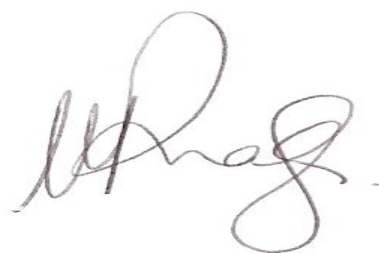# DECLARATION

I hereby declare that the whole thesis, unless specifically indicated to the contrary

in the text, is my own original work and has not been submitted at any other university

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NOMBULELO PRINCESS MAGULA

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AYESHA AHMED MOTALA

# METABOLIC COMPLICATIONS OF ANTIRETROVIRAL THERAPY (ART) IN A SOUTH AFRICAN BLACK POPULATION

SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE

DEGREE OF DOCTOR OF PHILOSOPHY

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SOUTH AFRICA

# DEDICATION

Abi, Siphe and Palesa: for the time you sacrificed to be with your mom while she was working away at the computer;

Abi, for your teen years that I worked so hard through and still turning out into the young lady that you have.

Biza, for all your support and encouragement through it all.

Mama no Tata, for the woman I am today.

I am eternally grateful.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| ACTG | Aids Clinical Trials Group |
| ADA | American Diabetes Association |
| ALP | Alkaline phosphatase |
| ALT | Alanine transaminase |
| ART | Antiretroviral therapy |
| BMI | Body mass index |
| BREC | Biomedical Research Ethics Committee |
| BSCL | Berardinelli-Seip congenital lipodystrophy |
| DAD | Data Collection on Adverse Events of Anti-HIV Drugs |
| DBP | Diastolic blood pressure |
| DM | Diabetes Mellitus |
| DXA | Dual Energy Xray Absorptiometry |
| FPLD1 | Kobberling familial partial lipodystrophy |
| FPLD2 | Familial partial lipodystrophy of the Dunnigan type |
| FRAM | Fat Redistribution and Metabolic Change in HIV Infection |

| | |
|---|---|
| GGT | Gamma glutamyl transferase |
| $HbA_{1c}$ | Haemoglobin $A_{1c}$ |
| HDL | High density lipoprotein cholesterol |
| HIV | Human Immunodeficiency Virus |
| IFG | Impaired fasting glucose |
| IGT | Impaired glucose tolerance |
| JNC | Joint National Committee |
| KEH | King Edward VIII Hospital |
| LDL | Low density lipoprotein cholesterol |
| NNRT | Non-nucleoside reverse transcriptase inhibitors |
| NRTI | Nucleoside reverse transcriptase inhibitors |
| OGTT | Oral glucose tolerance test |
| PI | Protease inhibitor |
| PPAR | Peroxisome proliferator-activated receptor gamma |
| SBP | Systolic blood pressure |
| SREBP | Sterol Regulatory Element-binding Proteins |
| VCT | Voluntary Counseling and Testing |
| WHO | World Health Organization |

# ABSTRACT

## Aims

To determine the prevalence and incidence of lipodystrophy (fat distribution [lipoatrophy and lipohypertrophy] and metabolic complications [insulin resistance-dysglycaemia and dyslipidemia]) in HIV-1 infected adult subjects of second generation Zulu descent at baseline and during 24 months of follow-up on antiretroviral therapy (ART).

## Methods

The total study group included three groups: HIV infected ART naive patients eligible for ART (HIV-ART, n=150), age, gender and ethnically matched HIV infected not eligible for ART (HIV-no ART, n=88) and HIV negative (control, n=88) subjects. All participants had demographic, anthropometric, biochemical and radiological assessments at baseline; in addition, the HIV-ART group had follow-up assessments for 24 months on ART (tenofovir, lamivudine and nevirapine or efavirenz). Fat distribution was assessed using FRAM questionnaires, computerized tomography (CT) scans and dual energy absorptiometry X-ray (DXA). Disorders of glycaemia (diabetes mellitus (DM), impaired glucose tolerance and impaired fasting glucose) were defined using WHO criteria. Total, LDL, HDL cholesterol and triglycerides were measured for each group; CD4 cell count and HIV RNA for group 2 and 3, at baseline, 3, 6, 12, 18 and 24 months. Poisson approximations estimated incidence of disorders of glycaemia.

## Results

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At baseline, when compared with the control group, the mean BMI (kg/m$^2$) was significantly lower in the HIV-ART and HIV-no ART subjects (26.4 vs. 28.6 vs. 29.1; p =0.01). Prevalence of lipoatrophy as measured by participant and physician examination questionnaires was similar in the three groups. Visceral and subcutaneous fat area by CT scan were similar between the groups but limb and trunk fat mass by DXA scan was significantly lower in the HIV-ART compared to control subjects. In the HIV-ART group, at the 24 month follow-up, there was a significant mean reduction in HIV RNA (p<0.0001) and increase in CD4 cell count (p<0.0001). The mean BMI increased to 29.4 kg/m$^2$ and no lipoatrophy developed; DXA scan showed a 33.6% increase in trunk fat mass (mean difference 4.2 kg, p <0.0001) and 30.8% increase in total fat mass (mean difference 9.4 kg, p < 0.0001); visceral (p 0.005) and subcutaneous (p 0.0002) fat area also increased.

At baseline, the prevalence of DM was 0% in HIV-ART and HIV-no ART and 4.9% in control subjects (p 0.005); the prevalence of "any dysglycaemia" was 3.7% in HIV-ART and HIV-no ART compared to 8.6% in control subjects. When compared with group 1, mean values in group 3 were lower for the following serum lipids: total cholesterol (p<0.0001), LDL (p=0.0007) and HDL (p<0.0001). There was no difference in mean total triglycerides in the three groups (p=0.3).  During follow-up, in the HIV-ART group, using glucose-based WHO criteria, the incidence of diabetes mellitus was 2.3 per 100 person year follow-up (PYFU) and of "any dysglycaemia" 7.6 per 100 PYFU. The only independent predictor of DM was visceral: subcutaneous fat ratio measured by CT scan (HR 2.95 [95% CI 1.25-6.98], p 0.01). Significant predictors for development of "any dysglycaemia" included systolic blood pressure (HR 1.04 [95%CI 1.02-1.07], p=0.0006), serum albumin (HR 0.85 [95% CI 0.76-0.94], p=0.002), CD4 cell count (HR 0.988 [95%CI 0.978-0.997], p=0.01) and efavirenz (HR 6.27 [95%CI 1.65-23.80], p=0.01) Serum total (p<0.0001), LDL (p<0.0001) and HDL-cholesterol (p<0.0001) increased significantly during follow-up.

**Conclusion:**

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In this cohort of South Africans with HIV-1 infection, at baseline (prior to ART) there was no significant fat redistribution or lipoatrophy and an absent to low prevalence of dysglycaemia. In the follow-up study, ART use was not associated with lipoatrophy although there was significant increase in BMI and in limb and trunk fat mass by DXA scan. ART was associated with increased incidence of dysglycaemia. These findings underscore the importance of clinical monitoring on ART. The association of efavirenz with dysglycaemia warrants further evaluation.

# CHAPTER ONE: INTRODUCTION AND BACKGROUND

## 1.1    Lipodystrophy

The advent of combination antiretroviral therapy (ART) for the management of people infected with the human immunodeficiency virus (HIV) has substantially reduced the number of opportunistic infections and mortality. This success is related to the reduction in the viral load and immune function recovery (1-3).

Despite the clinical and immunological benefits of ART, survival with ART appears to be associated with changes in metabolic function and body composition described as lipodystrophy. Generally, lipodystrophic syndromes may be classified as genetic or acquired, generalized or partial. The genetic forms of lipodystrophy include Berardinelli-Seip congenital lipodystrophy (BSCL), familial partial lipodystrophy of the Dunnigan type (FPLD2) and Kobberling familial partial lipodystrophy (FPLD1) among others. Acquired forms of lipodystrophy include the metabolic syndrome (syndrome X) and HIV-related lipodystrophy linked to antiretroviral therapy (4).

HIV-related lipodystrophy is a term that has been used to collectively describe fat accumulation (lipohypertrophy), subcutaneous fat wasting (lipoatrophy), dyslipidemia and insulin resistance (5). Lipohypertrophy has been shown to develop within the abdomen, dorso-cervical fat pad ("buffalo hump"), anterior neck and breasts among participants receiving ART (6). Lipoatrophy involves the loss of subcutaneous fat in the face, arms, legs, abdomen, and/or buttocks. In contrast to the traditional wasting syndrome of advancing HIV disease, lipoatrophy is distinguished by the preferential loss of fat tissue without substantial loss of lean tissue mass and by the fact that it most frequently occurs among participants who are responding to HIV therapy (7, 8).

HIV-related lipodystrophy emerged soon after the wide-spread adoption of ART in resource-rich countries and it was initially attributed to the use of protease inhibitors (PI) (9). However, there were subsequent reports in patients that were treated with nucleoside reverse

transcriptase inhibitors (NRTIs), particularly thymidine analogues. The wide variation of prevalence of HIV-related lipodystrophy (2%-84%) previously reported may partly be explained by an absence of a consensus case definition of lipodystrophy (10).

Carr et. al (11) objectively defined lipodystrophy using a case definition that included the following characteristics: demographic (age and gender), clinical (duration of HIV infection, HIV disease clinical stage, waist:hip circumference ratio), metabolic (anion gap, HDL cholesterol), body composition (leg fat, trunk:limb fat ratio, intra-abdominal:subcutaneous abdominal fat ratio). This case definition had a 79% (95% CI 70-85) sensitivity and 80% (95% CI 71-87) specificity for the diagnosis of lipodystrophy.

The Study of Fat Redistribution and Metabolic Change in HIV infection (FRAM) (12) proposed a new methodology for evaluating the individual components of lipodystrophy. FRAM was initiated in the United States in 2000 to evaluate the prevalence and correlates of changes in fat distribution, insulin resistance and dyslipidemia in a geographically and ethnically diverse population of men and women infected with HIV compared with controls.

Using the FRAM tools, it was found that HIV infected men were more likely to have peripheral lipoatrophy in at least one site compared with controls (38% vs. 5%; p<0.001) (13). More HIV infected men than controls had clinical central lipoatrophy (8% vs. 3%; p=0.03), however, central lipohypertrophy was less frequent (40.2% vs. 55.9%; p=0.001). HIV infected women were more likely to have peripheral lipoatrophy in at least one site compared with controls (28%vs 4%, p < 0.001) (14). The prevalence of clinical central lipoatrophy was low for both HIV and control (6% vs 3%, respectively, p = 0.44) and the prevalence of central lipohypertrophy in at least one site was very high (62% vs 63%, p = 0.91). One or more of the individual components of lipodystrophy may occur in an individual.

The first study to examine the effect of NNRTIs and PIs on body composition in Africa, found increased central and reduced peripheral fat in association with ART (15). Increasing time on stavudine was associated with a decrease in leg fat mass. While changing from NNRTI to PI

(and substituting stavudine with zidovudine), reversed peripheral fat loss partially. In this study it would be difficult to attribute a specific ART agent as an etiologic factor as these are used as combination therapy; however, stavudine has been shown to be associated with fat redistribution (16).

In light of a lack of data from Africa available for use in diagnostic criteria for lipodystrophy, Abrahams et.al recently developed an objective measure to define lipoatrophy and lipohypertrophy (17). In this cross-sectional study, anthropometric measures were found to be as good as dual energy Xray absorptiometry (DXA) derived measures to diagnose lipoatrophy. A significant association between lipoatrophy and time on ART was found, in particular stavudine. Triceps skinfold thickness was found to be a predictor of lipoatrophy.

Micklesfield et. al compared waist measures and two DXA methods for assessing fat in the abdominal region to CT derived visceral fat in black vs. white South African women (18). This study found that DXA and anthropometric measurements are not able to distinguish between population groups.

The paucity of data in Africa on lipoatrophy and lipohypertrophy highlights the need for studies in Africa on measurement of fat redistribution in HIV infected subjects, in particular, longitudinal studies.

Metabolic complications that may be associated with the lipodystrophy syndrome include dyslipidemia (increase in total cholesterol, low density lipoprotein (LDL) cholesterol and triglyceride (Tg) levels), glucose homeostasis abnormalities, asymptomatic hyperlactatemia and bone demineralization (19). Other studies showed that lipid changes in HIV-infected patients include an early decrease in high density lipoprotein (HDL) cholesterol followed by a decrease in LDL cholesterol, with subsequent increase in Tg levels and very low density lipoprotein cholesterol (VLDL) in later stages (20). The pro-atherogenic effects of decreasing HDL, increasing Triglycerides and VLDL seem to outweigh the anti-atherogenic effect of decreasing LDL. Symptomatic lactic acidosis has also been described (21). Most of these factors are established risk factors for

cardiovascular disease and evidence shows that premature cardiovascular disease, cerebrovascular disease and endothelial dysfunction are possibly linked to both effects of the antiretroviral drugs and HIV infection itself (22-27). Further sequelae of lipodystrophy include poor quality of life related to the physical changes that accompany these complications. This may affect adherence to ART and lead to treatment failure. The signs of lipodystrophy usually appear progressively over a period of 18 to 24 months.

The investigation of fat redistribution, metabolic changes including insulin resistance and dyslipidemia has received much attention in developed countries where highly active antiretroviral therapy (ART) has been available for about three decades (12). The choices of antiretroviral drugs are much wider and agents with the least toxicities are chosen, with treatment options individualized.

Emergence of diabetes associated with the use of ART has also been linked with traditional risk factors such as age (28), gender (29), family history (29, 30), obesity and visceral adiposity(29), lipid disturbances and metabolic syndrome (31), found to confer risk. It may be possible that HIV infection accelerates development of diabetes in subjects that are already at risk as in the general population.

The Swiss HIV cohort and the French 10-year study reported an increasing incidence of diabetes with increasing age (28, 29). The majority of studies in developed countries included men and male sex was associated with a 60% higher relative risk of developing diabetes. In the D:A:D study, there was a two-fold higher rate in overweight (body mass index 26-30 kg/m$^2$) participants and four-fold higher rate in obese participants (body mass index >30 kg/m$^2$) compared to those with a normal body mass index (31).

The American Diabetes Association and World Health Organization have now included HbA$_{1c}$ in addition to the gold standard oral glucose tolerance test (32-34) in the diagnostic criteria for diabetes. Adjusted estimates in sub-Saharan populations have suggested inverse associations between HIV and HbA$_{1c}$, and positive associations between ART exposure and HbA$_{1c}$. The first

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study to examine how the inclusion of $HbA_{1c}$ in the definition of diabetes diagnosis impacts on the association between HIV and diabetes risk, used fasting glucose and $HbA_{1c}$ as part of criteria (35). HIV infection was found to be associated with a greater risk of diabetes. Inclusion of $HbA_{1c}$ in the diagnostic criteria increased the accuracy of the diabetes diagnosis while exclusion of confirmatory criteria overestimated the incidence (35). There is no data in Africa on the incidence of diabetes using the $HbA_{1c}$ criteria.

## 1.2    Antiretroviral Therapy in South Africa

HIV prevalence escalated in South Africa (SA) and ultimately treatment was provided in 2004 with later revision of treatment initiation CD 4 cell count cut-off and treatment regimen to address complications experienced by patients. The HIV-1 prevalence increased from 0.7% in 1990 to 29.5% in 2004 among women attending antenatal clinics in SA. Antiretroviral therapy was not available to the public health sector in the country over this period. SA currently bears the burden of having the largest population living with HIV-1 in any one country with an estimated 5.6 million of the 34 million people infected worldwide; consequently, the treatment program for HIV-1 infected people in this country is the largest in the world (36, 37).

The primary goal is to provide treatment to as many people as possible. SA reached the target goal of universal access to antiretroviral treatment set by WHO as the number of those receiving treatment reached 2 million in October 2012 (37). The major limitation to improving on this ambitious goal is that the treatment options remain narrow. Individualization of treatment is attempted within a very narrow spectrum of available antiretroviral therapies. In SA, as in many other resource-constrained countries, the ideal of using the least toxic antiretroviral therapies remains elusive. Evidence informing optimal treatment strategies is limited and many treatment related questions remain unanswered.

Treatment guidelines in SA are in line with the World Health Organization guidelines which recommend the use of one non-nucleoside reverse-transcriptase inhibitor (NNRTI) and two nucleoside reverse transcriptase inhibitors (NRTI) as part of ART in the initial treatment of HIV-1

infected patients in resource constrained countries. Protease inhibitors (PI) are included in the second line of treatment, largely for patients who experience treatment failure or NNRTI related complications. Antiretroviral associated toxicities are one of the deterrents for treatment uptake and adherence by some patients.

When SA rolled out antiretroviral therapy in the public sector in 2004 for the first time, first line therapy included stavudine, lamivudine (NRTI'S) and nevirapine or efavirenz (NNRTI). The choice of stavudine as the NRTI in the first line treatment was dictated by cost despite severe metabolic complications associated with this agent. The government's delay in the provision of antiretroviral treatment while the epidemic was rising allowed for myths about the treatment to emerge. These myths consequently caused some patients to become apprehensive about taking treatment, when it was eventually offered. Treatment related complications also contributed to reluctance by some patients to take antiretroviral therapy.

The poor uptake of antiretroviral therapy was shown in the antiretroviral rollout program at King Edward VIII hospital where 25% of patients that were assessed to be eligible and offered antiretroviral therapy, never returned to the clinic to initiate antiretroviral therapy (38). The initial enthusiasm attributable to reduction in morbidity and mortality in those who started antiretroviral therapy was quickly dampened by reports of treatment related complications including lactic acidosis with associated high mortality rate,(39) immune reconstitution inflammatory syndrome, (40) and experiences of stigma reported by patients in relation to changing body habitus associated with taking antiretroviral treatment.

Soon after the rollout of antiretroviral treatment in the public sector, there were reports of metabolic toxicities related to ART (39, 41). The SA antiretroviral treatment program was subsequently characterized by stepwise changes in antiretroviral agents offered in response to treatment related complications. The first strategy adopted in response to metabolic toxicities and associated increased morbidity and mortality was to reduce the dose of stavudine used. This strategy followed the amendment to the WHO treatment guidelines for adults and adolescents (42).

The amendment to the WHO guidelines was informed by findings of a systematic review that demonstrated that there was no significant difference in virologic efficacy associated with the use of low dose stavudine compared with the standard dose of 40 mg BD for patients weighing 60 kg or more and 30 mg BD for patients weighing less than 60kg (43). A Cochrane systematic review was designed to evaluate the evidence supporting the use of low dose stavudine for reducing stavudine related metabolic and other toxicities with no compromise on efficacy (44). This review showed that trials that compared low dose with high dose stavudine had only included patients in developed countries, already on ART with sustained virologic suppression prior to randomization to low or high dose stavudine (16). No trials that included patients that were treatment naïve with high viral loads were identified, yet the stavudine dose reduction strategy was subsequently applied to patients that were treatment naïve, with high viral loads in developing countries. The national guidelines currently recommend stavudine at low dose, where it is indicated (45).

The next strategy to reduce metabolic complications targeted replacement of stavudine. The rank order of the NRTI's ability to cause mitochondrial toxicity in vitro is greatest with zalcitabine, followed in declining order by didanosine, stavudine and zidovudine. The least toxic agents are abacavir, lamivudine and tenofovir whose toxicity is considered to be the same (46). The NRTI agents available in the public sector at the start of the antiretroviral rollout program were the thymidine analogues and included stavudine, lamivudine, zidovudine and didanosine. These thymidine analogue NRTI agents are associated with more metabolic complications.

Access to non-thymidine NRTI analogues such as tenofovir and abacavir was limited until 2010. The replacement of stavudine with the non-thymidine analogue NRTI tenofovir was the next strategy by the South African government for reducing ART related metabolic toxicities. According to the SA National ART guidelines with effect from 2010, patients who were enrolled for treatment after this time were treated with an ART regimen that included tenofovir instead of stavudine. However, access to tenofovir remained a challenge in certain parts of the country with intermittent reports of stock-outs. Patients who were treated with stavudine prior to the change in guidelines are continued on stavudine except where stavudine-related complications are identified. The current strategy is to

change patients who are taking stavudine to tenofovir-based fixed drug combination. Stavudine remains in the guidelines for specific indications and whether phasing it out completely is sustainable has been a cause for much debate (47-49).

In light of evidence that initiation of ART at higher CD 4 cell counts is associated with better outcomes (50), the SA treatment program increased the CD 4 cell count for initiating treatment from 200 to 350 cells/mm$^3$. The cost of getting treatment to all who need it as the CD4 cell count for starting treatment has been increased to 350cells/mm$^3$ may dictate continued use of stavudine as alternative agents are more expensive (51). In January 2015 the national department of health guidelines increased the threshold for starting ART further to 500 cells/mm$^3$.

## 1.3   Cardiovascular Risk Factors

While specific agents are proposed to be the culprits for causing lipodystrophy, the interplay between ART, HIV and host factors may explain why some patients develop lipodystrophy while others are spared. A number of genes have been implicated in the development of HIV lipodystrophy. The Aids Clinical Trials Group (ACTG) 384 study of participants randomized to regimens that included didanosine-stavudine or zidovudine-lamivudine showed that individuals that were heterozygous carriers of the common hemochromatosis HFE 187C>G polymorphism experienced significantly less limb fat loss than non-carriers (52). Therefore this genetic polymorphism that affects iron transport may confer some protection against lipoatrophy among those treated with ART.

Peroxisome proliferator- activated receptor- gamma (PPAR)-gamma has been investigated in the pathogenesis of lipodystrophy as it is a transcription factor that is necessary for the development of mature adipocytes. PPAR-gamma expression may be induced by sterol regulatory element-binding protein 1 (SREBP-1), C/EBP-beta and delta (53). The expression of PPAR-gamma and SREBP-1c has been shown to be decreased in subcutaneous adipose tissue of patients with ART-associated lipodystrophy compared with ART-treated patients without

lipodystrophy (54). Among 178 South African Zulu subjects with type 2 diabetes and 200 healthy ethnically matched controls, common variants in the PPAR-gamma gene was not found (55). The plasma concentrations of adiponectin are reduced in both insulin-resistant conditions and in type 2 diabetes. Recently, low levels have also been demonstrated in non-HIV and HIV associated lipodystrophies (56-58).

While the interplay between ART, HIV and host factors remains unknown, it has been shown that the more advanced HIV disease is associated with a higher risk of complications. In general, initiation of ART at a higher CD 4 cell count is associated with better outcomes (50). The CD 4 cell count for initiating ART was then increased from 200cells/mm$^3$ and below to 350cells/mm$^3$ and below (45).

To date, much of the available literature on the prevalence and incidence of HIV-associated metabolic complications are those from developed countries, with little information from developing countries with resource-constrained settings. Developing countries, including in Africa, are set to have the greatest increase in non-communicable diseases such as diabetes over the next 20 years (59).

Estimates from the International Diabetes Federation on diabetes for 2030 suggest that the number of adults with diabetes will expand by 54% for the world and 90% or larger for Africa (34). A study in SA predicted that diabetes prevalence will increase irrespective of effects of HIV/AIDS on population growth rates (60). Estimates from the World Health Organisation on ischaemic heart disease for 2030 predict that it will be among the first three causes of death in the HIV population in low-income countries (61). In studies that controlled for traditional cardiovascular disease risk factors when comparing carotid intima media thickness (IMT), two studies found increased IMT in HIV-infected patients relative to controls (62, 63). The Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study suggests that the risk of a myocardial infarction is more than doubled among HIV infected patients with diabetes (64).

Observational studies in Europe and North America have shown higher rates of coronary heart disease compared to the general population (65, 66). Several studies have shown a link between coronary heart disease and exposure to PIs. Two observational studies have shown that with sufficient exposure to PIs, duration of exposure to PIs was associated with an increased risk for myocardial infarctions (67, 68).

In subjects who are ART naïve, there was a suggestion that HIV infection could promote atherosclerosis through mechanisms including: immune activation, chronic inflammation, coagulation disorders and lipid disorders (69).

Diabetes and HIV infection have both been independently associated with an increased risk of developing atherosclerosis. The association between HIV and diabetes is unclear. A higher incidence has been found in some studies (70, 71), while others have shown a similar (72, 73) and also lower (74) risk compared to uninfected controls.The intersection of the HIV epidemic, increasing use of antiretroviral therapies, rising prevalence of diabetes and dyslipidaemias are likely to bring about a new epidemic of ischaemic heart disease in a population that previously did not suffer much from ischaemic heart diseases.

The goal of this study was to evaluate the prevalence and incidence of lipodystrophy and associated metabolic complications among HIV infected subjects. These metabolic complications, if present and coupled with traditional risk factors, would constitute significant risk for cardiovascular disease in this population. Knowledge gained from examination of the prevalence and incidence of these complications will contribute to the design of  strategies for preventing or reducing  the risk factors for metabolic disorders and consequences thereof.

## 1.4    Aims and Objectives

**Aim of study:**

To determine the prevalence and incidence of lipodystrophy (fat distribution [lipoatrophy and lipohypertrophy] and metabolic complications [insulin resistance-dysglycaemia and dyslipidemia]) in adult HIV-1 infected subjects of second generation Zulu descent at baseline and during 24 months of follow-up on antiretroviral therapy (ART).

**Specific Objectives**

**Primary:**

To determine, in a group of healthy HIV-infected ART naïve subjects (study group) and an age and gender matched healthy non HIV-infected subjects (control group)

- Fat distribution patterns and prevalence of lipodystrophy

To evaluate, in a cohort of study subjects commenced on ART and followed up prospectively for at least 24 months

- The incidence of lipodystrophy
- The determinants for the development of lipodystrophy

**Secondary:**

To evaluate, in the cohort commenced on ART, the following:

- Clinical, immunological and virologic response to ART
- Effect of switching HIV drug regimens in a subgroup of subjects who develop lipodystrophy
- 

**Hypothesis**

It is hypothesized that lipodystrophy (lipo-atrophy, fat accumulation, insulin resistance and dyslipidemia) and associated metabolic complications is common in black patients receiving ART .

In order to prove this hypothesis, an ethnically homogeneous cohort of black patients treated within the South African Antiretroviral Treatment Rollout Program was investigated and compared with healthy HIV-1 infected patients with high CD 4 cell counts and HIV negative volunteers in order to elucidate the interplay between HIV-1, ART and host factors.

# CHAPTER TWO: RESEARCH DESIGN AND METHODS

## 2.1.     Section A

The study was conducted at King Edward VIII Hospital (KEH), Durban, KwaZulu-Natal. KEH is a teaching hospital of the Nelson R Mandela School of Medicine of the University of KwaZulu-Natal (UKZN) that serves a predominantly black population. Recruitment started at the KEH Voluntary Counselling and Testing centers. Study subjects were also recruited from patients presenting to the HIV clinic for initiation of combination antiretroviral therapy (ART) at KEH. Staff members of KEH and the Nelson R Mandela School of Medicine, UKZN including students were also invited to participate. Once clinical counselling about the HIV results was completed, individuals were informed about the study and invited to participate.

During the screening visit, the study coordinator and physician reviewed the study protocol with potential study participants. Eligibility criteria were evaluated and informed consent (Appendix C) obtained in either Zulu or English from those who met the criteria the inclusion criteria . Approval for the study was obtained from the Biomedical Research Ethics Committee (BREC) of the Nelson R Mandela School of Medicine, University of KwaZulu-Natal. BREC Reference number BF 096/09.

The study was conducted in two steps (Figure 2.1). Step 1 was  a cross-sectional design that determined the patterns of fat distribution and prevalence of lipodystrophy in a group of healthy HIV-infected, ART naïve subjects (study group) and an age and gender matched, healthy non HIV-infected volunteers (control group). Step 2 of the study was a prospective cohort design that evaluated the incidence of lipodystrophy and associated metabolic complications in a cohort of black patients who were ART naïve and commencing ART within the South African Antiretroviral Treatment Program and followed up for at least 24 months. This study examined individual components of

lipodystrophy in this population using the basic principles of the Study of Fat Redistribution and Metabolic Change in HIV Infection (FRAM) (12).

The major objectives of FRAM were to study each component of the lipodystrophy syndrome, determine the associations among the body fat depots, determine the factors associated with fat distribution in HIV infection by separately examining factors associated with the volume of each regional subcutaneous or visceral adipose tissue depot in comparison with controls, determine the HIV and non HIV-related factors associated with insulin resistance and dyslipidemias (among HIV studies, the ability to examine the association of regional subcutaneous and visceral adipose tissue and metabolic abnormalities is unique to FRAM) (12). Permission was obtained from the FRAM investigators to use the FRAM tools to investigate the individual components of lipodystrophy in this study population. This study differs from FRAM in the homogeneity of the population studied. Second generation Zulu people were enrolled to enhance the ethnic homogeneity. This study is unique in studying the regional and visceral adipose tissue distribution and metabolic abnormalities in an ethnically homogeneous population and using a cross-sectional and prospective design.

### 2.1.1. Study Design

Step 1 was a cross-sectional design that compared the fat distribution patterns and metabolic profile among HIV infected participants, including those who were healthy and not requiring ART and those requiring ART, with ethnic, age and gender matched healthy non HIV infected subjects. The relationship of lipoatrophy, lipohypertrophy, insulin resistance (disorders of glycaemia) and dyslipidemia with HIV and host factors was investigated by comparing measurements in participants with HIV-1 infection, with measurements from HIV negative participants

Step 2 was a prospective cohort design of participants who were commencing ART. The cohort was followed up at defined time intervals for a minimum duration of 24 months to evaluate the rate of development of lipoatrophy, lipohypertrophy, insulin

resistance (disorders of glycaemia) and dyslipidemia. The relationship between HIV and ART was investigated by comparing baseline measurements in HIV infected participants prior to starting ART with measurements at follow up to 24 months. Blood samples were also stored for later determination of genetic host factors associated with development of the metabolic complications.

**Figure 2.1: Study Design Flow Diagram**

**Step 1**

**Step 2**

Participants presenting at KEH and VCT centers in Durban

→ HIV +

Eligible for ART

(Study group)

→ HIV +

Not eligible for ART

(Study group)

Volunteers → HIV–

(Control group)

HIV+

Eligible for ART

Baseline, 3, 6, 12, 18 and 24 months;

1. ART outcomes

2. Examine incidence and determinants of lipodystrophy

**Outcomes:**

A. lipodystrophy development (by FRAM questionnaire

B. change in:

i. Fat distribution (by CT scan and Dexa scan measures)

ii. Insulin sensitivity (by OGTT)

iii. Lipid levels

iv) Other metabolic changes

At 0, 3, 6, 12, 18 and 24 months

Lipodystrophy in study subjects (not eligible for ART) vs control (HIV-) group

### 2.1.2. Study Population

**Step1**

The study group included South African black men and women with HIV infection, who were of Zulu descent at least second generation with both parents and grandparents being of Zulu descent. Recruited participants had to be 18 years and older, willing and able to provide signed informed consent.

These participants had to have HIV infection. Those who had a positive urine pregnancy test at the time of consent and those unable to offer informed consent were excluded.

The control group included age and gender matched HIV negative healthy volunteers who were also of second generation Zulu descent.

**Step 2**

Step 2 of the study included males and females aged 18 years and older of second generation Zulu descent who were HIV infected and eligible to start ART within the government rollout program. Those who were pregnant as determined by a urine pregnancy test at the time of consent, planning to become pregnant within the next 3 months or unable to give informed consent were excluded. Recruitment for step 1 and 2 of the study took place at Voluntary Counselling and Testing centers at KEH and other clinics within the hospital. Consecutive patients presenting for starting ART at the KEH HIV clinic were informed about the study, assessed for eligibility and invited to participate in the study.

Participants were categorized into three groups, HIV negative for group 1, HIV infected and not eligible to start combination antiretroviral therapy (ART) for group 2 and HIV infected and eligible to start ART for group 3. The eligibility for ART in asymptomatic patients depends on the level of the CD4 cell count. In 2011, the CD4 cell count cut-off for ART eligibility in South Africa changed from $\geq$ 200 cells/mm$^3$ to $\leq$ 350 cells/mm$^3$ (75-77). Eligibility for group 2 was then changed from CD4 cell count > 200 cells/mm$^3$ to CD4 cell count > 350 cells/mm$^3$ and

for group 3 changed from CD4 cell count of $\leq$ 200 cells/mm$^3$ to CD4 cell count of $\leq$ 350 cells/mm$^3$ according to eligibility of initiation of ART.

## 2.2.     Section B

### 2.2.1.  Study Procedures

Study procedures included a self-administered questionnaire, a detailed history and full physical examination, laboratory and radiological (imaging) tests (Section B, D).

### 2.2.2.  Demographic Information

The following demographic information was collected: age, gender, marital status, highest level of education, occupation.

### 2.2.3.  Medical History

Medical history included the following:

- History of opportunistic infections

- Personal and family history of diabetes

- Personal or family history of ischemic heart disease

- History of co-morbidities

- Level of physical activity

- Smoking, alcohol and illicit drug use

- Perception of any weight change or body changes

- Medication History:

o        Antiretroviral agents

o        Prescribed or non-prescribed agents that might affect body composition

- Anabolic agents

- Agents for dyslipidaemia

o    Antihypertensive agents

o    History of insulin use

All participants were requested to complete the self-administered FRAM questionnaire on body fat changes at several locations: the cheeks next to the nose, the lateral aspect of the face, or the legs, arms, buttocks (peripheral sites), back, chest, neck or abdomen.

### 2.2.4. Physical Examination

Each participant received a full examination by the study physician. A physical examination included measurement of blood pressure, urine examination, anthropometry and completion of the physician administered FRAM questionnaire. Two physicians examined participants and completed the questionnaires.

#### 2.2.4.1.    Blood Pressure

The blood pressure was recorded after the subject was seated for at least 5 minutes. American heart Association (AHA) guidelines for measuring blood pressure were applied. for measuring blood pressure were applied (78). Two readings were taken 5 minutes apart. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC7) criteria were used to categorize levels of blood pressure (79).

### 2.2.4.2. Body Mass Index

Body Mass Index (BMI) was calculated as: weight (kg)/ height (m)$^2$

**Table 2.1: WHO classification for BMI (80)**

| BMI(kg/m$^2$) | Classification |
| --- | --- |
| <18.50 | Underweight |
| 18.50 – 24.99 | Average |
| 25.00 – 29.99 | Overweight |
| $\geq$ 30.00 | Obese |

### 2.2.4.3. Other Anthropometry

Anthropometric measurements were conducted as per the A5142 Aids Clinical Trials Group study protocol at visits specified in study procedures and the methods are described in Appendix C.

http://aactg.s-3.com/members/download/other/metabolic/CIRCmeasures.doc.

Measurements included hip, waist, mid-arm, mid-thigh, neck and chest circumferences. Lipomas and skin folds were examined. A skinfold caliper was used to measure skin folds (triceps, mid-thigh, abdominal, sub-scapular).

## 2.2.4.4. Laboratory Tests

Oral Glucose Tolerance Test (OGTT) was performed using WHO criteria(33). For the OGTT, venous blood samples were obtained after an overnight fast (0 – minute sample) and 2 hours after ingestion of 75g glucose monohydrate dissolved in 250 ml water, for measurement of plasma glucose and serum insulin. Plasma glucose was measured by a glucose oxidase method and serum insulin by an immuno-enzymetric assay.

The WHO and American Diabetes Association (32) criteria for the diagnosis of diabetes mellitus and other categories of hyperglycaemia were applied based on OGTT results (Table 2.2 and 2.3). In addition, HbA$_{1c}$ criteria for diabetes were also applied. The synchron system was used to measure HbA$_{1c}$. This system uses two unique cartridges, haemoglobin (Hb) and A$_{1c}$ to determine A$_{1c}$ concentration as a percentage of total Hb. Haemoglobin reagent is used to measure total haemoglobin concentration by a calorimetric method (81).

**Table 2.2: WHO Diagnostic Criteria for Diabetes Mellitus and Categories of Hyperglycaemia(33)**

**Oral glucose tolerance test**

| Category | Venous plasma concentration, (mmol/l ) |
|---|---|
| **Diabetes Mellitus** | |
| Fasting *or* | $\geq 7.0$ |
| 2-h post glucose load | $\geq 11.1$ |
| **Impaired Glucose Tolerance (IGT)** | |
| Fasting (if measured) *and* | $< 7.0$ |
| 2-h post glucose load | $\geq 7.8$ |
| **Impaired Fasting Glycaemia (IFG)** | |
| Fasting | $\geq 6.1$ and |
| and (if measured) | $< 7.0$ |
| 2-h post glucose load | $<7.8$ |

| **HbA$_{1c}$ (%)** | |
|---|---|
| **Diabetes Mellitus** | $\geq 6.5$ |

**Table 2.3: American Diabetes Association Criteria for Diagnosis of Diabetes (32)**

| Category | Venous Plasma Concentration, mmol/l |
|---|---|
| **Diabetes Mellitus** | |
| Hb A$_{1C}$ | $\geq$ 6.5% or |
| Fasting plasma glucose (mmol/l) | $\geq$ 7.0 |
| 2-hour plasma glucose (mmol/l) | $\geq$ 11.1 |
| Classic symptoms of hyperglycaemia | |
| or hyperglycaemic crisis with a random plasma | |
| glucose (mmol/l) | $\geq$ 11.1 |
| **Impaired Fasting Glucose** | |
| Fasting plasma glucose (mmol/l) | 5.6 – 6.9 |
| **Impaired glucose tolerance** | |
| 2- hour post glucose (mmol/l) | 7.8 – 11.0 |

Fasting serum total cholesterol, total triglycerides, high density lipoproteins (HDL) cholesterol, low density lipoproteins (LDL) cholesterol were measured by an enzymatic calorimetric method with kits from Boehringer Mannheim (Mannheim Germany). LDL cholesterol was calculated by the Friedewald formula(82). The American Association of Clinical Endocrinologist's guidelines for management of dyslipidemia and prevention of atherosclerosis (83) was used to categorise the severity of the elevation of lipids.

Serum total protein and albumin was determined using the synchron system. This system determines total protein by a biuret method and albumin concentration by bichromatic digital endpoint methodology using bromocresol purple reagent (84). ALP reagent was used to measure alkaline phosphatase activity by a kinetic rate method using 2-amino-2—methyl-1-propanol (AMP) buffer (85). ALT and gamma GT reagents were used to measure alanine transaminase and gamma glutamyl transferase, respectively in serum by an enzymatic rate method (86). Iron concentration was measured by a timed-endpoint method using the Fe reagent. C-RP reagent was used to measure C-reactive protein by a turbidimetric method. The Jaffe' method was used to measure creatinine (87). The laboratory tests performed were those related to lipodystrophy, HIV infection and metabolic diseases, including inflammatory markers and liver functions. The FBC, iron studies were done because $HbA_{1c}$ was used as a diagnostic criterion. Liver function because of its association with metabolic syndrome (insulin resistance), urea and creatinine because $HbA_{1c}$ was measured; inflammatory markers because of their association with dysglycaemia.

### 2.2.4.5. Sampling Methods

Venous blood was drawn from a forearm vein and collected into:

Tubes containing sodium flouride for plasma glucose estimation (1 ml)

Plain tubes for:

- Serum fasting lipid profile: high density lipoprotein cholesterol, low density lipoprotein cholesterol, total cholesterol, triglycerides)

- Serum Inflammatory markers (CRP)

- 

- Serum liver function tests

- Serum hepatitis B and C

- Serum urea and creatinine

- Serum cortisol, uric acid, lactate

- HIV RNA

EDTA tubes for:
- full blood count

- CD4 and CD8 cell count

- DNA extraction (10ml) for genetic analysis

The above tests were performed on group 2 and 3 at baseline. In group 3, the tests were performed at baseline, 3, 6, 12, 18 and 24 months. Group 1 had all tests at baseline except for CD4 cell count and HIV RNA.

### 2.2.4.6. Imaging

- Chest radiography using standard protocols at KEH.

- Computer tomography imaging through the umbilicus (fourth lumbar vertebra) to measure abdomen as well as intra-abdominal fat area, mid-upper arm and mid-thigh subcutaneous fat areas.

- Dual X-ray Absorptiometry (DXA) for measurement of body fat composition.

### 2.2.4.6.1.    DUAL X-Ray ABSORPTIOMETRY PROTOCOL

Dual energy x-ray absorptiometry (DXA) scans were performed to measure regional fat and lean mass body composition. DXA scans were performed with a total body scanner (QDR-200 Hilogic, Waltham, MA) generating X-rays at two energy levels (40 and 70 kVp). Participants were asked to lie down on the device, and the X-ray passed through the body in a fine beam.  A series of transverse scans were made from head to toe at 1 cm intervals. Data were collected for approximately 120 pixel elements per transverse scan, with pixel size being approximately 5x10 mm.   The total scan area with this instrument is approximately 60x200 cm.  Scan speed is 16 cm/s or 8 cm/s if body weight is greater than 70 kg, for a maximum scan time of 25 minutes. Percent body fat was derived from the DXA using computer algorithms provided by the manufacturer. Compartmentalization of the body using the software programs with the instrument, allows evaluation of individual components of the body.  Fat and lean components of the trunk versus the appendages were evaluated.

### 2.2.4.6.2.    COMPUTER TOMOGRAPHY PROTOCOL

Regional fat distribution was evaluated by computer tomography (CT). Subjects were imaged on a TOSHIBA AQUILION 64 SCANNER.  All subjects were positioned supine with their arms crossed over their thorax away from the field of view.  A lateral and frontal scanogram was obtained to localize the L4/L5 interspace level. This level corresponds to the

level of the umbilicus. A single unenhanced helical acquisition was obtained at that level using the following scan parameters: 120 kV; 120 mA; scanning time 0.5sec; slice thickness 5mm.

CT data was then analysed using a dedicated fat measurement software programme (AQUILION FAT INDEX VIEW). A fixed CT attenuation threshold value of -150 to -30 HU was set for fat density in accordance with international standard reference. Total fat area ($cm^2$); subcutaneous fat area ($cm^2$); visceral fat area ($cm^2$); and the visceral : subcutaneous fat area ratio were calculated. Results were verified by two independent radiologists blinded to the study cohort groups.

## 2.3.    Section C

### 2.3.1.  Statistical Analysis

The sample size was based on the lipoatrophy outcome. To detect a 20% difference between HIV negative and HIV positive participants at 95% confidence interval and 80% power, the estimated sample size was 78 participants in each arm for step 1. To yield a precision of 12 to 28% assuming an incidence of 20% in the HIV infected population developing lipodystrophy, and to account for a 10% attrition rate and about 16% mortality rate in those started on ART, the sample size was estimated at 125 participants for step 2.

Statistical analysis was performed using SAS (version 9.3; SAS Institute Inc., Cary, NC, USA). All variables were examined using descriptive statistics-measures of central tendency [means and medians], dispersion [standard deviations, inter-quartile ranges] for continuous variables, frequency counts and marginal percentages with 95% confidence intervals for categorical variables. Means ($\pm$) standard deviation (SD) were used to depict central tendencies of variables that are normally distributed and medians (Interquartile range) for variables that are not normally distributed. Univariate and bivariate analysis were performed to describe baseline characteristics and proportions of participants with lipodystrophy and metabolic complications.

Student's t-test and ANOVA test were used for bivariate analysis of normally distributed continuous variables with two and three comparisons respectively. Wilcoxon and Kruskal Wallis were used for bivariate analysis of non-normally distributed continuous variables with two and three comparisons respectively. Fischer's exact test and chi squared test were used to calculate p-values for prevalence where there were fewer than 5 events in a cell or more events respectively. A p value of $< 0.05$ was regarded as statistically significant.

Multivariate models were developed to examine the determinants of lipodystrophy development. Possible confounders were tested for their relationship with both independent and dependent variables, and those not related to both at $p<0.2$ were dropped from consideration in logistic or linear regression as necessary.

Paired Student's t-test was used to calculate the mean difference between follow-up and baseline (24 months – baseline mean). Differences in means were calculated for data that was available at baseline and follow-up. Mcnemar test was used to test differences in categorical variables at baseline compared with 24 months follow-up.

$\beta$ estimate (95% confidence interval) measured rate of change over time at follow up testing linear trend using the linear mixed model, P tested whether the slope is = or ≠ zero.

Lipoatrophy was defined as concordance between participant report of fat loss and examination finding of wasting at each corresponding site. Lipohypertrophy was defined as concordance of participant report of fat gain and examination finding of fat accumulation at each corresponding site(88). Concordance between participant report and physician examination for fat distribution (lipoatrophy and lipohypertrophy) was measured using Mcnemar test.

Least square estimates were used to show means for each of the measured fat areas by gender andgroup.

The incidence of diabetes mellitus (DM), impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) after starting combination antiretroviral therapy (ART) was measured.

Time at risk was calculated as from ART initiation date to estimated date of DM, IGT, IFG, dysglycaemia or last visit date. Poisson approximations were used to calculate confidence intervals (CIs) for DM, IGT, IFG and dysglycaemia incidence. Cox proportional hazards regression was used to identify predictors of incident DM, IGT and IFG at univariate and multivariate level.

**Table 2.5: Overview of Data Collection**

| Examination | Imaging | Laboratory | Questionnaire |
|---|---|---|---|
| **History**<br>• diabetes<br>• hypertension<br>• stroke<br>• amputation<br>• ischaemic heart disease<br>• tobacco smoking<br>• alcohol consumption<br>• leisure physical activity<br>**Medication**<br>• anabolic agents<br>• hypolipidemic agents<br>• antihypertensive angents<br>• insulin use | **CT scan**<br>• L4/L5<br>• Mid-thigh<br>• Mid upper arm | • HIV RNA level<br>• CD4 cell count | Participant administered on fat changes around<br>• face<br>• cheeks<br>• neck<br>• breasts<br>• chest<br>• upper back<br>• waist<br>• buttocks<br>• arms<br>• legs<br>• abdomen |
| **WHO staging**<br><br>**Physical examination** | **Dexa scan, Fat distribution, BMC, FAT, Lean**<br>• L arm<br>• R arm<br>• L ribs<br>• R ribs<br>• T spine<br>• L spine<br>• Pelvis<br>• L leg<br>• R leg<br>• Subtotal | • **Oral glucose tolerance test**<br>• glucose (0 hour)<br>• Insulin   (0 hour)<br>• glucose (2 hour)<br>• insulin   (2 hour)<br>• HBA1c | |

| | | |
|---|---|---|
| **Blood pressure measurements**<br><br>Pulse<br><br>Temperature | **Lipids**<br>• total cholesterol<br>• triglycerides<br>• HDL cholesterol<br>• LDL cholesterol | **Physician administered on visual inspection of body sites**<br>• face<br>• cheeks<br>• neck<br>• upper back<br>• chest<br>• abdomen<br>• buttocks<br>• legs<br>• arms |
| **Anthropometric measurements:**<br>circumferences (cm)<br>• Mid-arm<br>• neck<br>• chest<br>• waist<br>• hip<br>• mid-thigh | • lactate<br>• CRP<br>• urate<br>• creatinine kinase<br>• lactate<br>    dehydrogenase<br>• cortisol<br>• iron<br>• transferrin<br>• ferritin | |
| **Skinfolds**<br>• triceps<br>• sub-scapular<br>• abdominal<br>• mid-thigh | **Urea and electrolytes**<br>•sodium<br>•potassium<br>•chloride<br>•bicarbonate<br>•urea | |

•creatinine

anion gap

**Liver function tests**

•total protein

•albumin

•total bilirubin

•alanine transaminase

•alkaline phosphatase

•gamma

   glutamyltransferase

**Full blood count**

•white cell count

•haemoglobin

•MCH

•MCV

•platelets

Calcium

Corrected Calcium

Magnesium

Phosphate

## Baseline Characteristics and Fat Distribution

### 3.1 Enrolment

Screening began in April 2009 at Voluntary Counselling and Testing (VCT) Centers at King Edward VIII Hospital (KEH) and other outpatient clinics at KEH.

Of the 530 individuals screened, 326 individuals were eligible to participate. Reasons for exclusion included failure to return to the clinic after screening (n=120), not being second generation Zulu (n=14), prior or concurrent use of antiretroviral therapy (n=20), co-infection with tuberculosis (n=27), concurrent malignancy (n=3), Figure 3.1 shows outcomes of the enrolment process.

Subjects were categorized into three groups: Group 1, HIV negative (n=88), Group 2, HIV infected and not eligible to start combination antiretroviral therapy (ART) (n=88) and Group 3, HIV infected and eligible to start ART (n=150). The eligibility for ART in asymptomatic patients depends on the level of the CD4 cell count. In 2011, the CD4 cell count cut-off for ART eligibility in South Africa changed from $\leq$ 200 cells/mm$^3$ to $\leq$ 350 cells/mm$^3$. Eligibility for group 2 was then changed from CD4 cell count > 200 cells/mm$^3$ to CD4 cell count > 350 cells/mm$^3$ and for group 3 changed from CD4 cell count $\leq$ 200 cells/mm$^3$ to CD4 cell count $\leq$ 350 cells/mm$^3$ according to eligibility of initiation of ART.

**Figure 3.1: Study Enrolment Flow Chart**



35
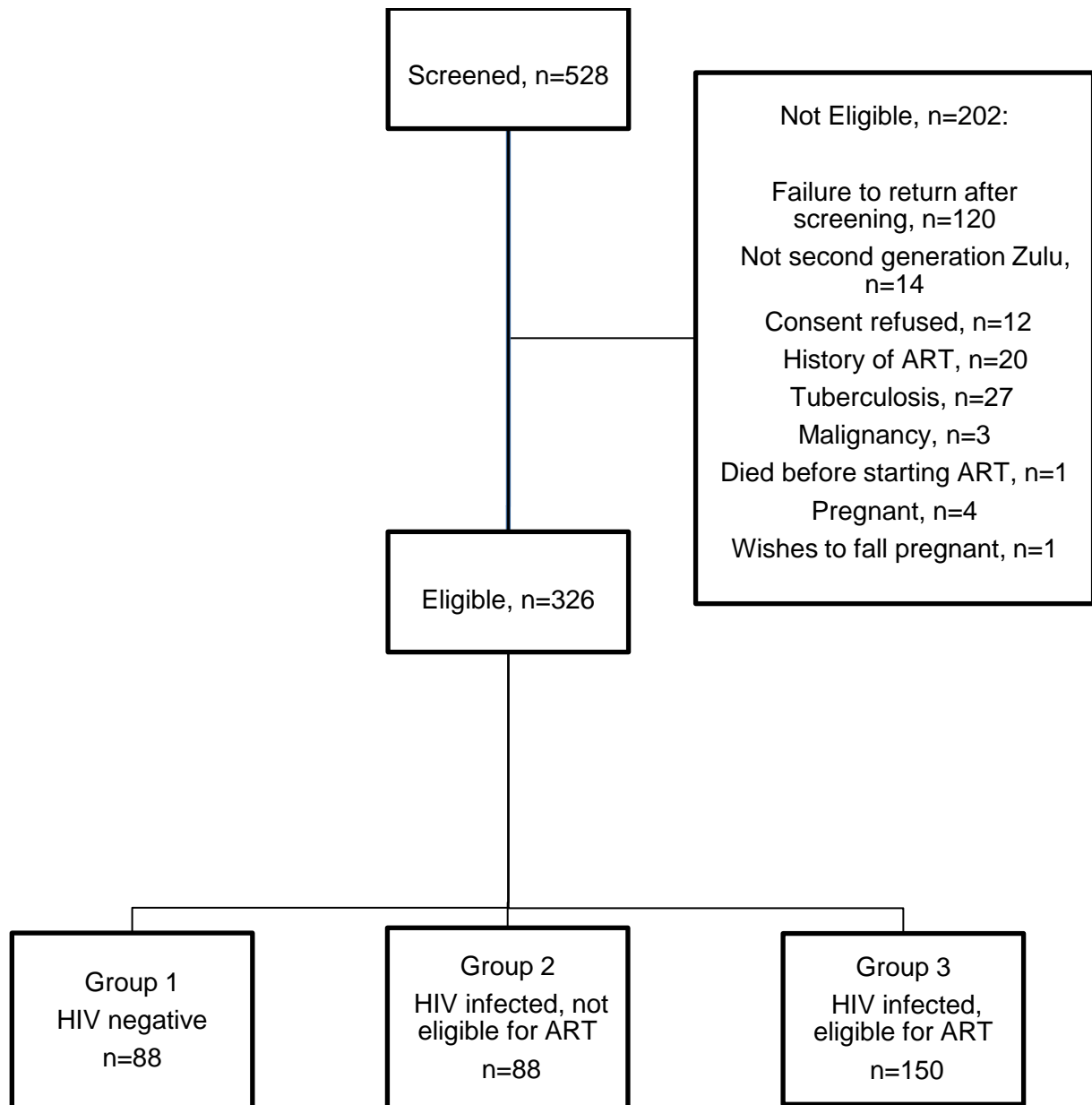
## 3.3. Demographic and Baseline Characteristics

### 3.3.1. Demographic

Table 3.1 shows the characteristics of the study group and control group. There was no difference in mean age between the groups (p=0.9) and there were more women in all groups: 65.91% for group 1 vs. 65.91% for group 2 vs. 68.0% for group 3. The majority of participants in all the groups had at least high school education, were unemployed and unmarried. When compared with group 1, group 3 had a lower frequency of smoking and alcohol use. The majority of participants in all the groups reported to have light occupational and no leisure physical activity. No study subjects were taking anabolic or lipid lowering agents. None of the subjects reported a history of stroke or heart disease. The frequency of familial diabetes was higher in group 3 and personal history of hypertension lower, when compared to group 1 although the difference was not significant.

Table 3.2 shows the clinical characteristics of the study groups and controls. When compared with group 1, mean values in group 2 and group 3 were lower for the following variables: systolic blood pressure (p=0.02) and BMI (p=0.01). Fewer subjects in group 3 had hypertension compared to group 1 and fewer subjects in group 3 were obese (27.1%) compared to group 1 (41.4%). Although the mean waist, chest circumference and waist to hip ratio were lower in group 3 compared to group 1, the difference was not significant. The following mean skin folds were lower in group 3 compared to group 1: sub-scapular, abdominal and mid-thigh but the difference was not significant.

**Table 3.1: History and Demographic characteristics of the study groups at baseline**

| Variable | Group 1 HIV negative n=88 | Group 2 HIV positive not starting ART, n=88 | Group 3 HIV positive starting ART, n=150 | P |
|---|---|---|---|---|
| Age,years ,mean $\pm$ SD | 37.0 $\pm$ 14.5 | 37.6 $\pm$ 9.1 | 36.9 $\pm$ 9.1 | 0.9 |
| Female | 58 (65.9) | 58 (65.9) | 102 (68.0) | 0.9 |
| **Marital status** | | | | 0.3 |
| Single | 61(69.3) | 67(79.8) | 112(76.7) | |
| Married | 23(26.1) | 16(19.1) | 25(17.1) | |
| Cohabitating | 1 (1.1) | 1(1.2) | 4(2.7) | |
| Widowed | 3 (3.4) | 0(0.00) | 5(3.4) | |
| **Education** | | | | 0.8 |
| Primary education | 12(13.8) | 12(14.1) | 28(19.7) | |
| High school education | 61(70.1) | 63(74.1) | 98(69.0) | |
| Tertiary education | 12(13.8) | 8 (9.4) | 12(8.5) | |
| No education | 2(2.3) | 2(2.4) | 4(2.8) | |
| Employed | 19 (30.2) | 24(37.5) | 59(41.3) | 0.3 |
| **Lifestyle** | | | | |
| Tobacco smoking | 18 (20.7) | 18 (21.2) | 24 (17.9) | 0.6 |
| Alcohol | 29 (34.1) | 33 (38.8) | 40 (27.0) | 0.01 |
| **Physical activity** | | | | |
| *Occupational* | | | | 0.6 |
| Sedentary | 3 (3.8) | 5 (6.0) | 6 (4.96) | |
| Light | 32 (40.0) | 29 (35.4) | 56 (46.3) | |
| Moderate | 32 (40.0) | 26 (31.7) | 37 (30.6) | |
| Heavy | 13 (16.3) | 22 (26.8) | 22 (18.2) | |

Continued next page

**Table 3.1 cont.: History and Demographic characteristics of the study groups at baseline**

| Variable | Group 1<br>HIV negative<br>n=88 | Group 2<br>HIV positive<br>not starting<br>ART, n=88 | Group 3<br>HIV positive<br>starting ART,<br>n=150 | P |
|---|---|---|---|---|
| *Leisure* | | | | 0.97 |
| Sedentary | 47 (55.95) | 46 (56.1) | 79 (55.6) | |
| Light | 14 (16.7) | 11 (13.4) | 112.68 (12.7) | |
| Moderate | 9 (10.7) | 10 (12.2) | 15 (10.6) | |
| Heavy | 14 (16.7) | 15 (18.3) | 30 (21.1) | |
| **Personal Medical** | | | | |
| **History** | | | | |
| Hypertension | 11 (13.4) | 4 (5.1) | 10 (7.1) | 0.3 |
| Stroke | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| Cardiomyopathy | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| Ischaemic heart disease | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| **Drugs** | | | | - |
| Anabolic agents | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| Lipid lowering agents | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| **Family History** | | | | |
| Familial diabetes | 17 (19.3) | 18 (21.2) | 36 (24.3) | 0.7 |
| Mother | 11 (12.5) | 12 (14.1) | 24 (16.6) | 0.9 |
| Father | 7 (7.95) | 7 (8.3) | 16 (10.9) | 0.9 |
| Sister | 3 (3.5) | 3 (3.5) | 2 (1.4) | 0.7 |
| Brother | 1 (1.2) | 3 (3.5) | 5 (3.4) | 0.8 |

Results expressed as n (%) except where specified. P values for comparison between group 1 vs. group 2 vs. group 3.

**Table 3.2: Clinical Characteristics**

| Variable | Group 1<br>**HIV negative**<br>n=88 | Group 2<br>**HIV positive not starting ART**<br>n=88 | Group 3<br>**HIV positive starting ART**<br>n=150 | P |
|---|---|---|---|---|
| Blood pressure (mmHg) | | | | |
| Systolic | 118.9 ± 21.8 | 115.66 ± 17.2 | 112.12 ± 16.8 | 0.02 |
| Diastolic | 72.9 ± 12.5 | 72.36 ± 11.2 | 70.93 ± 10.7 | 0.39 |
| Normal BP, n(%) | 48 (55.8) | 48 (59.3) | 102 (70.8) | 0.08 |
| High normal BP, n(%) | 20 (23.5) | 24 (29.6) | 33 (22.9) | |
| Hypertension | 18 (20.9) | 9 (11.1) | 9 (6.2) | |
| Stage 1 hypertension, n(%) | 12 (14.0) | 7 (8.6) | 6 (4.2) | |
| Stage 2 hypertension, n(%) | 6 (7.0) | 2 (2.5) | 3 (2.1) | |
| Missing | 2 (2.3) | 7 (7.9) | 6 (4.2) | |
| | | | | |
| **Anthropometric measurements** | | | | |
| Weight (kg) | 75.68 ± 19.0 | 74.49 ± 18.5 | 69.52 ± 15.8 | 0.02 |
| Height ($m^2$) | 1.62 ± 0.07 | 1.62 ± 0.08 | 1.63 ± 0.1 | 0.7 |
| Body mass index(kg/ $m^2$) | 29.13 ± 7.9 | 28.62 ± 7.8 | 26.41 ± 6.2 | 0.01 |
| *Body mass index categories* | | | | |
| Underweight, n (%) | 1 ( 1.2) | 0 (0.0) | 4 (2.8) | 0.07 |
| Normal, n (%) | 32 (36.8) | 32 (37.2) | 67 (46.5) | |
| Overweight, n (%) | 18( 20.7) | 29 (33.7) | 34 (23.6) | |
| Obese, n (%) | 36( 41.4) | 25 (29.1) | 39 (27.1) | |

Continued on next page

**Table 3.2 cont.: Clinical Characteristics**

| Variable | Group 1<br>HIV negative<br>n=88 | Group 2<br>HIV positive not starting ART<br>n=88 | Group 3<br>HIV positive starting ART<br>n=150 | P |
|---|---|---|---|---|
| *Circumference (cm)* | | | | |
| Waist | 93.8 ± 18.6 | 91.52 ± 16.1 | 92.45 ± 17.1 | 0.7 |
| Hip | 106.4 ± 17.8 | 105.75 ±13.9 | 106.15 ± 14.7 | 0.97 |
| Waist: Hip ratio | 0.88 ± 0.1 | 0.87 ± 0.1 | 0.87 ± 0.1 | 0.4 |
| Waist: Height ratio | 58.3 ± 12.4 | 56.79 ± 10.5 | 56.95 ± 11.2 | 0.6 |
| Mid-arm | 33.0(28.0 - 36.7) | 32.0(29.1 - 35.3) | 32.3(29.4 - 35.9) | 0.5 |
| Neck | 36.3 ± 3.9 | 35.7 ± 3.1 | 35.3 ± 3.5 | 0.2 |
| Chest | 96.9 ± 11.6 | 95.6 ± 11.5 | 91.8 ± 9.3 | 0.4 |
| Mid-thigh | 55.0 (49.0 -61.97) | 55.5 (49.5 - 61.2) | 55.4 (50.2 - 61.95) | 0.6 |
| **Skin folds (mm)** | | | | |
| Triceps | 23.6 ± 14.1 | 21.1 ± 12.6 | 23.3 ± 13.2 | 0.4 |
| Sub-scapular | 21.8 ± 12.8 | 18.8 ± 12.4 | 17.5 ± 12.1 | 0.2 |
| Abdominal | 26.98 ± 14.8 | 23.4 ± 13.5 | 25.2 ± 14.2 | 0.3 |
| Mid-thigh | 31.7 ± 16.7 | 28.5 ± 16.8 | 28.7 ± 15.95 | 0.4 |
| **HIV Parameters** | | | | |
| CD4 cell count, cells/mm$^3$ | N/A | 404.5(343.0 - 531.5) | 132.0(64.0 - 193.0) | 0.0001 |
| HIV RNA (log$_{10}$) | N/A | 4.3 ± 0.9 | 4.85 ± 0.9 | 0.002 |

Results expressed as means ± SD or median (IQR) except where specified. P values for comparison between group 1 vs. group 2 vs. group 3.

## 3.3. Fat loss and gain

### 3.3.1. Participant self-report

Study subjects completed a participant administered questionnaire and the physician administered questionnaire was completed as part of physical examination. Figure 3.2, 3.3 and table 3.3 show participants' report of fat loss and fat gain over the past five years. In comparison to group 1, group 3 reported more fat loss in the following peripheral sites: face (p=0.01), cheeks (p=0.03) and neck (p=0.03); more fat loss in central sites was also reported by group 3 for chest (p=0.01) and waist (p=0.003). Group 3 reported less fat gain compared to group 1 in the following peripheral sites: face, cheeks, arms, neck and buttocks. Less central fat gain (chest and waist) was reported by group 3 compared to group 1. Although peripheral fat loss was higher in group 3 in arms, legs and buttocks, the difference was not significant.

**Figure 3.2: Participant administered questionnaire on fat loss at baseline**
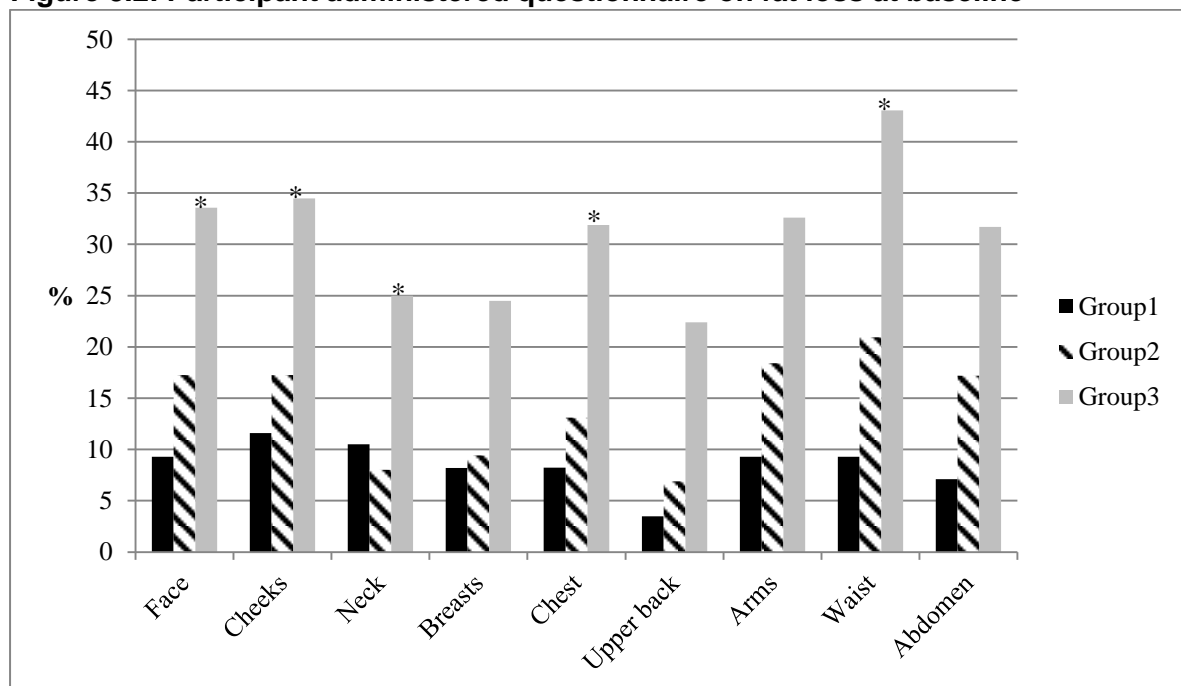


[*] P < 0.05 comparing fat loss in the study groups. Group1 = HIV negative, Group2 = HIV infected not starting ART, Group3 = HIV infected starting ART

**Figure 3.3: Participant administered questionnaire on fat gain at baseline**
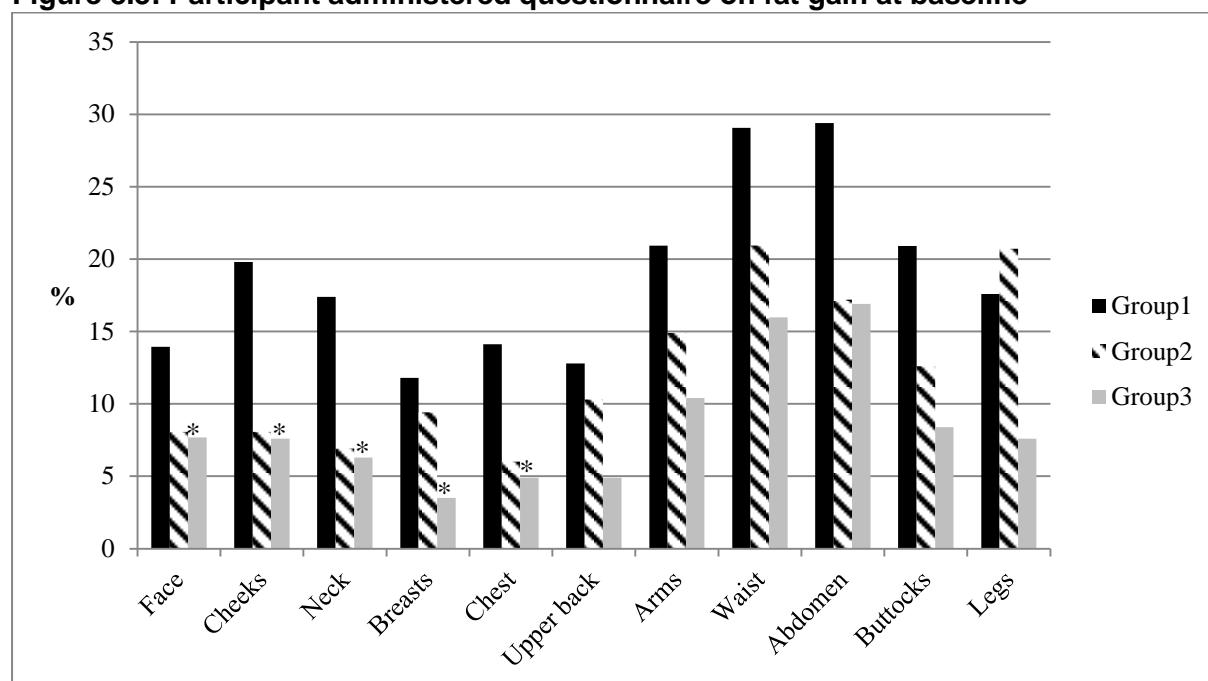


$^*$ P < 0.05 for comparison between the groups. Group1 = HIV negative, Group2 = HIV infected not starting ART, Group3 = HIV infected starting ART

### 3.3.2. Physician evaluation report

Figure 3.4, 3.5 and table 3.4 show physician examination report of wasting and fat accumulation. Group 3 was found to have more individuals with lipoatrophy and fewer with lipohypertrophy on general appearance compared to group 1 (p=0.03). More individuals in group 3 were found to have reduced fat in the peripheral sites (face, cheeks, neck, arms, buttocks and legs) and central sites (chest, upper back and abdomen), although the difference was not significant. Fewer individuals in group 3 were found to have peripheral fat accumulation (face, cheeks, neck, arms and buttocks) and central fat accumulation (chest, upper back and abdomen).

**Figure 3.4: Physician examination report of wasting at baseline**
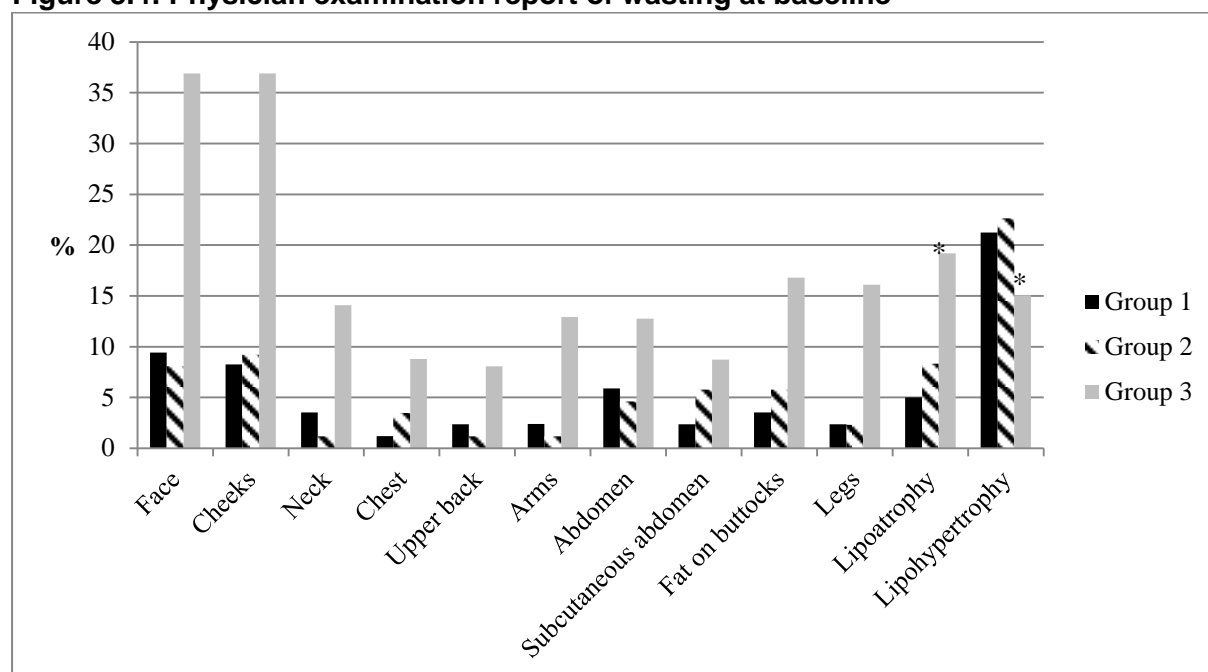


* P < 0.05 for comparison between the groups. Group1 = HIV negative, Group2 = HIV infected not starting ART, Group3 = HIV infected starting ART

**Figure 3.5: Physician examination report of fat accumulation at baseline**
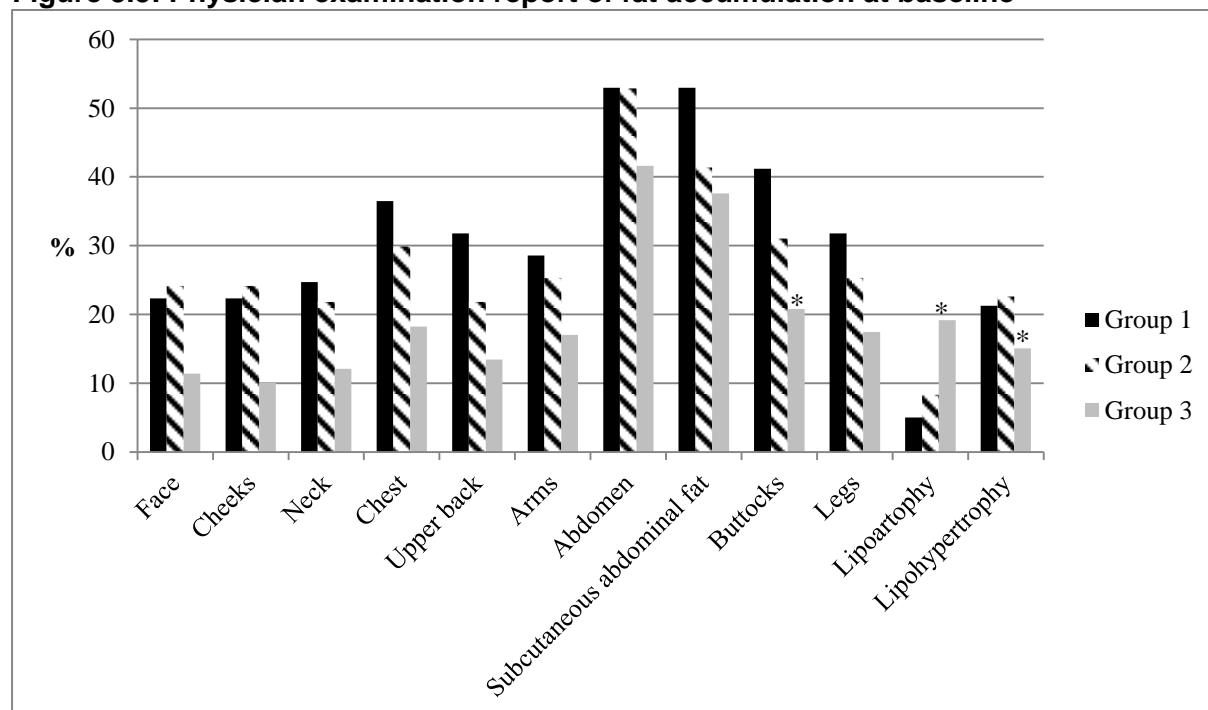


* P < 0.05 for comparison between the groups. Group1 = HIV negative, Group2 = HIV infected not starting ART, Group3 = HIV infected starting ART

**Table 3.3: Participants' report of fat loss and fat gain at baseline**

| Variable | Group 1 HIV negative n=88 | Group 2 HIV positive not starting ART n=88 | Group 3 HIV positive starting ART n=150 | P |
|---|---|---|---|---|
| **Shape of face** | | | | 0.01 |
| No change | 63(73.3) | 64(73.6) | 82(57.3) | |
| Loss | 8(9.3) | 15(17.2) | 48(33.6) | |
|   Mild | 6(75.0) | 13(86.7) | 30(62.5) | |
|   Moderate | 2(25.0) | 2(13.3) | 16(33.3) | |
|   Severe | 0(0.0) | 0(0.0) | 2(4.2) | |
| Gain | 12(13.95) | 7(8.1) | 11(7.7) | |
|   Mild | 9(75.0) | 2(28.6) | 7(63.6) | |
|   Moderate | 3(25.0) | 5(71.4) | 4(36.4) | |
|   Severe | 0(0.0) | 0(0.0) | 0(0.0) | |
| Don't know | 3(3.5) | 1(1.1) | 2(1.3) | |
| Missing | 3(3.5) | 1(1.1) | 7(4.9) | |
| **Fat in cheeks** | | | | 0.03 |
| No change | 56(65.1) | 64(73.6) | 80(55.2) | |
| Loss | 10(11.6) | 15(17.2) | 50(34.5) | |
|   Mild | 8(80.0) | 12(80.0) | 31(62.00) | |
|   Moderate | 2(20.0) | 2(13.3) | 16(32.0) | |
|   Severe | 0(0.00) | 1(6.7) | 3(6.0) | |
| Gain | 17(19.8) | 7(8.1) | 11(7.6) | |
|   Mild | 14(82.4) | 2(28.6) | 7(63.6) | |
|   Moderate | 2(11.8) | 5(71.4) | 4(36.4) | |
|   Severe | 1(5.9) | 0(0.0) | 0(0.0) | |
| Don't know | 3(3.5) | 0(0.0) | 3(2.1) | |
| Missing Data | 2(2.3) | 2(2.3) | 7(4.0) | |

Continued on next page

**Table 3.3 cont.: Participants' report of fat loss and fat gain at baseline**

| Variable | Group 1 HIV negative n=88 | Group 2 HIV positive not starting ART n=88 | Group 3 HIV positive starting ART n=150 | P |
|---|---|---|---|---|
| **Fat on neck** | | | | 0.03 |
| No change | 59(68.6) | 72(83.7) | 99(68.8) | |
| Loss | 9(10.5) | 7(8.1) | 36(25.0) | |
|     Mild | 7(77.8) | 5(71.4) | 21(58.3) | |
|     Moderate | 2(22.2) | 2(28.6) | 12(33.3) | |
|     Severe | 0(0.0) | 0(0.0) | 3(8.33) | |
| Gain | 15(17.4) | 6(6.9) | 9(6.3) | |
|     Mild | 7(77.8) | 3(50.0) | 5(56.4) | |
|     Moderate | 2(22.2) | 3(50.0) | 4(44.4) | |
|     Severe | 0(0.0) | 0(0.0) | 5(55.6) | |
| Don't know | 3(3.5) | 1(1.2) | 0(0.0) | |
| Missing | 2 (2.3) | 2(2.3) | 6(6.8) | |
| **Fat on breasts** | | | | 0.2 |
| No change | 66(79.5) | 67(78.8) | 100(69.9) | |
| Loss | 7(8.4) | 8(9.4) | 35(24.5) | |
|     Mild | 5(71.4) | 6(75.0) | 19(54.3) | |
|     Moderate | 2(28.6) | 2(25.0) | 13(37.1) | |
|     Severe | 0(0.0) | 0(0.0) | 3(8.6) | |
| Gain | 10(12.0) | 8(9.4) | 5(3.5) | |
|     Mild | 7(70.0) | 3(37.5) | 2(40.0) | |
|     Moderate | 3(30.0) | 5(62.5) | 3(60.0) | |
|     Severe | 0(0.0) | 0(0.0) | 0(0.0) | |
| Don't know | 0(0.0) | 3(3.4) | 7(4.7) | |
| Missing Data | 5(5.7) | 2(2.4) | 3(2.1) | |

Continued on next page

Table 3.3 cont.: Participants' report of fat loss and fat gain at baseline

|  | Group 1 | Group 2 | Group 3 | P |
|---|---|---|---|---|
|  | HIV negative n=88 | HIV positive not starting ART n=88 | HIV positive starting ART n=150 |  |
| **Fat on front of chest** |  |  |  | 0.01 |
| No change | 64(76.1) | 66(78.6) | 89(61.8) |  |
| Loss | 7(8.3) | 11(13.1) | 46(31.9) |  |
|    Mild | 6(85.7) | 7(63.6) | 29(63.0) |  |
|    Moderate | 1(14.3) | 1(14.3) | 15(32.6) |  |
|    Severe | 0(0.0) | 0(0.0) | 2(4.4) |  |
| Gain | 12(14.3) | 5(6.0) | 7(4.9) |  |
|    Mild | 8(66.7) | 2(40.0) | 5(71.4) |  |
|    Moderate | 4(33.3) | 3(60.0) | 2(28.6) |  |
|    Severe | 0(0.0) | 0(0.0) | 0(0.0) |  |
| Don't know | 1(1.2) | 2(2.4) | 2(1.4) |  |
| Missing | 4(1.2) | 4(4.5) | 6(4.0) |  |
| **Fat on upper back** |  |  |  | 0.1 |
| No change | 70(81.4) | 71(81.6) | 101(70.6) |  |
| Loss | 3(3.5) | 6(6.9) | 32(22.4) |  |
|    Mild | 2(66.7) | 4(66.7) | 19(59.4) |  |
|    Moderate | 1(33.3) | 2(33.3) | 12(37.5) |  |
|    Severe | 0(0.0) | 0(0.0) | 1(3.1) |  |
| Gain | 11(12.8) | 9(10.3) | 7(4.9) |  |
|    Mild | 6(54.6) | 2(22.2) | 5(71.4) |  |
|    Moderate | 5(45.5) | 7(77.8) | 2(28.6) |  |
|    Severe |  |  |  |  |
| Don't know | 2(2.3) | 0(0.0) | 4(2.7) |  |
| Missing | 2 (2.3) | 2 (2.3) | 6 (4.0) |  |

Continued on next page

Table 3.3 cont.: Participants' report of fat loss and fat gain at baseline

| | Group 1 | Group 2 | Group 3 | P |
|---|---|---|---|---|
| | HIV negative n=88 | HIV positive not starting ART n=88 | HIV positive starting ART n=150 | |
| **Fat on arms** | | | | 0.08 |
| No change | 58(67.4) | 57(65.5) | 78(54.2) | |
| Loss | 8(9.3) | 16(18.4) | 47(32.6) | |
|    Mild | 6(75.0) | 11(68.8) | 27(57.5) | |
|    Moderate | 2(25.0) | 5(31.3) | 16(34.0) | |
|    Severe | 0(0.0) | 0(0.0) | 4(8.5) | |
| Gain | 18(20.9) | 13(14.9) | 15(10.4) | |
|    Mild | 13(72.2) | 6(46.2) | 10(66.7) | |
|    Moderate | 5(27.8) | 6(46.2) | 5(33.3) | |
|    Severe | 0(0.0) | 1(7.7) | 0(0.0) | |
| Don't know | 2(2.3) | 1(1.1) | 4(2.8) | |
| Missing | 2(2.3) | 1(1.2) | 6(4.0) | |
| **Size of waist** | | | | 0.003 |
| No change | 51(59.3) | 48(55.8) | 55(38.2) | |
| Loss | 8(9.3) | 18(20.9) | 62(43.1) | |
|    Mild | 5(62.5) | 10(55.6) | 36(58.1) | |
|    Moderate | 3(37.5) | 7(38.9) | 22(35.5) | |
|    Severe | 0(0.0) | 1(5.6) | 4(6.5) | |
| Gain | 25(29.1) | 18(20.9) | 23(15.97) | |
|    Mild | 14(56.0) | 6(33.3) | 16(69.6) | |
|    Moderate | 10(40.0) | 12(66.7) | 7(30.4) | |
|    Severe | 1(4.0) | 0(0.0) | 0(0.0) | |
| Don't know | 2(2.3) | 2(2.3) | 3(2.1) | |
| Missing Data | 2(2.3) | 2(2.3) | 7(4.7) | |

Continued on next page

Table 3.3 cont.: Participants' report of fat loss and fat gain at baseline

| | Group 1 HIV negative n=88 | Group 2 HIV positive not starting ART n=88 | Group 3 HIV positive starting ART n=150 | P |
|---|---|---|---|---|
| **Fat in abdomen** | | | | 0.1 |
| No change | 51(60.0) | 54(62.1) | 71(49.3) | |
| Loss | 6(7.1) | 15(17.2) | 45(31.3) | |
| Mild | 5(83.3) | 8(53.3) | 26(57.8) | |
| Moderate | 1(16.7) | 7(46.7) | 17(37.8) | |
| Severe | 0(0.0) | 0(0.0) | 2(4.4) | |
| Gain | 25(29.4) | 15(17.2) | 24(16.7) | |
| Mild | 13(52.0) | 6(40.0) | 18(75.0) | |
| Moderate | 11(44.0) | 9(60.0) | 6(25.0) | |
| Severe | 1(4.0) | 0(0.0) | 0(0.0) | |
| Don't know | 3(3.5) | 3(3.5) | 4(2.8) | |
| Missing | 3 (3.5) | 1 (1.5) | 6 (4.0) | |
| **Fat on buttocks** | | | | 0.2 |
| No change | 55(63.9) | 59(67.8) | 80(55.9) | |
| Loss | 12(14.0) | 16(18.4) | 48(33.6) | |
| Mild | 9(75.0) | 9(56.3) | 26(54.17) | |
| Moderate | 3(25.0) | 7(43.8) | 18(37.5) | |
| Severe | 0(0.0) | 0(0.0) | 4(8.3) | |
| Gain | 18(20.9) | 11(12.6) | 12(8.4) | |
| Mild | 12(66.7) | 6(54.6) | 7(58.3) | |
| Moderate | 5(27.8) | 5(45.5) | 5(41.7) | |
| Severe | 1(5.6) | 0(0.0) | 0(0.0) | |
| Don't know | 1(1.2) | 1(1.2) | 3(2.0) | |
| Missing | 2(2.3) | 1(1.2) | 7(4.7) | |

Continued on next page

Table 3.3 cont.: Participants' report of fat loss and fat gain at baseline

| Variable | Group 1 HIV negative n=88 | Group 2 HIV positive not starting ART n=88 | Group 3 HIV positive starting ART n=150 | P |
|---|---|---|---|---|
| **Fat on legs** | | | | 0.08 |
| No change | 59(69.4) | 62(71.3) | 84(58.3) | |
| Loss | 9(10.6) | 16(18.4) | 48(33.3) | |
|    Mild | 6(66.7) | 11(68.7) | 30(62.5) | |
|    Moderate | 3(33.3) | 5(31.3) | 15(31.3) | |
|    Severe | 0(0.0) | 0 (0.0) | 3(6.2) | |
| Gain | 15(17.6) | 8(20.7) | 11(7.6) | |
|    Mild | 11(73.3) | 5(62.5) | 6(54.5) | |
|    Moderate | 4(26.7) | 3(37.5) | 5(45.5) | |
|    Severe | | | | |
| Don't know | 2(2.4) | 1(1.2) | 1(0.7) | |
| Missing | 3(3.4) | 1(1.2) | 6(4.0) | |
| **Fatty lumps** | | | | 0.7 |
| Fatty lumps, none | 68(97.1) | 75(98.7) | 133(97.1) | |
| Fatty Lumps, yes | 2(2.3) | 1(1.3) | 4(2.9) | |
| Lumps on Neck | 1(50.0) | 1(100.0) | 0(0.0) | |
| Lumps on Belly | 1(50.0) | 0(0.0) | 1(25.0) | |
| Lumps on other | 0(0.0) | 0(0.0) | 1(25.0) | |
| Fatty lumps, Don't know | 0(0.0) | 0(0.0) | 1(0.7) | |
| Missing | 18(20.5) | 12 (13.6) | 13 (8.7) | |

Results expressed as n (%). P value compares no change vs. change (fat loss or fat gain)

**Table 3.4: Physician examination report of wasting and fat accumulation at baseline**

| Variable | Group 1<br>HIV negative<br>n=88 | Group 2<br>HIV positive not starting ART<br>n=88 | Group 3<br>HIV positive starting ART<br>n=150 | P |
|---|---|---|---|---|
| **Shape of face** | | | | |
| Normal | 58(68.2) | 60 (68.9) | 78(52.4) | |
| Wasting | 8(9.4) | 7(8.0) | 55(36.9) | 0.3 |
|   Mild | 8(100.0) | 6(85.7) | 36(65.5) | |
|   Moderate | 0(0.0) | 1(14.3) | 18(32.7) | |
|   Severe | 0(0.0) | 0(0.0) | 1(1.8) | |
| Fat accumulation | 19(22.4) | 20(22.9) | 17(11.4) | 0.1 |
|   Mild | 9(47.4) | 15(71.4) | 15(88.2) | |
|   Moderate | 9(47.4) | 3(14.3) | 2(11.8) | |
|   Severe | 1(5.3) | 2(9.3) | 0(0.0) | |
| Missing | 3(3.4) | 1(1.1) | 0(0.0) | |
| **Fat on cheeks** | | | | |
| Normal | 59(69.4) | 58(66.7) | 80(53.7) | |
| Wasting | 7(8.2) | 8(9.2) | 55(36.9) | 0.1 |
|   Mild | 7(100.0) | 7(87.5) | 36(65.5) | |
|   Moderate | 0(0.0) | 1(12.5) | 19(34.6) | |
|   Severe | 0(0.0) | 0(0.0) | 0(0.0) | |
| Fat accumulation | 19(22.4) | 21(24.1) | 15(10.1) | 0.1 |
|   Mild | 9(47.4) | 15(71.4) | 13(86.7) | |
|   Moderate | 9(47.4) | 4(19.5) | 2(13.3) | |
|   Severe | 1(5.3) | 2(9.5) | 0(0.0) | |
| Missing Data | 3(3.4) | 1(1.1) | 0(0.0) | |

Continued on next page

**Table 3.4 cont.: Physician examination report of wasting and fat accumulation at baseline**

| Variable | Group 1<br><br>HIV negative n=88 | Group 2<br><br>HIV positive not starting ART n=88 | Group 3<br><br>HIV positive starting ART n=150 | P |
|---|---|---|---|---|
| **Fat on neck** | | | | |
| Normal | 61(71.8) | 67(77.0) | 110(73.8) | |
| Wasting | 3(3.5) | 1(1.2) | 21(14.1) | 0.1 |
| Mild | 3(100.0) | 0(0.0) | 15(71.4) | |
| Moderate | 0(0.0) | 1(100.0) | 6(28.6) | |
| Severe | 0(0.0) | 0(0.0) | 0(0.0) | |
| Fat accumulation | 21(24.7) | 19(21.8) | 18(12.1) | 0.2 |
| Mild | 12(57.1) | 15(78.95) | 11(61.1) | |
| Moderate | 6(28.6) | 2(10.5) | 7(38.9) | |
| Severe | 3(14.3) | 2(10.5) | 0(0.0) | |
| Missing | 3 (3.4) | 1 (1.1) | 1 (0.7) | |
| **Fat on front of chest** | | | | |
| Normal | 53(62.4) | 58(66.7) | 108(72.97) | |
| Wasting | 1(1.2) | 3(3.5) | 13(8.8) | 0.7 |
| Mild | 1(100.0) | 2(66.7) | 8(61.5) | |
| Moderate | 0(0.0) | 1(33.3) | 5(38.5) | |
| Severe | 0(0.0) | 0(0.0) | 0(0.0) | |
| Fat accumulation | 31(36.5) | 26(29.9) | 27(18.2) | 0.9 |
| Mild | 14(45.2) | 13(50.0) | 14(51.9) | |
| Moderate | 14(45.2) | 11(42.3) | 12(44.4) | |
| Severe | 3(9.7) | 2(7.7) | 1(3.7) | |
| Missing Data | 3(3.4) | 1(1.1) | 2(1.4) | |

Continued on next page

**Table 3.4 cont.: Physician examination report of wasting and fat accumulation at baseline**

| Variable | Group 1<br>HIV negative<br>n=88 | Group 2<br>HIV positive not starting ART n=88 | Group 3<br>HIV positive starting ART n=150 | P |
|---|---|---|---|---|
| **Fat on the upper back** | | | | |
| Normal | 55(65.5) | 67(77.0) | 117(78.5) | |
| Wasting | 2(2.4) | 1(1.2) | 12(8.1) | 0.4 |
|    Mild | 2(100.0) | 0(0.0) | 8(66.7) | |
|    Moderate | 0(0.0) | 1(1.0) | 4(33.3) | |
|    Severe | 0(0.0) | 0(0.0) | 0(0.0) | |
| Fat accumulation | 27(32.1) | 19(21.8) | 20(13.4) | 0.1 |
|    Mild | 12(44.4) | 14(73.7) | 11(55.0) | |
|    Moderate | 12(44.4) | 3(15.8) | 9(45.0) | |
|    Severe | 3(11.1) | 2(10.5) | 0(0.0) | |
|  Missing | 4(4.5) | 1(1.1) | 1(0.7) | |
| **Fat on arms** | | | | |
| Normal | 57(68.7) | 62(72.9) | 102(69.4) | |
| Wasting | 2(2.4) | 1(1.2) | 19(13.0) | 0.4 |
|    Mild | 1(100.0) | 0(0.0) | 13(68.4) | |
|    Moderate | 0(0.0) | 1(100.0) | 6(31.6) | |
|    Severe | 0(0.0) | 0(0.0) | 0(0.0) | |
| Fat accumulation | 24(28.9) | 22(25.9) | 25(17.0) | 0.3 |
|    Mild | 9(37.5) | 13(59.1) | 13(5) | |
|    Moderate | 13(54.2) | 7(31.8) | 12(48.0) | |
|    Severe | 2(8.3) | 2(9.1) | 0(0.0) | |
| Missing | 6(5.7) | 3 (3.4) | 2 (2.7) | |

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**Table 3.4 cont.: Physician examination report of wasting and fat accumulation at baseline**

| Variable | Group 1 HIV negative n=88 | Group 2 HIV positive not starting ART n=88 | Group 3 HIV positive starting ART n=150 | P |
|---|---|---|---|---|
| **Fat in abdomen** | | | | |
| Normal | 35(41.2) | 36(41.4) | 66(44.3) | |
| Wasting | 5(5.9) | 4(4.6) | 19(12.8) | 0.2 |
|   Mild | 5(100.0) | 2(50.0) | 15(78.95) | |
|   Moderate | 0(0.0) | 2(50.0) | 4(21.1) | |
|   Severe | 0(0.0) | 0(0.0) | 0(0.0) | |
| Fat accumulation | 45(52.9) | 46(52.9) | 62(41.6) | 0.05 |
|   Mild | 11(24.4) | 25(54.4) | 29(46.8) | |
|   Moderate | 30(66.7) | 17(36.96) | 29(46.8) | |
|   Severe | 4(8.9) | 4(8.7) | 4(6.5) | |
| Missing | 3(3.4) | 2(2.3) | 3(1.3) | |
| **Subcutaneous abdominal fat** | | | | |
| Normal | 38(44.7) | 45(52.9) | 79(53.0) | 0.5 |
| Wasting | 2(2.4) | 5(5.8) | 13(8.7) | |
|   Mild | 2(100.0) | 3(60.0) | 10(76.9) | |
|   Moderate | 0(0.0) | 2(40.0) | 3(23.1) | |
|   Severe | 0(0.0) | 0(0.0) | 0(0.0) | |
| Fat accumulation | 45(52.9) | 36(41.4) | 56(37.6) | |
|   Mild | 14(31.1) | 17(47.2) | 27(48.2) | |
|   Moderate | 26(57.8) | 16(44.4) | 26(46.4) | |
|   Severe | 5(11.1) | 3(8.3) | 3(5.4) | |
| Missing | 3(3.4) | 2(2.3) | 2(1.3) | |

Continued on next page

**Table 3.4 cont.: Physician examination report of wasting and fat accumulation at baseline**

| Variable | Group 1<br><br>HIV negative n=88 | Group 2<br><br>HIV positive not starting ART n=88 | Group 3<br><br>HIV positive starting ART n=150 | P |
|---|---|---|---|---|
| **Fat on buttocks** | | | | |
| Normal | 47(55.3) | 55(63.2) | 92(61.7) | 0.95 |
| Wasting | 3(3.5) | 5(5.8) | 25(16.8) | |
| Mild | 2(66.7) | 4(80.0) | 16(64.0) | |
| Moderate | 1(33.3) | 1(20.0) | 8(32.0) | |
| Severe | 0(0.0) | 0(0.0) | 1(4.0) | |
| Fat accumulation | 35(41.2) | 27(31.0) | 31(20.8) | 0.07 |
| Mild | 11(31.4) | 15(55.6) | 19(61.3) | |
| Moderate | 20(57.1) | 10(37.0) | 12(38.7) | |
| Severe | 4(11.4) | 2(7.4) | 0(0.0) | |
| Missing | 3 (3.4) | 1 (1.5) | 2 (1.3) | |
| **Fat on legs** | | | | |
| Normal | 55(65.5) | 63(72.4) | 100(67.1) | |
| Wasting | 2(2.35) | 2(2.3) | 24(16.1) | 0.5 |
| Mild | 2(100.0) | 1(50.0) | 16(66.7) | |
| Moderate | 0(0.0) | 1(50.0) | 7(29.2) | |
| Severe | 0(0.0) | 0(0.0) | 1(4.2) | |
| Fat accumulation | 27(32.1) | 22(25.3) | 26(17.5) | 0.09 |
| Mild | 9(33.3) | 14(63.6) | 13(50.0) | |
| Moderate | 14(51.9) | 6(27.3) | 13(50.0) | |
| Severe | 4(14.8) | 2(9.1) | 0(0.0) | |
| Missing | 4(4.5) | 1(1.5) | 0(0.0) | |

**Table 3.4 cont.: Physician examination report of wasting and fat accumulation at baseline**

| Variable | Group 1 HIV negative n=88 | Group 2 HIV positive not starting ART n=88 | Group 3 HIV positive starting ART n=150 | P |
|---|---|---|---|---|
| **General appearance** | | | | 0.03 |
| (1) Lipoatrophy | 4(5.0) | 7(8.3) | 28(19.2) | |
| (2) Lipohypertrophy | 17(21.3) | 19(22.6) | 22(15.1) | |
| Both (1) & (2) | 4(5.0) | 5(5.95) | 13(8.9) | |
| Neither (1) or (2) | 55(68.8) | 52(61.9) | 83(56.9) | |
| Missing | 8 (9.0) | 5 (5.7) | 4 (2.7) | |

Results expressed as n (%). P value compares no change vs. change

(fat loss or fat gain)

### 3.3.3. Concordance between participant report and physician examination

Concordance between participant self-report and physician examination was higher for face and cheek lipoatrophy in group 3 compared to group 1 (Figure 3.6) and lower for lipohypertrophy (Figure 3.7).

Both central (neck, chest, upper back, and abdomen) (p=0.0002) and peripheral (face, cheeks, arms, buttocks, legs) (p=0.003) lipohypertrophy were significantly lower in group 3 compared to group 1 (Figure 3.8). Peripheral lipoatrophy was higher in group 3 compared to group 1 while central lipoatrophy was lower in group 3 compared to group 1; however, the difference was not significant.

### 3.3.4. Association between lipoatrophy and lipohypertrophy

The presence of peripheral lipoatrophy was associated with a reduced likelihood of central lipohypertrophy (OR 0.22, 95% CI 0.08 – 0.66, p=0.01) for all subjects. There was no association between peripheral lipoatrophy and central lipohypertrophy in HIV-1 infected subjects starting ART (OR 1.10, 95% CI 0.5 – 2.3, p=0.7). Peripheral lipoatrophy was associated with central lipoatrophy (OR 118.84, 95% CI 34.08 – 414.45, p<0.0001) for all subjects; at group level, HIV-1 infected subjects starting ART had a three-fold likelihood of having peripheral and central lipoatrophy although this did not reach statistical significance (OR 3.4, 95% CI 1.09 – 10.4, p=0.06).

Peripheral lipohypertrophy was associated with central lipohypertrophy (OR 92.6, 95% CI 35.0 – 245.0, p<0.0001) for all subjects; at group level, there was no association between central and peripheral lipohypertrophy (OR 0.67, 95% CI 0.18 – 2.45, p=0.3). Central lipohypertrophy in HIV infected patients starting ART was negatively associated with trunk fat measured by DXA scan (OR 0.2, 95% CI 0.08 – 0.54, p=0.01) and visceral fat measured by CT scan (OR 0.1, 95% CI 0.05 – 0.4, p=0.002).

**Table 3.5: Association between lipoatrophy and lipohypertrophy at baseline**

| | OR (95% CI) | p | OR (95% CI) | p |
|---|---|---|---|---|
| | **Peripheral lipoatrophy vs. central lipohypertrophy** | | **Peripheral lipohypertrophy vs. central lipohypertrophy** | |
| All | 0.22 (0.08 – 0.66) | 0.01 | 92.6 (35.0 – 245.0) | <0.0001 |
| Group 3 vs. group 1 | 1.10 (0.5 – 2.3) | 0.7 | 0.67 (0.18 – 2.45) | 0.3 |
| | Peripheral lipoatrophy vs. central lipoatrophy | | | |
| All | 118.84 (34.08 – 414.45) | <0.0001 | | |
| Group 3 vs. group 1 | 3.4 (1.09 – 10.4) | 0.1 | | |

OR: Odds ratio, CI: Confidence interval. All: group 1, group 2 and group 3. Group1 = HIV negative, Group2 = HIV infected not starting ART, Group3 = HIV infected starting ART

**Figure 3.6: Prevalence of lipoatrophy by concordance between participant report and physician examination at baseline**
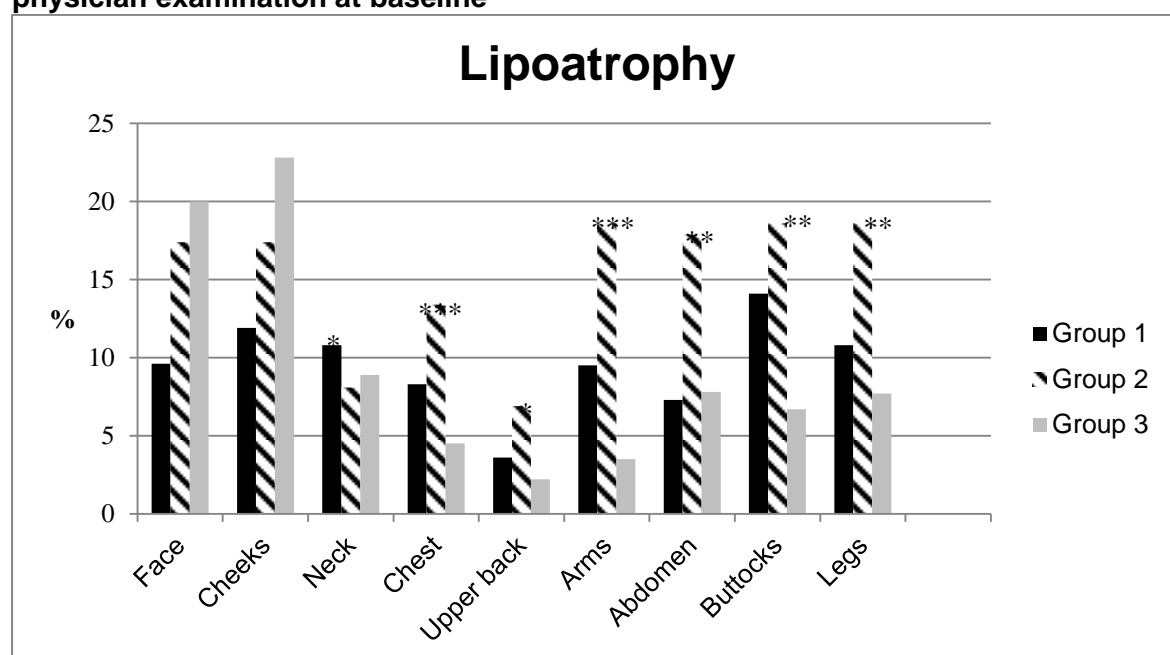


Group1 = HIV negative, Group2 = HIV infected not starting ART, Group3 = HIV infected starting ART. *p<0.05, **p<0.01, ***p<0.001 vs. Group 1

**Figure 3.7: Prevalence of lipohypertrophy by concordance between participant report and physician examination at baseline.**
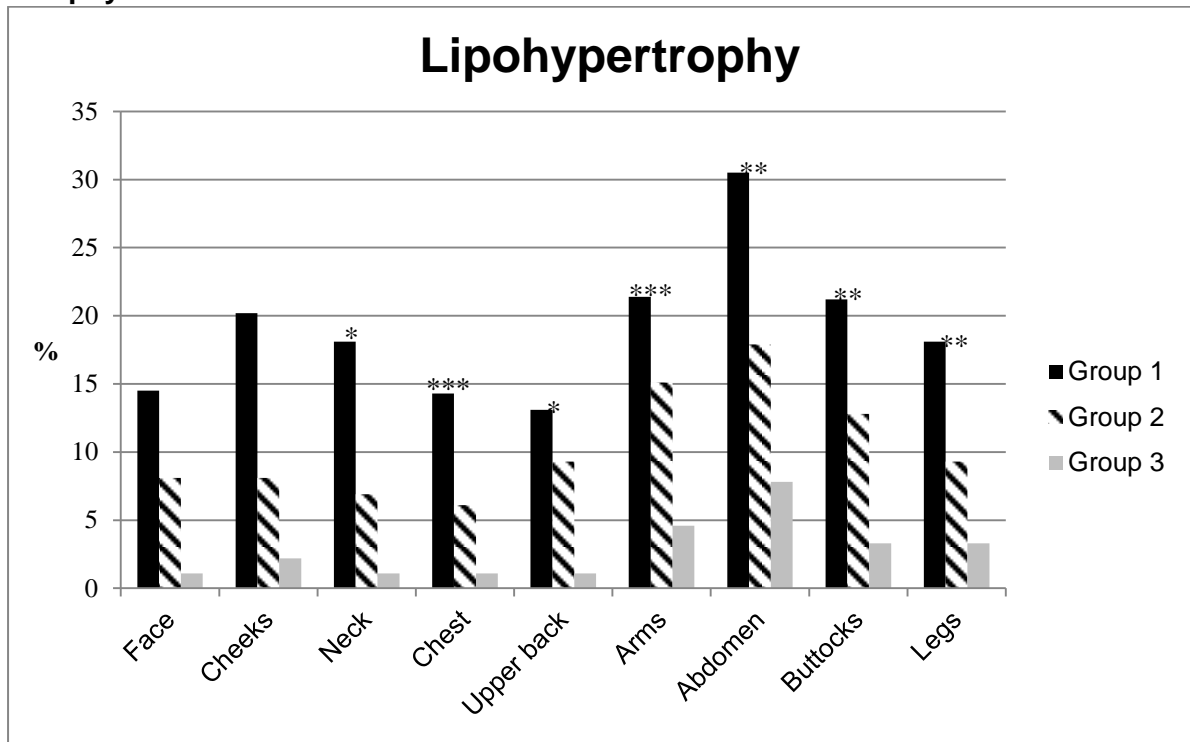


Group1 = HIV negative, Group2 = HIV infected not starting ART, Group3 = HIV infected starting ART *p<0.05, **p<0.01, ***p<0.001 vs. Group 1

**Figure 3.8: Fat distribution at baseline by participant report and physician examination concordance**
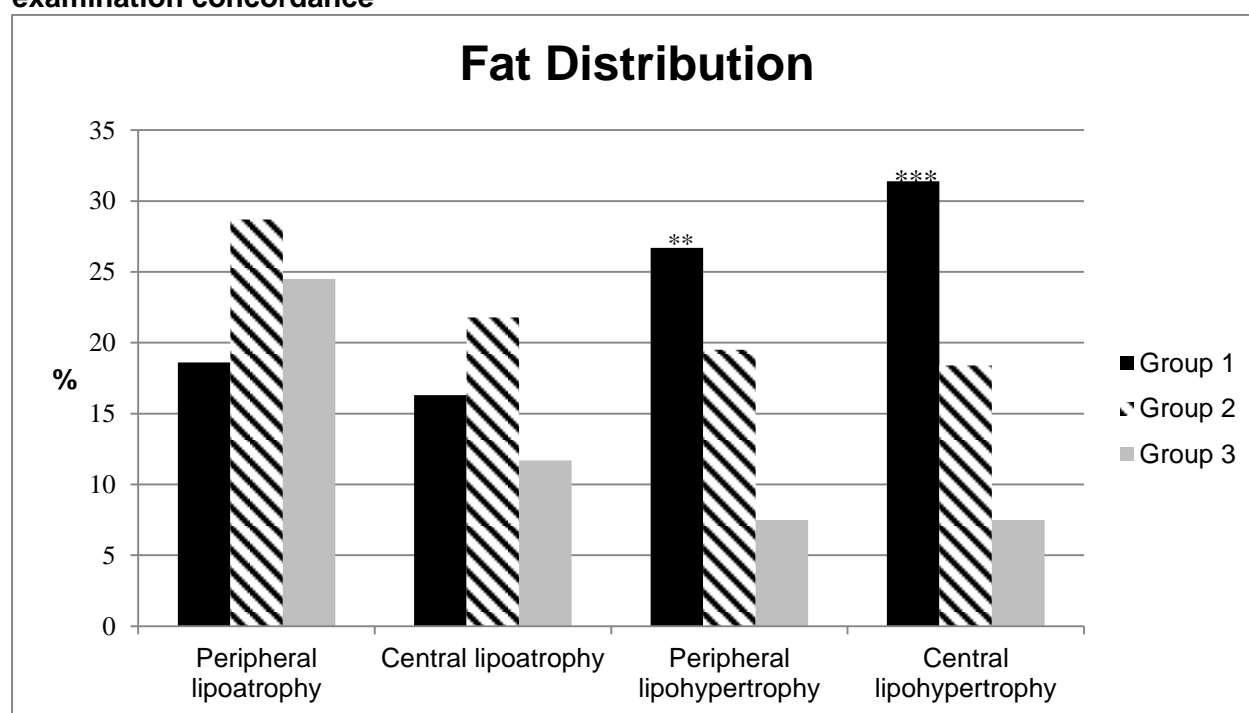


Group1 = HIV negative, Group2 = HIV infected not starting ART, Group3 = HIV infected starting ART [**]p 0.003, [***]p 0.0003 vs. Group 1

**Table 3.6: Lipoatrophy by participant report and physician examination concordance at baseline**

| | Face | Cheeks | Neck | Chest | Upper back | Arms | Abdomen | Buttocks | Legs |
|---|---|---|---|---|---|---|---|---|---|
| Group 1 | 8(9.6) | 10(11.9) | 9(10.8) | 7(8.3) | 3(3.6) | 8(9.5) | 6(7.3) | 12(14.1) | 9(10.8) |
| Group 2 | 15(17.4) | 15(17.4) | 7(8.1) | 11(13.4) | 6(6.9) | 16(18.6) | 15(17.9) | 16(18.6) | 16(18.6) |
| Group 3 | 18(20) | 21(22.8) | 8(8.9) | 4(4.5) | 2(2.2) | 3(3.5) | 7(7.8) | 6(6.7) | 7(7.7) |
| p | 0.47 | 0.76 | 0.03 | <0.0001 | 0.02 | 0.0004 | 0.004 | 0.005 | 0.002 |

Results expressed as n(%). Group1 = HIV negative, Group2 = HIV infected not starting ART,

Group3 = HIV infected starting ART

**Table 3.7: Lipohypertrophy by participant report and physician examination concordance at baseline**

| | Face | Cheeks | Neck | Chest | Upper back | Arms | Abdomen | Buttocks | Legs |
|---|---|---|---|---|---|---|---|---|---|
| Group 1 | 12(14.5) | 17(20.2) | 15(18.1) | 12(14.3) | 11(13.1) | 18(21.4) | 25(30.5) | 18(21.2) | 15(18.1) |
| Group 2 | 7(8.1) | 7(8.1) | 6(6.9) | 5(6.1) | 8(9.3) | 13(15.1) | 15(17.9) | 11(12.8) | 8(9.3) |
| Group 3 | 1(1.1) | 2(2.2) | 1(1.1) | 1(1.1) | 1(1.1) | 4(4.6) | 7(7.8) | 3(3.3) | 3(3.3) |
| p | 0.47 | 0.76 | 0.03 | <0.0001 | 0.02 | 0.0004 | 0.004 | 0.005 | 0.002 |

Results expressed as n(%). Group1 = HIV negative, Group2 = HIV infected not starting ART,

Group3 = HIV infected starting ART

**Table 3.8: Fat distribution by participant and physician examination concordance at baseline**

| | Peripheral Lipoatrophy | Central lipoatrophy | Peripheral Lipohypertrophy | Central lipohypertrophy |
|---|---|---|---|---|
| Group 1 | 16(18.6) | 14(16.3) | 23(26.7) | 27(31.4) |
| Group 2 | 25(28.7) | 19(21.8) | 17(19.5) | 16(18.4) |
| Group 3 | 23(24.5) | 11(11.7) | 7(7.5) | 7(7.5) |
| p | 0.3 | 0.2 | 0.003 | 0.0002 |

Results expressed as n(%). Group1 = HIV negative, Group2 = HIV infected not starting ART, Group3 = HIV infected starting ART. Peripheral: face, cheeks, arms, buttocks, legs; central: neck, chest, upper back, and abdomen

## Laboratory Characteristics

### 4.1. Glycaemic indices

The 1998 World Health Organisation (WHO) and 2009 American Diabetes Association (ADA) criteria for disorders of glycaemia were used to determine the prevalence of diabetes, impaired glucose tolerance (IGT) and impaired fasting glucose (IFG), using both glucose based (oral glucose tolerance test) and $HbA_{1c}$. Table 4.1, Figures 4.1 and 2 show the glycaemic categories in the three groups: HIV negative, group 1; HIV infected not starting ART, group 2 and HIV infected starting ART, group 3.

When glucose-based criteria were applied, using the 1998 WHO criteria, the prevalence of diabetes was 4.94% in group 1 and 0% in group 2 and group 3 (p=0.005). IGT rates were 3.7%, 2.4% and 2.96%, in groups 1, 2 and 3, respectively. The prevalence of dysglycaemia (diabetes, + IGT + IFG) was 8.6%, 3.6% and 3.7% in groups 1, 2 and 3 respectively, p=0.2 (Table 4.1 and Figure 4.1). Using ADA criteria, the prevalence of diabetes and IGT was similar to those with WHO criteria (Table 4.2 and Figure 4.2). However, IFG and dysglycaemia prevalence was higher with ADA than WHO criteria for all three groups.

Using $HbA_{1c}$ criteria for disorders of glycaemia (ADA and WHO), only 1 subject in group 1 was classed as having diabetes. Using ADA cut-points, only 2 subjects, both in group 3, were classed as pre-diabetes.

When stratified according to body mass index (BMI) (Table 4.2), obese individuals had the highest prevalence of dysglycaemia using both WHO criteria (diabetes 3.2%, impaired glucose tolerance 5.4% and impaired fasting glucose 2.2%) and ADA criteria (diabetes 3.2%, impaired glucose tolerance 5.4% and impaired fasting glucose 6.5%). None

of the underweight individuals had diabetes, IGT or IFG by either criterion. In those with normal BMI, 2.5% had IGT and 0% had diabetes or IFG according to WHO criteria, whereas 2.5% had IGT and 1.7% had IFG according to ADA.

**Table 4.1: Glycaemic indices at baseline**

| Variable | Group 1<br>HIV negative<br>n=88 | Group 2<br>HIV infected, not starting ART<br>n=88 | Group 3<br>HIV infected, starting ART<br>n=150 | P |
|---|---|---|---|---|
| **Plasma glucose (mmol/l)** | | | | |
| 0 – min | 5.0 $\pm$ 0.9 | 4.8 $\pm$ 0.4 | 4.8 $\pm$ 0.4 | 0.8 |
| 120 – min | 5.6 $\pm$ 2.3 | 4.8 $\pm$ 1.3 | 5.2 $\pm$ 1.1 | 0.3 |
| HbA$_{1c}$ | 3.97 $\pm$ 0.7 | 3.95 $\pm$ 0.6 | 3.98 $\pm$ 0.7 | 0.95 |
| | **Categories of glycaemia (WHO)** | | | |
| **Glucose-based (OGTT)** | | | | |
| Diabetes | 4(4.9) | 0(0.0) | 0(0.0) | 0.005 |
| IGT | 3(3.7) | 2(2.4) | 4(2.96) | 0.9 |
| IFG | 0(0.0) | 1(1.2) | 1(0.7) | 1.0 |
| Dysglycaemia | 7(8.6) | 3(3.6) | 5(3.7) | 0.2 |
| Missing | 7(7.9) | 4(4.5) | 15(10.0) | |
| **HbA$_{1c}$-based** | | | | |
| Diabetes ($\geq$6.5%) | 1(1.2) | 0(0.0) | 0(0.0) | 0.3 |
| Missing | 18(20.4) | 13(14.8) | 16(10.7) | |
| | **Categories of glycaemia (ADA)** | | | |
| **Glucose-based (OGTT)** | | | | |
| Diabetes | 4(4.9) | 0(0.0) | 0(0.0) | 0.005 |
| IGT | 3(3.7) | 2(2.4) | 4(2.96) | 0.9 |
| IFG | 3(3.7) | 3(3.6) | 5(3.7) | 1.0 |
| Dysglycaemia | 10(12.3) | 5(6.0) | 9(6.6) | 0.4 |
| Missing | 7(7.9) | 4(4.5) | 15(10.0) | |
| **HbA$_{1c}$-based** | | | | |
| Diabetes ($\geq$6.5%) | 1(1.2) | 0(0.0) | 0(0.0) | 0.3 |
| Pre diabetes* | 0(0.0) | 0(0.0) | 2(1.3) | 0.3 |
| Missing | 18(20.4) | 13(14.8) | 16(10.7) | |

Results expressed as mean $\pm$ SD or n (%). WHO: World Health Organization; ADA: American Diabetic Association; IGT: Impaired Glucose Tolerance; IFG: Impaired Fasting Glucose; HbA$_{1c}$: Haemoglobin A$_{1c}$; *HbA$_{1c}$ 5.7- 6.4mmol/l; OGTT: oral glucose tolerance test

**Figure 4.1: Prevalence of dysglycaemia using glucose-based World Health Organization (WHO) criteria in the study subjects\*(n=326)**
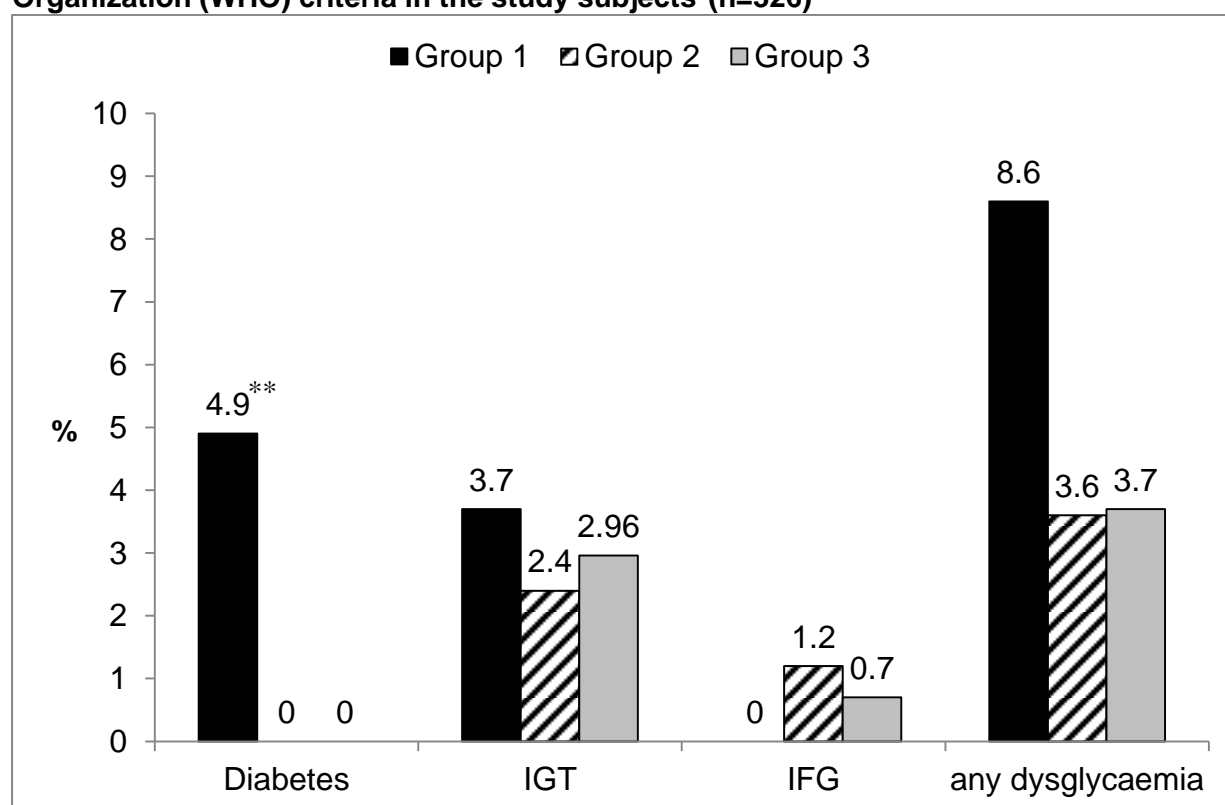


\*Group1: HIV negative; Group2: HIV infected not starting ART; Group3: HIV infected starting ART. IGT: Impaired glucose tolerance; IFG: Impaired fasting glucose.

\*\*$p < 0.01$ group 3 vs. group 1

**Figure 4.2: Prevalence of dysglycaemia by American Diabetes Association (ADA) criteria using glucose based criteria in study subjects* (n= 326)**
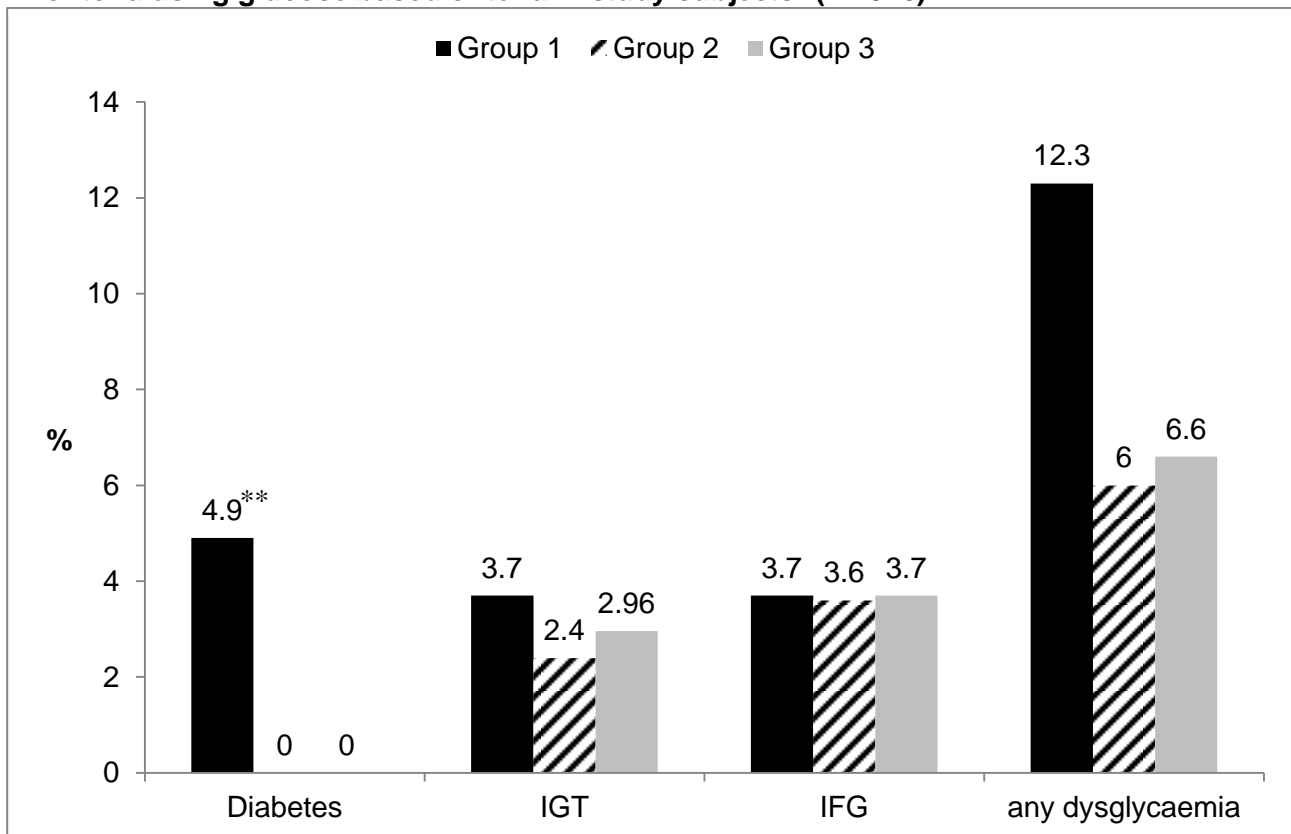


*Group1: HIV negative; Group2: HIV infected not starting ART; Group3: HIV infected starting ART. IGT: Impaired glucose tolerance; IFG: Impaired fasting glucose. **$p < 0.01$ group 3 vs. group 1.

**Table 4.2: Glycaemic categories according to body mass index at baseline (n: 293)**

| | n | Underweight | Normal | Overweight | Obese | P |
|---|---|---|---|---|---|---|
| **Categories of glycaemia (WHO)** | | | | | | |
| Diabetes | 4 | 0(0.0) | 0(0.0) | 1(1.3) | 3(3.2) | 0.2 |
| IGT | 9 | 0(0.0) | 3(2.5) | 1(1.3) | 5(5.4) | 0.4 |
| IFG | 2 | 0(0.0) | 0(0.0) | 0(0.0) | 2(2.2) | 0.2 |
| **Categories of glycaemia (ADA)** | | | | | | |
| Diabetes | 4 | 0(0.0) | 0(0.0) | 1(1.3) | 3(3.2) | 0.2 |
| IGT | 9 | 0(0.0) | 3(2.5) | 1(1.3) | 5(5.4) | 0.4 |
| IFG | 11 | 0(0.0) | 2(1.7) | 3(3.9) | 6(6.5) | 0.3 |

Results expressed as n (%). Body mass index (BMI) (kg/m$^2$): underweight < 18.5, normal 18.5-24.99, overweight 25.0-29.99; obese $\geq$ 30.0

## 4.2. Lipid Measurements

When compared with group 1, mean values in group 3 were lower for the following serum lipids: total cholesterol (p<0.0001), LDL (p= 0.0007) and HDL (p<0.0001) (Table 4.3). There was no difference in mean total triglycerides in the three groups (p=0.3). When using the American Association of Clinical Endocrinologists guidelines for management of dyslipidemia and prevention of atherosclerosis (83), no subject in group 2 or group 3 had high risk levels of total cholesterol compared with 3.9% in group 1, p=0.01. Compared to group 1, more subjects in group 3 had high cardiovascular disease risk levels of HDL (low HDL), for both males and females (p<0.0001).

**Table 4.3: Serum lipids at baseline**

| Variable | Group 1 HIV negative n=88 | Group 2 HIV infected, not starting ART n=88 | Group 3 HIV infected, starting ART n=150 | P |
|---|---|---|---|---|
| Serum lipids (mmol/l): | | | | |
| Total cholesterol | 4.1 $\pm$ 1.0 | 3.9 $\pm$0.8 | 3.5 $\pm$0.9 | <0.0001 |
| Total triglycerides | 0.9 $\pm$0.6 | 0.9 $\pm$ 0.5 | 0.97$\pm$0.6 | 0.3 |
| LDL | 2.6 $\pm$ 0.8 | 2.6 $\pm$ 0.6 | 2.2 $\pm$ 0.8 | 0.0007 |
| HDL | 1.15 + 0.26 | 0.94 + 0.26 | 0.82 + 0.29 | <0.0001 |
| **Grading of serum lipid abnormalities (mmol/l)** | | | | |
| Total cholesterol | | | | 0.006 |
| Optimal | 66 (84.6) | 74 (96.1) | 125 (96.2) | |
| Borderline | 9 (11.5) | 3 (3.9) | 5  (3.9) | |
| High risk | 3 (3.9) | 0 (0.0) | 0  (0.0) | |
| Missing | 10 (11.4) | 11(12.5) | 20(13.3) | |
| Total triglycerides | | | | 0.99 |
| Optimal | 69 (90.8) | 69 (90.8) | 116 (91.3) | |
| Borderline | 4 (5.3) | 4 (5.3) | 7  (5.5) | |
| High risk | 3 (3.95) | 3 (3.95) | 4  (3.2) | |
| Missing | 12(0.0) | 11(12.5) | 23(0.0) | |
| LDL | | | | 0.2 |
| Optimal | 52 (86.7) | 63 (94.0) | 104 (91.2) | |
| Borderline | 6 (10.0) | 4 (5.97) | 9 (7.89) | |
| High risk | 0 (0.0) | 0 (0.0) | 1 ( 0.9) | |
| Very High risk | 2 (3.3) | 0 (0.0) | 0 ( 0.0) | |
| Missing | 28(0.0) | 21(23.9) | 36 (24.0) | |

Continued on next page

**Table 4.3 cont.: Serum lipids at baseline**

| Variable | Group 1 HIV negative n=88 | Group 2 HIV infected, not starting ART n=88 | Group 3 HIV infected, starting ART n=150 | P |
|---|---|---|---|---|
| HDL (Female) | | | | <0.0001 |
| Optimal | 7 (12.7) | 0 (0.0) | 2 (2.3) | |
| Borderline | 7 (12.7) | 4 (7.4) | 3 (3.5) | |
| High risk | 38 (74.6) | 45 (92.6) | 81 (94.2) | |
| HDL (Male) | | | | <0.0001 |
| Optimal | 2 (8.0) | 2 (7.4) | 0 (0.0) | |
| Borderline | 16 (64.0) | 4 (14.8) | 5 (12.2) | |
| High risk | 7 (28.0) | 21 (77.8) | 36 (87.8) | |
| Missing | 11 (12.5) | 12 (13.6) | 23 (15.3) | |

Results expressed as mean $\pm$ SD or n (%). P values for comparison between group 1 vs. group 2 vs. group 3. LDL: Low density lipoprotein cholesterol; HDL: High density lipoprotein cholesterol

## 4.3. Inflammatory markers/haematological and other blood tests

Table 4.4 shows laboratory parameters of the three groups. Serum urea and creatinine were within reference range in all three groups. Group 3 had the highest levels of total protein ($p<0.0001$), globulin ($p <0.0001$), C-reactive protein ($p<0.0001$) and cortisol ($p=0.005$) but had the lowest mean levels of iron ($p<0.0001$), transferrin ($p<0.0001$), haemoglobin ($p<0.0001$) and lymphocyte count ($p<0.0001$).

**Table 4.4: Inflammatory Markers and other blood tests at baseline**

| Variable | Group 1 HIV negative n=88 | Group 2 HIV positive not starting ART, n=88 | Group 3 HIV positive starting ART n=150 | P |
|---|---|---|---|---|
| **Renal Function** | | | | |
| Bicarbonate (mmol/l) | 26.3±2.8 | 25.2±2.2 | 24.6±2.5 | <0.0001 |
| Chloride (mmol/l) | 104.1±4.9 | 103.9±2.9 | 104.4±3.9 | 0.5 |
| Urea (mmol/l) | 3.3±1.1 | 3.4±1.1 | 3.5±1.3 | 0.6 |
| Creatinine (µmol/l) | 65.0(58.0-79.0) | 66.0(54.0-75.0) | 66.0(56.0-78.0) | 0.7 |
| Anion gap | 13.5±2.7 | 12.3±2.9 | 11.35±3.12 | <0.0001 |
| Calcium (mmol/l) | 2.31(2.3-2.4) | 2.26(2.2-2.3) | 2.21(2.1-2.3) | <0.0001 |
| Magnesium (mmol/l) | 0.9(0.8-0.9) | 0.9(0.8-0.9) | 0.9(0.8-0.9) | 0.2 |
| Phosphate (mmo/l) | 1.1±0.2 | 1.1±0.2 | 1.1±0.2 | 0.3 |
| **Liver function** | | | | |
| Total protein(g/L) | 73.9±4.8 | 82.98±11.74 | 86.80±9.72 | <0.0001 |
| Albumin(g/L) | 40.1±3.7 | 37.12±3.74 | 34.38±5.59 | <0.0001 |
| Globulin(g/L) | 33.6±4.3 | 46.21±9.82 | 51.95±11.71 | <0.0001 |
| Total bilirubin | 8.9±3.4 | 7.2±3.5 | 7.9±4.9 | 0.02 |
| ALT(U/l) | 16.0(14.0-21.0) | 19.0(15.0-24.0) | 21.0(16.0-29.0) | 0.0001 |
| ALP | 61.5±19.2 | 60.6±20.2 | 66.95±29.3 | 0.09 |
| GGT | 23.5±17.6 | 24.4±17.8 | 30.7±32.1 | 0.1 |
| **Full blood count** | | | | |
| Haemoglobin (g/dL) | 12.9±2.3 | 12.0±1.9 | 11.1±1.97 | <0.0001 |
| Platelet count (x$10^9$/L) | 276.1±52.2 | 271.61±99.22 | 249.9±82.1 | 0.03 |
| White cell count (x$10^9$/L) | 5.5(4.2-7.4) | 5.6(4.5-6.9) | 4.29(3.3-5.2) | <0.0001 |
| Lymphocytes (x$10^9$/L) | 1.97(1.7-2.5) | 2.0(1.6-2.3) | 1.4(0.9-2.0) | <0.0001 |

Continued on next page

**Table 4.4 cont.: Inflammatory Markers and other blood tests at baseline**

| Variable | Group 1 HIV negative n=88 | Group 2 HIV positive not starting ART, n=88 | Group 3 HIV positive starting ART n=150 | P |
|---|---|---|---|---|
| **Inflammatory Markers** | | | | |
| CRP*(mg/L) | 5.9 ± 8.0 | 6.0 ± 7.1 | 19.5 ± 36.3 | <0.0001 |
| Lactate (mmol/l) | 1.7(1.4-2.5) | 1.4(1.0-1.9) | 1.3(1.0-1.8) | <0.0001 |
| Uric acid(mmol/L) | 0.27(0.23-0.31) | 0.30(0.25-0.35) | 0.28(0.23-0.34) | 0.05 |
| Cortisol(nmol/L) | 258.6±106.9 | 263.2±103.3 | 305.6±131.3 | <0.005 |
| Iron(umol/L) | 13.3(10.3-17.9) | 11.5(7.7-13.7) | 9.95(6.0-13.5) | <0.0001 |
| Transferrin(g/L) | 2.7(2.5-3.0) | 2.5(2.2-2.8) | 2.2(1.9-2.6) | <0.0001 |
| Saturation (%) | 19.0(12.0-25.0) | 19.5(14.0-24.0) | 17.0(10.0-25.0) | 0.5 |
| Ferritin(ug/L) | 57.0(30.0-122.0) | 57.5(28.0-98.0) | 90.0(28.0-204.0) | 0.07 |

Results expressed as mean ± SD or median (IQR). P values for comparison between group 1 vs. group 2 vs. group 3. *C-reactive protein, ALT: serum alanine aminotransferase, ALP: serum alkaline phosphatase, GGT: serum gamma glutamyl transferase

## 4.4. Risk factors associated with diabetes and dysglycaemia at baseline.

Risk factors for diabetes were assessed only for HIV negative subjects (n=88) since the prevalence of diabetes was 0% for each of each group of the HIV infected subjects. Risk factors for dysglycaemia was measured for all groups (n=326).

### 4.4.1. Diabetes

Univariate analysis (Table 4.5) showed that significant variables associated with diabetes were the following: systolic blood pressure (p=0.001), diastolic blood pressure (p=0.002), triglyceride (p=0.01), total cholesterol (p=0.03), cortisol (p=0.01) and visceral fat area (p=0.04).

In multivariate analysis (Table 4.6), the significant independent risk factors associated with diabetes were systolic blood pressure (p=0.02) and triglycerides (p=0.04) after adjusting for body mass index.

### 4.4.2. Dysglycaemia (Diabetes or IGT or IFG)

Univariate analysis (Table 4.5) showed that significant factors associated with dysglycaemia were age (p=0.004), systolic blood pressure (p<0.0001), diastolic blood pressure (p=0.0004), mid-arm circumference (p=0.04), triglycerides (p=0.0004), total cholesterol (p=0.02), cortisol (p=0.0004), visceral fat area (p=0.003) and visceral: subcutaneous fat area (p=0.02).

In multivariate analysis (Table 4.6), independent risk factors associated with dysglycaemia (WHO criteria) were systolic blood pressure (p=0.003), serum triglycerides (p=0.02) and visceral: subcutaneous fat ratio (p=0.008) after adjusting for body mass index. Using ADA criteria, systolic blood pressure (OR 1.05 95%CI 1.02-1.09, p=0.004) and visceral subcutaneous fat area (OR 6.2 95%CI 1.5 to 25.6, p=0.01) were significantly associated with dysglycaemia after adjusting for body mass index.

**Table 4.5: Univariate analysis for risk factors associated with Diabetes and dysglycaemia**[*]

| | Univariate analysis | | | |
| | Diabetes | | any dysglycaemia | |
| Variable | OR (95%CI) | p | OR (95% CI) | p |
|---|---|---|---|---|
| Age | 1.06 (0.97-1.1) | 0.2 | 1.08(1.02-1.13) | 0.004 |
| Gender | 1.99 (0.28-14.3) | 0.5 | 0.4(0.15 - 1.20) | 0.1 |
| Systolic BP | 1.4 (1.1 - 1.7) | 0.001 | 1.29(1.15 - 1.44) | <0.0001 |
| Diastolic BP | 1.9 (1.3 - 2.7) | 0.002 | 1.44(1.18 - 1.76) | 0.0004 |
| Body mass index | 2.3 (0.4-15.3) | 0.4 | 2.1 (0.8-5.5) | 0.1 |
| Circumferences: | | | | |
| Waist | 1.04 (0.99 - 1.1) | 0.1 | 1.02(0.99-1.05) | 0.2 |
| Mid-thigh | 0.99(0.92-1.1) | 0.9 | 0.99(0.94-1.04) | 0.6 |
| Hip | 1.01(0.95-1.07) | 0.8 | 0.99(0.96-1.03) | 0.8 |
| Mid-arm | 1.1 (0.99 - 1.3) | 0.07 | 1.08(1.00-1.17) | 0.04 |
| Waist to height ratio | 1.05(0.98-1.1) | 0.2 | not estimatable | 0.004 |
| Familial diabetes | 1.2 (0.1 to 11.4) | 0.9 | 1.8(0.6-5.5) | 0.3 |

Continued on next page

**Table 4.5 cont.: Univariate analysis for risk factors associated with Diabetes and dysglycaemia[*]**

| | Univariate analysis | | | |
| --- | --- | --- | --- | --- |
| | **Diabetes** | | **any dysglycaemia** | |
| LDL | 1.3 (0.2 to 7.2) | 0.8 | 1.4(0.66-2.82) | 0.4 |
| HDL | 1.3 (0.1 to 26.7) | 0.8 | 0.49(0.08-2.83) | 0.4 |
| Triglyceride | 2.99 (1.3 to 6.96) | 0.01 | 2.79(1.44-5.4) | 0.002 |
| Total cholesterol | 2.9 (1.1 to 7.1) | 0.03 | 0.55(0.32-0.92) | 0.02 |
| Globulin | 0.9 (0.9 to 1.0) | 0.1 | 0.98(0.94-1.02) | 0.3 |
| Cortisol | 1.0 (1.0 to 1.0) | 0.01 | 1.01(1.0 - 1.01) | 0.0004 |
| CD4 cell count | | | 0.998(0.994-1.002) | 0.4 |
| [†]Clinical central hypertrophy | 0.2(0.02 to 4.07) | 0.3 | 0.6(0.12 to 3.3) | 0.6 |
| [‡]Visceral fat area | 1.014(1.001 to 1.03) | 0.04 | 1.01(1.005 to 1.023) | 0.003 |
| [‡]Subcutaneous fat area | 1.001(0.99 to 1.008) | 0.8 | 0.998(0.99 to 1.003) | 0.4 |
| [‡]Visceral:subcutaneous fat ratio | 2.89(0.23 to 37.30) | 0.4 | 5.16(1.36 to 19.58) | 0.02 |

CI: Confidence interval; BP: blood pressure; LDL: low density lipoprotein; HDL: high density lipoprotein. [*]Diabetes or impaired glucose tolerance (IGT) or impaired fasting glucose (IFG). [†]Participant and physician examination questionnaire; [‡]Computerized tomography scan measurements

**Table 4.6: Bivariate and Multivariate analysis for risk factors associated with diabetes mellitus and dysglycaemia[*] at baseline**

| | Multivariate analysis | | | |
| | [*]Diabetes | | [†]any dysglycaemia | |
| Variable | OR (95%CI) | p | OR (95% CI) | p |
|---|---|---|---|---|
| Systolic BP | 1.06(1.01-1.06) | 0.02 | 1.07(1.02-1.12) | 0.003 |
| Triglycerides | 4.95(1.10-22.24) | 0.04 | 3.42(1.18-9.94) | 0.02 |
| Visceral:subcutaneous fat | | | 15.6(2.07 to 117.9) | 0.008 |

BP: blood pressure; [*]World Health Organisation (WHO) and American Diabetes Association glucose-based criteria;
[†]WHO glucose-based criteria; [*]Diabetes or IGT or IFG

# CHAPTER FIVE: LONGITUDINAL STEP

## FOLLOW-UP STUDY OF GROUP 3 SUBJECTS
## (HIV INFECTED AND ELIGIBLE FOR ART)

## 5.1.      Follow up and response rate

Group 3 subjects were commenced on combination antiretroviral therapy (ART) and followed up for 24 months or longer. Of the 150 (M: F; 48:102) subjects who participated at baseline, 97 (M: F; 33:64) returned for the 24 months visit, with an overall follow up (response) rate of 97/150 (64.7%) (Figure 5.1).

Figure 5.2 outlines the details of the follow up. Of the 53 subjects who did not have complete 24 month visit, 8 (15.1%) were known to have died, 4(7.5%) fell pregnant and 41(77.4%) were lost to follow up. The largest proportion of loss to follow-up (16/41, 39.0 %) and known deaths (6/8; 75.0%) occurred in the first three months after commencement of ART.

Baseline characteristics of the group that did not complete the 24 month visit follow up (n=53) were similar to those of the group that completed follow up (n=97) as shown in Table 5.1, except for the difference in employment status. The majority of subjects in the group that did not complete follow up were unemployed compared to the majority of employed subjects in the group that completed follow up (p=0.002).

For the total group, the known mortality rate was 5.3% (8/150) and pregnancy rate was 3.9% (4/102). Forty subjects missed visits during the course of the study, again with the highest proportion (<50%) for the 3 month visit, with declining rates for subsequent visits.

**Figure 5.1: Overall response rate of Group 3 (HIV infected and eligible for ART)**

Baseline

**n =150**

↓

24 Months

Follow-up complete

**n = 97**

(64.7%)

Not followed-up

**n = 53**

(35.0%)
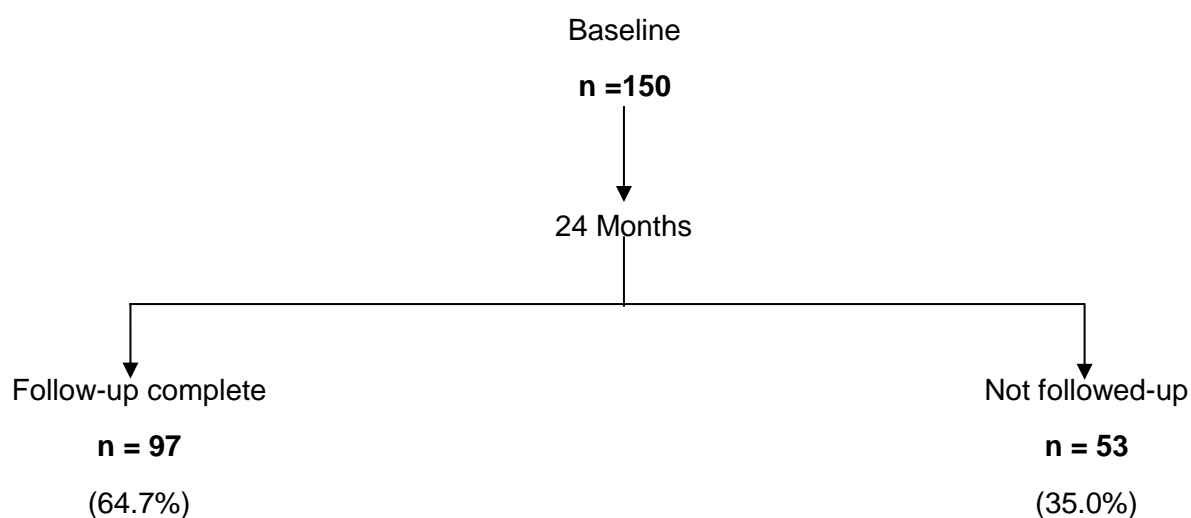
**Figure 5.2: Follow up of Group 3 (HIV infected and eligible for ART)**

Baseline
**n = 150**

n = 47
Loss to follow-up 16
Died 6
Missed visit 25

↓

3 months
**n = 103**

n = 21
Loss to follow-up 8
Pregnant 1
Missed visit 12

↓

6 months
**n = 107**

n = 11
Loss to follow-up 6
Died 1
Missed visit 4

↓

12 months
**n = 108**

n = 15
Loss to follow-up 5
Pregnant 1
Missed visit 9

↓

18 months
**n = 97**

n =9
Loss to follow-up 6
Pregnant 2
Died 1

↓
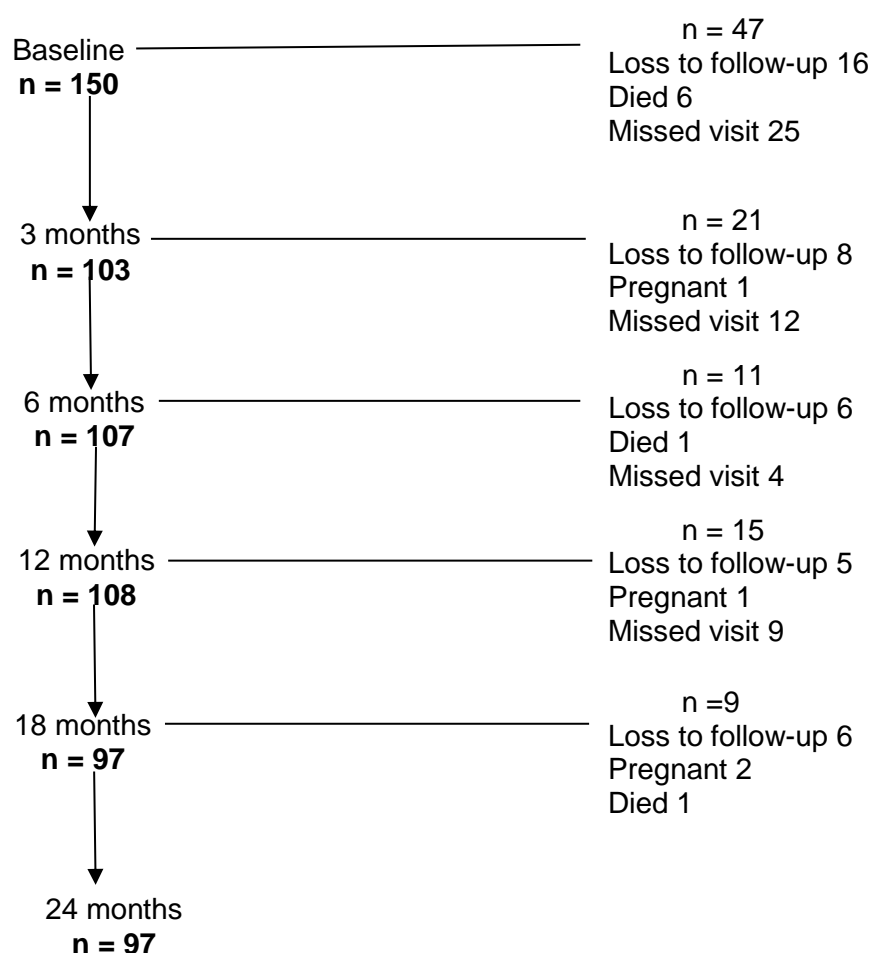
24 months
**n = 97**

**Table 5.1: Baseline characteristics of Group 3 subjects that completed vs. not completed 24 month visit follow-up**

| Variable | 24 month follow-up complete n=97 | 24 month follow-up incomplete n=53 | p |
|---|---|---|---|
| Age | 37.5±9.1 | 36.0±9.3 | 0.4 |
| Female | 64(65.98) | 38(71.7) | 0.5 |
| Marital Status | | | 0.5 |
| Single | 71(73.2) | 41(77.4) | |
| Married | 15(15.5) | 10(18.9) | |
| Divorced | 4(4.1) | 0(0.0) | |
| Cohabitating | 3(3.1) | 1(1.9) | |
| Widowed | 4(4.1) | 1(1.9) | |
| Education | | | 0.4 |
| Primary | 19(20.9) | 9(17.7) | |
| High school | 64(70.3) | 34(66.7) | |
| Tertiary | 7(7.7) | 5(9.8) | |
| No education | 1(1.1) | 3(5.9) | |
| Employed | 47(50.5) | 12(24.0) | 0.002 |
| Body mass index | 26.6±5.9 | 26.1±6.7 | 0.7 |
| Body mass index categories | | | 0.8 |
| Underweight | 3(3.3) | 1(1.9) | |
| Normal | 42(45.7) | 25(48.1) | |
| Overweight | 20(21.7) | 14(26.9) | |
| Obese | 27(29.4) | 12(23.1) | |
| Laboratory | | | |
| CD4 cell count(cells/mm$^3$) | 142±82.6 | 135.2±97.5 | 0.7 |
| log HIV RNA load | 4.7±1.0 | 4.9±0.8 | 0.2 |
| Haemoglobin | 11.3±1.9 | 10.8±2.1 | 0.1 |
| Albumin | 34.9±5.0 | 33.4±6.3 | 0.1 |
| Treatment allocation | | | |
| Efavirenz | 49(50.5) | 27(50.9) | 0.96 |
| Nevirapine | 48(49.5) | 26(49.1) | 0.96 |
| Tenofovir | 97(100.0) | 53(100.0) | - |
| Lamivudine | 97(100.0) | 53(100.0) | - |

Data expressed as mean±SD or n(%)

## 5.2. Treatment Allocation

Table 5.2 shows treatment allocation for group 3. Combination antiretroviral therapy included two nucleoside reverse transcriptase inhibitors, (lamivudine 150mg BD and tenofovir disoproxil fumarate (TDF) 300mg nocte) and a non-nucleoside reverse transcriptase inhibitor (either nevirapine 200mg BD or efavirenz 600mg nocte). More women 66/102(64.7%) were prescribed nevirapine and efavirenz was prescribed to the majority of men 40/48(83.3%). Efavirenz was avoided in women of child-bearing age because of its presumed teratogenic effects and in men who worked shifts because of its neuro-psychiatric effects which requires that it is taken at bed-time.

No participants were commenced on a protease inhibitor as first line treatment as per national treatment guidelines but three participants were switched to ritonavir boosted lopinavir combined with lamivudine and zidovudine at 18 months follow up for HIV treatment failure. All participants with CD4 cell count less than 200cells/mm$^3$ were prescribed prophylactic co-trimoxazole to prevent opportunistic infections until the CD 4 cell count was sustained above 200cells/mm$^3$.

**Table 5.2: Antiretroviral treatment (ART) in group 3 subjects**

| Variable | All patients n=150 | Male n=48 | Female n=102 | P |
|---|---|---|---|---|
| Efavirenz | 76 (50.7) | 40 (83.3) | 36 (35.3) | <0.0001 |
| Nevirapine | 74 (49.3) | 8 (16.7) | 66(64.7) | <0.0001 |
| Lamivudine | 150 (100.0) | 48 (100.0) | 102 (100.0) | |
| Tenofovir | 150 (100.0) | 48 (100.0) | 102 (100.0) | |

Results expressed as n (%)

## 5.3. Characteristics at baseline and at follow-up

### 5.3.1. Clinical

The clinical characteristics of group 3 from baseline through 24 months follow up are shown in Table 5.3. When mean values at 24 months on ART were compared with baseline prior to commencing ART, there was a significant increase in mean systolic ($p<0.0001$) and diastolic ($p=0.05$) blood pressure, weight ($p < 0.0001$) and body mass index ($p < 0.0001$). There was an increase in the following measurements from baseline prior to commencing ART through 24 months on ART: circumferences (waist, hip, mid-arm, neck and mid-thigh) and skinfolds (triceps, sub-scapular and mid-thigh); however, the differences were not significant.

When the mean difference between 24 months follow-up and baseline was measured only for those who completed follow up, there was a significant mean difference (increase) in weight ($p<0.0001$) and body mass index ($p<0.0001$) (Table 5.4). There were no significant mean differences for other clinical and anthropometric measurements.

**Table 5.3: Clinical Characteristics at follow up on ART in group 3 subjects**

| Variable | Baseline n=150 | 3 Months n=103 | 6 Months n=107 | 12 Months n=108 | 18 Months n=97 | 24 Months n=97 | β estimate [95%CI] | P |
|---|---|---|---|---|---|---|---|---|
| Blood pressure (mmHg) | | | | | | | | |
| Systolic | 112.1 $\pm$ 16.8 | 112.2 $\pm$ 12.7 | 112.2 $\pm$ 12.1 | 116.3 $\pm$ 15.8 | 118.97 $\pm$ 15.9 | 118.95 $\pm$ 15.2 | 1.2[0.6-1.8] | <0.0001 |
| Diastolic | 70.9 $\pm$ 10.7 | 70.9 $\pm$ 9.5 | 70.1 $\pm$ 8.5 | 72.5 $\pm$ 10.8 | 73.2 $\pm$ 10.6 | 73.6 $\pm$ 11.4 | 0.4[-0.007-0.8] | 0.05 |
| Normal BP, n(%) | 102 (70.8) | 69 (70.4) | 75 (77.3) | 57 (58.2) | 44 (50.6) | 51 (54.8) | | |
| High Normal BP, n(%) | 33 (22.9) | 25 (25.5) | 17 (17.5) | 30 (30.6) | 30 (34.5) | 29 (31.2) | | |
| Stage 1 hypertension, n(%) | 6 (4.2) | 4 (4.1) | 4 (4.1) | 8 (8.2) | 11 (12.6) | 9 (9.7) | | |
| Stage 2 hypertension, n(%) | 3 (2.1) | 0 (0.00) | 1 (1.0) | 3 (3.1) | 2 (2.3) | 4 (4.3) | | |
| Missing | 3 (2.0) | 9 (8.4) | 10 (9.4) | 9 (8.3) | 8 (8.3) | 4 (4.1) | | |
| Anthropometric measurements | | | | | | | | |
| Weight (kg) | 69.5 $\pm$ 15.8 | 71.2 $\pm$ 18.1 | 72.2 $\pm$ 16.7 | 73.6 $\pm$ 18.1 | 75.9 $\pm$ 19.4 | 78.1 $\pm$ 18.1 | 1.3[0.9-1.7] | <0.0001 |
| Height (m) | 1.6 $\pm$ 0.1 | 1.6 $\pm$ 0.1 | 1.6 $\pm$ 0.1 | 1.6 $\pm$ 0.1 | 1.6 $\pm$ 0.1 | 1.6 $\pm$ 0.1 | | |
| Body mass index (kg/m$^2$) | 26.4 $\pm$ 6.2 | 27.2 $\pm$ 7.1 | 27.2 $\pm$ 6.2 | 28.1 $\pm$ 6.7 | 28.7 $\pm$ 7.1 | 29.4 $\pm$ 7.0 | 0.5[0.3-0.6] | <0.0001 |
| Underweight, n (%) | 4 (2.8) | 2 (2.1) | 2 (2.1) | 4 (4.3) | 3 (3.9) | 3 (3.2) | | |
| Normal, n (%) | 67 (46.5) | 42 (43.3) | 44 (45.8) | 32 (34.0) | 29 (37.2) | 29 (30.5) | | |
| Overweight, n (%) | 34 (23.6) | 24 (24.7) | 19 (19.8) | 22 (23.4) | 16 (20.5) | 25 (26.3) | | |
| Obese, n (%) | 39 (27.1) | 29 (29.9) | 31 (32.3) | 36 (38.3) | 31 (86.5) | 38 (40.0) | | |
| Missing, n(%) | 3 (2.0) | 0 (0.0) | 11 (10.3) | 13 (12.0) | 18 (18.6) | 2 (2.1) | | |

Continued on next page

Table 5.3 cont.: Clinical Characteristics at follow up on ART in group 3 subjects

| Variable | Baseline n=150 | 3 Months n=103 | 6 Months n=107 | 12 Months n=108 | 18 Months n=97 | 24 Months n=97 | β estimate [95%CI] | P |
|---|---|---|---|---|---|---|---|---|
| Circumference (cm): | | | | | | | | |
| Waist | 92.5 ± 17.1 | 91.3 ± 25.2 | 91.5 ± 14.9 | 89.3 ± 15.3 | 90.1 ± 15.5 | 93.5 ± 15.9 | -0.06[-0.9-0.8] | 0.9 |
| Hip | 106.2 ± 14.7 | 102.97 ± 14.9 | 105.6 ± 13.3 | 101.9 ± 13.9 | 103.1 ± 14.2 | 106.8 ± 14.4 | -0.06[-0.8-0.07] | 0.9 |
| Waist: Hip ratio | 0.9 ± 0.1 | 0.9 ± 0.3 | 0.9 ± 0.1 | 0.9 ± 0.1 | 0.87 ± 0.07 | 0.9 ± 0.1 | -0.0007[-0.006-0.004] | 0.8 |
| Waist: Height | 56.95 ± 11.2 | 56.5 ± 16.9 | 56.4 ± 10.3 | 55.8 ± 10.3 | 55.8 ± 9.95 | 57.4 ± 10.5 | 0.03[-0.5-0.5] | 0.9 |
| Mid-arm | 32.3 (29.4-35.9) | 31.9 (27.9-34.9) | 32.2 (28.9-35.4) | 30.8 (28.2-34.8) | 31.3 (27.6-34.9) | 33.0(29.5–36.5) | 0.02[-0.2-0.3] | 0.9 |
| Neck | 35.3 ± 3.5 | 34.6 ± 3.45 | 35.1 ± 3.7 | 34.7 ± 2.7 | 35.1 ± 3.9 | 36.6 ± 11.0 | 0.1[-0.2-0.4] | 0.4 |
| Chest | 94.8 ± 10.6 | 92.9 ± 10.8 | 93.8 ± 10.3 | 92.7 ± 9.8 | 92.4 ± 11.68 | 95.9 ± 10.2 | 0.04[-0.5-0.5] | 0.9 |
| Mid-thigh | 55.3 (50.2-61.95) | 53.1 (48.4-59.9) | 54.5 (49.95-61.4) | 52.6 (48.0-61.2) | 53.3 (48.2-61.3) | 57.1(50.3-64.2) | 0.07[-0.4-0.5] | 0.8 |
| Skin folds | | | | | | | | |
| Triceps | 23.3 ± 13.2 | 20.5 ± 11.4 | 21.8 ± 12.3 | 20.7 ± 12.3 | 20.6 ± 12.6 | 23.8 ± 14.3 | -0.01[-0.08-0.06] | 0.8 |
| Sub-scapula | 19.3 ± 11.97 | 17.6 ± 10.3 | 19.5 ± 12.2 | 17.5 ± 11.6 | 17.4 ± 11.2 | 20.0 ± 12.6 | -0.01[-0.07-0.04] | 0.7 |
| Abdominal | 25.2 ± 14.2 | 22.8 ± 13.2 | 24.6 ± 12.8 | 22.1 ± 12.95 | 23.1 ± 12.97 | 23.7 ± 13.4 | -0.03[-0.1-0.03] | 0.3 |
| Mid-thigh | 32.7 ± 17.1 | 30.8 ± 16.0 | 32.2 ± 16.2 | 29.9 ± 17.2 | 29.9 ± 16. 6 | 33.0 ± 18.6 | -0.03[-0.1-0.055] | 0.5 |

Data expressed as means ± SD or median (IQR) except where specified. BP: Blood pressure. β estimate measures rate of change over time, testing linear trend using linear mixed model.CI: Confidence interval

## 5.4. Fat loss and gain at follow up

### 5.4.1. Participant self-report

A participant administered questionnaire was completed at baseline, month 12 and month 24 (Figure 5.3, Tables 5.3 and 5.7). Figure 5.3 shows the participants' report of fat gain compared to fat loss from five years prior to baseline (before commencing ART) to 24 months on ART. Participants reported more fat gain than fat loss at the 24 month follow up visit on ART in peripheral sites: face (p<0.0001), cheeks (p<0.0001), arms (p<0.0001), buttocks (p<0.0001), legs (p<0.0001). Similarly, more fat gain than loss was reported at the 12 month visits for these sites. More fat gain than loss was also reported on follow up in the central sites: neck (p<0.0001), upper back (p<0.0001), front of chest (p<0.0001), breasts (p<0.0001), waist (p<0.0001) and abdomen (p<0.0001).

The proportion of those with fat loss at peripheral sites (face, cheeks, neck, arms and legs) at baseline compared with the proportion of those with fat loss at peripheral sites at 24 months follow up was not different except for fat on buttocks (p=0.03). The proportion of those with fat gain at central sites (chest, breasts, abdomen and upper back) at baseline was not different from the proportion of those with fat gain at central sites at 24 months follow up. The majority of participants reported no change in fat distribution at baseline and at 24 months follow up (Table 5.7).

**Table 5.4: Characteristics at baseline vs. at 24 month follow up in group 3 subjects on ART***

| Variable | Baseline | | 24 months | | Mean Difference | P |
|---|---|---|---|---|---|---|
| | Mean±SD | n | Mean±SD | n | | |
| Blood pressure (mmHg) | | | | | | |
| Systolic | 115.2 ± 16.9 | 91 | 118.95 ± 15.2 | 91 | 3.8 ± 17.99 | 0.1 |
| Diastolic | 72.4 ± 11.1 | 90 | 73.6 ± 11.4 | 90 | 0.9 ± 11.95 | 0.5 |
| **Anthropometric measurements** | | | | | | |
| Weight (kg) | 70.7 ± 15.3 | 92 | 78.1 ± 18.1 | 93 | 7.8 ± 8.9 | <0.0001 |
| Height (m) | 1.6 ± 0.1 | 92 | 1.6 ± 0.1 | 94 | - | - |
| BMI(kg/m$^2$) | 26.6 ± 6.0 | 92 | 29.4 ± 7.0 | 93 | 2.9 ± 3.3 | <0.0001 |
| **Circumference (cm):** | | | | | | |
| Waist | 91.2 ± 16.7 | 91 | 93.5 ± 15.9 | 93 | 1.7 ± 23.1 | 0.5 |
| Hip | 104.6 ± 14.1 | 92 | 106.8 ± 14.4 | 93 | 1.9 ± 21.0 | 0.3 |
| Waist: Hip ratio | 0.9 ± 0.1 | 91 | 0.9 ± 0.1 | 93 | 0.0 ± 0.1 | 0.8 |
| Waist: Height | 55.8 ± 10.7 | 89 | 57.4 ± 10.5 | 93 | 1.5 ± 14.0 | 0.3 |
| Mid-arm | 31.9(28.6 - 35.5) | 92 | 33.0(29.5 – 36.5) | 93 | 0.8 ± 7.4 | 0.3 |
| Neck | 35.3 ± 3.3 | 92 | 36.6 ± 11.0 | 93 | 1.2 ± 11.3 | 0.3 |
| Chest | 94.3 ± 10.4 | 91 | 95.9 ± 10.2 | 93 | 1.3 ± 15.4 | 0.4 |
| Mid-thigh | 55.3(50.0 – 60.2) | 91 | 57.1(50.3 - 64.2) | 93 | 2.1 ± 14.2 | 0.2 |
| **Skin folds (mm)** | | | | | | |
| Triceps | 21.5 ± 12.2 | 92 | 23.8 ± 14.3 | 93 | 2.0 ± 18.9 | 0.3 |
| Sub-scapular | 18.7 ± 12.2 | 92 | 20.0 ± 12.6 | 93 | 0.8 ± 16.7 | 0.6 |
| Abdomen | 23.9 ± 14.2 | 92 | 23.7 ± 13.4 | 92 | -1.0 ± 19.92 | 0.6 |
| Mid-thigh | 30.5 ± 16.9 | 91 | 33.0 ± 18.6 | 93 | 2.1 ± 24.5 | 0.4 |

Data expressed as mean ± SD. Paired Student's t-test calculated mean difference (24 months-baseline mean) for data available at baseline and at follow up. *97 subjects completed follow up; n= number for which data was available for each variable. BMI: body mass index.

### 5.4.2. Physician evaluation report

A physician administered questionnaire was completed as part of physical examination. (Figure 5.4, Table 5.7) shows the report of fat distribution found on examination of all participants (n: 150) at baseline and those with data at 24 months follow up (n: 97). There was increased fat found on examination at the 24 month visit after commencing ART at peripheral sites: face ($p<0.0001$), cheeks ($p<0.0001$), neck ($p<0.0001$), upper back ($p=0.03$), arms ($p=0.04$). Increased fat was also found at these sites at the 12 month visit.

Although there was more increased fat in other peripheral sites (buttocks and legs), the difference was not significant at the 24 month visit. Increased fat was also found in central sites: chest ($p=0.02$) with a similar trend found at the 12 month visit ($p<0.0001$). Increased fat in the abdomen ($p=0.9$) and subcutaneous abdominal area ($p=0.9$), although more than fat loss on examination at the 24 month visits, was not significantly different. However, significantly increased fat compared to fat loss was found at both sites at the 12 month visit ($p<0.0001$). More lipohypertrophy ($p<0.0001$) than lipoatrophy was found on general appearance at the 12 and 24 month visits after commencing ART, however, the majority of participants were found to have neither lipohypertrophy nor lipoatrophy at both visits (Figure 5.5).

When the proportion of those with fat loss found on examination at peripheral sites at baseline was compared to that at 24 months follow up, no differences were noted (Figure 5.6). There were also no differences in the proportion of those with fat gain on examination at central sites compared with the proportion of those with fat gain at central sites at 24 months follow up. The majority of subjects were found to have no change in fat distribution both at baseline and at 24 months follow up (Figure 5.7).

**Figure 5.3: Fat distribution participant administered questionnaire at follow-up in group 3 subjects (n: 97)**
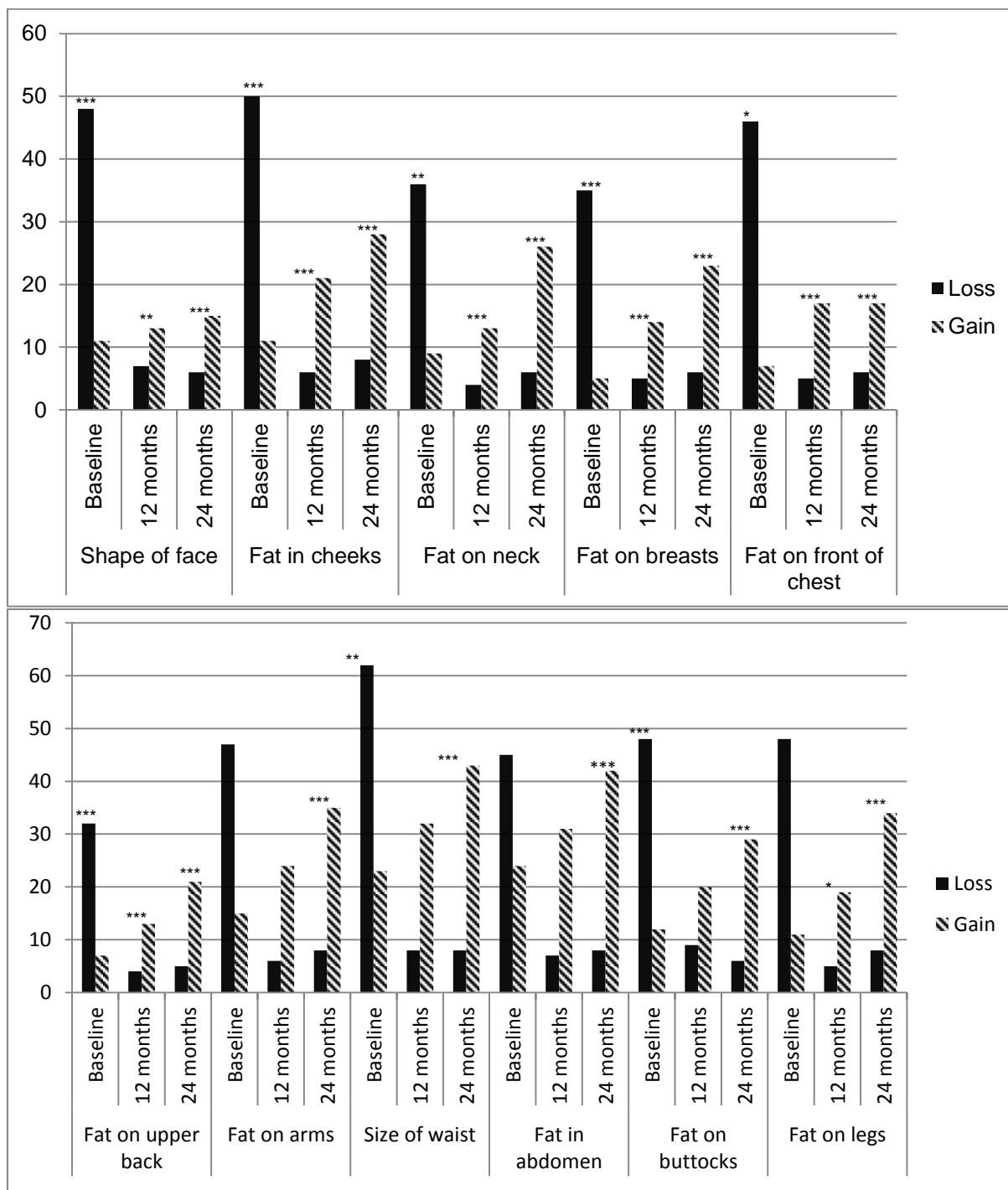


*P<0.05, **P<0.01, ***P<0.001 for comparison between fat gain and fat loss during follow up on ART

**Figure 5.4: Fat distribution physician administered questionnaire at baseline and during follow-up (n: 97)**
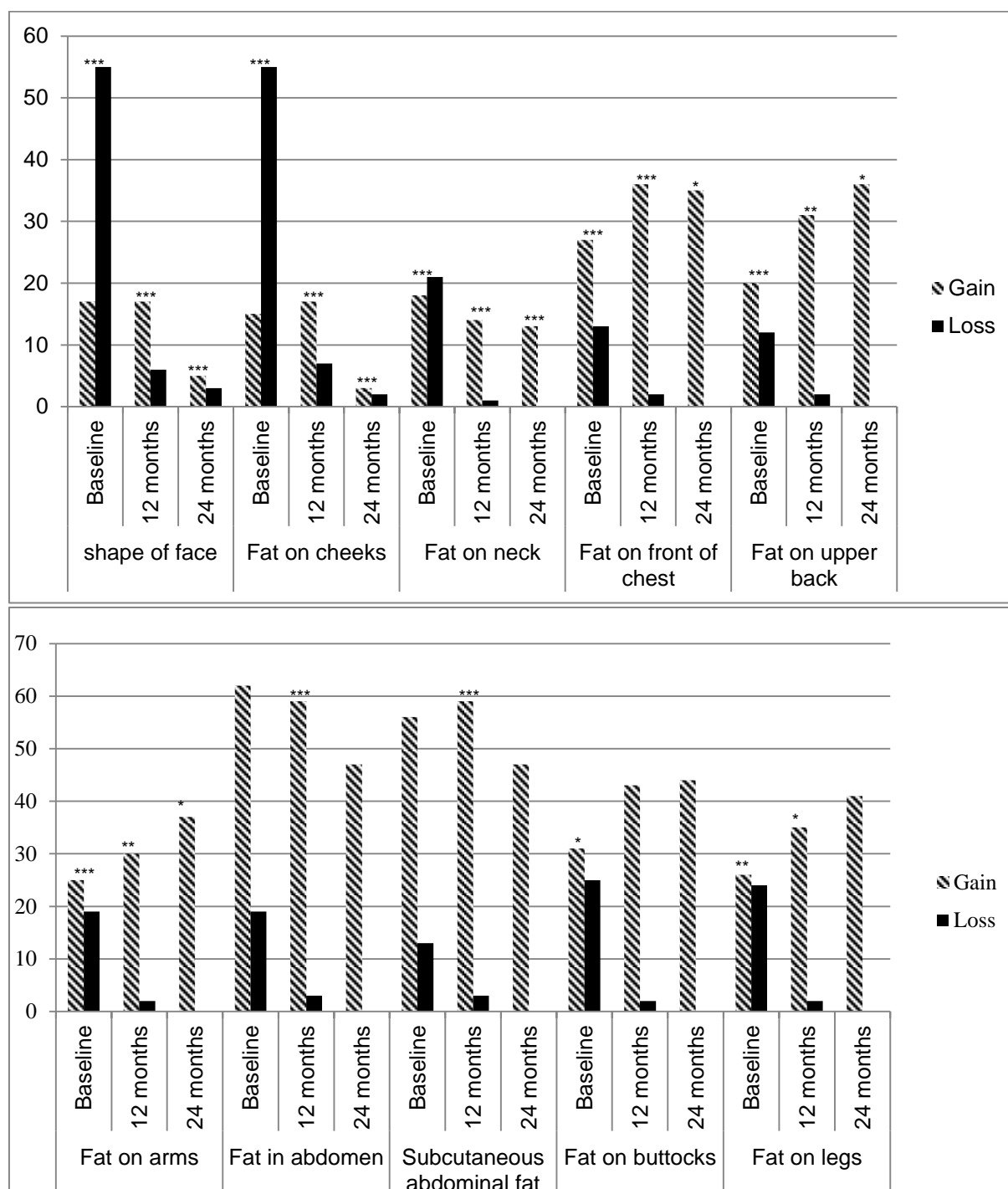


*P<0.05, **P<0.01, ***P<0.001 for comparison between fat gain and fat loss during 24 months follow up on ART

**Figure 5.5: Lipoatrophy vs. lipohypertrophy on physical examination (general appearance) at baseline and during follow up of Group 3 subjects**
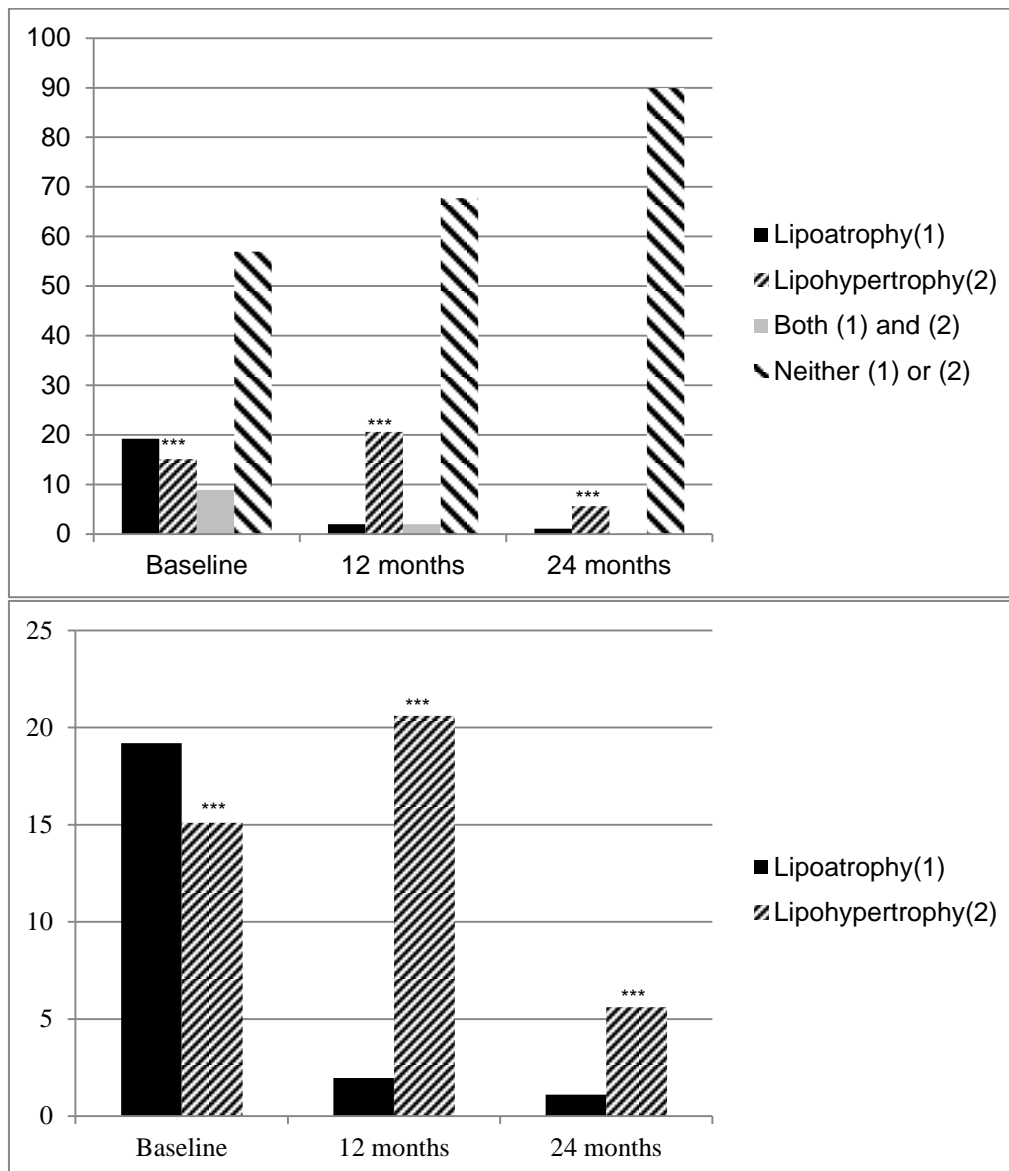


***P<0.001 for comparison between lipoatrophy vs. lipohypertrophy

**Figure 5.6: Participants administered questionnaire on fat redistribution (fat loss and fat gain) at baseline and at 24 months in group 3 subjects**
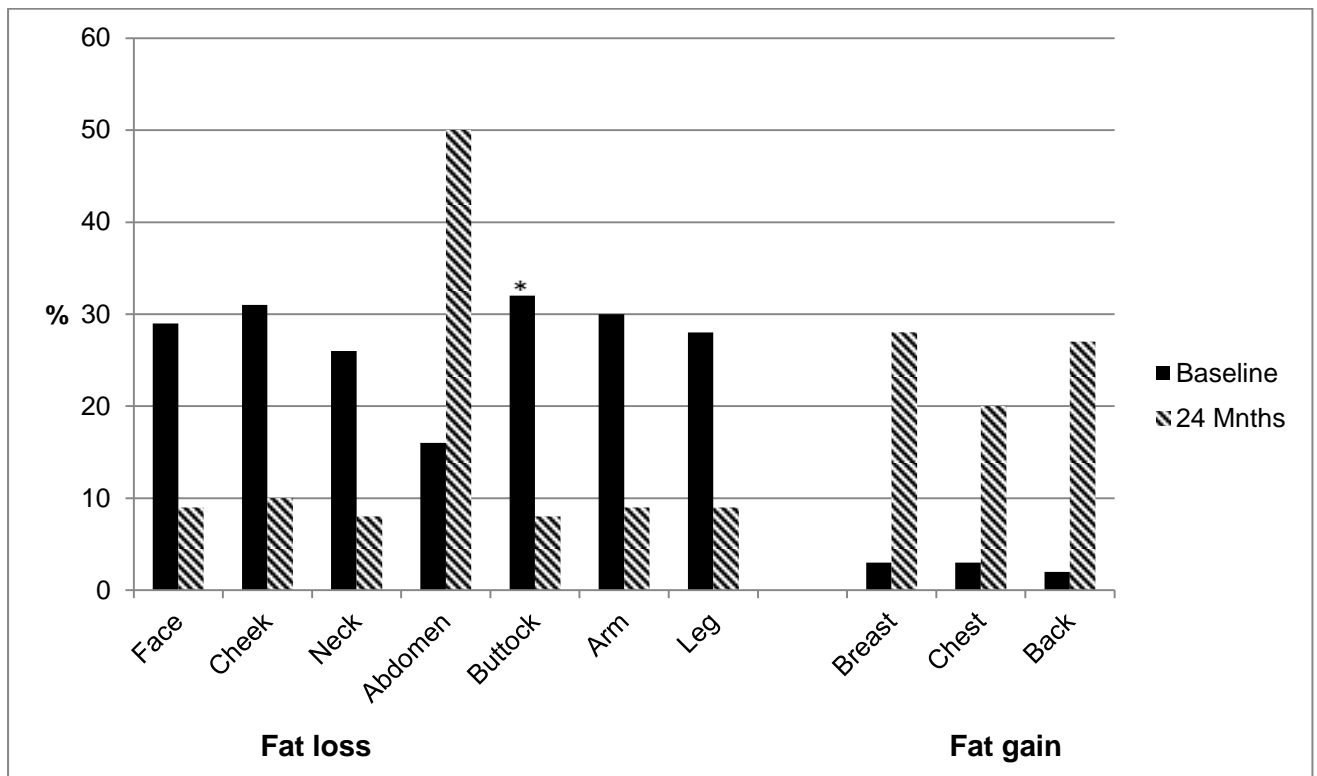


*P<0.05 baseline vs. 24 months follow up

**Figure 5.8: Physician examination general appearance at baseline and at the 24 months follow-up**
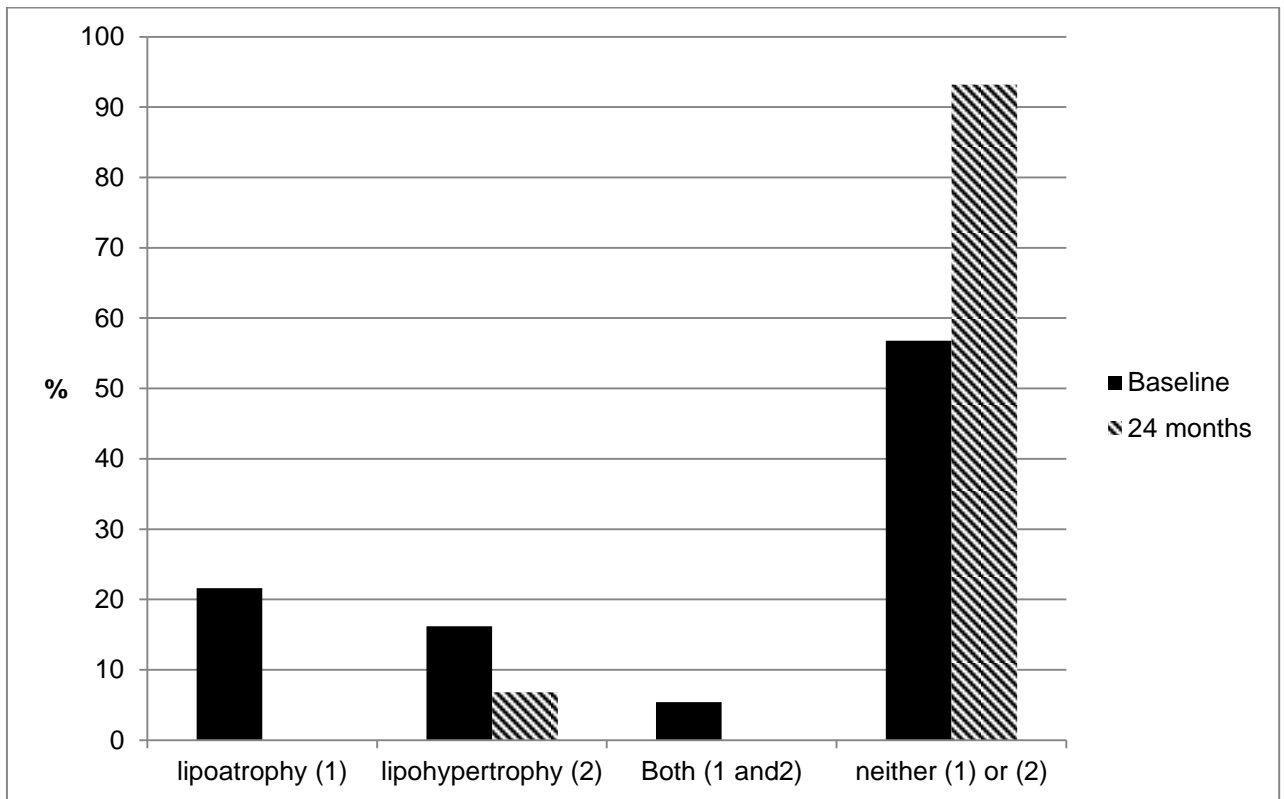
**Table 5.7: Participant report of fat distribution at baseline compared with 24 month follow-up of Group 3 subjects (n: 76)**

| Baseline | 24 months | | | |
|---|---|---|---|---|
| | Loss | Gain | No change | p |
| Fat on face | | | | 0.6 |
| loss | 1(1.8) | 1(1.8) | 14(24.6) | |
| gain | 0(0.0) | 1(1.8) | 2(3.5) | |
| No change | 4(7.0) | 10(17.5) | 23(40.4) | |
| Fat on cheek | | | | 0.5 |
| loss | 3(4.2) | 7(9.7) | 12(16.7) | |
| gain | 1(1.4) | 2(2.8) | 0(0.0) | |
| No change | 3(4.2) | 15(20.8) | 27(37.5) | |
| Fat on neck | | | | 0.8 |
| loss | 1(1.5) | 4(5.97) | 12(1) | |
| gain | 0(0) | 0(0.0) | 22.99) | |
| No change | 4(5.97) | 17(25.4) | 26(38.8) | |
| Fat on breast | | | | 0.9 |
| loss | 2(3.1) | 5(7.7) | 9(13.9) | |
| gain | 0(0.0) | 1(1.5) | 1(1.5) | |
| No change | 3(4.6) | 12(18.5) | 31(47.7) | |
| Fat on chest | | | | 0.3 |
| loss | 3(4.6) | 1(1.5) | 14(21.5) | |
| gain | 0(0.0) | 0(0.0) | 2(3.1) | |
| No change | 2(3.1) | 12(18.5) | 30(46.2) | |
| Fat on upper back | | | | 0.2 |
| loss | 2(3.1) | 3(4.6) | 9(13.9) | |
| gain | 0(0.0) | 1(1.5) | 0(0.0) | |
| No change | 1(1.5) | 13(20.0) | 33(50.77) | |
| Don't know | 1(1.5) | 0(0.0) | 1(1.5) | |

Continued on next page

**Table 5.7 cont.: Participant report of fat distribution at baseline compared with 24 month follow-up of Group 3 subjects (n: 76)**

| Baseline | 24 months | | | p |
|---|---|---|---|---|
| | Loss | Gain | No change | |
| loss | 4(5.8) | 6(8.7) | 11(15.9) | |
| gain | 0(0.0) | 9(13.0) | 2(2.9) | |
| No change | 2(2.9) | 19(27.5) | 15(21.7) | |
| Fat on  buttocks | | | | 0.03 |
| loss | 4(5.97) | 7(10.5) | 10(14.9) | |
| gain | 0(0.0) | 3(4.5) | 2(2.99) | |
| No change | 1(1.5) | 12(17.9) | 26(38.8) | |
| Don't know | | | 1(1.5) | |
| Fat on arm | | | | 0.3 |
| loss | 4(5.97) | 6(8.96) | 10(14.9) | |
| gain | 0(0.0) | 3(4.5) | 2(2.99) | |
| No change | 2(2.99) | 18(26.9) | 22(32.8) | |
| Fat on leg | | | | |
| loss | 4(5.9) | 5(7.4) | 10(14.7) | |
| gain | 0(0.0) | 2(2.9) | 1(1.5) | |
| No change | 2(2.9) | 19(27.9) | 24(35.3) | |

Results expressed as n(%). P value compares fat gain vs. fat gain, fat loss vs. fat loss, no change vs. no change at 24 months follow up

**Table 5.8: Physician examination of fat distribution at baseline compared with 24 months follow-up of Group 3 subjects (n: 76)**

| Baseline | 24 months | | | p |
| --- | --- | --- | --- | --- |
| | loss | gain | No change | |
| Fat on face | | | | 0.1 |
|   loss | 2(2.6) | 0(0.0) | 27(35.1) | |
|   gain | 0(0.0) | 0(0.0) | 8(10.4) | |
|   No change | 0(0.0) | 4(5.2) | 36(46.8) | |
| Fat on cheek | | | | 0.4 |
|   loss | 1(1.3) | 0(0.0) | 27(35.1) | |
|   gain | 0(0.0) | 0(0.0) | 8(10.4) | |
|   No change | 0(0.0) | 3(3.9) | 38(49.4) | |
| Fat on neck | | | | 0.3 |
|   loss | | 0(0.0) | 11(14.3) | |
|   gain | | 1(1.3) | 7(9.1) | |
|   No change | | 11(14.3) | 47(61.0) | |
| Fat on chest | | | | 0.04 |
|   loss | | 0(0.0) | 8(10.7) | |
|   gain | | 8(10.7) | 7(9.3) | |
|   No change | | 22(29.3) | 30(40.0) | |
| Fat on back | | | | 0.05 |
|   loss | | 0(0.0) | 5(6.5) | |
|   gain | | 6(7.8) | 3(3.9) | |
|   No change | | 26(33.8) | 37(48.1) | |
|   Don't know | | | | |
| Fat on abdomen | | | | <0.0001 |
|   loss | | 0(0.0) | 11(14.5) | |
|   gain | | 23(30.3) | 5(6.6) | |
|   No change | | 20(26.3) | 17(22.4) | |

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**Table 5.8 cont.: Physician examination of fat distribution at baseline compared with 24 months follow-up of Group 3 subjects (n: 76)**

| Baseline | 24 months | | | p |
|---|---|---|---|---|
| | loss | gain | No change | |
| loss | | 1(1.3) | 12(15.8) | |
| gain | | 12(15.8) | 5(6.6) | |
| No change | | 26(34.2) | 20(26.3) | |
| Fat on arm | | | | 0.09 |
| loss | | 1(1.4) | 8(10.8) | |
| gain | | 8(10.8) | 6(8.1) | |
| No change | | 21(28.4) | 30(40.5) | |
| Fat on leg | | | | 0.01 |
| loss | 4(5.9) | 5(7.4) | 10(14.7) | |
| gain | 0(0.0) | 2(2.9) | 1(1.5) | |
| No change | 2(2.9) | 19(27.9) | 24(35.3) | |

Results expressed as n (%). P value compares fat gain vs. fat gain, fat loss vs. fat loss, no change vs. no change at follow up

## 5.5. Concordance between participant report and physician examination

Lipoatrophy was not found in any participants by self-report or physical examination at 24 months of follow up on ART (Figure 5.9, Tables 5.9 and 5.10). At 24 months, there was a high concordance between participant report and physician examination for "no change" in fat distribution at all sites except for abdomen, where there was a high concordance for "lipohypertrophy" (Figure 5.10, Tables 5.10 and 5.11). There was significantly higher concordance for any "peripheral lipohytrophy" (p<0.001) and any "central lipohypertrophy" (p<0.001) (Figure 5.9, Table 5.9).

**Figure 5.9: Concordance between participant report and physician examination for fat distribution in   group 3 subjects at the 24 month follow-up (n: 66)**
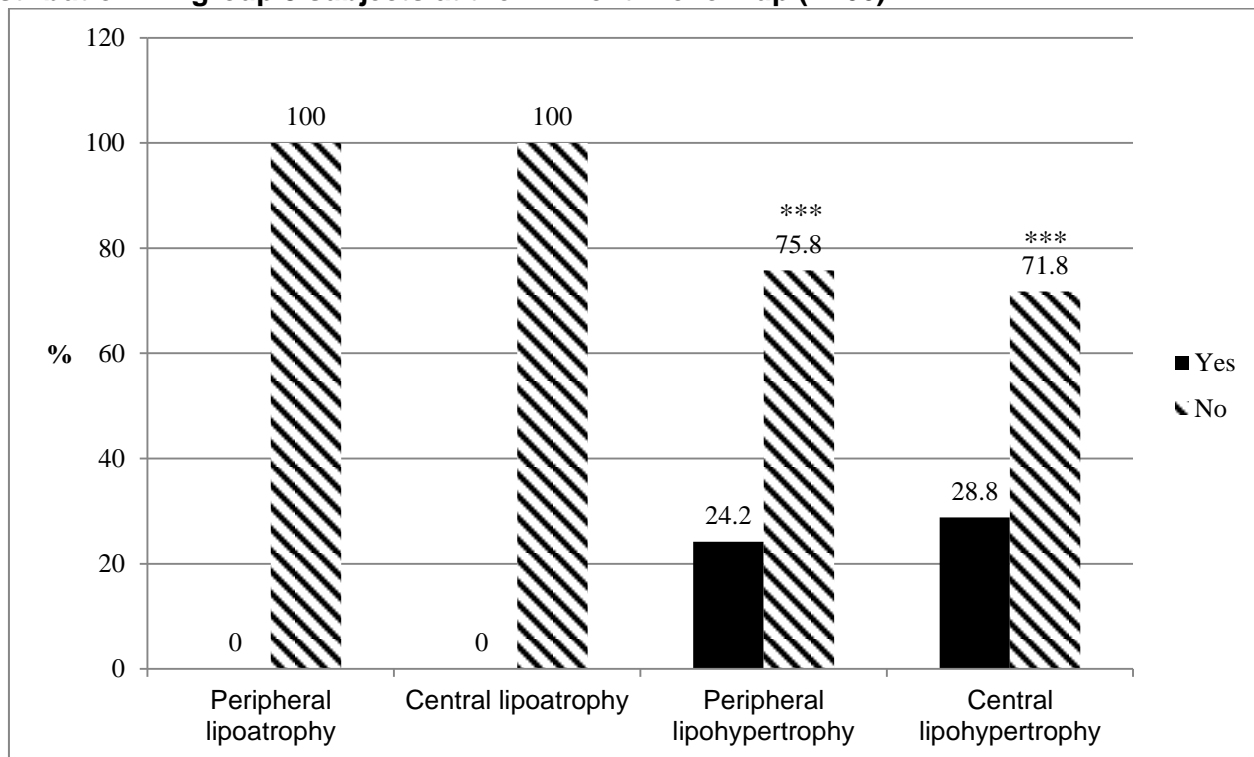


Peripheral lipoatrophy/lipohypertrophy: face, cheeks, arms, buttocks, legs; central lipoatrophy/lipohypertrophy: neck, chest, upper back, abdomen. [***]p<0.001: yes vs. no

**Table 5.9: Concordance between participant report and physician examination for fat distribution in group 3 subjects at the 24 month follow-up (n: 66)**

| | Peripheral lipoatrophy | Central lipoatrophy | Peripheral lipohypertrophy | Central lipohypertrophy |
|---|---|---|---|---|
| Yes | 0(0.0) | 0(0.0) | 16(24.2) | 19(28.8) |
| *No | 66(100.0) | 66(100) | 50(75.8) | 47(71.8) |
| p | | | <0.0001 | 0.0006 |

Results expressed as n(%). *Subjects with no change at 24 months of ART included. Peripheral: face, cheeks, arms, buttocks, legs; central: neck, chest, upper back, and abdomen

**Table 5.10: Concordance between participant report and physician examination of fat distribution at 24 months in group 3 subjects (n: 62)**

| Participant | Physician loss | gain | No change | Total |
|---|---|---|---|---|
| **Fat on face** | | | | |
| Loss | - | 0(0.0) | 5(9.8) | 5(9.8) |
| Gain | - | 0(0.0) | 10(19.6) | 10(19.6) |
| No change | - | 0(0.0) | 35(68.6) | 35(68.6) |
| Total | - | 0(0.0) | 50(100.0) | |
| **Fat on cheek** | | | | |
| Loss | - | 0(0.0) | 7(11.3) | 7(11.3) |
| Gain | - | 2(3.2) | 18(29.0) | 21(33.9) |
| No change | - | 0(0.0) | 33(53.2) | 33(53.2) |
| Total | - | 2(3.2) | 59(95.2) | |
| **Fat on neck** | | | | |
| Loss | - | 1(1.7) | 4(6.8) | 5(8.5) |
| Gain | - | 6(10.2) | 13(22.0) | 19(32.2) |
| No change | - | 3(5.1) | 31(52.5) | 34(57.6) |
| Total | - | 10(16.95) | 49(83.1) | |
| **Fat on chest** | | | | |
| Loss | - | 2(3.5) | 3(5.3) | 5(8.8) |
| Gain | - | 7(12.3) | 6(10.5) | 13(22.8) |
| No change | - | 12(21.1) | 26(45.6) | 38(66.7) |
| Total | - | 21(36.8) | 36(63.2) | |
| **Fat on back** | | | | |
| Loss | - | 1(1.7) | 3(5.1) | 4(6.8) |
| Gain | - | 9(15.3) | 9(15.3) | 18(30.5) |
| No change | - | 14(23.7) | 22(37.3) | 36(61.0) |
| Total | - | 24(40.7) | 35(59.3) | |
| **Fat on abdomen** | | | | |
| Loss | - | 3(4.8) | 3(4.8) | 6(9.5) |
| Gain | - | 19(30.2) | 14(22.2) | 33(52.4) |
| No change | - | 12(19.1) | 11(17.5) | 23(36.5) |
| Total | - | 34(53.97) | 29(46.03) | |
| **Fat on buttock** | | | | |
| Loss | - | 3(5.0) | 2(3.3) | 5(8.3) |
| Gain | - | 11(18.3) | 11(18.3) | 22(36.7) |
| No change | - | 16(26.7) | 16(26.7) | 32(53.3) |
| Total | - | 30(50.0) | 30(50.0) | |

**Table 5.8 cont.: Physician examination of fat distribution at baseline compared with 24 months follow-up of Group 3 subjects (n: 76)**

| Participant | | Physician | | | |
|---|---|---|---|---|---|
| | | loss | gain | No change | Total |
| Loss | - | | 3(4.9) | 3(4.9) | 6(9.8) |
| Gain | - | | 14(22.95) | 13(21.3) | 27(44.3) |
| No change | - | | 9(14.8) | 19(31.2) | 28(45.9) |
| Total | - | | 26(42.6) | 35(57.4) | |
| **Fat on leg** | | | | | |
| Loss | - | | 4(6.6) | 2(3.3) | 6(9.8) |
| Gain | - | | 14(22.95) | 12(19.7) | 26(42.6) |
| No change | - | | 11(18.0) | 17(27.9) | 28(45.9) |
| Total | - | | 29(47.5) | 32(52.5) | |

Results expressed as n (%).

**Figure 5.10: Concordance between participant report and physician examination for fat distribution in group 3 subjects (n: 62) at the 24 month follow up**
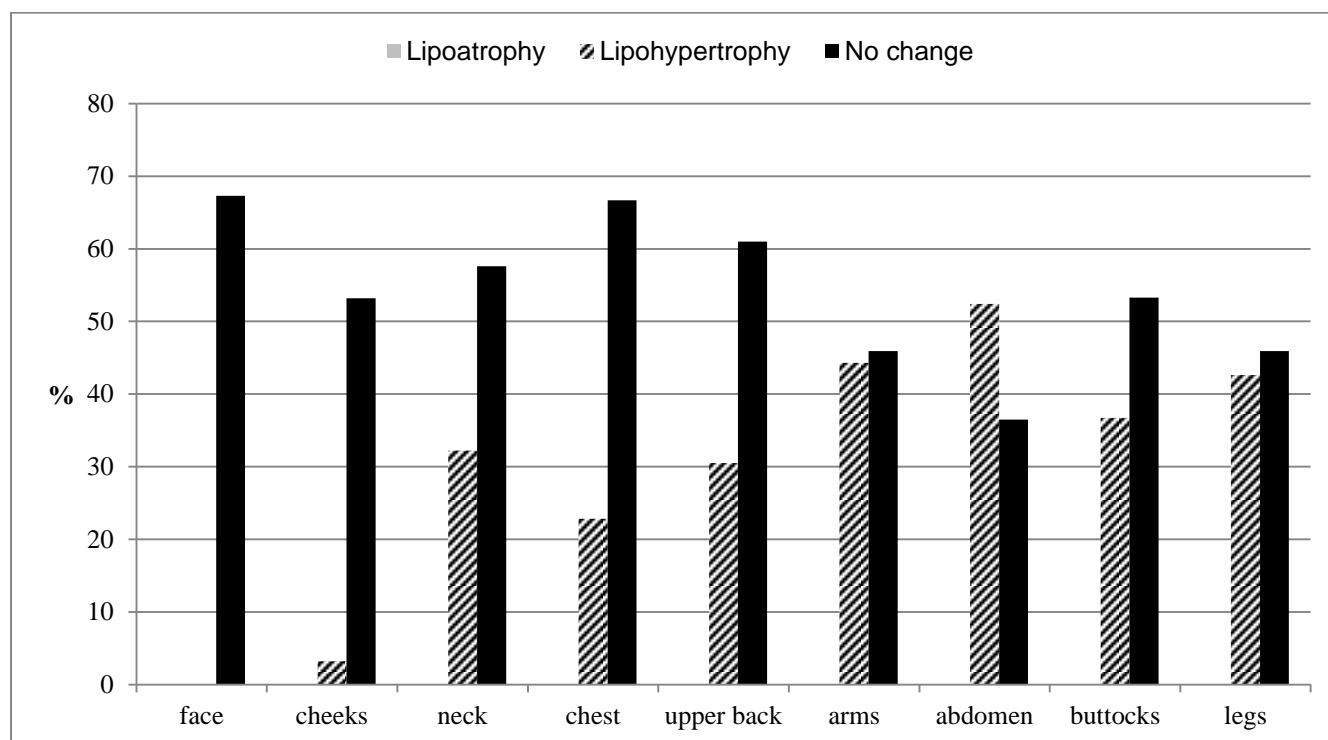


Lipoatrophy: fat loss; Lipohypertrophy: fat gain

**Table 5.11: Concordance between participant report and physical examination for clinical lipoatrophy and lipohypertrophy in group 3 subjects at the 24 month follow-up**

|  | face | cheeks | neck | chest | upper back | arms | abdomen | buttocks | legs |
|---|---|---|---|---|---|---|---|---|---|
| Lipoatrophy | - | - | - | - | - | - | - | - | - |
| Lipohypertrophy | 0(0.0) | 2(3.2) | 19(32.2) | 13(22.8) | 18(30.5) | 27(44.3) | 33(52.4) | 22(36.7) | 26(42.6) |
| No change | 35(68.6) | 33(53.2) | 34(57.6) | 38(66.7) | 36(61.0) | 28(45.9) | 23(36.5) | 32(53.3) | 28(45.9) |

Results expressed as n (%).

## 5.6. Associations with peripheral lipohypertrophy

There was an association between central and peripheral lipohypertrophy (OR 172.5 [95%CI 17.9 to >999.99] p<0.0001) (Table 5.12). Furthermore, of 16 subjects with peripheral lipohypertrophy and 19 subjects with central lipohypertrophy, 15 of them had both central and peripheral lipohypertrophy. There was no association between central lipohypertrophy and visceral fat, subcutaneous fat area, visceral: subcutaneous fat or total fat area as measured by CT scan.

**Table 5.12: Associations with central lipohypertrophy**

| Variable | Central lipohypertrophy | |
| --- | --- | --- |
| | OR(95% CI) | p |
| Peripheral lipohypertrophy | 172.5 (17.5 to >999.99) | <0.0001 |
| Visceral fat | 1.01 (0.99 to 1.04) | 0.2 |
| Subcutaneous fat | 1.003 (0.99 to 1.01) | 0.3 |
| Visceral: subcutaneous fat | 0.3 (0.004 to 19.8) | 0.6 |
| Total fat | 1.003 (0.998 to 1.009) | 0.2 |

CI: Confidence interval

# CHAPTER SIX: INCIDENCE OF DIABETES MELLITUS AND OTHER DISORDERS OF GLYCAEMIA (DYSGLYCAEMIA) IN SUBJECTS COMMENCED ON ART

The 1998 World Health Organisation (WHO) and 2011 American Diabetes Association (ADA) criteria for disorders of glycaemia were used to determine the incidence of diabetes mellitus (DM), impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) after commencing combination antiretroviral therapy (ART).

## 6.1. Follow-up OGTT

Figure 6.1 shows the follow-up OGTT results in the group that commenced ART (Group 3). By year 1, there were 6, 3 and 2 subjects with IFG, IGT and DM, respectively. By year 2, there were 3, 5 and 3 subjects with IFG, IGT and DM, respectively.

Of the five subjects who developed DM by the end of the study period, 1 had IGT at baseline and DM at 3 months, OGTT was normal at the end of follow-up with no drug therapy. Four subjects had normal OGTT at baseline; of these, 1 developed DM at 6 months and was subsequently lost to follow up; 1 developed DM at year 2, 1 had IFG at year 1 and developed DM at year 2; 1 had IGT at year 1 and DM at year 2.

Eight subjects developed IFG during the study period. At baseline, 7 had normal OGTT - 5 developed IFG at year 1; of these, 4 had normal OGTT at year 2 and one developed DM at year 2; one developed IFG at year 2 and one had IGT at 18 months and IFG at year 2. One subject had IFG at baseline, with IFG persisting at year 2.

Of the 12 subjects that developed IGT during the study period, 4 had IGT at baseline - 1 persisted as IGT by year 1 but had normal OGTT at year 2, 1 developed DM at year 1 and 2 had normal OGTT at year 1. Seven subjects had normal OGTT at baseline - 2

developed IGT at year 1 with one of these developing DM at year 2 and one having normal

OGTT at year 2; 4 subjects developed IGT at year 2 and one developed IGT at 18 months.

**Figure 6.1. Follow-up oral glucose tolerance test (OGTT) in group 3 subjects during the study period**
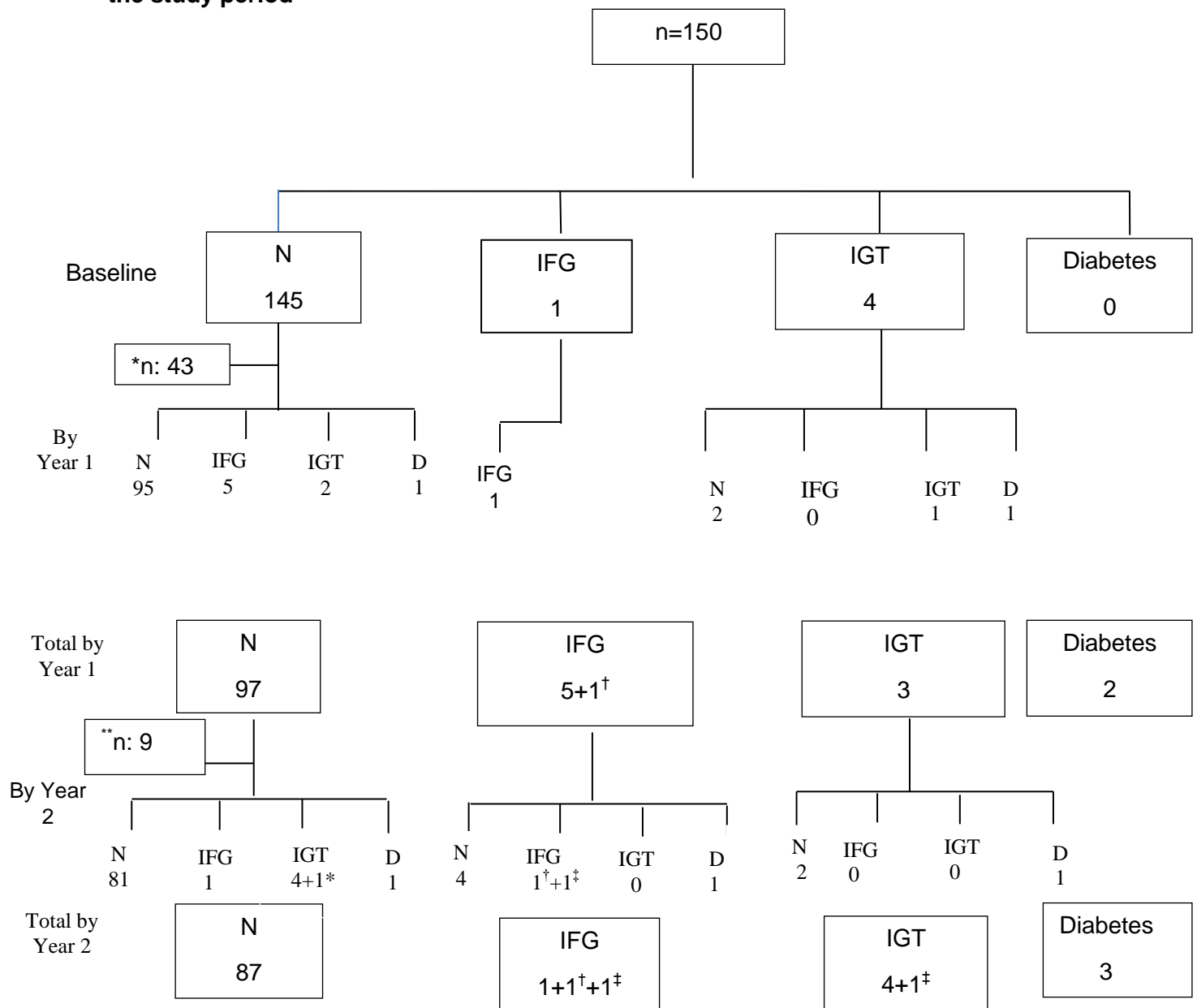


N: normal glucose tolerance; IFG: impaired fasting glucose; IGT: impaired glucose tolerance; D: diabetes. *34 loss to follow up: 2 pregnant, 7 died. **6 loss to follow up: 2 pregnant, 1 died. †IFG at baseline persisted as IFG at year 2; ‡OGTT normal at baseline, IGT at 18 months and IFG at year 2.

## 6.2. Incidence of disorders of glycaemia (dysglycaemia)

Time at risk was calculated "from ART initiation date" to the estimated date of DM, IGT, IFG, dysglycaemia (any disorder of glycaemia / DM or IGT or IFG), impaired glucose regulation (IGR) / pre-diabetes (IGT or IFG) or last visit date. The subjects with IFG (n=1) and IGT (n=4) at baseline were excluded from the incidence analysis of IFG and IGT, respectively, but were included in the incidence analysis for DM. Poisson approximations were used to calculate confidence intervals (CIs) for incidence of DM, IGT, IFG, any disorder of glycaemia or impaired glucose regulation (IGR). Table 6.1 shows the incidence rate of DM, IGT, IFG, IGR and dysglycaemia in the group that commenced ART (Group 3). Baseline covariates in Tables 6.2-6.7 were used to model the risk factors for any disorder. Cox proportional hazards regression was used to identify predictors of incident glycaemic abnormality (Table 6.8).

Of 150 HIV infected persons that were commenced on ART (group 3), 5 developed DM during 221.9 person-years follow up (PYFU) using glucose based criteria with an incidence rate of 2.3 cases per 100 PYFU (95% CI 0.7 – 5.3) (Figure 6.2). When $HbA_{1c}$ criteria were applied, 8 of 150 participants developed DM during 211.9 PYFU (incidence: 3.8 cases per 100 PYFU [95% CI 1.6 – 7.4]) (Figure 6.3).

Seven participants developed IGT during 220.1 PYFU (incidence: 3.2 cases per 100 PYFU [95% CI 1.3 – 6.6]) (Figure 6.4) and seven participants developed IFG during 215.1 PYFU (incidence: 3.2 cases per 100 PYFU [95% CI 1.3 – 6.6]) (Figure 6.5).

Thirteen persons developed IGR (IGT or IFG) by glucose based criteria (incidence: 6.1 cases per 214.6 PYFU [95%CI 3.2 – 10.4]) with one of these persons developing both IGT and IFG (Figure 6.6).

Of 150 persons followed up, 16 developed any disorder of glycaemia (DM or IGT or IFG) during 211.6 PYFU (incidence: 7.6 cases per 100 PYFU [95% CI 4.3 – 12.3]) (Figure 6.7).

**Table 6.1: Incidence rate of diabetes mellitus, impaired glucose tolerance, impaired fasting glucose, impaired glucose regulation/pre-diabetes and any disorder of glycaemia in group 3 subjects (n=150)**

| | No. patients with events | No. of Person-Yr (n) | Incidence Rate /100 Person-Yr (95% CI) |
|---|---|---|---|
| **Diabetes Mellitus** | | | |
| **Plasma glucose criteria (WHO and ADA)** | | | |
| All patients | 5 | 221.9 (150) | 2.3 (0.7 to 5.3) |
| Male | 4 | 68.7 (48) | 5.8 (1.6 to 14.9) |
| Female | 1 | 153.2 (102) | 0.7 (0 to 3.6) |
| **HbA$_{1c}$criteria (WHO and ADA)** | | | |
| All | 8 | 211.9(150) | 3.8 (1.6 to 7.4) |
| Male | 2 | 70(48) | 2.86 (0.3 to 10.3) |
| Female | 6 | 141.91(102) | 4.22 (1.6 to 9.2) |
| **Impaired glucose tolerance** | | | |
| All patients | 7 | 220.1 (146) | 3.2 (1.3 to 6.6) |
| Male | 4 | 66.95 (46) | 4.5 (0.92 to 13.1) |
| Female | 3 | 153.1 (100) | 2.6 (0.7 to 6.7) |
| **Impaired fasting glucose** | | | |
| All patients | 7 | 215.1(149) | 3.2 ( 1.3 to 6.6) |
| Male | 4 | 65.1 (47) | 6.1 (1.7 to 15.7) |
| Female | 3 | 153.4 (102) | 1.96 (0.4 to 5.7) |
| **IGR\*: Impaired fasting glucose or Impaired glucose tolerance** | | | |
| All | 13 | 214.6(150) | 6.1(3.2 to 10.4) |
| Male | 7 | 63.4(42) | 11.0(4.4 to 22.7) |
| Female | 6 | 151.2(102) | 3.96(1.5 to 8.6) |
| **Any Dysglycaemia[†]** | | | |
| All | 16 | 211.6(150) | 7.6(4.3 to 12.3) |
| Male | 9 | 61.2(48) | 14.7(6.7 to 27.9) |
| Female | 7 | 150.5(102) | 4.7(1.9 to 9.6) |

*Impaired glucose regulation/pre-diabetes; [†]Diabetes Mellitus or Impaired glucose tolerance or Impaired fasting glucose

**Figure 6.2: Incidence of diabetes mellitus using OGTT criteria during 24 months follow-up on ART**
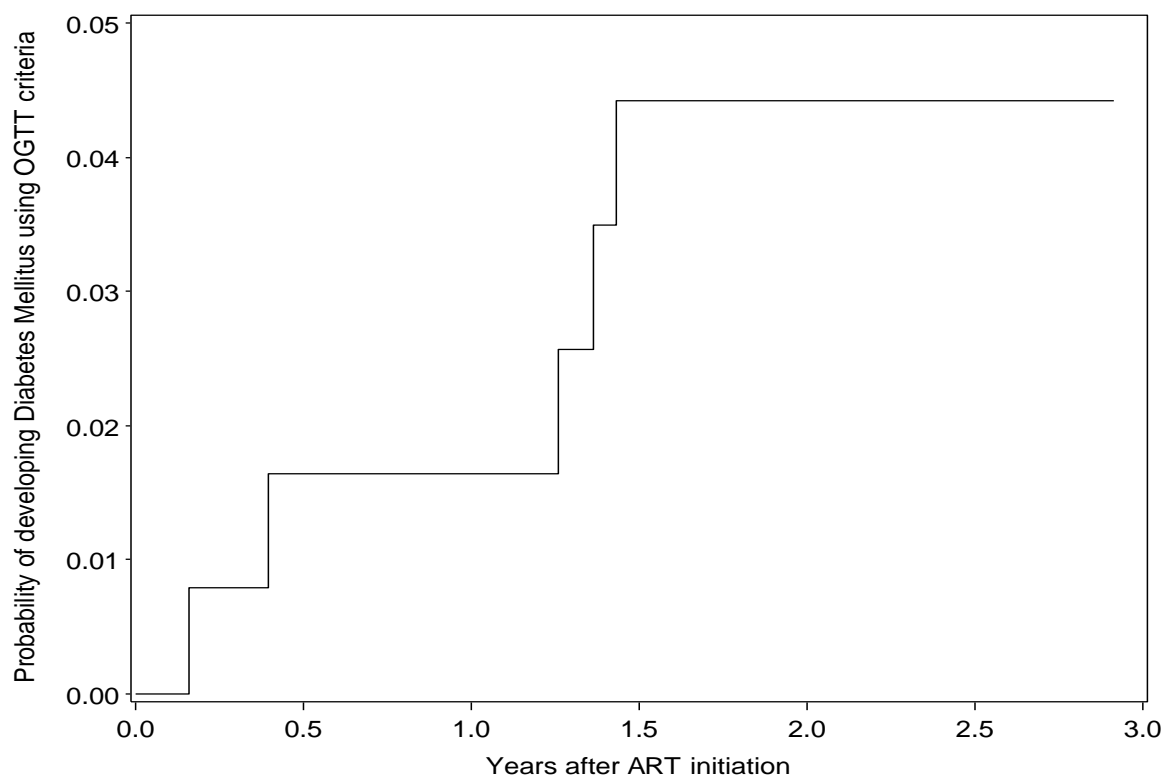
**Figure 6.3: Incidence of diabetes mellitus using HbA$_{1c}$criteria during 24 months follow up on ART**
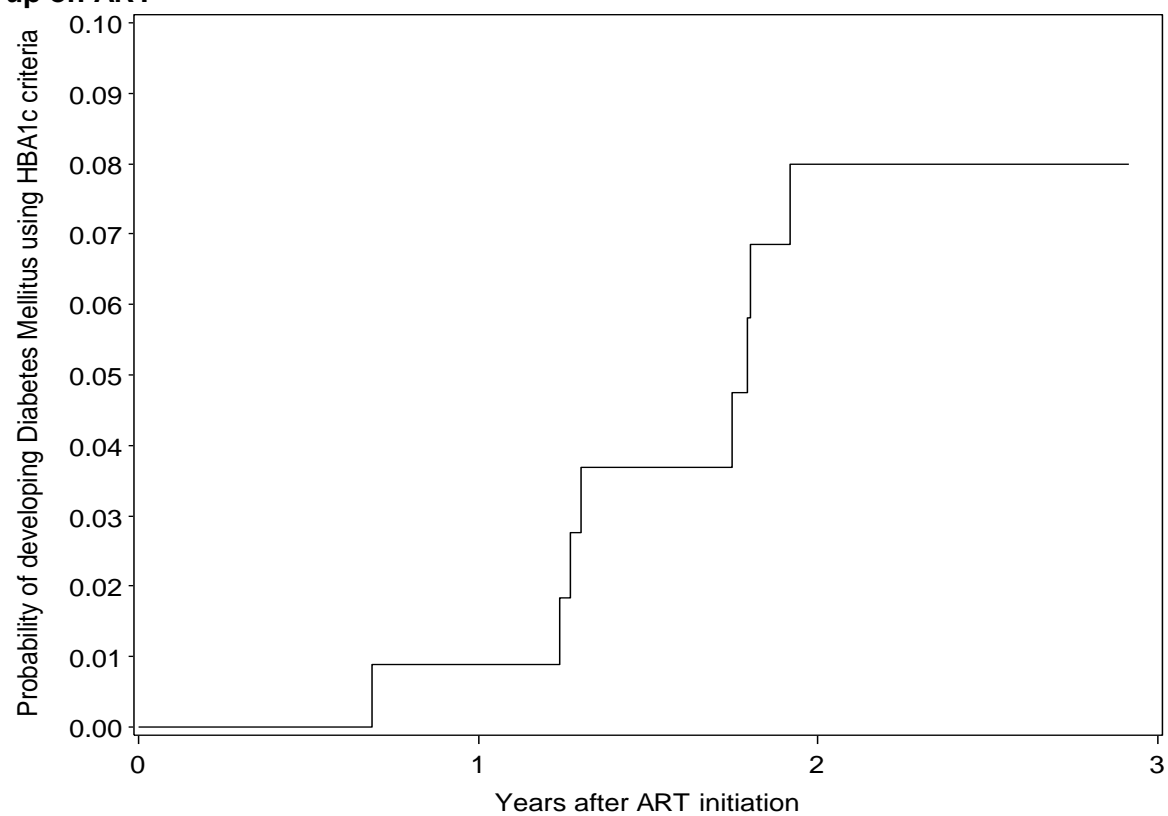
**Figure 6.4: Incidence of impaired glucose tolerance (IGT) during 24 months follow-up on ART**
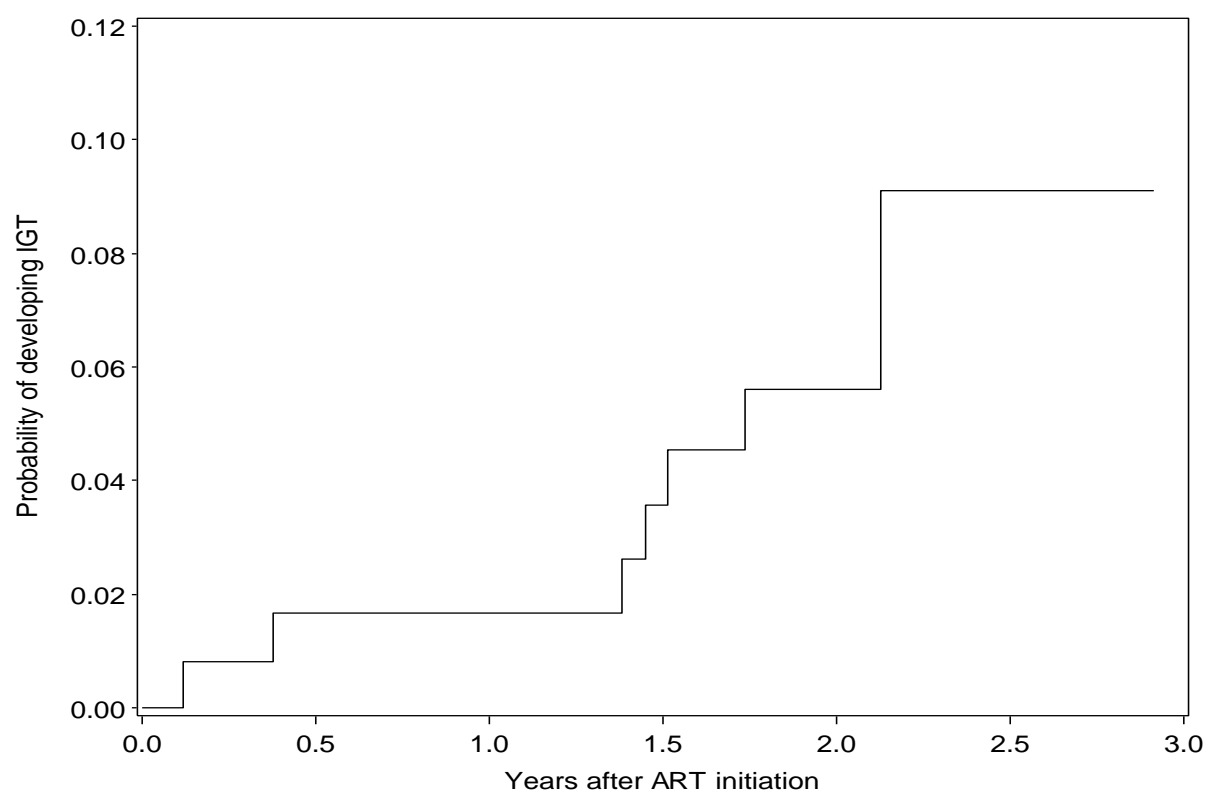
**Figure 6.5: Incidence of impaired fasting glucose (IFG) during 24 months follow-up on ART**
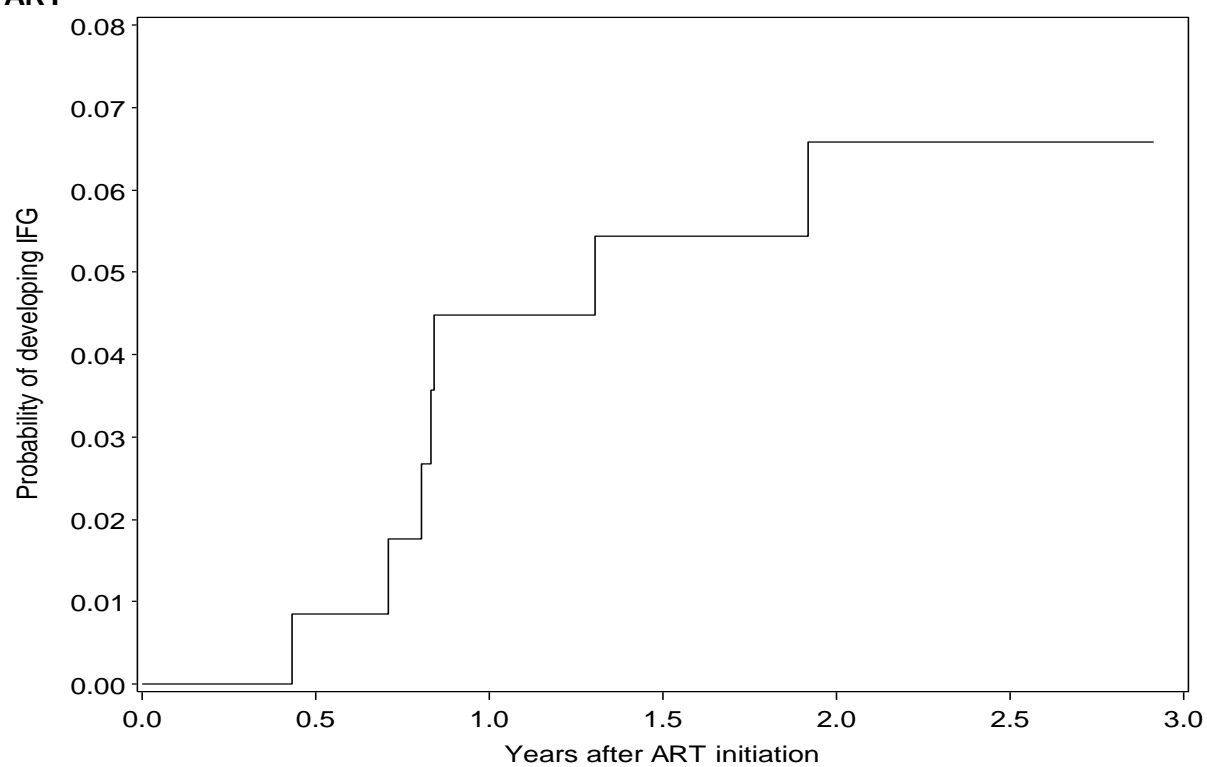
**Figure 6.6: Incidence of impaired glucose regulation/pre-diabetes (IGT or IFG) during 24 months follow-up on ART**
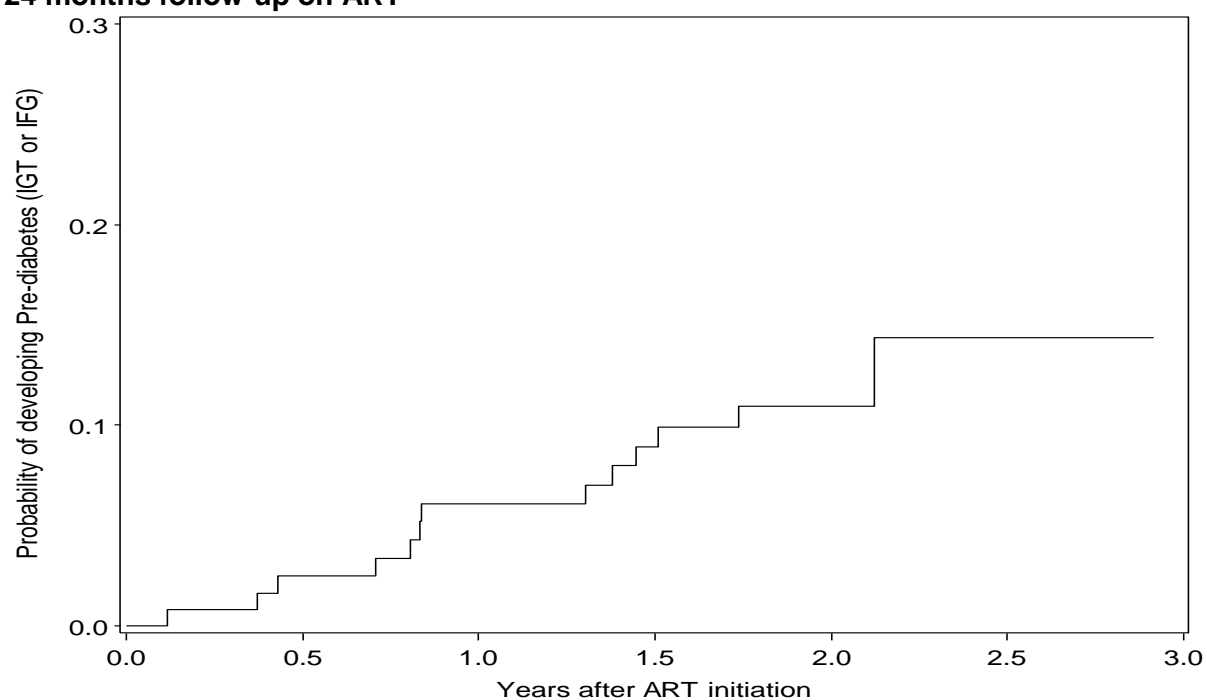
**Figure 6.7: Incidence of dysglycaemia (DM or IGT or IFG) during 24 months follow-up on ART**
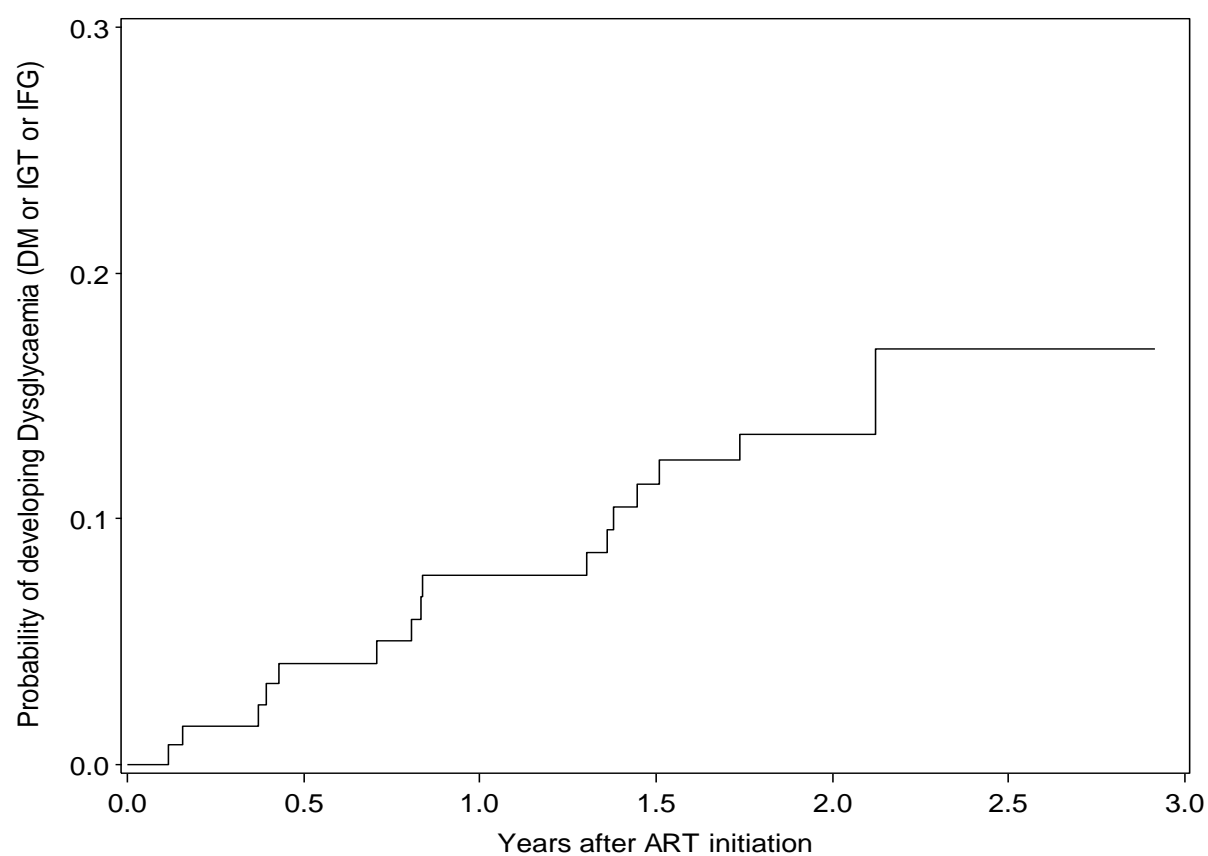
## 6.3. Risk Factors Analysis

Analysis of risk factors for disorders of glycaemia is shown in Tables 6.2 – 6.44.

### 6.3.1. Risk factors for diabetes mellitus (DM)

Tables 6.2 - 6.8 show univariate analysis and baseline characteristics of subjects who did and did not develop DM during follow up on combination ART using glucose-based criteria.When compared with the group which did not develop DM, the group that developed DM had more males (p=0.02), more exposure to efavirenz (p=0.02), higher systolic (p=0.04) and diastolic (p=0.003) blood pressure and visceral: subcutaneous fat ratio on CT scan (p<0.0001). No significant difference was observed between the two groups for any other demographic, clinical, laboratory or radiological variable (Tables 6.2- 6.8).

In univariate analysis, variables significantly associated withincidence of DM were diastolic blood pressure (p=0.01) and visceral: subcutaneous fat area ratio on CT scan (p <0.0001).

In multivariate analysis, the only significant risk factor (predictor) for development of DM was visceral: subcutaneous fat ratio (HR 2.95[95%CI 1.25-6.98], p=0.01). A unit higher visceral: subcutaneous fat ratio was associated with a nearly three-fold risk of developing DM using glucose-based criteria.

When $HbA_{1c}$ criteria was used (Tables 6.9 – 6.15), comparison of the group which developed DM with the group that did not develop DM showed that subjects who developed DM had more familial DM (p=0.04), higher mean serum urea (p=0.02) and lower serum albumin (p=0.03). In univariate analysis, variables that were significantly associated with the incidence of DM were serum albumin (p=0.02) and magnesium (p=0.02).

In multivariate analysis the only significant risk factor associated with the development of DM using $HbA_{1c}$ criteria was haemoglobin (OR 0.61[95%CI 0.39-0.96],

p=0.03) i.e. a unit higher haemoglobin was associated with a 39% lower risk of developing

DM using HbA$_{1c}$ criteria (Table 6.44).

**Table 6.2: Baseline demographic characteristics in the group developing Diabetes Mellitus vs. not developing diabetes mellitus by OGTT criteria during 24 months of follow up on ART**

| Variable | Developing Diabetes Mellitus (OGTT criteria), n=5 | Not Developing Diabetes Mellitus (OGTT), n= 145 | p | HR(95% CI) | #p |
|---|---|---|---|---|---|
| Gender | | | | | |
|    Male | 4(8.3) | 44(91.7) | 0.02 | 8.89(0.99-79.60) | 0.05 |
|    Female | 1(0.98) | 101(9.0) | | | |
| Treatment allocation | | | | | |
|    Efavirenz | 5(3.33) | 71(47.3) | 0.02 | Not estimatable | 0.9 |
|    Nevirapine | 0(0.00) | 74(49.3) | 0.02 | Not estimatable | 0.9 |
| BMI | | | | | |
| >30 kg/m2 | 1(0.70) | 38(26.57) | 0.7 | 0.62(0.07-5.57) | 0.7 |
| Familial DM | 1(0.7) | 34(23.8) | 0.8 | 0.64(0.07-5.72) | 0.7 |
| Smoker | 1(0.7) | 18(12.6) | 0.8 | 1.69(0.19-15.16) | 0.6 |
| Occupational physical activity | | | 0.7 | | |
|    Sedentary | 0(0.0) | 4(3.1) | | 1 | |
|    Light | 3(2.3) | 55(42.6) | | Not estimatable | 0.9 |
|    Moderate | 1(0.8) | 41(31.8) | | Not estimatable | 0.9 |
|    Heavy | 0(0.0) | 24(18.6) | | 1 | |
| Leisure physical activity | | | | | |
|    Sedentary | 3(2.2) | 74(53.6) | | 1 | |
|    Light | 0(0.0) | 17(12.3) | | Not estimatable | 0.9 |
|    Moderate | 0(0.0) | 14(10.1) | | Not estimatable | 0.9 |
|    Heavy | 2(1.5) | 28(20.3) | | 2.05(0.34-12.29) | 0.4 |

Data are n(%). OGTT: oral glucose tolerance test, BMI: body mass index, DM: diabetes mellitus. p: chi-square test for differences between the two groups, HR: hazard ratio, CI: confidence interval, #p: differences at univariate level using Cox proportional hazards

**Table 6.3: Baseline clinical characteristics in the group developing Diabetes Mellitus vs. not developing Diabetes Mellitus by OGTT criteria during 24 months follow up***

| Variable | Developing DM (OGTT) | | Not developing DM | | p | Univariate analysis | *p |
|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | | HR (95% CI) | |
| Age | 5 | 39.8±6.1 | 142 | 36.8±9.3 | 0.5 | 1.03(0.94-1.12) | 0.5 |
| Blood pressure (mmHg) | | | | | | | |
| Systolic | 5 | 126.8±17.4 | 136 | 111.4±16.6 | 0.04 | 1.0(1.00-1.1 | 0.1 |
| Diastolic | 5 | 84.8±12.1 | 135 | 70.3±10.4 | 0.003 | 1.1(1.0-1.1) | 0.01 |
| **Anthropometric measurements** | | | | | | | |
| Weight (kg) | 5 | 64.4±16.9 | 141 | 69.0±15.5 | 0.5 | 0.98(0.92-1.04) | 0.4 |
| Body mass index (kg/m$^2$) | 5 | 23.1±7.2 | 141 | 26.3±6.0 | 0.2 | 0.9(0.7-1.1) | 0.2 |
| **Circumference (cm):** | | | | | | | |
| Waist | 5 | 90.8±24.4 | 135 | 92.7±16.9 | 0.8 | 1.00(0.95-1.1) | 0.92 |
| Hip | 5 | 106.2±26.4 | 135 | 106.2±14.3 | 0.9 | 1.0(0.95-1.1) | 0.9 |
| Waist: Hip ratio | 5 | 0.85±0.04 | 134 | 0.87±0.08 | 0.6 | 0.1(0.0-915.5) | 0.6 |
| Waist: Height ratio | 5 | 54.9±18.7 | 132 | 57.2±10.9 | 0.7 | 0.99(0.91-1.1) | 0.8 |
| Mid-arm | 5 | 31.0±6.8 | 136 | 32.8±4.98 | 0.4 | 0.94(0.8-1.1) | 0.5 |
| Neck | 5 | 34.1±3.0 | 136 | 35.4±3.6 | 0.4 | 0.9(0.6-1.2) | 0.4 |
| Chest | 5 | 90.2±13.7 | 135 | 95.1±10.5 | 0.3 | 0.96(0.9-1.1) | 0.4 |
| Mid-thigh | 5 | 48.98±22.0 | 132 | 56.7±9.7 | 0.1 | 0.93(0.8-1.0) | 0.1 |

Continued on next page

| Variable | Developing DM (OGTT) | | Not developing DM | | p | Univariate analysis | *p |
|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | | HR (95% CI) | |
| **Skin folds** | | | | | | | |
| Triceps | 5 | 19.5±16.0 | 135 | 23.6±13.2 | 0.5 | 0.98(0.91-1.1) | 0.6 |
| Sub-scapular | 5 | 13.8±9.1 | 134 | 19.6±12.1 | 0.3 | 0.95(0.9-1.1) | 0.4 |
| Abdomen | 5 | 19.4±17.0 | 134 | 25.5±14.2 | 0.4 | 0.97(0.90-1.0) | 0.4 |
| Mid-thigh | 5 | 28.6±23.8 | 133 | 33.0±16.99 | 0.6 | 0.99(0.94-1.0) | 0.7 |

Data are mean ± SD or n(%).n= number for which data was available for each variable.DM: Diabetes Mellitus. OGTT: Oral glucose tolerance test. BP: Blood pressure.[*]5 subjects developed DM and 145 did not develop DM; p: Student's t test for differences between the two groups.HR: Hazard ratio, CI: Confidence interval.[#]p: differences at univariate level using Cox proportional hazards.

**Table 6.4 Baseline laboratory characteristics in the group developing Diabetes Mellitus vs. not developing Diabetes Mellitus by OGTT criteria during 24 months follow up***

| Variable | Developing DM (OGTT) | | Not developing DM | | p | Univariate analysis | #p |
|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | | HR (95%CI) | |
| **Plasma glucose (mmol/l)** | | | | | | | |
| 0 hour | 5 | 4.5±0.4 | 138 | 4.8±0.5 | 0.2 | 0.2(0.0-1.2) | 0.1 |
| 2 hour | 5 | 5.3±2.5 | 130 | 5.2+1.1 | 0.9 | 1.2(0.5-2.8) | 0.7 |
| HbA$_{1c}$ | 5 | 4.2±0.6 | 127 | 3.96+0.7 | 0.5 | 1.7(0.4-7.1) | 0.5 |
| **Serum lipids (mmol/l)** | | | | | | | |
| Total cholesterol | 5 | 2.9±0.7 | 135 | 3.51±0.89 | 0.1 | 0.4(0.1-1.4) | 0.1 |
| Total triglycerides | 5 | 0.7±0.2 | 133 | 0.97±0.57 | 0.4 | 0.3(0.0-3.8) | 0.3 |
| LDL | 5 | 1.80±0.48 | 120 | 2.24±0.76 | 0.2 | 0.5(0.1-1.7) | 0.3 |
| HDL | 5 | 0.75±0.35 | 132 | 0.82±0.29 | 0.6 | 0.4(0.0-13.3) | 0.6 |
| **HIV Parameters** | | | | | | | |
| CD4+ cell count (cells/mm$^3$ ) | 5 | 100.2±78.7 | 139 | 140.31±88.7 | 0.3 | 0.99(0.98-1.0) | 0.2 |
| HIV RNA, copies/ml | 4 | 4.15±2.14 | 123 | 4.8±0.9 | 0.2 | 0.7(0.3-1.4) | 0.3 |
| **Full blood count** | | | | | | | |
| Haemoglobin (g/dL) | 5 | 10.5±1.9 | 141 | 11.1±1.98 | 0.5 | 0.8(0.5-1.3) | 0.3 |
| Platelet count (x10$^9$/L) | 5 | 217.6±48.7 | 141 | 251.4±83.4 | 0.4 | 1.00(0.98-1.0) | 0.4 |
| White cell count (x10$^9$/L) | 5 | 4.1±0.6 | 141 | 4.5±1.7 | 0.5 | 0.9(0.5-1.6) | 0.6 |
| Lymphocytes (x10$^9$/L) | 5 | 1.3±0.5 | 141 | 2.04±4.23 | 0.7 | 0.7(0.2-2.2) | 0.5 |

Data are mean ± SD or n(%).n= number for which data was available for each variable.DM: Diabetes Mellitus. LDL: low density lipoprotein, HDL: high density lipoprotein, HbA$_{1c}$: haemoglobinA$_{1c}$.*5 subjects developed DM and 145 did not develop DM; p: Student's t-test for differences between the two groups.HR: Hazard ratio, CI: Confidence interval.#p: differences at univariate level using Cox proportional hazards.

**Table 6.5: Baseline laboratory characteristics in the group developing Diabetes Mellitus vs. not developing Diabetes Mellitus by OGTT criteria during 24 months follow up***

| Variable | Developing DM (OGTT) | | Not developing DM | | p | Univariate analysis | #p |
|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | | HR (95%CI) | |
| **Renal function** | | | | | | | |
| Bicarbonate (mmol/l) | 5 | 24.6+3.21 | 140 | 24.6+2.5 | 0.9 | 0.97(0.7-1.4) | 0.9 |
| Chloride (mmol/l) | 5 | 103.4+5.3 | 140 | 104.5+3.9 | 0.5 | 0.9(0.7-1.1) | 0.3 |
| Urea (mmol/l) | 5 | 4.5+1.5 | 140 | 3.5+1.3 | 0.1 | 1.4(0.91-2.3) | 0.1 |
| Creatinine (mmol/l) | 5 | 76.0+11.8 | 140 | 68.9+19.1 | 0.4 | 1.0(0.98-1.1) | 0.3 |
| Anion gap | 5 | 9.6+2.4 | 140 | 11.4+3.1 | 0.2 | 0.8(0.6-1.1) | 0.2 |
| Calcium (mmol/l) | 4 | 2.2+0.1 | 136 | 2.2+0.1 | 0.5 | 0.0(0.0-112.0) | 0.4 |
| Magnesium (mmol/l) | 4 | 0.88+0.11 | 133 | 0.90+0.23 | 0.8 | 0.5(0.0-630.4) | 0.8 |
| Phosphate (mmo/l) | 4 | 1.13+0.21 | 134 | 1.12+0.19 | 0.9 | 1.7(0.0-308.3) | 0.8 |
| **Liver function** | | | | | | | |
| Total protein (g/L) | 5 | 90.2+11.9 | 139 | 86.6+9.7 | 0.4 | 1.0(0.95-1.1) | 0.6 |
| Albumin (g/L) | 5 | 30.4+5.3 | 139 | 34.6+5.5 | 0.1 | 0.9(0.8-1.0) | 0.1 |
| Globulin (g/L) | 5 | 59.8+11.99 | 139 | 52.0+11.8 | 0.1 | 1.0(0.98-1.1) | 0.2 |
| Total bilirubin | 5 | 6.2+2.86 | 140 | 7.9+4.9 | 0.5 | 0.90(0.7-1.2) | 0.5 |
| Alanine amino transferase (U/l) | 5 | 26.8+5.5 | 138 | 26.0+18.3 | 0.9 | 1.0(0.96-1.0) | 0.9 |
| Serum alkaline phosphatase | 5 | 80.6+24.7 | 139 | 65.9+28.2 | 0.3 | 1.0(1.00-1.0) | 0.1 |
| Serum gamma glutamyl transferase | 5 | 41.6+22.5 | 139 | 28.5+29.6 | 0.3 | 1.0(1.00-1.1) | 0.1 |

Data are mean ± SD or n(%).n= number for which data was available for each variable. DM: Diabetes Mellitus. OGTT: Oral glucose tolerance test. *5 subjects developed DM and 145 did not develop DM; p: Student's t-test for differences between the two groups. HR: Hazard ratio, CI: Confidence interval.#p: differences at univariate level using Cox proportional hazards.

**Table 6.6: Baseline laboratory characteristics (inflammatory markers) in the group developing Diabetes Mellitus vs. not developing Diabetes Mellitus by OGTT criteria during 24 months follow up\***

| Variable | Developing DM (OGTT) | | Not developing DM | | p | Univariate analysis | #p |
|---|---|---|---|---|---|---|---|
| | n | Mean$\pm$SD | n | Mean$\pm$SD | | HR (95%CI) | |
| **Inflammatory Markers** | | | | | | | |
| CRP (mg/L) | 5 | 13.0$\pm$9.97 | 128 | 19.9$\pm$37.2 | 0.7 | 1.00(0.96-1.0) | 0.95 |
| Lactate (mmol/l) | 3 | 1.4$\pm$0.2 | 131 | 1.51$\pm$0.79 | 0.9 | 0.8(0.2-3.8) | 0.8 |
| Uric acid (mmol/L) | 4 | 0.30$\pm$0.08 | 104 | 0.29$\pm$0.10 | 0.8 | 1.99(0.0-4854.6) | 0.9 |
| Cortisol (nmol/L) | 5 | 280.6$\pm$73.3 | 125 | 306.5+133.7 | 0.7 | 1.00(0.99-1.0) | 0.9 |
| Iron (umol/L) | 2 | 5.6$\pm$0.4 | 127 | 10.2$\pm$5.1 | 0.2 | 0.7(0.4-1.2) | 0.2 |
| Transferrin (g/L) | 2 | 2.4$\pm$0.97 | 115 | 2.25$\pm$0.55 | 0.6 | 1.9(0.2-20.1) | 0.6 |
| Saturation (%) | 2 | 10.0$\pm$2.8 | 112 | 151.8$\pm$190.4 | 0.2 | 0.8(0.6-1.1) | 0.2 |
| Ferritin (ug/L) | 2 | 259.0$\pm$219.2 | 85 | 18.2$\pm$9.2 | 0.4 | 1.0(1.00-1.0) | 0.4 |

Data are mean $\pm$ SD or n(%).n= number for which data was available for each variable.DM: Diabetes Mellitus. OGTT: Oral glucose tolerance test. *5 subjects developed DM and 145 did not develop DM; p: Student's t-test for differences between the two groups. HR: Hazard ratio, CI: Confidence interval.#p: differences at univariate level using Cox proportional hazards.

**Table 6.7: Baseline DXA findings in the group developing Diabetes Mellitus vs. not developing Diabetes Mellitus by OGTT criteria during 24 months follow up***

| Variable | Developing DM (OGTT) | | Not developing DM | | p | Univariate analysis | #p |
|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | | HR (95%CI) | |
| **Fat, by DXA (kg)** | | | | | | | |
| Total mass | 5 | 62384.4±16344.8 | 130 | 68063.4±15191.0 | 0.4 | 1.0(1.0-1.0) | 0.4 |
| Total fat | 5 | 12300.4±10499.6 | 130 | 21467.8±11582.6 | 0.1 | 1.00(1.00-1.0) | 0.1 |
| Left arm | 5 | 731.8±600.0 | 130 | 1255.8±736.0 | 0.1 | 1.00(1.00-1.0) | 0.1 |
| Right arm | 5 | 656.7±544.9 | 130 | 1246.9±712.4 | 0.1 | 1.00(1.00-1.0) | 0.1 |
| Left leg | 5 | 2821.2±2670.27 | 130 | 4701.8+2429.5 | 0.1 | 1.0(1.00-1.0) | 0.1 |
| Right leg | 5 | 2769.6±2846.4 | 130 | 4851.9±2553.5 | 0.1 | 1.0(1.00-1.0) | 0.1 |
| Trunk | 5 | 4529.9±3944.5 | 130 | 8590.6±5521.97 | 0.1 | 1.0(1.00-1.0) | 0.1 |
| **Lean, by DXA (kg)** | | | | | | | |
| Total | 5 | 50083.98±9927.98 | 130 | 46595.6±9094.5 | 0.4 | 1.0(1.0-1.0) | 0.5 |
| Left arm | 5 | 2814.1±586.2 | 130 | 2516.8±739.0 | 0.4 | 1.0(1.00-1.0) | 0.5 |
| Right arm | 5 | 3099.6±629.3 | 130 | 2645.5±754.5 | 0.2 | 1.0(1.0-1.0) | 0.3 |
| Left leg | 5 | 8089.5±1681.7 | 130 | 7873.7±1816.3 | 0.8 | 1.0(1.0-1.0) | 0.91 |
| Right leg | 5 | 8125.7±1272.9 | 130 | 7935.7±1816.1 | 0.8 | 1.0(1.0-1.0) | 0.93 |
| Trunk | 5 | 24342.7±5697.2 | 130 | 22015.2+4045.9 | 0.2 | 1.0(1.0-1.0) | 0.3 |
| Total BMD | 5 | 1.19±0.14 | 130 | 1.10±0.1 | 0.2 | 71.8(0.04-137521.3) | 0.3 |

Data are mean ± SD or n(%).n= number for which data was available for each variable.DM: Diabetes Mellitus. OGTT: Oral glucose tolerance test. DXA: dual energy Xray absorptiometry,BMD: bone mineral density.*5 subjects developed DM and 145 did not develop DM; p: Student's t-test for differences between the two groups. HR: Hazard ratio, CI: Confidence interval.#p: differences at univariate level using Cox proportional hazards.

**Table 6.8: Baseline CT scan findings in the group developing Diabetes Mellitus vs. not developing Diabetes Mellitus by OGTT criteria during 24 months follow up\***

| Variable | Developing DM (OGTT) | | Not developing DM | | p | Univariate analysis | #p |
|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | | HR (95%CI) | |
| **Fat distribution, CT scan** | | | | | | | |
| Total fat area | 4 | 125.1±173.2 | 116 | 298.7±202.9 | 0.1 | 0.99(0.98-1.0) | 0.1 |
| Visceral fat area | 4 | 31.4±23.3 | 116 | 62.9±49.7 | 0.2 | 0.97(0.92-1.0) | 0.2 |
| Visceral: subcutaneous ratio | 4 | 2.54±2.44 | 116 | 0.393±0.396 | <0.0001 | 3.4(1.9-6.2) | <0.0001 |
| Subcutaneous fat area | 4 | 93.7±151.2 | 116 | 235.7±170.4 | 0.1 | 0.99(0.98-1.0) | 0.1 |
| Waist size | 4 | 82.8±12.5 | 113 | 94.6±16.6 | 0.2 | 0.96(0.9-1.0) | 0.2 |

Data are mean ± SD or n(%).n= number for which data was available for each variable.DM: Diabetes Mellitus. Oral glucose tolerance test.\*5 subjects developed DM and 145 did not develop DM; p: Student's t-test for differences between the two groups. HR: Hazard ratio, CI: Confidence interval.#p: differences at univariate level using Cox proportional hazards

**Table 6.9: Baseline demographic characteristics of group developing Diabetes Mellitus by HbA$_{1c}$criteria vs. not developing Diabetes Mellitus during 24 months follow up on ART**

| Variable | Developing Diabetes Mellitus (HbA$_{1c}$ criteria), n=8 | Not Developing Diabetes Mellitus (HbA$_{1c}$), n=142 | p | HR (95% CI) | [#]p |
|---|---|---|---|---|---|
| Gender | | | | | |
| Male | 2 (4.2) | 46(95.8) | 0.3 | 0.35(0.04-2.87) | 0.3 |
| Female | 6 (6.1) | 93(93.9) | | | |
| Treatment allocation | | | | | |
| Efavirenz | 4(2.67) | 72(48.0) | 0.7 | 0.79(0.18-3.53) | 0.8 |
| Nevirapine | 4(2.67) | 70(46.67) | 0.7 | 1.27(0.28-5.67) | 0.8 |
| BMI | | | | | |
| >30 kg/m$^2$ | 3(2.10) | 4(2.80) | 0.4 | 1.9(0.42-8.33) | 0.4 |
| Familial DM | 4(2.8) | 31(21.7) | 0.04 | 3.66(0.82-16.35) | 0.1 |
| Smoker | 2(1.4) | 17(11.9) | 0.4 | 2.45(0.47-12.62) | 0.3 |
| Occupationalphysical activity | | | | | |
| Sedentary | 0(0.0) | 0(0.0) | | | |
| Light | 0(0.0) | 4(3.1) | | Not estimatable | |
| Moderate | 3(2.3) | 55(42.6) | | 0.49(0.10-2.46) | 0.4 |
| Heavy | 1(0.8) | 41(31.8) | | 0.23(0.02-2.22) | 0.2 |
| | 3(2.3) | 21(16.3) | | 1 | |
| Leisure physical activity | | | | | |
| Sedentary | 6(4.4) | 71(51.5) | | 1 | |
| Light | 0(0.0) | 17(12.3) | | Not estimatable | 0.9 |
| Moderate | 0(0.0) | 14(10.1) | | Not estimatable | 0.9 |
| Heavy | 0(0.0) | 30(21.7) | | Not estimatable | 0.3 |

Data are n(%), HbA$_{1c}$: haemoglobinA$_{1c}$, DM: diabetes mellitus.p: chi-square test for differences between the two groups. HR: hazard ratio,

CI: confidence interval.[#]p: differences at univariate level using Cox proportional hazards

**Table 6.10: Baseline clinical characteristics ofthose developing Diabetes Mellitus vs. not developing Diabetes Mellitus by HbA$_{1c}$ criteria during 24 month follow up in group 3 subjects on ART***

| Variable | Developing DM (HbA$_{1c}$) n | Mean±SD | | Not developing DM n | Mean±SD | p | Univariate analysis HR(95%CI) | #p |
|---|---|---|---|---|---|---|---|---|
| Age | 8 | 40.4±9.1 | | 142 | 36.6±9.1 | 0.3 | 1.03(0.96-1.11) | 0.8 |
| **Blood pressure (mmHg)** | | | | | | | | |
| Systolic | 7 | 118.1±12.4 | | 136 | 111.9±17.0 | 0.3 | 1.01(0.97-1.05) | 0.6 |
| Diastolic | 7 | 69.8±10.4 | | 133 | 70.98±10.9 | 0.3 | 0.98(0.91-1.1) | 0.6 |
| **Anthropometric measurements** | | | | | | | | |
| Weight (kg) | 8 | 72.4±15.9 | | 138 | 68.9±15.99 | 0.6 | 1.01(0.97-1.05) | 0.7 |
| Body mass index (kg/m$^2$) | 8 | 29.1±6.8 | | 138 | 26.0±6.2 | 0.2 | 1.1(0.97-1.2) | 0.2 |
| **Circumference (cm):** | | | | | | | | |
| Waist | 8 | 90.5±17.7 | | 132 | 92.3±16.9 | 0.7 | 0.99(0.95-1.04) | 0.8 |
| Hip | 8 | 102.1±12.5 | | 132 | 106.2±14.8 | 0.4 | 0.99(0.94-1.0) | 0.6 |
| Waist: Hip ratio | 8 | 0.88±0.1 | | 131 | 0.87±0.08 | 0.7 | 2.9(0-46011.6) | 0.8 |
| Waist: Height ratio | 7 | 56.6±12.2 | | 130 | 56.8±11.0 | 0.9 | 1.0(0.94-1.1) | 0.93 |
| Mid-arm | 8 | 30.2±3.98 | | 133 | 32.8±4.9 | 0.1 | 0.91(0.8-1.1) | 0.2 |
| Neck | 8 | 33.6±2.3 | | 133 | 35.4±3.6 | 0.2 | 0.8(0.6-1.06) | 0.1 |
| Chest | 8 | 90.1±7.6 | | 132 | 94.9±10.7 | 0.2 | 0.96(0.9-1.03) | 0.3 |
| Mid-thigh | 8 | 54.9±6.7 | | 129 | 56.4±10.5 | 0.7 | 0.99(0.92-1.07) | 0.9 |
| **Skin folds** | | | | | | | | |
| Triceps | 8 | 17.4±9.2 | | 132 | 23.5±13.3 | 0.2 | 0.97(0.91-1.0) | 0.3 |
| Sub-scapular | 8 | 18.95±18.7 | | 131 | 19.0±11.3 | 0.9 | 1.00(0.95-1.1) | 0.95 |
| Abdomen | 8 | 23.9±16.7 | | 131 | 25.2±14.1 | 0.8 | 1.00(0.95-1.0) | 0.9 |
| Mid-thigh | 8 | 25.3±11.99 | | 130 | 33.1±17.3 | 0.2 | 0.98(0.94-1.0) | 0.3 |

Data are mean ± SD or n(%).n= number for which data was available for each variable.DM: Diabetes Mellitus.HbA$_{1c}$: haemoglobinA$_{1c}$*8 subjects developed DM and 142 did not develop DM; p: Student's t-test for differences between the two groups. HR: Hazard ratio, CI: Confidence interval.#p: differences at univariate level using Cox proportional hazards.

**Table 6.11: Baseline laboratory characteristics of those developing Diabetes Mellitus vs. not developing Diabetes Mellitus by HbA$_{1c}$ criteria during 24 month follow up in group 3 subjects on ART***

| Variable | Developing DM (HbA$_{1c}$) | | Not developing DM | | p | Univariate analysis | #p |
|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | | HR(95%CI) | |
| **Plasma glucose (mmol/l)** | | | | | | | |
| 0 hour | 8 | 5.0±0.5 | 134 | 4.8±0.5 | 0.2 | 2.5(0.7-9.1) | 0.2 |
| 2 hour | 8 | 5.7±0.6 | 125 | 5.1±1.2 | 0.2 | 1.6(0.91-2.9) | 0.1 |
| HbA$_{1c}$ | 8 | 4.2±0.9 | 126 | 3.97±0.67 | 0.3 | 1.98(0.6-6.6) | 0.3 |
| **Serum lipids (mmol/l)** | | | | | | | |
| Total cholesterol | 8 | 3.51±0.73 | 132 | 3.51±0.89 | 0.95 | 1.2(0.5-2.5) | 0.7 |
| Total triglycerides | 8 | 1.27±0.76 | 130 | 0.95±0.57 | 0.1 | 1.5(0.8-2.8) | 0.2 |
| LDL | 8 | 2.32±0.85 | 129 | 2.23±0.75 | 0.1 | 1.4(0.5-3.7) | 0.6 |
| HDL | 6 | 0.68±0.10 | 118 | 0.84±0.29 | 0.8 | 0.1(0.0-2.4) | 0.2 |
| **HIV Parameters** | | | | | | | |
| CD4+ cell count (cells/mm3 ) | 8 | 162.8±117.9 | 136 | 139.5±86.4 | 0.4 | 1.0(0.99-1.01) | 0.4 |
| HIV RNA, copies/ml | 7 | 4.93±0.98 | 120 | 4.73±0.93 | 0.6 | 1.4(0.6-3.20) | 0.4 |
| **Full blood count** | | | | | | | |
| Haemoglobin (g/dL) | 8 | 10.1±1.9 | 138 | 11.2±2.0 | 0.1 | 0.7(0.5-1.0) | 0.07 |
| Platelet count (x10$^9$/L) | 8 | 222.1±100.1 | 138 | 251.5±80.4 | 0.3 | 1.00(0.99-1.0) | 0.3 |
| White cell count (x10$^9$/L) | 8 | 4.7±0.8 | 138 | 4.5±1.7 | 0.8 | 1.2(0.8-1.8) | 0.5 |
| Lymphocytes (x10$^9$/L) | 8 | 1.8±0.9 | 138 | 2.0±4.3 | 0.9 | 0.97(0.8-1.2) | 0.8 |

Data are mean ± SD or n(%).n= number for which data was available for each variable.DM: Diabetes Mellitus.HbA$_{1c}$: haemoglobinA$_{1c}$.*8 subjects developed DM and 142 did not develop DM.p: Student's t-test for differences between the two groups. HR: Hazard ratio, CI: Confidence interval.#p: differences at univariate level using Cox proportional hazards

**Table 6.12: Baseline laboratory characteristics of those developing DM vs. not developing DM by HbA$_{1c}$ criteria during 24 month follow up in group 3 subjects on ART\***

| Variable | Developing DM (HbA$_{1c}$) | | Not developing DM | | p | Univariate analysis | #p |
|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | | HR(95%CI) | |
| **Renal function** | | | | | | | |
| Bicarbonate (mmol/l) | 8 | 24.1±3.0 | 137 | 24.7±2.5 | 0.6 | 0.9(0.7-1.2) | 0.4 |
| Chloride (mmol/l) | 8 | 104.9±3.8 | 137 | 104.4±3.9 | 0.8 | 1.0(0.8-1.2) | 0.8 |
| Urea (mmol/l) | 8 | 4.5±1.5 | 137 | 3.5±1.3 | 0.02 | 1.4(1.00-2.0) | 0.1 |
| Creatinine (mmol/l) | 8 | 74.8±28.7 | 137 | 68.8±18.4 | 0.4 | 1.0(0.99-1.1) | 0.2 |
| Anion gap | 8 | 11.4±3.1 | 137 | 11.4±3.0 | 0.9 | 1.0(0.8-1.3) | 0.9 |
| Calcium (mmol/l) | 8 | 2.13±0.12 | 132 | 2.21±0.12 | 0.1 | 0.0(0.0-2.1) | 0.1 |
| Magnesium (mmol/l) | 7 | 0.82±0.09 | 130 | 0.91±0.23 | 0.3 | 0.0(0.0-0.1) | 0.02 |
| Phosphate (mmo/l) | 7 | 1.10±0.15 | 131 | 1.11±0.19 | 0.9 | 0.93(0.02-56.16) | 0.9 |
| **Liver function** | | | | | | | |
| Total protein (g/L) | 7 | 87.7±6.6 | 137 | 86.6±10.0 | 0.8 | 1.0(0.93-1.1) | 0.96 |
| Albumin (g/L) | 7 | 29.9±6.5 | 137 | 34.9±5.2 | 0.03 | 0.87(0.78-0.98) | 0.02 |
| Globulin (g/L) | 7 | 57.9±8.7 | 137 | 51.7±11.9 | 0.2 | 1.0(0.98-1.1) | 0.3 |
| Total bilirubin | 7 | 4.9±3.4 | 138 | 7.9±4.7 | 0.1 | 0.8(0.6-1.0) | 0.05 |
| Alanine amino transferase (U/l) | 7 | 17.7±2.98 | 136 | 26.2±17.8 | 0.2 | 0.92(0.8-1.04) | 0.2 |
| Serum alkaline phosphatase | 7 | 58.0±11.7 | 137 | 66.5±28.6 | 0.4 | 0.99(0.95-1.03) | 0.5 |
| Serum gamma glutamyl transferase | 7 | 16.1±5.2 | 137 | 30.3±31.4 | 0.2 | 0.99(0.95-1.02) | 0.1 |

Data are mean ± SD or n(%).n= number for which data was available for each variable.DM: Diabetes Mellitus.HbA$_{1c}$: haemoglobinA$_{1c}$.*8 subjects developed DM and 142 did not develop DM; p: Student's t-test for differences between the two groups. HR: Hazard ratio, CI: Confidence interval.#p: differences at univariate level using Cox proportional hazards.

**Table 6.13: Baseline laboratory characteristics(inflammatory markers) of those developing DM vs. not developing DM by HbA$_{1c}$ criteria during 24 month follow up in group 3 subjects on ART\***

| Variable | Developing DM (HbA$_{1c}$) | | Not developing DM | | p | Univariate analysis | #p |
|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | | HR(95%CI) | |
| **Inflammatory Markers** | | | | | | | |
| CRP (mg/L) | 7 | 11.14±10.59 | 126 | 19.25±37.2 | 0.5 | 1.00(0.96-1.0) | 0.8 |
| Lactate (mmol/l) | 5 | 1.1±0.3 | 129 | 1.5±0.8 | 0.2 | 0.2(0.02-2.5) | 0.2 |
| Uric acid (mmol/L) | 4 | 0.35±0.12 | 105 | 0.29±0.1 | 0.3 | 9.3(0.07-1236.3) | 0.4 |
| Cortisol (nmol/L) | 8 | 278.9±123.7 | 121 | 305.4±129.1 | 0.6 | 0.99(0.99-1.01) | 0.8 |
| Iron (umol/L) | 8 | 8.6±4.3 | 122 | 10.3±5.0 | 0.3 | 0.9(0.8-1.1) | 0.2 |
| Transferrin (g/L) | 8 | 2.0±0.7 | 110 | 2.3±0.5 | 0.3 | 0.4(0.1-2.0) | 0.3 |
| Saturation (%) | 8 | 19.0±12.0 | 105 | 143.4±186.9 | 0.8 | 0.99(0.91-1.1) | 0.9 |
| Ferritin (ug/L) | 7 | 235.6±203.6 | 81 | 18.1±8.8 | 0.2 | 1.0(1.00-1.0) | 0.9 |

Data are mean ± SD or n(%).n= number for which data was available for each variable. DM: Diabetes Mellitus.HbA$_{1c}$: haemoglobinA$_{1c}$.*8 subjects developed DM and 142 did not develop DM; p: Student's t-test for differences between the two groups. HR: Hazard ratio, CI: Confidence interval.#p: differences at univariate level using Cox proportional hazards.

**Table 6.14: BaselineDXA scan findings in the group developing DM vs. not developing DM by HbA$_{1c}$ criteria during 24 month follow up in group 3 subjects on ART***

| Variable | Developing DM (HbA$_{1c}$) | | Not developing DM | | p | Univariate analysis | [#]p |
|---|---|---|---|---|---|---|---|
| | n | Mean$\pm$SD | n | Mean$\pm$SD | | HR(95%CI) | |
| **Fat, by DXA (kg)** | | | | | | | |
| Total mass | 5 | 76452.5$\pm$18380.3 | 130 | 67725.0$\pm$15613.7 | 0.2 | 1.0(1.0-1.0) | 0.3 |
| Total fat | 5 | 29176.2$\pm$14762.8 | 130 | 20822.7$\pm$11978.8 | 0.1 | 1.0(1.0-1.0) | 0.1 |
| Left arm | 5 | 1813.1$\pm$1002.8 | 130 | 1207.7$\pm$732.9 | 0.1 | 1.0(1.0-1.0) | 0.1 |
| Right arm | 5 | 1736.3$\pm$995.0 | 130 | 1198.0$\pm$711.6 | 0.1 | 1.0(1.0-1.0) | 0.1 |
| Left leg | 5 | 5974.8$\pm$2804.6 | 130 | 4586.9$\pm$2523.1 | 0.2 | 1.0(1.0-1.0) | 0.3 |
| Right leg | 5 | 6055.5$\pm$2863.4 | 130 | 4740.9$\pm$2666.9 | 0.3 | 1.0(1.0-1.0) | 0.3 |
| Trunk | 5 | 12685.2$\pm$7225.5 | 130 | 8270.5$\pm$5695.2 | 0.1 | 1.0(1.0-1.0) | 0.1 |
| **Lean, by DXA (kg)** | | | | | | | |
| Total body | 5 | 47276.3$\pm$9982.6 | 130 | 46902.3$\pm$9303.6 | 0.9 | 1.0(1.0-1.0) | 0.93 |
| Left arm | 5 | 2422.3$\pm$692.2 | 130 | 2552.6$\pm$758.9 | 0.7 | 1.0(1.00-1.0) | 0.6 |
| Right arm | 5 | 2523.1$\pm$681.9 | 130 | 2688.9$\pm$778.7 | 0.6 | 1.0(1.00-1.0) | 0.5 |
| Left leg | 5 | 8016.6$\pm$1828.7 | 130 | 7915.2$\pm$1848.5 | 0.9 | 1.0(1.0-1.0) | 0.95 |
| Right leg | 5 | 8141.5$\pm$1771.9 | 130 | 7978.1$\pm$1842.4 | 0.8 | 1.0(1.0-1.0) | 0.98 |
| Trunk | 5 | 22535.1$\pm$4734.9 | 130 | 22150.3$\pm$4162.5 | 0.9 | 1.0(1.0-1.0) | 0.96 |
| Total BMD | 5 | 1.08$\pm$0.12 | 130 | 1.1$\pm$0.1 | 0.3 | 0.0(0-18.7) | 0.7 |

Data are mean $\pm$ SD or n(%).n= number for which data was available for each variable.DM: Diabetes Mellitus.HbA$_{1c}$: haemoglobinA$_{1c}$. [*]8 subjects developed DM and 142 did not develop DM.p: Student's t-test for differences between the two groups. HR: Hazard ratio, CI: Confidence interval.[#]p: differences at univariate level using Cox proportional hazards.

**Table 6.15: BaselineCT scan findings in the group developing DM vs. not developing DM by HbA$_{1c}$ criteria during 24 month follow up in group 3 subjects on ART***

| Variable | Developing DM (HbA1c) | | Not developing DM | | p | Univariate analysis | |
|---|---|---|---|---|---|---|---|
| | n | Mean$\pm$SD | n | Mean$\pm$SD | | HR(95%CI) | [#]p |
| **Fat distribution, CT scan** | | | | | | | |
| Total Fat Area | 7 | 312.8$\pm$207.7 | 113 | 289.6$\pm$208.6 | 0.8 | 1.0(0.99-1.01) | 0.7 |
| Visceral Fat Area | 7 | 68.7$\pm$66.6 | 113 | 60.1$\pm$47.0 | 0.7 | 1.0(0.99-1.0) | 0.7 |
| Visceral: subcutaneous fat ratio | 7 | 0.3$\pm$0.2 | 113 | 0.5$\pm$0.7 | 0.5 | 0.3(0.01-8.8) | 0.5 |
| Subcutaneous fat area | 7 | 244.1$\pm$148.3 | 113 | 229.3$\pm$178.6 | 0.9 | 1.0(1.00-1.0) | 0.8 |
| Waist size | 7 | 96.8$\pm$15.4 | 110 | 93.9$\pm$17.2 | 0.7 | 1.01(0.97-1.05) | 0.7 |

Data are mean $\pm$ SD or n(%).n= number for which data was available for each variable. DM: Diabetes Mellitus. HbA$_{1c}$: haemoglobinA$_{1c}$[*]8 subjects developed DM and 142 did not develop DM. p: Student's t-test for differences between the two groups. HR: Hazard ratio, CI: Confidence interval.[#]p: differences at univariate level using Cox proportional hazards.

### 6.3.2. Risk factors for Impaired Glucose Tolerance (IGT)

Tables 6.16 – 6.22 shows univariate analysis and baseline characteristicsof subjects who did and did not develop impaired glucose tolerance during follow up. When compared with the group that did not develop IGT, subjects who developed IGT had significantly higher mean systolic (p=0.003) and diastolic (p=0.01) blood pressure, alanine amino transferase (p=0.04) and visceral: subcutaneous fat ratio (p=0.002) on CT scan. No significant difference was observed between the two groups for other demographic, clinical, laboratory or radiological variables (Tables 6.16 – 6.22)

In univariate analysis, variables that were significantly associated with development of IGT were systolic (p=0.01) and diastolic (p=0.01) blood pressure, alanine amino transferase (p=0.003) and visceral: subcutaneous fat ratio (p=0.002) on computerized tomography scan (Tables 6.16 – 6.22).

In multivariate analysis (Table 6.44), risk factors that predicted development of IGT were systolic blood pressure (HR 1.05[95%CI1.01 – 1.09], p=0.01) and visceral: subcutaneous fat ratio (HR 8.16[95%CI 1.53 – 43.53], p=0.01). One unit of systolic blood pressure predicted a five percent higher risk and a unit of visceral: subcutaneous fat ratio predicted an eight fold higher risk of developing IGT. When treatment allocation was adjusted for, systolic blood pressure (HR 1.04 [95%CI1.01 – 1.07], p=0.01) and visceral: subcutaneous fat ratio (HR 4.89 [95%CI 1.60 – 14.92], p=0.005) remained the risk factors significantly associated with development of IGT.

**Table 6.16: Baseline demographic characteristics of those developing impaired glucose tolerance vs. not developing impaired glucose tolerance during 24 months of ART**

| Variable | Developing impaired glucose tolerance, n=7 | Not Developing impaired glucose tolerance, n=139 | p | HR (95% CI) | [#]p |
|---|---|---|---|---|---|
| Gender | | | | | |
| Male | 3(42.9) | 43(30.9) | 0.51 | 1.62(0.36-7.26) | 0.53 |
| Female | 4(57.1) | 96(69.1) | | 0.61(0.14-2.76) | 0.53 |
| **Treatment allocation** | | | | | |
| Efavirenz | 4(57.1) | 68(48.9) | 0.67 | 1.45(0.33-6.49) | 0.63 |
| Nevirapine | 3(42.9) | 71(51.1) | 0.67 | 0.69(0.15-3.08) | 0.63 |
| BMI | | | | | |
| >30 kg/m | 2(28.6) | 36(27.3) | 0.94 | 1.08(0.21-5.62) | 0.93 |
| Familial DM | 2(28.6) | 32(24.2) | 0.8 | 1.01(0.27-3.81) | 0.9 |
| Smoker | 1(14.3) | 18(13.6) | 0.05 | 0.77(0.09-6.13) | 0.9 |
| Occupational physical activity | | | | | |
| Sedentary | 0(0.0) | 4(3.4) | | Not estimatable | 1.0 |
| Light | 2(28.6) | 54(45.8) | | Not estimatable | 1.0 |
| Moderate | 4(57.1) | 36(30.5) | | Not estimatable | 1.0 |
| Heavy | 1(14.3) | 23(19.5) | | Not estimatable | 1.0 |

Continued on next page

**Table 6.16 cont.: Baseline demographic characteristics of those developing impaired glucose tolerance vs. not developing impaired glucose tolerance during 24 months of ART**

| Variable | Developing impaired glucose tolerance, n=7 | Not Developing impaired glucose tolerance, n=139 | p | HR (95% CI) | #p |
|---|---|---|---|---|---|
| Leisure physical activity | | | | | |
| Sedentary | 5(71.4) | 69(54.3) | | | |
| Light | 0(0.0) | 17(13.4) | | Not estimatable | 1.0 |
| Moderate | 1(14.3) | 13(10.2) | | 1.06(0.12-9.05) | 1.0 |
| Heavy | 1(14.3) | 28(22.1) | | 0.64(0.08-5.51) | 1.0 |

Data are n(%), BMI: body mass index, DM: diabetes mellitus, HR: hazard ratio, CI: confidence interval, DM: diabetes mellitus. p: chi-square test for differences between the two groups, #p: differences at univariate level using Cox proportional hazards

**Table 6.17: Baseline clinical characteristics of those developing impaired glucose tolerance vs. not developing impaired glucose tolerance during 24 months follow up in group 3 subjects on ART***

| Variable | Developing IGT | | Not developing IGT | | p | Univariate analysis | #p |
|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | | HR (95%CI) | |
| Age | 7 | 39.5±7.7 | 139 | 36.6±9.2 | 0.4 | 1.04(0.98-1.11) | 0.2 |
| Blood pressure (mmHg) | | | | | | | |
| Systolic | 7 | 129.9±23.5 | 132 | 110.98±15.8 | 0.003 | 1.03(1.01-1.1) | 0.01 |
| Diastolic | 6 | 81.8 ± 11.1 | 123 | 70.4±10.5 | 0.01 | 1.1(1.01-1.1) | 0.01 |
| **Anthropometric measurements** | | | | | | | |
| Weight (kg) | 7 | 67.6 ±16.5 | 138 | 69.3±16.1 | 0.8 | 0.99(0.95-1.03) | 0.6 |
| Body mass index (kg/m$^2$) | 7 | 26.95±7.3 | 138 | 26.3±6.2 | 0.8 | 1.01(0.90-1.1) | 0.8 |
| **Circumference (cm):** | | | | | | | |
| Waist | 7 | 89.5±18.7 | 131 | 92.8±17.3 | 0.6 | 0.99(0.95-1.0) | 0.8 |
| Hip | 7 | 98.4±13.7 | 131 | 106.8±14.8 | 0.1 | 0.96(0.90-1.0) | 0.2 |
| Waist: Hip ratio | 7 | 0.9±0.1 | 130 | 0.86±0.1 | 0.2 | NE | 0.2 |
| Waist: Height ratio | 7 | 56.3±12.2 | 128 | 57.1±11.3 | 0.8 | 0.98(0.94-1.1) | 0.98 |
| Mid-arm | 7 | 29.2±5.0 | 132 | 32.9±5.0 | 0.06 | 0.8(0.7-1.02) | 0.08 |
| Neck | 7 | 35.0±2.2 | 132 | 35.3±3.6 | 0.9 | 0.96(0.8-1.2) | 0.7 |
| Chest | 6 | 88.4±9.6 | 132 | 95.1±10.7 | 0.1 | 0.93(0.8-1.0) | 0.1 |
| Mid-thigh | 6 | 49.4±8.6 | 129 | 56.8±10.4 | 0.09 | 0.94(0.9-1.0) | 0.07 |
| **Skin folds** | | | | | | | |
| Triceps | 7 | 16.0±9.2 | 131 | 24.0±13.3 | 0.1 | 0.95(0.9-1.0) | 0.2 |
| Sub-scapular | 11 | 18.4±19.95 | 130 | 19.5±11.6 | 0.8 | 0.99(0.94-1.1) | 0.9 |
| Abdomen | 11 | 20.4±18.7 | 130 | 25.8±14.0 | 0.3 | 0.98(0.92-1.0) | 0.4 |
| Mid-thigh | 11 | 25.8±14.7 | 129 | 33.6±17.2 | 0.2 | 0.98(0.93-1.0) | 0.4 |

Data are mean ± SD. IGT: impaired glucose tolerance. HR: Hazard ratio, CI: Confidence interval. BP: Blood pressure. *7 subjects developed impaired glucose tolerance (IGT) and 139 did not develop IGT; n= number for which data was available for each variable.p: Student's t-test for differences between the two groups, #p: differences at univariate level using Cox proportional hazards. NE: Not estimatable

**Table 6.18: Baseline laboratory characteristics of those developing impaired glucose tolerance vs. not developing impaired glucose tolerance during 24 months follow up in group 3 subjects on ART***

| Variable | Developing IGT | | Not developing IGT | | p | Univariate analysis | [#]p |
| --- | --- | --- | --- | --- | --- | --- | --- |
| | n | Mean±SD | n | Mean±SD | | HR (95% CI) | |
| **Plasma glucose (mmol/l)** | | | | | | | |
| 0 hour | 7 | 4.9±0.2 | 133 | 4.8±0.5 | 0.4 | 1.8(0.4-7.6) | 0.4 |
| 2 hour | 7 | 5.4±1.8 | 124 | 5.1±1.0 | 0.5 | 1.4(0.6-2.9) | 0.4 |
| $HbA_{1c}$ | 7 | 3.96±0.5 | 124 | 3.98±0.69 | 0.9 | 0.88(0.3-2.7) | 0.8 |
| **Serum lipids (mmol/l)** | | | | | | | |
| Total cholesterol | 6 | 3.71± 0.75 | 132 | 3.49±0.87 | 0.5 | 1.5(0.6-3.7) | 0.4 |
| Total triglycerides | 6 | 0.94±0.34 | 130 | 0.96±0.58 | 0.9 | 0.87(0.2-3.6) | 0.8 |
| LDL | 6 | 2.59±0.83 | 116 | 2.21±0.73 | 0.2 | 2.2(0.77-6.4) | 0.1 |
| HDL | 6 | 0.67±0.29 | 129 | 0.84±0.28 | 0.2 | 0.1(0.004-3.3) | 0.2 |
| **HIV Parameters** | | | | | | | |
| CD4+ cell count (cells/mm3) | 7 | 100.71±61.9 | 136 | 144.46±88.1 | 0.2 | 0.99(0.98-1.0) | 0.2 |
| HIV RNA, copies/ml | 6 | 4.38± 1.73 | 120 | 4.75±0.88[7] | 0.3 | 0.8(0.4-1.6) | 0.5 |
| **Full blood count** | | | | | | | |
| Haemoglobin (g/dL) | 7 | 11.2±1.7 | 138 | 11.2±1.93 | 0.96 | 0.99(0.7-1.5) | 0.96 |
| Platelet count ($x10^9$/L) | 7 | 253.6±130.5 | 138 | 249.9±79.1 | 0.9 | 1.0(0.99-1.0) | 0.8 |
| White cell count ($x10^9$/L) | 7 | 4.6±1.7 | 138 | 4.5±1.6 | 0.8 | 1.1(0.7-1.8) | 0.6 |
| Lymphocytes ($x10^9$/L) | 7 | 1.3±0.91 | 138 | 2.1±4.3 | 0.6 | 0.6(0.2-1.8) | 0.4 |

Data are mean ± SD. IGT:Impaired glucose tolerance,$HbA_{1c}$: haemoglobin$A_{1c}$, LDL: low density lipoprotein, HDL: high density lipoprotein,HR: Hazard ratio, CI: Confidence interval.[*]7 subjects developed IGT and 139 did not develop IGT; n= number for which data was available for each variable.p: Student's t-test for differences between the two groups, [#]p: differences at univariate level using Cox proportional hazards

**Table 6.19: Baseline laboratory characteristics of those developing impaired glucose tolerance vs. not developing impaired glucose tolerance during 24 months follow up in group 3 subjects on ART***

| Variable [#] | Developing IGT | | Not developing IGT | | p | Univariate analysis | [#]p |
|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | | HR (95%CI) | |
| **Renal function** | | | | | | | |
| Bicarbonate (mmol/l) | 7 | 24.0±2.2 | 137 | 24.6±2.5 | 0.5 | 0.9(0.6-1.2) | 0.3 |
| Chloride (mmol/l) | 7 | 103.3±4.9 | 137 | 104.7±3.7 | 0.3 | 0.9(0.7-1.1) | 0.2 |
| Urea (mmol/l) | 7 | 3.6±1.2 | 137 | 3.5±1.3 | 0.9 | 1.0(0.6-1.7) | 0.9 |
| Creatinine (mmol/l) | 7 | 70.7±17.3 | 137 | 68.4±18.6 | 0.8 | 1.0(0.97-1.1) | 0.6 |
| Anion gap | 7 | 11.7 ± 2.4 | 137 | 11.3±3.1 | 0.8 | 1.0(0.8-1.3) | 0.7 |
| Calcium (mmol/l) | 7 | 2.2±0.1 | 132 | 2.2±0.1 | 0.2 | 0.0(0.00-8.7) | 0.2 |
| Magnesium (mmol/l) | 7 | 1.07±0.2 | 129 | 0.9±0.2 | 0.05 | 3.6(0.8-16.4) | 0.1 |
| Phosphate (mmo/l) | 7 | 1.1±0.2 | 130 | 1.1±0.2 | 0.9 | 1.1(0.0-54.6) | 0.96 |
| **Liver function** | | | | | | | |
| Total protein (g/L) | 7 | 86.4±10.5 | 136 | 87.0±9.7 | 0.9 | 0.99(0.9-1.0) | 0.8 |
| Albumin (g/L) | 7 | 32.0±6.1 | 136 | 34.8±5.3 | 0.2 | 0.9(0.8-1.03) | 0.1 |
| Globulin (g/L) | 7 | 54.4±7.2 | 136 | 52.2±12.1 | 0.6 | 1.01(0.96-1.1) | 0.7 |
| Total bilirubin | 7 | 6.86±2.79 | 137 | 7.8±4.8 | 0.6 | 0.96(0.8-1.2) | 0.6 |
| Alanine amino transferase (U/l) | 7 | 40.3±38.9 | 135 | 25.3±16.7 | 0.04 | 1.03(1.00-1.05) | 0.02 |
| Serum alkaline phosphatase | 7 | 61.1±20.7 | 136 | 65.99±26.7 | 0.6 | 0.99(0.96-1.03) | 0.8 |
| Serum gamma glutamyl transferase | 7 | 31.6±29.1 | 136 | 29.8±32.3 | 0.9 | 1.01(0.99-1.04) | 0.4 |

Data are mean ± SD.IGT: impaired glucose tolerance,HR: Hazard ratio, CI: Confidence interval.*7 subjects developed IGT and 139 did not develop IGT; n= number for which data was available for each variable.p: Student's t-test for differences between the two groups, [#]p: differences at univariate level using Cox proportional hazards

**Table 6.20: Baseline laboratory characteristics of those developing impaired glucose tolerance vs. not developing impaired glucose tolerance during 24 months follow up in group 3 subjects on ART***

| Variable | Developing IGT | | Not developing IGT | | | Univariate analysis | [#]p |
|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | p | HR (95%CI) | |
| **Inflammatory Markers** | | | | | | | |
| CRP (mg/L) | 7 | 20.0± 36.4 | 125 | 16.97±31.88 | 0.8 | 1.01(0.99-1.03) | 0.4 |
| Lactate (mmol/l) | 6 | 1.3±0.7 | 126 | 1.5±0.8 | 0.6 | 0.7(0.2-2.4) | 0.5 |
| Uric acid (mmol/L) | 4 | 0.3±0.1 | 103 | 0.29±0.10 | 0.4 | 6.9(0.03-1792) | 0.5 |
| Cortisol (nmol/L) | 7 | 325.1±234.0 | 120 | 300.6±117.0 | 0.6 | 1.00(0.99-1.01) | 0.2 |
| Iron (umol/L) | 6 | 10.6±4.5 | 124 | 10.3±5.0 | 0.9 | 1.01(0.9-1.1) | 0.95 |
| Transferrin (g/L) | 5 | 2.24±0.4 | 113 | 2.3±0.6 | 0.9 | 1.2(0.2-6.9) | 0.9 |
| Saturation (%) | 5 | 15.6±7.5 | 83 | 18.3±9.2 | 0.5 | 0.95(0.9-1.1) | 0.4 |
| Ferritin (ug/L) | 6 | 209.0±232.6 | 107 | 149.94±186.6 | 0.5 | 0.99(0.99-1.0) | 0.6 |

Data are mean ± SD. IGT: impaired glucose tolerance,HR: Hazard ratio, CI: Confidence interval. BP: Blood pressure. [*]7 subjects developed IGT and 139 did not develop IGT; n= number for which data was available for each variable.p: Student's t-test for differences between the two groups, [#]p: differences at univariate level using Cox proportional hazards; CRP: C-reactive protein

**Table 6.21: BaselineDXA scan findings of those developing impaired glucose tolerance vs. not developing impaired glucose tolerance during 24 months follow up in group 3 subjects on ART***

| Variable | Developing IGT | | Not developing IGT | | p | Univariate analysis | #p |
|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | | HR (95%CI) | |
| **Fat, by DXA (kg)** | | | | | | | |
| Whole body total | 7 | 20183.1±16063.8 | 0.8 | 21483.3±11885.97 | 0.8 | 1.0(1.0-1.0) | 0.7 |
| Left arm | 7 | 1366.6±1145.5 | 0.7 | 1240.3±725.1 | 0.7 | 1.0(0.99-1.0) | 0.7 |
| Right arm | 7 | 1307.2±1083.9 | 0.8 | 1232.3±707.5 | 0.8 | 1.0(0.99-1.0) | 0.8 |
| Left leg | 7 | 3806.9±3033.1 | 0.3 | 4736.6±2488.1 | 0.3 | 1.0(0.99-1.0) | 0.3 |
| Right leg | 7 | 3885.6±3198.5 | 0.3 | 4894.8±2621.0 | 0.3 | 1.0(0.99-1.0) | 0.3 |
| Trunk | 7 | 9025.9±7530.7 | 0.8 | 8555.3±5713.7 | 0.8 | 1.0(1.0-1.0) | 0.8 |
| **Lean, by DXA (kg)** | | | | | | | |
| Whole body total | 7 | 46096.2± 3693.1 | 0.8 | 46901.5±9524.6 | 0.8 | 1.0(1.0-1.0) | 0.7 |
| Left arm | 7 | 2603.6±457.1 | 0.8 | 2539.2±771.97 | 0.8 | 1.0(0.99-1.0) | 0.9 |
| Right arm | 7 | 2753.8±380.7 | 0.8 | 2672.0.3±793.5 | 0.8 | 1.0(0.99-1.0) | 0.9 |
| Left leg | 7 | 7644.6±980.0 | 0.8 | 7930.8±1883.5 | 0.7 | 1.0(0.99-1.0) | 0.6 |
| Right leg | 7 | 7736.7±898.2 | 0.7 | 7989.9±1876.6 | 0.7 | 1.0(1.0-1.0) | 0.7 |
| Trunk | 7 | 21987.6±1400.2 | 0.8 | 22139.9±4265.1 | 0.9 | 1.0(1.0-1.0) | 0.8 |
| Total BMD | 7 | 1.1±0.1 | 0.3 | 1.1±0.1 | 0.9 | 0.3(0.0-400.9) | 0.7 |

Data are mean ± SD. IGT: impaired glucose tolerance. HR: Hazard ratio, CI: Confidence interval. BP: Blood pressure. *7 subjects developed IGT and 139 did not develop IGT; n= number for which data was available for each variable. p: Student's t-test for differences between the two groups, #p: differences at univariate level using Cox proportional hazards

**Table 6.22: Baseline CT scan findings of those developing impaired glucose tolerance vs. not developing impaired glucose tolerance during 24 months follow up in group 3 subjects on ART***

| Variable | Developing IGT | | Not developing IGT | | p | Univariate analysis | #p |
|---|---|---|---|---|---|---|---|
| | n | Mean$\pm$SD | n | Mean$\pm$SD | | HR (95%CI) | |
| **Fat distribution, CT scan** | | | | | | | |
| Total Fat Area | 6 | 329.3$\pm$244.8 | 113 | 291.9$\pm$207.4 | 0.7 | 1.00(0.99-1.01) | 0.8 |
| Visceral Fat Area | 6 | 74.9$\pm$53.5 | 113 | 59.7$\pm$48.0 | 0.5 | 1.0(0.99-1.0) | 0.4 |
| Visceral: subcutaneous fat ratio | 6 | 1.07$\pm$1.65 | 113 | 0.4$\pm$0.4 | 0.002 | 3.76(1.6-8.7) | 0.002 |
| Subcutaneous fat area | 6 | 254.4$\pm$186.8 | 113 | 231.96$\pm$176.3 | 0.8 | 1.00(0.99-1.0) | 0.7 |
| Waist size | 6 | 100.1$\pm$16.2 | 110 | 94.0$\pm$17.2 | 0.4 | 1.0(0.98-1.1) | 0.3 |

Data are mean $\pm$ SD. IGT: impaired glucose tolerance,HR: Hazard ratio, CI: Confidence interval.*7 subjects developed IGT and 139 did not develop IGT; n= number for which data was available for each variable.p: Student's t-test for differences between the two groups, #p: differences at univariate level using Cox proportional hazards

### 6.3.3. Risk factors for impaired fasting glucose (IFG)

Tables 6.23 – 6.29 show univariate analysis and baseline characteristics of subjects who did and who did not develop impaired fasting glucose. Compared with subjects that did not develop IFG, subjects who developed IFG had the following: more familial DM (p=0.03), anthropometry – higher mean chest (p=0.04), and waist (p=0.02) circumference, waist: hip ratio (p=0.01) and abdominal (p=0.03) skinfolds; biochemistry – higher serum bicarbonate (p=0.02), ferritin (p=0.04) and lower platelet count (p=0.02). No significant difference was observed between the two groups for any other demographic, clinical, laboratory or radiological variables (Tables 6.23 – 6.29).

In univariate analysis, variables that were significantly associated with development of IFG were the following: anthropometry –mid-arm (p=0.01), chest (p=0.03), and waist (p=0.004) circumference, subscapular (p=0.02) and abdominal (p=0.03) skinfolds; biochemistry – serum bicarbonate (p=0.03), alkaline phosphatase (p=0.01) and platelet count (p=0.02).

In multivariate analysis, the risk factors that predicted development of IFG were gender (HR 0.15 [95%CI0.02 – 0.89], p=0.04), waist circumference (HR 1.09 [95%CI 1.03 - 1.15], p=0.003) and serum alkaline phosphatase (HR 1.05 [95%CI1.02 – 1.08], p=0.002). Females had an 85% lower risk of developing IFG, while one unit higher waist circumference and serum alkaline phosphatase were associated with a 9% and 5% greater risk, respectively. (Table 6.44)

**Table 6.23: Baseline characteristics of those developing impaired fasting glucose vs. group with no impaired fasting glucose during 24 months follow up in group 3 subjects on ART***

| Variable | Developing impaired fasting glucose, n=7 | No impaired fasting glucose, n=142 | p | Univariate analysis HR(95% CI) | [#]p |
|---|---|---|---|---|---|
| Gender | | | | | |
| Male | 4(57.1) | 43(30.3) | 0.14 | 3.15(0.70-14.07) | 0.13 |
| Female | 3 (42.9) | 99(69.7) | | 0.32(0.07-1.42) | 0.13 |
| Treatment allocation | | | | | |
| Efavirenz | 6(85.7) | 69(48.6) | 0.06 | 6.76(0.81-56.11)) | 0.08 |
| Nevirapine | 1(14.3) | 73(51.4) | 0.06 | 0.15(0.02-1.23) | 0.08 |
| BMI | | | | | |
| >30 kg/m$^2$ | 1(16.7) | 37(27.2) | 0.57 | 0.50(0.06-4.26) | 0.52 |
| Familial DM | 4(57.1) | 30(22.2) | 0.03 | 4.82(1.51-20.17) | 0.2 |
| Smoking | 2(28.6) | 17(12.6) | 0.41 | 2.66(0.52-13.72) | 0.2 |
| Occupational physical activity | | | 0.8 | | |
| Sedentary | 0(0.0) | 4(3.3) | | NE | 1.00 |
| Light | 3(50.0) | 54(44.3) | | NE | 1.00 |
| Moderate | 1(16.7) | 41(33.6) | | NE | 1.00 |
| Heavy | 2(33.3) | 22(18.0) | | NE | 1.00 |
| Leisure physical activity | | | 0.5 | | |
| Sedentary | 3(42.9) | 74(56.9) | | 1 | |
| Light | 1(14.3) | 15(11.5) | | 1.45(0.15-1.98) | 0.75 |
| Moderate | 0(0.0) | 14(10.8) | | NE | 1.00 |
| Heavy | 3(42.9) | 27(20.8) | | 3.47(0.70-17.17) | 0.13 |

Data are n(%), BMI: body mass index, DM: diabetes mellitus, HR: hazard ratio, CI: confidence interval. p: chi-square test for differences between the two groups, [#]p: differences at univariate level using Cox proportional hazards. NE: Not estimatable

**Table 6.24: Baseline clinical characteristics of the group developing impaired fasting glucose vs. group with no impaired fasting glucose during 24 months follow up in group 3 subjects on ART***

| Variable | Impaired fasting glucose | | No impaired fastingglucose | | p | Univariate analysis | #p |
|---|---|---|---|---|---|---|---|
| | n | Mean+SD | n | Mean+SD | | HR(95% CI) | |
| Age | 7 | 41.0+6.9 | 142 | 36.6+9.1 | 0.2 | 1.1(0.99-1.13) | 0.08 |
| Blood pressure (mmHg) | | | | | | | |
| Systolic | 6 | 116.25+14.0 | 136 | 111.4+16.2 | 0.5 | 1.01(0.97-1.1) | 0.7 |
| Diastolic | 6 | 69.5+8.0 | 135 | 70.7+10.5 | 0.8 | 0.98(0.91-1.1) | 0.7 |
| **Anthropometric measurements** | | | | | | | |
| Weight (kg) | 7 | 67.91.7+13.4 | 141 | 68.95+15.97 | 0.9 | 1.01(0.97-1.05) | 0.6 |
| Body mass index (kg/m$^2$) | 7 | 24.6+6.1 | 141 | 26.2+6.2 | 0.5 | 0.94(0.82-1.1) | 0.4 |
| **Circumference (cm)** | | | | | | | |
| Waist | 7 | 106.7+20.5 | 134 | 91.5+16.5 | 0.02 | 1.06(1.012-1.11) | 0.004 |
| Hip | 7 | 112.2+13.4 | 134 | 105.8+14.7 | 0.3 | 1.04(0.99-1.09) | 0.1 |
| Waist: hip ratio | 7 | 0.94+0.1 | 133 | 0.86+0.1 | 0.01 | NE | - |
| Waist: height ratio | 6 | 64.0+15.4 | 132 | 56.5+10.8 | 0.1 | 1.1(0.99-1.1) | 0.07 |
| Mid-arm | 7 | 35.7+5.9 | 135 | 32.5+4.9 | 0.09 | 1.1(0.99-1.3) | 0.01 |
| Neck | 7 | 37.3+3.8 | 135 | 35.1+3.4 | 0.1 | 1.2(1.00-1.5) | 0.05 |
| Chest | 7 | 102.4+11.8 | 134 | 94.2+10.3 | 0.04 | 1.1(1.01-1.2) | 0.03 |
| Mid-thigh | 7 | 56.97+15.98 | 131 | 56.3+9.92 | 0.9 | 1.03(0.97-1.10) | 0.4 |
| **Skin folds** | | | | | | | |
| Triceps | 7 | 27.6+11.5 | 134 | 22.95+13.2 | 0.4 | 1.05(1.01-1.09) | 0.07 |
| Sub-scapular | 7 | 27.1+20.0 | 133 | 18.7+11.2 | 0.07 | 1.06(1.01-1.09) | 0.02 |
| Abdomen | 7 | 36.3+18.7 | 133 | 24.4+13.6 | 0.03 | 1.05(1.00-1.1) | 0.03 |
| Mid-thigh | 7 | 33.9+17.6 | 132 | 32.6+17.1 | 0.8 | 1.01(0.97-1.1) | 0.7 |

Data are mean + SD.IFG: impaired fasting glucose. HR: Hazard ratio, CI: Confidence interval.*7 subjects developed IFG and 142 did not develop IFG; n= number for which data was available for each variable.p: Student's t-test for differences between the two groups, #p: differences at univariate level using Cox proportional hazards. NE: Not estimatable.

**Table 6.25: Baseline laboratory characteristics of those developing impaired fasting glucose vs. group with no impaired fasting glucose during 24 month follow up in group 3 subjects on ART***

| Variable | Impaired fasting glucose | | No impaired fasting glucose | | p | Univariate analysis | #p |
|---|---|---|---|---|---|---|---|
| | n | Mean+SD | n | Mean±SD | | HR(95% CI) | |
| **Plasma glucose (mmol/l)** | | | | | | | |
| 0 hour | 7 | 4.71±0.5 | 136 | 4.8±0.5 | 0.7 | 0.5(0.08-3.7) | 0.5 |
| 2 hour | 7 | 4.8±1.4 | 127 | 5.2±1.1 | 0.4 | 0.8(0.4-1.6) | 0.5 |
| HbA$_{1c}$ | 7 | 3.7±1.1 | 126 | 4.0±0.7 | 0.3 | 0.5(0.2-1.5) | 0.2 |
| **Serum lipids (mmol/l)** | | | | | | | |
| Total cholesterol | 7 | 2.95±0.59 | 134 | 3.51±0.89 | 0.1 | 0.4(0.1-1.2) | 0.4 |
| Total triglycerides | 7 | 0.98±0.36 | 132 | 0.97±0.59 | 0.9 | 0.99(0.3-3.3) | 0.99 |
| LDL | 6 | 1.71±0.69 | 119 | 2.24±0.74 | 0.0 | 0.4(0.1-1.3) | 0.1 |
| HDL | 7 | 0.71±0.15 | 131 | 0.83±0.29 | 0.3 | 0.2(0.0-4.3) | 0.3 |
| **HIV Parameters** | | | | | | | |
| CD4 cell count(cells/mm3 ) | 7 | 119.4±126.2 | 139 | 140.5±86.2 | 0.5 | 0.6(0.3-1.0) | 0.06 |
| HIV RNA, copies/ml | 6 | 3.9±1.6 | 123 | 4.8±0.9 | 0.0 | 0.7(0.4-1.2) | 0.2 |
| **Full blood count** | | | | | | | |
| Haemoglobin (g/dL) | 7 | 10.6±2.6 | 141 | 11.1±1.9 | 0.5 | 0.8(0.5-1.3) | 0.4 |
| Platelet count (x10$^9$/L) | 7 | 182.2±89.9 | 141 | 253.91±79.8 | 0.0 | 0.99(0.98-0.99) | 0.02 |
| White cell count (x10$^9$/L) | 7 | 4.0±1.8 | 141 | 4.5±1.7 | 0.5 | 0.9(0.5-1.5) | 0.6 |
| Lymphocytes (x10$^9$/L) | 7 | 1.3±0.9 | 141 | 2.0±4.2 | 0.7 | 0.7(0.3-1.9) | 0.5 |

Data are mean ± SD. IFG: impaired fasting glucose. HR: Hazard ratio, CI: Confidence interval.LDL: low density lipoprotein, HDL: high density lipoprotein. *7 subjects developed IFG and 142 did not develop IFG; n= number for which data was available for each variable.p: Student's t-test for differences between the two groups, #p: differences at univariate level using Cox proportional hazards

**Table 6.26: Baseline laboratory characteristics of those developing impaired fasting glucose vs. group with no impaired fasting glucose during 24 months follow up in group 3 subjects on ART\***

| Variable | Impaired fasting glucose | | No impaired fasting glucose | | p | Univariate analysis | [#]p |
|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | | HR(95% CI) | |
| **Renal function** | | | | | | | |
| Bicarbonate (mmol/l) | 7 | 26.7±1.5 | 140 | 24.5±2.5 | 0.02 | 1.6(1.0-2.3) | 0.03 |
| Chloride (mmol/l) | 7 | 105.0±3.4 | 140 | 104.5±3.94 | 0.7 | 1.00(0.8-1.2) | 0.9 |
| Urea (mmol/l) | 7 | 3.0±1.6 | 140 | 3.5±1.3 | 0.3 | 0.7(0.3-1.4) | 0.3 |
| Creatinine (mmol/l) | 7 | 62.7±19.9 | 140 | 69.2±18.6 | 0.4 | 0.98(0.9-1.0) | 0.4 |
| Anion gap | 7 | 10.7±2.8 | 140 | 11.3±3.1 | 0.6 | 0.95(0.7-1.2) | 0.7 |
| Calcium (mmol/l) | 6 | 2.2±0.2 | 136 | 2.2±0.1 | 0.4 | 0.03(0.24.8) | 0.3 |
| Magnesium (mmol/l) | 6 | 0.8±0.1 | 133 | 0.9±0.9 | 0.5 | 0.0(0.0-289.9) | 0.3 |
| Phosphate (mmo/l) | 6 | 1.1±0.0 | 134 | 1.1±0.2 | 0.9 | 0.9(0.0-65.3) | 0.9 |
| **Liver function** | | | | | | | |
| Total protein (g/L) | 7 | 85.7±7.9 | 139 | 86.8±9.92 | 0.8 | 0.98(0.9-1.1) | 0.6 |
| Albumin (g/L) | 7 | 34.1±3.7 | 139 | 34.4±5.7 | 0.9 | 0.98(0.9-1.1) | 0.8 |
| Globulin (g/L) | 7 | 51.6±9.3 | 139 | 52.4±12.0 | 0.9 | 0.99(0.93-1.1) | 0.8 |
| Total bilirubin | 7 | 6.3±4.7 | 140 | 7.93±4.9 | 0.4 | 0.89(0.7-1.1) | 0.4 |
| ALT (U/l) | 7 | 22.3±6.1 | 138 | 26.8±19.3 | 0.5 | 0.99(0.93-1.0) | 0.6 |
| ALP | 7 | 87.6±46.8 | 139 | 65.8±28.1 | 0.06 | 1.02(1.01-1.05) | 0.01 |
| GGT | 7 | 39.0±40.3 | 139 | 30.2±31.9 | 0.5 | 1.0(0.99-1.0) | 0.06 |

Data are mean ± SD.IFG: impaired fasting glucose,HR: Hazard ratio, CI: Confidence interval.*7 subjects developed impaired fasting glucose (IFG) and 142 did not develop IFG; n= number for which data was available for each variable.p: Student's t-test for differences between the two groups, [#]p: differences at univariate level using Cox proportional hazards,ALT: serum alanine transaminase,ALP: serum alkaline phosphatase,GGT: serum gamma glutamyl transferase

**Table 6.27: Baseline laboratory characteristics of those developing impaired fasting glucose vs. group with no impaired fasting glucose during 24 months follow up in group 3 subjects on ART***

| Variable | Impaired fasting glucose | | No impaired fasting glucose | | p | Univariate analysis | #p |
|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | | HR(95% CI) | |
| **Inflammatory Markers** | | | | | | | |
| CRP (mg/L) | 6 | 11.3±10.5 | 129 | 19.99±37.1 | 0.6 | 0.99(0.96-1.0) | 0.8 |
| Lactate (mmol/l) | 6 | 1.4±0.6 | 129 | 1.5±0.8 | 0.6 | 0.7(0.2-2.3) | 0.6 |
| Uric acid (mmol/L) | 4 | 0.2±0.1 | 106 | 0.29±0.1 | 0.2 | 0.0(0.0-17.8) | 0.1 |
| Cortisol (nmol/L) | 7 | 315.1±116.7 | 123 | 302.8±131.1 | 0.8 | 1.0(0.99-1.0) | 0.4 |
| Iron (umol/L) | 6 | 8.9±4.5 | 125 | 10.2±5.0 | 0.5 | 0.9(0.8-1.1) | 0.4 |
| Transferrin (g/L) | 6 | 2.4±0.7 | 113 | 2.2±0.6 | 0.5 | 1.8(0.4-7.6) | 0.4 |
| Saturation (%) | 4 | 21.3±9.5 | 85 | 17.91±9.1 | 0.5 | 1.0(1.0-1.0) | 0.3 |
| Ferritin (ug/L) | 5 | 327.6±519.8 | 109 | 145.3±162.4 | 0.04 | 1.0(0.9-1.1) | 0.7 |

Data are mean ± SD. IFG: impaired fasting glucose. HR: Hazard ratio, CI: Confidence interval. BP: *7 subjects developed IFG and 142 did not develop IFG; n= number for which data was available for each variable.p: Student's t-test for differences between the two groups, #p: differences at univariate level using Cox proportional hazards

**Table 6.28: BaselineDXA scan of the group developing impaired fasting glucose vs. group with no impaired fasting glucose during 24 months follow up in group 3 subjects on ART***

| Variable | Impaired fasting glucose | | No impaired fasting glucose | | p | Univariate analysis | #p |
|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | | HR(95% CI) | |
| **Body composition, DXA (kg)** | | | | | | | |
| Body cell mass | 6 | 68351.5±17296.9 | 130 | 68039.0±15600.6 | 0.96 | 1.0(1.0-1.0) | 0.98 |
| **Fat** | | | | | | | |
| Total | 6 | 18167.7±14194.1 | 130 | 21419.3±12129.6 | 0.5 | 1.0(1.0-1.0) | 0.5 |
| Left arm | 6 | 1154.4±987.2 | 130 | 1247.1±746.93 | 0.8 | 1.0(0.99-1.0) | 0.7 |
| Right arm | 6 | 1107.5±997.6 | 130 | 1236.3±723.6 | 0.7 | 1.0(0.99-1.0) | 0.6 |
| Left leg | 6 | 3797.5±2626.1 | 130 | 4698.3±2538.3 | 0.4 | 1.0(0.99-1.0) | 0.3 |
| Right leg | 6 | 3906.3±2664.1 | 130 | 4845.9±2674.7 | 0.4 | 1.0(0.99-1.0) | 0.3 |
| Trunk | 6 | 7279.9±6982.6 | 130 | 8578.3±5808.7 | 0.6 | 1.0(1.0-1.0) | 0.6 |
| **Lean** | | | | | | | |
| Total | 6 | 50183.8±10052.1 | 130 | 46619.7±9015.4 | 0.3 | 1.0(0.99-1.0) | 0.4 |
| Left arm | 6 | 2789.0±606.3 | 130 | 2520.1±740.9 | 0.4 | 1.0(0.99-1.0) | 0.4 |
| Right arm | 6 | 3035.5±618.6 | 130 | 2648.0±757.4 | 0.2 | 1.0(1.0-1.0) | 0.3 |
| Left leg | 6 | 8263.2±1624.9 | 130 | 7879.7±1815.8 | 0.6 | 1.0(1.0-1.0) | 0.7 |
| Right leg | 6 | 7960.7±1349.1 | 130 | 7957.7±1814.4 | 0.99 | 1.0(1.0-1.0) | 0.96 |
| Trunk | 6 | 24360.6±5785.9 | 130 | 22017.4±3991.7 | 0.2 | 1.0(1.0-1.0) | 0.2 |
| Total BMD | 6 | 1.2±0.1 | 130 | 1.1±0.1 | 0.02 | 756.8(0.94-607181.5) | 0.05 |

Data are mean ± SD. IFG: impaired fasting glucose. HR: Hazard ratio, CI: Confidence interval.*7 subjects developed IFG and 142 did not develop IFG; n= number for which data was available for each variable.p: Student's t-test for differences between the two groups, #p: differences at univariate level using Cox proportional hazards

**Table 6.29: BaselineCT scan findings of the group developing impaired fasting glucose vs. group with no impaired fasting glucose during 24 month follow up in group 3 subjects on ART\***

| Variable | Impaired fasting glucose | | No impaired fasting glucose | | p | Univariate analysis | [#]p |
|---|---|---|---|---|---|---|---|
| | n | Mean$\pm$SD | n | Mean$\pm$SD | | HR(95%CI) | |
| **Fat distribution, CT scan** | | | | | | | |
| Total fat area | 6 | 213.4$\pm$200.9 | 116 | 295.6$\pm$208.5 | 0.3 | 0.99(0.99-1.0) | 0.4 |
| Visceral fat area | 6 | 56.9$\pm$61.4 | 116 | 59.3$\pm$39.5 | 0.9 | 1.00(0.98-1.02) | 0.96 |
| Visceral: subcutaneous fat ratio | 6 | 0.4$\pm$0.3 | 116 | 0.5$\pm$0.7 | 0.9 | 1.2(0.6-2.5) | 0.7 |
| Subcutaneous fat area | 6 | 156.5$\pm$145.8 | 116 | 236.2$\pm$178.7 | 0.3 | 0.99(0.99-1.0) | 0.3 |
| Waist size | 6 | 88.1$\pm$14.3 | 113 | 94.4$\pm$17.2 | 0.4 | 0.98(0.93-1.0) | 0.4 |

Data are mean $\pm$ SD. IFG: impaired fasting glucose. HR: Hazard ratio, CI: Confidence interval. [*]7 subjects developed IFG and 142 did not develop IFG; n= number for which data was available for each variable.p: Student's t-test for differences between the two groups, [#]p: differences at univariate level using Cox proportional hazards

### 6.3.4. Risk factors for impaired glucose regulation (IGR)/Pre-diabetes

Tables 6.30 – 6.36 shows univariate analysis and baseline characteristics of subjects who did and those who did not develop impaired glucose regulation (IGR)/Pre-diabetes. When compared with the group that did not develop IGR, subjects who developed IGR had higher systolic blood pressure (p=0.03), waist: hip ratio (p=0.03), lower log HIV RNA (p=0.03) and platelet count (p=0.02). No significant difference was observed between the two groups for any other demographic, clinical, laboratory or radiological variables (Table 6.30 – 6.36).

In univariate analysis, variables that were significantly associated with development of IGR were serum alkaline phosphatase (p=0.04), GGT (p=0.02), platelet count (p=0.04) and log HIV RNA (p=0.03) (Tables 6.30 – 6.36).

In multivariate analysis,variables that predicted development of IGR were gender (HR 0.24 [95%CI 0.07-0.88],p=0.03) and serum albumin (HR 0.85[95%CI0.76-0.96], p=0.01). Females had a 76% lower risk of developing IGR and one unit higher serum albumin was associated with a 15% lower risk (Table 6.44)

**Table 6.30: Baseline demographic characteristics of those developing impaired glucose regulation (IGT or IFG) vs. not developing impaired glucose regulation during 24 months follow up in group 3 subjects on ART***

| Variable | Developing IGT or IFG, n=13 | No IGT or IFG, n=137 | p | HR(95% CI) | #p |
|---|---|---|---|---|---|
| Gender | | | | | |
| Male | 7(53.9) | 41(29.9) | 0.08 | 2.71(0.91-8.07) | 0.07 |
| Female | 6(46.2) | 96(70.1) | | 0.37(0.12-1.10) | 0.07 |
| Treatment allocation | | | | | |
| Efavirenz | 9(69.2) | 67(48.9) | 0.2 | 2.56(0.80-8.32) | 0.1 |
| Nevirapine | 4(30.8) | 70(51.1) | 0.2 | 0.39(0.12-1.27) | 0.1 |
| BMI | | | | | |
| >30 kg/m$^2$ | 2(16.7) | 37(28.2) | 0.4 | 0.50(0.11-2.29) | 0.4 |
| Familial DM | 6(46.2) | 29(22.3) | 0.06 | 2.32(0.92-5.88) | 0.1 |
| Smoker | 3(23.1) | 16(12.3) | 0.2 | 1.65(0.47-5.87) | 0.01 |
| Occupational physical activity | | | 0.7 | | |
| Sedentary | 0(0.0) | 4(3.4) | | NE | 1.00 |
| Light | 5(41.7) | 53(45.3) | | NE | 1.00 |
| Moderate | 5(41.7) | 37(31.6) | | NE | 1.00 |
| Heavy | 2(16.7) | 22(18.8) | | NE | 1.00 |

Continued on next page

**Table 6.30cont.: Baseline demographic characteristics of those developing impaired glucose regulation (IGT or IFG) vs. not developing impaired glucose regulation during 24 months follow up in group 3 subjects on ART\***

| Variable | Developing IGT or IFG, n=13 | No IGT or IFG, n=137 | p | HR(95% CI) | #p |
|---|---|---|---|---|---|
| Leisure physical activity | | | | | |
| Sedentary | 7(53.9) | 70(56.0) | 0.8 | | |
| Light | 1(7.7) | 16(12.8) | | 0.59(0.07-4.82) | 0.6 |
| Moderate | 1(7.7) | 13(10.4) | | 0.73(0.07-5.98) | 0.8 |
| Heavy | 4(30.8) | 26(20.8) | | 1.99(0.57-6.79) | 0.3 |

Data are n(%), BMI: body mass index, DM: diabetes mellitus, HR: hazard ratio, CI: confidence interval. p: chi-square test for differences between the two groups, #p: differences at univariate level using Cox proportional hazards. NE: not estimable.

**Table 6.31: Baseline clinical characteristics of those developing impaired glucose regulation (IGT or IFG) vs. not developing impaired glucose regulation during 24 months follow up in group 3 subjects on ART***

| Variable | IGT or IFG | | No IGT or IFG | | p | Univariate analysis | #p |
|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | | HR (95%CI) | |
| Age | 13 | 41.0±6.9 | 137 | 36.6±9.1 | 0.2 | 1.03(0.99-1.095 | 0.4 |
| Blood pressure (mmHg) | | | | | | | |
| Systolic | 12 | 121.96±19.2 | 131 | 111.1±16.3 | 0.03 | 1.02(0.99-1.05) | 0.1 |
| Diastolic | 11 | 75.0±11.5 | 131 | 70.6±10.6 | 0.2 | 1.03(0.98-1.08) | 0.3 |
| **Anthropometric measurements** | | | | | | | |
| Weight (kg) | 13 | 66.3±13.0 | 136 | 69.4±16.3 | 0.5 | 0.99(0.97-1.03) | 0.7 |
| Body mass index (kg/m$^2$) | 13 | 24.9±6.1 | 136 | 26.4±6.3 | 0.4 | 0.95(0.86-1.07) | 0.4 |
| Circumference (cm): | | | | | | | |
| Waist | 13 | 95.8±19.8 | 129 | 92.2±16.0 | 0.5 | 1.02(0.99-1.05) | 0.3 |
| Hip | 13 | 103.96±14.6 | 129 | 106.4±14.8 | 0.6 | 0.99(0.96-1.04) | 0.8 |
| Waist: Hip ratio | 13 | 0.91±0.1 | 128 | 0.86±0.1 | 0.03 | NE | 0.1 |
| Waist: Height ratio | 12 | 58.0±12.6 | 127 | 56.9±11.1 | 0.7 | 1.02(0.97-1.07) | 0.5 |
| Mid-arm | 13 | 32.1±6.4 | 130 | 32.8±4.9 | 0.7 | 0.99(0.88-1.1) | 0.9 |
| Neck | 13 | 35.9±3.2 | 130 | 35.2±3.6 | 0.5 | 1.06(0.91-1.2) | 0.4 |
| Chest | 12 | 95.2±12.7 | 130 | 94.8±10.4 | 0.9 | 1.01(0.96-1.07) | 0.7 |
| Mid-thigh | 12 | 52.8±13.5 | 127 | 56.8±9.90 | 0.2 | 0.96(0.89-1.03) | 0.2 |
| **Skin folds** | | | | | | | |
| Triceps | 13 | 21.08+11.8 | 129 | 23.6+13.4 | 0.5 | 0.99(0.95-1.04) | 0.8 |
| Sub-scapular | 13 | 19.7+16.7 | 128 | 19.3+11.5 | 0.9 | 1.01(0.96-1.05) | 0.8 |
| Abdomen | 13 | 25.9+18.2 | 128 | 25.1+13.8 | 0.9 | 1.01(0.97-1.05) | 0.7 |
| Mid-thigh | 13 | 28.5+16.1 | 127 | 33.3+17.2 | 0.3 | 0.99(0.96-1.02) | 0.5 |

Data are mean ± SD. IGT: impaired glucose tolerance, IFG: impaired fasting glucose. HR: Hazard ratio, CI: Confidence interval.* 13 subjects developed impaired glucose regulation (IGT or IFG) and 137 did not develop impaired glucose regulation; n= number for which data was available for each variable. p: Student's t-test for differences between the two groups, #p: differences at univariate level using Cox proportional hazards. NE: Not estimatable

**Table 6.32: Baseline laboratory characteristics of those developing impaired glucose regulation (IGT or IFG) vs. not developing impaired glucose regulation during 24 months follow up in group 3 subjects on ART***

| Variable [#]p | IGT or IFG | | No IGT or IFG | | p | Univariate analysis | [#]p |
|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | | HR(95%CI) | |
| **Plasma glucose (mmol/l)** | | | | | | | |
| 0 hour | 13 | 4.8±0.4 | 131 | 4.8±0.5 | 0.8 | 0.99(03-3.4) | 0.98 |
| 2 hour | 13 | 5.0±1.3 | 122 | 5.2±1.1 | 0.6 | 0.89(0.5-1.5) | 0.7 |
| HbA$_{1c}$ | 13 | 3.9±0.8 | 121 | 3.99±0.7 | 0.6 | 0.71(0.3-1.6) | 0.4 |
| **Serum lipids (mmol/l)** | | | | | | | |
| Total cholesterol | 12 | 3.24±0.75 | 130 | 3.52±0.90 | 0.3 | 0.71(0.4-1.4) | 0.3 |
| Total triglycerides | 12 | 0.98±0.35 | 128 | 0.97±0.59 | 0.97 | 0.96(0.4-2.4) | 0.9 |
| LDL | 11 | 2.08±0.86 | 115 | 2.24±0.74 | 0.1 | 0.79(0.4-1.8) | 0.6 |
| HDL | 12 | 0.69±0.23 | 127 | 0.83±0.29 | 0.5 | 0.1(0.01-1.5) | 0.1 |
| **HIV Parameters** | | | | | | | |
| CD4+ cell count (cells/mm$^3$ ) | 13 | 103.1±96.1 | 134 | 143.2±86.5 | 0.1 | 0.99(0.99-1.00) | 0.08 |
| HIV RNA, copies/ml | 11 | 4.2±1.7 | 119 | 4.8±0.8 | 0.03 | 0.54(0.3-0.95) | 0.03 |
| **Full blood count** | | | | | | | |
| Haemoglobin (g/dL) | 13 | 10.8±2.2 | 136 | 11.2±1.95 | 0.5 | 0.9(0.64-1.02) | 0.3 |
| Platelet count (x10$^9$/L) | 13 | 215.5±118.6 | 136 | 252.8±77.5 | 0.02 | 0.99(0.99-1.00) | 0.04 |
| White cell count (x10$^9$/L) | 13 | 4.3±1.7 | 136 | 4.5±1.7 | 0.1 | 0.99(0.7-1.4) | 0.9 |
| Lymphocytes (x10$^9$/L | 13 | 1.3±0.9 | 136 | 2.1±4.3 | 0.5 | 0.61(0.31-1.18) | 0.1 |

Data are mean ± SD. IGT: impaired glucose tolerance, IFG: impaired fasting glucose. HR: Hazard ratio, CI: Confidence interval.[*] 13 subjects developed impaired glucose regulation (IGT or IFG) and 137 did not develop impaired glucose regulation; n= number for which data was available for each variable. p: Student's t-test for differences between the two groups, [#]p: differences at univariate level using Cox proportional hazards

**Table 6.33: Baseline laboratory characteristics of those developing impaired glucose regulation (IGT or IFG) vs. not developing impaired glucose regulation during 24 months follow up in group 3 subjects on ART***

| Variable | IGTor IFG | | No IGT or IFG | | p | Univariate analysis | #p |
| | n | Mean±SD | n | Mean±SD | | HR(95%CI) | |
|---|---|---|---|---|---|---|---|
| **Renal Function** | | | | | | | |
| Bicarbonate (mmol/l) | 13 | 25.2±2.3 | 135 | 24.5±2.5 | 0.3 | 1.1(0.85-1.4) | 0.5 |
| Chloride (mmol/l) | 13 | 104.4±4.2 | 135 | 104.5±3.9 | 0.9 | 0.96(0.83-1.1) | 0.6 |
| Urea (mmol/l) | 13 | 3.4±1.5 | 135 | 3.5±1.3 | 0.3 | 1.1(0.85-1.4) | 0.5 |
| Creatinine (mmol/l) | 13 | 66.8±19.2 | 135 | 69.3±18.8 | 0.9 | 0.96(0.83-1.1) | 0.6 |
| Anion gap | 13 | 11.1±2.6 | 135 | 11.3±3.2 | 0.7 | 0.90(0.58-1.4) | 0.6 |
| Calcium (mmol/l) | 12 | 2.2±0.1 | 131 | 2.2±0.1 | 0.7 | 0.99(0.97-1.0) | 0.9 |
| Magnesium (mmol/l) | 12 | 0.98±0.4 | 128 | 0.9±0.2 | 0.8 | 0.98(0.83-1.2) | 0.8 |
| Phosphate (mmo/l) | 12 | 1.1±0.2 | 129 | 1.1±0.2 | 0.2 | 0.02(0.00-1.9) | 0.09 |
| **Liver function** | | | | | | | |
| Total protein (g/L) | 13 | 85.9±7.3 | 134 | 86.8±10.0 | 0.2 | 2.4(0.54-10.4) | |
| Albumin (g/L) | 13 | 32.8±5.1 | 134 | 34.5±5.6 | 0.9 | 1.2(0.06-22.2) | |
| Globulin (g/L) | 13 | 53.01±8.5 | 134 | 52.2±12.2 | 0.8 | 0.98(0.93-1.04) | 0.6 |
| Total bilirubin | 13 | 6.2±3.7 | 135 | 8.0±4.9 | 0.3 | 0.93(0.85-1.02) | 0.1 |
| Alanine amino transferase (U/l) | 13 | 32.3±29.2 | 133 | 26.0±17.6 | 0.8 | 1.00(0.96-1.05) | 0.9 |
| ALP | 13 | 74.8±38.9 | 134 | 66.2±28.2 | 0.2 | 0.9(0.75-1.07) | 0.2 |
| GGT | 13 | 36.8±34.8 | 134 | 30.1±31.9 | 0.3 | 1.02(0.99-1.04) | 0.1 |

Data are mean + SD. IGT: impaired glucose tolerance, IFG: impaired fasting glucose. HR: Hazard ratio, CI: Confidence interval.* 13 subjects developed impaired glucose regulation (IGT or IFG) and 137 did not develop impaired glucose regulation; n= number for which data was available for each variable. p: Student's t-test for differences between the two groups, #p: differences at univariate level using Cox proportional hazards

**Table 6.34: Baseline laboratory characteristics (inflammatory markers) of group developing impaired glucose regulation (IGT or IFG) vs. group not developing impaired glucose regulation during 24 months follow up in group 3 subjects on ART***

| Variable | IGT or IFG | | No IGT or IFG | | P | Univariate analysis | [#]p |
|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | | HR(95% CI) | |
| CRP (mg/L) | 12 | 16.8±28.0 | 124 | 19.8±37.0 | 0.8 | 1.01(0.99-1.02) | 0.6 |
| Lactate (mmol/l) | 11 | 1.4±0.6 | 125 | 1.5±0.8 | 0.5 | 0.72(0.30-1.7) | 0.5 |
| Uric acid (mmol/L) | 8 | 0.3±0.1 | 102 | 0.3±0.1 | 0.7 | 0.15(0.00-807.9) | 0.7 |
| Cortisol (nmol/L) | 13 | 328.2±182.3 | 118 | 302.9±125.3 | 0.5 | 1.00(1.00-1.01) | 0.07 |
| Iron (umol/L) | 11 | 9.5±4.5 | 121 | 10.3±5.1 | 0.6 | 0.95(0.84-1.09) | 0.5 |
| Transferrin (g/L) | 10 | 2.3±0.6 | 110 | 2.2±0.6 | 0.6 | 1.5(0.5-4.96) | 0.5 |
| Saturation (%) | 10 | 16.2±8.6 | 81 | 18.1±9.1 | 0.9 | 0.98(0.91-1.07) | 0.7 |
| Ferritin (ug/L) | 10 | 209.5±373.9 | 105 | 148.8±164.2 | 0.3 | 1.00(0.99-1.00) | 0.2 |

Data are mean ± SD. IGT: impaired glucose tolerance, IFG: impaired fasting glucose. HR: Hazard ratio, CI: Confidence interval.[*] 13 subjects developed impaired glucose regulation (IGT or IFG) and 137 did not develop impaired glucose regulation; n= number for which data was available for each variable. p: Student's t-test for differences between the two groups, [#]p: differences at univariate level using Cox proportional hazards

**Table 6.35: Baseline DXA scan findings of group developing impaired glucose regulation (IGT or IFG) vs. group not developing impaired glucose regulation during 24 months follow up in group 3 subjects on ART***

| Variable | IGT or IFG | | No IGT or IFG | | p | Univariate analysis | #p |
|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | | HR(95%CI) | |
| Total mass | 12 | 64867.75±14960.55 | 125 | 68600.8±15859.3 | 0.4 | 1.00(1.00-1.00) | 0.4 |
| **Fat (g)** | | | | | | | |
| Total | 12 | 16988.22±12680.28 | 125 | 21739.1±12072.7 | 0.2 | 1.00(1.00-1.00) | 0.2 |
| Left arm | 12 | 1120.3±927.2 | 125 | 1255.5±736.4 | 0.6 | 1.00(0.99-1.00) | 0.5 |
| Right arm | 12 | 1061.8±878.6 | 125 | 1248.9±717.3 | 0.4 | 1.00(0.99-1.00) | 0.4 |
| Left leg | 12 | 3383.2±2378.8 | 125 | 4784.3±2518.8 | 0.1 | 1.00(0.99-1.00) | 0.05 |
| Right leg | 12 | 3459.1±2471.9 | 125 | 4935.8±2652.5 | 0.1 | 1.00(.099-1.00) | 0.05 |
| Trunk | 12 | 7134.9±6106.7 | 125 | 8693.4±5815.2 | 0.4 | 1.00(1.00-1.00) | 0.4 |
| **Lean (g)** | | | | | | | |
| Total | 12 | 47879.5±7623.9 | 125 | 46861.7±9408.2 | 0.7 | 1.00(1.00-1.00) | 0.8 |
| Left arm | 12 | 2703.4±536.9 | 125 | 2530.3±766.9 | 0.4 | 1.00(1.00-1.00) | 0.5 |
| Right arm | 12 | 2904.3±519.9 | 125 | 2657.4±786.8 | 0.3 | 1.00(1.00-1.00) | 0.4 |
| Left leg | 12 | 7871.2±1336.8 | 125 | 7932.2±1876.6 | 0.9 | 1.00(1.00-1.00) | 0.9 |
| Right leg | 12 | 7777.5±1107.5 | 125 | 8009.2±1878.9 | 0.7 | 1.00(1.00-1.00) | 0.6 |
| Trunk | 12 | 23060.1±4234.7 | 125 | 22109.8±4158.6 | 0.7 | 1.00(1.00-1.00) | 0.5 |
| Total BMD | 12 | 1.2±0.1 | 125 | 1.1±0.1 | 0.1 | 37.3(0.254-5490.8) | 0.2 |

Data are mean ± SD. IGT: impaired glucose tolerance, IFG: impaired fasting glucose. HR: Hazard ratio, CI: Confidence interval.* 13 subjects developed impaired glucose regulation (IGT or IFG) and 137 did not develop impaired glucose regulation; n= number for which data was available for each variable. p: Student's t-test for differences between the two groups, #p: differences at univariate level using Cox proportional hazards

**Table 6.36: Baseline CT scan findings of group developing impaired glucose regulation (IGT or IFG) vs. group not developing impaired glucose regulation during 24 months follow up in group 3 subjects on ART***

| [#]Variable | IGT or IFG | | No IGT or IFG | | p | Univariate analysis | [#]p |
|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | | HR(95%CI) | |
| **Fat distribution, CT scan** | | | | | | | |
| Total fat area | 11 | 241.6±197.2 | 112 | 299.7±211.0 | 0.4 | 0.99(0.99-1.00) | 0.4 |
| Visceral fat area | 11 | 56.5±47.2 | 112 | 62.2±49.6 | 0.7 | 0.99(0.99-1.01) | 0.8 |
| Visceral: subcutaneous fat | 11 | 0.8±1.2 | 112 | 0.4±0.5 | 0.1 | 1.5(0.93-2.3) | 0.09 |
| Subcutaneous fat area | 11 | 185.1±159.7 | 112 | 237.3±178.4 | 0.4 | 0.99(0.99-1.00) | 0.4 |
| Waist size | 11 | 92.2±15.1 | 109 | 94.6±17.6 | 0.7 | 0.99(0.96-1.03) | 0.7 |

Data are mean ± SD. IGT: impaired glucose tolerance, IFG: impaired fasting glucose. HR: Hazard ratio, CI: Confidence interval.[*] 13 subjects developed hyperglycaemia (IGT or IFG) and 137 did not develop hyperglycaemia; n= number for which data was available for each variable. p: Student's t-test for differences between the two groups, [#]p: differences at univariate level using Cox proportional hazards

## 6.3.5. Risk factors for any disorder of glycaemia (Diabetes Mellitus or impaired glucose tolerance or impaired fasting glucose)

Table 6.37 – 6.43 shows univariate analysis and baseline characteristics of subjects who did and who did not develop any disorder of glycaemia/ dysglycaemia (DM or IGT or IFG). When compared with the group that did not develop dysglycaemia, the group that developed dysglycaemia had the following: more males (p=0.03) and more exposure to efavirenz (p=0.04), higher systolic (p=0.003) and diastolic (p=0.01) blood pressure,visceral:subcutaneous fat ratio (p=0.0006) on CT scan,with lower serum HDL-cholesterol (p=0.02), left (p=0.04) and right (p=0.04) leg fat area on DXA scan. No significant difference was observed between the two groups for any other demographic, clinical, laboratory or radiological variables (Tables 6.37-6.43).

In univariate analysis, variables that were significantly associated with development of dysglycaemia were the following: gender (p=0.03), serum HDL-cholesterol (p=0.01), GGT (p=0.03), CD4 cell count (p=0.04), left (p=0.03) and right (p=0.02) leg fat on DXA scan and visceral: subcutaneous fat ratio (p <0.0001) on CT scan (Tables 6.37- 6.43).

In multivariate analysis, risk factors that predicted the development of dysglycaemia were systolic blood pressure (HR 1.04[95%CI 1.02-1.07], p=0.0006), serum albumin (HR 0.85 [95% CI 0.76-0.94], p=0.002), CD4 cell count (HR 0.988[95%CI 0.978-0.997], p=0.01) and efavirenz (HR 6.27[95%CI 1.65-23.80], p=0.01).One unit higher systolic blood pressure was associated with a 4% greater risk of developing dysglycaemia and exposure to efavirenz as part of ART was associated with a six-fold risk. Higher serum albumin and CD 4 cell count were associated with a lower risk of developing dysglycaemia (Table 6.44).

**Table 6.37: Baseline characteristics of group developing any disorder of glycaemia vs. group not developing any disorder of glycaemia during 24 months follow up in group 3 subjects on ART***

| Variable | Developing any disorder of glycaemia, n=16 | Not developing any disorder of glycaemia, n=134 | p | Univariate analysis HR(95% CI) | #p |
|---|---|---|---|---|---|
| Gender | | | | | |
| Male | 9(56.3) | 39(29.1) | 0.03 | 3.09(1.15-8.30) | 0.03 |
| Female | 7(43.8) | 95(70.9) | | 0.37(0.12-1.10) | 0.07 |
| **Treatment allocation** | | | | | |
| Efavirenz | 12(75.0) | 64(7.8) | 0.04 | 2.56(0.79-8.32) | 0.1 |
| Nevirapine | 4(25.0) | 70(52.2) | 0.04 | 0.39(0.12-1.27) | 0.1 |
| BMI | | | | | |
| >30 kg/m$^2$ | 3(20.0) | 36(28.1) | 0.5 | 0.62(0.17-2.20) | 0.5 |
| Familial DM | 6(46.2) | 29(22.3) | 0.06 | 1.92(0.79-4.70) | 0.2 |
| Smoker | 3(23.1) | 16(12.3) | 0.2 | 1.42(1.42-4.95) | 0.6 |

Continued on next page

**Table 6.37 cont.: Baseline characteristics of group developing any disorder of glycaemia vs. group not developing any disorder of glycaemia during 24 months follow up in group 3 subjects on ART\***

| Variable | Developing any disorder of glycaemia, n=16 | Not developing any disorder of glycaemia, n=134 | p | Univariate analysis HR(95% CI) | #p |
|---|---|---|---|---|---|
| Occupational physical activity | | | 0.7 | | |
| Sedentary | 0(0.0)) | 4(3.4) | | Not estimable | 1.00 |
| Light | 5(41.7) | 53(45.3) | | Not estimable | 0.99 |
| Moderate | 5(41.7) | 37(31.6) | | Not estimable | 0.99 |
| Heavy | 2(16.7) | 22(18.8) | | Not estimable | 0.99 |
| Leisure physical activity | | | 0.8 | | |
| Sedentary | 7(53.9) | 70(56.0) | | | |
| Light | 1(7.7) | 16(12.8) | | 0.59(0.07-4.82) | 0.62 |
| Moderate | 1(7.7) | 13 (10.4) | | 0.74(0.09-5.98) | 0.77 |
| Heavy | 4(30.8) | 26(20.8) | | 1.99(0.58-6.79) | 0.27 |

Data are n(%), BMI: body mass index, DM: diabetes mellitus, HR: hazard ratio, CI: confidence interval. p: chi-square test for differences between the two groups, #p: differences at univariate level using Cox proportional hazards

**Table 6.38: Baseline clinical characteristics of group developing any disorder of glycaemia (Diabetes Mellitus or impaired glucose tolerance or impaired fasting glucose) vs. group not developing any disorder of glycaemia during 24 months follow up***

| Variable | DM or IGT or IFG | | No DM or IGTor IFG | | P | Univariate analysis | [#]p |
|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | | HR(95%CI) | |
| Age | 16 | 39.4±6.1 | 134 | 36.6±9.4 | 0.2 | 1.05(1.001-1.09) | 0.05 |
| **Blood pressure (mmHg)** | | | | | | | |
| Systolic | 15 | 124.2± 19.2 | 128 | 110.6±15.97 | 0.003 | 1.01(.96-1.01) | 0.07 |
| Diastolic | 14 | 77.8±11.7 | 128 | 70.2±10.5 | 0.01 | 0.98(0.9-1.07) | 0.07 |
| **Anthropometric measurements** | | | | | | | |
| Weight (kg) | 16 | 65.9±12.5 | 133 | 69.5±16.4 | 0.3 | 0.99(0.97-1.03) | 0.8 |
| Body mass index (kg/m2) | 16 | 24.7±6.3 | 133 | 26.4±6.3 | 0.3 | 0.9(0.8-1.09) | 0.4 |
| **Circumference (cm):** | | | | | | | |
| Waist | 16 | 96.7±20.6 | 126 | 91.96±16.6 | 0.3 | 1.02(0.99-1.05) | 0.2 |
| Hip | 16 | 106.4±17.6 | 126 | 106.2±14.4 | 0.9 | 1.01(0.97-1.04) | 0.7 |
| Waist: Hip ratio | 16 | 0.9±0.1 | 125 | 0.9±0.1 | 0.06 | 1098.4(0.8-1553150) | 0.06 |
| Waist: Height ratio | 15 | 58.6±14.4 | 124 | 56.8±10.8 | 0.6 | 1.02(0.98-1.07) | 0.4 |
| Mid-arm | 16 | 32.6±6.1 | 127 | 32.8±4.9 | 0.9 | 1.01(0.91-1.12) | 0.9 |
| Neck | 16 | 35.5±3.3 | 127 | 35.3±3.6 | 0.7 | 1.02(0.9-1.2) | 0.8 |
| Chest | 15 | 95.2±12.98 | 127 | 94.8±10.4 | 0.9 | 1.01(0.96-1.06) | 0.7 |
| Mid-thigh | 15 | 54.4±14.5 | 124 | 56.7±9.7 | 0.4 | 0.98(0.93-1.04) | 0.5 |
| **Skin folds** | | | | | | | |
| Triceps | 16 | 21.9±12.6 | 126 | 23.6±13.3 | 0.6 | 0.99(0.96-1.04) | 0.9 |
| Sub-scapular | 16 | 19.4±15.3 | 125 | 19.2±11.6 | 0.98 | 1.00(0.96-1.04) | 0.9 |
| Abdomen | 16 | 25.7±17.9 | 125 | 25.2±13.8 | 0.9 | 1.01(0.97-1.04) | 0.7 |
| Mid-thigh | 16 | 30.8±17.4 | 124 | 33.1±17.1 | 0.6 | 0.99(0.97-1.03) | 0.9 |

Data are mean ± SD. DM: diabetes mellitus, IGT: impaired glucose tolerance, IFG: impaired fasting glucose. HR: Hazard ratio, CI: Confidence interval.[*]16 subjects developed any disorder of glycaemia and 134 did not develop any disorder of glycaemia; n= number for which data was available for each variable.p: Student's t-test for differences between the two groups, [#]p: differences at univariate level using Cox proportional hazards

**Table 6.39: Baseline laboratory characteristics of group developing any disorder of glycaemia (Diabetes Mellitus or impaired glucose tolerance or impaired fasting glucose) vs. group not developing any disorder of glycaemia during 24 months follow up***

| Variable | DM or IGT or IFG | | No DM or IGTor IFG | | p | Univariate analysis | [#]p |
|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | | HR(95% CI) | |
| Plasma glucose (mmol/l) | | | | | | | |
| 0 hour | 16 | 4.78+0.4 | 128 | 4.8+0.5 | 0.9 | 0.8(0.3-2.5) | 0.7 |
| 2 hour | 16 | 5.36+1.5 | 119 | 5.1+1.0 | 0.5 | 1.3(0.8-2.09) | 0.3 |
| HbA1c | 16 | 3.98+0.8 | 118 | 4.0+0.7 | 0.98 | 0.9(0.4-2.08) | 0.9 |
| Serum lipids (mmol/l) | | | | | | | |
| Total cholesterol | 15 | 3.15+0.77 | 127 | 3.54+0.86 | 0.1 | 0.6(0.3-1.2) | 0.1 |
| Total triglycerides | 15 | 0.95+0.33 | 125 | 0.96+0.60 | 0.9 | 0.9(0.4-2.2) | 0.8 |
| LDL | 14 | 2.04+0.80 | 112 | 2.25+0.72 | 0.3 | 0.7(0.4-1.64) | 0.4 |
| HDL | 15 | 0.66+0.23 | 124 | 0.85+0.28 | 0.02 | 0.07(0.01-0.61) | 0.01 |
| HIV Parameters | | | | | | | |
| CD4+ cell count (cells/mm3 ) | 16 | 101.8+91.96 | 131 | 146.4+86.4 | 0.06 | 0.99(0.99-1.0) | 0.04 |
| HIV RNA, copies/ml | 14 | 4.4+1.6 | 116 | 4.8+0.8 | 0.1 | 0.7(0.4-1.2) | 0.2 |
| Full blood count | | | | | | | |
| Haemoglobin (g/dL) | 16 | 10.5+2.1 | 133 | 11.2+1.8 | 0.2 | 0.79(0.6-1.0) | 0.08 |
| Platelet count (x109/L) | 16 | 220.4+107.6 | 133 | 254.4+76.7 | 0.1 | 0.99(0.99-1.00) | 0.1 |
| White cell count (x109/L) | 16 | 4.2+1.6 | 133 | 4.5+1.6 | 0.4 | 0.9(0.7-1.3) | 0.7 |
| Lymphocytes (x109/L) | 16 | 1.3+0.83 | 133 | 2.1+4.4 | 0.4 | 0.6(0.3-1.2) | 0.1 |

Data are mean ± SD. DM: diabetes mellitus, IGT: impaired glucose tolerance, IFG: impaired fasting glucose. HR: Hazard ratio, CI: Confidence interval.[*]16 subjects developed any disorder of glycaemia and 134 did not develop any disorder of glycaemia; n= number for which data was available for each variable.p: Student's t-test for differences between the two groups, [#]p: differences at univariate level using Cox proportional hazards.

**Table 6.40: Baseline laboratory characteristics of the group developing any disorder of glycaemia (Diabetes Mellitus or impaired glucose tolerance or impaired fasting glucose) vs. group not developing any disorder of glycaemia during 24 months follow up***

| Variable | DM or IGT or IFG | | No DM or IGTor IFG | | p | Univariate analysis | #p |
|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | | HR(95% CI) | |
| **Renal function** | | | | | | | |
| Bicarbonate (mmol/l) | 16 | 25.3+2.3 | 132 | 24.5+2.5 | 0.3 | 1.1(0.88-1.4) | 0.4 |
| Chloride (mmol/l) | 16 | 103.9+4.7 | 132 | 104.6+3.8 | 0.6 | 0.9(0.8-1.1) | 0.2 |
| Urea (mmol/l) | 16 | 3.5+1.4 | 132 | 3.5+1.3 | 0.9 | 0.97(0.7-1.4) | 0.9 |
| Creatinine (mmol/l) | 16 | 68.30+17.5 | 132 | 68.3+18.4 | 0.9 | 1.00(0.97-1.0) | 0.8 |
| Anion gap | 16 | 10.9+2.6 | 132 | 11.4+3.1 | 0.5 | 0.96(0.83-1.13) | 0.6 |
| Calcium (mmol/l) | 15 | 2.2+0.1 | 128 | 2.2+0.1 | 0.2 | 0.04(0.00-2.4) | 0.1 |
| Magnesium (mmol/l) | 15 | 0.96+0.4 | 125 | 0.89+0.2 | 0.2 | 2.1(0.5-8.7) | 0.3 |
| Phosphate (mmo/l) | 15 | 1.1+0.2 | 126 | 1.1+0.2 | 0.8 | 0.84(0.06-12.0) | 0.9 |
| **Liver function** | | | | | | | |
| Total protein (g/L) | 16 | 88.0+8.8 | 131 | 86.92+9.7 | 0.6 | 1.01(0.96-1.1) | 0.9 |
| Albumin (g/L) | 16 | 32.6+4.7 | 131 | 34.88+5.4 | 0.2 | 0.92(0.85-1.0) | 0.06 |
| Globulin (g/L) | 16 | 55.4+9.97 | 131 | 52.0+12.1 | 0.3 | 1.02(0.98-1.05) | 0.4 |
| Total bilirubin | 16 | 6.4+3.5 | 132 | 7.88+4.8 | 0.2 | 0.91(0.8-1.1) | 0.2 |
| ALT (U/l) | 16 | 31.4+26.3 | 130 | 25.4+17.2 | 0.2 | 1.02(0.99-1.04) | 0.1 |
| ALP | 16 | 73.0+35.63 | 131 | 64.7+25.0 | 0.4 | 1.01(0.99-1.03) | 0.06 |
| GGT | 16 | 34.8+31.6 | 131 | 29.2+32.2 | 0.6 | 1.01(1.00-1.03) | 0.03 |

Data are mean ± SD. DM: diabetes mellitus, IGT: impaired glucose tolerance, IFG: impaired fasting glucose. HR: Hazard ratio, CI: Confidence interval.*16 subjects developed any disorder of glycaemia and 134 did not develop any disorder of glycaemia; n= number for which data was available for each variable. p: Student's t-test for differences between the two groups, #p: differences at univariate level using Cox proportional hazards. ALT: Serum alanine transaminase; ALP: Serum alkaline phosphatase; GGT: Serum gamma glutamyl transaminase

**Table 6.41: Baseline laboratory characteristics (inflammatory marker) of group developing any disorder of glycaemia (Diabetes Mellitus or impaired glucose tolerance or impaired fasting glucose) vs. group not developing any disorder of glycaemia during 24 months follow up***

| Variable | DM or IGT or IFG | | No DM or IGTor IFG | | p | Univariate analysis | [#]p |
|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | | HR(95% CI) | |
| **Inflammatory Markers** | | | | | | | |
| CRP (mg/L) | 15 | 16.53+25.3 | 121 | 17.29+32.82 | 0.7 | 1.01(0.99-1.02) | 0.6 |
| Lactate (mmol/l) | 13 | 1.4+0.8 | 123 | 1.5+0.8 | 0.5 | 0.7(0.3-1.62) | 0.4 |
| Uric acid (mmol/L) | 11 | 0.27+0.08 | 99 | 0.29+0.10 | 0.6 | 0.12(0.00-224.4) | 0.6 |
| Cortisol (nmol/L) | 16 | 324.9+163.5 | 115 | 296.6+116.2 | 0.5 | 1.00(1.00-1.01) | 0.07 |
| Iron (umol/L) | 13 | 8.92+4.4 | 119 | 10.4+5.0 | 0.3 | 0.92(0.82-1.05) | 0.2 |
| Transferrin (g/L) | 12 | 2.3+0.6 | 108 | 2.3+0.5 | 0.5 | 1.62(0.56-4.7) | 0.4 |
| Saturation (%) | 10 | 16.2+8.6 | 79 | 18.4+9.2 | 0.5 | 0.96(0.89-1.04) | 0.3 |
| Ferritin (ug/L) | 14 | 228.1+360.1 | 101 | 137.6+153.6 | 0.2 | 1.00(1.00-1.00) | 0.1 |

Data are mean + SD. DM: diabetes mellitus, IGT: impaired glucose tolerance, IFG: impaired fasting glucose. HR: Hazard ratio, CI: Confidence interval.[*]16 subjects developed any disorder of glycaemia and 134 did not develop any disorder of glycaemia; n= number for which data was available for each variable.p: Student's t-test for differences between the two groups, [#]p: differences at univariate level using Cox proportional hazards

**Table 6.42: Baseline DXA scan findings of the group developing any disorder of glycaemia (Diabetes Mellitus or impaired glucose tolerance or impaired fasting glucose) vs. group not developing any disorder of glycaemia during 24 months follow up***

| Variable | DM or IGT or IFG | | No DM or IGTor IFG | | p | Univariate analysis | [#]p |
|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | | HR(95%CI) | |
| Body composition, DXA | | | | | | | |
| Total mass | 15 | 64316.4+14187.0 | 122 | 68760.4+15934.01 | 0.3 | 1.00(1.00-1.00) | 0.2 |
| Fat (kg) | | | | | | | |
| Total | 15 | 16430.4+12308.9 | 122 | 21924.5+12048.23 | 0.1 | 0.99(0.99-1.00) | 0.2 |
| Left arm | 15 | 1063.5+872.8 | 122 | 1265.83+736.8 | 0.3 | 1.00(.99-1.00) | 0.2 |
| Right arm | 15 | 1002.4+828.4 | 122 | 1260.84+716.8 | 0.2 | 0.99(.99-1.00) | 0.2 |
| Left leg | 15 | 3404.9+2453.5 | 122 | 4816.0+2505.5 | 0.04 | 1.00(.99-1.00) | 0.03 |
| Right leg | 15 | 3462.0+2575.7 | 122 | 4971.8+2635.1 | 0.04 | 1.00(0.99-1.00) | 0.02 |
| Trunk | 15 | 6680.8+5766.99 | 122 | 8787.5+5824.7 | 0.2 | 1.00(1.00-1.00) | 0.5 |
| Lean (kg) | | | | | | | |
| Total | 15 | 47885.95+6925.71 | 122 | 46835.9+9508.7 | 0.7 | 1.00(1.00-1.00) | 0.8 |
| Left arm | 15 | 2722.9+485.8 | 122 | 2523.6+774.6 | 0.3 | 1.00(.99-1.00) | 0.4 |
| Right arm | 15 | 2920.4+481.4 | 122 | 2649.4+793.4 | 0.2 | 1.00(1.00-1.00) | 0.3 |
| Left leg | 15 | 7878.8+1206.1 | 122 | 7932.8+1898.2 | 0.9 | 1.00(1.00-1.00) | 0.8 |
| Right leg | 15 | 7832.8+1011.5 | 122 | 8008.1+1900.7 | 0.7 | 1.00(1.00-1.00) | 0.96 |
| Trunk | 15 | 22979.7+3906.8 | 122 | 22096.4+4193.1 | 0.4 | 1.00(1.00-1.00) | 0.7 |
| Total bone mineral density | 15 | 1.2+0.1 | 122 | 1.1+0.1 | 0.06 | 1.00(1.00-1.00) | 0.4 |

Data are mean ± SD. DM: diabetes mellitus, IGT: impaired glucose tolerance, IFG: impaired fasting glucose. HR: Hazard ratio, CI: Confidence interval.[*]16subjects developed any disorder of glycaemia and 134 did not develop any disorder of glycaemia; n= number for which data was available for each variable.p: Student's t-test for differences between the two groups, [#]p: differences at univariate level using Cox proportional hazards

**Table 6.43: Baseline radiologycharacteristics of group developing any disorder of glycaemia (Diabetes Mellitus or impaired glucose tolerance or impaired fasting glucose) vs. group not developing any disorder of glycaemia during 24 months follow up***

| Variable | Diabetes or IGTor IFG | | No Diabetes or IGT or IFG | | | Univariate analysis | |
|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | p | HR(95%CI) | [#]p |
| **Fat distribution, CT scan** | | | | | | | |
| Total fat area | 14 | 224.9+191.2 | 109 | 303.4+211.1 | 0.2 | 0.99(099.-1.0) | 0.2 |
| Visceral fat area | 14 | 52.8 +42.8 | 109 | 62.9+50.1 | 0.5 | 0.99(0.98-1.01) | 0.6 |
| Visceral: subcutaneous fat ratio | 14 | 1.0+1.6 | 109 | 0.4+0.4 | 0.0006 | 3.2(1.9-5.5) | <0.0001 |
| Subcutaneous fat area | 14 | 172.1+157.1 | 109 | 240.4+178.4 | 0.2 | 0.99(0.99-1.0) | 0.2 |
| Waist size | 14 | 90.5+14.9 | 106 | 94.9+17.6 | 0.4 | 1.00(0.98-1.0) | 0.8 |

Data are mean ± SD. DM: diabetes mellitus, IGT: impaired glucose tolerance, IFG: impaired fasting glucose. HR: Hazard ratio, CI: Confidence interval. [*]16subjects developed any disorder of glycaemia and 134 did not develop any disorder of glycaemia; n= number for which data was available for each variable. p: Student's t-test for differences between the two groups, [#]p: differences at univariate level using Cox proportional hazards

**Table 6.44: Multivariate models for incident Diabetes Mellitus, Impaired Glucose tolerance and Impaired Fasting glucose**

| Variable | HR(95%CI) | p |
|---|:---:|:---:|
| **Diabetes Mellitus (OGTT criteria)** | | |
| Age | 1.01(0.86-1.18) | 0.9 |
| Gender | 1.73(0.07-40.88) | 0.7 |
| Systolic blood pressure | 1.02(0.96-1.08) | 0.6 |
| Visceral:subcutaneous fat | 2.95(1.25-6.98) | 0.01 |
| Efavirenz | not estimatable | 0.9 |
| **Diabetes Mellitus (HbA1c criteria)** | | |
| Age | 1.03(0.95-1.11) | 0.5 |
| Gender | 1.76(0.21-14.93) | 0.6 |
| Systolic blood pressure | 1.02(0.98-1.06) | 0.3 |
| Haemoglobin | 0.61(0.39-0.96) | 0.03 |
| **Impaired Glucose Tolerance** | | |
| Systolic blood pressure | 1.05(1.01 to 1.09) | 0.01 |
| Visceral:subcutaneous fat | 8.16(1.53 to 43.53) | 0.01 |
| **Impaired Fasting Glucose** | | |
| Gender | 0.15(0.02 to 0.89) | 0.04 |
| Waist | 1.09(1.03 to1.15) | 0.003 |
| Serum alkaline phosphatase | 1.05(1.02 to 1.08) | 0.002 |

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**Table 6.44 cont.: Multivariate models for incident Diabetes Mellitus, Impaired Glucose tolerance and Impaired Fasting glucose**

| Variable | HR(95%CI) | p |
|---|:---:|:---:|
| **Impaired glucose regulation/Pre-diabetes[#]** | | |
| Gender | 0.24(0.07 to 0.88) | 0.03 |
| Albumin | 0.85(0.76-0.96) | 0.01 |
| **Dysglycaemia (any disorder of glycaemia)[*]** | | |
| Systolic blood pressure | 1.04(1.02-1.07) | 0.0006 |
| Albumin | 0.85(0.76-0.94) | 0.002 |
| CD4 cell count | 0.988(0.978-0.997) | 0.01 |
| Efavirenz | 6.27(1.65-23.80) | 0.01 |

CI: Confidence interval, *Diabetes Mellitus or Impaired glucose tolerance or impaired fasting glucose, #Impaired glucose tolerance or impaired fasting glucose. OGTT: oral glucose tolerance test, $HbA_{1c}$: Haemoglobin$A_{1c}$

## 6.4. Changes in laboratory variables in group 3 subjects who completed 24 month visit on ART

The results of laboratory tests were evaluated in group 3 subjects on ART who completed 24 months visit (Tables 6.45 – 6.47). There was a significant increase in mean CD 4 cell count (p< 0.0001) and a reduction in mean log HIV viral load (p < 0.0001),$HbA_{1c}$ (p=0.0003), serum total cholesterol (p<0.0001), LDL cholesterol(p<0.0001) and HDL cholesterol (p<0.0001) also increased significantly (Tables 6.45 - 6.47, Figure 6.8).

When compared with baseline, at 24 months, the mean $HbA_{1c}$ (p=0.0003),serum total cholesterol (p<0.0001), LDL cholesterol (p<0.0001) were significantly higher and HDL cholesterol lower, however, no difference was observed for risk categories for serum lipids. (Tables 6.49 – 6.50)

There was an increase in mean serum creatinine (p=0.0003), albumin (p<0.0001), GGT (p=0.001), haemoglobin (p<0.0001), and a decrease in total protein (p<0.0001), globulins (p<0.0001) at 24 months on ART (Tables 6.48 – 6.49). Linear trend using the linear mixed model showed that these variables changed similarly over time.

**Table 6.45: Laboratory characteristics during follow up on ART in group 3 subjects**

| Variable | Baseline (n = 150) | 3 Months (n = 103) | 6Months (n = 107) | 12 Months (n = 108) | 18 Months (n = 97) | 24 Months (n = 97) | P | P |
|---|---|---|---|---|---|---|---|---|
| Plasma glucose (mmol/l) | | | | | | | | |
| 0 hour | $4.8 \pm 0.4$ | $5.0 \pm 0.6$ | $5.0 \pm 0.5$ | $5.0 \pm 0.5$ | $5.0 \pm 0.7$ | $4.95 \pm 0.6$ | 0.07 | |
| 2 hour | $5.2 \pm 1.2$ | $5.3 \pm 1.0$ | $5.2 \pm 0.9$ | $5.1 \pm 0.9$ | $5.3 \pm 1.2$ | $5.2 \pm 1.2$ | | 0.9 |
| $HbA_{1c}$ | $4.0 \pm 0.7$ | $3.8 \pm 0.5$ | $3.8 \pm 0.6$ | $3.9 \pm 0.8$ | $4.1 \pm 1.0$ | $4.5 \pm 1.1$ | | 0.0002 |
| **HIV Parameters** | | | | | | | | |
| CD4 cell count (cells/mm$^3$) | 132.0(64.0-92.0) | 218.0(146.0-289.0) | 223.5(158.5-14.0) | 257.0(175.5-355.5) | 279.0(180.0-382.0) | 344.0(209.0-416.0) | <0.0001 | |
| HIV RNA, (log$_{10}$) | $4.8 \pm 0.9$ | $1.9 \pm 1.2$ | $1.7 \pm 1.2$ | $1.5 \pm 1.2$ | $1.4 \pm 1.2$ | $1.3 \pm 0.9$ | <0.0001 | |

Data expressed as mean $\pm$ SD or median (IQR).

**Figure 6.8: Immunological and Virological response after 24 months of ART in group 3 subjects**
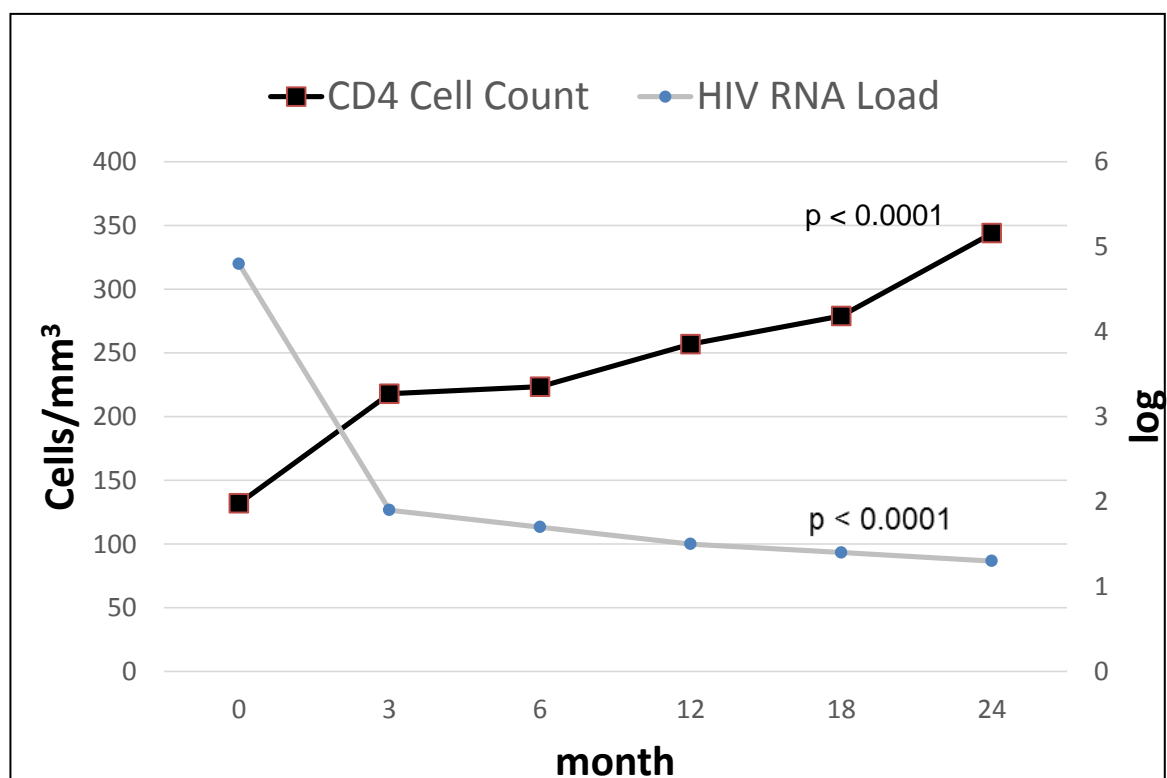
**Table 6.46: Laboratory characteristics (serum lipids) during follow up on ART in group 3 subjects**

| Variable | Baseline (n = 150) | 3 Months (n = 103) | 6Months (n = 107) | 12 Months (n = 108) | 18 Months (n = 97) | 24 Months (n = 97) | p |
|---|---|---|---|---|---|---|---|
| **Serum lipids (mmol/l)** | | | | | | | |
| Total cholesterol | 3.5 ± 0.9 | 3.9 ± 0.9 | 4.1 ± 0.8 | 4.1 ± 0.9 | 4.3 ± 0.9 | 4.3 ± 0.8 | <0.0001 |
| Total triglycerides | 1.0 ± 0.6 | 1.0 ± 0.5 | 0.9 ± 0.5 | 1.0 ± 0.8 | 1.0 ± 0.7 | 1.0 ± 0.7 | 0.5 |
| LDL | 2.2 ± 0.8 | 2.4 ± 0.7 | 2.4 ± 0.8 | 2.3 ± 0.8 | 2.6 ± 1.3 | 2.5 ± 1.7 | <0.0001 |
| HDL | 0.8 ± 0.3 | 1.1 ± 0.4 | 1.2 ± 0.3 | 1.2 ± 0.4 | 1.4 ± 0.9 | 1.3 ± 0.3 | <0.0001 |
| **Grading of abnormality of serum lipids (mmol/l)** | | | | | | | |
| Total cholesterol | | | | | | | |
| Optimal | 125 (96.2) | 83 (89.3) | 82 (89.1) | 87 (87.0) | 82 (84.5) | 79 (85.9) | |
| Borderline | 5 (3.9) | 9 (9.7) | 7 (7.6) | 11 (11.0) | 12 (12.4) | 13 (14.1) | |
| High risk | 0 (0.0) | 1(1.1) | 3 (3.3) | 2 (2.0) | 3 (3.1) | 0 (0.0) | |
| Missing | 20 (13.3) | 10 (9.7) | 13 (12.2) | 8 (7.4) | 1 (1.0) | 4 (4.2) | |
| Total Triglycerides | | | | | | | |
| Optimal | 116 (91.3) | 86 (92.5) | 85 (92.4) | 92 (92.9) | 86 (89.6) | 79 (87.8) | |
| Borderline | 7 (5.5) | 5 (5.4) | 3 (3.3) | 3 (3.0) | 6 (6.2) | 7 (7.8) | |
| High risk | 4 (3.1) | 2 (2.2) | 4 (4.4) | 3 (3.0) | 4 (4.1) | 4 (4.4) | |
| Very High risk | | | | 1 (1.01) | | | |
| Missing | 23 (15.3) | 10 (9.7) | 13 (12.2) | 9 (8.3) | 2 (2.1) | 6 (6.3) | |

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Table 6.46 cont.: Laboratory characteristics (serum lipids) during follow up on ART in group 3 subjects

| Variable | Baseline (n = 150) | 3 Months (n = 103) | 6Months (n = 107) | 12 Months (n = 108) | 18 Months (n = 97) | 24 Months (n = 97) | p |
|---|---|---|---|---|---|---|---|
| LDL | | | | | | | |
| Optimal | 104 (91.2) | 78 (91.8) | 71 (88.8) | 61 (89.7) | 64 (90.1) | 55 (88.71) | |
| Borderline | 9 (7.9) | 6 (7.1) | 6 (7.5) | 6 (8.8) | 6 (8.5) | 7 (11.29) | |
| High risk | 1 (0.9) | 0 (0.0) | 3 (3.8) | 1 ( 1.5) | 2 (2.8) | 0 (0.0) | |
| Very High risk | | 1 (1.2) | | | 1 (1.4) | | |
| Missing | 36 (24.0) | 18 (17.5) | 25 (23.4) | 40 (37.0) | 25 (25.8) | 34 (35.4) | |
| HDL Female | | | | | | | |
| Optimal | 2 (1.3) | 8 (7.8) | 8 (7.5) | 15 (13.9) | 21 (21.6) | 17 (17.5) | |
| Borderline | 3 (2.0) | 16 (15.5) | 15 (14.0) | 16 (14.8) | 18 (18.6) | 21 (21.6) | |
| High risk | 81 (54.0) | 40 (38.8) | 37 (34.6) | 34 (31.5) | 25 (25.8) | 23 (23.7) | |
| HDL Male | | | | | | | |
| Optimal | 0 (0.0) | 0 (0.0) | 2 (1.9) | 0 (0.0) | 2 (2.1) | 2 (2.1) | |
| Borderline | 5 (3.3) | 10 (6.7) | 12 (11.2) | 15 (13.9) | 17 (17.5) | 17 (17.5) | |
| High risk | 36 (24.0) | 19 (18.4) | 17 (15.9) | 18 (16.7) | 13 (13.4) | 11 (11.3) | |
| Missing | 23 (15.3) | 10 (9.7) | 14 (13.1) | 10 (9.3) | 2 (2.1) | 5 (5.2) | |

Data expressed as mean $\pm$ SD or n (%).LDL: Low density lipoprotein cholesterol; HDL: High density lipoprotein cholesterol

**Table 6.47: Laboratory characteristics during follow up on ART in group 3 subjects**

| Variable | Baseline (n = 150) | 3 Months (n = 103) | 6Months (n = 107) | 12 Months (n = 108) | 18 Months (n = 97) | 24 Months (n = 97) | P |
|---|---|---|---|---|---|---|---|
| **Renal Function** | | | | | | | |
| Bicarbonate (mmol/l) | 24.6 ± 2.5 | 24.3 ± 2.6 | 24.1 ± 2.9 | 24.9 ± 2.3 | 24.5 ± 2.7 | 24.4 ± 2.6 | 0.7 |
| Chloride (mmol/l) | 104.5 + 3.9 | 105.0 + 3.2 | 103.9 + 10.8 | 103.6 + 10.0 | 104.5 + 3.4 | 104.1 + 2.9 | 0.3 |
| Urea (mmol/l) | 3.5 + 1.3 | 3.8 + 1.3 | 3.4 + 1.2 | 3.4 + 1.1 | 3.6 + 1.6 | 3.95 + 5.5 | 0.8 |
| Creatinine (µmol/l) | 66.0(56.0-78.0) | 64.0(58.0-78.0) | 67.5(57.0-78.5) | 67.0(58.0-77.0) | 68.0(59.0-81.0) | 72.0(63.0-83.0) | 0.0001 |
| Anion gap | 11.3 ± 3.1 | 12.5 ± 3.2 | 12.7 ± 2.8 | 12.8 ± 2.7 | 13.4 ± 3.1 | 13.6 ± 2.4 | <0.0001 |
| Calcium (mmol/l) | 2.2 (2.1-2.3) | 2.25 (2.17-2.3) | 2.23 (2.18-2.29) | 2.24 (2.18-2.30) | 2.22 (2.17-2.29 | 2.24 (2.18-2.29) | 0.6 |
| Magnesium (mmol/l) | 0.9 (0.8-0.9) | 0.87 (0.83-0.92) | 0.90(0.83-0.94) | 0.89 (0.85-0.95) | 0.88 (0.83-0.92) | 0.87 (0.83-0.93) | 0.3 |
| Phosphate (mmo/l) | 1.1 ± 0.2 | 1.07 ± 0.2 | 1.03 ± 0.2 | 1.04 ± 0.3 | 1.05 ± 0.3 | 1.02 ± 0.2 | 0.0007 |
| **Liver function** | | | | | | | |
| Total protein(g/L) | 86.7 ± 9.8 | 82.8 ± 8.0 | 81.4 ± 7.6 | 79.8 ± 10.2 | 78.4 ± 6.6 | 78.3 ± 7.2 | <0.0001 |
| Albumin(g/L) | 34.3 ± 5.6 | 36.4 ± 5.0 | 37.6 ± 4.4 | 38.4 ± 4.4 | 38.3 ± 3.9 | 39.2 ± 3.5 | <0.0001 |
| Globulin(g/L) | 51.9 ± 11.7 | 46.5 ± 10.1 | 43.9 ± 9.2 | 42.4 ± 9.1 | 40.1 ± 7.8 | 38.98 ± 8.8 | <0.0001 |
| Total bilirubin | 7.9 ± 4.9 | 6.1 ± 4.1 | 5.8 ± 3.2 | 6.0 ± 3.6 | 6.3 ± 3.2 | 6.8 ± 3.2 | 0.1 |
| Alanine    amino | 21.0(16.0-29.0) | 25.0(22.0-33.0) | 24.5(20.0-31.0) | 25.0(20.0-33.0) | 22.0(20.0-27.0) | 22.0(18.5-28.5) | 0.7 |
| Alkaline phosphatase | 66.96+29.3 | 81.0(65.0-99.0) | 83.5(68.0-103.0) | 85.0(68.0-112) | 88.0(69.0-107.5) | 84.0(67.0-111.0) | 0.0001 |
| GGT | 21.0(15.0-3.0) | 37.0(27.0-57.0) | 33.0(25.0-49.0) | 33.0(23.0-48.0) | 34.5(24.0-47.5) | 31.0(24.0-46.0) | <0.0001 |
| **Full blood count** | | | | | | | |
| Haemoglobin (g/dL) | 11.1 ± 2.0 | 11.6 ± 1.9 | 11.6 ± 2.3 | 12.0 ± 1.8 | 12.1 ± 2.3 | 12.6 ± 1.6 | <0.0001 |
| Platelet count (x$10^9$/L) | 249.6 ± 82.0 | 269.7 ± 74.4 | 268.3 ± 70.8 | 272.0 ± 64.6 | 267.0± 70.5 | 260.0 ± 67.2 | 0.09 |
| White    cell    count | 4.3 (3.3-5.2) | 4.2 (3.3-5.1) | 4.1 (3.4-5.3) | 4.3 (3.5-5.2) | 4.2 (3.3-5.7) | 4.4 (3.5-5.4) | 0.2 |

Data expressed as mean±SD or median (IQR). GGT: gamma glutamyl transferase.

**Table 6.48: Laboratory Characteristics (inflammatory markers) at follow up on ART in group 3 subjects**

| Variable | Baseline (n = 150) | 3 Months (n = 103) | 6Months (n = 107) | 12 Months (n = 108) | 18 Months (n = 97) | 24 Months (n = 97) | p |
|---|---|---|---|---|---|---|---|
| **Inflammatory Markers** | | | | | | | |
| CRP(mg/L) | 14.9+24.3 | 10.6+33.4 | 10.0+8.0 | 10.2+14.6 | 8.3+9.3 | 12.1+26.2 | 0.05 |
| Lactate (mmol/l) | 1.3 (1.0-1.8) | 1.3 (1.0-2.1) | 1.5 (1.2-2.3) | 1.50 (1.1-1.9) | 1.3 (1.0-1.7) | 1.2 (1.0-1.6) | 0.4 |
| Uric acid (mmol/L) | 0.27(0.23-0.31) | 0.24(0.20-0.28) | 0.24 (0.20-0.30) | 0.23 (0.20-0.28) | 0.22 (0.19-0.28) | 0.24 (0.18-0.29) | <0.0001 |
| Cortisol (nmol/L) | 305.4 ± 131.3 | 279.4 ± 96.5 | 301.6 ± 106.3 | 292.8 ± 117.2 | 298.5 ± 113.7 | 307.8 ± 140.2 | 0.4 |
| Iron (umol/L) | 10.0(6.0-13.5) | 10.3(7.3-14.6) | 11.9(7.8-15.6) | 11.3(8.1-14.9) | 9.9(6.7-14.2) | 11.9(8.4-15.7) | 0.008 |
| Transferrin(g/L) | 2.2 (1.9-2.6) | 2.5 (2.0-2.8) | 2.5 (2.2-3.1) | 2.6 (2.3-3.1) | 2.7 (2.4-3.0) | 2.6(2.3-3.0) | <0.0001 |
| Saturation (%) | 17.0(10.0-25.0) | 17.0(12.0-23.0) | 20.0(11.0-27.5) | 18.0(13.0-23.0) | 15.0(9.0-24.0) | 20.0(12.00-26.00) | 0.5 |
| Ferritin (ug/L) | 93.0(32.0-226.0) | 37.0(13.0-99.0) | 34.0(11.0-87.0) | 38.0(15.0-87.0) | 28.5(16.0-65.0) | 34.0(17.0-65.0) | <0.0001 |

Data expressed as mean+SD or median (IQR)

**Table 6.49: Characteristics at baseline vs. at 24 month follow up in group 3 subjects on ART\***

| Variable | Baseline | | 24 months | | Mean difference | p |
|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | | |
| **Plasma glucose (mmol/l)** | | | | | | |
| 0 hour | 93 | 4.8 ± 0.4 | 95 | 4.9 ± 0.6 | 0.1±0.8 | 0.2 |
| 2 hour | 89 | 5.1 ± 1.1 | 95 | 5.9 ± 1.2 | 0.1±1.6 | 0.5 |
| $HbA_{1c}$ | 85 | 4.0 ± 0.6 | 80 | 4.6 ± 1.1 | 0.6±1.3 | 0.0003 |
| **Serum lipids (mmol/l)** | | | | | | |
| Total cholesterol | 91 | 3.4 ± 0.9 | 93 | 4.3 ± 0.8 | 0.8±0.7 | <0.0001 |
| Total triglycerides | 90 | 0.98 ± 0.6 | 93 | 1.0 ± 0.7 | 0.04±0.9 | 0.6 |
| LDL | 82 | 2.2 ± 0.8 | 63 | 2.5 ± 0.7 | 0.4±0.6 | <0.0001 |
| HDL | 88 | 0.8 ± 0.3 | 92 | 1.3 ± 0.3 | 0.5±0.3 | <0.0001 |
| **HIV Parameters** | | | | | | |
| CD4 cell count(cells/$mm^3$) | 95 | 142.0±82.6 | 93 | 337 + 165.9 | 199±132 | <0.0001 |
| HIV RNA($log_{10}$) | 82 | 4.7 ± 1.0 | 87 | 1.3 ± 0.9 | -3.3±1.3 | <0.0001 |

LDL: Low density lipoprotein cholesterol; HDL: High density lipoprotein cholesterol. p: Paired Student's t-test.Calculated mean difference (24 months-baseline mean) for data available at baseline and at follow up.*97 subjects completed follow up; n= number for which data was available for each variable

**Table 6.50: Categories of serum lipids at baseline vs. at 24 month follow up in group 3 subjects on ART***

| Baseline | 24 months | | | | p |
|---|---|---|---|---|---|
| | Optimal | Borderline | High risk | Total | |
| Total cholesterol | | | | | 0.01 |
| optimal | 68(83.95) | 11(13.6) | | 79(97.5) | |
| borderline | 1(1.2) | 1(1.2) | | | |
| high risk | | | | | |
| Total | 69(85.2) | 12(14.8) | | 81(100.0) | |
| LDL | | | | | 0.5 |
| optimal | 43(87.8) | 2(4.1) | | 45(91.8) | |
| borderline | 0(0.0) | 4(8.2) | | | |
| high risk | | | | | |
| Total | 43(87.8) | 6(12.2) | | 49(100.0) | |
| Triglyceride | | | | | 0.95 |
| optimal | 65(84.4) | 3(3.9) | 2(2.6) | 70(90.9) | |
| borderline | 3(3.9) | 1(1.3) | 1(1.3) | 5(6.5) | |
| high risk | 1(1.3) | 1(1.3) | 0(0.0) | 2(2.6) | |
| Total | 69(89.6) | 5(6.5) | 0(0.0) | 77(100.0) | |
| HDL(male) | | | | | |
| optimal | | | | | |
| borderline | 2(8.3) | 2(8.3) | 0(0.0) | 4(16.7) | NE |
| high risk | 0(0.0) | 13(54.2) | 7(29.2) | 20(83.3) | |
| Total | 2(8.3) | 15(62.5) | 7(29.2) | 24(100.0) | |
| HDL(female) | | | | | |
| optimal | | | | | |
| borderline | 2(3.7) | 0(0.0) | 0(0.0) | 2(3.7) | NE |
| high risk | 12(22.2) | 18(33.3) | 22(40.7) | 52(96.3) | |
| Total | 14(25.9) | 18(33.3) | 22(40.7) | 54(100.0) | |

Data expressed as n (%). LDL: Low density lipoprotein cholesterol; HDL: High density lipoprotein cholesterol. P: Mcnemar test for difference at baseline compared to 24 months follow up. NE: not estimatable

**Table 6.51: Laboratory characteristics at baseline vs. at 24 month follow up in group 3 subjects on ART[*]**

| Variable | Baseline | | 24 months | | | |
|---|---|---|---|---|---|---|
| | n | Mean±SD | n | mean±SD | Mean difference | p |
| **Renal function** | | | | | | |
| Bicarbonate (mmol/l) | 96 | 24.9 ± 2.4 | 96 | 24.4 ± 2.6 | -0.4 ± 3.2 | 0.2 |
| Chloride (mmol/l) | 96 | 104.8 ± 3.7 | 96 | 104.2 ± 2.9 | -0.6 ± 4.1 | 0.1 |
| Urea (mmol/l) | 96 | 3.6 ± 1.4 | 96 | 3.94 ± 5.2 | 0.3±5.1 | 0.5 |
| Creatinine (mmol/l) | 96 | 68.4 ± 17.2 | 96 | 74.3 ± 17.1 | 5.9±15.4 | 0.0003 |
| Anion gap | 96 | 11.3 ±3.2 | 96 | 13.6 ± 2.4 | 2.3±3.9 | <0.0001 |
| Calcium (mmol/l) | 92 | 2.2 ±0.1 | 95 | 2.2 ± 0.1 | 0.02 ± 0.2 | 0.3 |
| Magnesium (mmol/l) | 90 | 0.9 ± 0.2 | 95 | 0.9 ± 0.2 | -0.001 ± 0.3 | 0.97 |
| Phosphate (mmo/l) | 91 | 1.1 ±0.2 | 95 | 1.0 ±0.2 | -0.1 ± 0.2 | 0.0001 |
| **Liver function** | | | | | | |
| Total protein(g/L) | 95 | 88.1 ± 10.2 | 96 | 78.3 ± 7.2 | -9.8±9.2 | <0.0001 |
| Albumin(g/L) | 95 | 34.92 ± 5.0 | 96 | 39.2 ± 3.5 | 4.3±4.2 | <0.0001 |
| Globulin(g/L) | 82 | 52.6 ± 12.5 | 91 | 38.98 ± 8.7 | -13.8 ± 9.7 | <0.0001 |
| Total bilirubin | 95 | 7.9 ± 4.9 | 94 | 6.8±3.2 | -1.2 ± 5.7 | 0.04 |
| ALT(U/l) | 96 | 26 ± 18.7 | 95 | 24.8±9.99 | -1.2±19.99 | 0.6 |
| ALP(U/L) | 96 | 63.0 ± 23.0 | 95 | 91.3±32.2 | 28.9±27.3 | <0.0001 |
| GGT (U/L) | 95 | 26.1 ± 18.5 | 96 | 46.9±68.2 | 21.3±60.5 | 0.0009 |

Continued on next page

**Table 6.51 cont.: Laboratory characteristics at baseline vs. at 24 month follow up in group 3 subjects on ART**

**Full blood count**

| | | | | | | |
|---|---|---|---|---|---|---|
| Haemoglobin (g/dL) | 96 | 11.3 ± 1.9 | 96 | 12.6±1.6 | 1.2±1.6 | <0.0001 |
| Platelet count (x109/L) | 96 | 244.1 ± 85.4 | 96 | 259.8.6+66.8 | 15.7±85.6 | 0.07 |
| White cell count (x109/L) | 96 | 4.3 ± 1.5 | 96 | 4.6±1.5 | 0.3±1.6 | 0.1 |
| Lymphocytes (x109/L) | 96 | 1.9 ± 3.1 | 86 | 2.3±6.6 | 0.4±7.2 | 0.6 |

**Inflammatory Markers**

| | | | | | | |
|---|---|---|---|---|---|---|
| CRP(mg/L) | 85 | 13.99 ± 24.7 | 91 | 12.1 ± 26.1 | -3.2 ±27.1 | |
| Lactate (mmol/l) | 89 | 1.6 ± 0.7 | 95 | 1.4 + 0.95 | -0.2 ± 1.3 | 0.2 |
| Uric acid(mmol/L) | 72 | 0.30 ± 0.1 | 74 | 0.29 + 0.1 | -0.1 ± 0.1 | 0.0001 |
| Cortisol(nmol/L) | 86 | 288.2 ± 116.4 | 87 | 310.5 ± 141.7 | 8.9±178.5 | 0.3 |
| Iron(umol/L) | 86 | 10.6 + 4.9 | 94 | 12.8 ± 6.2 | 2.2±7.5 | 0.005 |
| Transferrin(g/L) | 76 | 2.2±0.5 | 93 | 2.7 ± 0.5 | 0.4±0.5 | <0.0001 |
| Saturation (%) | 60 | 19.2± 8.9 | 85 | 20.3±10.8 | 1.8± 12.0 | 0.3 |
| Ferritin(ug/L) | 78 | 148.97±199.98 | 87 | 56.1 ± 73.7 | 94.6±182 | <0.0001 |

Paired Student's t-test. calculated mean difference (24 months-baseline mean) for data available at baseline and at follow up.*97 subjects completed follow up; n= number for which data was available for each variable; ALT: Serum alkaline alanine transaminase; ALP: Serum alkaline phosphatase; GGT: Serum gamma glutamyl transferase.

# CHAPTER SEVEN: RADIOLOGY EXAMINATION AT BASELINE

Dual energy X-ray absorptiometry (DXA) scans were performed to measure regional fat and lean mass body composition.

## 7.1. Fat distribution by DUAL Energy X-ray Absorptiometry (DXA)

Table 7.1 shows baseline regional fat and lean body mass distribution in HIV negative control subjects (group 1), HIV infected subjects not starting ART (group 2) and HIV infected

subjects starting ART (group 3). When compared with group 1, subjects in group 3 had significantly lower mean fat mass for the following regions: total (p=0.0009), left arm (p=0.005), right arm (p=0.0007), left leg (p=0.008), right leg (p=0.009), trunk (p=0.0004). Body cell mass was lower in group 3 when compared to group 1 (p=0.0008). Compared to group 1, group 3 subjects had a lower lean mass in the right leg region (p=0.03); however, lean mass was not significantly different between the three groups in the rest of the regions measured.

**Table 7.1: Regional fat and lean body mass distribution, DXA scan at baseline**

| Variable | Group1 (n = 88) | Group2 (n = 88) | Group3 (n = 150) | p |
|---|---|---|---|---|
| Body cell mass (g) | 75052.9 ± 18926.5 | 75014.2 ± 18441.3 | 67192.9 ± 15215.2 | 0.0008 |
| **Fat mass (g)** | | | | |
| Total | 26858.8 ± 15436.01 | 26206.7 ± 14261.7 | 20396.7 ± 11176.5 | 0.0009 |
| Left arm | 1551.98± 939.6 | 1531.8 ± 965.4 | 1198.0 ± 713.2 | 0.005 |
| Right arm | 1584 ± 920.4 | 1536.8 ± 925.2 | 1173.7 ± 671.4 | 0.0007 |
| Left leg | 5450.2 ± 3052.3 | 5525.3 ± 2724.0 | 4475.7 ± 2320.3 | 0.008 |
| Right leg | 5621.95 ± 3113.2 | 5635.5 ± 2803.6 | 4605.5 ± 2428.6 | 0.009 |
| Trunk | 11658.4 ± 7740.8 | 11034.1 ± 7253.7 | 8145.1 ± 5422.6 | 0.0004 |
| **Lean mass (g)** | | | | |
| Total | 48194.0 ± 7503.6 | 48807.4± 8088.3 | 46796.2 ± 9370.5 | 0.2 |
| L Arm | 2647.8 ± 695.5 | 2668.1± 669.5 | 2524.9 ± 761.6 | 0.2 |
| R Arm | 2846.7 ± 629.7 | 2797.8 ± 693.8 | 2655..3 ± 770.4 | 0.1 |
| L Leg | 8301.1 ± 1583.9 | 8328.3 ± 1593.2 | 7874.3 ± 1873.9 | 0.1 |
| R Leg | 8510.6 ± 1680.0 | 8382.2 ± 1670.6 | 7920.1 ± 1835.8 | 0.03 |
| Trunk | 22254.6 ± 3418.1 | 22959.9 ± 3789.1 | 22201.3 ± 4205.8 | 0.3 |
| Total BMD | 1.1 ± 0.1 | 1.12 ± 0.1 | 1.14 ± 0.11 | 0.1 |
| Missing | 9 (10.2) | 7 (7.9) | 13 (8.7) | |

Data expressed as Mean±SD. Group 1: HIV negative, Group 2: HIV infected not starting ART, Group 3: HIV infected starting ART. BMD: bone mineral density

Using multiple linear regression, age and fasting glucose were positively associated with fat mass for left arm, right arm, trunk and total fat mass (Table 7.2); right leg and left leg fat mass were positively associated with age only. A unit higher fasting plasma glucose was associated with a nearly 2kg higher total body fat mass and 1kg higher trunk fat mass. Men had 15kg lower total body fat mass and 6kg lower trunk fat mass when compared to females. Group 3 had nearly 6kg lower total body fat mass and 3kg lower trunk fat when compared with group 1.

In multiple linear regression, the effect of age, gender, glucose and group on fat mass at baseline was estimated (Table 7.2 and 7.3). Least square estimates were used to show means for each of the measured fat areas by gender and group (Table 7.2). In all measured regions, the fat mass was higher in females and lower in group 3 subjects.

There was a significant positive association between fat mass and CD4 cell count for each measured area for groups 2 and 3, but no significant association with HIV viral load (Table 7.3).

**Table 7.2: Multivariate analysis on fat mass distribution by DXA scan in Group 1, 2 and 3 at baseline (n=297)**

| | Multiple linear regression | | | | Least Square Estimates | | |
|---|---|---|---|---|---|---|---|
| Variable | Estimate | Standard Error | p | Variable | Estimate | Standard Error | p |
| **Fat mass (g)** | | | | | | | |
| **Left arm** | | | | | | | |
| Group 1 | reference | | | Group 1 | 1412.99 | 78.1302 | <0.0001 |
| Group 2 | -275.07 | 94.1348 | 0.0038 | Group 2 | 1354.34 | 75.6234 | <0.0001 |
| Group 3 | -333.71 | 96.7593 | 0.0007 | Group 3 | 1079.27 | 58.8644 | <0.0001 |
| Female | reference | | | Female | 1757.55 | 49.7748 | <0.0001 |
| Male | -950.7 | 82.4193 | <0.0001 | Male | 806.85 | 66.8874 | <0.0001 |
| Age | 19.8456 | 3.7422 | <0.0001 | | | | |
| Glucose | 153.77 | 62.1835 | 0.01 | | | | |
| **Right arm** | | | | | | | |
| Group 1 | reference | | | Group 1 | 1430.56 | 76.1382 | <0.0001 |
| Group 2 | -286.19 | 91.7348 | 0.002 | Group 2 | 1360.22 | 73.6954 | <0.0001 |
| Group 3 | -356.53 | 94.2924 | 0.0002 | Group 3 | 1074.03 | 57.3637 | <0.0001 |
| Female | reference | | | Female | 1753.64 | 48.5058 | <0.0001 |
| Male | -930.73 | 80.318 | <0.0001 | Male | 822.9 | 65.1821 | <0.0001 |
| Age | 19.2134 | 3.6468 | <0.0001 | | | | |
| Glucose | 163.91 | 60.5982 | 0.0073 | | | | |
| **Left leg** | | | | | | | |
| Group 1 | reference | | | Group 1 | 4948.2 | 237.39 | <0.0001 |
| Group 2 | -867.99 | 286.02 | 0.0026 | Group 2 | 4925.92 | 229.77 | <0.0001 |
| Group 3 | -890.27 | 293.99 | 0.0027 | Group 3 | 4057.93 | 178.85 | <0.0001 |
| Female | reference | | | Female | 6373.26 | 151.24 | <0.0001 |
| Male | -3458.48 | 250.42 | <0.0001 | Male | 2914.77 | 203.23 | <0.0001 |
| Age | 62.5871 | 11.3704 | <0.0001 | | | | |
| **Right leg** | | | | | | | |
| Group 1 | reference | | | Group 1 | 5077.01 | 245.45 | <0.0001 |
| Group 2 | -833.42 | 295.73 | 0.0052 | Group 2 | 5009.06 | 237.58 | <0.0001 |
| Group 3 | -901.37 | 303.98 | 0.0007 | Group 3 | 4175.64 | 184.93 | <0.0001 |
| Female | reference | | | Female | 6549.36 | 156.37 | |
| Male | -3590.91 | 258.93 | <0.0001 | Male | 2958.45 | 210.13 | <0.0001 |
| Age | 63.6791 | 11.7567 | <0.0001 | | | | <0.0001 |
| **Trunk** | | | | | | | |
| Group 1 | reference | | | Group 1 | 10569 | 627.7 | <0.0001 |
| Group 2 | -2469.74 | 756.28 | 0.0012 | Group 2 | 9907.2 | 607.56 | <0.0001 |
| Group 3 | -3131.74 | 777.37 | <0.0001 | Group 3 | 7437.46 | 472.92 | <0.0001 |
| Female | reference | | | Female | 12553 | 399.89 | <0.0001 |
| Male | -6496.67 | 662.16 | <0.0001 | Male | 6056.28 | 537.37 | <0.0001 |
| Age | 200.81 | 30.0653 | <0.0001 | | | | |
| Glucose | 1174.88 | 499.58 | 0.0194 | | | | |

Continued on next page

**Table 7.2 cont.: Multivariate analysis on fat mass distribution by DXA scan in Group 1, 2 and 3 at baseline (n=297)**

**Total**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Group 1 | reference | | | Group 1 | 24435.0 | 1216.79 | <0.0001 |
| Group 2 | -4846.14 | 1466.04 | 0.0011 | Group 2 | 23512.0 | 1177.75 | <0.0001 |
| Group 3 | -5769.31 | 1506.91 | <0.0001 | Group 3 | 18666.0 | 916.75 | <0.0001 |
| Female | reference | | | Female | 29873.0 | 775.18 | <0.0001 |
| Male | -15338.0 | 1283.59 | <0.0001 | Male | 14535.0 | 1041.69 | <0.0001 |
| Age | 368.36 | 58.2812 | <0.0001 | | | | |
| Glucose | 1952.04 | 968.44 | 0.0448 | | | | |

Group 1: HIV negative, Group 2: HIV infected not starting ART, Group 3: HIV infected starting ART.

Least square estimates for measuring means for each measured area by group and gender.

**Table 7.3: Multivariate analysis on the association between DXA scan fat distribution and HIV-1 infection measures for Group 2 and 3 at baseline (n=218)**

| Variable | Multiple linear regression | | |
|---|---|---|---|
| | Estimate | Standard Error | p |
| **Left arm** | | | |
| CD 4 count | 0.9211 | 0.2877 | 0.0016 |
| HIV RNA | -0.00026 | 0.000160 | 0.1018 |
| | | | |
| **Right arm** | | | |
| CD 4 count | 0.9942 | 0.2752 | 0.0004 |
| HIV RNA | -0.00026 | 0.000153 | 0.0914 |
| | | | |
| **Left leg** | | | |
| CD 4 count | 2.3502 | 0.9005 | 0.0098 |
| HIV RNA | -0.00072 | 0.000501 | 0.1543 |
| | | | |
| **Right leg** | | | |
| CD 4 count | 2.4260 | 0.9337 | 0.0101 |
| HIV RNA | -0.00074 | 0.000519 | 0.1546 |
| | | | |
| **Trunk** | | | |
| CD 4 count | 7.7377 | 2.2125 | 0.0006 |
| HIV RNA | -0.00167 | 0.001230 | 0.1763 |
| | | | |
| **Total** | | | |
| CD 4 cell count | 14.6271 | 4.4676 | 0.0013 |
| HIV RNA | -0.00367 | 0.002484 | 0.1409 |

Group 2: HIV infected subjects not starting ART, Group 3: HIV infected subjects starting ART.

## 7.2. Abdominal fat distribution by CT scan

Table 7.4 shows the fat distribution in the abdominal region for the three groups at baseline. No significant differences in fat distribution were found between the groups for each of the measured areas: total fat, visceral and subcutaneous fat and waist size.

Table 7.4: Fat distribution, CT scan at baseline

| Variable | Group 1 (n = 88) | Group 2 (n = 88) | Group 3 (n = 150) | p |
|---|---|---|---|---|
| Total Fat Area | 338.01 $\pm$ 227.94 | 336.23 $\pm$ 211.85 | 294.48 $\pm$ 209.72 | 0.3 |
| Visceral Fat Area | 72.86 $\pm$ 56.44 | 79.97 $\pm$ 55.45 | 61.71 $\pm$ 49.25 | 0.1 |
| Sub Fat Area | 0.37 $\pm$ 0.32 | 0.43 $\pm$ 0.33 | 0.46 $\pm$ 0.67 | 0.5 |
| Subcutaneous Fat Area | 265.16 $\pm$ 187.96 | 256.25 $\pm$ 174.48 | 232.61 $\pm$ 176.85 | 0.4 |
| Waist Size | 107.72 $\pm$ 85.57 | 99.09 $\pm$ 16.78 | 94.40 $\pm$ 17.32 | 0.2 |
| Missing | 22 (25.0) | 27 (30.7) | 28 (31.8) | |

Data expressed as Mean$\pm$SD or n(%). Group 1: HIV negative, Group 2: HIV infected not starting ART, and Group 3: HIV infected starting ART.

# CHAPTER EIGHT: RADIOLOGY EXAMINATION DURING FOLLOW-UP IN GROUP 3 SUBJECTS

## 8.1. Fat distribution by DUAL Energy X-ray Absorptiometry

Dual energy X-ray absorptiometry (DXA) scans were performed to measure regional fat and lean mass body composition at baseline and follow-up (12 months and 24 months).

Table 8.1, Figure 8.1 and 8.2 show regional fat and lean mass body composition in group 3 (HIV infected starting ART) subjects during the study period. There was an increase in right arm, left arm, right leg, left leg, trunk and total fat mass during the 24 months of follow up.

At all measured regions, fat mass was significantly higher at 24 months than at baseline (Table 8.2). There was no significant change in lean mass for any of the measured regions during the study period.

There was a significant increase in fat mass by DXA scan in all the measured regions at 24 months compared with baseline; however, the increase at 12 months was not significant. Trunk and total fat mass were 4kg and 9kg, higher respectively, at 24 months on ART compared to baseline prior to initiation of ART (Table 8.3).

At baseline, in the multivariate linear mixed models, age and gender were significantly associated with fat mass. Fat mass in all measured regions was significantly higher in older subjects while males had a significantly lower fat mass in all measured regions compared to females (Table 8.4).

**The increase in fat mass during the study period was higher in men (vs. women) and lower with increasing age (Table 8.5). Although subjects on efavirenz had a higher fat mass in all the regions, the increase over time was lower in those taking efavirenz compared to nevirapine in all regions except for right and left arms. Subjects with higher CD4 cell counts had a higher fat mass in all regions at baseline but the increase in fat mass over time was significantly lower with increasing CD 4 cell count.Table 8.1: Fat distribution by DXA scan for Group 3 during 24 months of follow-up.**

| | Baseline (n=137) | 12 months (n= 70) | 24 months (n=87) | p |
|---|---|---|---|---|
| **Fat mass (g)** | | | | |
| Total | 21322.94+12153.63 | 27907.54+12763.11 | 30400.10+13444.71 | <0.0001 |
| Left arm | 1243.68+751.96 | 1558.58+743.09 | 1777.89+828.75 | <0.0001 |
| Right arm | 1232.54+731.01 | 1559.53+743.79 | 1788.14+837.03 | <0.0001 |
| Left leg | 4661.53+2529.91 | 6054.13+2732.32 | 6421.64+2776.76 | <0.0001 |
| Right leg | 4806.47+2661.69 | 6266.79+2848.07 | 6719.50+2915.70 | <0.0001 |
| Trunk | 8556.88+5834.77 | 11439.56+6082.27 | 12592.89+6579.15 | <0.0001 |
| **Lean mass(g)** | | | | |
| Total | 46950.85+9246.00 | 48070.34+9969.38 | 47132.96+9462.62 | 0.78 |
| Left arm | 2545.46+749.68 | 2634.39+815.47 | 2614.81+791.81 | <0.0001 |
| Right arm | 2679.04+768.89 | 2764.79+812.67 | 2789.12+805.94 | <0.0001 |
| Left leg | 7926.85+1831.85 | 8204.32+2008.20 | 7999.69+1803.51 | 0.68 |
| Right leg | 7988.93+ 1822.80 | 8315.09+1996.65 | 8132.73+1856.10 | 0.68 |
| Trunk | 22193.07+4158.25 | 22616.09+4493.41 | 22124.85+4271.81 | 0.33 |

Data    Mean+SD.    Group    3:    HIV-1    infected    subjects    starting    ART.    g:    gram.

**Figure 8.1: Fat mass distribution by Dual Energy X-ray absorptiometry during follow-up in group 3**
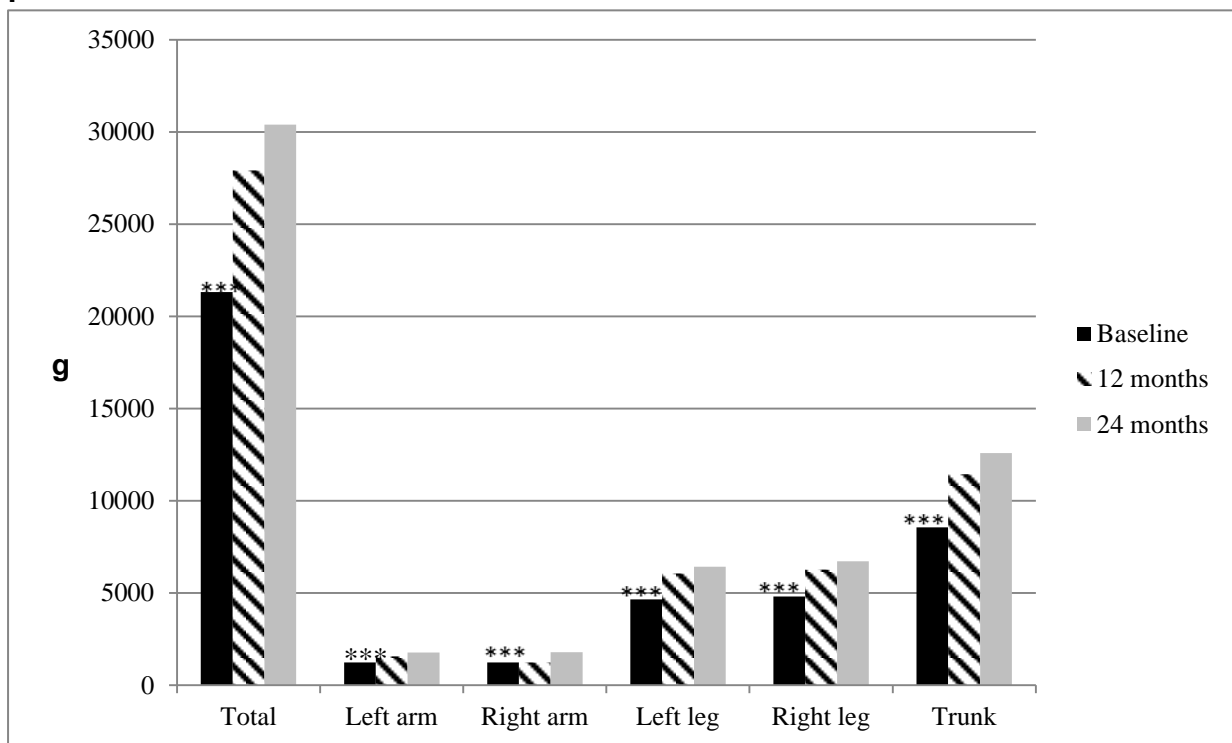
.



***P<0.001: baseline vs. other time points: change in fat mass during follow-up using linear mixed models. g: gram.

**Figure 8.2: Lean mass distribution by Dual Energy X-ray absorptiometry during follow-up in group 3.**
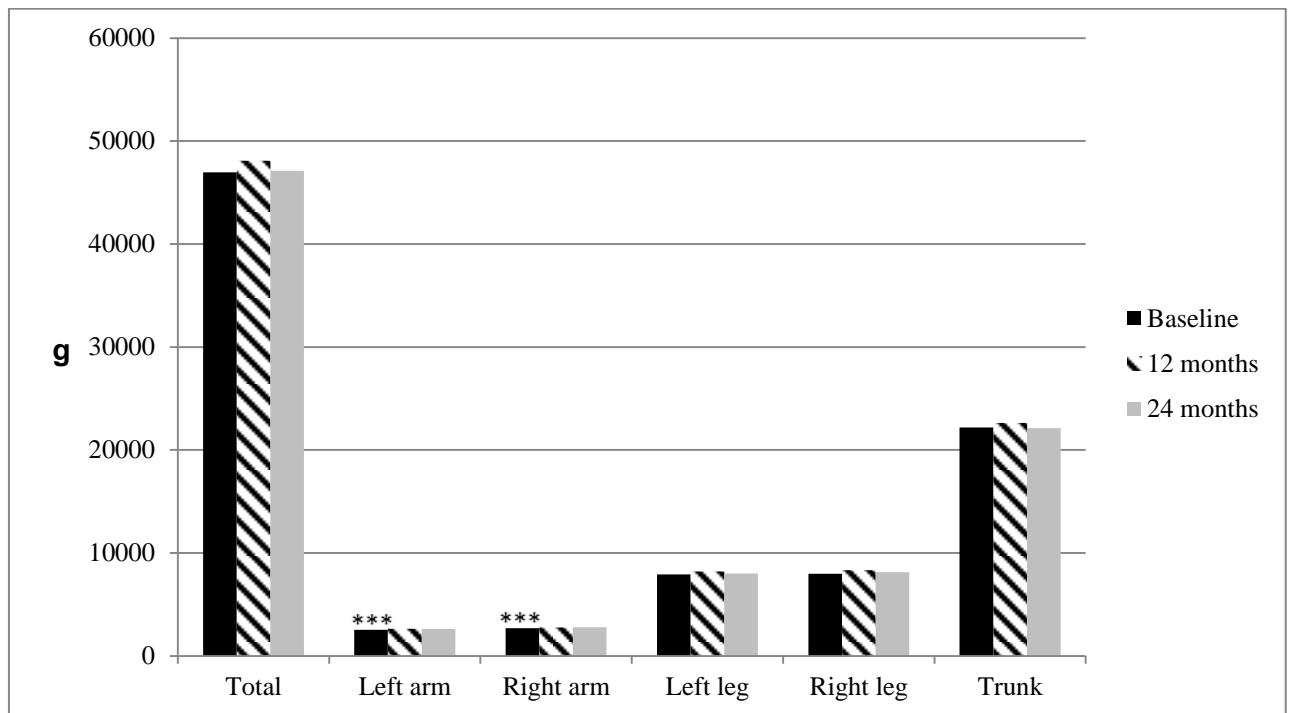


***P<0.001baseline vs. other time points: change in fat mass during follow-up using linear mixed models. g: gram.

**Table 8.2: Fat mass by DXA scan at baseline and at the 24 months follow-up in group 3 subjects on ART[*].**

| | Baseline (n= 87) | 24 Months (n=87) | Mean Difference | p |
|---|---|---|---|---|
| **Fat mass (g)** | | | | |
| Total | 21036.6 $\pm$ 11920.4 | 30400.1 $\pm$ 13444.7 | 9363.5 $\pm$ 6778.2 | <0.0001 |
| Left arm | 1256.5 $\pm$ 780.0 | 1777.9 $\pm$ 828.8 | 521.4 $\pm$ 424 | <0.0001 |
| Right arm | 1222.1 $\pm$ 746.4 | 1788.1 $\pm$ 837.0 | 566 $\pm$ 465.3 | <0.0001 |
| Left leg | 4606.2 $\pm$ 2449.3 | 6421.6 $\pm$ 2776.8 | 1815.4 $\pm$ 1308.1 | <0.0001 |
| Right leg | 4768.99 $\pm$ 2604.96 | 6719.5 $\pm$ 2915.7 | 1950.5 $\pm$ 1396.3 | <0.0001 |
| Trunk | 8361 $\pm$ 5617.7 | 12592.9 $\pm$ 6579.2 | 4231.9 $\pm$ 3446.6 | <0.0001 |
| **Lean mass (g)** | | | | |
| Total | 47275.1 $\pm$ 9182.5 | 47132.96 $\pm$ 9462.6 | -142.1 $\pm$ 2848.4 | 0.6 |
| Left arm | 2592.6 $\pm$ 754.0 | 2614.8 $\pm$ 791.8 | 22.2 $\pm$ 232.8 | 0.4 |
| Right arm | 2750.7 $\pm$ 776.0 | 2789.1 $\pm$ 805.9 | 38.4 $\pm$ 240.5 | 0.1 |
| Left leg | 8000.0 $\pm$ 1816.8 | 7999.7 $\pm$ 1803.5 | -0.4 $\pm$ 663.8 | 0.996 |
| Right leg | 8026.2 $\pm$ 1828.5 | 8132.7 $\pm$ 1856.1 | 106.5 $\pm$ 743.6 | 0.2 |
| Trunk | 22285.4 $\pm$ 4127.2 | 22124.9 $\pm$ 4271.8 | -160.6 $\pm$ 1419.2 | 0.3 |

p:paired Student's t-test. Calculated mean difference (24 months-baseline mean) for data available at baseline and at follow up. g: gram.

**Table 8.3: Multivariate linear mixed models analysis on the change of fat mass distribution by DXA scan during follow-up in group 3 subjects during 24 months follow-up on ART.**

| | Linear Mixed Model | | | Least Square Estimates | | |
|---|---|---|---|---|---|---|
| Variable | Estimate | Standard Error | p | Variable | Estimate | Standard Error |
| **Fat mass (g)** | | | | | | |
| **Left arm** | | | | | | |
| Visit 1 | reference | | | Visit 1 | 1243.68 | 66.0772 |
| Visit 2 | 219.30 | 124.18 | 0.0794 | Visit 2 | 1558.58 | 92.4406 |
| Visit 3 | 534.21 | 106.03 | <0.0001 | Visit 3 | 1777.89 | 82.9186 |
| **Right arm** | | | | | | |
| Visit 1 | reference | | | Visit 1 | 1232.54 | 65.5118 |
| Visit 2 | 228.61 | 123.12 | 0.0652 | Visit 2 | 1559.53 | 91.6497 |
| Visit 3 | 555.59 | 105.12 | <0.0001 | Visit 3 | 1788.14 | 82.2092 |
| **Left leg** | | | | | | |
| Visit 1 | reference | | | Visit 1 | 4661.53 | 226.69 |
| Visit 2 | 367.52 | 426.02 | 0.3896 | Visit 2 | 6054.13 | 317.13 |
| Visit 3 | 1760.12 | 363.74 | <0.0001 | Visit 3 | 6421.64 | 284.47 |
| **Right leg** | | | | | | |
| Visit 1 | reference | | | Visit 1 | 4806.47 | 237.79 |
| Visit 2 | 452.71 | 446.89 | 0.3126 | Visit 2 | 6266.79 | 332.67 |
| Visit 3 | 1913.04 | 381.56 | <0.0001 | Visit 3 | 6719.50 | 298.40 |
| **Trunk** | | | | | | |
| Visit 1 | reference | | | Visit 1 | 8556.88 | 523.01 |
| Visit 2 | 1153.33 | 982.90 | 0.2424 | Visit 2 | 11440 | 731.68 |
| Visit 3 | 4036.01 | 839.22 | <0.0001 | Visit 3 | 12593 | 656.31 |
| **Total** | | | | | | |
| Visit 1 | reference | | | Visit 1 | 21323 | 1084.32 |
| Visit 2 | 2492.57 | 2037.79 | 0.2231 | Visit 2 | 27908 | 1516.95 |
| Visit 3 | 9077.17 | 1739.90 | <0.0001 | Visit 3 | 30400 | 1360.69 |

Visit 1= baseline, n=137; Visit 2 = 12months, n=70; Visit 3= 24 months, n=87. Multivariate linear mixed least square estimates for measuring means for each measured area during follow-up. g: gram.

**Table 8.4: Multivariate linear mixed models analysis on the factors associated with fat mass distribution by DXA scan in Group 3 at baseline.**

| Variable | Linear Mixed Model | | |
|---|---|---|---|
| | Estimate | Standard Error | p |
| **Fat mass (g)** | | | |
| **Left arm** | | | |
| Female | Reference | | |
| Male | -899.35 | 8.7978 | <0.0001 |
| Age | 33.3038 | 94.1348 | 0.0038 |
| Efavirenz | 5.3572 | 177.46 | 0.9760 |
| CD4 count | -0.08765 | 0.8691 | 0.9199 |
| **Right arm** | | | |
| Female | Reference | | |
| Male | -876.07 | 183.35 | 0.0002 |
| Age | 32.4395 | 9.1000 | <0.0001 |
| Efavirenz | -50974 | 183.56 | 0.9779 |
| CD4 cell count | 0.04757 | 0.8989 | 0.9579 |
| **Left leg** | | | |
| Female | Reference | | |
| Male | -3485.30 | 579.21 | <0.0001 |
| Age | 86.7458 | 28.7467 | 0.0034 |
| Efavirenz | 27.4262 | 579.86 | 0.9624 |
| CD4 cell count | -3.6214 | 2.8398 | 0.2059 |
| **Right leg** | | | |
| Female | Reference | | |
| Male | -3607.80 | 607.89 | <0.0001 |
| Age | 93.8041 | 30.1705 | 0.0026 |
| Efavirenz | 38.0507 | 608.58 | <0.9503 |
| CD4cell count | -4.2053 | 2.9804 | 0.1621 |
| **Trunk** | | | |
| Female | Reference | | |
| Male | -5167.59 | 1516.56 | 0.0010 |
| Age | 304.08 | 75.2685 | 0.0001 |
| Efavirenz | 429.30 | 1518.26 | 0.7781 |
| CD4 cell count | -2.5170 | 7.4355 | 0.7359 |
| **Total** | | | |
| Female | Reference | | |
| Male | -5167.59 | 1516.56 | 0.0001 |
| Age | 304.08 | 75.2685 | 0.0001 |
| Efavirenz | 429.30 | 1518.26 | 0.7781 |
| CD4 cell count | -2.5170 | 7.4355 | 0.7359 |

g:gram. Efavirenz compared with nevirapine as reference.

**Table 8.5: Univariate and multivariate linear mixed models analysis on the factors associated with fat mass distribution by DXA during 24 months follow-up in Group 3 .**

| Variable | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|
| | Estimate | Standard error | p | Estimate | Standard error | p |
| **Left arm fat** | | | | | | |
| Age | 0.4696 | 0.09426 | <0.0001 | 0.4781 | 0.07599 | <0.0001 |
| Age*visit | -0.01306 | 0.01135 | 0.25 | 0.008022 | 0.01264 | 0.53 |
| Female | Reference | | | Reference | | |
| Male | -10.8223 | 1.2785 | <0.0001 | -11.3974 | 1.3305 | <0.0001 |
| Female*visit | Reference | | | Reference | | |
| Male*visit | 0.9275 | 0.2203 | <0.0001 | 1.6504 | 0.5526 | 0.004 |
| Nevirapine | Reference | | | Reference | | |
| Efavirenz | 30.9934 | 1.3065 | <0.0001 | -0.9681 | 1.5303 | 0.53 |
| Nevirapine*visit | Reference | | | Reference | | |
| Efavirenz*visit | 0.01864 | 0.2085 | 0.93 | -0.5755 | 0.2573 | 0.03 |
| CD4 count | 0.03038 | 0.005234 | <0.0001 | 0.01441 | 0.004534 | 0.002 |
| CD4 count*visit | -0.00444 | 0.000877 | <0.0001 | -0.00177 | 0.000803 | 0.03 |
| **Right arm fat** | | | | | | |
| Age | 0.4596 | 0.09324 | <0.0001 | 0.4647 | 0.07474 | <0.0001 |
| Age*visit | -0.01189 | 0.01231 | 0.34 | 0.008407 | 0.01400 | 0.55 |
| Female | Reference | | | | | |
| Male | -11.2433 | 1.3116 | <0.0001 | -11.9000 | 1.3643 | <0.0001 |
| Female*visit | Reference | | | | | |
| Male*visit | 0.9280 | 0.2398 | 0.0002 | 1.8278 | 0.6118 | 0.004 |
| Nevirapine | Reference | | | Reference | | |
| Efavirenz | 30.7778 | 1.2898 | <0.0001 | -0.7628 | 1.5153 | 0.62 |
| Nevirapine*visit | Reference | | | Reference | | |
| Efavirenz*visit | 0.02874 | 0.2258 | 0.899 | -0.5460 | 0.2849 | 0.09 |
| CD4 count | 0.03444 | 0.005688 | <0.0001 | 0.01579 | 0.004763 | 0.001 |
| CD4 count*visit | -0.00525 | 0.000957 | <0.0001 | -0.00216 | 0.000862 | 0.01 |
| **Left leg fat** | | | | | | |
| Age | 0.7211 | 0.1727 | <0.0001 | 94.2276 | 19.6701 | <0.0001 |
| Age*visit | -0.01999 | 0.01935 | 0.30 | 6.8388 | 3.3525 | 0.04 |
| Female | Reference | | | | | |

| Variable | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|
| | Estimate | Standard error | p | Estimate | Standard error | p |
| Male | -18.5806 | 2.2636 | <0.0001 | -2205.99 | 325.64 | <0.0001 |
| Female*visit | Reference | | | Reference | | |
| Male*visit | 1.2613 | 0.3856 | 0.00 | 200.05 | 146.55 | 0.12 |
| Nevirapine | Reference | | | Reference | | |
| Efavirenz | 60.8320 | 2.3353 | <0.0001 | -86.2663 | 392.61 | 0.83 |
| Nevirapine*visit | Reference | | | Reference | | |
| Efavirenz*visit | -0.1626 | 0.3545 | 0.65 | -182.78 | 67.7614 | 0.009 |
| CD4 count | 0.05676 | 0.008575 | <0.0001 | 4.2896 | 1.1013 | 0.0002 |
| CD4 count*visit | -0.00941 | 0.001435 | <0.0001 | -0.7307 | 0.1947 | 0.0003 |
| **Right leg fat** | | | | | | |
| Age | 0.7063 | 0.1808 | 0.0002 | 0.7092 | 0.1466 | <0.0001 |
| Age*visit | -0.01444 | 0.02008 | 0.47 | 0.02790 | 0.02236 | 0.22 |
| Female | Reference | | | Reference | | |
| Male | -20.0573 | 2.3438 | <0.0001 | -20.4569 | 2.4743 | <0.0001 |
| Female*visit | Reference | | | Reference | | |
| Male*visit | 1.3470 | 0.3977 | 0.001 | 2.8483 | 0.9778 | 0.005 |
| Nevirapine | Reference | | | Reference | | |
| Efavirenz | 61.4186 | 2.4278 | <0.0001 | -1.0505 | 2.9357 | 0.72 |
| Nevirapine*visit | Reference | | | Reference | | |
| Efavirenz*visit | -0.2200 | 0.3666 | <0.0001 | -1.2947 | 0.4553 | 0.006 |
| CD4 count | 0.06246 | 0.009088 | <0.0001 | 0.03454 | 0.008236 | <0.0001 |
| CD4 count*visit | -0.01010 | 0.001516 | <0.0001 | -0.00561 | 0.001441 | 0.0002 |
| **Trunk fat** | | | | | | |
| Age | 1.4696 | 0.2744 | <0.0001 | 1.4518 | 0.2433 | <0.0001 |
| Age*visit | -0.02285 | 0.03346 | 0.496 | 0.04060 | 0.03659 | 0.27 |
| Female | Reference | | | Reference | | |
| Male | -25.9527 | 3.8823 | <0.0001 | -27.9211 | 4.0610 | <0.0001 |
| Female*visit | Reference | | | Reference | | |
| Male*visit | 2.4352 | 0.6389 | 0.0003 | 4.6806 | 1.5986 | 0.005 |
| Nevirapine | Reference | | | Reference | | |
| Efavirenz | 81.5953 | 3.8758 | <0.0001 | -0.6461 | 4.8656 | 0.894 |
| Nevirapine*visit | Reference | | | Reference | | |

| Variable | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|
| | Estimate | Standard error | p | Estimate | Standard error | p |
| Efavirenz*visit | -0.1229 | 0.6126 | 0.84 | -1.9375 | 0.7451 | 0.01 |
| CD4 count | 0.09572 | 0.01488 | <0.0001 | 0.04632 | 0.01328 | 0.0008 |
| CD4 count*visit | -0.01561 | 0.002501 | <0.0001 | -0.00731 | 0.002321 | 0.002 |
| | | | | | | |
| **Whole body total fat** | | | | | | |
| age | 1.8313 | 0.3745 | <0.0001 | 1.8235 | 0.3133 | <0.0001 |
| Age*visit | -0.03339 | 0.04435 | <0.0001 | 0.05601 | 0.04922 | 0.26 |
| Female | Reference | | | Reference | | |
| Male | -39.1663 | 5.1046 | <0.0001 | -41.5098 | 5.3264 | <0.0001 |
| Female*visit | Reference | | | Reference | | |
| Male*visit | 3.2050 | 0.8616 | 0.0004 | 6.5819 | 2.1499 | 0.003 |
| Nevirapine | Reference | | | Reference | | |
| Efavirenz | 130.57 | 5.1951 | <0.0001 | 1.8095 | 6.2815 | 0.77 |
| Nevirapine*visit | Reference | | | Reference | | |
| Efavirenz*visit | -0.2277 | 0.8121 | 0.78 | 2.6567 | 1.0022 | 0.0097 |
| CD4 count | 0.1258 | 0.01962 | <0.0001 | 0.06913 | 0.01755 | 0.03 |
| CD4 count*visit | -0.02045 | 0.003290 | <0.0001 | -0.01106 | 0.003086 | 0.02 |

## 8.2. Abdominal Fat Distribution by CT scan

Table 8.6 shows the fat area distribution at the various time points of the study. Visceral, subcutaneous and total fat area measured by CT scan through L4 spine increased significantly at the 24 month follow up compared to baseline. The mean difference using paired Student's t-test between baseline and 24 months also shows a significant increase in fat area (Table 8.7).

**Table 8.6: Fat area distribution by CT scan through L4 spine during 24 months follow up in group 3 subjects.**

| Variable | Baseline (n = 122) | 12 Months (n = 53) | 24 Months (n = 42) | p |
|---|---|---|---|---|
| Total fat | $294.5 \pm 209.7$ | $325.6 \pm 195.1$ | $334.4 \pm 212.9$ | <0.0001 |
| Visceral fat | $61.7 \pm 49.3$ | $68.4 \pm 54.2$ | $71.0 \pm 50.1$ | 0.0025 |
| Subcutaneous:visceral fat | $0.5 \pm 0.7$ | $0.4 \pm 0.3$ | $0.4 \pm 0.5$ | 0.2014 |
| Subcutaneous fat | $232.6 \pm 176.9$ | $255.8 \pm 169.97$ | $262.6 \pm 180.8$ | <0.0001 |
| Waist size | $94.4 \pm 17.3$ | $98.1 \pm 16.4$ | $98.8 \pm 17.1$ | <0.0001 |

Data expressed as Mean$\pm$SD

**Table 8.7: Abdominal fat distribution by CT scan for baseline and 24 months in group 3 subjects on ART**

| Variable | Baseline (n =35 ) | 24 Months (n =35 ) | Mean Difference (n=35) | p |
|---|---|---|---|---|
| Total fat area | 263.7 + 211.4 | 340.6 + 216.99 | 75.6 + 106.7 | 0.0002 |
| Visceral fat area | 53.9 + 38.9 | 67.6 + 45.6 | 13.5 + 26.5 | 0.005 |
| Subcutaneous: visceral fat area ratio | 0.6 + 1.1 | 0.4 + 0.5 | 0.2 + 0.1 | 0.09 |
| Subcutaneous fat area | 209.3 + 181.7 | 272.0 + 186.1 | 61.8 + 88.8 | 0.0002 |
| Waist size | 91.7 + 14.6 | 98.4 + 17.1 | 6.3 + 9.3 | 0.0005 |

Calculated mean difference (24 months-baseline mean) for data available at baseline and at follow up. p: Paired Student's t-test. *97 subjects completed follow up; n= number for which data was available for each variable

# CHAPTER NINE: DISCUSSION

This study on the metabolic complications of antiretroviral therapy (ART) in South Africans of Zulu descent has highlighted several observations, both in the cross-sectional and prospective study and in relation to HIV-1 infection, antiretroviral therapy and HIV negative controls.

## 9.1. FAT DISTRIBUTION

### 9.1.1. Cross sectional / Baseline

No significant fat redistribution was found on participant report, physical examination and DXA scan in the HIV-1 infected subjects starting ART (HIV-ART), HIV-1 infected subjects not starting ART (HIV-no ART) and HIV negative control subjects (controls). The three groups studied were similar with respect to total fat, visceral and subcutaneous fat and waist size as measured by CT scan through L4.

There was a positive association between peripheral and central lipoatrophy and between peripheral and central lipohypertrophy in the HIV-1 infected subjects and in HIV negative controls. All HIV infected subjects were ART naïve at baseline. The prevalence of overweight/obesity (BMI $\geq$ 25 kg/m$^2$) was 50.7% in HIV-ART compared with 62.0% in controls. HIV-ART subjects had peripheral lipoatrophy by participant and physician concordance which was similar to that found in controls. Physician examination findings showed no difference in fat loss and fat gain between HIV- ART and control subjects.

Physician examination findings were supported by findings of no difference between the groups for anthropometric measurements, i.e. circumference and skin fold measurements for all sites measured. Therefore, fat distribution did not distinguish HIV-1 infected from control subjects. The study and control subjects were of the same ethnicity, similar for other demographic characteristics and all had mean BMI in overweight/obese category, it is therefore probable that HIV infection did not affect fat distribution in this cohort.

In an earlier study on a cohort also with a high prevalence of overweight/obesity, Mulligan et al reported that peripheral lipoatrophy was significantly higher in HIV infected women on ART than in HIV infected women not taking ART and HIV negative control subjects (89). Use of ART (stavudine or indinavir) in HIV infected men, the majority of whom were white, was also associated with lipoatrophy (90). Furthermore, in the Tien and Pallela studies, lipoatrophy distinguished HIV infected from control subjects (91, 92). Other investigators found peripheral lipoatrophy in association with central lipohypertrophy in patients on ART (5, 10, 11, 93). While the finding of peripheral lipoatrophy in association with central lipohypertrophy fitted with the syndrome recognized as lipodystrophy at the time, the use of uni-directional instruments may have contributed to the lack of identifying peripheral lipoatrophy that may have been associated with central lipoatrophy

In contrast to findings by physician examination and anthropometric measurements, there was a significant difference in fat mass measured by DXA scan in HIV-1 infected (HIV-ART and HIV-no ART) compared with control subjects. The finding of significantly lower fat mass prevalence at all measured regions (arms, legs, trunk and total fat mass) by DXA scan in HIV- ART subjects is consistent with a significantly lower central and peripheral lipohypertrophy prevalence based on the FRAM questionnaire (12). Peripheral and central lipoatrophy has also been found in HIV infected subjects by other investigators using FRAM questionnaires (7, 89). Central lipohypertrophy was significantly associated with trunk fat measured by DXA scan in this study. The association between central lipohypertrophy and trunk fat mass using DXA (88) and MRI scan (90) has been shown previously. However, DXA scan is not able to distinguish subcutaneous from visceral fat, therefore fat distribution in these compartments cannot be determined with DXA scan alone. In a cross-sectional study in S. Africa, visceral fat mass was estimated from measurement of waist circumference and abdominal skinfold thickness, but no radiology was performed (94). In

that study, subjects with dysglycaemia had significantly more visceral fat and peripheral wasting.

Trunk fat by magnetic resonance imaging (MRI) scan was found to be significantly lower in women on a non-PI-based ART regimen compared to women on PI-based ART regimen (88) suggesting a link between PI therapy and increased trunk fat.

The significantly lower fat mass on DXA scan, coupled with a significantly lower central and peripheral lipohypertrophy found clinically, suggests a reduction in fat mass both peripherally and centrally (i.e. global fat mass reduction).

In addition, no differences were found between the groups when compared for distribution of abdominal subcutaneous and visceral adipose tissue measured using CT scan. Therefore while there was fat mass reduction observed globally, no fat redistribution was found clinically or radiologically in HIV- ART subjects.

It has been postulated that in ART naïve participants, lipoatrophy and lipohypertrophy may be reflective of HIV-associated wasting or age-associated obesity (95). Other investigators have distinguished lipoatrophy from the traditional wasting syndrome of HIV disease by preferential loss of fat tissue without substantial loss of lean tissue mass and frequently among patients responding to ART (7, 96). This would suggest that fat reduction in our cohort is likely related to lipoatrophy and not HIV-1 associated fat wasting; there was significantly lower fat mass in HIV-ART prior to ART compared to controls with similar lean mass in the groups. FRAM investigators reported that in both men and women, peripheral lipoatrophy occurred more commonly in HIV infected subjects and this was not associated with a reciprocal increase in visceral or trunk fat (88, 90). Those findings are similar to the findings of the current study and are likely attributable to use of a bi-directional instrument (12).

The prevalence of overweight/obesity in HIV-ART, HIV-no ART and control subjects was 50.7%, 62.8% and 62.1% respectively. This is consistent with national estimates

reported for South Africa in the Global Burden of disease study in which the prevalence of overweight/obese in males and females older than 20 years was 38.8% and 69.3%, respectively, for the period 1980 to 2013 (97).

In a cohort of predominantly African American males, Lakey et al also found a prevalence of 52% in ART naïve subjects; however, in that study the prevalence was much higher in control subjects (91%) (98). Mulligan et al reported a higher prevalence of overweight/obesity of 68% in HIV infected women and 75% in HIV negative controls (99). This may be accounted for by the fact that HIV infected subjects in the Mulligan study were on ART, while they were ART naïve in the current study; in addition, prevalence was reported by gender in that study and men had a BMI in the normal range (90).

Consistent with a significantly lower BMI in HIV-ART subjects, DXA scan showed significantly lower fat mass in HIV-ART and HIV-no ART subjects, both peripherally and centrally compared to controls. There was no significant difference between the groups in lean mass except for the right leg lean mass. It might be argued that the lack of difference between the groups on assessment of lipoatrophy was accounted for by the relatively high BMI in the HIV-1 infected subjects; however, DXA scan measurement has shown objectively that there is a significantly lower fat mass in the HIV- ART compared to control subjects.

Similar findings were reported in the Women's Interagency study (WIHS) in which, when compared with control subjects, those with HIV infection had significantly lower total, trunk and leg fat mass but similar lean mass; also, the majority of participants in that study were overweight (89). The Lipodystrophy Case Definition Study also found a correlation between anthropometric, DXA and CT scan measurements. (11)

In multivariate linear regression analysis, factors positively associated with higher fat mass were older age and higher fasting plasma glucose. Men had significantly lower fat mass than females, consistent with the prevalence of overweight/obese for South Africa in the global burden of disease report (97). Fat mass was higher at higher CD4 cell counts in the HIV-ART and HIV-no ART subjects. In contrast to previous studies, there was no

association between CRP and trunk fat mass (100). BMI, visceral adipose tissue, subcutaneous adipose tissue have been previously shown to predict serum CRP levels in both HIV-infected and noninfected adults (101).

Lean mass measured by DXA scan was similar in HIV- ART and control subjects for all regions except for the right leg region which was lower. This pattern of fat loss supports the notion of preferential loss in fat tissue without substantial loss in lean tissue mass that is characteristic of HIV associated lipoatrophy (7, 8). The significantly lower fat mass, with no difference in lean mass between the groups may suggest that the loss in fat mass is due to HIV infection itself as subjects were ART naïve. Reduction in fat mass measured on DXA scan is probably a phenomenon that occurred prior to any change in lean mass in the HIV-1 infected subjects, which was not different to that in the control subjects at this stage. The difference in BMI between the groups is likely accounted for by the significantly lower fat mass measured by DXA scan in the HIV- ART compared to control subjects.

The finding of significantly lower fat mass in HIV-ART and HIV-no ART compared to control subjects in all regions measured by DXA scan, albeit with lower peripheral and central lipoatrophy on clinical examination supports the findings of the study of Fat Distribution in Women with HIV infection (88) and Fat Distribution in Men with HIV infection (13) (FRAM). The present study also found a lack of association between peripheral lipoatrophy and central lipohypertrophy, consistent with findings of the FRAM studies (13, 88). Additionally, in the present study there was significantly lower fat mass in the limbs and trunk by DXA scan which also points to central and peripheral lipoatrophy.

The first study to report HIV associated lipodystrophy or fat re-distribution was a physical examination report of fat wasting in the face, arms or legs with or without central obesity (9). This and several subsequent studies utilized uni-directional questionnaires designed to investigate the presence of peripheral lipoatrophy and central lipohypertrophy (9, 11, 30, 102-111). Therefore, such questionnaires were not able to identify fat loss centrally and fat gain peripherally.

In order to improve assessment of lipodystrophy, the HIV Lipodystrophy Case definition Study Group investigators developed a case definition for lipodystrophy using methods similar to that used to develop rheumatological syndrome case definitions (112). Variables included in the case definition for lipodystrophy were age, sex, duration of HIV infection, HIV disease clinical stage, ratio of waist to hip circumference, anion gap, HDL cholesterol, ratio of trunk to limb fat, intra-abdominal to extra-abdominal fat ratio and percentage of leg fat. The model had 79% (95% CI 70 – 85) sensitivity and 80% (95% CI 71 – 87) specificity for HIV lipodystrophy case definition. (112)

The Study of Fat Redistribution and Metabolic Change in HIV Infection (FRAM) developed a different instrument that identified changes that did not anticipate the presence of peripheral lipoatrophy and central lipohypertrophy a priori (12). The current study utilized the same bi-directional questionnaires employed by the FRAM study (12) that are designed to identify the presence of lipoatrophy or lipohypertrophy whether it is peripheral or central, i.e. it was possible to identify peripheral lipoatrophy that is not associated with central lipohypertrophy.

Peripheral lipoatrophy has been shown in many studies to be associated with central lipohypertrophy and attributable mainly to ART, in particular protease inhibitors (PI) and thymidine analogue nucleoside reverse transcriptase inhibitors (tNRTIs). These changes, however, have also been demonstrated in ART naïve HIV infected participants (95).

It is likely that although the lipoatrophy by participant and physician concordance was similar in HIV- ART and control subjects, the significantly lower fat mass on DXA scan in the former group with no difference in lean mass may be associated with the entity of lipoatrophy secondary to HIV-1 infection prior to initiation of ART.

The significantly lower fat mass on DXA scan in the limbs and trunk in HIV- ART compared to control subjects suggests a global reduction in fat mass. These findings are supported by the finding of a lack of association between peripheral lipoatrophy and central lipohypertrophy in this study.

The prevalence rate of overweight/obesity in the study subjects and controls is high, consistent with the high prevalence in the general population. Older age, female gender, higher fasting plasma glucose and higher CD 4 cell count were associated with higher fat mass on DXA scan. Obesity and increase in fat mass in HIV infected subjects has been described in the literature mainly as lipohypertrophy, with most studies reporting this lipohypertrophy as occurring mainly in the trunk. In the ACTG study A55224s, use of co-formulated tenofovir/emtricitabine NRTI was associated with an increase in trunk fat of about 25% (113).The ACTG 5142 showed that patients on all ART combinations including NRTI-sparing regimens containing efavirenz and lopinavir, had a 27% increase in trunk fat after 96 weeks of treatment (114). Lipohypertrophy combined with lipoatrophy was associated with older age, use of protease inhibitors and duration of stavudine therapy in a study of HIV infected subjects, the majority of whom were Caucasian males (115).

In a study that compared metabolic data before and after initiation of ART that contained a protease inhibitor, glucose and insulin levels increased without any changes in body fat distribution as measured by DXA scan (116). In a study that included predominantly Caucasian males, older age and diabetes were associated with all parameters of adiposity; BMI, waist circumference and waist to hip ratio (29).

## 9.2. Fat Distribution at follow up for Group 3 subjects on ART

The main findings in this step of the study were an increase in fat mass and body mass index without fat re-distribution after 24 months of ART. Instead of lipoatrophy, there was central (24.2%) and peripheral (28.8%) lipohypertrophy as measured by the FRAM questionnaire(12) at 24 months with a concomitant significant increase in body weight, body mass index and fat mass (limbs, trunk and total) by DXA scan at 24 months on ART. Central lipohypertrophy was accompanied by a significant increase in visceral and subcutaneous fat area measured by CT scan. However, there was no significant difference in anthropometric

measurements of all circumferences and skin folds between baseline and 24 months follow-up on ART.

The absence of any lipoatrophy in this cohort deserves comment. It is possible that the bidirectional nature of the questionnaire utilized allowed for the findings of peripheral and central lipohypertrophy on participant and physician examination concordance, as such a questionnaire did not a priori anticipate the lack of association between peripheral lipoatrophy and central lipohypertrophy. In addition, there is evidence that ART (in particular, thymidine analogues and protease inhibitors) is a risk factor for development of lipoatrophy and fat re-distribution. The absence of lipoatrophy in this study is likely explained by the replacement in the South African treatment program of thymidine analogue nucleoside reverse transcriptase inhibitors (NRTI) stavudine by non-thymidine nucleotide analogue Tenofovir disoproxil fumarate (TDF) in 2009. First line treatment in South Africa also does not include protease inhibitors.

In the present study, all subjects were treated with TDF, lamivudine and efavirenz or nevirapine. At baseline, ART naïve subjects starting ART had a significantly lower fat mass compared to HIV negative controls by DXA scan. There was a significant increase in fat mass from baseline to 24 months follow-up on ART. A prospective, randomized trial conducted in 81 centers comparing TDF and stavudine, each in combination with lamivudine and efavirenz for treatment of naïve patients showed equivalence in virologic outcome, significantly favourable lipodystrophy frequency and greater limb fat at 24 months and 36 months by DXA among those treated with TDF compared to stavudine (117). However, in that study, baseline fat mass was not measured.

In an observational study such as this, it is difficult to ascribe an etiologic factor to the increase in fat mass. The effect could be related to immune recovery and restoration to health or specific drug effect. However, the low fat mass compared to HIV negative controls at baseline and the increase with ART could suggest immune recovery secondary to a

metabolically safe TDF containing regimen. TDF is a weak inhibitor of mitochondrial DNA polymerase gamma in vitro, with little effect on mitochondrial content in multiple cell types.(46) This might explain the increase in fat mass, and not lipoatrophy, related to TDF use. By contrast, lipoatrophy associated with stavudine may be related to mitochondrial injury resulting from inhibition of mitochondrial DNA polymerase gamma within adipocytes and depletion of mitochondrial DNA.

Development of significant peripheral and central lipohypertrophy was accompanied by an increase in body mass index following 24 months of ART. The increase in the proportion of overweight/obese from baseline to 24 months on ART (50.7% to 66.3%) was consistent with that reported by Lakey et.al. of (52.0% to 66.0%) albeit over 12 months of ART (98). In the current study, the proportion that was obese at 24 months of ART was similar to that in HIV negative control subjects at baseline (40.0% vs. 41.4%) and with obesity prevalence of 42.0% in women 20 years and older for South Africa (97).

The development of obesity was accompanied by a significant rise in systolic blood pressure and an increase in a proportion with stage 1 hypertension from 4.2% to 9.7%, approaching the prevalence of stage 1 hypertension at baseline in HIV negative controls of 13.6%. Overall, hypertension increased from 6.3% to 14% after 24 months follow-up. Lakey et.al. also showed an increase in the proportion with hypertension and/or dyslipidemia from 49% pre-treatment to 74% after 12 months of ART among patients classified as overweight/obese. The higher systolic blood pressure and body mass index suggest an increased risk of cardiovascular disease in these patients.

In the HIV-ART group, DXA scan showed a mean increase in trunk fat mass of 33.6% (mean difference 4.2 kg, p <0.0001) and total fat mass increase of 30.8% (mean difference 9.4 kg, p < 0.0001); CT scan showed a significant increase in visceral and subcutaneous fat area after 24 months of ART. The combination of central and peripheral lipohypertrophy, higher BMI, higher fat mass on DXA scan at all sites (limbs, trunk and total)

and increase in fat mass area through L4 spine (total, visceral, subcutaneous fat area and waist size) on CT scan following 24 months of ART indicate generalized increase in adiposity with ART, and are probably all related to the increase in BMI.

The visceral compartment has been shown to be the most common site for fat accumulation in treated HIV-1 infected patients; dorsocervical, hepatic, cardiac, intrathoracic and subcutaneous regions may also be affected (118-120). Of the agents used in the present study, efavirenz is one that has been implicated in the development of lipoatrophy through its ability to inhibit mitochondrial function and adipocyte differentiation (121, 122) However, no lipoatrophy was observed on treatment in the present study.

In our study, there was a significant increase in fat mass in all measured regions from prior to starting ART and during 24 months of follow-up on ART. To our knowledge, there are no previous studies that have reported on fat measurement from pre-treatment to end of follow-up on ART. Most studies measured fat gain when reversing lipoatrophy after a drug switch from an offending drug. Fat mass increases of 10 to 42% following switching off NRTI have been reported (123-128).

Although fat mass was lower in males at baseline prior to starting ART, during follow-up, there was a higher fat mass in males compared to females. At baseline, fat mass was higher with older age and higher CD4 cell count; however, the rate of increase was lower with increasing CD4 cell count and increasing age during follow-up.

It appears that the significant immune reconstitution (mean increase CD4 cell count of 199 cells/mm$^3$) and virological suppression (mean reduction of 3.3 log HIV RNA) was accompanied by an increased BMI and fat mass on DXA scan to the degree seen in HIV negative controls at baseline. Pre-ART mean BMI was 26.4 kg/m$^2$ and this increased to 29.4 kg/m$^2$ after 24 months follow-up, similar to the mean BMI of 29.1 kg/m$^2$ in HIV negative controls at baseline. After 24 months of follow-up, fat mass in limb and trunk measured by DXA scan was higher in older subjects and females, similar to findings in the general

population. At 24 months after ART, HIV-ART subjects had obesity and hypertension rates similar to those found in HIV negative controls at baseline.

In the general population there is an increase in all-cause mortality and risk of morbidity from diabetes, hypertension, hyperlipidaemia and cardiovascular disease related to obesity (27). Obesity in the HIV infected population has not received as much attention as fat re-distribution in spite of increasing frequency of cardiovascular disease in this population. Unmeasured risk factors such as obesity may contribute to the underestimation of cardiovascular disease risk in HIV infected subjects receiving ART when the Framingham cardiovascular disease risk-prediction model for the general population is used (129).

In a study that included HIV infected subjects and HIV negative controls (FRAM study) and which used the Framingham risk score, increased visceral adipose tissue was associated a higher cardiovascular disease risk in HIV infected subjects compared to controls. Peripheral lipoatrophy (as measured by leg subcutaneous adipose tissue) was associated with an increased cardiovascular risk in HIV infected subjects while low leg subcutaneous adipose tissue was associated with a low cardiovascular risk in controls (130). In the current study, the significantly increased trunk fat mass, visceral and subcutaneous fat area from baseline to 24 months on ART, will likely increase cardiovascular risk. Estimating cardiovascular risk is therefore important and in a cohort that had no prior exposure to ART, identifying specific drug effect might be possible.

## 9.3. Diabetes and Dysglycaemia

### 9.3.1. Baseline

This study has investigated glucose homeostasis in an ethnically homogeneous, HIV-1 infected population. At baseline, using both World Health Organization (WHO) and American Diabetes Association (ADA) glucose-based criteria, none of the HIV-1 infected subjects (whether or not initiated on ART) had diabetes while a significantly higher prevalence of diabetes (4.9%) was found in control subjects. In HIV-1 infected subjects

whether or not initiating ART, the prevalence of dysglycaemia was 3.7% compared to 8.6% HIV negative control subjects.

There is a paucity of data on the prevalence of diabetes in HIV-1 infected, ART naïve patients. (72, 131, 132) A study that compared 2565 HIV infected, ART-naïve participants in the Terry Beirn Community Programs for Clinical Research on AIDS (CPCRA) with 6585 HIV negative participants from the National Health and Nutrition Examination Survey (NHANES)(72) reported a diabetes prevalence of 3.3% in subjects (CPCRA) compared with 4.8% in HIV negative control subjects (NHANES). A meta-analysis of up to 6064 study participant data from SSA found no evidence of association between HIV infection and fasting glucose or HbA$_{1c}$ (133).

Low diabetes prevalence in HIV infected, ART naïve patients has previously been reported. (131, 132) Kilby et. al. found a prevalence of hyperglycaemia of 1.9% in ART naïve HIV infected patients, after reviewing blood glucose results from 1392 medical records. This was probably the first report of the frequency of hyperglycaemia in HIV infected patients. In a subset of those who developed diabetes and had been treated with megesterol for weight loss, there was an association between hyperglycaemia and megesterol use. Although not directly comparable, El-Sadr et al reported that more advanced HIV disease was associated with less favourable glucose homeostatic profile.(132)

Consistent with the findings in this study, Galli et al reported diabetes prevalence of 0.8% in a subset of 368 HIV infected ART-naïve subjects. (134). By contrast, the Multicenter AIDS Cohort Study (MACS) found a higher prevalence among 157 HIV-infected, ART naïve men (7%) (70); however, in that study, the rate of diabetes (5%) in HIV negative controls was similar to the current study (70).

The prevalence of diabetes in HIV infected ART naïve subjects in the present study is consistent with that found in previous studies although demographic characteristics differ between these studies. The majority of available data is in cohorts that are predominantly

men and Caucasian (70 – 100%) (70, 72, 134), white (83%) (70) and in developed countries (Italy, USA) (70, 72, 134). The present study was set in a developing country; the proportion of females was (66%) and 100% black. The significance of the ethnic distribution is that it has been postulated that black people may metabolize antiretroviral drugs differently and because the background prevalence may be different; also, ART has been shown to influence insulin sensitivity.

Demographic characteristics (older age, black race or Asian ethnicity, male gender), higher body mass index, lower serum HDL cholesterol, higher serum triglyceride are factors that have been associated with diabetes in HIV infected patients as in the general population (72, 74, 132, 135, 136). However, there is paucity of data in developing countries.

The diabetes prevalence in the HIV negative controls in this study is similar to the diabetes prevalence of 5.3% using oral glucose tolerance test in the same province in 1993 (Omar 1993). Since the controls were recruited from a population at similar risk for acquiring HIV infection (presenting at voluntary counselling centers to test for HIV infection), with no significant differences in demographic characteristics with the HIV infected group, it is likely that the significant difference in prevalence between the two groups is related to effects of HIV infection and not demographic characteristics.

When abnormalities in glucose homeostasis were first recognized after the use of ART, earlier studies focused investigations on patients who were taking ART. While this was unavoidable as this treatment related complications were not anticipated, it precluded assessment of the role of HIV infection *per se* on glucose metabolism. The low prevalence of diabetes in ART naïve subjects in the present study and other studies suggests a relatively low association between HIV infection and diabetes perhaps on the basis of reduction in BMI associated with HIV infection.

Traditional risk factors for diabetes identified in univariate analysis in the present study were systolic and diastolic blood pressure, serum total cholesterol and triglycerides.

Other "non-traditional" factors identified were serum cortisol and visceral fat area. In multivariate analysis the significant independent risk factors associated with diabetes were systolic blood pressure and serum triglycerides. Other traditional risk factors for diabetes that were investigated (age, gender, waist circumference) were not significant in either univariate or multivariate analysis.

Traditional risk factors for diabetes (older age, higher BMI and hypertension) as occurs in the general population were identified among HIV-infected subjects in the Galli study (134) (72). El-Sadr study also found that older age and higher BMI was associated with higher glucose levels and evidence of insulin resistance (132). In that study, mean plasma glucose was described without the categories of disorders of glycaemia and insulin resistance was calculated using the homeostasis model of assessment (HOMA).

Non-traditional risk factors for diabetes have been examined in several studies, in particular, protease inhibitors, stavudine and hepatitis C.(30, 137) (138) Brar et al found a trend towards a higher prevalence of diabetes in patients co-infected with HIV and Hepatitis C (72). In a study on African-Americans with HIV-1 infection, there was an absence (0%) of diabetes in subjects who were not treated with PIs as opposed to 12% in those on PI treatment (137). PI treatment was not examined in multivariate analysis in this study.

The varying prevalence of diabetes in different studies is probably partly related to different methods and criteria for the diagnosis of diabetes. Definition of diabetes in various studies has ranged from self-report of diabetes or use of anti-diabetic medications, (72, 134, 139) random blood glucose, (131, 140) fasting blood glucose (70, 132, 134) to oral glucose tolerance test (29, 141) and glycated haemoglobin (HbA$_{1c}$). (140) Retrospective (131)and cross-sectional (70, 72, 140, 141) designs have been employed to describe diabetes prevalence, each with its inherent limitations.

There is a paucity of data on the prevalence of dysglycaemia (any disorder of glycaemia) in HIV-infected subjects and in particular, ART naïve HIV infected patients as

most studies have determined the prevalence of diabetes and other disorders of glycaemia using either history of diabetes, anti-diabetic medication use or fasting plasma glucose.

The present study defined disorders of glycaemia using glucose-based criteria using both World Health Organization (WHO) and American Diabetes criteria (ADA). Performing an oral glucose tolerance test has an advantage of diagnosing impaired glucose tolerance that cannot be diagnosed with fasting plasma glucose alone. In addition to diabetes, the prevalence of impaired glucose tolerance (2.96%) and impaired fasting glucose (0.7%) were also determined in HIV-ART subjects. The prevalence of any dysglycaemia was 3.7% in the HIV-ART and 3.6% in HIV-no ART subjects, using WHO glucose-based criteria compared to 8.6% in controls and even higher using the ADA glucose-based criteria (6.6% in HIV-ART vs. 12.3% in control subjects).

In a cross-sectional study in the Western Cape, using glucose-based ADA criteria in HIV-infected ART naïve subjects, the prevalence of dysglycaemia (21.9%) was much higher than in this study (6.6%) (141) The widely different prevalence in dysglycaemia is unexplained.

At baseline, when compared with HIV-infected subjects, HIV negative control subjects had a higher prevalence of DM and dysglycaemia; in addition, systolic BP and BMI were significantly higher. When the total study group (study and control subjects) were categorized according to BMI, although the glycaemic categories were not significantly different between the BMI categories, none of the underweight subjects had dysglycaemia while a higher proportion of overweight/obese subjects had dysglycaemia when compared to those with a normal BMI (Chapter 4, Table 2).

Regarding risk factors associated with dysglycaemia, in univariate analysis, significant risk factors were age, systolic and diastolic blood pressure, serum total cholesterol and triglycerides. Other significant risk factors were mid-arm circumference, visceral fat area, visceral: subcutaneous fat area ratio and serum cortisol. In multivariate

analysis independent risk factors associated with dysglycaemia using the WHO criteria were systolic blood pressure, serum triglycerides and visceral: subcutaneous fat ratio. Using ADA criteria, systolic blood pressure and visceral: subcutaneous fat area were significantly associated with dysglycaemia. Traditional risk factors (age, gender, waist circumference) were not significantly associated with dysglycaemia in multivariate analysis.

In multivariate analysis, fat mass measured by DXA scan was positively associated with fasting plasma glucose. A unit higher fasting plasma glucose was associated with a nearly 2kg higher total body fat mass and 1kg higher trunk fat mass.

Diabetes and dysglycaemia prevalence was higher in HIV negative participants compared to HIV-1 infected subjects. It is therefore possible that the significantly lower BMI, lower body fat (including lower trunk fat) and absence of the traditional fat redistribution (peripheral lipoatrophy and central lipohypertrophy), may have been protective against diabetes in subjects with HIV-1 infection. Independent risk factors associated with diabetes after adjusting for body mass index were systolic blood pressure and serum triglycerides, while age or gender did not emerge as independent risk associates.

As pointed out earlier, previous studies on the prevalence of diabetes or dysglycaemia in HIV-infected ART naïve subjects were in cohorts that were predominantly male, (72, 134) and white, (70, 131) in developed countries. Available prevalence data from sub-Saharan Africa is limited to those on HIV-infected subjects taking ART (140, 142) or HIV-infected subjects on ART compared with ART naïve subjects. (141) To our knowledge, this is the first study from a developing country to report metabolic changes in a predominantly female, ethnically homogeneous, black population that was HIV infected, ART naïve compared with HIV negative controls.

Cohorts in developing and developed countries differ not only in ethnic characteristics and gender distribution, but also in treatment combinations offered as developed countries may use ART combinations that include protease inhibitors (PI) in first

line therapy, whereas PIs are reserved for second-line therapy in developing countries (45). Treatment options are dictated by availability of resources. It is therefore crucial that data on metabolic complications in developing countries is described as this cannot be simply be extrapolated from developed countries.

## 9.4. Prospective

### 9.4.1. Incidence of glycaemic disorders

This study describes, for the first time within the South African government antiretroviral programme, the incidence of diabetes mellitus (DM), impaired glucose tolerance (IGT), impaired fasting glucose (IFG), impaired glucose regulation (IGR/IGT or IFG) and any dysglycaemia (DM or IGT or IFG) among subjects initiated on ART and followed-up prospectively over 24 months. As stated above, at baseline, there was an absence (0%) of diabetes in HIV-ART and 4.9% in the control group. Using glucose-based WHO criteria, the incidence of DM on ART was 2.3 per 100 person years follow up (PYFU). The incidence per 100 PYFU for IGT was 3.2, of IFG, 3.2, of IGR 6.1 and of "any dysglycaemia" 7.6.

The only independent predictive risk for DM was the visceral: subcutaneous fat ratio measured by DXA scan at baseline. One unit increase in the ratio associated with a nearly three-fold risk of developing DM. Independent predictive risks for IGT were systolic blood pressure and visceral:subcutaneous fat ratio. One unit of systolic blood pressure predicted a five percent higher risk and a unit of visceral: subcutaneous fat ratio predicted an eight fold higher risk of developing IGT. Visceral fat has been shown to be a risk factor for diabetes and cardiovascular disease, therefore the visceral: subcutaneous fat ratio risk implies that the volume of visceral fat in relation to subcutaneous fat is also important (143). Independent predictive risks for IFG were gender, waist circumference and serum alkaline phosphatase. Females had an 85% lower risk of developing IFG, while one unit higher waist circumference

and serum alkaline phosphatase were associated with a 9% and 5% greater risk, respectively. Independent risks for IGR were gender and serum albumin. Females had a 76% lower risk of developing IGR and one unit higher serum albumin was associated with a 15% lower risk.

### 9.4.2. Diabetes

The incidence of DM in the present study is similar to that found in the Women's Interagency study (WIHS) conducted in adult women in 6 US cities from 2000-2006 (2.3 per 100 PYFU vs 2.5 and 2.89 per 100 PYFU among subjects receiving ART containing PI and non-PI containing ART)(71). In an earlier investigation of the WIHS from 1994 to 1998, a period that spans introduction of PIs, the incidence rate of DM was similar (2.8/100 PYFU) (139). Although the incidence of DM in this study is similar to those of the two WIHS (1994-1998 and 2000-2006), those studies used less sensitive methods to diagnose DM. Definition of DM in the WIHS 1994-1998 was based on the reported occurrence of DM since the previous study visit (enquiry every 12 months) or any new illness (enquiry every 6 months) or by review of the complete list of medications (obtained every 6 months); from 2000-2006, it was based on fasting glucose $\geq$ 7.0 mmol/l, reporting antidiabetic medication or reporting DM diagnosis (with subsequent confirmation by fasting glucose $\geq$ 7.0 mmol/l or reported antidiabetic medication).

On the other hand, the incidence rate of DM found in this study is lower than the rate found in the Multicenter AIDS Cohort Study (MACS) (70) (2.3 per 100 PYFU vs. 4.7 per 100 PYFU among subjects receiving ART) but higher than the incidence rate from the D: A: D study (Data Collection on Adverse Events of Anti-HIV Drugs) (5.72 per 1000 PYFU among subjects receiving ART) and from the APROCO-COPILOTE cohort (14.1 per 1000 PYFU among subjects receiving ART) (29, 31).

In the MACS study, DM was defined as a fasting glucose $\geq$ 7.0 mmol/l, self-reported DM or current self-reported use of antidiabetic medication, each of which was ascertained at

each semi-annual visit. While the MACS study showed a higher incidence rate compared to the present study and the WIHS, the method was, however, less sensitive compared to one used in the present study. Case detection of DM in the present study was based on a fasting plasma glucose $\geq$ 7.0 mmol/l and/or 2 hour post glucose load $\geq$ 11.1 mmol/l (oral glucose tolerance test (OGTT), HBA1c $\geq$ 6.5 according to the World Health Organization and American Diabetic Association criteria for diagnosis of DM. Tests were conducted at baseline, 3, 6, 12, 18 and 24 months.

The study by Tien et al is the only study to report the incidence of diabetes based on the $HbA_{1c}$ (ADA, WHO criteria) (35). That study found that inclusion of $HbA_{1c}$ in the diagnostic criteria increases diagnostic accuracy for diabetes. The study was conducted within a large cohort of HIV infected and uninfected women in the United States.

To our knowledge, the present study is the first to examine the incidence of diabetes using $HbA_{1c}$ criterion (ADA, WHO) for definition of diabetes, in a developing country among HIV-infected subjects. Using $HbA_{1c}$ criteria for diagnosis of DM (WHO, ADA) the incidence rate was 3.8 cases per 100 PYFU [95% CI 1.6 – 7.4] during 211.9 PYFU, higher than incidence rate of 2.3 cases per 100 PYFU using OGTT. The difference in incidence rate may be related to the $HbA_{1c}$ being more sensitive or a falsely elevated rate because of anaemia associated with more significant HIV disease at the start of ART. Haemoglobin increased significantly during the 24 months of follow-up. Clearly there is a need for further studies that evaluate DM incidence using $HbA_{1c}$ criteria.

Earlier studies that used a participant report only (WIHS 1994-1998) and participant report with fasting glucose (WIHS 2000-2006), found similar incidence rates with the present study which used a fasting and 2 hour post glucose load test at more frequent intervals. This may be accounted by the association between protease inhibitors (PI) (used in the WIHS study) and hyperglycaemia, impaired glucose tolerance and insulin resistance reported with the earlier generation PIs. WIHS found that PI use was an independent risk factor for self-reported DM. The somewhat more rigorous methods used in the present study and the fact

PI therapy was not used might explain the incidence rate that is similar to studies that used less sensitive methods with PI therapy.

Thymidine analogues neucleoside reverse transcriptase inhibitors (tNRTI) stavudine and zidovudine were significantly associated with diabetes in the D: A: D study.(135) It has been proposed that NRTI toxicities are caused by cellular mitochondrial DNA depletion and subsequent mitochondrial dysfunction (144-147). The severity of NRTI-associated toxicity depends on the degree of mitochondrial DNA depletion. The rank order of NRTI according to their ability to cause mitochondrial dysfunction in vitro is zalicitabine > didanosine > stavudine > zidovudine > abacavir = lamivudine = tenofovir (46). Stavudine was part of the first line regimen when ART was introduced in the public sector of South Africa and was reported to be associated with high rates of lactic acidosis and neuropathy.(39) Stavudine-related toxicities in South Africa led first to reduction in stavudine dose as recommended by the WHO(42) and eventually to its withdrawal as part of first line therapy in 2009 (45). Stavudine continues to be used based on the clinical condition; it is deemed a relatively safer option and is used in patients with anaemia (zidovudine contraindicated), renal disease or concomitant use of nephrotoxic drugs (tenofovir contraindicated).

This study was conducted at a time when thymidine analogue NRTI stavudine had been withdrawn from first-line therapy of those commencing ART and replaced with the non-thymidine analogue NRTI tenofovir.  Tenofovir has been reported to cause the least mitochondrial toxicity which is implicated to cause insulin resistance and lipodystrophy. This might explain an incidence rate in this study that is lower than that found in the MACS study which used fasting blood glucose, and similar to the WIHS which used self-report (presumably less sensitive method) in the case definition of DM.

The only independent risk factor associated with development of DM using the glucose-based criteria in this study was visceral: subcutaneous fat area ratio on CT scan, a finding that has not previously been reported, to our knowledge. Although visceral fat mass

on its own did not emerge as a risk, the ratio of visceral to subcutaneous fat mass indicates that the relationship between the two contributes to the risk by virtue of being components of trunk mass.

When $HbA_{1c}$ criteria for diagnosis of DM was used, haemoglobin was an independent risk factor for development of DM (HR 0.61[0.39-0.96], p=0.03). A unit increase in haemoglobin was associated with a 39% less risk of developing DM. To our knowledge, the association of haemoglobin as a risk for diabetes in HIV infected subjects has not previously been described. A caveat to this is that anaemia may give a falsely elevated $HbA_{1c}$ since haemoglobin levels and red cell turnover may be altered by HIV infection itself and/or ART exposure. Although there was a significant increase in the mean haemoglobin from baseline through follow-up in this cohort, the mean haemoglobin remained within the normal range throughout follow-up.

Higher BMI, older age, nadir CD4 cell count of $\leq$ 300 cells/mm$^3$ among HIV-infected subjects taking ART were factors associated with a higher risk of incident diabetes in a study of men, more than 80% of whom were white. (70) Other risk factors for incident diabetes that have been identified include prior exposure to indinavir, stavudine and didanosine. (29) Of significance in that study is that the risk persisted even after drug withdrawal. The Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study showed an increased risk associated with the use of stavudine and zidovudine and a lesser risk associated with nevirapine and ritonavir. (135) Cumulative exposure to NRTI was associated with increased incidence of diabetes in the Tien study. (71)

### 9.4.3. Impaired glucose tolerance

At baseline, the prevalence of IGT was similar in the HIV-ART subjects and HIV negative controls (2.9% vs. 3.7%). The incidence in HIV-ART subjects was 3.2 per 100 PYFU.

Regarding risk factors, in univariate analysis, systolic and diastolic blood pressure, alanine amino transferase and visceral: subcutaneous fat ratio on CT scan, were significantly associated with development of IGT.

In multivariate analysis, the only traditional risk factor associated with development of IGT was systolic blood pressure at baseline. One unit of systolic blood pressure predicted a five percent higher risk of developing IGT. This finding is in keeping with that for diabetes and dysglycaemia in this study and the general population. Visceral: subcutaneous fat ratio was a non-traditional risk factor that predicted development of IGT. A unit of visceral: subcutaneous fat ratio predicted an eight fold higher risk of developing IGT. This risk is probably a reflection of higher visceral fat mass association with impaired glucose tolerance although visceral fat mass on its own did not emerge as a risk factor as described for diabetes above.

To our knowledge, there is no prior report of IGT incidence and its risk factors in HIV infected subjects taking ART as most studies examined fasting glucose alone. Also, there have been no follow-up studies of glucose tolerance in the general population in SSA.

### 9.4.4. Impaired fasting glucose

At baseline, the prevalence of impaired fasting glucose (IFG) was similar in HIV-ART and control subjects (3.7% vs. 3.7%). The incidence of IFG in HIV-ART subjects was 3.2 per 100 PYFU.

In univariate analysis, the following risk factors were associated with development of IFG: anthropometry – mid arm, chest and waist circumference, subscapular and abdominal

skinfolds; biochemistry – serum bicarbonate, alkaline phosphatase and platelet count were factors that were significantly associated with development of IFG.

In multivariate analysis, risk factors that predicted development of IFG were gender, and waist circumference. Females had an 85% lower risk of developing IFG, while one unit higher waist circumference was associated with a 9% greater risk. One unit higher serum alkaline phosphatase (ALP) was associated with a 5% greater risk. No follow-up studies of impaired fasting glucose in subjects on ART have been reported.

### 9.4.5. Any dysglycaemia

The incidence of DM, impaired glucose tolerance, impaired fasting glucose was estimated as dysglycaemia as well as in individual categories. The incidence of "any dysglycaemia" was 7.6 cases per 100 PYFU [95% CI 4.3 – 12.3) during 211.6 PYFU. It was considered important not only to study the categories separately but also together because IGT and IFG are precursors of DM and combining the categories highlights risk factors for future cardiometabolic disease that might not be apparent if analysed separately. This high incidence rate of dysglycaemia underscores the importance of investigating the association of ART with any disorder of glycaemia.

Independent risk factors for incident dysglycaemia were systolic blood pressure (HR 1.04[1.02-1.07], p=0.0006), serum albumin (HR 0.85[0.76-0.94], p=0.0003), CD4 cell count (HR 0.988[0.978-0.997], p=0.01) and efavirenz (HR 6.27[1.65-23.80], p=0.01). A unit increase in systolic blood pressure was associated with a 4% higher risk and a unit increase in serum albumin and CD4 cell count were associated with a 15% and 1% lower risk of developing dysglycaemia, respectively. Exposure to efavirenz as part of ART was associated with a nearly six-fold risk of developing dysglycaemia. Furthermore, all subjects who developed DM using glucose-based criteria were treated with efavirenz compared to nevirapine (p=0.02).

This is probably the first report of incident dysglycaemia among HIV-1 infected subjects starting ART. Higher serum albumin and CD4 cell count is likely related to restoration of health and an indirect link with risk for dysglycaemia. In this study, improvement in CD4 cell count was accompanied by a significant increase in BMI and systolic blood pressure; both established risk factors for dysglycaemia.

To our knowledge, this is the first report of efavirenz as a predictive risk factor for the development of new-onset dysglycaemia. This finding concurs with that of a cross-sectional study conducted within the South African National Department of Health Antiretroviral Program and using the same criteria for dysglycaemia; that study showed that efavirenz was significantly associated with the prevalence of dysglycaemia (141). Efavirenz was combined with stavudine in some of the subjects in the Dave study and therefore the combination might have contributed to the prevalence of dysglycaemia. In the present study, none of the subjects were treated with stavudine, making it highly unlikely that stavudine was a contributor.

Previous studies that tested the association of NNRTI or efavirenz with fasting plasma glucose did so in subjects that had previously been treated with a protease inhibitor or different combination NRTIs of varying metabolic risk (148-150). Thus effects of the previous exposure to a protease inhibitor or NRTI with a high metabolic risk might influence glucose metabolism. In the Swiss Cohort Study treatment with a combination of an NRTI and NNRTI was not shown to be associated with DM but there was an association with black ethnicity (73).

The association of efavirenz with dysglycaemia in South Africa may be due to ART drugs being metabolised differently in Africans as was reported in a study that found higher steady state levels of efavirenz in association with CYP2B6*16 among Africans compared to Swedes and Turks (151). Higher steady state efavirenz plasma levels in Africans associated with this polymorphism might explain different drug response and adverse drug reactions.

The significant increase in body mass index, reduction in the globulin fraction (decreasing total protein and increasing serum albumin) and increasing haemoglobin are consistent with health restoration that is supported by the significant increase in CD4 cell count and reduction in HIV-1 viral load. These findings suggest that use of ART in these subjects was effective and the development of disorders of glycaemia accompany the health restoration by ART. HIV-infected subjects might therefore develop DM following immune reconstitution, either in association with ART or because their risks become similar to that of HIV-negative controls. Therefore, the incidence rate in treated HIV-1 infected subjects in this population might approximate that of the HIV negative population as they achieve restoration to good health.

## 9.5. Dyslipidaemia

### 9.5.1. Baseline

In terms of risk stratification for cardiovascular disease(83), "high-risk" total cholesterol levels were found in 3.9% of HIV negative control subjects but in none (0%) of the HIV-1 infected group. The prevalence of "high-risk" HDL (low HDL) was higher in HIV-1 ART subjects than in control subjects, both in women (94% vs. 74.6%) and in men (56% vs. 28%).

Findings in this study of low total cholesterol, low density lipoprotein (LDL)-cholesterol and high density lipoprotein (HDL)-cholesterol in HIV infected group with advanced disease compared to HIV negative subjects are consistent with findings in previous studies. Low values of total cholesterol, LDL-cholesterol and HDL-cholesterol with high levels of serum triglycerides in HIV-1 infected patients prior to initiation of ART compared to HIV negative subjects have previously been reported. (20, 132, 152-155)

In contrast to previous reports, this study found that mean serum triglycerides were similar in the HIV-1 infected and controls subjects. The combination of high serum triglycerides and low HDL-cholesterol levels are established risk factors for cardiovascular disease. In this study though, the lower HDL-cholesterol level in the HIV-1 infected subjects was accompanied by serum triglycerides that were not significantly different from the controls. The lower HDL cholesterol in the HIV-ART subjects might reflect lower levels of exercise in this group and the similar triglyceride levels might reflect dietary patterns and lack of impact of HIV on serum .triglycerides. Serum triglycerides and total cholesterol were both found significantly associated with diabetes and dysglycaemia in univariate analysis. Serum triglyceride levels had a nearly 5-fold and 3-fold risk associated with diabetes and dysglycaemia, respectively.

### 9.5.2. Prospective

Changes in serum lipid profiles were determined during 24 months of ART.

In terms of risk stratification for cardiovascular disease (83), "high-risk" total cholesterol levels increased from 0% at baseline to 2% at 12 months and returned to 0% by 24 months in HIV-ART subjects. "High-risk" HDL (low HDL) decreased from 94.2% at baseline to 43.3% at 24 months in HIV-1 ART female subjects and from 56.3% at baseline to 35.4% at 24 months in HIV-1 ART male subjects. "High risk" LDL cholesterol was 0.9% at baseline and 0% at 24 months.

There was a significant increase in serum total cholesterol, LDL-cholesterol and HDL-cholesterol during 24 months follow-up, with no significant increase in serum triglycerides during this period. Increase in total cholesterol, LDL-cholesterol, and HDL-cholesterol have been shown following NNRTI based ART, with modest increase in serum triglycerides (156). Our findings are similar to those reported by Leth et al, in which HIV infected patients starting ART on a regimen containing efavirenz or nevirapine were followed-up for 48 months. There was a significant increase in HDL cholesterol, accompanied by increase in total cholesterol, LDL-cholesterol and triglycerides; the proportion HDL-cholesterol increase was significantly larger in the nevirapine treatment group compared to the efavirenz treatment group. The unfavourable increase in total cholesterol, LDL-cholesterol and triglycerides in that study was thought not to be related to stavudine since stavudine use was high throughout follow-up (96% for nevirapine group and 98% treatment group). The increase in HDL-cholesterol might be related to the effect of ART (efavirenz or nevirapine) or to health restoration as indicated by a significant HIV RNA suppression and increase in CD4 cell count in our study. The current study did not use an agent that is known to have a deleterious effect on lipid metabolism, viz. stavudine.

Nucleotide reverse transcriptase inhibitors (viz. tenofovir) are associated with a better lipid profile compared to nucleoside reverse transcriptase inhibitors (viz. stavudine), in

particular thymidine analogues. In a study of HIV infected patients from 81 sites in the United States, South America and Europe, treated with tenofovir or stavudine and compared with placebo, larger increases in total cholesterol, LDL-cholesterol and triglycerides occurred with stavudine compared with tenofovir (117).

Tenofovir is a preferred agent for first line therapy because of its more favourable toxicity profile.(157) The modest effect of ART on this cohort may be related to the use of non-thymidine analogue nucleotide reverse transcriptase inhibitor (viz. tenofovir) instead of the previously used thymidine analogue nucleoside reverse transcriptase inhibitor (viz. stavudine). Thymidine analogue nucleoside reverse transcriptase inhibitors have been associated with accumulation within adipocytes. There is an associated mitochondrial dysfunction and depletion of mitochondrial DNA, partly due to inhibition of DNA polymerase-ɣ. NNRTI efavirenz has also been shown to inhibit mitochondrial dysfunction and adipocyte dysfunction.

The move from the metabolically toxic stavudine (44) to TDF by the S. African National treatment program might have significantly contributed to improvement in HDL cholesterol and lack of a significant increase in serum triglycerides. However, the change from choosing an NNRTI between efavirenz and nevirapine (as in the present study) to only using efavirenz as part of the fixed dose combination first line therapy in the new national treatment program, might result in adverse lipid profiles with associated increase in future cardiovascular risk.

## 9.6. Study Strengths

The main strength of the study is the cross-sectional as well as prospective design. In addition, there was ethnic homogeneity of the HIV-1 infected (starting and not starting ART) and HIV negative controls.

The tool used to measure fat distribution was bi-directional as it did not *a priori* anticipate an association between peripheral lipoatrophy and central lipohypertrophy and it was more objective than in earlier studies.

The assessment of glycaemia was based on both plasma glucose and $HbA_{1c}$ and included the use of OGTT. The use of OGTT allowed for identification of disorders of glycaemia (IGT, any dysglycaemia) that may not have been identified with the use of fasting plasma glucose or $HbA_{1c}$ alone.

The study was conducted within the National treatment program at a time when changes to treatment policies were implemented. It was possible, after ethics approval, to implement changes such as increasing the CD4 cell cut-off for initiating ART from < 200 cells/$mm^3$ to $\leq$ 350 cells/$mm^3$. The study was also started soon after discontinuation of the notoriously toxic thymidine analogue NRTI stavudine and its replacement by non-thymidine nucleotide analogue tenofovir which is relatively less toxic.

The backbone non-Nucleoside Reverse Transcriptase Inhibitor used within the National program during the study was either Nevirapine or Efavirenz. The option was chosen based largely on consideration for teratogenicity or neuropsychiatric effects against efavirenz in which case nevirapine was the chosen NNRTI. Soon after the study was completed, the National treatment program rolled out the fixed drug combination. The backbone NNRTI option in the fixed drug combined pill is efavirenz only.

Although the sequences of events in the history of the largest ART program in the world were unplanned, the timing of the study in the history of the program deserves

comment. The comparison of nevirapine and efavirenz within the treatment program could only have been possible while the two agents were used within the National treatment program. This study documented treatment effects after discontinuation of a drug that had been reported to have metabolic toxicity (stavudine), but prior to discontinuation of the alternative NNRTI (nevirapine) within the program which. While the hepatotoxic effects of Nevirapine are well documented, this and other studies have shown nevirapine to have less metabolic toxicity compared to efavirenz which is now the sole NNRTI used in first line treatment for the National treatment program. Efavirenz is now the sole NNRTI used within the National antiretroviral treatment program because of the considered advantage of fixed combination therapy.

## 9.7. Study Limitations

The main limitation of the study is the lack of a control group in the prospective arm of the study. Changes that occurred with respect to fat distribution and metabolic changes have therefore not been compared with changes that occurred in the HIV negative controls over the same period. These are changes that may have been related to diet, exercise, age and other factors unrelated to HIV-1 infection or ART. Failure to follow-up the control group investigated in the cross-sectional step of the study was largely related to resource constraints.

Loss to follow-up, while it might reflect the real life scenario within National ART programs, reduced the strength of the study findings. However, comparison between subjects that completed the study and those that did not complete the study showed that these groups were similar except for employment status. The majority of the subjects who did not complete the study were unemployed.  It may be that those that were unemployed had poor food security and might have died. Furthermore, they may have been unable to return for their routine clinic visits because of lack of transport money and eventually stopped taking treatment or chose to access care closer to their homes.

## 9.8. Conclusions

The 20th century witnessed increasing epidemics of non-communicable disease and towards the turn of the century, the HIV epidemic became the main cause of morbidity and mortality. Introduction of antiretroviral therapy has significantly reduced mortality related to HIV infection. However, as HIV-infected people are living longer, non-communicable disease are now re-emerging in this population. As the 21st century matures, the convergence of the non-communicable disease epidemic with the HIV epidemic can be anticipated. Our study highlights some signals of this convergence.

This study has shown a high prevalence of overweight/obesity among HIV-1 infected subjects prior to starting ART. Following initiation of ART, there was a significant increase in BMI, systolic blood pressure, limb and trunk fat mass by DXA scan, visceral and subcutaneous fat on CT scan.

No fat re-distribution was found using clinical and radiological measures (DXA and CT scan) both prior to starting ART and during follow-up on ART.

This study found an absence of diabetes in HIV-infected patients prior to starting ART and development of diabetes after initiation of ART. The prevalence of dysglycaemia was significantly lower in HIV-infected subjects prior to starting ART compared to HIV negative controls.

Visceral: subcutaneous fat ratio is a risk factor for diabetes found in this study that has not been previously reported. Systolic blood pressure, serum albumin, CD4 cell count and use of efavirenz were risk factors associated with incident dysglycaemia in this study.

This study reports, for the first time, the association of efavirenz and incident dysglycaemia.

## 9.9. Implications for Practice

This study highlights the importance of blood pressure, body mass index, glucose and serum lipid monitoring for HIV-1 infected patients treated with ART in order to detect early onset of treatment related complications. Furthermore, patient education regarding the development of hypertension, overweight/obesity and diabetes including non-pharmacological interventions such as exercise and dietary control should be instituted early. HIV care providers must be trained to monitor for these complications.

## 9.10. Implications for Research

The impact of overweight/obesity, hypertension, diabetes and unfavourable lipid profile in HIV-infected subjects on cardiovascular disease in this population that traditionally has a lower risk of cardiovascular disease needs to be elucidated further.

Further studies in developing countries that investigate the incidence of diabetes in HIV-1 infected subjects compared with HIV negative controls are needed. The effect of efavirenz on dysglycaemia in HIV-1 infected subjects needs further confirmation. Future work will include genetic analysis.

# CHAPTER 10:APPENDICES

Appendix A

**Questionnaire** 1

## FRAM PHYSICAL EXAM
(Interviewer Administered)

___ / ___ / _____ EXAM DATE

MM    DD    YYYY

1.    The participant's **CHEEKS,** just **lateral** to the **NOSE AND MOUTH** are:

| Severely<br>Fat | Moderately<br>Fat | Mildly<br>Fat | Normal | Mildly<br>Wasted | Moderately<br>Wasted | Severely<br>Wasted |
|---|---|---|---|---|---|---|
| 1☐ | 2☐ | 3☐ | 4☐ | 5☐ | 6☐ | 7☐ |

2.  The participant's **FACE SHAPE** is:

| Severely<br>Round | Moderately<br>Round | Mildly<br>Round | Normal | Mildly<br>Thin | Moderately<br>Thin | Severely<br>Thin |
|---|---|---|---|---|---|---|
| 1☐ | 2☐ | 3☐ | 4☐ | 5☐ | 6☐ | 7☐ |

3.  The participant's **NECK** is:

| Severely<br>Fat | Moderately<br>Fat | Mildly<br>Fat | Normal | Mildly<br>Wasted | Moderately<br>Wasted | Severely<br>Wasted |
|---|---|---|---|---|---|---|
| 1☐ | 2☐ | 3☐ | 4☐ | 5☐ | 6☐ | 7☐ |

4. The participant's **UPPER BACK** is:

| Severely<br>Fat | Moderately<br>Fat | Mildly<br>Fat | Normal | Mildly<br>Wasted | Moderately<br>Wasted | Severely<br>Wasted |
|---|---|---|---|---|---|---|

| 1☐ | 2☐ | 3☐ | 4☐ | 5☐ | 6☐ | 7☐ |

5.  The participant's **CHEST** is:

| Severely Fat | Moderately Fat | Mildly Fat | Normal | Mildly Wasted | Moderately Wasted | Severely Wasted |
|---|---|---|---|---|---|---|
| 1☐ | 2☐ | 3☐ | 4☐ | 5☐ | 6☐ | 7☐ |

6.  The participant's **ABDOMEN** is:

| Severely Protuberant | Moderately Protuberant | Mildly Protuberant | Normal | Mildly Slender | Moderately Slender | Severely Slender |
|---|---|---|---|---|---|---|
| 1 ☐☐ | 2 | 3 | 4 | 5 | 6 | 7 |

7.  The participant's **SUBCUTANEOUS ABDOMINAL FAT** is:

| Severely Fat | Moderately Fat | Mildly Fat | Average | Mildly Wasted | Moderately Wasted | Severely Wasted |
|---|---|---|---|---|---|---|
| 1☐ | 2☐ | 3☐ | 4☐ | 5☐ | 6☐ | 7☐ |

8.  The participant's **BUTTOCKS** are:

| Severely Fat | Moderately Fat | Mildly Fat | Normal | Mildly Wasted | Moderately Wasted | Severely Wasted |
|---|---|---|---|---|---|---|
| 1☐ | 2☐ | 3☐ | 4☐ | 5☐ | 6☐ | 7☐ |

9.  The participant's **LEGS** are:

| Severely Fat | Moderately Fat | Mildly Fat | Normal | Mildly Wasted | Moderately Wasted | Severely Wasted |
|---|---|---|---|---|---|---|
| 1☐ | 2☐ | 3☐ | 4☐ | 5☐ | 6☐ | 7☐ |

10. The participant's **ARMS** are:

| Severely Fat | Moderately Fat | Mildly Fat | Normal | Mildly Wasted | Moderately Wasted | Severely Wasted |
|---|---|---|---|---|---|---|
| 1☐ | 2☐ | 3☐ | 4☐ | 5☐ | 6☐ | 7☐ |

11. In general, does the participant appear to have:

| Lipoatrophy (fat loss) | Lipohypertrophy (fat accumulation) | Both Lipoatrophy and Lipohypertrophy | Neither Lipoatrophy nor Lipohypertrophy |
|---|---|---|---|
| 1☐ | 2☐ | 3☐ | 4☐ |

**Questionnaire 2**

## FRAM PHYSICAL EXAM
(Participant Administered)

The following two items are examples of types of questions included on this form. Please tell us about changes you may have noticed in your body in the **last five years**.

Example 1. Has there been a change in the amount of fat in your **ANKLES**?

☐ No

☐ Yes

☐ Don't know

---

1a.    If YES, what type of change?

| Severely Increased | Moderately Increased | Mildly Increased | Mildly Decreased | Moderately Decreased | Severely Decreased |
|:---:|:---:|:---:|:---:|:---:|:---:|
| ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 | ☐ 6 |

1b. When did you first notice the change?

____/_____
MM/ YYYY

---

Example 2.  Has there been any change in the shape of your **FINGERS**?

Please tell us about changes you may have noticed in your body in the **last five years**.

☐ No  ―――――――

☐ Yes

---

2a.     If YES, what type of change?

| Severely Rounder | Moderately Rounder | Mildly Rounder | Mildly Thinner | Moderately Thinner | Severely Thinner |
|---|---|---|---|---|---|
| ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 | ☐ 6 |

2b. When did you first notice the change?

___/_____
MMYYYY

---

1.     Has there been a change in the amount of fat in your **CHEEKS**, just **next to** your **NOSE AND MOUTH**?

☐ No

☐ Yes ―――――┐

☐ Don't know

---

1a.     If YES, what type of change?

| Severely Increased | Moderately Increased | Mildly Increased | Mildly Decreased | Moderately Decreased | Severely Decreased |
|---|---|---|---|---|---|
| ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 | ☐ 6 |

1b. When did you first notice the change?

___/_____
MM/YYYY

2.    Has there been any change in the shape of your **FACE**?

☐ No

☐ Yes ⟶

☐ Don't know

---

2a.    If YES, what type of change?

| Severely Rounder | Moderately Rounder | Mildly Rounder | Mildly Thinner | Moderately Thinner | Severely Thinner |
|---|---|---|---|---|---|
| ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 | ☐ 6 |

2b. When did you first notice the change?

___/_____
MM / YYYY

---

3.    Has there been a change in the amount of fat on your **NECK**?

☐ No

☐ Yes ⟶

☐ Don't know

---

3a.    If YES, what type of change?

| Severely Increased | Moderately Increased | Mildly Increased | Mildly Decreased | Moderately Decreased | Severely Decreased |
|---|---|---|---|---|---|
| ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 | ☐ 6 |

3b. When did you first notice the change?

___/_____
MM / YYYY

4.    Has there been a change in the size of one or both of your **BREASTS** other than related to pregnancy or nursing?

☐ No

☐ Yes ─────────────────┐

☐ Don't know

4a.    If YES, what type of change?

| Severely Larger | Moderately Larger | Mildly Larger | Mildly Smaller | Moderately Smaller | Severely Smaller |
|---|---|---|---|---|---|
| ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 | ☐ 6 |

4b. When did you notice the change?

___ / _____

MM / YYYY

5.    Has there been a change in the fat on the front of your **CHEST**?

☐ No

☐ Yes ─────────────┐

☐ Don't know

5a.    If YES, what type of change?

| Severely Increased | Moderately Increased | Mildly Increased | Mildly Decreased | Moderately Decreased | Severely Decreased |
|---|---|---|---|---|---|
| ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 | ☐ 6 |

5b. When did you first notice the change?

___/_____

MM/YYYY

6.    Has there been a change in the fat on your **UPPER BACK**?

☐ No

☐ Yes ─────────┐

☐ Don't know ────┘

---
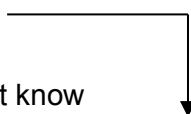
6a.  If YES, what type of change?

| Severely Increased | Moderately Increased | Mildly Increased | Mildly Decreased | Moderately Decreased | Severely Decreased |
|---|---|---|---|---|---|
| ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 | ☐ 6 |

6b. When did you first notice the change?

___/_____
MM/YYYY

7.  Has there been a change in the size of your **WAIST**?

☐ No

☐ Yes ────────┐

☐ Don't know

7a.  If YES, what type of change?

| Severely Larger | Moderately Larger | Mildly Larger | Mildly Smaller | Moderately Smaller | Severely Smaller |
|:---:|:---:|:---:|:---:|:---:|:---:|
| ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 | ☐ 6 |

7b. When did you first notice the change?

___/_____
MM  YYYY

8.  Has there been a change in the amount of fat on your **BUTTOCKS**?

☐ No

☐ Yes ────────┐

☐ Don't know

8a.  If YES, what type of change?

| Severely Increased | Moderately Increased | Mildly Increased | Mildly Decreased | Moderately Decreased | Severely Decreased |
|:---:|:---:|:---:|:---:|:---:|:---:|
| ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 | ☐ 6 |

8b. When did you first notice the change?

___/_____
MM                                                              YYYY

9. Has there been a change in the amount of fat on your **ARMS**?

☐ No

☐ Yes ─────────────┐

☐ Don't know        ▼

---

9a.  If YES, what type of change?

| Severely Increased | Moderately Increased | Mildly Increased | Mildly Decreased | Moderately Decreased | Severely Decreased |
|:---:|:---:|:---:|:---:|:---:|:---:|
| ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 | ☐ 6 |

9b. When did you first notice the change?

___/_____
MM/YYYY

---

10. Has there been a change in the amount of fat on your **LEGS**?

☐ No

☐ Yes ———————————┐

☐ Don't know

10a. If YES, what type of change?

| Severely Increased | Moderately Increased | Mildly Increased | Mildly Decreased | Moderately Decreased | Severely Decreased |
|---|---|---|---|---|---|
| ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 | ☐ 6 |

10b. When did you first notice the change?

___/_____

MM/YYYY

11. Has there been a change in your **BELLY or ABDOMINAL FAT**?

☐ No

☐ Yes ———————————┐

☐ Don't know

11a. If YES, what type of change?

| Severely Increased | Moderately Increased | Mildly Increased | Mildly Decreased | Moderately Decreased | Severely Decreased |
|---|---|---|---|---|---|
| ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 | ☐ 6 |

11b. When did you first notice the change?

___/_____

MM  YYYY

12.   Have you noticed any other **new areas of fatty lumps**?

☐ No

☐ Yes

☐ Don't know

---

12a.  If YES, where on your body have you noticed this?  (Please mark all that apply.)

☐ Neck

☐ Back

☐ Chest

☐ Arms

☐ Belly

☐ Buttocks

☐ Legs

☐ Other (Please specify): _____

Appendix B

**Anthropometric Measurements**

**Procedures for measurement of circumferences**

General Information

1. Subject should be dressed in underwear, socks, and a hospital gown; all outer clothing should be removed.

2. Use non-stretchable, cloth or vinyl measuring tape that measures in centimeters or millimeters and is at least one half inch in width.

3. Make sure the tape does not compress the tissues during the measurement.

4. Measuring tape should always be read at eye level.

5. All measurements should be made in triplicate.

Hip Circumference:

1. The subject should be standing erect but relaxed.

2. Ask the subject not to try to hold in the stomach during the measurements.

3. Viewing the subject from the side, visually identify the widest width of the hip. The hospital gown may be held to conform to the subject's contour; the widest point is generally where there is maximal protuberance of the buttocks.

4. Measure circumference at that point, making sure the measuring tape is exactly parallel to the floor. Record the result in cm to the nearest millimeter.

5. Repeat this procedure twice, so that in all 3 measurements are performed.

Waist Circumferences:

The subject should be standing erect but relaxed.

1. Ask the subject not to try to hold in the stomach during the measurements.

2. All measurements should be made after subject has exhaled.

3. The usual method is to measure the smallest circumference around the waist. However, this measurement is not sufficient in individuals with increased abdominal girth. Therefore, we obtain three different circumferences:

   a) Minimal waist (conventional): Viewing the subject from the front or rear, identify the smallest width of the waist; measure circumference at that point.

   b) Umbilicus waist: Measure circumference at the level of the navel.

   c) Midwaist: Locate the upper border of the right ilium and measure the waist circumference at this level. The tape measure should be parallel to the floor.

4. In each case, the measuring tape should be parallel to the floor during the measurement.

5. Perform each measurement in triplicate, recording results in cm to the nearest mm.

Mid-arm Circumference:

1. All measurements should be performed on the right arm unless there is a specific reason why this is not possible.  At the time of the first measurement, note the side used in the source document and use the same side for all subsequent measurements.  To correctly locate the midarm region, upper arm length should first be measured.

2. Ask the subject to bend the arm at right angle with the palm facing upward.

3. Locate the acromial process on shoulder blade.  It may help to slide your fingers along the clavicle to find the acromial process.

4. Locate the olecranon process, which is the tip of the elbow.

5. Using a measuring tape, measure down the posterior aspect of the arm between these two points, being careful to keep the tape straight by holding it slightly away from the two end points if necessary.

6. Divide the length by 2, and mark this midpoint on the arm with a pen.

7. Ask the subject to relax the arm at his/her side with the palm facing inward.  Make certain that the subject is not flexing the muscles in the arm.

8. Place the measuring tape around the arm at this midpoint, holding the tape horizontal to the floor (and, therefore, perpendicular to the length of the arm).

9. The tape should be touching the skin continuously and should follow the contours of the tissue (i.e., no gaps), but it should not compress the skin or tissue.

10. Record measurement in cm to the nearest mm.

11. Repeat the circumference measurement twice and record.  In all, the measurement should be made 3 times.

Mid-thigh Circumference:

1. As is the case with the midarm circumference, this measurement should always be made on the right side, unless doing so is impossible. To correctly locate the midthigh region, upper leg length should first be measured.

2. Ask the subject to sit on a chair, exam table, or bed, with the knee bent at a 90° angle.

3. Locate the midpoint of the upper border of the patella (kneecap).

4. Locate the inguinal crease, just below the anterior superior iliac spine. An easily identifiable landmark is the tendon that moves when the leg is flexed upward slightly.

5. Measure the length between these two marks.

6. Divide the length by 2, and mark this midpoint on the top of the thigh, making sure the tape measure remains straight.

7. Ask the subject to stand, with the foot of the right leg slightly forward from that of the left leg. The knee of the right leg should be flexed slightly, and all of the weight should be on the left leg.

8. Ask the subject not to flex the muscles in the thigh.

9. Measure circumference across the midpoint, holding the tape perpendicular to the length of the thigh.

10. Record measurement in cm to the nearest mm.

11. Repeat the circumference measurement twice and record. In all, the measurement should be made 3 times.

Neck Circumference:

The subject should be sitting erect but relaxed.

1. Locate and mark the vertebra prominens (C7) on the posterior aspect of the neck. The vertebra prominens is the first spinous process palpated when the fingers are moved downward along the midline of the posterior neck.

2. Locate and mark the laryngeal prominence of the thyroid cartilage. The laryngeal prominence is the most protruding midline structure on the anterior surface of the neck in men and is often called the Adam's apple. In women, the laryngeal prominence of the thyroid cartilage is less visible, but can be palpated between the hyoid bone and the cricoid cartilage.

3. Place the tape measure at the back of the neck just above the vertebra prominens (C7) and bring the tape measure circumferentially around to a point just under the laryngeal prominence. This should be a smooth, downward sloping circumference measurement along the skin contour. There should be no slack or give in the tape. Record the measurement in cm to the nearest mm.

4. Repeat this procedure two more times, so that in all, three measurements are performed.

Chest Circumference:

1. The subject should be standing erect, feet apart at shoulder width.

2. The chest should be bare; however, women may wear a strapless bra. If a woman wears a bra for the first measurement, she should wear one for all subsequent measurements; and if she does not wear a bra at baseline, she should not wear one for all subsequent measurements.

3. The tape should be parallel to the floor at the level of the $4^{th}$ costosternal joints.

4. The measurement should be taken at the end of a normal expiration.

5. Record the measurement in cm to the nearest mm.

6. Repeat the measurement twice and record all three measurements.

# CONSENT DOCUMENTS

**<u>INFORMATION GIVEN TO PARTICIPANTS</u>**

# <u>INFORMATION DOCUMENT</u>

**Study title: Metabolic complications of HAART in a South African Black Population**

**Dear Participant**

**Introduction:**

We, the researchers at the University of KwaZulu-Natal, are doing research on the distribution of fat in the body, sugar in the blood and fat in the blood. Research is just the process to learn the answer to a question. I am doing this research for a higher degree called the PhD. In this study we want to learn if people without HIV infection have changes in the body that may lead to the development of diabetes and heart disease; or whether this is a problem that may be found in those with HIV infection or those receiving treatment used for treating HIV infection. The factors we are investigating include changes in the distribution of fat in the body, fat in the blood or sugar in the blood.

Treatment for HIV has been shown to be effective in reducing the amount of the HIV virus in the blood, thus prolonging life and improving the quality of life. However it has also been shown in parts of the world where this form of treatment has been available for longer periods that there may be some complications related to this treatment. Therefore we want to perform this study in our own population to evaluate whether the body changes seen among people infected with HIV are as a consequence of HIV infection per se, therapy for treating the disease or perhaps factors in the body that are unrelated to HIV infection and may be found in those without HIV infection.

For those requiring antiretroviral therapy, the treatment being offered in the study is used for people who are treated for HIV infection, however, some of the tests that will be conducted are not part of routine care. Some of these tests are aimed at answering research questions relating to the possible causes or associations with treatment complications.

**Invitation to participate:** You are invited to participate in this important research study.

**What is involved in the study** – The first question that this study seeks to answer is whether body fat distribution changes seen among patients with HIV infection is associated with HIV infection or whether these changes may be found even among people that are not infected with HIV. To answer this question we will compare fat distribution patterns among those that are HIV infected with those that are not infected.

The next question relates to whether antiretroviral therapy is responsible for the body fat distribution changes and related complications among those treated with this form of therapy. This question will be answered by comparing body fat distribution before starting antiretroviral treatment with distribution patterns after 24 months on treatment.

The study will be conducted in two steps. Eighty eight participants both HIV infected and uninfected will be enrolled in each arm of step 1 of the study. Procedures for this first step of the study will be conducted once. One hundred fifty participants will be enrolled in step 2 of the study; these will be treated with HAART, monitored and followed up for a period of up to 24 months.

Each participant will complete a self-administered questionnaire that will include questions relating to body fat distribution changes; receive a physical examination and have blood and Xray tests performed. One tube of blood will be stored for possible future genetic tests based on the outcomes of the research. These procedures will be conducted at baseline for all participants and for those followed up in step 2 of the study at month 3, 6, 12, 18 and 24; further tests which include blood tests and X-ray and a special X-ray called CAT scan (CT) scan, Dual X-Ray Absorpitometry (DXA), will be performed at baseline, month 12 and 24. About 2 spoons of urine sample will be stored for tests on urine markers or metabolism that may become available in the future.

Risks **of being involved in the study are the same as for those receiving antiretroviral treatment outside of the study in relation to treatment related complications as similar treatment will be used.**

**Potential Benefits** of being in the study for those enrolled for step 1 of the study, include the fact that you will receive free counseling to help you cope with your disease and education on how you can stay healthy for a long period of time. If you are at a stage where you require antiretroviral therapy, the opportunity to be started on antiretroviral therapy will be made available to you. If you are enrolled in the first step of the study and are found not to have HIV infection, you will receive free education to help you stay HIV free.

If you are enrolled for step 2 of the study, you will receive close monitoring of your disease and treatment related complications. Ongoing counseling to help you cope with both the treatment and disease will be offered.

Since the treatment being offered is the same as that offered to all those infected with HIV, there is no real alternative to treatment that could be offered. Some of the tests that will be performed will be for study purposes and these might help in identifying complications early before they fully manifest.

**As a participant, you will be given important information about the study while you are involved in the project and after the study results are available.**

**Participation is voluntary**, that refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled as a participant. You may discontinue participation at any time without penalty loss of benefits to which you are otherwise entitled.

**Reimbursements** for "out of pocket" expenses: Participants will be reimbursed R100 for travel costs, refreshments and other expenses incurred in relation to the study.

**Confidentiality**: Every effort will be made to keep personal information confidential. Absolute confidentiality cannot be guaranteed. Personal information may be disclosed if

required by law. Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the Research Ethics Committee,

_____

Data Safety Monitoring Committee and the Medicines Control Council (where appropriate).

If results are published, your personal information will not be used and therefore you will remain anonymous.

Contact details of researcher/s – for further information or reporting of study related adverse events, please contact Dr Nombulelo Magula on 031 260 4238 or email: magulan@ukzn.ac.za


**Contact details of BREC Administrator or Chair** – for reporting of complaints/ problems:

**Biomedical Research Ethics, Research Office, UKZN, Private Bag X54001, Durban 4000**

Telephone:     +27 (0) 31 260 4769 / 260 1074

Fax:              +27 (0) 31 260 2384

Administrator: Ms P NgwenyaEmail:          ngwenyap@ukzn.ac.za

Chair: Email:  Prof D R Wassenaar          c/o ngwenyap@ukzn.ac.za


The following must be included in the consent form when applicable:


a.    A statement that the particular treatment or procedure may involve risks to the subject (or to the embryo or fetus, if the subject is or may become pregnant) that are currently unforeseeable.
b.    Anticipated circumstances under which participation may be terminated by the investigator without the participant's consent.
c.    Any additional costs to the participant that may result from participation in the research.
d.    The consequences of a participant's decision to withdraw from the research and procedures for orderly termination of participation by the participant.
e.    A statement that significant new findings developed during the course of the research which may relate to the participant's willingness to continue participation will be provided to the participant.
f.     Where genetic tests are to be done, a separate information sheet and consent form will be made available.
If applicable, a statement that specimens will be stored for future research pertaining to the specific research question being studied. Specify how long specimens will be stored for, where they will be stored, whether they will be shipped out of South Africa, whether samples will be anonymized. If stored for future genetic testing, a further signed separate consent form is required.

# CONSENT DOCUMENT

## Consent to Participate in Research

Greeting: Dear Participant

My name is Dr Nombulelo Magula. I am studying for a higher degree called PhD at the University of KwaZulu-Natal, Nelson R Mandela School of Medicine and I am conducting research on the distribution of fat in the body, sugar in the blood and fat in the blood.

You have been asked to participate in this research study to assess fat distribution patterns, sugar and fat in the blood among people with HIV infection who are not on antiretroviral therapy compared with those that are not HIV infected; and those infected with HIV who are on antiretroviral therapy. As part of the study, you will be asked to complete a self-administered questionnaire that will include questions relating to body fat distribution changes; receive a physical examination and have laboratory and radiological tests performed. One anonymized tube of blood will be stored for possible future genetic tests based on the outcomes of the research. A sample of urine will be stored for future metabolite tests. The blood will be stored at a Doris Duke Medical Research Institute laboratory at the University of KwaZulu-Natal. All your information will be kept confidentially. Excess sample material will be destroyed after five years or sooner if you specifically so requested.

You have been informed about the study by Dr Nombulelo Magula.

You may contact Dr Magula at King Edward VIII Hospital or by telephone on 031 260 4238 at any time if you have questions about the research or if you are injured as a result of the research. You may contact the **Biomedical Research Ethics Office** on **031-260 4769 or 260 1074** if you have questions about your rights as a research participant.

Your participation in this research is voluntary, and you will not be penalized or lose benefits if you refuse to participate or decide to stop at any time.

If you agree to participate, you will be given a signed copy of this document and the participant information sheet which is a written summary of the research. The research study, including the above information, has been described to me orally. I understand what my involvement in the study means and I voluntarily agree to participate. I have been given an opportunity to ask any questions that I might have about participation in the study.

_____  _____

**Signature of Participant**                    **Date**


_____  _____

**Signature of Witness**                         **Date**

**(Where applicable)**


_____  _____

**Signature of Translator**                      **Date**

**(Where applicable)**

# CONSENT DOCUMENT

**Consent to Participate in Research**          **Study ID:**

You have been asked to participate in a research study:  **Metabolic Complications of Highly Active Antiretroviral Therapy in a South African Black Population.**

Your signatures below are needed to indicate that you agree to participate in this research project.

I have been informed about the study by **Dr N Magula.**

I have been informed about any available compensation or medical treatment if injury occurs as a result of study-related procedures.

_____   _____          _____

**Name (PRINT)**                    **Signature of Participant**             **Date**

I understand that my participation in this research is voluntary, and I will not be penalized or lose benefits if I refuse to participate or decide to stop.

_____   _____          _____

**Name (PRINT)**                    **Signature of Participant**             **Date**

I understand that some of my blood will be collected and used for examination of genes that may be associated with the distribution of fat in the body, sugar in the blood and fat in the blood. The research will be conducted at the Nelson R Mandela School of Medicine, University of KwaZulu-Natal.

_____   _____          _____

**Name (PRINT)**                    **Signature of Participant**             **Date**

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I agree that some of my DNA may be stored for up to 5 years such that if further studies on genes that may influence the distribution of fat in the body, sugar in the blood and fat in the blood are needed, the stored DNA can be used for that purpose. Disposal of DNA is done by incineration.


_____      _____      _____

**Name (PRINT)**                    **Signature of Participant**              **Date**


The research study, including the above information, has been described to me orally. I understand what my involvement in the study means and I voluntarily agree to participate.


_____      _____      _____

**Name (PRINT)**                    **Signature of Participant**              **Date**


**Contact details of the Biomedical Research Ethics Administration Research Office:**

(For reporting of any complaints or problems)


University of KwaZulu-Natal

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**Contact details of Dr N Magula:**

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Congella

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Email:        magulan@ukzn.ac.za

isiZulu Consent Versions

## **ULWAZI OLUNIKEZWA ABABAMBE IQHAZA**

### **UMQULU WOLWAZI**

**Isihloko socwaningo: Izinkinga zenhliziyo ezithinta indlela eyejwayelekile yokukhiqizeka kwamandla nokukhula kumuntu ze-HAART emphakathini onsundu waseNingizimu-Afrika**

**Mbambi weqhaza othandekayo**

**Isingeniso**

Thina bacwaningi baseNyuvesi yaKwaZulu-Natali, senza ucwaningo ngokusabalala kwamafutha emzimbeni, ushukela egazini kanye namafutha egazini. Ucwaningo luyinqubo nje ejwayelekile yokufunda ngempendulo embuzweni. Ngenza lolu cwaningo njengengxenye yeziqu eziphakeme ezibizwa nge-PhD. Kulolu cwaningo sifuna ukuthola ukuthi kungabe abantu abangenalo igciwane lesandulela ngculazi (HIV) baba nalo yini ushintsho emizimbeni yabo engaholela ekutheni kudaleke isifo sikashukela kanjalo nesifo senhliziyo, noma lena yinkinga engatholakala kulabo abanegciwane lesandulela ngculazi (HIV) noma labo abasebenzisa imishanguzo elwisana negciwane lesandulela ngculazi (HIV). Izimo esizicwaningayo zibandakanya ukusabalala kwamafutha emzimbeni, amafutha egazini kanye noshukela egazini.

Imishanguzo elwisana negciwane lesandulela ngculazi (HIV) iboniswe njengephumelelayo ekunciphiseni izinga legciwane lesandulela ngculazi egazini, ngalokho yandisa iminyaka yokuphila futhi ithuthukisa nezinga lokuphila kulabo abakhahlanyezwe yiogciwane. Ngakolunye uhlangothi kuphinde kwavela ezingxenyeni zomhlaba lapho lolu hlobo lomshanguzo lube khona isikhathi eside, lokho okungaze kudale izinkinga ezithile ezihlobene nalo mshanguzo. Ngakho-ke sifuna ukwenza lolu cwaningo emphakathini wakithi ukuhlonza ukuthi ngabe uguquko emzimbeni olubonakala phakathi kwabantu abanesandulela ngculazi (HIV) ngenxa yomthelela wegciwane, unyango lokwelapha isifo noma-ke izimpawu emzimbeni ezingahlobene negciwane lesandulela ngculazi (HIV) futhi nangatholakala kulabo abangenalo igciwane lesandulela ngculazi (HIV).

Kulabo abadinga ukwelashwa ngemishanguzo yesandulela ngculazi, ukwelashwa okunikezelwayo ocwaningweni kusetshenziselwa abantu abelashelwa igciwane lesandulela ngculazi, kepha-ke, ezinye zezivivinyo ezizokwenziwa aziyona ingxenye yokunakekelwa okwejwayelekile. Ezinye zalezi zivivinyo zihlose ukuphendula imibuzo ehlobene nezimbangela ezilindelekile noma ubudlelwano nezinkinga zokwelashwa.

**Isimemo sokubamba iqhaza:** Uyamenywa ukuba ubambe iqhaza kulolu cwaningo olubalulekile.

**Yini ebandakanyekayo kulolu cwaningo –** Umbuzo wokuqala lolu cwaningo oluhlose ukuwuphendula ukuthi kungabe izinguquko ezidalwa wukusabalala kwamafutha emzimbeni olubonakala ezigulini ezinegciwane lesandulela ngculazi luhlobene negciwane lesandulela ngculazi noma lezi zinguquko zingatholakala naphakathi kwabantu abangenali igciwane lesandulela ngculazi. Ukuze siphendule lo mbuzo sizoqhathanisa izindledlana zokusabalala kwamafutha kulabo abanegciwane lesandulela ngculazi kanjalo nalabo abangenalo igciwane.

Umbuzo olandelayo uhlobene nokuthi kungabe ukwelapha ngemishanguzo elwisana negciwane lesandulela ngculazi yikona yini okudala izinguquko zokusabalala kwamafutha emzimbeni kanjalo nezinkinga ezihlobene nalezo kulabo abelashwa ngalolu hlobo lonyango. Lo mbuzo uzophenduleka ngokuthi kuqhathaniswe ukusabalala kwamafutha emzimbeni ngaphambi kokuqalisa ukwelashwa ngemishanguzo elwisana nesandulela ngculazi, kanjalo nezindledlana zokusabalala eziqhubeka emva kwezinyanga ezingamashumi amabili nane zokwelashwa.

Ucwaningo luzokwenziwa kulandelwa amahlandla amabili. Ababambe iqhaza abangamashumi ayisishiyagalombili nesishiyagalombili abanegciwane nalabo abangenalo bazoba yingxenye yehlandla lokuqala locwaningo. Izinqubo zaleli hlandla lokuqala locwaningo zizokwenziwa kanye. Ababambe iqhaza abayikhulu namashumi ayisihlanu bazoba yingxenye yehlandla lesibili locwaningo, labo bazokwelashwa ngohlelo lwe-HAART, oluzobhekelelwa lubuye lulandelelwe esikhathini esingafinyelela ezinyangeni ezingamashumi amabili nane.

Yilowo nalowo obambe iqhaza uyogcwalisa uhla lwemibuzo ehleliwe olunikezelwa ngabaqhuba ucwaningo eyobandakanya imibuzo ehlobene nezinguquko ezidalwa wukusabalala kwamafutha emzimbeni, uyophinde ahlolwe emzimbeni abuye ahlolwe igazi enze ne X-ray. Ishubhu elilodwa legazi liyogcinwa ukuze lisetshenziswe ekuhlolweni kwangomuso kuye ngemiphumela yocwaningo. Lezi zinqubo zizolandelwa ngokufana kubo bonke ababambe iqhaza kanye nalabo abalandelelwayo ehlandlelni lesibili locwaningo, ukuhlola okwengeziwe kuyobandakanya ukuhlolwa kwegazi kanye ne-X-ray kanye ne-X-ray eyisipesheli ebizwa nge-CAT scan (CT), lokho kuyokwenziwa enyangeni yesi-3, yesi-6, ye-12, ye-18 kanye neyama-24.

**Izingozi zokuzibandakanya kulolu cwaningo ziyafana nalezo zalabo abathola ukwelashwa ngomshanguzo olwisana negciwane lesandulela ngculazi egazini ngaphandle kocwaningo kuhlobene nezinkinga ezihambisana nokwelashwa, njengalokhu kwefana ukwelashwa okuzosetshenziswa.**

**Imihlomulo elindelekile** yokuzibandakanya kulolu cwaningo kulabo abasohleni lwehlandla lokuqala locwaningo, ibandakanya iqiniso lokuthi uyothola ukwelulekwa kwamahhala ukuze ubhekane nesifo kanye nesifundo sokuthi ungahlala kanjani uphilile isikhathi eside. Uma kwenzeka ukuthi usezingeni lokuthi udinga ukwelashwa ngemishanguzo elwisana negciwane lesandulela ngculazi, ithuba lokuthi uqale ukusebenzisa imishanguzo liyonikezelwa kuwe. Uma usohleni lwababambe iqhaza ehlandleni lokuqala futhi utholakala ungenalo igciwane lesandulela ngculazi,

uyothola isifundo samahhala esiyokusiza ukuthi uhlale uzivikele ekuhlaselweni yigciwane lesandulela ngculazi.

Uma usohleni lwehlandla lesibili locwaningo, uyothola ukulandelelwa okuseduze kwesifo kanye nokwelashwa okubhekelele ubungozi begciwane. Uyokwelulekwa ukuze usizakale ekubhekaneni nokwelashwa kanye nesifo.

Ngenxa yokuthi ukwelashwa okunikezelwayo kufana ncimishi naloko okunikezelwa kulabo abanegciwane lesandulela ngculazi, akukho okunye ukwelashwa okuyobuye kusetshenziswe uma kwehluleka loko obekusetshenziswe kuqala. Okunye ukuhlolwa okuyokwenziwa kuyobe kungokwezinhloso zocwaningo futhi lokho kuyosiza ukuhlonza izinkinga kusenesikhathi ngaphambi kokuthi zibe nomthelela ongemuhle.

**Njengobambe iqhaza, uyonikezelwa ngemininingwane ebalulekile yocwaningo ngesikhathi uqhubeka nokuba yingxenye yocwaningo nangasemuva kokuphuma kwemiphumela yocwaningo.**

**Ukubamba iqhaza kulolu cwaningo akuphoqelekile,** ukwala ukuba yingxenye yocwaningo akuhambisani nesijeziso noma nokulahlekelwa yimihlomulo obufanele ukuyithola njengobambe iqhaza kulolu cwaningo. Ungakhetha ukuphonsa ithawula ngesikhathi ucwaningo luqhubeka ngaphandle kwesijeziso nokulahlekelwa yimihlomulo obekufanele uyithole njengobambe iqhaza.

**Izinxephezelo,** zezindleko "eziphume ephaketheni": Ababambe iqhaza bayonxeshezelwa ngamarandi ayikhulu (R 100, 00) okuyizindleko zokugibela, ukudla kanye nezinye izindleko ezimayelana nocwaningo.

**Ukugcinwa kwemininingwane iyimfihlo:** Kuyokwenziwa yonke imizamo ukugcina imininingwane ebucayi iyimfihlo. Ukugcinwa okupheleleyo kwemininingwane iyimfihlo akuqinisekisiwe. Imininingwane ebucayi ingavezwa uma idingwa ngabomthetho.

Izinhlangano ezingenza inhlolovo noma zikopishe amarekhodi ocwaningo lwakho ukuqinisekisa ubuqiniso bawo nokuhlaziya ulwazi oluthokakele zibandakanya Ikomidi Lemigomo Yokwenza Ucwaningo (Research Ethics Committee)

Ikomidi Elilandelela Ukuphepha Kolwazi oluqoqiwe (Data Safety Monitoring Committee), kanye noMkhandlu Wokulawulwa Kokusetshenziswa Kwemithi (Medicines Control Council) (Lapho kudingekile)

**Uma kwenzeka imininingwane ishicilelwa, imininingwane yakho ngeke isetshenziswe, ngakho uyohlala ungumuntu ongaziwa.**

**Imininingwane yabacwaningi – Mayelana nolwazi olwengeziwe noma ukubika ngezigameko ezimbi ezihlobene nocwaningo, thintana noDkt. Nombulelo Magula kule nombolo: 031 260 4238 noma i-email: [magulan@ukzn.ac.za](mailto:magulan@ukzn.ac.za)**

**Imininingwane NgoMqondisi we-BREC noma uSihlalo – Ukudlulisa izikhalo noma izinkinga:**

**Ihhovisi le-Biomedical Research Ethics, UKZN, Private Bag X54001, Durban 4000**

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Lokhu okulandelayo kumele kubandakanywe kwifomu yemvume uma kudingekile:

a. Isitatimende sokuthi ukwelashwa okuthile noma inqubo elandelwe ingabandakanya ubungozi kulabo ababandakanyekayo (noma ehlulini noma embungwini, uma lowo obambe iqhaza ekhulelwe noma ekhulelwa) obungeke bubonakale okwamanje.

b. Izimo ezilindelekile lapho ukubamba iqhaza kungaphazanyiswa yilowo oqhuba ucwaningo ngaphandle kwesivumelwano nobambe iqhaza.

c. Nanoma yiziphi izindleko ezengeziwe kulowo obambe iqhaza ezingaba yimiphumela yokubamba iqhaza kulolu ocwaningweni.

d. Imiphumela yokuhoxa kwalowo obambe iqhaza ocwaningweni kanye nemigomo elandelwe yokuhoxa ocwaningweni kwalowo obambe iqhaza.

e. Isitatimende sokuthi ulwazi olusha olutholakele noluthuthuke ngesikhathi ucwaningo luqhubeka nolungaholela ekutheni lowo obambe iqhaza abe nesifiso sokuqhubeka abambe iqhaza, siyonikezelwa kulowo obambe iqhaza.

f. Lapho kuhlolwa izinhlayiyana ezidlulisa ufuzo, elinye ishidi leminingwane nefomu yemvume kuyonikezelwa.

Uma kunesidingo, isitatimende sokuthi amasampula ocwaningo ayogcinelwa ucwaningo lwangomuso oluthinta umbuzo othile wocwaningo olwenziwayo. Futhi luyocacisa ukuthi amasampula ayogcinwa isikhathi esingakanani, nokuthi ayogcinwa isikhathi esingakanani, nanokuthi ngabe amasampula ayoyeqa yini imingcele yaseNingizimu-Afrika, nokuthi amasampula kungabe ayohlala engaziwa yini lapho ethathwe khona. Uma egcinelwe ukuhlolwa kwezinhlayiyana ezidlulisa ufuzo, kuyodingeka kube khona imvume eyengeziwe esayinwayo.

# UMQULU WEMVUME

## Imvume yokubamba iqhaza ocwaningweni

Ukubingelela: Mbambi weqhaza othandekayo

Igama lami nginguNombulelo Magula, Ngenza iziqu eziphakeme ezibizwa nge-PhD eNyuvesi yaKwaZulu-Natali, Esikoleni Sezifundo Zokwelapha, i-*Nelson Mandela School of Medicine*, ngenza ucwaningo ngokusabalala kwamafutha emzimbeni, ushukela egazini kanye namafutha egazini.

Uyacelwa ukuba ubambe iqhaza kulolu cwaningo ukuhlola indlela elandelwa wukusabalala kwamafutha, ushukela namafutha egazini kubantu abanegciwane lesadulela ngculazi abangelashwa ngemishanguzo elwisana negciwane lesandulela ngculazi kuqhathaniswa nalabo abangakakhahlanyezwa yigciwane; kanye nalabo abakhahlanyezwe yigciwane lesandulela ngculazi abasebenzisa imishanguzo elwisana negciwane lesandulela ngculazi egazini. Njengengxenye yocwaningo, uyocelwa ukuba ugcwalise uhla lwemibuzo ehleliwe oyonikezwa yona ngabaqhuba ucwaningo oluyobandakanya imibuzo ehlobene nezinguquko ezidalwa wukusabalala kwamafutha emzimbeni, uyophinda uhlolwe emzimbeni bese kwenziwa nezivivinyo eziyogcinwa emagunjaneni okugcina ucwaningo lwesayensi kanjalo nocwaningo lwama-X-ray. Igazi lakho liyothathwa futhi ukuhlola i-*DNA* njengoba lokhu kungahlotshaniswa nesimo ucwaningo olwenziwa phezu kwaso. Ishubhu elilodwa lwegazi luyogcinwa lungenagama kwenzelwa ukuhlolwa kwangomuso kwezinhlayiyana ezidlulisa ufuzo ezihlobene noma ezincike emiphumeleni yalolu cwaningo. Igazi liyogcinwa egunjaneni lokugcinwa kocwaningo lwesayensi Lwezokwelapha, i-Doris Duke Medical Research Institute eYunivesithi yaKwaZulu-Natali. Yonke imininingwane yakho iyogcinwa iyimfihlo. Amasampula aseleyo ayocekelwa phansi emva kweminyaka emihlanu noma ngaphansi kwaleyo minyaka uma unesicelo sokuthi kwenzeke kanjalo

Ulwazi olumayelana nocwaningo uluthole ngoNombulelo Magula

Ungaxhumana noDkt. Magula e-King Edward VIII Hospital noma ngocingo ku: 031 260 4238 nanganoma yisiphi isikhathi uma unemibuzo mayelana nocwaningo noma uma uthole ukulimala okuthile ngenxa yokubamba iqhaza ocwaningweni.

Ungaxhumana **neHhovisi, i-Biomedical Research Ethics Office,** kule nombolo: **031-260 4769 noma ku: 260 1074** uma unemibuzo emayelana namalungelo akho njengobambe iqhaza ocwaningweni.

Akuphoqelekile ukubamba iqhaza kulolu cwaningo, futhi ngeke ujeziswe noma ulahlekelwe yimihlomulo uma wala ukubamba iqhaza noma ukhetha ukuhoxa ocwaningweni nangananoma yisiphi isikhathi.

Uma uvuma ukubamba iqhaza, uyonikezwa ikhophi esayiniwe yalo mqulu kanye neshidi lemininingwane yokubamba iqhaza eyisifinyezo esibhalwe phansi socwaningo.

Lolu cwaningo, kubandakanya ulwazi olungenhla, luchazwe kabanzi kimina ngomlomo. Ngiyaqonda ukuthi kusho ukuthini ukubamba kwami iqhaza kulolu cwaningo futhi ngivuma ngaphandle kokuphoqwa ukubamba iqhaza kulolu cwaningo. Nginikiwe ithuba lokubuza noma iyiphi imibuzo engingaba nayo mayelana nokubamba kwami iqhaza kulolu cwaningo.

_____ _____
**Isiginesha yobambe iqhaza** **Usuku**


_____ _____
**Isiginesha kafakazi** **Usuku**
**(uma kufanele)**


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**Isiginesha yomhumushi** **Usuku**
**(uma kufanele)**

# UMQULU WEMVUME

**Imvume yokubamba iqhaza ocwaningweni** **Inombolo Yomfundi:**

Ucelwe ukuba ubambe iqhaza ocwaningweni, olusihloko sithi: **Metabolic Complications of Highly Active Antiretroviral Therapy in a South African Black Population**

Amasiginesha akho angezansi ayadingeka ukuveza ubufakazi bokuthi uyavumelana nokubamba iqhaza kulolu cwaningo.

Ngazisiwe ngocwaningo ngu**Dkt. N. Magula.**

Ngazisiwe mayelana nazo zonke izinxephezelo noma ukwelashwa ngemithi uma kwenzeka ingozi edalwe yindlela okuqhutshwe ngayo ucwaningo.

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**Igama (Ngokugqamile)** **Isiginesha yobambe iqhaza** **Usuku**

Ngiyaqonda ukuthi akuphoqelekile ukubamba kwami iqhaza kulolu cwaningo, futhi ngeke ngijeziswe noma ngilahlekelwe yimihlomulo uma ngala ukubamba iqhaza kulolu cwaningo noma ngikhetha ukuhoxa.

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**Igama (Ngokugqamile)** **Isiginesha yobambe iqhaza** **Usuku**

Ngiyaqonda ukuthi elinye lamagazi ami liyoqoqwa futhi lisetshenziswe ukuhlola izinhlayiyana ezidlulisa ufuzo ezihlobene nokusabalala kwamafutha emzimbeni, ushukela egazini kanye namafutha egazini. Ucwaningo luyokwenziwa eSikoleni Sezokwelapha saseNyuvesi yaKwaZulu-Natali, i-*Nelson Mandela School of Medicine*.

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**Igama (Ngokugqamile)** **Isiginesha yobambe iqhaza** **Usuku**

Ngiyavuma ukuthi ezinye izingxenye ze-DNA yami ziyogcinwa isikhathi esingafinyelela eminyakeni emihlanu, kangangokuthi uma kunocwaningo olwengeziwe lwezinhlayiyana ezidlulisa ufuzo ezingahlobana nokusabalala kwamafutha egazini, ushukela egazini kanye namafutha egazini, i-DNA egciniwe ingasetshenziselwa lezo zinhloso. Ukulahlwa kwe-DNA kwenziwa ngokushiswa.

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**Igama (Ngokugqamile)** **Isiginesha yobambe iqhaza** **Usuku**

Ucwaningo, kubandakanya nemininingwane engenhla, kuchaziwe kimi ngomlomo. Ngiyaqonda ukuthi ukuzibandakanya kwami kulolu cwaningo kusho ukuthi ngiyazivumela ukubamba iqhaza.

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_____
**Igama (Ngokugqamile)        Isiginesha yobambe iqhaza        Usuku**


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