The mitochondria synthesizes ATP via oxidation of metabolites using the tricarboxylic acid cycle and the catabolism of fatty acids via β-oxidation, through a series of chemical reactions known as the electron transport chain (ETC), and oxidative phosphorylation (Barve et al., 2010). Interactions in the ETC can be disrupted by sudden changes in electrons combining with reactive-oxygen containing molecules resulting in the formation of ROS. Higher levels of ROS may accumulate and disrupt complexes I and III of the ETC, resulting in a damaged mitochondrial genome (Reyskens and Essop, 2014). Proteins, lipids or DNA may interact with ROS and become damaged due to the inhibition of antioxidants (glutathione peroxide and superoxide) in the mitochondria (Barve et al., 2010). Although PIs have received much attention in deducing underlying mechanisms associated with IR and DM, to a lesser extent have these mechanisms been contrasted with NRTIs.

Nuclease reverse transcriptase inhibitors, the first line of drugs in developing countries, have been strongly associated with the development of IR and subsequently DM2 (Idiculla et al., 2011). De Wit et al. (2008) indicated that the incidence of DM2 correlated with the use of d4T in combined ART; furthermore, AZT and ddI was found to increase the risk of developing DM2. Stavudine is a nucleoside thymidine analogue which requires phosphorylation by cellular kinases. It acts by inhibiting HIV reverse

transcriptase by competing with deoxythymidine triphosphate as a substrate and incorporating it into the viral cDNA, resulting in chain termination. During this chain reaction it also inhibits human cellular DNA gamma and beta polymerases subsequently resulting in the reduction in the synthesis of mitochondrial DNA (mtDNA) (Wood, 2006). It has been reported that in the general population, genetically associated mitochondrial dysfunction is a major factor in the development of IR and DM (Paik and Kotler, 2011). Therefore, it is likely that the effects of both genetic and acquired mitochondrial dysfunction place this HAART population at increased risk of developing DM as compared to the general population.

### 1.2.5 The effect of NRTIS and NNRTIS on glucose metabolism

The effect of HAART on glucose metabolism resulting in DM2 is not clearly elucidated; however, thymidine based NRTIs and PIs are of major concern (Petoumenos et al., 2012). Thymidine NRTIs such as AZT combined with LPV/r were found to reduce insulin sensitivity with up to 25% increasing the risk for IR, however no IR was found when combined with NVP as a backbone. Fleishman et al. (2007), hypothesized that NRTIs induce cumulative effects resulting in development of DM2; whereas, PIs induce acute metabolic effects. Nucleoside reverse transcriptase inhibitors inhibit the enzymes that replicate the virus including DNA polymerase which replicates mtDNA. In cases where NRTIs are erroneously incorporated into mtDNA, they attenuate the corresponding section (Barve et al., 2010) making the section non-functional. Under normal circumstances, the mitochondrial DNA can replace mis-incorporated pieces from the mtDNA with new pieces. However, mechanisms underlying NRTI-dependent inhibition of DNA polymerase on repair of mtDNA are still not clear (Barve et al., 2010). Nucleoside reverse transcriptase inhibitors induce inhibition of DNA polymerase causing reductions in mtDNA levels resulting in defective electron transport and an increase in oxidative stress in the mitochondria through the production of ROS. Oxidative stress further damages mtDNA leading to cumulative damages to the mitochondrial genome (Barve et al., 2010).

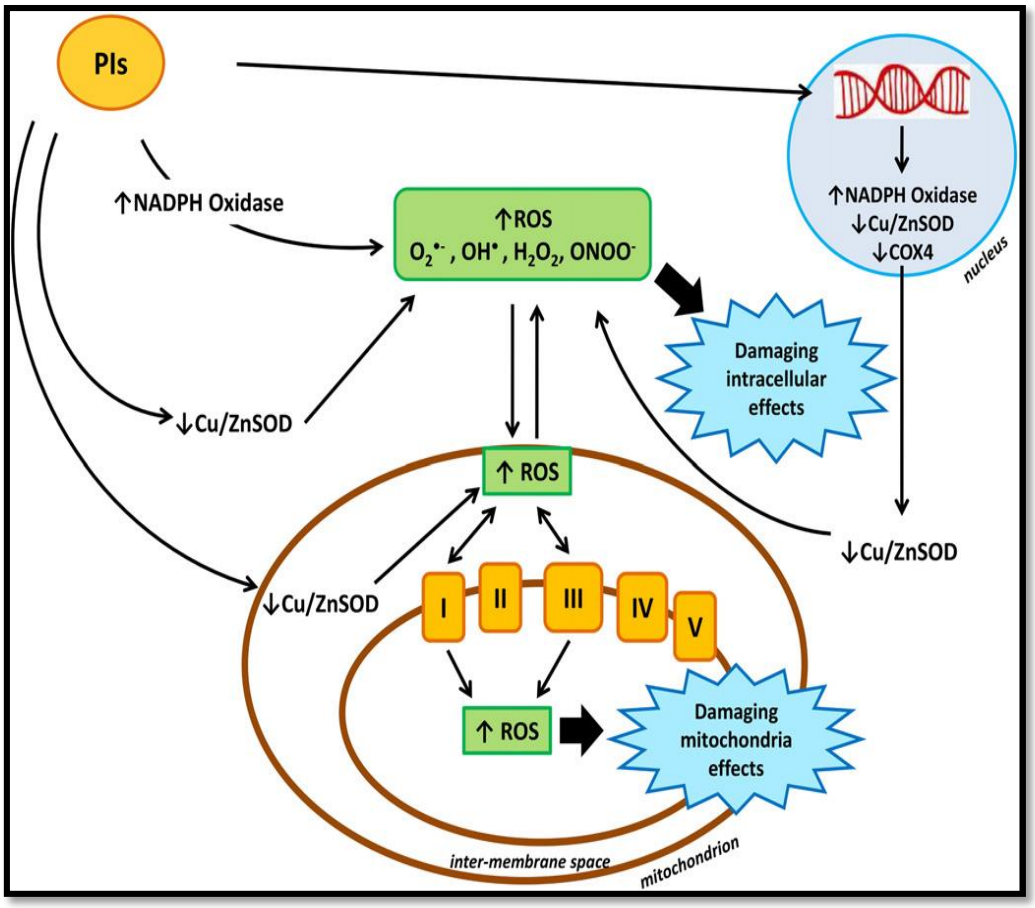



**Figure 1.4:** A multifaceted response resulting in PI induced mitochondrial toxicity through the production of ROS; reactive oxygen species (ROS), O2•− superoxide free radical, OH•—hydroxyl radical, $H_2O_2$—hydrogen peroxide, ONOO−—peroxynitrite, Cu/Zn SOD—copper/zinc superoxide dismutase, COX4: cytochrome c oxidase 4, NADPH—nicotinamide adenine dinucleotide phosphate hydrogen (Reyskens and Essop, 2014).

Metabolic disorders arising due to NRTIs have not been clearly explained; however, a holistic approach involving NRTIs and PIs are implicated in these adverse events (Flint et al., 2009). The pathophysiology of each specific group of drugs becomes extremely difficult to explain especially when the drugs are used in combination (Butt et al., 2009). Although HAART outcomes have proven successful in the clinical management of the disease, it has reached a point where the long term effects of these regimens should ideally have a dual focus; its impact on the carbohydrate metabolism and the lipid metabolism.

### 1.2.6 HAART is associated with increased blood lipid levels

#### 1.2.6.1 Overview of the lipid metabolism

Plasma constituents of lipoprotein complexes include TC, HDL, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), LDL and TG (Maritz, 2006). Lipoproteins are particles that mediate the delivery of hydrophobic lipids to various tissues through the circulation (Poirier and Despres, 2003). Chylomicrons are structurally large light particles; however, they have a low density. They are of different sizes ranging between 75–1200 nm. One chylomicron consist of 90% neutral lipids with composition of triglycerides being more compared to small particles of cholesteryl esters (Demignot et al., 2014). Chylomicrons and their remnants are the largest lipoproteins. They are mainly produced by the intestine and they transport dietary fats (Semenkovich, 2012).

Chylomicrons have been identified as contributors to the development of metabolic diseases such as cardiovascular diseases, DM2 and dyslipidaemia through co-existence of obesity (Demignot et al., 2014). Chylomicrons are stabilized by phospholipids, cholesterol and lipoproteins such as apolipoproteins (Demignot et al., 2014). Apolipoproteins (Apo) are a group of proteins that mediate lipid transport across plasma (Das, 2010). They are designated in alphabetical order, numerals and romans, for example, AI, AII, C2 (Das, 2010; Demignot et al., 2014). Chylomicron remnants consist of apoB48, apoE, apoAI, apoAII, apoAIV, apoCII and apoCIII (Semenkovich, 2012).

Absorption of dietary lipids involves enterocytes of the jejunum. Triglycerides are hydrolysed in the stomach by gastric lipase and then enter the lumen of the small intestine where they are further hydrolysed by pancreatic lipase into fatty acids and 2 monoaclylglycerol. Phospholipids are produced by bile, from dietary sources they are hydrolysed by pancreatic phospholipase A2 into lysophosphatydylcholine and fatty acids (Demignot et al., 2014). Fatty acids can be formed as free fatty acids by the adipocytes which are non-esterified or they may also be formed by the intestine as esterified fatty acids subsequently leading to the production of triglycerides (Wang et al., 2008).

Triglycerides that are produced from chylomicrons are hydrolysed by lipoprotein lipase into fatty acids that are mobilized in the circulation as a source of energy. The chylomicrons that are not hydrolysed into triglycerides become bound to LDL receptors

in the liver where it has a high specific affinity for apoE (Demignot et al., 2014). Low density lipoprotein receptors facilitate the removal of LDL and other particles of VLDL and IDL by binding to apoB100 and apoE. Furthermore, the LDL receptor maintains this activity in the liver through regulating the sterol regulatory element binding proteins (SREBPs) (Semenkovich, 2012).

The process of dietary lipid absorption occurs in the small intestine precisely in the jejunum lumen. Fat becomes absorbed as triglycerides which are moved out of the enterocytes as chylomicrons that are triglyceride-rich lipoproteins (Demignot et al., 2014). Transport of lipoproteins involves two major metabolic pathways. They are mediated via exogeneous and endogeneous pathways. Exogeneous pathways transports dietary lipids to the peripheral pathways and endogeneous pathways transports to the liver and periphery (Figure 1.5) (Das, 2010).

In the exogenous pathway, dietary fats become hydrolysed in the GIT to free cholesteryl ester and triglycerides which are then bound to apoB48 (Semenkovich, 2012). These lipids become packaged together with several apolipoproteins, phospholipids and unesterified cholesterol into nascent chylomicrons which are transported into the blood (Das, 2010). Transport of these particles to adipose tissue and muscle is mediated by activation of lipoprotein lipase (LPL), by binding to apoCII and then triglycerides become hydrolysed into fatty acids which are transported for storage in adipose tissue or muscles for energy (Semenkovich, 2012). Chylomicrons are degraded to smaller chylomicron remnants which bind to hepatic parenchymal cells mediated by apoE receptors, where they are rapidly removed from the circulation (Das, 2010).

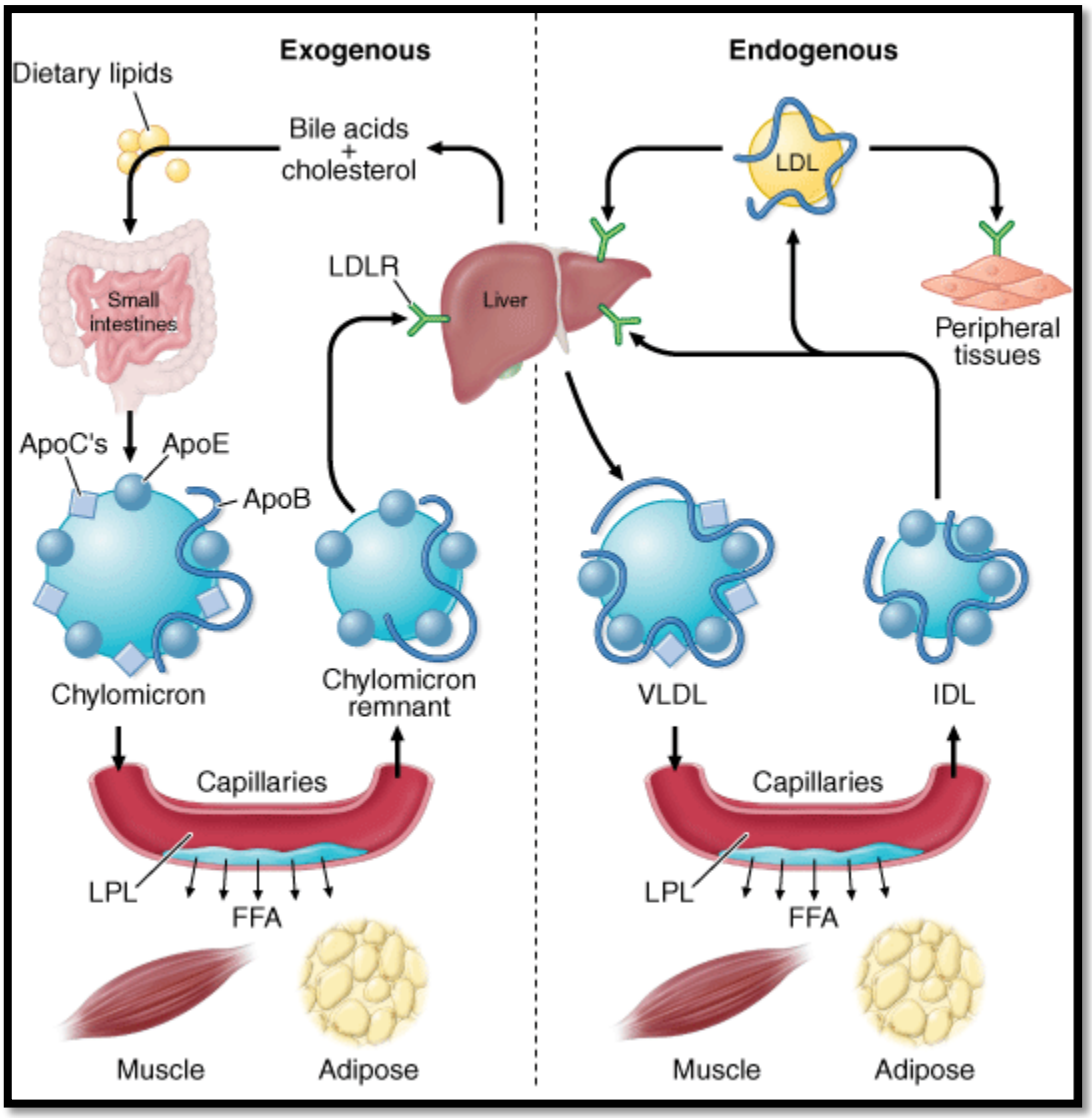



**Figure 1.5:** The two metabolic pathways involved in lipid transport, intermediate density lipoprotein (IDL), very low density lipoproteins (VLDL), LDL, HDL and free fatty acids (Das, 2010).

In the endogenous pathway, fats stored in the liver are further metabolized into lipid species that are stored in hepatocytes or exported as lipoproteins (Semenkovich, 2012). Triglycerides and cholesterol are transported to different tissues through synthesis of VLDL in the liver (Das, 2010). Nascent VLDL containing apoB100 is then released in plasma, where it acquires apo E, apo CII, and apo CIII. Hydrolysis of triglycerides mediated by LPL results in release of fatty acids, it also causes loss of phospholipids and apolipoproteins to HDL (Semenkovich, 2012). Very low density lipoproteins become converted to intermediate density lipoprotein (IDL) and subsequently LDL mediated by apoE (Das, 2010). Most of the LDL is cleared from

plasma through LDL receptor mediated pathways, however small remnants of VLDL, IDL and LDL become atherogenic as they are able to cross the membrane into the arterial walls resulting in diseases such as atherosclerosis (Semenkovich, 2012).

Excess cholesterol is transported back to the liver which is packaged in bile for excretion (Das, 2010). This process involves reverse cholesterol transport. High density lipoproteins are synthesized by the liver and intestines as a phospholipid disc bound to apo AI and apo AII (Semenkovich, 2012). This lipoprotein interacts with the liver by binding to scavenger receptor 1 (SR-B1) which mediates the transfer of cholesteryl ester from HDL to the liver (Das, 2010; Semenkovich, 2012). In addition, it may also transfer cholesteryl ester to apo B100 containing lipoproteins such as VLDL converting to IDL and then LDL after transporting to the liver (Semenkovich, 2012). The three pathways involved in lipid transport may be of great importance when looking into the underlying mechanisms associated with lipid disorders, especially dyslipidaemia.

#### 1.2.6.2 Prevalence of dyslipidaemia in HAART treated patients

Lipid disorders have been observed in HIV patients before the HAART era; however these abnormalities are becoming endemic in HIV positive Patients receiving HAART (Minnaar, 2008). The prevalence of dyslipidaemia is associated with risk factors such as older age and with HIV related factors such as viral load and baseline hyperlipidaemia (Tsiodras et al., 2000).

Saves et al. (2002), found that the prevalence of dyslipidaemia was as high at month 12 as it was at month 20; however, the findings indicated a decrease in TC and LDL from baseline to follow-up; an increase in TG and low HDL levels observed at month 12 compared to month 20. Bekolo et al. (2014) conducted a study in Cameroon on HIV HAART patients that were on first line regime consisting of AZT+3TC+NVP. They found that the prevalence of hypertriglyceridaemia was 51.8%; increased LDL-C was 33.3% and hypercholesterolemia was 29.8 %. These findings suggest that although PIs have been implicated in the development of metabolic disorders, the effects of first line regimen drugs such as AZT which were implicated in similar effects as stavudine cannot be disregarded. Therefore they concluded that HAART duration of more than two years was associated with poor lipid profiles.

### 1.2.7 Mechanism of altered lipid metabolism in HAART treated patients

Pathogenic mechanisms associated with dyslipidaemia have been postulated. Common mechanisms associated with hyperlipidaemia include increased intra-hepatic production and an impaired clearance of lipids from the blood stream (Calza et al., 2008). These mechanisms suggest PIs induce the inhibition of Cis-9 retinoic acid by erroneously binding to CRABP-1 leading to the increased apoptosis of peripheral adipocytes, decreased lipid storage and an increase in lipid mobilization in the blood stream. Furthermore, PIs may impair lipoprotein receptor related protein (LRP) pathways thus interfering with fatty acid storage in the adipocytes (Calza et al., 2008). Figure 1.6 illustrates the pathways that may be affected by PIs, resulting in the development of dyslipidaemia (Das, 2010).

#### 1.2.7.1 The effect of PIs on lipid metabolism

The effect of PIs on the lipid metabolism is characterized by high levels of triglycerides (TG), total-cholesterol (TC) and LDL-cholesterol, and low levels of HDL-cholesterol (Brennan-Benson, 2009). The use of ritonavir has been associated with severely elevated serum TC and TG levels in both seronegative and HIV positive patients (Noor et al., 2001). High rates of lipid abnormalities have been detected in patients receiving PIs (Calza et al., 2004).

Hyperlipidaemia is found in 70–80% of patients receiving a PI-boosted regimen. Newer PIs such as atazanavir were found to improve lipid parameters in dyslipidaemic patients as compared to the LPV/r PI (Souza et al., 2013). Ritonavir is strongly associated with hyperlipidaemia with a 7.2 fold risk of hypertriglyceridaemia as compared to other PIs (Hester, 2012).

Protease inhibitors are associated with hypercholesterolemia, hypertriglyceridaemia, elevated LDL-C and decreased HDL-C concentrations (Souza et al., 2013). The effect of PIs on cholesterol levels is suggested to be regimen-specific. Drug-specific mechanisms include direct stimulation of the formation of VLDL lipoproteins or decreased lipoprotein lipase activity and changes in mobilization of lipid stores (Tsiodras et al., 2010).

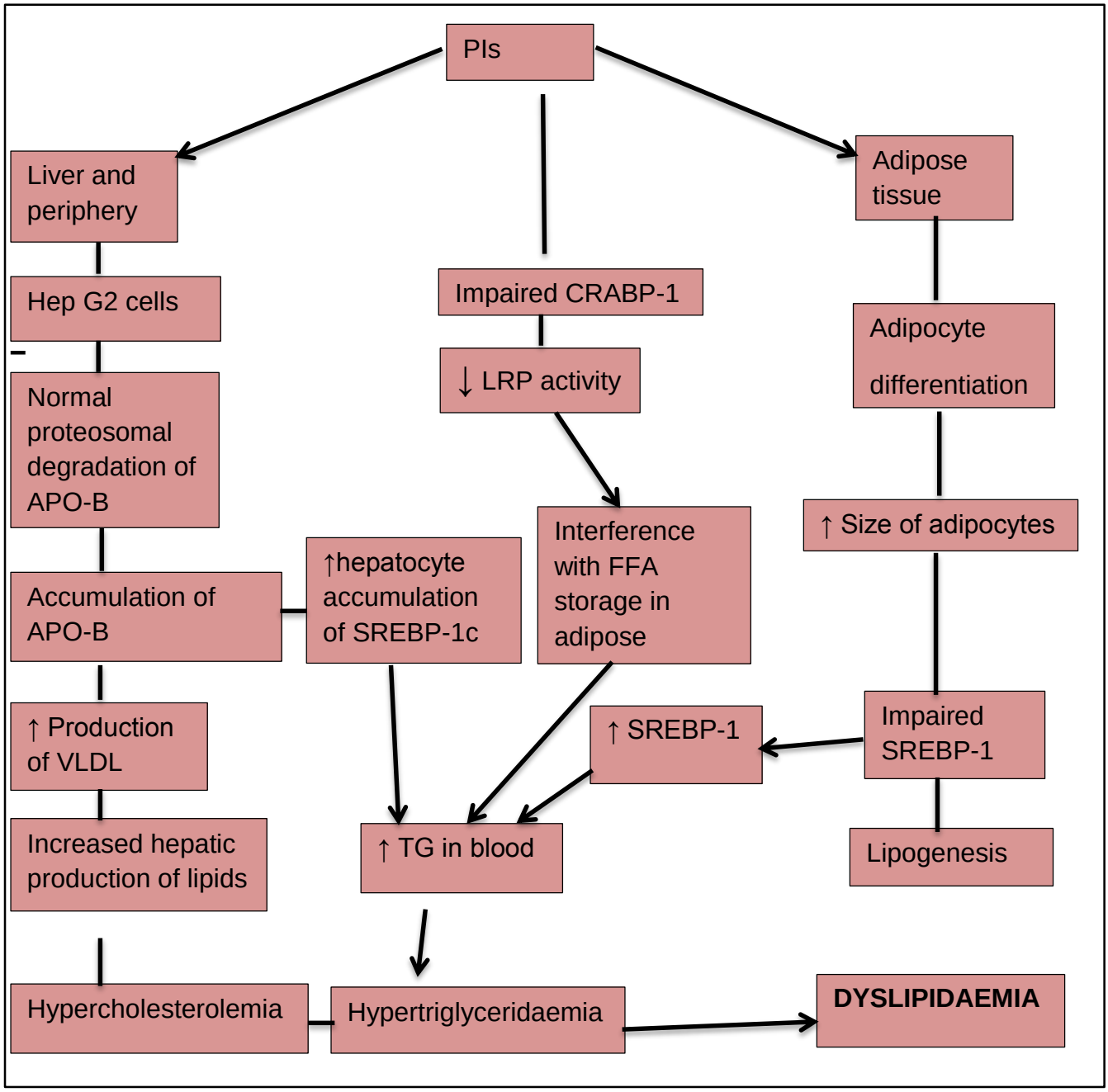



**Figure 1.6:** Mechanisms associated with PI induced dyslipidaemia. Cytoplasmic retinoic acid binding protein type 1 (CRABP-1); Lipoprotein receptor related protein (LRP); Sterol regulatory element binding protein–1 (SREBP–1); Apolipoprotein–B (APO–B–) (adapted from Das, 2010).

In this case PIs may inhibit proteosomal degradation of nascent apolipoprotein B, resulting in increased levels of VLDL lipoproteins. In the liver, an up-regulation of metabolic pathways may also lead to overproduction of VLDL caused by intra-hepatic

accumulation of nuclear transcription factors involved in the metabolism of apolipoprotein B (Das, 2010).

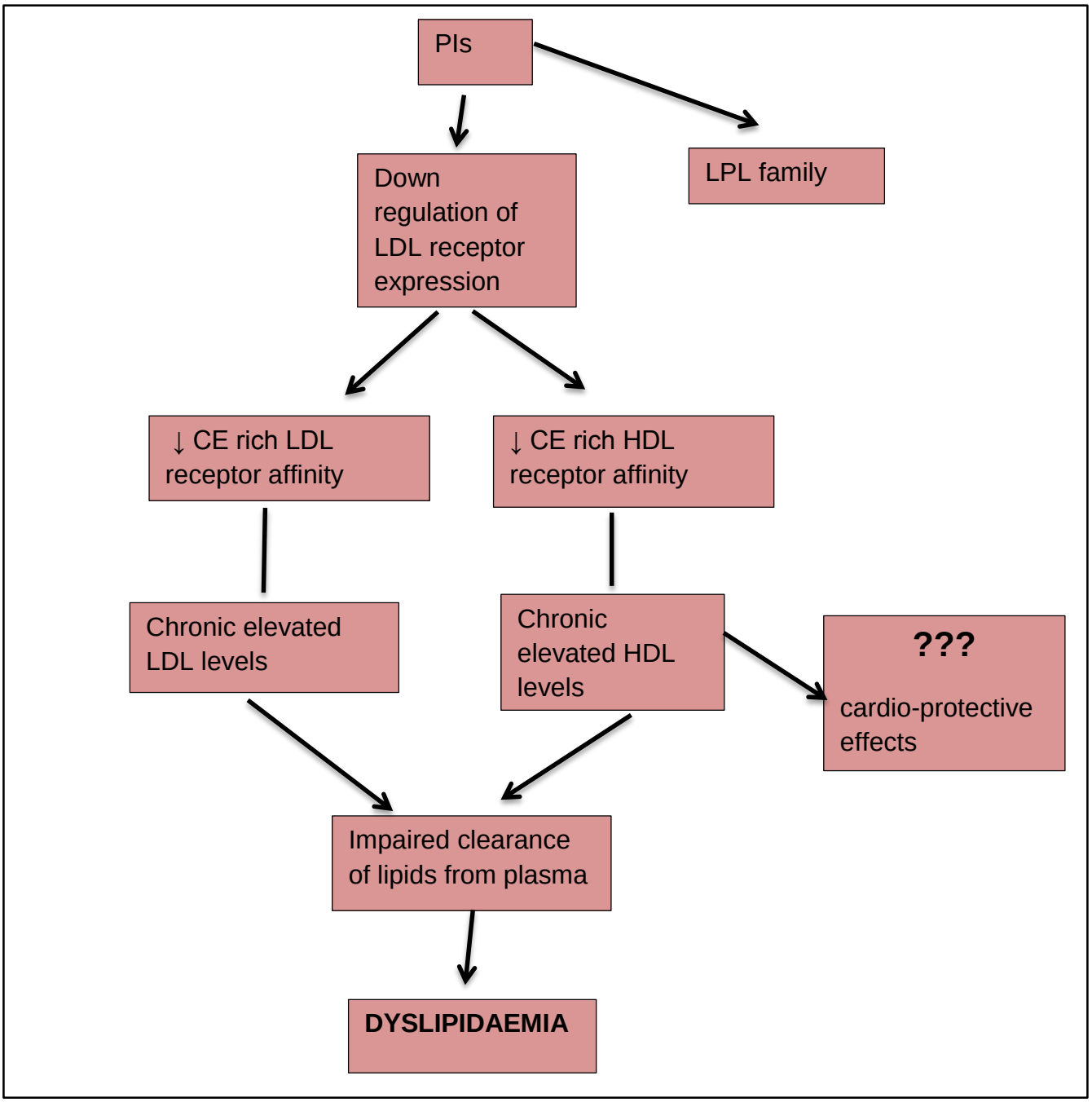



**Figure 1.7:** Mechanisms associated with impaired lipid clearance resulting in dyslipidaemia. Cholesteryl esters (CE); Lipoprotein lipase (LPL).

Suggested mechanisms of PI induced dyslipidaemia include proteosome dysfunction in the liver and/or adipocytes. Protease inhibitors such as ritonavir, nelfinavir and saquinavir inhibits certain components of proteosomal activity. Inhibition of

proteosomal activity is dependent on the concentration of the drugs where effects of inhibition can be found within therapeutic doses (Barve et al., 2010). Proteosomal degradation of apo B may result in accumulation of apo B in Hep G2 cells thus increasing the production of VLDL subsequently resulting in hypertriglyceridaemia.

In cases where indinavir impairs proteasome function, proteins such as sterol regulatory element-binding protein-1 (SREBP)-1 may accumulate in the cell nucleus resulting in hyperactivity of the genes (Figure 1.6). Proteosomes regulate these proteins through degradation. Furthermore, these proteins play a significant role in deposition of fat molecules in the liver (Barve et al., 2010). In adipocytes, SREBP-1 promotes lipogenesis and adipocyte differentiation. Accumulation of SREBP-1 will increase the size of adipocytes therefore increasing production of VLDL from triglycerides (Das, 2010).

The down regulation of SREBP-1 (Figure 1.7), due to drug induced depletion may lead to halted peroxisome proliferator activator receptor gamma (PPAR-ɣ) expression, resulting in impaired adipocyte differentiation and induction of adipocyte apoptosis mainly in the peripheral subcutaneous fat depots. Morphological changes observed include lipoatrophy in the limbs and facial fat (Barve et al., 2010).

#### 1.2.7.2 The effect of NRTIs and NNRTIs on lipid metabolism

Nucleoside reverse transcriptase inhibitors have been significantly associated with the development of lipoatrophy and lipohypertrophy resulting in lipodystrophy (Subbaraman et al., 2007). The use of d4T, AZT, ddI or EFV has been associated with dyslipidaemia (Calza et al., 2004). The development of lipodystrophy has been strongly associated with the dose and duration of HAART use (Tanwani and Mokshagundam, 2003). Reports on the effects of NRTIs on the lipid metabolism have shown variable effects. Nucleoside reverse transcriptase inhibitors have been found to suppress synthesis of mitochondrial DNA subsequently leading to reduced oxidative phosphorylation resulting in dyslipidaemia (Libonati et al., 2012). Patients using TDF+3TC were found to exhibit lower serum concentrations of LDL-C, TC and TG as compared to patients using AZT+3TC, d4T+3TC or ddI+3TC (Souza et al., 2013). These findings have led to improvements in the choice of NRTIs in patients initiating HAART. Furthermore, the effects of NNRTIs have also shown to be moderate in alteration of lipid metabolism.

There has been consistent reports on NNRTIs exhibiting favourable lipid profiles especially in patients using NVP (Minaar, 2008; Souza et al., 2013). Non-nucleoside reverse transcriptase inhibitors have been found to alter lipid profiles; however, to a minimal effect with the exception of NVP. Patients taking EFV were found to have higher levels of triglycerides and HDL-C compared to patients taking NVP (Libonati et al., 2012). Minnaar (2008) hypothesized that the mechanism involving NVP in increasing HDL-C thus resulting in some form of protection from cardiovascular diseases. The mechanism involves NVP stimulating synthesis of apo AI which is a protein component of HDL and its presence maintains the size, shape and function of HDL. Furthermore, interaction of apo AI with ATP-binding cassette transporter A1 (ABCA1), promotes cholesterol efflux which stimulates the synthesis of HDL thus resulting in increased circulating levels of HDL. Although the current HAART regimen (FDC) consist of EFV and not NVP as a NNRTIs, there is a need for further investigation on the effects of EFV when contained in the new FDC.

#### 1.2.7.3 The link between leptin and abnormal lipid metabolism

Since its discovery in 1994 (Zhang et al., 1994), leptin has become known as an adipocyte derived hormone involved in energy homeostasis and its ability to decrease insulin resistance through its central effects on the hypothalamus and peripheral effects on fatty acid oxidation (Mantzoros, 1999). Leptin is mainly produced in white adipose tissue; however, it is also expressed in other organs such as the placenta, brain, bone, thyroid etc. (Polyzos and Mantzoros, 2015). This hormone marks its 20$^{th}$ anniversary, yet its effects are still poorly understood due to its versatility in the different organs.

Leptin levels fluctuate according to nutrient intake, thus during fasting a sudden drop in leptin levels is encountered (Park and Ahima, 2015). Leptin was thought to be a hormone that would reduce the pandemic obesity however the outcome was not achieved. This was due to leptin resistance observed in obese individuals making it difficult to achieve weight loss (Paz-Filho et al., 2015). These findings have led to extensive research that was done to investigate the underlying mechanisms associated with leptin resistance/tolerance. Obese individuals have been found to exhibit high levels of leptin; however, these high levels are unable to reduce adiposity indicating leptin resistance. In contrast leptin deficiency in obese rats and individuals

have been associated with insulin resistance, DM and other components of metabolic syndrome (Park and Ahima, 2015). It was found that there is an overlap between leptin and insulin signalling pathways thus it might be an explanation to the mechanisms underlying leptin affecting insulin pathways resulting in insulin resistance. The src homology (SH) was found to interact with leptin and insulin pathways through Janus kinase (JAK) 2 and IRS-1 and IRS-2 which are mediated in response to leptin. Impairment of the src homology 2 domain-containing adapter protein B (SH2B) due to mutations leads to hyperleptinaemia and hyperinsulinaemia, thus leptin administration improves insulin sensitivity (Polyzos and Mantzoros, 2015). These effects were distinguished via central and peripheral pathways after the effect of leptin administration was studied in rats.

In non-obese diabetic mice, peripheral leptin administration was found to supress glucagon thus inhibiting the effects of glucagon in raising blood glucose levels. Similarly, central effects of synthetic leptin decreased glucagon and glucose through insulin-dependent mechanisms (Park and Ahima, 2015). Leptin does this by inhibiting expression of gene that mediates insulin signalling pathways (Paz-Filho et al., 2015). In a Turkish cohort study it was observed that leptin administration resulted in normal glucose, insulin and lipid levels. They also found that a diabetic showed a decrease in glucose levels from 7.3 mmol/L to 4.3 mmol/L after treatment with synthetic leptin (Paz-Filho et al., 2015). Leptin mediates pancreatic beta cell function by reducing expression of insulin genes; stimulating beta cell proliferation; inhibiting insulin secretion and inhibiting beta cell apoptosis (Paz-Filho et al., 2015).

In the lipid metabolism, leptin administration was found to stimulate de novo lipogenesis and lipolysis via sympathetic system through central effects. Leptin also stimulated fatty acid oxidation by up-regulating peroxisome proliferator-activated receptor gamma-coactivator 1a and decreases triglyceride stores in adipose tissue and liver (Park and Ahima, 2015). Effects of leptin were further investigated in HIV positive patients on HAART; resulting in the focus on recombinant leptin therapy in this population.

Fasting leptin levels were found to correlate with total body fat concentrations in HIV positive patients (Tsiodras and Mantzoros, 2006). Hypoleptinaemia is significantly associated with severe lipodystrophy and hypertriglyceridaemia (Oral et al., 2002). Leptin replacement therapy significantly decreases visceral fat; however, it does not improve peripheral lipoatrophy thus it result in abnormal fat distribution (Calmy et al.,

2008). Oral et al. (2002) found that leptin deficiency in HIV positive patients plays a role in the development of insulin resistance; however, after four months of leptin replacement therapy glycaemic control was significantly improved. Findings from various human studies (Zhang et al., 1994; Oral et al., 2002; Depaoli et al., 2010; Mantzoros, 2012), have led to the discovery of a recombinant leptin therapy in individuals with a deficiency in leptin. Further support for this is the recent approval of leptin (Metreleptin™) as recombinant therapy to treat congenital leptin deficiency and lipodystrophy (Paz-Filho et al., 2015).

#### 1.2.7.4 HIV/HAART associated lipodystrophy syndrome and leptin replacement therapy

HIV/HAART associated lipodystrophy syndrome (HALS) is a combination of lipid abnormalities, lipodystrophy and impaired glucose tolerance in HIV positive patients receiving HAART (Depaoli et al., 2010). Leptin levels decline with weight loss and patients with HALS are found to have very low levels of circulating leptin. Physiological leptin concentrations between (0.04–0.08 mg/kg/day) were found to improve insulin sensitivity and glucose tolerance and a significant weight loss (Paruthi et al., 2013). The discovery and use of recombinant leptin have led to speculations that it would shed light in solving problems that have been of concern especially in HALS patients.

Leptin replacement therapy significantly decreases visceral fat; however, it does not improve peripheral lipoatrophy thus it results in abnormal fat distribution (Calmy et al., 2008). Depaoli et al. (2010) filed a patent on their invention of the therapeutic use of leptin, leptin analogs, and leptin derivatives for the treatment of lipoatrophy and its associated metabolic abnormalities. They found that the use of recombinant leptin in patients who developed a deficiency in leptin and or leptin resistance significantly improved their metabolic profiles especially in patients who had baseline leptin levels of <4 ng/ml.

Al-Fadhli et al. (2014), investigated 30 patients who were on HAART and grouped them into group I (patients on HAART with low viral load and $CD4^+$ count) and group II (patients on HAART with low viral load and high $CD4^+$ count). They found that IR was prominent in group II with a possible explanation of the use of PIs inhibiting GLUT-4 and glucose transport. Furthermore, they concluded that due to the high serum leptin levels observed in patients with high $CD4^+$ count, there is a correlation

with the latter and fasting serum insulin and homeostatic model assessment for insulin resistance (HOMA-IR) indicating that HAART may lead to decreased levels of leptin which may impair immunological recovery.

Hormones such as adiponectin are associated with IR, visceral adiposity, percentage body fat, W: H ratio and BMI. Hyperleptinaemia was found to be associated with IR especially in females; however, the study could not reveal the pathogenesis of IR through leptin pathways (Arama et al., 2013).

Fasting leptin levels correlate with total body fat concentrations in HIV positive patients (Tsiodras and Mantzoros, 2006). Hypoleptinaemia is significantly associated with severe lipodystrophy and hypertriglyceridaemia (Oral et al., 2002). On the contrary Ketlogetswe et al. (2014), documented that serum leptin levels in HAART patients with lipodystrophy are comparable to the levels in HAART patients without lipodystrophy, and that of HIV uninfected populations as leptin levels are determined by total body fat mass irrespective of body fat distribution.

### 1.2.8 HAART IS ASSOCIATED WITH ALTERED BODY COMPOSITION

#### 1.2.8.1 Prevalence of overweight, obesity and abdominal obesity in HAART treated patients

Obesity is defined by the WHO as the excessive accumulation of fat in the body, resulting in adverse health effects such as the development of metabolic diseases (Osuji et al., 2010). Diseases associated with obesity has became a major public health concern in both developed and developing countries (Crowther and Norris, 2012). The prevalence of obesity is evident in countries undergoing nutritional and economic transition (Smit, 2012).

Manifestations of obesity include abdominal obesity, which is a form of obesity associated with the metabolic syndrome and it presents as increased waist circumference (Ntyintyane et al., 2006). Studies conducted in SSA have focused on under nutrition (Osuji et al., 2010); and found that there are no defined cut off points to assess waist circumference for the diagnosis of metabolic syndrome in SSA (Crowther and Norris, 2012). Waist circumfernce has been identified as a variable that distingushes the presence or absence of metabolic syndrome (Ntyintyane et al., 2006). Changes in body composition have become the basis of assessing risk of individuals in developing metabolic diseases.

The prevalence of overweight was reported to be as high as 60% in African urban women with approximately 56% and 29% of South African females and males being overweight and obese respectively (Sengwayo et al., 2012). These rates raise concerns in the South African HIV population as they are already showing an altered metabolic profile, it seems that the prevalence of overweight and obesity will be higher in this population due to other confounding factors such as exposure to HAART.

Altered anthropometric parameters were found to be associated with long term exposure to PIs resulting in increased WC and BMI stemming from metabolic derailment of glucose and lipid metabolism (Reyskens and Essop, 2014). Ali (2014) found a decrease in all body composition parameters (i.e BMI, total body fat, visceral fat and subcutaneous fat) regardless of treatment option. These findings suggest that a combination of factors including HIV itself, HAART and duration on HAART may attribute to body morphology alterations.

In South Africa, it was found that the mean BMI was higher in ARV group and non HIV positive group (general population) than in HIV positive ART naïve patients (Awotedu et al., 2010). In one study, body morphology changes were reported to be regimen specific (Sreekantamurthy et al., 2014). Similarly Nell et al, (2015) conducted a study on gender based differences in anthropometric profiles of HAART patients. The patients were divided into three groups; Human immunodeficiency virus positive HAART naïve (control), >3 years on HAART and <3 years on HAART. Their findings showed that there was an increased prevalence of obesity among females (56%) compared to males (29%); however, they indicated that the majority of females were obese before initiation of HAART and this increase in overweight and obesity among the female population is rather a problem in the general South African black population. Furthermore, it was found that the prevalence of abdominal obesity (WC>88 females and >102 males) was higher (39.5%) in the ARV group compared to the other groups (31.7%) (Awotedu et al., 2010). The mean W:H ratio was higher in >3 years HAART group compared to the other groups suggesting that the longer these patients are on HAART increases their risk for cardio-metabolic diseases (Nell et al., 2015).

Similarly a study investigating the effect of the duration of HAART on body composition and metabolism found that with an increasing number of years on HAART, patients developed significantly higher prevalence of overweight and obesity including abdominal fat accumulation; hypertension and hypercholesterolemia. These risk

factors were also observed in clusters in one individual thereby increasing their risk for metabolic syndrome and cardiometabolic risks (Ekali et al., 2013).

#### 1.2.8.2 HIV/HAART associated lipodystrophy syndrome body fat distribution

HIV-lipodystrophy is a syndrome which is characterized by an increase in visceral adipose tissue as compared to subcutaneous tissue (Barbaro and Lacobellis, 2009). Lipodystrophy is a syndrome that is associated with a mixed pattern of lipoatrophy (fat loss) and lipohypertrophy (fat accumulation) (Libonati et al., 2012). Lipodystrophy is mostly associated with hyperlipidaemia and insulin resistance. Clinical manifestations of lipoatrophy indicate localized subcutaneous fat loss in the face, arms, legs, and buttocks (George, 2006). Lipohypertrophy individuals usually present a central visceral fat accumulation in the abdomen, breasts, and dorso-ventral region (buffalo hump) (Grinspoon and Carr, 2005).

Lipodystrophic patients usually presents predominantly with upper body fat distribution where increases in WC are usually observed in both males and females (Libonati et al., 2012). Sreekantamurthy et al. (2014) found a significantly increased W:H ratio (0.91) amongst patients receiving PIs as compared to ART naïve patients (0.87) indicating upper body fat distribution (central obesity) in these patients.

## 1.3 RESEARCH PROBLEM

In light of the preceding literature, it can be concluded that long-term treatment with HAART may have negative effects on the glucose and lipid metabolism of HIV positive patients, resulting in dysglycaemia and dyslipidaemia, thus increasing their risk for the development of chronic diseases of lifestyle. Extensive studies on the effect of duration on HAART in fasting blood glucose and triglycerides are yet to be explored and the study took the opportunity to investigate these effects. Therefore, it is important for the Department of Health to be aware of the current state of affairs. This was the first study of this nature that was conducted in the Capricorn District. Furthermore, the studies conducted in parts of South Africa with regard to metabolic diseases that develop in HIV positive patients taking HAART, did not emphasize on the effect of other hormones (e.g., leptin), which also reflect a significant role in disruptions of glucose and lipid metabolism.

## 1.4 AIM AND OBJECTIVES

### 1.4.1 Aim of the study

To investigate the effect of long-term (>12 months) use of different regimens of HAART on glucose and lipid metabolism in HIV positive patients at clinics in the Polokwane Local Municipality, Limpopo Province, South Africa.

### 1.4.2 Objectives

The objectives of the study were to:

i. Assess the effect of HAART on body composition (waist circumference [WC], hip circumference [HC], waist to hip ratio [W:H] and body mass index [BMI]);
ii. Assess the effect of HAART at different intervals (T0 and T6–T12) on fasting blood levels of total cholesterol (TC), HDL-C, LDL-C, triglycerides, leptin and glucose, insulin.
iii. Determine which regimens in HAART have an effect on, body composition BMI, WC, HC and W: H; total cholesterol, HDL-C, LDL-C, triglycerides, leptin and glucose, insulin.

## 1.5 OUTLINE OF THE CHAPTERS

The outline of the various chapters are as follows. Chapter one deals with the background and literature review relevant to the contents and research focus of this dissertation. Chapter two outlines the procedures that were followed including sampling method(s), the study design, scope, data collection and pilot study. Chapters three, four and five focusses on the results and their discussions. Chapter three deals specifically with demographics, anthropometry and HAART; Chapter four with the association between the lipid metabolism and HAART, and Chapter five with the impact of HAART on the carbohydrate metabolism. Chapter six summarises the major findings and highlights recommendations. Ma

## 1.6 SCOPE OF THE STUDY

This study investigates the effects of long term use of HAART on the lipid and glucose metabolism and body composition. It is a prospective cohort study with baseline data collection done on patients who have been on HAART for 12 months or more. The 12

month cut off as inclusion criteria was used to enrol patients who have been on HAART for longer periods of years. Furthermore, the follow up was done after 6–12 months to observe any changes in the selected variables measured at baseline, to further validate the findings associated with these drugs. Due to the small sample sizes, difficulties were incurred in retaining follow-ups at month 6 and month 12 specifically. Patients do not visit clinics every month. Often one visit every three months is allowed. Since the researcher was not working at the clinics full time, it was in some cases difficult to follow-up patients at specific intervals. Furthermore patients often changed dates of their visits without informing the researcher. The 6–12 months cut-off was decided upon to increase the statistical range (i.e few groups of patients were followed-up at month six, seven, eight, nine and 12), therefore these groups were combined into one big group. Fasting blood samples were used to determine glucose and lipid variables and anthropometric measurements were taken using the International standard method. This study is limited to 3 clinics in the Polokwane local municipality; however, those clinics are the main HIV clinics attached to the public hospitals and a tertiary hospital, this was done in order to have a pool of patients from different areas in the municipality.

**CHAPTER 2**

# METHODS AND MATERIALS

## 2.1 RESEARCH METHODLOGY

This prospective cohort study was conducted over a period of 18 months (September 2013–May 2015). The data was collected at 12 months or more (baseline) and follow-up after 6–12 from initial baseline (Appendix A).

### 2.1.1 Study area

This study included three HIV clinics: Phela-O-Phediše (Mankweng), Tirisano (Polokwane) and Thekganang (Seshego) in the Polokwane Local Municipality (PLM), Limpopo Province. Their selection was based on their close proximity to provincial hospitals in the above-mentioned regions.

### 2.1.2 Study population

In this study, HIV-positive patients on HAART receiving treatment at the designated clinics were considered eligible. The inclusion criteria comprised of HIV positive males and females aged 18–60 receiving HAART for 12 months or longer. The exclusion criteria were: patients who were on HAART for less than 12 months, the use of any drugs known to affect lipid and glucose metabolism, pregnant females at the time of study, use of glucocorticoids, and body weight loss in the past 6 months, history of AIDS defining illnesses. Furthermore patients with clinical anaemia or haemoglobin levels of less than 10 and 12 gm/dL in females and males (Das, 2010) respectively will be excluded because, Masaisa et al. (2011), found that anaemia is a known AIDS complication associated with a lower BMI.

### 2.1.3 Sample size and technique

A sample size of 144 $HIV^+$ patients receiving HAART were required for the study. This sample size was calculated based on the formula described by Kasiulevičius et al. (2006) and the assumption on the prevalence of dyslipidaemia, which is reported to

be significantly affected by duration of HAART. Confidence interval of 95% and sampling error of 5% was used for the equation.

$$n = \frac{Z^2 p(1-p)}{e^2}$$

Where n is the sample size, Z confidence interval (95%), p is the prevalence of dyslipidaemia among patients who have been on HAART, and e is the sampling error. Thirty subjects were randomly selected from the sample for the pilot study and a total of 144 $HIV^+$ patients were sought for the prospective cohort study. The sample was selected disproportional from clinics. Consecutive sampling was used to select the study participants.

### 2.1.4 Attrition rate

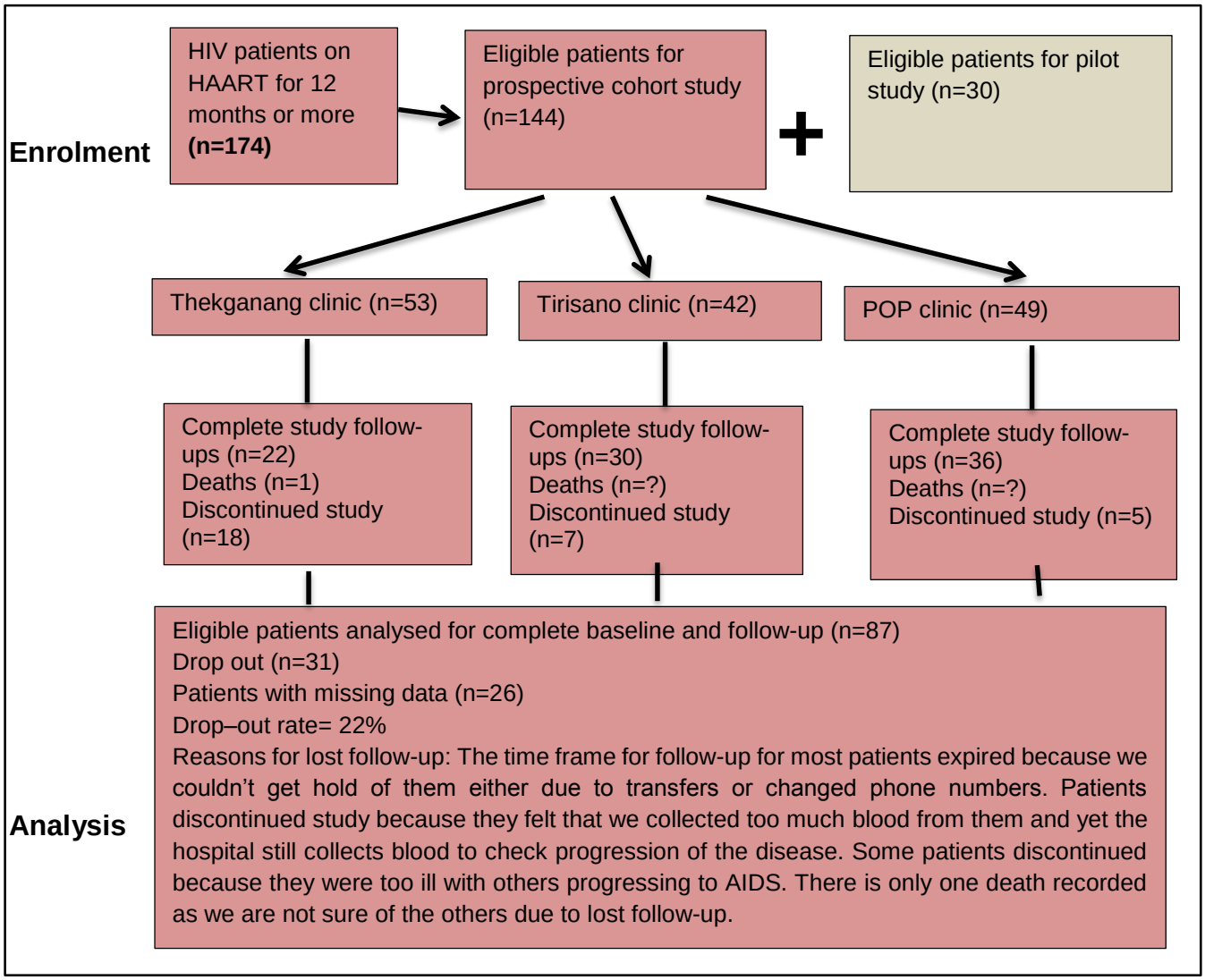



**Figure 2.1:** Distribution and attrition of participants at baseline and follow-up.

### 2.1.5 Data collection

Sampling took approximately 18 months and was conducted twice (T0 and T6–T12). A self-reported questionnaire was used to collect demographic and medical data (Appendix A). The medical history of the patient was retrieved from the patient files and added to the questionnaire. Patients were required to fast overnight for 10–12 hours.

#### 2.1.5.1 Medical history

The patient files were used to obtain the following information:

- History of Cardiovascular diseases: The information on history of cardiovascular diseases was collected from the patient files, this was done to validate that the patients participating in the study had no history of metabolic diseases which have shown to increase the risk of developing cardiovascular diseases
- Existence of other metabolic diseases such as hypertension, diabetes mellitus, dyslipidaemia, insulin resistance etc. This information was used to classify patients whether they were eligible to participate in the study. This information was used to validate the exclusion criteria.
- Use of other medications for diseases such as asthma, epilepsy, arthritis and psychiatric were noted. This information is crucial when interpreting findings. In order for the study to address the aim, it is important to also note any drug-drug interactions that might affect the outcome of the results.

### 2.1.6 Organizational procedure

A formal letter for permission to visit the clinics to make prior arrangements was submitted to the Department of Health and Social Development. Each clinic was visited according to the schedule that their patients follow, for treatment collection. A follow-up visit to the clinics was done to randomly identify subjects, get informed consent from the subjects (Appendix C) and to collect contact particulars. Subjects were briefed about the study verbally and written consent was obtained. A day before the clinic visit, subject were reminded via a phone call to fast for at least ten hours.

The clinics had five days scheduled in a week for patients to collect their treatment; therefore, subjects per clinic were divided into five days for sampling. During the months of September and October 2013 Tirisano and Thekganang clinic were visited on alternating days and 49 patients were sampled during these months. During March–July 2014, 57 patients were sampled at Thekganang clinic and 38 patients at Phela O Phedise clinic for the duration of the period. The follow-up for 6–12 months was done per clinic. On the day of performing the tests 5–15 subjects were sampled due to the limited number of qualified registered staff nurses that were available to assist with drawing blood. Subjects received a fruit and sugar free cold drink or fruit juice after completing the measurements.

### 2.1.7 Pilot study

A pre-test of the data collection method was conducted to test for viability and reliability of the study. The pre-test provided information on how much time would be needed to administer the questionnaire and conduct the test. A population group of 30 (17%) subjects from the sample were randomly selected to conduct the pilot study. The sample for the pilot study was selected at random from the clinics (i.e., ten patients on HAART for 12 months or more were selected from each clinic). The selected subjects were not included in the prospective cohort study. The questionnaire was given to the patients in each clinic. All anthropometric measurements and biological markers were piloted. The results from the pilot study are not part of the results attained in the prospective cohort study.

### 2.1.8 Anthropometric measurements

All the anthropometric measurements were undertaken by the researcher and trained field workers. The researcher is a level one anthropometrist and all methods were standardized by the researcher before collecting data. Anthropometric measurements including, WC, hip circumference, weight and height were taken for the determination of body fat distribution and BMI. The criteria by Seedat and Rayner (2012) was used to determine abdominal obesity. Anthropometric measurements were taken using the International Standards for Anthropometric Assessment (Marfell-Jones et al., 2006). Weight was measured using a calibrated electronic scale (DH–2008B), with subjects

wearing minimal clothing. Height was taken using a Stadiometer (Leicester portable Stadiometer), where the head of the subject was placed in the Frankfort plane with the body standing upright. Waist and hip circumference was measured using a Lufkin Executive Thinline steel anthropometric measuring tape, with subjects wearing minimal clothing on the abdominal and thigh regions.

### 2.1.9 Blood collection

After an overnight fast, a registered nurse collected venous blood between seven and ten during clinic visits. Whole blood was collected in BD Vacutainer® tubes containing no anticoagulant. The samples were allowed to clot. Samples were stored at room temperature until complete clotting and then they were centrifuged at 3000 rpm for ten minutes, to allow separation of serum from whole blood. Within two hours after centrifugation, 500 µL of cell-free sample was transferred to a storage tube. The tube was tightly closed immediately. Samples were then stored in a bio-freezer at -80°C for further analysis.

Plasma glucose was measured from venous blood collected in BD Vacutainer® tubes containing potassium oxalate/sodium fluoride. Serum and plasma samples were centrifuged for 20 minutes at 3000 rpm; where after serum samples were stored at –80ºC until analysis. Glucose was measured within four hours, because it was previously indicated that freezing plasma samples reduced the reliability of the glucose levels (Clark et al., 1995).

### 2.1.10 Biochemical analysis

All variables were analysed using automated machines. The researcher prepared plasma and serum samples from whole blood. Preparation of samples involved centrifuging whole blood at 3000 rpm for ten minutes to separate plasma and serum in the specified vacutainer tubes. Machines were calibrated by researcher before commencement of analysis.

2.1.10.1 Insulin

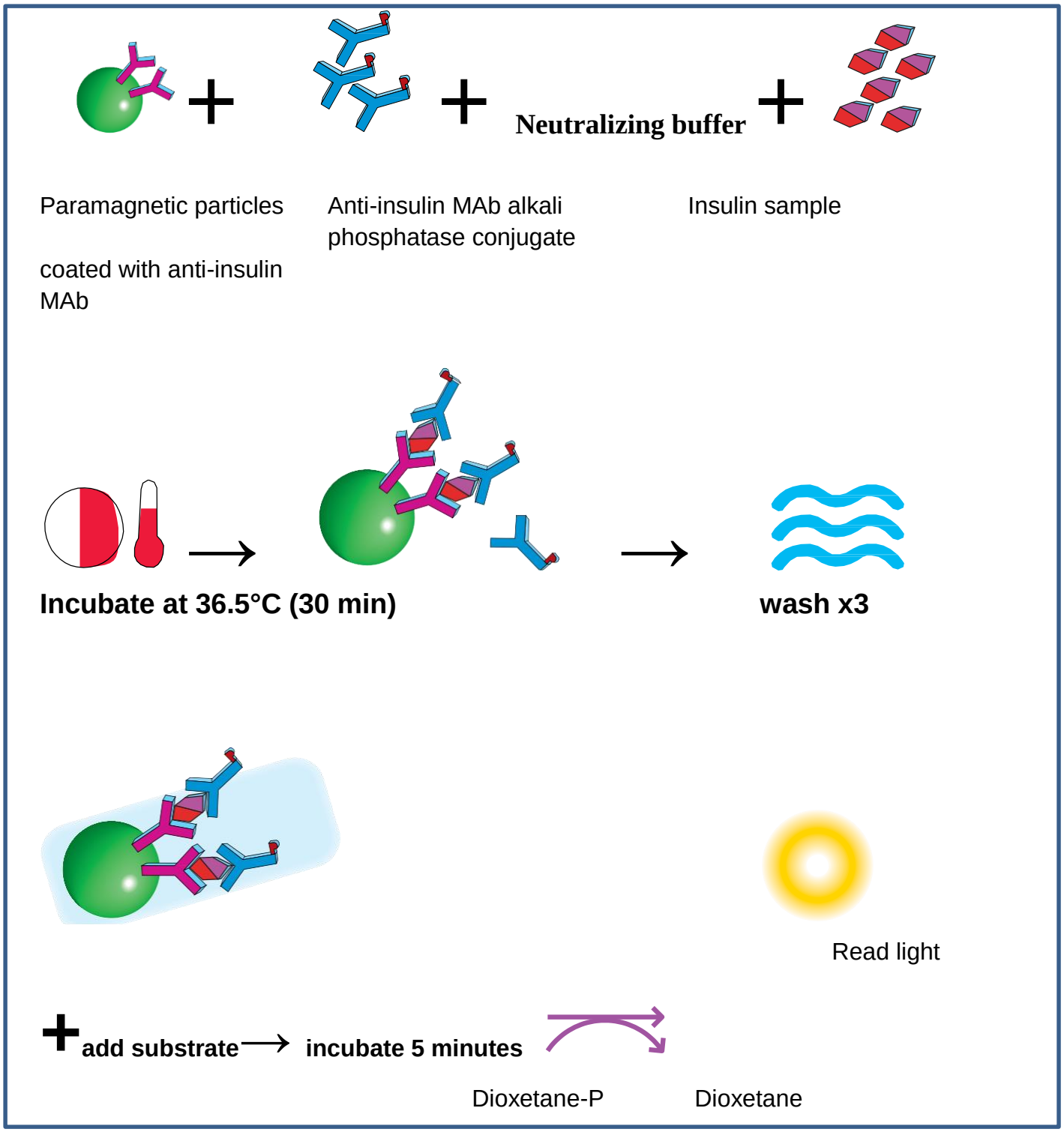



**Figure 2.2**: One-step sandwich technique (Beckman Coulter, Inc. 2000).

Insulin levels were determined using the Access Ultrasensitive Insulin assay (Beckman Coulter, Inc.). This assay was done using an automated analyser. The Access Ultrasensitive Insulin assay is a simultaneous one-step immune-enzymatic

("sandwich") assay. The researcher added samples to a reaction vessel along with mouse monoclonal anti-insulin alkaline phosphatase conjugate and paramagnetic particles coated with mouse monoclonal anti-insulin antibody 7, 8, 9. The serum insulin was bound to the antibody on the solid phase, while the conjugate reacted with a different antigenic site on the insulin molecule. After incubation in a reaction vessel, materials bound to the solid phase were held in a magnetic field while unbound materials were washed away. Then, the chemiluminescent substrate Lumi Phos* 530 was added to the vessel and light generated by the reaction was measured with a luminometer. The light production was directly proportional to the concentration of insulin in the sample. The concentration of insulin in the sample was determined from a stored, multi-point calibration curve. To calculate insulin resistance (IR) the HOMA-IR formula was used:

IR = fasting plasma glucose x fasting plasma insulin (miu/L)/ 22.5

#### 2.1.10.2 Leptin

Leptin levels were determined using a Radioimmunoassay (RIA) Millipore™ HL-81K kit. The samples were analysed using a wizard™2 gamma counter. The researcher followed the three day procedure outlined herein. Assay set-up (day one): for the preparation of the assay, 300 µL of Assay Buffer were pipetted to the Non-Specific Binding (NSB) tubes (3–4), 200 µL to reference (Bo) tubes (5–6), and 100 µL to tubes 7 through the end of the assay. Hundred microliter of standards and quality controls and 100 µL of each sample were pipetted in duplicates. Hundred microliter of 125 I-Human Leptin was pipetted to all tubes and 100 µL of Human Leptin antibody pipetted to all tubes except total count tubes (1–2) and NSB tubes (3–4). Furthermore, the samples were vortexed and incubated overnight (24 hours) at 4°C. Day two: 100 µL of precipitating reagent (4°C) was added to all tubes (except total count tubes. The tubes were then vortexed and incubated for 20 minutes at 4°C. Centrifugation was done for all tubes [except total count tubes (1–2)] for 20 minutes at 2,000 xg. The supernatant of all tubes except total count tubes (1–2) were decanted immediately, and tubes were drained for at least 15–60 seconds, and excess liquid from lip of tubes was blotted. Furthermore, all tubes were counted in gamma counter for one minute.

Human Leptin in unknown samples was calculated using automated data reduction procedures in ng/mL

**Conversion of rpm to xg**:

xg = (1.12 x 10-5) (r) (rpm)2.
r = radial distance in cm (from axis of rotation to the bottom of the tube)
rpm = rotational velocity of the rotor

2.1.10.3 Total cholesterol

Total serum cholesterol levels were measured using the cholesterol liquicolor system reagent for Humastar 600 test kit (CHOD-PAP Method). The enzyme reagent consists of phosphate buffer (pH 6.5) (100 mmol/L), 4-Aminophenazone (0.3 mmol/L), phenol (5 mmol/L), peroxidase (> 5 KU/l), cholesterolesterase (> 150 U/l), cholesteroloxidase (> 100 U/l) and sodium azide (0.05 %). Total cholesterol and HDL-C are stable for four days at 2–8°C. Extended stabilities at –20°C are three months for total and 7–14 days for HDL-C. Table 2.1 indicates volumes that were pipetted into cuvettes (Table 2.1).

**Table 2.1:** Volumes pipetted into cuvettes for the determination of total cholesterol.

| | Reagent blank | Sample or Standard |
|---|---|---|
| Sample/ standard | _ | 10 µl |
| Enzyme reagent | 1000 µl | 1000 µl |

The cuvettes were incubated at 37ºC for five minutes. The absorbance of the sample and standard against the reagent blank were measured at 500 nm within 60 minutes. Serum total cholesterol values were expressed in mmol/L.

### 2.1.10.4 High density lipoprotein cholesterol

Serum HDL-C levels (mmol/L) were measured using the HDL-C liquicolor system reagent for Humastar 600 test kit (Enzymatic colorimetric test). Table 2.2 indicates the volumes that were pipetted into cuvettes.

**Table 2.2:** Procedure for determination of concentration for high density lipoprotein-cholesterol.

| Pipetted into cuvettes | Reagent blank | Sample |
|---|---|---|
| Water | 10 µl | _ |
| Calibrator/sample | _ | 10 µl |
| Enzymes | 750 µl | 750 µl |
| Mix gently and incubate at 37ºC for 5 minutes | | |
| Substrate | 250 µl | 250 µl |
| Mix gently and incubate at 37ºC and read the absorbance (570–610 nm) of samples against reagent blank after 5 minutes. | | |

### 2.1.10.5 Low density lipoprotein cholesterol

Low density lipoprotein cholesterol was measured indirectly by using the directly measured TC and HDL-C in an estimated Friedewald equation.

Friedewald equation: Friedewald-LDL-C (mmol/L) **=TC – HDL-C – TG/2.2** (Vujovic et al., 2010).

### 2.1.10.6 Triglycerides

Serum triglyceride levels were measured using the triglyceride liquicolor system reagent for Humastar 600 test kit (GPO-PAP Method). The following volumes were pipetted into cuvettes (Table 2.4).

The cuvettes were then incubated at 37ºC for five minutes. The absorbance of the sample and standard versus the reagent blank was read at 500 nm within 60 minutes. Serum total cholesterol values were expressed in mmol/L.

**Table 2.3:** Procedure for the determination of concentration for triglyceride levels.

| | Reagent blank | Sample or standard |
|---|---|---|
| Reagent | _ | 10 µl |
| Sample/ standard | 1000 µl | 1000 µl |

2.1.10.7 Glucose

Plasma glucose levels were measured using the glucose liquicolor system reagent for Humastar 600 test kit (GOD-PAP Method). The following volumes were pipetted into cuvettes (Table 2.5), for plasma sample preparation.

**Table 2.4:** Procedure for plasma sample preparation.

| Pipette into test tubes | Semi-micro | |
|---|---|---|
| Sample | 50 µl | _ |
| STD | _ | 50 µl |
| Deproteinizing solution | 500 µl | _ |
| Distilled water | _ | 500 µl |

The samples were centrifuged at high speed for ten minutes. The volumes in Table 2.6 were pipetted in cuvettes for the determination of plasma glucose concentrations.

**Table 2.5:** Procedure for the determination of plasma glucose concentrations.

| Cuvettes | Reagent Blank | Sample | Standard (STD) |
|---|---|---|---|
| Supernatant or Diluted standard | _ | 50 µl | 50 µl |
| Distilled water | 50 µl | _ | _ |
| Enzyme reagent | 1000 µl | 1000 µl | 1000 µl |
| The samples were incubated for 5 minutes at 37ºC. | | | |

The absorbance of the sample and standard versus the reagent blank were measured within 60 minutes. Absorbance was measured at 500 nm, and concentration of glucose were expressed in mmol/L.

2.1.10.8 South African cut off values

**Table 2.6:** The current South African cut offs for the assessment of risk of non–communicable diseases.

| Body mass index (BMI) | Cut-off value | | Reference |
|---|---|---|---|
| | | | Seedat and Rayner., 2012 |
| Underweight | <18.5 kg/m$^2$ | | |
| Normal weight | 18.5-24.9 kg/m$^2$ | | |
| Pre-obese (overweight) | 25.0-29.9 kg/m$^2$ | | |
| Obese | ≥30.0 kg/m$^2$ | | |
| Abdominal obesity WC | Ideal | Substantial risk | Seedat and Rayner., 2012 |
| Females WC | <80 cm | >88 cm | |
| Males WC | <94 cm | >102 cm | |
| Waist to hip ratio | | | Peer et al., 2015 |
| Females | <0.85 | >0.85 | |
| Males | <1.0 | >1.0 | |
| Fasting lipids | Recommended levels in SA | Metabolic abnormality | Seedat and Rayner., 2012 |
| TC | Normal | Hypercholesterolemia | |
| | <5.0 mmol/L | ≥5.0 mmol/L | |
| Triglycerides | Normal | Hypertriglyceridaemia | |
| | <1.7 mmol/L | ≥1.7 mmol/L | |
| HDL-C | Normal: Males: ≥1.04 mmol/L Females:≥1.3 mmol/L | | |
| | | | |
| LDL-C Friedewald | Ideal | High risk for CVD | Peer et al., 2015; Seedat and Rayner., 2012 |
| | <3.0 | >3.0 | |
| Fasting glucose | Normal | Impaired fasting glucose | Diabetes Mellitus |
| | ≤5.6 mmol/L | 5.6- 6.9 mmol/L | ≥7.0 mmol/L |
| HOMA IR | Normal | Insulin resistance | |
| | <2.5 miu/L | ≥ 2.5 miu/L | Awotedu et al., 2010 |
| Leptin | Normal | Hyperleptinaemia | hypoleptinaemia |
| | <15 ng/ml (Askari et al., 2010) | >15 ng/ml (Askari et al., 2010) | ≤4 ng/ml (Depaoli et al., 2010) |

## 2.2 STATISTICAL ANALYSIS

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) program version 23. The distribution of data was tested for normality using histograms and line graphs. Data distributed normally was presented as mean (standard error mean) and data without a normal distribution was presented as median inter quartile range (IQR). The researcher captured the data on a spread sheet file, cleaned the data and consulted three statisticians for further analysis of the data. Descriptive statistics were presented as means and standard error mean (SEM) for continuous variables and frequencies and percentages for categorical variables. Comparison of quantitative variables was performed using chi-square test for categorical variables, while the paired Student's t-test was used for continuous data. A p-value of less than 0.05 was considered statistically significant.

## 2.3 RELIABILITY, VALIDITY AND OBJECTIVITY

Validity and reliability was maintained by ensuring that all the field workers were trained by skilled personnel (level one anthropometrist). Repeat measurements were taken on all anthropometric measurements, and if the two measurements had a vast difference of >5%, a third measurement was taken. The questionnaire was validated by conducting a pilot study to ensure the feasibility and reliability of the study.

## 2.4 BIAS

The medical history of the patients was extracted from the patient files, which are used for diagnostic purposes and would not necessarily provide accurate information on the history of the patient. Therefore to validate the records, reliance was mostly on chronic medication that the patient was taking. All the anthropometric measurements were undertaken by the researcher and trained field workers. The researcher is a level one anthropometrist and all methods were standardized by the researcher before collecting data. All the field workers attended training on how to take anthropometric measurements, as these require high precision. Two trained field workers were required to assist taking measurements in an individual to ensure accuracy. The questionnaire was designed after an extensive review of similar studies existing in the literature and an expert biostatistian was consulted to review and confirm suitability of

questionnaire for use. The researcher trained all field workers on how to use the questionnaire.

## 2.5 ETHICAL CONSIDERATIONS

Ethics approval (Appendix D) was granted by the University of Limpopo Ethics Committee (MREC), for approval (MEDUNSA Campus). Permission to perform the study at the selected clinics was granted by the senior manager of primary health care at the Department of Health and Social Development, Capricorn District. Informed consent was obtained after all aspects, as stipulated in “MREC application form” were discussed.

# CHAPTER 3

# RESULTS AND DISCUSSION: DEMOGRAPHICS, ANTHROPOMETRY AND HAART

## 3.1 INTRODUCTION

This chapter presents the results of the study regarding the demographic characteristics of the participants, their anthropometric profiles, as well as the different HAART regimens followed by them. Throughout the chapter baseline characteristics are compared with follow-up at 6–12 months. Findings that were found to be significant between the two intervals were further analysed.

### 3.1.1 Demographic characteristics of the study participants

**Table 3.1**: Age distribution of HAART patients according to gender.

| Age (years) | Total population | Males | Females |
|---|---|---|---|
| 18–30 | 13% | 8% | 14.8% |
| 31–40 | 36% | 23% | 41% |
| 41–50 | 37% | 54% | 29.5% |
| 51–60 | 14% | 15% | 13% |

The study comprised of males and females aged 18–60 years, and age was distributed normally. The majority of the participants were middle aged adults 31–50 years old. The mean age for the study group was 41.5 years±8.9 SD. Majority of males were aged 41–50 years which indicates an increased risk for the development of CDL as in the general population CDL start to prevail around the ages of 40 years (Steyn, 2006). Females were dominant in the ages 31-40 years. The percentage ages 18–30 years were comparable to the ages 51–60 years, this suggests that HIV is becoming more prevalent in the younger adults as it is in the older adults and that the burden of disease in South Africa will increase in the near future.

**Table 3.2:** Medical history and gender distribution among HAART patients.

| Gender | Percentage |
|---|---|
| Males | 70% |
| Females | 30% |
| Medical history | Percentage |
| Hypertension | 4.6% |
| Arthritis | 1.2% |
| Psychiatric | 1.2% |
| Not on an medication except HAART | 81.6% |
| No record | 9.2% |

Among the 144 participants that gave consent to participate in the study, 87 [61 (70%) females and 26 (30%) males] had all relevant biochemical and anthropometric measurements done at baseline and follow-up. Gender based differences were observed in anthropometric measurements. The medical history retrieved from the clinic files, showed that 82% of the patients were not on any chronic medication. However, the remaining 7% of the patients were on treatment for diseases such as hypertension, arthritis and psychiatric conditions. Hypertension is one of the chronic diseases that can co-exist with other CDL, therefore patients that are on hypertension treatment are at increased risk for developing other chronic diseases of lifestyle such as diabetes mellitus and obesity (Steyn, 2006). The risk is further exacerbated by exposure to drugs such as PIs. Patients that are on HAART have increased risk of developing hypertension with the prevalence being more than the general population (Julius, 2010). Therefore the incidence of hypertension in the HIV population poses a greater risk of developing cardiovascular diseases.

**Table 3.3:** Distribution of different drug combinations among HAART patients at baseline and follow-up according to gender.

| | | Regimen 1A | Regimen 1B | Regimen 1C | Regimen 2A |
|---|---|---|---|---|---|
| Total population | Baseline | 59.8% | 12.6% | 9.2% | 18.4% |
| | Follow-up | 47.1% | 28.7% | 4.6% | 19.5% |
| | | | | | |
| Males | Baseline | 69.2% | 7.7% | 3.8% | 19.2% |
| | Follow-up | 46.2% | 34.6% | 0% | 19.2% |
| | | | | | |
| Females | Baseline | 55.7% | 14.8% | 11.5% | 18% |
| | Follow-up | 47.5% | 26.2% | 6.6% | 19.7% |

The first line regime consist of three combinations including [1A]; [1B] and [1C]. The triple FDC drug is combination [1B]. At follow-up an increase in the total population was observed with 28.7% taking this drug combination including participants that were switched from old regimen combinations. Stavudine is one of the drugs that was phased out in most clinical settings due to its toxicity; however, a few participants were still on d4T-based regime at the time of data collection, with 9.2% [1C] at baseline and a decreased 4.6% taking the drug combination at follow-up. The second line regime consist of four combinations [2A]. There were no differences in patients that were on the second line regime [2A] at baseline and follow-up; however, of the four combinations for second line regime a few individuals were taking ATV as a PI. Participants taking drug combinations in the second line regime were fewer compared to those in the first line regime.

**Table 3.4:** Distribution of different drug combinations at different HAART intervals at baseline and follow-up.

| Duration of HAART in months | Interval | Regimen 1A | Regimen 1B | Regimen 1C | Regimen 2A |
|---|---|---|---|---|---|
| 12-24 months | Baseline | 68% | 25% | 7% | 0% |
| | Follow-up | 22% | 78% | 0% | 0% |
| | | | | | |
| 25-48 months | Baseline | 59% | 4% | 7% | 30% |
| | Follow-up | 68% | 18% | 3% | 12% |
| | | | | | |
| 49-72 months | Baseline | 57% | 7% | 0% | 36% |
| | Follow-up | 40% | 25% | 5% | 30% |
| | | | | | |
| 73-96 months | Baseline | 46% | 8% | 23% | 23% |
| | Follow-up | 25% | 19% | 13% | 44% |
| | | | | | |
| 97+ months | Baseline | 60% | 20% | 20% | 0% |
| | Follow-up | 50% | 50% | 0% | 0% |

Participants were recruited based on the duration of HAART, those who were on HAART for 12 months or more were eligible to participate in the study. Table 3.4 indicate the number of HAART months and the different regime combinations that the participants were taking at baseline and follow-up. The distribution and duration of

drugs in the different HAART intervals becomes a very important aspect when assessing the effects of different drug combinations on the variables in question. Toxicities from being on the same drug regime for long periods of time may also contribute to metabolic derailment resulting in metabolic diseases.

It is clear from table 3.4 that as these patients progress with taking the drugs within the different years, they start to portray different profiles that may increase their risk for developing CDLs. There were no participants taking Lpv/r based regimens (2A) in the first 12–24 months; however, in the corresponding months between 25 months and 96 months, some patients were switched to the second line regime which is comprised of a PI. Furthermore, findings portray a trend that shows that patients on HAART tend to experience virologic resistance as early as 25–48 months as it is observed in table 3.4, at baseline and follow-up that patients are switched to PIs at the very early stage of the disease, which poses a great risk for the development of metabolic diseases. Moreover, at baseline the percentage of patients taking PIs was highest between months 49–72. However, at follow-up it was highest during months 73–96 thus increasing the risk of cardio-metabolic diseases in these patients. It is evidence of a shift in the trend as shown at follow-up, that the percentage of patients on combination [1A] and [1B] were more than patients on [2A] compared to baseline at months 97++. These findings supports further investigations to validate the effects of the new regimen (FDC) combination that was recently introduced.

**Table 3.5:** Changes in the body mass index over time.

| Body fat distribution | Baseline Mean (SEM) | Follow-Up Mean (SEM) | p-values |
|---|---|---|---|
| BMI (kg/m$^2$) | 25.4 (0.664) | 25.6 (0.731) | 0.275 |
| Duration of HAART (months) | 45.8 (2.93) | 55.6 (2.84) | <0.001 |

The changes in the BMI of the patients are summarised in Table 3.5. The mean BMI of the participants did not change at follow-up.

**Table 3.6:** Gender based changes in body fatness at baseline and follow-up.

| | Females (n=54) Mean (SEM) | | | Males (n=26) Mean (SEM) | | |
|---|---|---|---|---|---|---|
| | Baseline | Follow-Up | p-values | Baseline | Follow-Up | p-values |
| Waist (cm) | 82.2 (1.573) | 80.8 (1.531) | 0.04 | 84.5 (2.606) | 83.5 (2.407) | 0.33 |
| Hip (cm) | 101.4 (1.675) | 101.3 (1.686) | 0.91 | 95.3 (2.181) | 96.3 (2.069) | 0.34 |
| Waist-hip | 0.81 (0.012) | 0.79 (0.010) | 0.15 | 0.88 (0.017) | 0.87 (0.011) | 0.23 |

Table 3.6 summarises the changes in the waist, hip circumferences and waist to hip ratios for males and females. Among females (n=54), the waist circumference level decreased significantly (p=0.04). The hip circumference and waist to hip ratio for females remained unchanged. Similarly there was not a statistical difference between baseline and follow-up in the waist circumference, hip circumference and waist to hip ratio for males (n=26). Malangu (2014), reported that the mean WC 83.8±8.6 was higher in males compared to females 75.5±6.6. These findings are consistent with the present study findings, as the findings indicate that the mean WC was higher in males than in females.

**Table 3.7**: Gender based changes in body fatness in two lines of regimes.

| | Two NRTIs + one NNRTIs= first line regimen | | | One PI + two NRTIs= second line regimen | | |
|---|---|---|---|---|---|---|
| Body fat distribution | Baseline Mean (SEM) | Follow-Up Mean (SEM) | p-values | Baseline Mean (SEM) | Follow-Up Mean (SEM) | p-values |
| BMI ($kg/m^2$) | | | | | | |
| Males | 24.3 (1.180) | 24.3 (1.242) | 0.77 | 25.3 (2.042) | 24.6 (1.729) | 0.44 |
| Females | 25.6 (0.835) | 26.0 (0.938) | 0.16 | 26.4 (2.900) | 26.5 (3.252) | 0.83 |
| | | | | | | |
| WC (cm) | | | | | | |
| Males | 84.1 (3.074) | 86.1 (2.844) | 0.51 | 83.4 (4.627) | 84.2 (4.274) | 0.45 |
| Females | 83.1 (1.734) | 81.6 (1.701) | 0.04 | 77.8 (3.613) | 78.6 (3.580) | 0.45 |
| | | | | | | |
| HC (cm) | | | | | | |
| Males | 95.9 (2.523) | 96.0 (2.361) | 0.95 | 92.7 (4.346) | 97.7 (4.630) | 0.21 |
| Females | 101.6 (1.774) | 101.6 (1.734) | 0.97 | 102.9 (4.650) | 101.5 (5.025) | 0.09 |
| | | | | | | |
| W:H ratio | | | | | | |
| Males | 0.87 (0.016) | 0.87 (0.012) | 0.39 | 0.91 (0.061) | 0.86 (0.026) | 0.06 |
| Females | 0.82 (0.014) | 0.80 (0.012) | 0.06 | 0.75 (0.014) | 0.77 (0.016) | 0.09 |

Table 3.3 summarises the changes in body composition, during the study period, for first and second line regimes in males and females. As shown, the mean waist circumference significantly decreased in females that were on the first line regime. There were no statistical significant changes between baseline and follow-up in the two lines of regime among males and females for hip circumference and W:H ratio.

The mean BMI levels were higher at baseline and follow-up in the second line regime in females. Similarly Julius (2010) found that the mean BMI in females (25.7±5.6kg/$m^{2)}$ was higher than in males 23.1±3.2kg/$m^2$.

A recent study which focussed on patients at pre HAART and post HAART (> 3 years), found that part of the increase in BMI was attributed to the fact that by the time HIV patients initiate HAART, they are already overweight (Nell et al., 2015). The other reason is due to the use of drugs such as PIs which were found to increase BMI compared to the other treatment regimes. Altered anthropometric parameters were reported to be associated with long term exposure to PIs resulting in increased WC and BMI stemming from metabolic derailment of the glucose and lipid metabolism (Reyskens and Essop, 2014). Consistent with the present study findings, Ali (2014) found a decrease in all body composition parameters (i.e BMI, total body fat, visceral fat and subcutaneous fat) in patients taking first line regime, further validating the deleterious effects of PIs by increasing body fatness

These findings suggest that a combination of factors including HIV itself, exposure to HAART and duration on HAART may contribute to morphological alterations. Similarly a study investigating the impact of the duration of HAART on body composition and metabolism reported that an increased number of years on HAART is associated with a significantly higher prevalence of overweight and obesity. This included abdominal fat accumulation, as well as hypertension and hypercholesterolemia. These risk factors increases the possibility for metabolic syndrome and cardio metabolic risks (Ekali et al., 2013).

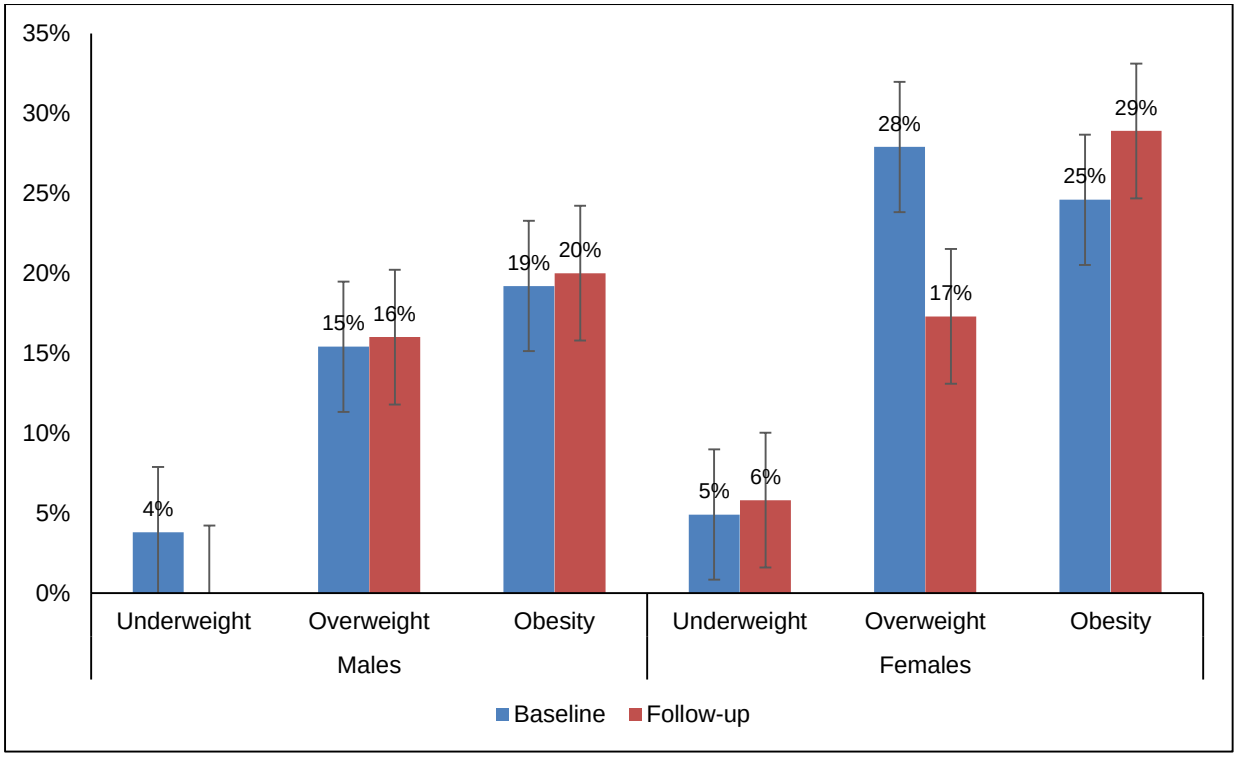
There was a significant decrease in WC in females at follow-up. However, the mean WC was higher in males than in females. These gender based disparities among patients taking HAART, were also reported by Nell et al. (2015). As expected, the mean HC was higher in females compared to males; however, development of lipodystrophy may lead to body fat distribution changes and is apparent especially in females. In normal circumstances females tend to have a pear-shaped body rather than the distinct apple-shaped form observed in males due to central adiposity.

In the present study there was not a significant increase in WC in males however it was observed to be higher at follow-up in both regimes.

**Table 3.8:** Changes in HAART duration over time in the two lines of regimes.

| | Two NRTIs + one NNRTIs= first line regimen | | | One PI + two NRTIs= second line regimen | | |
|---|---|---|---|---|---|---|
| Variables | Baseline Mean (SEM) | Follow-Up Mean (SEM) | p-values | Baseline Mean (SEM) | Follow-Up Mean (SEM) | p-values |
| Duration of HAART (months) | 43.2 (3.396) | 53.3 (3.312) | <0.001 | 56.2 (4.792) | 64.7 (4.471) | <0.001 |

The mean duration of HAART significantly increased from baseline to follow-up (Table 3.1); however, this finding was expected. In Table 3.8, the duration of HAART in patients on second line regime were significantly higher than those on first line regimen as it is also expected, because in the ARV roll out programme, NRTIs and NNRTIs are in the first line of defence in suppressing viral replication through inhibiting viral reverse transcriptase, from transcribing the single stranded RNA into a double stranded RNA. This is achieved by introducing NRTIs and NNRTIS into the system. When the virus becomes resistant due to mutations formed by multi drug resistant traits, PIs are introduced as the second line of defence where they inhibit cleavage of proteins and mature viral particles into the assembled Gag and Gag-pol multi protein complexes (Reyskens and Essop, 2014). The duration of HAART significantly increased BMI and WC. Although the data did not show specifically which combination is implicated, however it can be suggested that patients that were on second line regime were affected by longer HAART durations due to the significant decrease observed in patients taking first line regime. It seems that the longer durations on HAART in patients taking PI combinations increase the BMI and WC especially in males which further increases their risk for developing metabolic diseases.

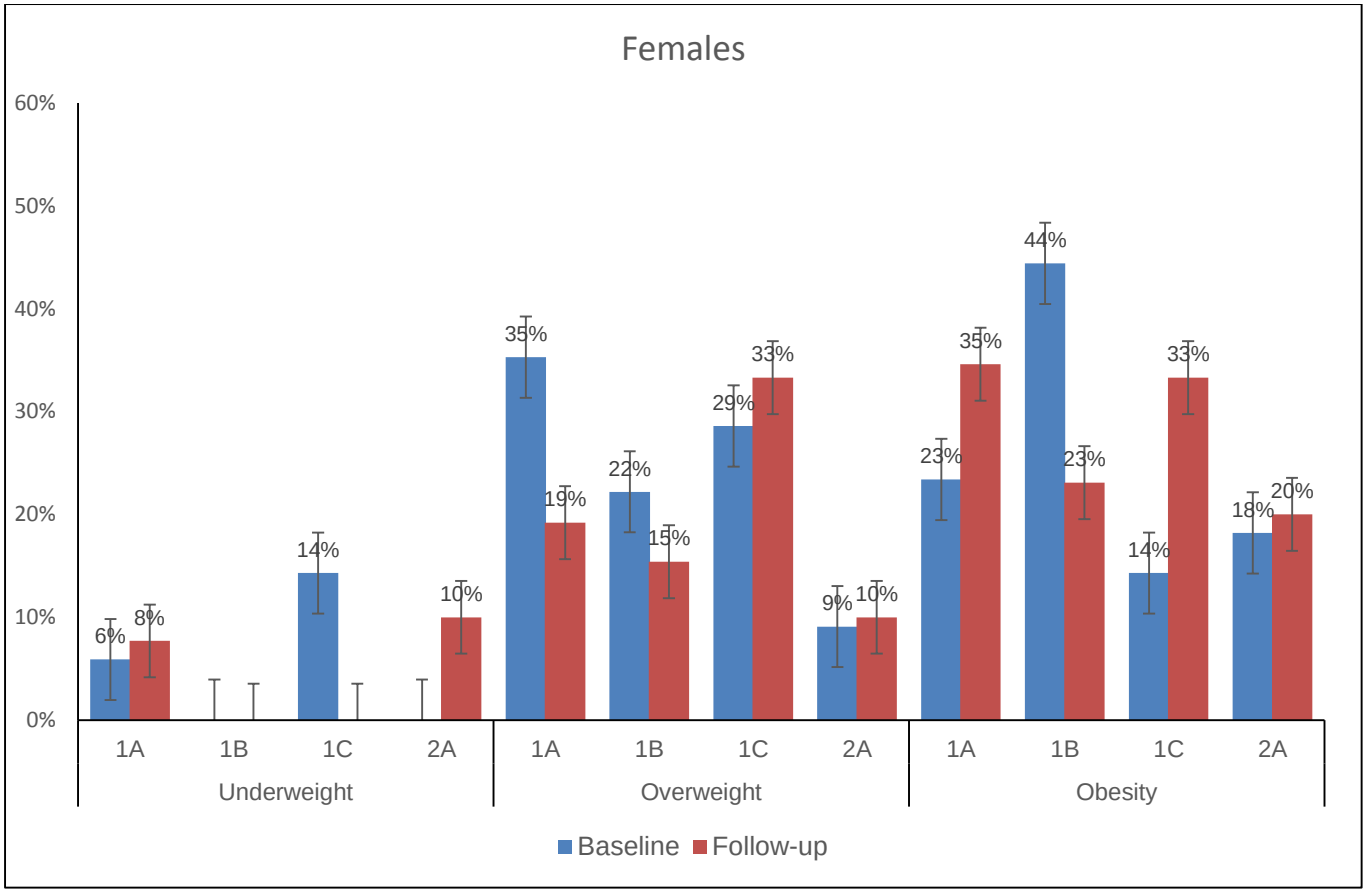



Significance in HAART regimens between males and females at baseline: p=0.39

Significance in HAART regimens between males and females at follow-up: p=0.44

**Figure 3.1**: The prevalence of body fatness in males and females.

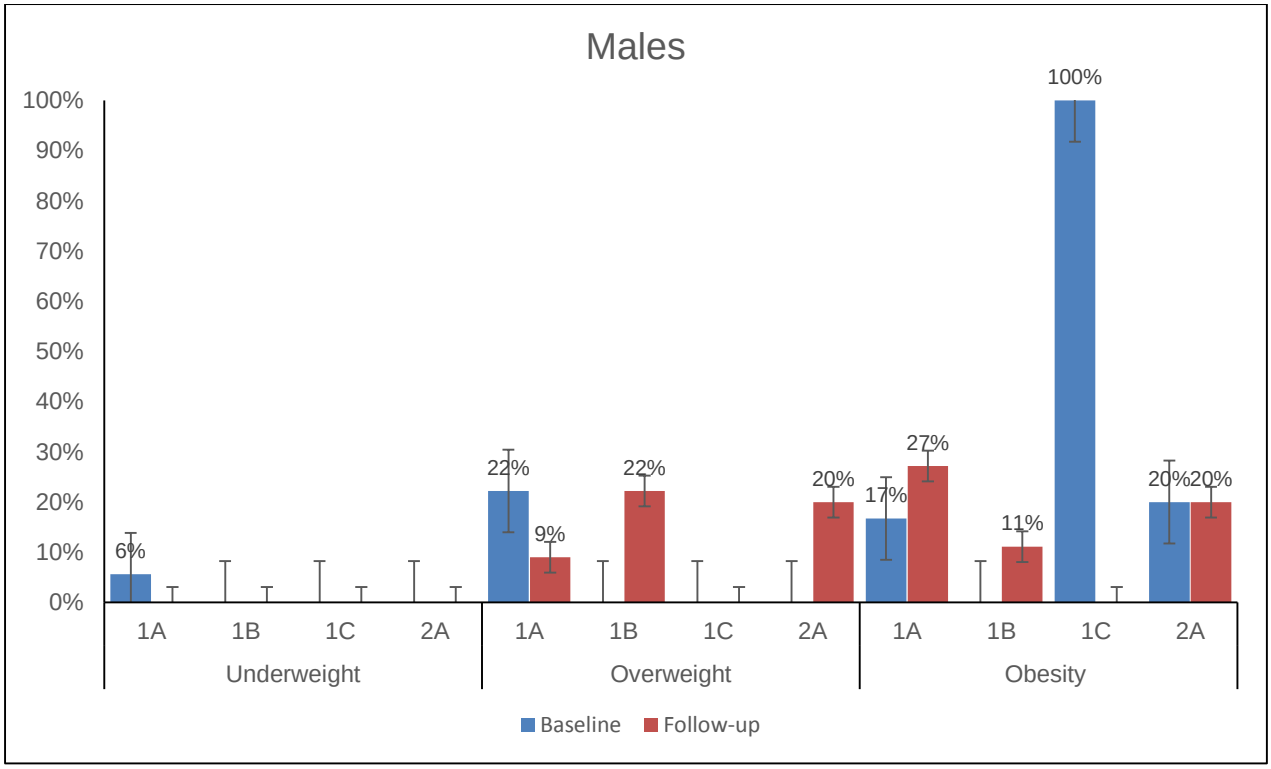
There were no statistical differences between baseline and follow-up in the prevalence of underweight, overweight and obesity associated with the use of HAART regimens. Overall the prevalence of overweight (BMI≥25.0-29.9 kg/m$^2$) and obesity (BMI≥30.0 kg/m$^2$) was higher in females than males. The findings are consistent with Julius (2010), where the study reported that the prevalence of overweight 30.8% (females) and 20.9% (males); obesity 19.8% (females) and 5.9% (males) was higher in females compared to males. It is important to note that the prevalence of underweight was also higher in females compared to males. Consistent with the study findings, Julius (2010), reported that the prevalence of underweight was higher in females (5.5%) than males (2.9%). In the general and HIV population, the prevalence of underweight is usually higher in males compared to females however in this study it was not the case. This may be due to the fact that majority of the sample constituted females (n=61) than males (n=26), therefore the prevalence is higher in females based on their sample size. This finding may further suggest wasting and other AIDS related complications in these individuals suggesting virologic failure.




Significance between regimens in females at baseline: p=0.41

Significance between regimens in females at follow-up: p=0.88

**Figure 3.2 (a)**: the prevalence of body fatness in females according to regimens.



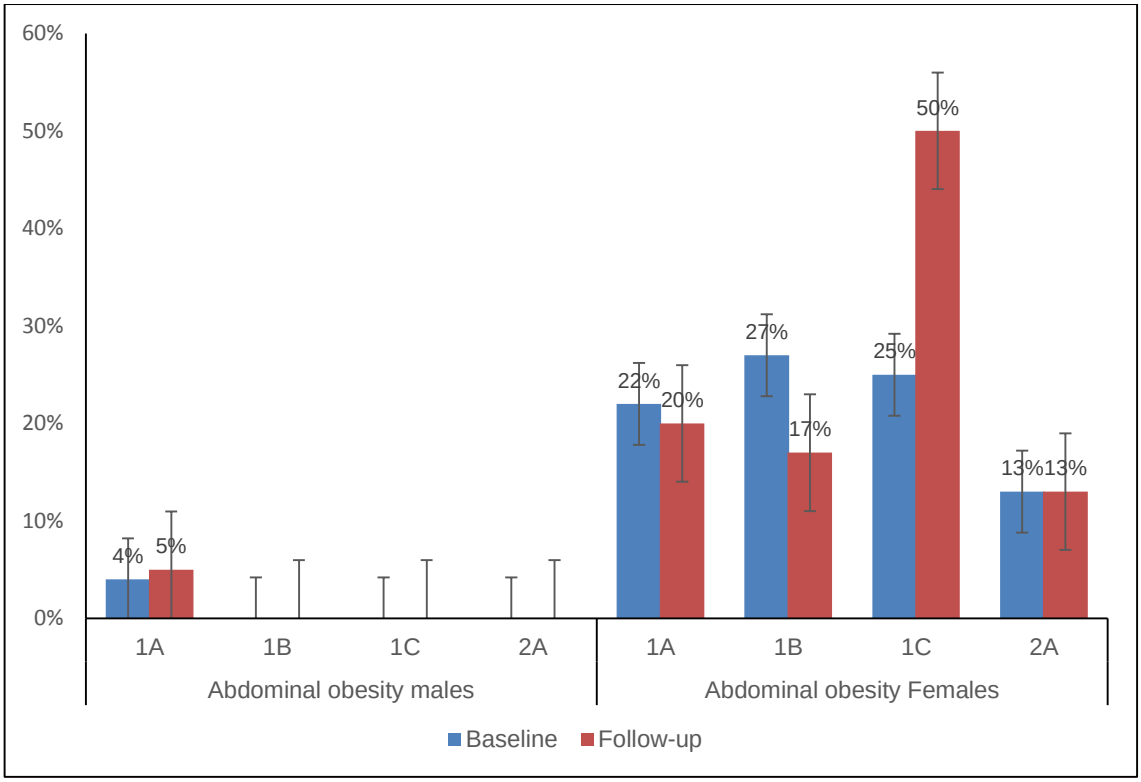

Significance between regimens in males at baseline: p=0.58

Significance between regimens in males at follow-up: p=0.87

**Figure 3.2 (b):** the prevalence of body fatness in males according to regimens.

Figure 3.2a and b shows the effects of different combinations of HAART regimen on body fatness at baseline and follow-up in females and males respectively. The prevalence of underweight was predominant in females as it persisted even at follow-up, however in males it diminished at follow-up suggesting regimen switch improved wasting in males. Consistent with a large body of evidence, obesity was prevalent in all patients that were on d4T-based regimens.

Consistent with the present study findings, Malangu (2014), reported that the prevalence of obesity was higher in females compared to males; furthermore, patients on PIs were significantly obese. The high prevalence of overweight and obesity raises a serious concern in these patients as these conditions are known risk factors for other CDL.



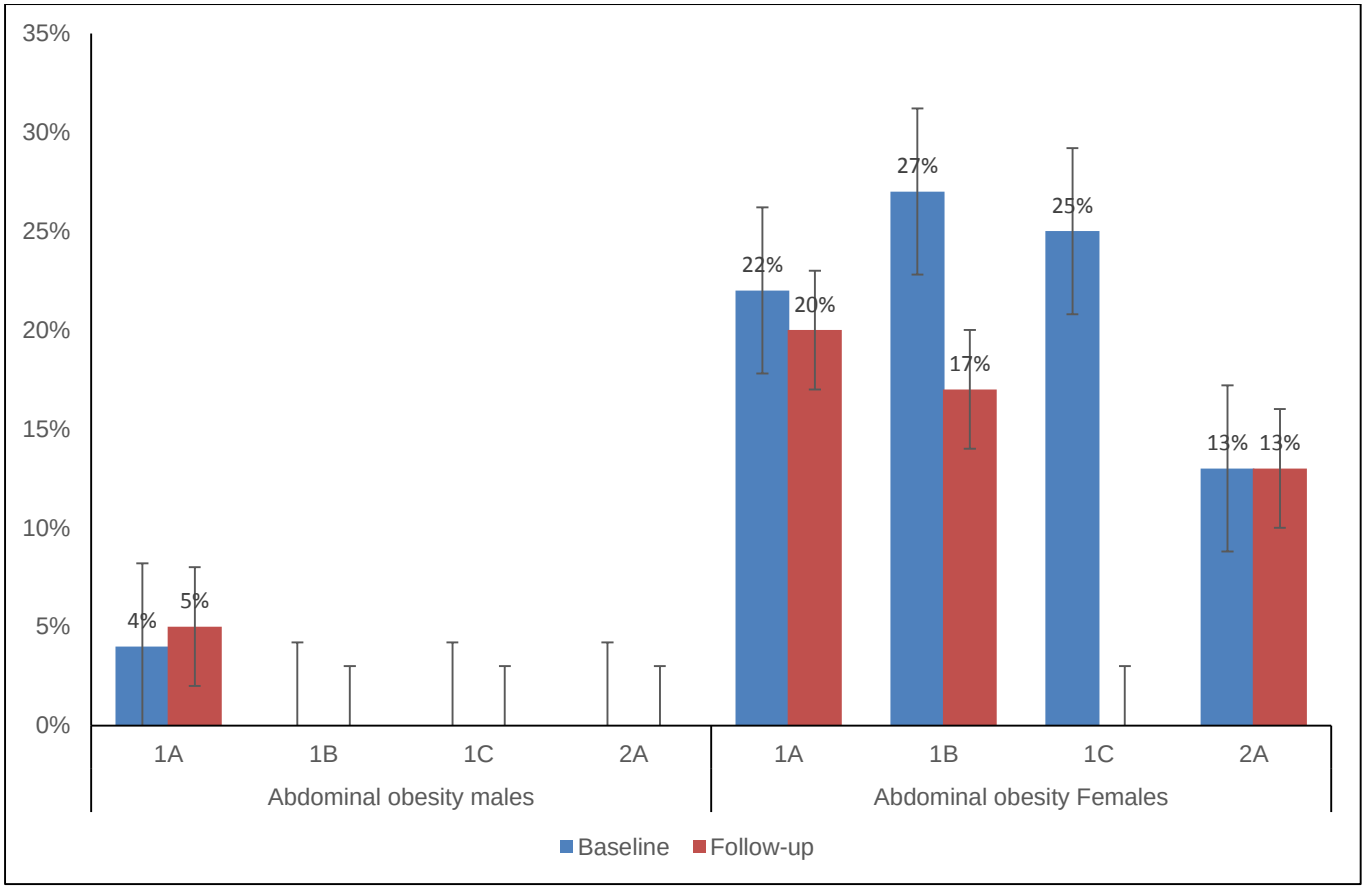

Significance between regimens in males and females at baseline: p=0.69

Significance between regimens in males and females at follow-up: p=0.64

**Figure 3.3:** The prevalence of abdominal obesity by gender in HAART patients.

The prevalence of abdominal obesity was more pronounced in females than in males (Figure 3.3). In contrast to the study findings, Malangu (2014), reported that the prevalence of abdominal obesity was higher in males (16.3%) compared to females (8.5%). Individuals that had central obesity had a cut-off BMI at ≥30 kg/m$^2$.

In Figure 3.3, the prevalence of abdominal obesity was subdivided to take into account the effect different drug combinations. Applying the NCEP III criteria (Awotedu et al., 2010), the prevalence of abdominal obesity in males was found only in patients taking [1A]. There were no males that met the criteria for abdominal obesity in the other drug combinations. In females it was found in all combinations at baseline and follow-up respectively. There were no statistical differences across all drug combinations in the prevalence of abdominal obesity in both genders. This might be due to the small sample sizes within the four regimen combinations.




Significance between regimens in males and females at baseline: p=0.45

Significance between regimens in males and females at follow-up: p=0.60

**Figure 3.4:** The prevalence of raised W:H ratio by gender in HAART patients.

The prevalence of raised W: H ratio was predominant in females compared to males however there was no statistical differences observed, this might be due to the small number of males compared to females. Furthermore, the prevalence doubled in females taking [1C]. Overall body morphology alterations were observed in patients taking d4T-based regimens (1C).

## 3.2 CONCLUSION

The present data suggests that patients with increased body fatness are consequently at increased risk of developing CDL. Obesity is one of the major risk factors associated with many clinical problems. Diseases associated with metabolic effects of excess adiposity include coronary heart disease, hypertension, type 2 diabetes mellitus and

lifestyle induced cancers. Mechanisms include increased release of free fatty acid and production of adipokines. Therefore accumulation of visceral fat increases the risk of developing IR, IGT, dyslipidaemia, hypertension and a prothrombotic and pro-inflammatory state (Goedecke et al., 2006). In a study by Nell et al., (2015), the high prevalence of overweight and obesity in HAART patients was attributed to patients being overweight before commencement of HAART. In the present study BMI was not measured before commencement of HAART. Furthermore, the findings indicate that females are more overweight and obese. Increased body fatness was observed in patients on d4T or AZT and PI-based regimens. Various studies have indicated that body morphology alterations may be regimen specific and that changes in body fat distribution may indicate HALS. However, the study did not measure the presence of lipodystrophy thus conclusions are deduced on existing literature. It is therefore important to conduct prospective studies that will consider HAART naïve patients and compare them with HAART patients to further validate the exact time frame when these regimens start to become toxic to the metabolism. The high prevalence of overweight and obesity in this study is worrying. Lipid profiles of obese individuals have been reported to vary according to ethnicity. For example, Goedecke et al, (2006) reported that in the general population, black South Africans portrayed lower profiles of hypercholesterolemia and high LDL-C levels compared to white and mixed ancestry South Africans. Furthermore lower prevalences (2.4%) of coronary heart diseases were reported in a sub population of black South Africans. This report validate that even though high prevalences of overweight and obesity are reported in this study, an interplay of individual genetic susceptibility, ethnicity, gender, HIV infection and exposure to drugs such as PIs may be linked as causal pathways in the increased risk of developing CDL in obese patients.

# CHAPTER 4

# RESULTS AND DISCUSSION: LIPID METABOLISM VS HAART

## 4.1 INTRODUCTION

This chapter presents the results of the study dealing with the impact of the different HAART regimens on the lipid metabolism. Results are presented as a lipid profile that include total cholesterol, LDL, HDL and triglycerides at baseline and follow-up at 6–12 months. In combination with the investigation into lipid profile alterations, leptin levels is also reported on for a sub-population. In addition, attention is also drawn to some of the most common lipid abnormalities found in these HAART patients. Findings that were significant between the two intervals were further analysed. The findings are further discussed and brought into context by comparing them with other studies and the impact of the findings on the current SA health context**.** Frequency distributions were done for each variable. Descriptive statistics were presented as means and standard error mean (SEM) for continuous variables and frequencies and percentages for categorical variables. Comparison of quantitative variables was performed using Chi-square test for categorical variables, while paired Student's t-test was used for continuous data. Total cholesterol and HDL were directly measured and LDL-C was indirectly measured by using the Friedawald equation to calculate it.

**Table 4.1:** Changes in lipid profiles over time.

| | Baseline Mean (SEM) | Follow-Up Mean (SEM) | p-values |
|---|---|---|---|
| Total cholesterol (mmol/L) | 5.3 (0.135) | 4.9 (0.118) | <0.001 |
| HDL-C (mmol/L) | 3.2 (0.080) | 3.2 (0.075) | 0.92 |
| LDL-C (mmol/L) | 1.5 (0.108) | 1.1 (0.101) | <0.001 |
| Triglycerides (mmol/L) | 1.4 (0.080) | 1.3 (0.063) | 0.30 |
| Leptin (ng/ml) | 11.36 (1.581) | 9.67 (1.193) | 0.01 |

Table 4.1 summarises the changes in lipid profiles over the period of the study. The mean value for total cholesterol significantly decreased p=<0.001. Furthermore, the levels of LDL-cholesterol decreased significantly p=<0.001. Though the study findings showed reductions in the mean levels of HDL-cholesterol and triglycerides; these findings were not statistically significant. These findings were higher than findings in another study conducted in South Africa (Julius, 2010). They reported mean TC 4.7 ±1.0 mmol/L; high density lipoprotein cholesterol 1.2±0.4 mmol/L; low density lipoprotein cholesterol 2.8±0.9 mmol/L.

**Table 4.2:** A comparison of lipid profiles of individuals on first and second line HAART regimes over time.

| | two NRTIs + one NNRTIs= first line regimen Mean (SEM) | | | one PI + two NRTIs= second line regimen Mean (SEM) | | |
|---|---|---|---|---|---|---|
| Variables | Baseline | Follow-up | p-values | Baseline | Follow-up | p-values |
| Total cholesterol (mmol/L) | 5.4 (0.153) | 4.9 (0.138) | <0.001 | 4.9 (0.268) | 4.8 (0.211) | 0.80 |
| HDL-C (mmol/L) | 3.3 (0.084) | 3.3 (0.082) | 0.60 | 2.7 (0.172) | 2.8 (0.158) | 0.40 |
| LDL-C (mmol/L) | 1.5 (0.124) | 1.1 (0.112) | <0.001 | 1.4 (0.219) | 1.2 (0.181) | 0.20 |
| Triglycerides (mmol/L) | 1.3 (0.089) | 1.2 (0.055) | 0.23 | 1.7 (0.147) | 1.8 (0.219) | 0.90 |
| Leptin (ng/ml) | 11.04 (1.829) | 9.71 (1.530) | 0.057 | 9.40 (3.322) | 4.95 (1.749) | 0.065 |

Table 4.2 shows the changes in lipid profiles over the period of the study in the two lines of regime. The mean total cholesterol significantly decreased p=0.001, in the first line regime. The mean LDL-cholesterol decreased significantly p=<0.001 in the first line regime. There were no statistical Though the study findings showed reductions in the mean levels of triglycerides and no changes in HDL-cholesterol between the two

treatment arms at baseline and follow-up; these findings were not statistically significant.

The mean TG were higher in the second line regime. It has been reported that PIs exert atherogenic effects on lipid profiles thus increasing the risk for cardiovascular diseases. Furthermore, it was reported that NNRTIs such as NVP exhibit anti atherogenic effects on lipid profiles; therefore, there was a significant decrease in the treatment regimes containing some NRTIs+NNRTIs. These findings may also suggest that the first line regime is favourable in terms of lipid profiles compared to the second line regime. Furthermore, the findings support the hypothesis postulated by Fleishman et al. (2007) that NRTIs induce cumulative effects compared to acute effects observed in patients on PIs.

**Table 4.3**: Lipid profiles portraying gender based differences in the two lines of regimes.

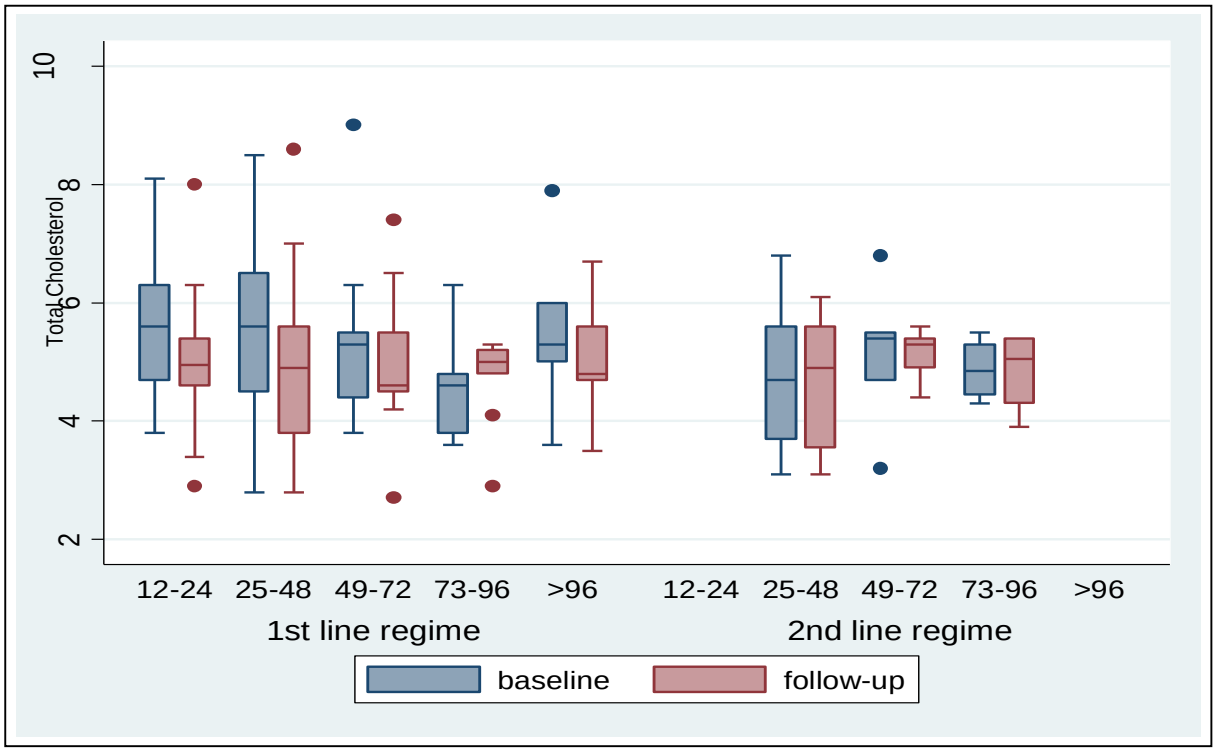
| | Two NRTIs + one NNRTIs= first line regimen Mean (SEM) | | | One PI + two NRTIs= second line regimen Mean (SEM) | | |
|---|---|---|---|---|---|---|
| Variables | Baseline | Follow-Up | p-values | Baseline | Follow-Up | p-values |
| TC (mmol/L) | | | | | | |
| Males | 5.1 (0.280) | 4.5 (0.260) | 0.02 | 5.1 (0.569) | 5.1 (0.416) | 1.00 |
| Females | 5.5 (0.181) | 5.0 (0.158) | 0.01 | 4.8 (0.310) | 4.7 (0.248) | 0.82 |
| | | | | | | |
| HDL (mmol/L) | | | | | | |
| Males | 3.1 (0.163) | 2.9 (0.128) | 0.22 | 2.6 (0.256) | 2.5 (0.117) | 0.64 |
| Females | 3.39 (0.096) | 3.38 (0.097) | 0.97 | 2.7 (0.225) | 2.9 (0.213) | 0.33 |
| | | | | | | |
| LDL (mmol/L) | | | | | | |
| Males | 1.4 (0.209) | 1.1 (0.226) | 0.04 | 1.5 (0.456) | 1.5 (0.251) | 1.00 |
| Females | 1.58 (0.153) | 1.13 (0.139) | 0.007 | 1.4 (0.258) | 1.0 (0.228) | 0.16 |
| | | | | | | |
| TG (mmol/L) | | | | | | |
| Males | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Females | 1.3 (0.154)<br>1.3 (0.109) | 1.2 (0.102)<br>1.2 (0.066) | 0.53<br>0.31 | 2.1 (0.265)<br>1.6 (0.161) | 2.3 (0.385)<br>1.5 (0.194) | 0.75<br>0.83 |
| | | | | | | |
| Leptin (ng /ml)<br>Males<br>Females | 14.2<br>(5.764)<br>10.3<br>(1.878) | 9.6 (2.030)<br>9.7 (1.853) | 0.6<br>0.7 | 14.6 (3.050)<br>11.4 (4.413) | 13.2<br>(1.700)<br>8.4 (2.084) | 0.5<br>0.4 |

The changes in lipid levels at baseline and follow-up show that there are several contributions to the development of dyslipidaemia in patients taking HAART. The lipid levels show significant decreases in TC and LDL-C. When levels were compared according to treatment arms the first line regime showed significant decreases in TC and LDL levels. These findings are consistent with Dillon et al. (2013), their findings suggested that ARV treatment exposure was associated with high HDL-C and LDL-C levels whereas hypertriglyceridemia and lower HDL were found in HIV positive ARV therapy naïve patients. Das (2010), reported that HIV ART group developed mild dyslipidaemia when compared to ART naïve and HIV negative groups not showing differences in TG and TC levels. However, in the PI group, the study reported high TG, LDL-C and IDL cholesterol. The present study findings show that severe dyslipidaemia was prevalent in the PI group.

The mean TC and LDL-C significantly decreased for the total population however when the data was further analysed according to gender the mean TC and LDL-C significantly decreased in both genders. Significant differences were found between males and females were TC and LDL-C levels decreased at follow-up in the first line regime. However, no significant differences were observed between the genders in the second line regime. These findings suggest that the decrease in TC and LDL-C is regimen specific and that it is not dependent on gender. The decrease in LDL-C cholesterol may partly be explained by the TC/HDL-C ratio. A TC/HDL-C ratio ≥5 may compensate for the increased levels of LDL-C, thus further reducing the risk for cardiovascular disease. However, no significant changes were observed in TC/HDL ratio. Furthermore, increased lipid changes were reported in participants taking ABC+3TC compared to those taking TDF+FTC combined with either EFV or ATV/r. Participants that were given ATV/r combined with TDF+FTC or ABC+3TC did not show

any significant changes in TC:HDL-C ratios. These findings suggest that the FDC may reduce the risk of CDL in these patients and may fuel advances in generating cost effective and safer regimens in the near future. The individual demographic and genetic characteristics may significantly contribute to the severity of dyslipidaemia in HAART patients experiencing HAART induced dyslipidaemia therefore it is clear from the table that even though significant decreases were observed in both males and females, females had higher TC and LDL-C levels.



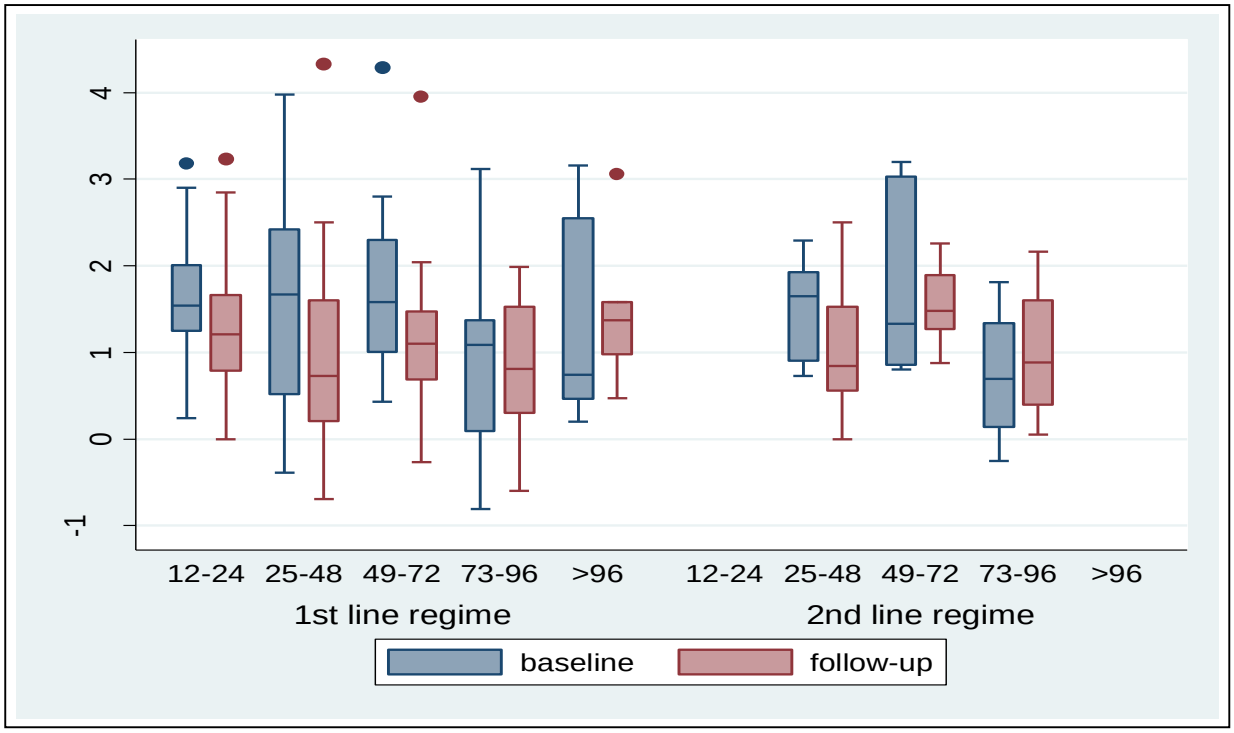

- **Significance at baseline TC**: 12-24 months p=0.471; 25-48 months p=0.673; 49-72 months p=0.627; 73-96 months p=0.419 and >97 months p=0.659; **Significance at follow-up TC**: 12-24 months p=0.722; 25-48 months p=NS; 49-72 months p=NS; 73-96 months p=NS and >97 months p=0.500

**Figure 4.1:** The effect of two lines of regime on TC at different HAART intervals.

Findings that were significant between the two intervals, in the two treatment regimes were further analysed. It was found that there was a difference in the effects of duration of HAART; however, these effects were not statistically significant. Thus being noted, from Figure 4.1, it is clear that TC levels at baseline are higher on average compared to follow-up in the first line regime. At baseline the median TC ranges from 4.5-5.5 mmol/L as compared to follow-up ranging from 4.5-5.0 mmol/L. looking at HAART duration, it is clear that as HAART years increase between 25-48 months to >96

months, 75% of patients at baseline have TC levels of 5.5 mmol/L or lower compared to only 25% of the population with TC levels below 5.0 mmol/L in the first line regime. Patients with TC ≥8.0 mmol/L and TC≤4.0 mmol/L indicated by the dots (outliers) were more pronounced at follow-up in the first line compared to the second line regime.

In the second line regime, median TC levels were <6.0 mmol/L at baseline and follow-up. There is a general distribution of high TC levels with most patients falling within the 75$^{th}$ percentile at baseline and follow-up respectively. The median is higher between months 49-72 and 73-96 compared to the first line regime. In the second line regime; the IQR is smaller on average however the median is higher compared to first line regime at both baseline and follow-up. Furthermore, most of the individuals fall within the 75$^{th}$ percentile which is worrying considering the small sample size in this group. There are only two outliers in this group compared to the first line regime making the variation of distribution smaller and increasing the risk of patients in this group for developing cardiovascular diseases.




- **Significance at baseline LDL**: 12-24 months p=0.782; 25-48 months p=0.858; 49-72 months **p=0.024**; 73-96 months p=0.738 and >97 months p=0.659; **Significance at follow-up LDL**: 12-24 months p=NS; 25-48 months p=NS; 49-72 months p=NS; 73-96 months p=NS and >97 months p=0.500

**Figure 4.2:** The effect of the two lines of regimen on LDL-C at different HAART intervals. (LDL-C in mmol/L vs period in months)

Low density lipoprotein cholesterol levels are higher on average at baseline compared to follow-up. The variability in first line regime is smaller in the second line regime. In the first line regime 50% of individuals have LDL-C values ≤ 1.5 mmol/L compared to 25% of individuals having LDL-C values ≤1.5 mmol/L. this indicates that higher LDL-C are observed in the second line regime. Significant decreases in LDL-C is observed between HAART months 49-72 in the first line regime. Figure 4.2 indicates that most patients fall within the 75$^{th}$ percentile at baseline in the first line regime between months 25-48. The median LDL-C levels showed fluctuations, however is higher between months 49-72. The median LDL-C was highest at >3.0 mmol/L in patients taking second line regime at months 49–72 at baseline; however, significantly reduced to just below 2.0 mmol/L. The patients with a median >3.0 mmol/L were more pronounced at follow-up compared to baseline only in the first line regimen. These findings indicate the cumulative effects of NRTIs over longer durations of HAART compared to PIs. Overall the median was higher at baseline compared to follow-up. The increase in median TC and LDL is more clustered between months 25–48 to >96 in first line regime. Although there was no statistical significance associating longer duration of HAART with high TC and LDL-C levels in the two lines of regime, the more than borderline high TC and LDL-C levels in these patients at baseline and follow-up cannot be ignored, as it poses a great risk on these patients developing cardiovascular diseases in the near future.

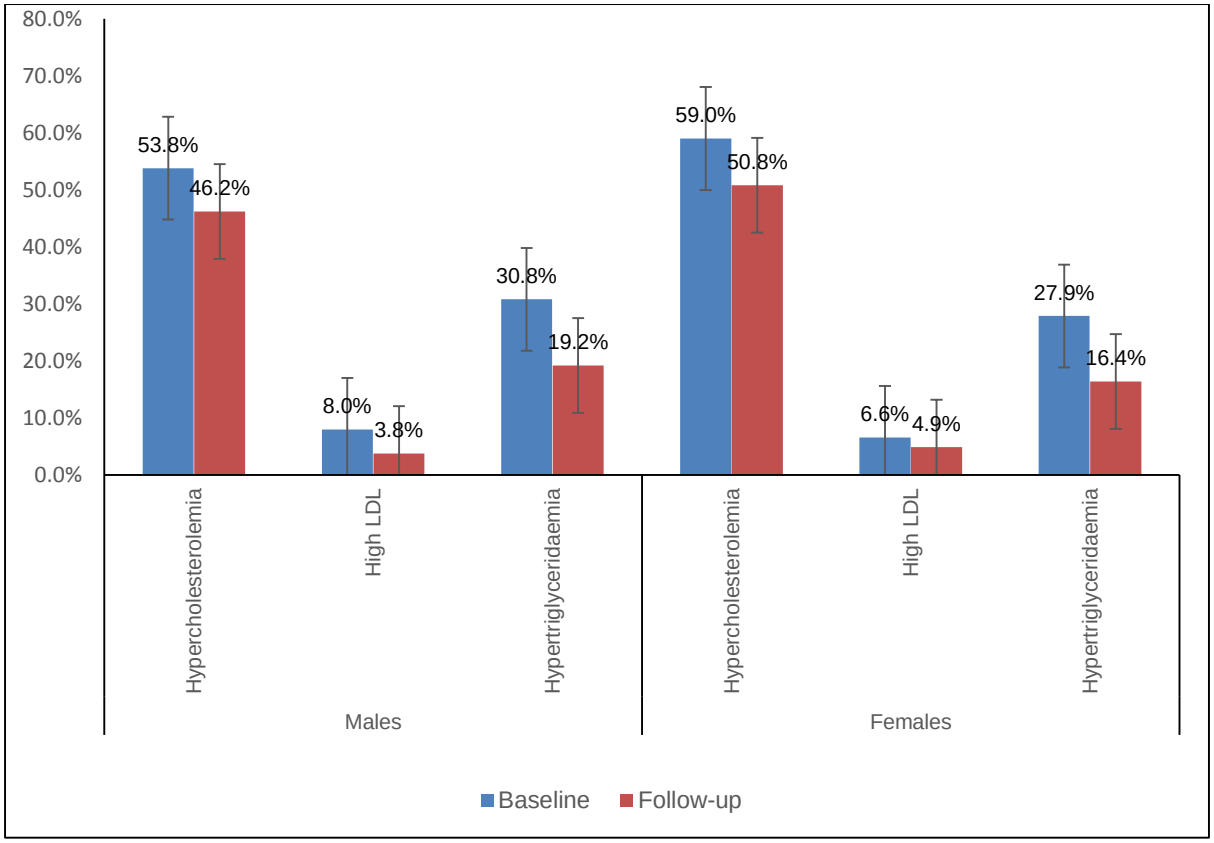
The duration of HAART is one of the major factors contributing to the metabolic derailment (Ali, 2014). A cohort study was conducted where the lipid profiles of 124 patients were measured before they commenced HAART and then followed-up for a period of three years. The findings indicated a significant increase in TC from pre-therapy to post-therapy after 3 years, ranging from 3% to 13%, a decrease in HDL-C from 21% pre-therapy to 17% post-therapy; an increase in LDL-C >2.1 mmol/L; from 2% pre-therapy to 5% post-therapy and an increase in triglycerides >1.7 mmol/L from 22% pre-therapy to 29% post-therapy (Duro et al., 2013). Although the present study did not investigate pre-therapy effects, it is clear that exposure to HAART as well as longer durations derail the metabolic pathways involved in lipid metabolism. Similarly, Malangu (2014), reported that the prevalence of hypertriglyceridaemia was

significantly higher in males (26.5%) than in females (14.5%). Furthermore, the prevalence of hypertriglyceridaemia was significantly higher in patients who had been on HAART for ≥60 months with a prevalence of 17.4% vs 12.5% and TC of 29.1% vs 18.3%.

**Table 4.4:** The effect of HAART duration on triglycerides and LDL-C by HAART combinations at baseline.

| Variables | HAART duration | Baseline P value |
|---|---|---|
| Triglycerides | 12–24 months | 0.03 |
| LDL-C | 49–72 months | 0.02 |

The duration of HAART between months 12–24 significantly increased TG (p=0.03). Furthermore, LDL-C levels were significantly increased between months 49–72 (p=0.02). Although the data did not show specifically which regimens is implicated, it seems that patients on PIs are implicated because it is evident of the significant decreases in TC and LDL-C in the first line regime and not in the second line regime. Furthermore, in the long run patients taking these regimens develop increased LDL-C; however, TG start to increase as early as 12–24 months. Malangu (2014), reported that patients who had been on HAART for ≥60 months had high TG and TC. These findings are not in agreement with the present study findings with regard to triglycerides.



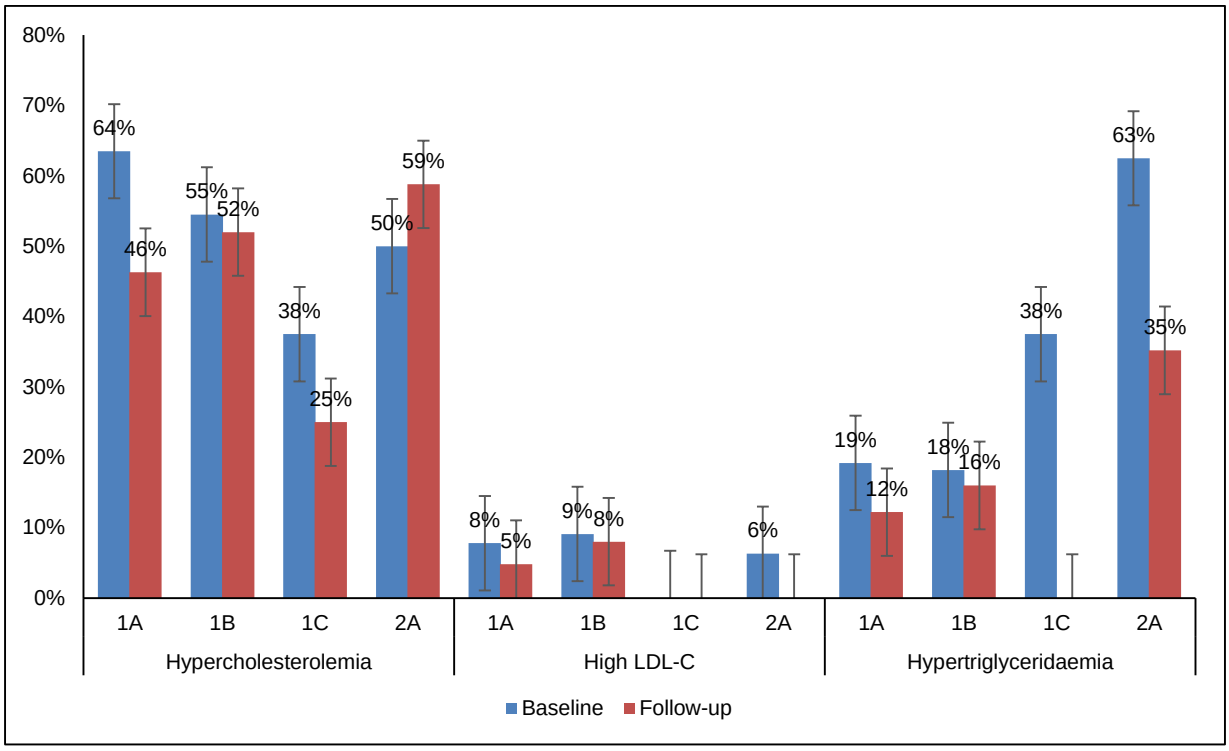

- Significance at baseline hypercholesterolemia in males and females: p=0.39
- Significance at follow-up hypercholesterolemia in males and females: p=0.59
- Significance at baseline high LDL levels in males and females: p=0.79
- Significance at follow-up high LDL in males and females: p=0.59
- Significance at baseline hypertriglyceridaemia in males and **females: p=0.007**

➢ Significance at follow-up hypertriglyceridaemia in males and females: p=0.14

**Figure 4.3**: The prevalence of dyslipidaemia in both genders at baseline and follow-up.

The prevalence of hypertriglyceridaemia was significantly higher in females compared to males. Consistent with the present study findings, Tadewos et al. (2012) found that the prevalence of raised TC was 43.4%, high LDL-C 33.6% and high TG 55.8%. The severity of dyslipidaemia in first line regime was determined by variation in HAART duration. Furthermore, they reported that the prevalence of high TC was higher in females (44%) than males (42.1%), low HDL in females (51.3%) and (47.4%) in males; high LDL 38.7% females and 23.7% in males; TG 57.9% in males and 54.7% in females TC /HDL –C ratio ≥5 males 42.1% and females 46.7%. Furthermore, Julius, (2010) found that the prevalence of dyslipidaemia was higher in females than males.

They reported that the prevalence of high TC was 6% females and 2% in males, TG 26% in females and 14% in males and LDL 11% in females and 7% in males. The prevalence of dyslipidaemia was high in the present study findings compared to previously mentioned studies, these high rates poses a great risk in the future health prospects of these patients.



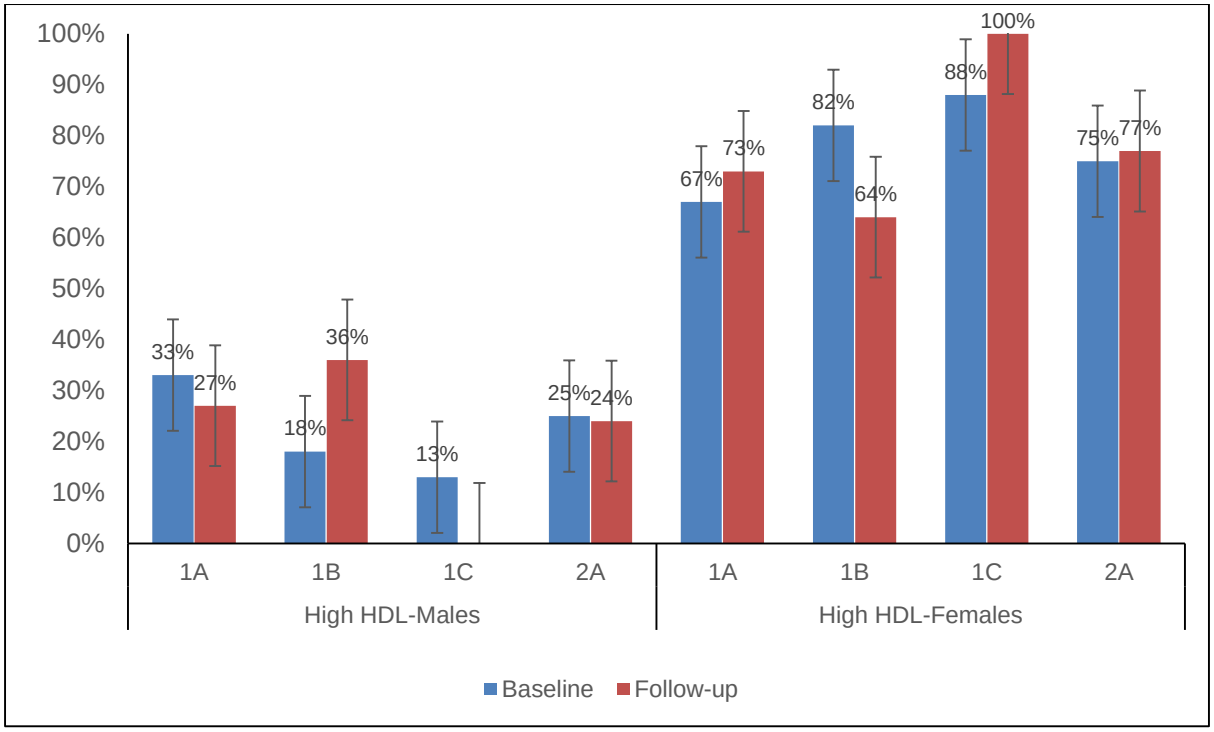

- Significance at baseline hypercholesterolemia: **Regimen 1A p=<0.001**; Regimen 1B p=0.921; Regimen 1C p=0.357 and Regimen 2A p=0.976
- Significance at follow-up hypercholesterolemia: **Regimen 1A p=<0.001**; Regimen 1B p=0.673; Regimen 1C p=0.793 and Regimen 2A p=0.837
- Significance at baseline high LDL levels: **Regimen 1A p=<0.001**; Regimen 1B p=0.515; Regimen 1C p=0.114 and Regimen 2A p=0.216
- Significance at follow-up high LDL: **Regimen 1A p=<0.001**; Regimen 1B p=0.127; Regimen 1C p=0.573 and Regimen 2A p=0.199

**Figure 4.4:** The prevalence of lipid abnormalities in HAART patients over time.

The prevalence of dyslipidaemia was observed by high TC, LDL and TG. Figure 4.4 shows the effects of different combinations of HAART regimen on lipid profiles at baseline and follow-up respectively. The use of NNRTI (NVP), NRTIs (such as ABC) and NtRIs (TDF) and the PI atazanavir (ATV) are considered favourable in terms of metabolic profiles (Petoumenos et al., 2012). Therapy constituted with d4T, AZT, ddl

or EFV have been associated with the development of dyslipidaemia also more pronounced in patients receiving PIs (Calza et al., 2008). From the graph it is clear that the prevalence of hypercholesterolemia decreased at follow-up in regimen 1A, however it is subsequently, higher in regimen 2A. The prevalence of high LDL-C levels significantly decreased in regimen 1A at follow-up and was diminished in combinations 1C and 2A this may partly be due to regimen switch indicating that patients switch to a different regimen with existing lipid abnormalities. Another reason may partly be due to the TC:HDL ratio which was not measured in this setting however it is clear from Figure 4.5 that the high HDL levels in combination 1A, 1C and 2A in females compensated for the TC:HDL ratio therefore the levels of LDL-C decreased significantly. Consistent with the present study findings, Souza et al. (2013) reported that patients using TDF+3TC were found to exhibit lower serum concentrations of LDL-C, TC and TG as compared to patients using AZT+3TC, d4T+3TC or ddl+3TC.

Overall the study findings are consistent with other studies; however, what can be deduced from the findings is that all drug combinations portrayed some type of lipid abnormality. The study was able to demonstrate that PI containing regimens, portrayed pronounced hypertriglyceridaemia especially at follow-up, considering the small number of patients taking the combinations compared to patients taking combination [1A] and [1B]. The high prevalence of hypertriglyceridaemia found in d4T and AZT containing combinations at baseline disappeared at follow-up suggesting that patients that were switched to other combinations moved into new regimens with existing lipid abnormalities.

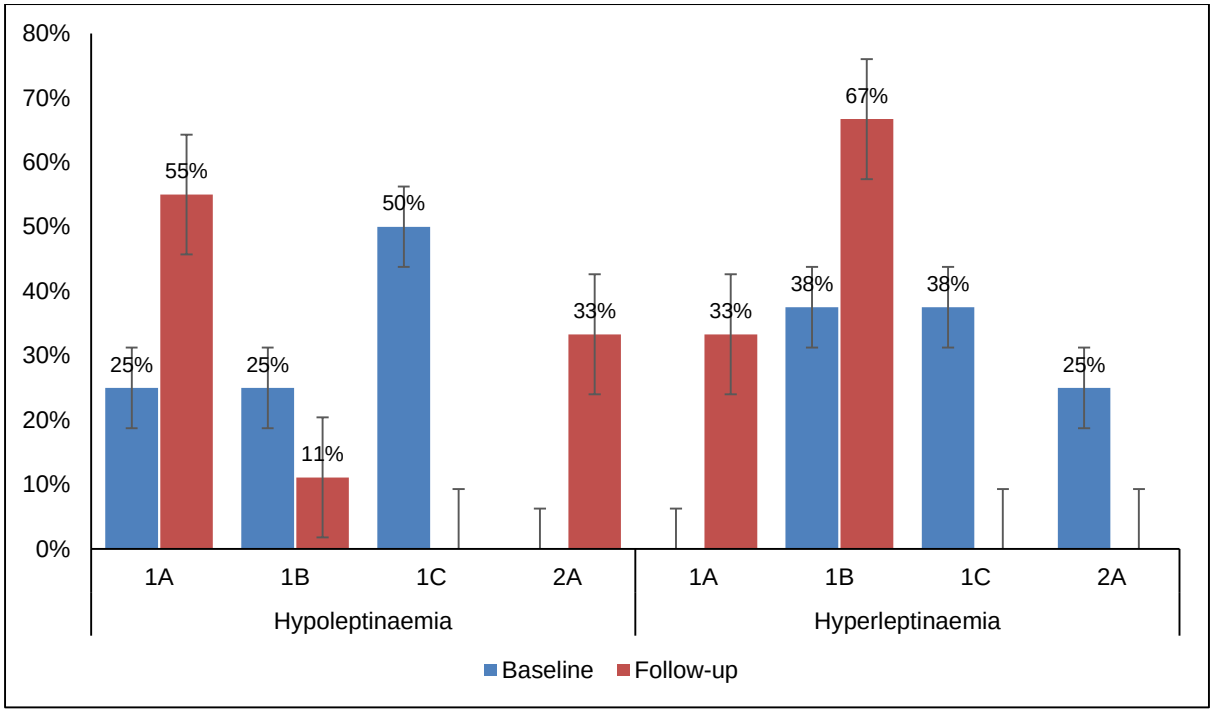



- Significance between regimens in males and females at baseline: p=0.54
- Significance between regimens in males and females at follow-up: p=0.46

**Figure 4.5:** The prevalence of high HDL-C levels in HAART patients over time.

Overall all patients had borderline high HDL-C levels. Higher HDL-C levels were found in females compared to males. Although borderline high HDL-C levels were found in all patients, it seems that they were less pronounced in males across the different drug combinations. However, using the new drug combination [1B], was accompanied by HDL-C levels that nearly doubled.

Duro et al. (2013), found that there were small increases in TC and TG and higher increase in HDL-C in patients taking NNRTIs compared to patients taking PIs. Furthermore, they suggested that NNRTIs contributed to a more favourable lipid profile. These findings partly elucidate the present study findings as shown in Table 4.2, where significant decreases in TC and LDL-C were only observed in patients taking combinations containing NNRTIs and NRTIs. The mechanism of NNRTIs increasing HDL levels involves differences in genetic predisposition. Furthermore, HDL was reported to exhibit antioxidant effects by inhibiting transcription of viral RNA into double stranded RNA. Thus elevated HDL levels in HAART patients provide some form of protection.




- Significance between regimens at baseline: p=0.162
- Significance between regimens at follow-up: p=0.210

**Figure 4.6**: The prevalence of hypoleptinaemia and hyperleptinaemia in HAART patients over time.

Figure 4.6 shows the effects of different combinations of HAART regimen on leptin levels at baseline and follow-up respectively. The prevalence of hypoleptinaemia at follow-up in patients taking combination (1B) may partly be explained by regimen switch as the prevalence of hypoleptinaemia in patients taking (1C) was reduced at follow-up. Currently in South Africa there are no defined cut-off values to classify individuals as hypoleptinaemic; however, with the large body of evidence on the therapeutic effects of synthetic leptin in patients with HALS, it would be beneficial to look into validating these effects in the South African population. Al-Fhadli et al. (2014) found that HOMA IR positively correlated with leptin levels and they suggested that this was due to compensatory hyperinsulinaemia as result of insulin resistance stimulating adipocytes to secrete leptin. However inhibition of glucose transport was found to reduce leptin levels in cultured adipocytes (Hruz, 2006). The study further hypothesized that hyperleptinaemia was due to hyperinsulinaemia in patients with high CD4+ counts. These findings are not in agreement with the present study findings as the study did not find any correlation between HOMA IR, insulin and leptin levels.

Hyperleptinaemia was more pronounced at follow-up in patients taking combination (1A), it seems that there is improved insulin sensitivity in this group of patients as a result of insulin signalling pathways directly stimulating leptin secretion (Hruz, 2006). This finding may be supported by the lower prevalence of IR (18.2% and 12.0%) at baseline and follow-up in patients taking combination (1B). The percentage of patients having normal and high leptin levels outweigh the percentage of hypoleptinaemic patients thus these findings further suggest that favourable profiles observed in patients taking combination (1B) may further reduce the risk of metabolic diseases compared to other combinations. However, high leptin levels may also be associated with leptin resistance and therefore leptin not being able to control body weight may seem to increase the risk of obesity and IR, thus increasing the burden of metabolic diseases in an individual.

## 4.2 CONCLUSION

The significant changes in lipids were only observed in the first line regime, it is noticeable that TC and LDL levels were higher at baseline in patients on first line regime compared to second line regime. However, the significant decrease in these levels became similar to the levels of second line regime at follow-up. These findings suggest that the regimen switch, where 15% of the patients moved to the FDC drug and to a lesser extent 2% of the patients were moved to the second line regimen had a significant influence on the lipid profiles of these patients.

The small body of evidence that exist on FDC has not been able to hypothesize mechanisms that might be associated with lower lipid levels, therefore these mechanisms may further be investigated in future studies. These findings further demonstrate the gaps in research that still needs to be addressed. The present study could not demonstrate that the effects of FDC will remain favourable even after longer durations. Furthermore, there were no significant changes in HDL-C levels in both treatment arms at baseline and follow-up. However, it is clear from Table 4.2 that HDL levels were increasing and higher in the first line regime suggesting the favourable lipid levels compared to second line regime due to the protective effects of high HDL levels. Unfortunately due to the small sample size in leptin analysis, not much statistical power can be achieved, in this regard future cohort studies with larger

sample sizes may be able to explore the effects of HAART on adipokines and their relationship within the different drug combinations.

Leptin levels were analysed for only a sub-group of the total population. Only 29 patients were included for leptin analysis for baseline and follow-up therefore the following results should be observed bearing in mind that the sample size was tremendously reduced due to shortage of samples to run for the entire study population.

The changes in leptin levels at baseline and follow-up did not show any significance even when compared within the two lines of regime, this may be due to the small sample size. Although mean leptin levels were lower at follow-up in both treatment regimes, the mean leptin levels were slightly higher in the PI group than NRTI/NNRTI group. At baseline the prevalence of hypoleptinaemia shows that the highest percentage was found in combination (1C) which is a d4T containing regimen. At follow-up the prevalence of hypoleptinaemia was observed in the PI group however not at baseline. Due to the low statistical power obtained from leptin data, it becomes difficult to extrapolate mechanisms associated with either hypoleptinaemia or hyperleptinaemia.

# CHAPTER 5

# RESULTS AND DISCUSSION: CARBOHYDRATE METABOLISM VS HAART

## 5.1 INTRODUCTION

This chapter presents the results obtained during the investigation into the impact of HAART on the carbohydrate metabolism. Baseline and follow-up at 6–12 months measurements of glucose and insulin are used to reflect on impaired fasting glucose, the prevalence of DM and IR in this specific cohort. Findings that were significant between the two intervals were further analysed. The findings are further discussed and brought into context by comparing with other studies and the impact of the findings on the current SA health context**.**

**Table 5.1:** A comparison of the changes observed in glucose and insulin levels overtime.

| | Baseline Mean (SEM) | Follow-up Mean (SEM) | p-values |
|---|---|---|---|
| Glucose (mmol/L) | 5.4 (0.231) | 5.4 (0.158) | 0.66 |
| Insulin (mmol/L) | 6.3 (0.411) | 8.0 (0.961) | 0.09 |
| HOMA IR (miu/L) | 1.51 (0.110) | 1.45 (0.197) | 0.78 |

Table 5.1 shows the changes in glucose and insulin, and HOMA IR during the study period. The mean HOMA IR decreased from 1.51±1.03 to 1.45±1.84. Though the study findings showed a reduction in the mean levels of glucose and HOMA IR, these findings were not statistically significant.

**Table 5.2:** Changes in glucose and HOMA IR over time in the two lines of regimes.

| | Two NRTIs + one NNRTIs= first line regimen Mean (SEM) | | | One PI + two NRTIs= second line regimen Mean (SEM) | | |
|---|---|---|---|---|---|---|
| Variables | Baseline | Follow-up | p-values | Baseline | Follow-up | p-values |
| Glucose (mmol/L) | 5.3 (0.181) | 5.2 (0.089) | 0.68 | 5.81 (0.933) | 5.80 (0.724) | 0.90 |
| Insulin (mmol/L) | 6.4 (0.484) | 8.3 (1.175) | 0.39 | 5.9 (0.700) | 6.8 (1.010) | 0.07 |
| HOMA IR (miu/L) | 1.52 (0.118) | 1.44 (0.240) | 0.76 | 1.49 (0.299) | 1.51 (0.231) | 0.94 |

As shown, overall the findings in table 5.2 were not significant however it is interesting to find that the mean value for HOMA IR was higher in the second line regime. The duration of HAART significantly increased glucose (p=0.04) between months 49–72 in the second line regime.

The prevalence of dysglycaemia was observed by borderline high fasting glucose levels subsequently increasing the risk for the development of IFG and DM. The study found that glucose was significantly increased at duration between 49–72 months. These findings are in contrast to the findings by Ekali et al. (2013), who reported that glucose was unaffected by HAART duration. Similar to the present study findings, Judith et al. (2007) observed the use of d4T and AZT in combination with either EFV or NVP resulting in significantly increased blood glucose levels after 12 months of treatment. Consistent with previously mentioned studies, Hester (2012) found that AZT and d4T were implicated in the pathogenesis of DM2 by mitochondrial toxicity resulting in lipoatrophy in the skeletal muscle and subcutaneous tissue.

It is evident from the graph that the prevalence of impaired fasting glucose and IR are higher than the prevalence of DM and that these abnormalities in the glucose metabolism may translate in increased prevalence of DM. Furthermore it increases the risk of developing CVD. Gender based disparities indicate that the prevalence of hyperglycaemia is more prevalent in males compared to females. Sachithanathan et al. (2013), reported that the prevalence of DM was 8% in an Ethiopian setting. Furthermore, the patients who were on HAART ≥4 years were at significantly high risk of developing DM. Similarly Malangu (2014), reported that patients who had been on

HAART for ≥60 months had significantly high fasting glucose levels (17.4% in males and 2.95% in females).

The prevalence of DM in a Johannesburg HIV clinic was 1%; however, the prevalence was markedly higher in this study population. It is difficult to deduce the causes of the differences in the prevalence of DM in the two studies. Therefore multiple contributing factors such as the stage of the virus, severity of disease, co-infections such as tuberculosis (TB), hepatitis, the effects of intra-drug switching, history of HAART induced metabolic diseases and HAART duration makes the analysis complex. Julius (2010), concluded that the disparity among the HIV population in Johannesburg and other studies may be due to modified factors such as genetic susceptibility, obesity rates and sedentariness.

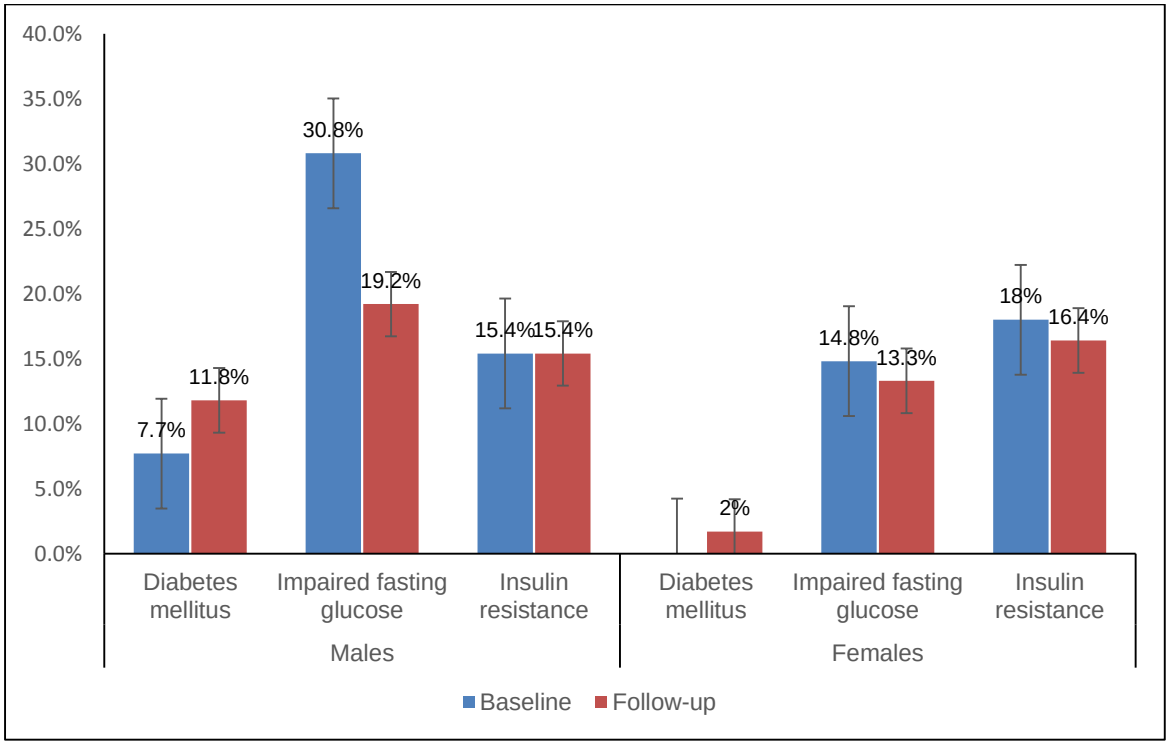
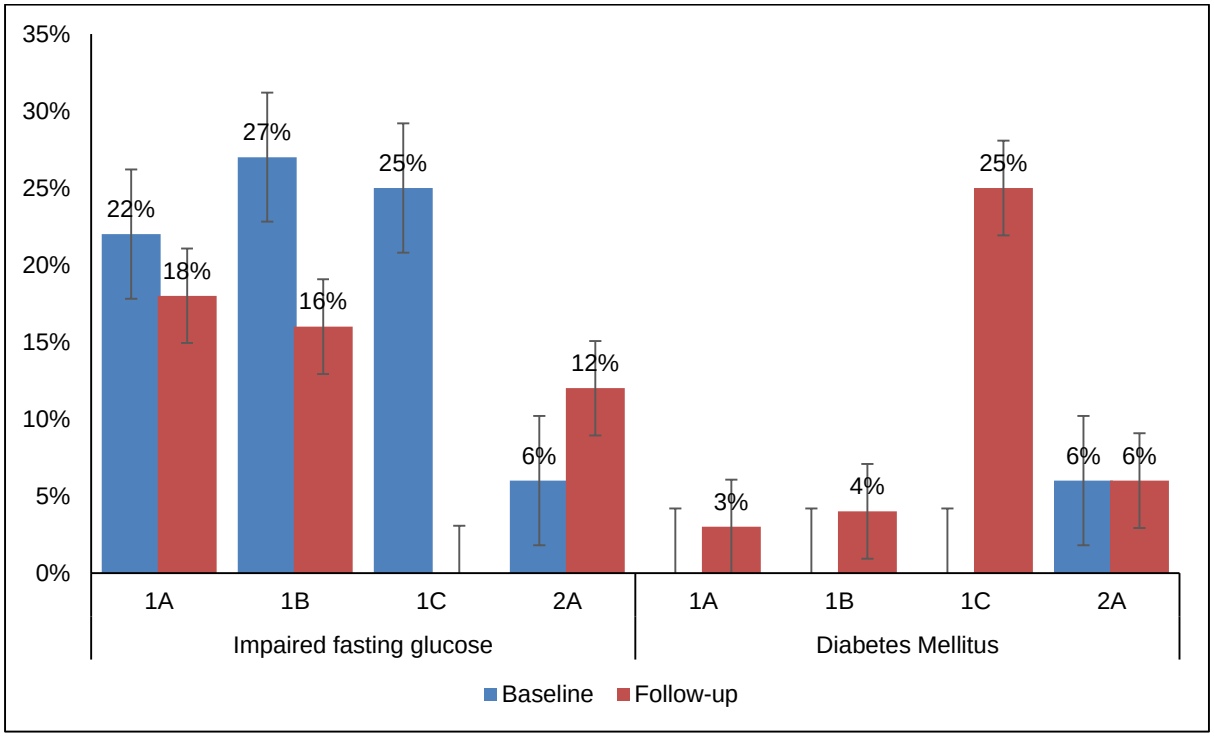



**Figure 5.1:** Frequency graph depicting the prevalence of impaired fasting glucose, insulin resistance and diabetes mellitus in males and females over time.

The impact of HAART on the prevalence of impaired fasting glucose and DM in this cohort is illustrated in Figure 5.1. Patients that met the criteria for diagnosis of DM were only found in the second line regime containing a PI at baseline and follow-up respectively. In the first line regime, the prevalence of DM was only found at follow-up. These findings raise concerns, because they indicate that duration of HAART is implicated as DM was more prevalent at follow-up than at baseline. The highest

prevalence was found in patients taking d4T-based regimens. Furthermore, the prevalence of DM remained unchanged in patients taking PI regimens however an increase in the impaired fasting glucose was found.



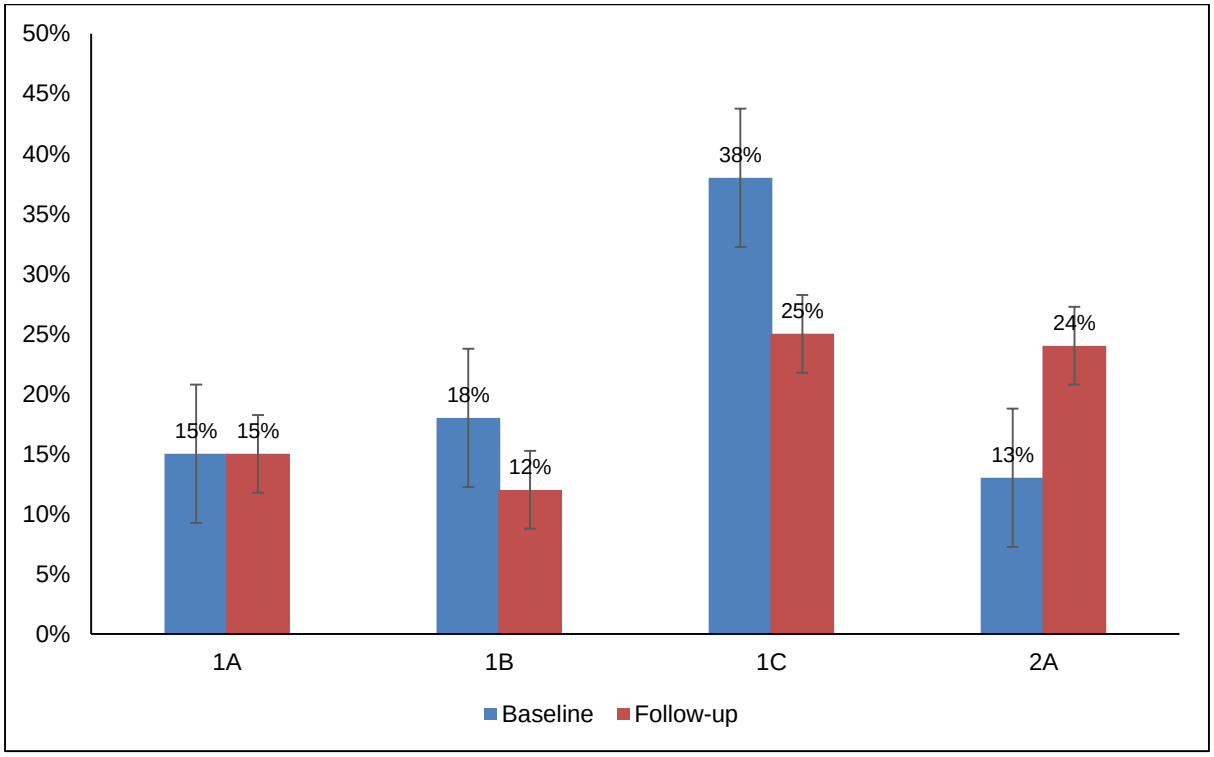

- Significance at baseline DM and IFG in males and females: p=0.71
- Significance at follow-up DM and IFG in males and females: p=0.55

**Figure 5.2:** The prevalence of impaired fasting glucose and diabetes mellitus in HAART patients over time.

The prevalence of IR was marked in patients taking combination [1C], it is clear that IR was prevalent in all drug combinations; however, that only the group taking d4T as thymidine backbone was elevated. The other combinations showed minimal effects in the development of IR and are thus comparable with the second line regime. Overall, there was a sharp decrease in the prevalence of IR in patients taking first line in contrast to the doubled increase observed for patients taking second line regime.

At baseline, the prevalence of DM was only found in PI containing regimen. The prevalence is relatively high compared to findings by Julius (2010), which was only one percent for the entire study population. At follow-up a marked increase (25%) in

the prevalence of DM was found in patients taking d4T and AZT containing regimens. This increase is more pronounced when compared to the prevalence in PI containing regimens (5.9%).




- Significance between regimens at baseline: p=0.443
- Significance between regimens at follow-up: p=0.728

**Figure 5.3:** The prevalence of IR in patients taking different HAART regimens over time.

The mechanisms underlying IR have been reviewed in the literature; however, what draws attention to this discussion is that the prevalence of IR has reached a staggering 37.5% in patients taking d4T which then decreases at follow-up. This finding deduce speculations that regimen switch to either FDC or PI containing regimens might have reduced the prevalence of IR in d4T containing regimens. Reports have been well documented on the effects of NRTIs regimen in inducing mitochondrial toxicity through inhibiting DNA polymerase activity.

It has been established that before the HAART-era, IR was rarely reported among HIV positive subjects. However, after initiation of HAART, reports on different regimens affecting insulin sensitivity were discovered. Feeney and Mallon (2011),

reported up to 35% reductions in insulin sensitivity in patients on PIs compared to NRTIs. They further reported a 24% reduction in insulin sensitivity with ritonavir boosted lopinavir regimens. Insulin resistance is one of the key factors playing a central role in the development of metabolic diseases such as DM2, cardiovascular disease, abdominal obesity and metabolic syndrome. Studies investigating the effects of individual drugs have been successful in pointing out the problematic drugs. However, very few studies have been able to point out a combination of drugs involved in inducing IR. Awotedu et al. (2010), found that the prevalence of IR was 12.8% and this finding is lower than the present study findings of 17.2% and 16.1% at baseline and follow-up respectively. Awotedu et al. (2010), found that the prevalence of IR markedly increased after introducing PIs in patients that were not taking PI containing regimens. These findings partly explain, the increase in HOMA IR at baseline and follow-up in patients taking PIs indicating the acute effects induced by PIs. Furthermore, in their study, linear regression model revealed that d4T was associated with IR which is evident in this study where the highest prevalence was reported in the d4T containing regimen. A multivariate analysis showed that only exposure to ART and metabolic syndrome were independent risk factors for development of IR in these patients.

**Table 5.3**: Correlations between insulin, HOMA and leptin over time.

| | Leptin T0 | Leptin T6-12 |
|---|---|---|
| Insulin T0 (mmol/L) | r=-0.382<br>P=0.06 | - |
| Insulin T6-12 (mmol/L) | - | r=-0.101<br>P=0.638 |
| | | |
| HOMA IR T0 | r=-0.105<br>P=0.624 | |
| HOMA IRT6-12 | - | r=-0.248<br>P=0.24 |

Pearson’s correlations (Table 5.4) were done to determine if there is any correlation between leptin levels, insulin and HOMA. There was no correlation between leptin levels, insulin and HOMA at baseline and follow-up respectively.

## 5.2 CONCLUSION

Overall, the prevalence of DM was pre-dominantly found at follow-up with the highest prevalence in patients taking d4T containing regimens; however, it cannot be disregarded that the prevalence of DM was present at baseline and follow-up in the PI-containing regimens which raises concerns about the effects of PIs on glucose metabolism. The prevalence of DM was 25% at follow-up in patients taking [1C], thus this indicates a similar trend of IR in patients taking combination [1C], and therefore patients that are insulin resistant may ultimately double the increase in development of DM2 which further places them at greater risk of developing cardio-metabolic traits and metabolic syndrome.

# CHAPTER 6

# CONCLUSION AND RECOMMENDATIONS

## 6.1 CONCLUSION

In this prospective cohort, the effects of different drug combinations were shown by comparing the effects of first line regime and second line regime in patients on HAART for 12 months or more. These effects were portrayed as contributions of several factors associated with HAART such as duration on treatment and the effects of drug combinations within the two treatment arms. Although factors associated with the progression of HIV itself were not particularly looked into. Factors including age, genetic predisposition, positive family history etc., may also be elucidated in the pathogenesis of HAART induced metabolic disorders.

The prevalence of hyperglycaemia in HIV positive patients reported in other studies was consistent with the present study. Reports indicate that the prevalence in South Africa is 23.5% (Dave et al., 2011), Uganda 16.3% (Omech et al., 2012), Rwanda 16–18% (Mutimara, 2007) and Kenya 21.4% (Manuthu, 2008).

Currently second line regime is believed to be more detrimental for blood lipid levels than first line regime; the results from this study showed that the prevalence of hypercholesterolaemia and high LDL-C levels were higher in the patients receiving second line regime compared to those receiving first line regime. The results of the current study are consistent with other study findings and they are cause for concern, regardless of the causes of the increased total cholesterol and LDL-C levels in these patients. Increased TC and LDL-C levels will place this already ill population at risk for developing non-communicable diseases and its complications. Therefore, it is strongly recommended that the Department of Health and Social Development should seriously consider inclusion of routine monitoring of patient lipograms in all treatment regimens of patients receiving HAART as they tend to fluctuate.

Furthermore, the study observed an alarming trend of increased body fatness and abdominal fat distribution in this population, in females. Since overweight and obesity are risk factors for most of the chronic diseases of lifestyle such as DM2; therefore, suggesting that the future health prospects of these patients are at stake.

There seems to be a direct relationship between the duration of HAART and increased body fatness. It is strongly recommended that more emphasis be placed on a healthy diet, physical activity and complications associated with abdominal fat distribution in the treatment of HIV positive patients on HAART.

Considering the quadruple burden of disease in SA, it is important that HAART patients should be monitored to effectively manage incidence of metabolic diseases in this population. The costs implicated in managing such diseases exceed the country's limited resources. In summary, HAART patients are at increased risk of developing dyslipidaemia and dysglycaemia after exposure to HAART especially PIs at longer durations.

One of the limitations for this study was that there was no control group consisting of HIV positive HAART naïve patients to further validate the effects of HAART. However, Mashinya et al., (2014) conducted a study in the Limpopo Province Dikgale area, which is a rural area around Mankweng situated under Capricorn district. It is therefore important to compare their study findings with the current study due to the populations being from the same area as the current study. Their sample size constituted 89 HIV-positive HAART naïve patients, furthermore they indicated that sample size was a limitation to the study. In summary they reported that the prevalence of DM was the same in the HIV positive HAART naïve group and the HIV negative control group (13.5%). Furthermore they found that glucose levels were significantly lower in females compared to males. These findings are consistent with the present study findings as the prevalence of DM was more in males compared to females. They also reported altered lipid profiles with significantly lower TC, HDL-C, non HDL-C and LDL-C in HIV-positive HAART naïve patients. Furthermore they reported HAART naïve patients were 2.3 times likely to have low HDL-C levels than HIV negative group. Therefore in the current study, HDL-C levels were all normal across all regimens indicating that HAART increases HDL-C levels. Moreover the previous study found significant decreases in TC, HDL-C, and LDL-C in HAART naïve patients. HAART was found to significantly reduce TC and LDL-C levels in patients taking 1$^{st}$ line regime but not in the PI group. These findings suggest that lipid alterations in HAART patients are primarily due to the use of PIs and that other factors such as HIV positive status, exposure to PIs in longer durations of HAART may determine the severity of dyslipidaemia in HAART patients.

## 6.2 LIMITATIONS OF THE STUDY

The findings of this study will not be applicable to all races as the population consisted of black Africans. Although this was not intended, we found that the majority of the patients attending the clinics selected were black Africans. There were no age and gender matched controls who were HIV negative or who were HIV positive and not on HAART due to the design of the study being a prospective cohort. The drop-out rate in the 6–12 month follow-up reduced the sample size; therefore, a low statistical power may be obtained within the different treatment arms when the initial baseline sample is matched with the follow-ups. The levels of LDL-C were calculated and not measured directly, underestimation of these levels using prediction equations such as the above mentioned may under diagnose LDL-C levels in predicting risk of CVD.

The study was conducted over a period of 18 months with follow-ups being only up to 12 months. Some patients were switched to the new FDC which made the analysis complex. Statistically gender-based differences were clearly demonstrated; however, the much smaller number of males compared to females reduced the significance of these findings. This is a general limitation in most clinical studies as males are less likely to attend clinics because the majority of them are sole breadwinners and thus unable to take leave from work (Nell et al., 2015). Due to the nature of the study, patients had to come fasting. Patients agreed to meet appointments only during their time to collect treatment making it a lengthy contact time resulting in participants reluctant to do follow-ups.

## 6.3 RECOMMENDATIONS

The following suggestions are recommended to the Department of Health:

- Start a joint project with the Department of Agriculture and Forestry; and Water Sanitation to provide vegetable and fruit seeds and also training the population on how to grow these plants and sustain them, especially in areas such as Limpopo where the land area is sufficient for plant production, as part of the problem may be that a lot of patients were not eating a healthy diet consisting of a variety of vegetables and fruits.
- Educate the HIV population on the importance of eating healthy and put more emphasis on the complications associated with unhealthy eating and physical inactivity.
- Weight loss interventions should be strictly emphasized with a priority on exercise and diet.
- Encouragement of patients to engage in several sport activities more frequently such as a 45 minute brisk walk every day.
- Encourage and promote the adequate utilizing of existing recreational facilities for improving weight management.
- Initiation of overweight and obesity campaigns among health centres.
- Integrate health care systems where all diseases may be managed under one roof rather HIV patients having their own clinics this will assist in managing all the diseases holistically as HIV is considered a chronic disease. This in turn will reduce the costs associated with treating clusters of metabolic abnormalities in one individual.
- Routine assessments of CDL risk factors such as body fatness, body fat distribution, lipograms and fasting blood glucose may be conducted. These procedures may be achieved by simple, inexpensive methods and are not time consuming. Fasting blood glucose and lipogram may be measured using a simple finger prick and this can be done whenever the patients collect their treatment.

- Routine measurements such as measuring weight and height are already in place in health facilities however WC and W: ratio can also be easily measured using a measuring tape.
- Body fat distribution can be easily measured using skinfolds. Training of personnel to conduct and monitor these assessments may further reduce the cost burden of treating these diseases when they start to prevail in the HIV population.

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## Appendix A

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1.** | **Personal information** | | | | | | | | | | | | | | |
| | Subject number | | Gender | | Male | | Age | | | | | | | | |
| **1.** | | | | | Female | | | | | | | | | | |
| **2.** | **Medical History** | | | | | | | | | | | | | | |
| **2.1** | **History of CDL?** | YES | | NO | | | | | | | | | | | |
| **2.1.1** | **If YES, specify** | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | |
| **2.2** | **Are you using HAART?** | | | | | | | | | YES | NO | | | | |
| **2.2.1** | **If YES, how long (in months)?** | | | | | | | | | | | | | | |
| **2.2.2** | **Describe the regimen** | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | |
| **2.3** | **If No to 2.2, are you using alternative treatment?** | | | | | | | | | YES | NO | | | | |
| **2.3.1** | **If YES, how long?** | | | | | | | | | | | | | | |
| **2.3.2** | **Describe the regimen** | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | |
| **3.** | **Survey data** | **Height** | **Weight** | **WC** | **HC** | **Waist: Hip ratio** | **Glucose (mmol/l)** | **Insulin (mmol/l)** | **Leptin (ng/ml)** | **Lipid profile** | | | | **BMI** | |
| | | | | | | | | | | **Total cholesterol** | **HDL-C** | **LDL-C** | **Triglycerides** | | |
| | **T0** | | | | | | | | | | | | | | |
| | **T6–12** | | | | | | | | | | | | | | |

## APPENDIX B

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

**ETHICS COMMITTEE**
**CLEARANCE CERTIFICATE**
**UNIVERSITY OF LIMPOPO**
**POLOKWANE MANKWENG HOSPITAL COMPLEX**

UNIVERSITY OF LIMPOPO

**PROJECT NUMBER** : PMREC – 71/2013

**TITLE** : The effect of highly active Anti-Retroviral treatment on glucose and lipid metabolism in human immunodeficiency virus positive patients at clinics in the Polokwane Local Municipality

**RESEARCHER** : Ms MM Mashao

**ALL PARTICIPANTS** : N/A

Supervisor : Dr M van Staden

**DATE CONSIDERED** : 07 May 2013

**DECISION OF COMMITTEE**

- Recommended for approval

**DATE** : 30 October 2013

**PROF A J MBOKAZI**
**Chairperson of Polokwane Mankweng Hospital Complex Ethics Committee**

**NOTE:** *The budget for research has to be considered separately. Ethics committee is not providing any funds for projects.*

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA
CONFIDENTIAL

# DEPARTMENT OF HEALTH AND SOCIAL DEVELOPMENT
## CAPRICORN DISTRICT

Ref. 6/3/1
Enq: Maumela M.B
Tel: (015) 290 9149/082 883 9922
Date: 10/05/2013

**TO: Ms M. M. Mashao**
**School of Molecular & Life Sciences**
**University of Limpopo**
**Sovenga**
**0727**

**PERMISSION TO CONDUCT RESEARCH STUDY ON THE EFFECT OF HIGHLY ACTIVE ANTI-RETROVIRAL TREATMENT ON GLUCOSE AND LIPID METABOLISM IN HIV POSITIVE PATIENTS.**

1. The above matter has reference.
2. We sincerely wish to acknowledge receipt of your approval letter to conduct Research study in Capricorn District.
3. The District wish to inform you that your request to conduct the above mentioned Research study in clinics around Polokwane Local Municipality has been granted.
4. Please, take note that our District representative in this regard will be Ms M.B Maumela, please; feel free to communicate with her.

Regards,

Phebjane 100513

**DISTRICT EXECUTIVE MANAGER** **DATE**

CONFIDENTIAL

Private Bag: X9530, Polokwane 0700, 34 Hans van Rensburg St. Polokwane 0700
Tel.: (015) 290 9000, Fax: (015) 291 5917 / 3934, Website: http/www.limpopo.gov.za

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UNIVERSITY OF LIMPOPO
Medunsa Campus

*MEDUNSA RESEARCH & ETHICS COMMITTEE*

*CLEARANCE CERTIFICATE*

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|---|---|
| **MEETING:** | **08/2012** |
| **PROJECT NUMBER:** | **MREC/ML/248/2012: PG** |
| **PROJECT:** | |
| **Title:** | The effect of highly active anti-retroviral treatment on glucose and lipid metabolism in human immunodeficiency virus positive patients at clinics in the Polokwane Local Municipality, Limpopo Province, South Africa |
| **Researcher:** | Ms MM Mashao |
| **Supervisor:** | Dr M van Staden |
| **Co-supervisor:** | Mr LJC Erasmus |
| **Department:** | Physiology and Environmental Health |
| **School:** | Molecular and Life Sciences |
| **Degree:** | Master of Science Physiology |

**DECISION OF THE COMMITTEE:**

MREC approved the project.

**DATE:** 11 October 2012

**PROF GA OGUNBANJO**
**CHAIRPERSON MREC**

The Medunsa Research Ethics Committee (MREC) for Health Research is registered with the US Department of Health and Human Services as an International Organisation **(IORG0004319)**, as an Institutional Review Board **(IRB00005122)**, and functions under a Federal Wide Assurance **(FWA00009419)**
Expiry date: **11 October 2016**

**Note:**
i) **Should any departure be contemplated from the research procedure as approved, the researcher(s) must re-submit the protocol to the committee.**
ii) **The budget for the research will be considered separately from the protocol.**
**PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.**

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