

**LIPID DISORDER IN HIV INFECTION:  
APOLIPOPROTEIN-B KINETICS, FAT DISTRIBUTION,  
INSULIN RESISTANCE AND ADIPOCYTOKINES IN  
PATIENTS TAKING PROTEASE INHIBITORS OR NON-  
NUCLEOSIDE REVERSE TRANSCRIPTASE  
INHIBITORS**

**By**

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## Abstract

### Background:

Antiretroviral (ARV) treatment of HIV infection is associated with disturbances in body fat distribution, dyslipidaemia, insulin resistance and diabetes. The exact mechanisms of these changes have not been fully understood.

Fat redistribution with loss or accumulation of excess fat in different regions can be associated with changes in adipocytokines. The adipocytokines also play key roles in glucose and fat metabolism.

### Objective:

To investigate the effect of HIV infection and ARV on apolipoprotein-B (apo-B) metabolism and body fat distribution.

To investigate the effect of adiponectin and other cytokines on apo-B metabolism, lipid changes, insulin resistance and body fat distribution.

### Methods

We performed a cross sectional study on 67 subjects who were HIV positive (n=55), or presumed HIV negative controls (n=12). The subjects were treatment naïve (n=15) or taking two nucleoside analogues plus either a protease inhibitor (PI, n=15), or a non-nucleoside reverse transcriptase inhibitor (NNRTI, n=25) for between 1 – 6 years.

Apo-B kinetics and lipid composition were measured following an infusion of a stable isotope  $^{13}\text{C}$  leucine after an overnight fast. Body fat distribution was measured by whole body dual energy X-ray absorptiometry (DEXA) scan and expressed in terms of lean body mass (LBM). Plasma adiponectin and leptin were measured by radioimmunoassay and cytokines levels were measured in a multiplex bead sandwich immunoassay.

Insulin Resistance was calculated using the Homeostasis Assessment Model ( $\text{HOMA}_{\text{IR}}$ ). The apo-B kinetics were calculated using a multicompartmental model with an intrahepatic delay function.

### Results

1. Apo-B kinetics: The very low density lipoprotein (VLDL), Intermediate density lipoprotein (IDL) and low density lipoprotein (LDL) apo-B fractional clearance rate (FCR) was reduced ( $p=0.005$ ) with longer circulating residence time of the different apo-B containing particles in the three lipoprotein compartments in all the HIV positive groups compared to HIV negative controls ( $p=0.05$ ). The HIV positive treatment groups had mild dyslipidaemia. The treatment naïve group and the HIV-negative control group did not have any difference in serum triglyceride (TG) and total cholesterol (TC). Compared to the control subjects, PI-treated patients exhibited raised serum TG, LDL cholesterol (LDL-C) and IDL cholesterol (IDL-C) that was not exhibited by nevirapine (NVP) or efavirenz (EFV) treatment groups. The delay in the clearance of apo-B was more marked in patients taking PI containing ritonavir.

2. Body fat distribution, insulin resistance and apo-B kinetics: Trunk fat, VLDL apoB absolute secretion rate (ASR), and insulin resistance were not different between groups. Peripheral fat was lower in the treated patients ( $P < 0.05$ ) and correlated with duration of therapy ( $r = -0.55$ ;  $P < 0.001$ ). There was a positive correlation between peripheral fat and VLDL apo-B FCR ( $P = 0.002$ ) and IDL apo-B FCR ( $P = 0.002$ ) and a negative correlation with VLDL apo-B pool size, VLDL cholesterol (VLDL-C), and triglyceride ( $P = 0.03$ ;  $P = 0.01$ ;  $P = 0.002$ ).

3. Effect of adiponectin and cytokines on apo-B metabolism, body fat distribution: Adiponectin (median [interquartile range]) was reduced in treatment naïve (5.4  $\mu\text{g/ml}$ , [4.7-8.5]), PI (5.0  $\mu\text{g/ml}$  [3.3-6.4]) and NNRTI (5.0  $\mu\text{g/ml}$ , [3.1-6.7]) groups compared to controls (9.7  $\mu\text{g/ml}$  [6.9-13.3],  $p < 0.05$ ). In all subjects adiponectin correlated positively with high density lipoprotein-cholesterol (HDL-C), VLDL, IDL and LDL apo-B FCR and limb fat/lean body mass (all  $p < 0.01$ ) and correlated negatively with plasma TG and HOMA ( $p < 0.001$ ). In a linear regression model which included HOMA, adiponectin level but not HOMA was predictive of VLDL, IDL and LDL apoB FCR and HDL cholesterol (all  $p < 0.05$ ). Other cytokines including TNF-alpha ( $\alpha$ ), IL-6, IL-8, and leptin did not have any effect on apo-B metabolism.

## Conclusion

This thesis highlights that in patients with mild dyslipidaemia reduced VLDL, IDL and LDL apoB FCR and consequently an increased circulating residence time of apo-B containing lipoproteins may be a primary abnormality in lipoprotein kinetics in HIV infection, possibly consequent to body fat redistribution. The same abnormalities were also found in patients treated with either a PI, or NNRTI containing HAART regimens. The association of apo-B FCR with regional fat distribution suggests a common mechanism for lipodystrophy and abnormal VLDL, IDL and LDL metabolism. The latter may be due to a decrease in lipoprotein lipase, down regulation of the LDL receptor or a combination of all these. Adiponectin may have a direct effect on lipoprotein metabolism, independent of insulin action. The increased residence time of apo-B containing lipoproteins will lead to increased LDL oxidation and atherogenesis thus contributing to the increased risk for cardiovascular disease (CVD) reported in these patient populations.

## **Dedications**

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## List of Symbols and abbreviation

<b>Name</b>	<b>ABBREVIATION</b>
a-Ketoisocaproate	a-KIC
Acquired Immunodeficiency Syndrome	AIDS
AIDS Clinical Trials Group	ACTG
Antiretroviral	ARV
Apolipoprotein-B	Apo-B
Apolipoprotein E2	apo E2
Apolipoprotein C-III	apo C-11
Bioelectrical Impedance Analysis	BIA
Cardiovascular Disease	CVD
Chemokine Co-Receptor-5	CCR-5
CXC chemokine receptor-4	CXCR4
Cholesteryl Ester Transfer Protein	CETP
Cholesterylesters	CE
Chromatography-Mass Spectrometry	GC-MS
Combination Anti-Retroviral Therapy	CART

## Chapter 1

Coronary Heart Disease	CHD
Cytoplasmic Retinoic Acid Binding Protein Type 1	CRABP-1
Data Collection on Adverse Events of Anti-HIV Drugs	D:A:D
Endoplasmic Reticulum	ER
Endothelial Lipase	EL
Ethylenediamine Tetraacetate	EDTA
Free Fatty Acids	FFA
Hepatic Lipase	HL
High Density Lipoprotein	HDL
Highly Active Anti-Retroviral Therapy	HAART
HIV Associated Nephropathy	HIVAN
HIV Out Patient Study	HOPS
Human Immunodeficiency Virus	HIV
Interferon	IFN
Interleukin-12	IL-12
Interleukin-6	IL-6
Interleukin-8	IL-8

## Chapter 1

Intermediate Density Lipoprotein	IDL
Lecithin-Cholesterol Acyltransferase	LCAT
Lipoatrophy	LA
Lipoprotein Lipase	LPL
Lipoprotein Receptor Related Protein	LRP
Low Density Lipoprotein	LDL
National Cholesterol Education Programme	NCEP
Non-nucleoside Reverse Transcriptase Inhibitors	NNRTIs
Nucleoside Reverse Transcription Inhibitors	NRTIs
Nucleotide Analogues (Nucleotide Reverse Transcriptase Inhibitors)	NtRTIs
Peroxisome proliferator activator receptor gamma	PPAR- $\gamma$
Phosphatidylinositol 3-Kinase	PI-3
Plasminogen Activator Inhibitor	PAI
Protease Inhibitors	PIs
Sexually transmitted diseases	STD
Triglyceride	TG

## **Chapter 1**

### **General Introduction**

#### **Section 1: HIV, AIDS and treatment**

##### **1.1.1 HIV overview**

Incidence of Human Immunodeficiency Virus (HIV) infection had grown to pandemic proportions soon after the first cases of acquired immunodeficiency syndrome (AIDS) were reported in 1981. Over 33 million people are now living with HIV in the world and in 2007 there were 2.7 million new HIV infections and 2 million HIV-related deaths world wide [1].

The estimated number of people living with HIV in the UK has increased from just over 16,000 in 1990 to over 77,000 in 2007 with more than a quarter (28 per cent) unaware of their infection [2]. HIV continues to be one of the most important communicable diseases in the UK. It is an infection associated with serious morbidity, high costs of treatment and care, significant mortality and number of potential years of life lost.

##### **1.1.2 Basic virology**

Human retroviruses HIV-1 and HIV-2 are members of the family of human retroviruses (Retroviridae) [3, 4]. They present as RNA viruses whose hallmark is the reverse transcription of genomic RNA to DNA by the enzyme reverse transcriptase. HIV-1 is the most common cause of AIDS worldwide. HIV-2 has been identified predominantly in western Africa [5]. It has around 40% sequence homology with HIV-1 type, but the prognosis of HIV-2 infection is much better than HIV-1.

Like any virus, HIV must use the cells of another organism – its host – to survive and reproduce.

### **1.1.3 Viral replication cycle**

Human immunodeficiency virus (HIV) is adapted to use the cells of the human immune system. The life cycle inside the host cells, CD-4 and macrophages has been summarised in the figure (figure 1.1)[6].

The virions enter the blood stream of a new host. A protein on the surface of the virus called gp120[7], has a high affinity for the CD4 receptor found on cells circulating in the blood called CD4+ or T helper cells. The virion attaches to the host cell. This binding is strengthened further by a co-receptor on the cell surface, called chemokine co-receptor type-5 (CCR-5) and CXCR chemokine receptor type-4 (CXCR-4)[8].

Once the virion has been bound to the host cell surface, its next task is to get inside. This is achieved through the fusion of the virus coat and the cell membrane.

Following fusion, the genetic material of the virus, which is RNA, is released into the cell, along with the viral enzymes, reverse transcriptase and integrase [9-11]. Reverse transcriptase reads the viral RNA and builds the corresponding DNA strands. The DNA copy is known as a provirus. Viral DNA then moves to the cell nucleus, where the cell's own genes, also made of DNA, are present. The viral enzyme, integrase, splices the strands of DNA into the host cell genome and helps the integration of the virion into the host genome [12-14].

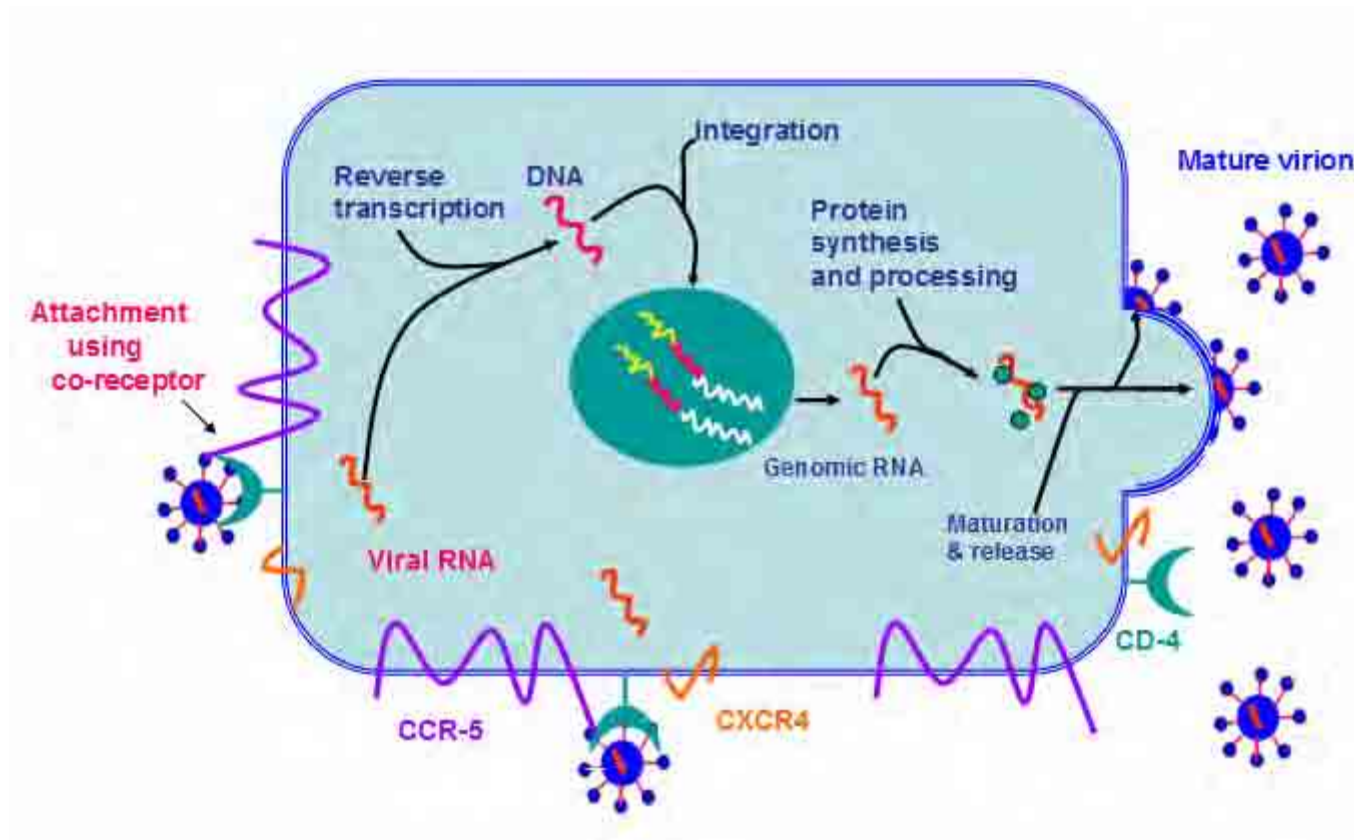
The proviral DNA can persist for many years in a latent state. When the host cell is activated as during cell division, the proviral DNA transcribes

into RNA, which is then translated into viral proteins and polyproteins.

Together, the RNA and these proteins then migrate to the inside of the host cell membrane, where they assemble into new virions.

Among the viral proteins is the enzyme, protease, which cleaves the polyproteins into smaller, functional proteins, thereby, allowing the new viral particles to mature [11]. Following assembly, the newly formed virions bud from the host cell surface, entering the bloodstream where they encounter uninfected CD4+ cells and begin another cycle.

Figure 1.1: The replication cycle of HIV [6] (Adapted from Fauci *et al*, 1996)





#### **1.1.4. Pathophysiology and natural history of the disease**

(figure 1.2)

The most common mode of transmission of HIV involves deposition of HIV on mucosal surfaces, especially the genital mucosa [15, 16]. Direct inoculation into the blood through intravenous (IV) needle sharing is also a common mode of HIV transmission [17]. Vertical transmission from mother to child is another mode of transmission [18, 19]. Following successful transmission of HIV from one individual to another, the course of subsequent infection is quite variable and dependent on a number of factors.

Throughout the course of HIV disease the virus replicates continuously. Up to 10 billion virions are produced and cleared every day. The half-life of an HIV-infected CD4 cell is about 1.3 days [20]. Most CD4 cells turn over rapidly, but some belong to a latent pool with a long half-life and the virus can remain there in a latent phase. Virus-specific CD4 cells, which are critical in maintaining an effective host immune response in chronic viral infections, are present early in HIV infection but are generally lost over time [21]. The patient's immune system keeps pace with this activity during the relatively clinical latent period. However, in the absence of effective antiretroviral treatment, the immune system ultimately reaches a point at which viral replication exceeds its ability to produce CD4 cells. The viral replication rates reach such a magnitude that lymphoid tissues are completely destroyed or nearly destroyed and this leads to a decline in immunologic function and the development of clinical disease manifestations, including opportunistic infections and neoplasms [20]. The rate of viral replication is thought to stabilize after primary infection at a

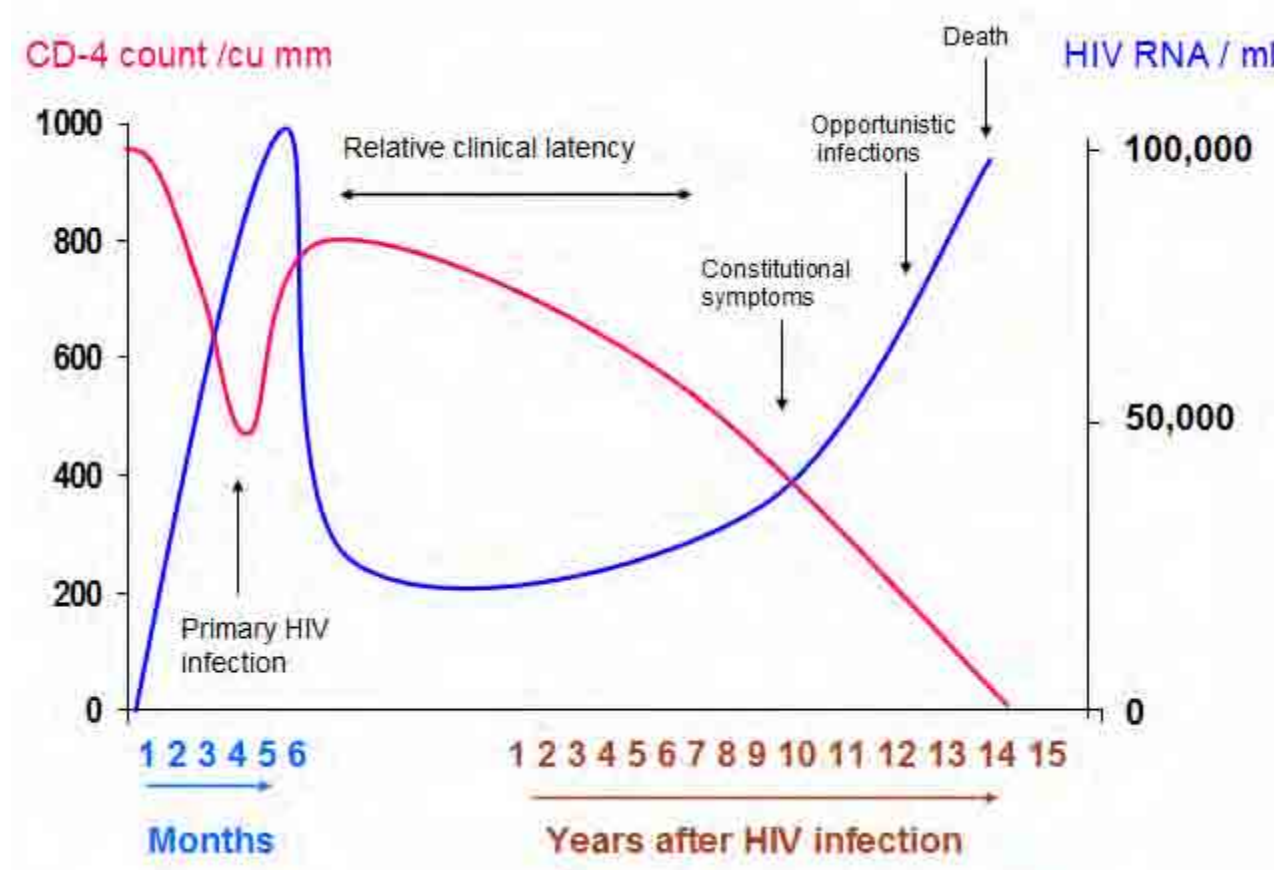
particular level or "set point"; this level may be maintained within a ten-fold range over months and possibly years.

Viruses or virus populations that can use only the chemokine co-receptor (CCR) -5 are termed R5 viruses and viruses or virus populations that can use only the CXCR4 chemokine co-receptor are termed X-4 viruses [22, 23]. Viruses using both populations are termed as dual type and the viral population containing both R5 and X4 or dual type are called mixed type. During the early stage of HIV-1 infection (as determined in subtype B HIV-1 infections), the R5 virus predominates. Over 80% of individuals with early stage HIV-1 infection have R5 virus. However, as HIV-1 disease progresses, the prevalence of CXCR4-utilizing viruses gradually increases. About 50% of people with HIV-1 that progress to AIDS demonstrate only R5 virus throughout the entire course of HIV-1 disease. The presence of X4 viruses has been associated with accelerated disease progression and a decrease in CD4 cells, but despite descriptions of in vitro cytopathogenicity of X4 viruses, no causal in vivo relationship or mechanism for this association has been proven. The role of different population of CD-4 cells either R5 or X4 or mixed type may have effect on metabolic changes, but has not been described yet.

Throughout the disease huge amounts of cytokines are liberated and the types and nature of the cytokines can vary at different stages. These cytokines may have effects on the different vital functions including the metabolic changes in the body.

Figure 1.2: Typical course of an untreated HIV-infected individual[24].

— : HIV RNA level    — : CD-4 count



### **1.1.5. Treatment of HIV**

The treatment of HIV infection is complex and changes rapidly as advances are made in basic science and clinical experience. Understanding of the different stages of viral replication and different enzymes used by the virus for their replication have helped to identify different agents that can block the function of the enzymes and thereby stopping the viral replication inside host cells. Various events in the HIV life cycle have been identified as potential targets for antiretroviral therapy [11]. The complexity of treatment regimens against HIV infection increases further with the rising number of available compounds and the growth in new information about their use [25-27].

Drugs of five different classes (table 1.1) are currently available in the UK, with several others close to release [25]. Inhibitors of HIV reverse transcriptase and of HIV protease are so far the most developed and most used in clinical practice in developed countries. Newer agents of these classes with better efficacy against the virus and activity against resistance viruses are in the pipeline of development. Drugs of longer half life with penetration into different body compartments with less systemic side effects to the host are coming into production. Drugs of higher antiviral potency with less side effects and less number of tablets could help improve patients' long needed adherence to treatment and longer survival.

#### **1.1.5.1. Reverse transcriptase inhibitors [9, 10]:**

These are of three types: nucleoside analogues (nucleoside reverse transcription inhibitors, NRTIs), nucleotide analogues (nucleotide reverse

transcriptase inhibitors, NtRTIs) and non-nucleoside analogues (non-nucleoside reverse transcriptase inhibitors, NNRTIs).

Nucleoside analogues (NRTIs) inhibit reverse transcription by binding to viral DNA and also act as DNA chain terminators. NRTIs need to be phosphorylated intracellularly for activity to occur. These were the first group of agents to be used against HIV, initially as monotherapy and later as dual drug combinations. Usually two drugs of this class are combined to provide the 'backbone' of a triple or combined regimen.

Nucleotide analogues (NtRTIs) have a similar mechanism of action but do not require intracellular phosphorylation for activity. Tenofovir, a monophosphorylated thymidine derivative, is the only licensed compound of this group.

Table 1.1: Antiretroviral drugs available in the UK [25]

Reverse Transcriptase Inhibitors		Protease Inhibitors (PI)	Entry Inhibitors	Integrase inhibitors
Nucleoside Reverse Transcriptase Inhibitors (NRTIs)	Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)			
Zidovudine (AZT) Lamivudine (3TC) Didanosine (ddI) Stavudine (d4T) Abacavir (ABC) **Tenofovir* (TDF) **Emtricitabine (FTC)	Nevirapine (NVP) Efavirenz (EFV) Delavardine **Etravirin (ETR)	Saquinavir (SQV) Ritonavir(RTV) Nelfinavir(NFV) Indinavir(IDV) Lopinavir(LPV) Amprenavir(APV) **Atazanavir(ATV) **Fos-amprenavir(FPV) **Daurunavir(DAV) **Tipranavir(TPV)	**Enfuvirtide (T20) **Maraviroc	**Raltegravir

\*NtRTI

\*\* These drugs were not available in the UK or licensed to use in the UK at the time of the study

#### **1.1.5.2. Non-nucleoside analogues (NNRTIs)**

These drugs interfere with reverse transcriptase by direct binding to the enzyme and are considered as a separate class because of an entirely different cross-resistance pattern and different mechanism of action [28]. They are generally small molecules that are widely disseminated throughout the body and have a long half-life.

#### **1.1.5.3. Protease inhibitors (PIs)**

These drugs act competitively on the HIV aspartyl protease enzyme, which is involved in the production of functional viral proteins and enzymes. In consequence, viral maturation is impaired and immature dysfunctional viral particles are produced. There are marked differences in toxicity, pharmacokinetics and resistance patterns which influence prescribing. Cross-resistance that means development of resistance occurring for more than one drug at the same time is common across the PI group, which makes it difficult to use the drugs sequentially. There appears to be no activity against human aspartyl proteases (*e.g.* renin), although there are clinically significant interactions with the cytochrome P-450 system. This is used to therapeutic advantage by using small doses of a PI, ritonavir that blocks the metabolism of other PIs and boosts the blood level of those drugs [27, 28].

#### **1.1.5.4. Entry inhibitors**

Chemokine co-receptor (CCR-5) blocker: It blocks the attachment of CCR-5 co-receptors and inhibits the virus to enter the CD-4 cells [22]. Currently maraviroc is a member of this class and licenced to be used in the UK.

Enfurvitide (T20). It is a peptide derived from HIV protein, GP41 that inhibits GP41-mediated fusion of HIV with the target cell [28].

#### **1.1.5.5. Integrase inhibitors**

Raltegravir: It is an integrase inhibitor [14]. Initial results appear to be very much effective and are now licensed to be used in treatment experienced patients [25]. Recent trials in treatment naïve patients [29] for using in treatment of naïve patients appear to be very encouraging.

#### **1.1.5.6. Newer drugs [30, 31]**

Newer NRTIs include Alovudine (MIV-310), and SPD-756 which appear to have activity against NRTI-resistant strains.

New NNRTIs [30-32] include TMC-278[33], RDEA 427, RDEA640[34] appear to have efficacy against NNRTI resistant virus and TMC -278 is expected to be licenced in UK by the end of 2010.

PRO 140, a monoclonal antibody with ability work when administered intravenously every 3 to 4 weeks has created lot of interest in phase II trial [35].

#### **1.1.6. Principles of treatment**

The ongoing HIV replication leads to damage of the immune system and progression to AIDS. The plasma HIV RNA levels reflect the magnitude of HIV replication. The CD4+ T-cell counts indicate the current level of competence of the immune system. The rates of disease progression differ among patients, and treatment decisions should be individualized based

on plasma HIV RNA levels and CD4+ T-cell counts. Maximal suppression of viral replication is a goal of therapy; the greater the suppression, the less likely the appearance of drug-resistance variants (quasispecies). The most effective therapeutic strategies involve simultaneous initiation of combinations of anti-HIV drugs active against the virus present in the patient's blood. This prevents the development of any specific mutation leading to resistance to any particular class and helps to achieve a sustained viral suppression. The combinations of drugs usually of two or more different classes are called Highly Active Anti-Retroviral Therapy (HAART) or Combination Anti-Retroviral Therapy (CART). In the UK, the treatment is usually considered when CD-4 count drops below 350 cells per dl of blood [25, 26].

#### **1.1.7. Toxicities of HAART**

It is well recognised that despite significant improvements in morbidity introduced by HAART [36], all classes of antiretroviral agents have multiple side effects (Table 1.2), some of which can be life threatening [37]. Specifically, the NRTI class has been implicated in peripheral neuropathy and lactic acidosis [38]. In addition, abacavir is associated with potentially fatal hypersensitivity reactions in ~5% of treated patients [39] and didanosine is associated with pancreatitis [40].



Table 1.2: Adverse events of drugs [28, 37]. Bold Words: Relatively common side effects

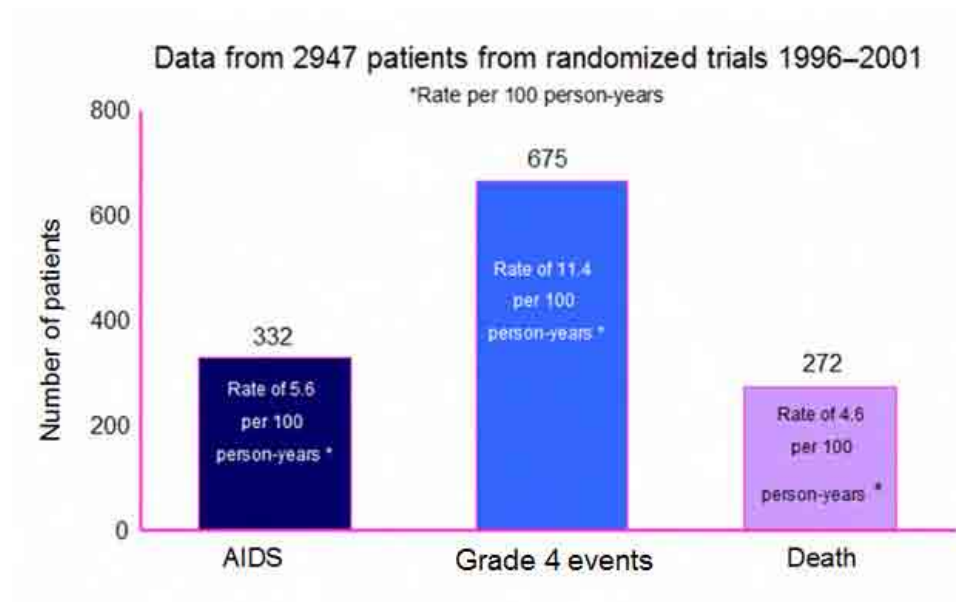
Drug	Side effects
Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTI)	
Abacavir	<b>Hypersensitivity reaction</b> , fever, rash, vomiting. Association with mitochondrial dysfunction and lactic acidosis
Didanosine/ddI	Nausea, diarrhoea, peripheral neuropathy, <b>pancreatitis</b> . Association with mitochondrial dysfunction and lactic acidosis, lipodystrophy.
Emtricitabine (FTC)	Headache, nausea, <b>skin pigmentation</b>
Lamivudine (3TC)	Nausea, headache, rash, peripheral neuropathy, myelosuppression, mitochondrial dysfunction & lactic acidosis
Stavudine/d4T	Polyneuropathy. Megaloblastic changes, mitochondrial dysfunction and lactic acidosis, <b>lipodystrophy</b>
Zidovudine/AZT	Nausea, headache, insomnia, <b>skin and nail pigmentation, myelosuppression, megaloblastic changes</b> . Association with mitochondrial dysfunction and lactic acidosis, <b>lipodystrophy</b>
Tenofovir	<b>Hypophosphataemia, renal toxicity</b> , osteopaenia
Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI)	
Efavirenz	Rash, Stevens-Johnson syndrome, <b>central nervous system effects (vivid dreams, agitation, hallucinations, and depression amongst others)</b> , fat re-distribution.
Nevirapine	<b>Rash</b> , Stevens-Johnson syndrome, <b>hepatic toxicity</b>
Etravirin	<b>Rash</b>
Protease Inhibitors (PI)	
Atazanavir	<b>Hyperbilirubinaemia</b> , nephrolithiasis
Indinavir	<b>Nephrolithiasis and crystalluria</b> , dry skin, nail dystrophy, alopecia, hyperbilirubinaemia, fat redistribution, raised plasma lipids, hyperglycaemia
Nelfinavir	<b>Diarrhoea, fat redistribution, abnormal plasma lipids, hyperglycaemia</b>
Ritonavir	<b>Nausea/vomiting, diarrhoea</b> , taste distortion, perioral paraesthesia, <b>fat redistribution</b> , hepatotoxicity, <b>abnormal plasma lipids hyperglycaemia</b> , pancreatitis
Saquinavir (soft gel)	<b>Nausea, diarrhoea</b> , abdominal pain, <b>fat redistribution</b> , abnormal plasma lipids, hyperglycaemia
Lopinavir	<b>Diarrhoea, nausea, fat redistribution, abnormal plasma lipids</b>
Fosamprenavir	Similar to amprenavir
Daurinavir	As ritonavir and indinavir with less severity
Tipranavir	Nausea, abnormal LFT, dyslipidemia
Entry Inhibitors	
Enfuvirtide (T20)	Reaction at the injection site, bacterial septicaemia
CCR-5 inhibitor (Maraviroc)	Rash, abnormal liver function test
Integrase Inhibitors	
Raltegravir	Rash, abnormal liver function test

### **1.1.8 Changing spectrum of HIV disease** (figure 1.3)

The continuous exposure of antivirals of different class with different side effect profile has led to a new trend of problems. Toxicity related problems have become an important threat to the morbidity and even mortality in HIV patients. Drug related serious toxicity has been found to be more common than AIDS or AIDS related morbidity in the era of HAART. In a large multicenter randomized treatment trial setting in the USA [41], 675 patients (11.4 per 100 person-years) developed drug related serious side effect described as grade 4 events and only 332 patients (5.6 per 100 person-years) developed an AIDS events (figure 1.3). Grade-4 events, were defined as non-AIDS related events considered to be severe or life threatening[42]. Of the 272 deaths, 153 (56.2%) developed grade-4 events, prior to death and cardiovascular disease (CVD) events were associated with greatest risk of death (figure 1.3). Besides cardiovascular disease, problems in disturbances of other organs have been recognised as well. While HIV associated nephropathy (HIVAN) has become less common with HAART some antiretrovirals have been associated with significant nephrotoxicity[43]. A protease inhibitor called Indinavir [44] has been reported to cause kidney stones in up to 43% of HIV-infected patients, and tenofovir [45, 46], a reverse transcriptase inhibitor has been reported to be associated with Fanconi-like renal tubular injury. Liver disorders have been found to be more common in patients who are co-infected with hepatitis B and C infections [47]. Mitochondrial dysfunction leading to pancreatitis, neuropathy and lactic acidemia has been found to be more common with some nucleoside analogues like stavudine and didanosine [48]. There is a greater incidence of osteoporosis in HIV-

infected women, both pre- and post-menopausal [49-53]. In a review of 20 cross-sectional studies, there was a 3.7 fold increased risk of osteoporosis in HIV positive individuals compared to HIV-ve population. Some reports suggest that effect in bone mineral density is more commonly associated with PI use [49, 51]. Skin rash and liver toxicity is more common with nevirapine [28].

Figure 1.3: Spectrum of HIV related serious illness: incidence of grade 4 events is higher than that of AIDS defining illnesses. CVD events were associated with greatest risk of death [41].



#### 1.1.9. Risk of cardiovascular disease (CVD) in HIV (table 1.3, figure 1.4)

There is a significant body of evidence suggesting that exposure to HAART, primarily Protease Inhibitors (PI)s [54-56], may be associated with a measurable increase in cardiovascular events (table 1.3). The French Hospital Database on HIV found that patients who are treated with PI for more than 30 months had twice the risk of developing MI than those with less drug exposure [56]. A review of claims for more than

28,000 HIV patients of younger age group (18-33 years) who were exposed to anti retroviral agents had twice the risk of CVD seen among age-matched, treatment naïve patients [57]. Klein *et al* [58] reported that the rate of hospitalisation for coronary artery disease among HIV patients regardless of whether on HAART or not was more than twice among their uninfected counterparts. The Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) Study Group recently reported outcomes demonstrating an increased incidence of myocardial infarction (MI) that was proportional to the cumulative duration of HAART (figure 1.4a), particularly PIs [54]. After adjusting for conventional risk factors, low HDL-cholesterol, total cholesterol and duration of exposure of HAART were associated with increased risk of CVD (figure 1.4b). There was a 16% increased risk of MI per additional year of HAART exposure over the first seven years of use [54]. The study very recently has suggested that recent use of abacavir and didanosine is associated with a higher risk of developing myocardial infarction, but not with stroke (figure 1.4c)[59]. The Frankfurt HIV-cohort study [60] showed increased incidence of myocardial infarctions in HIV-infected patients after HAART was introduced. However, there was no specific association with any particular class of drugs.

In a study conducted at Johns Hopkins University, cardiovascular event rates in a cohort of 2671 HIV-infected patients were approximately three times higher than expected compared with national coronary heart disease (CHD) and CVD event rates [61]. The traditional risk factors including age, hypertension and diabetes played an important role. The recently presented SMART (**S**trategies for **M**anagement of **A**nti-**R**etroviral **T**herapy)

study has shown excess risk of cardiovascular disease (CVD) among patients receiving intermittent antiretroviral therapy [62]. Sub analysis of the same study [63, 64] has shown that treatment interruptions were associated with lowering of all lipids including both total and HDL cholesterol levels, but rise of inflammatory and coagulation markers to a high magnitude which correlated with increase in HIV RNA level [65]. This leaves the reasons for observed elevated risk of cardiovascular events still uncertain, but inflammatory and atherogenic factors, such as pro-inflammatory cytokines driven by uncontrolled viral replication, may play an important role [66].

Uncontrolled viral replication may have effects as well. Further evidence of a pro-inflammatory role for HIV may be found in the results of the ACTG (AIDS Clinical Trials Group) A5102 study, which found that although interrupting antiretroviral therapy (ART) resulted in rapid improvements in lipid profiles, the risk of CVD increases [67]. In contrast some studies did not provide evidence of an association between ART and increased CVD events or mortality. The HIV Out Patient Study (HOPS) [68] found no association of CVD with specific antiretroviral (ARV) agents or classes but found a significantly increased association with the traditional risk factors. In a retrospective study [69] of CVD risk in 36,766 HIV-infected patients comprising a database of the Department of Veterans Affairs (VA), the rates of CVD hospital admissions and death did not increase with exposure to ART over 8.5 years. However, initiation of ART was correlated with a decline in overall morbidity and mortality.

Taken together, these data and some other data suggest that a small but significant and increasing risk exists for cardiovascular disease related to

HIV infection with or without antiretroviral use. The actual number of cardiovascular events remains small and must be placed in the overall perspective of the benefit of antiretroviral (ARV) therapies on immune function. However, the risk may rise with aging of the HIV population and longer duration of ARV exposure and metabolic abnormalities.

Table 1.3 Antiretroviral therapy and risk of CVD (CVD= cardiovascular disease; CHD=coronary heart disease;MI=myocardial infarction; HTN=hypertension;DM= diabetes mellitus; TC=total cholesterol; MI=myocardial infarction, P=prospective; R=retrospective) [54-56, 58, 60, 63, 69-72]

Study	Number	Study	Event	ARV	Effect	Traditional Risk Factors
D:A:D [54, 55]	23,490	P	345 MI	cART and PI	Yes	Smoking, age, gender, HTN, DM
French [56]	34,976	R	49 MI	PI	Yes	Age
Medi-Cal [57]	28,513	R	NA	ART	Risk with ART in 18-33 year olds	Not evaluated
Kaiser [58]	4408	R	86 MI	PIs	Risk of HIV+ vs HIV- No risk on PI	Not evaluated
Frankfurt [60]	4993	R	29 MI	ART	Yes	Age < 40 y
SMART [62]	54472	P	63 CHD	Intermittent ART	No – stopping therapy led to complication	Age
John Hopkins [61]	2671	Case Control	43 CHD	HIV+ vs HIV-	Yes	Age, HTN, DM
HOPS [68]	1807	P	84 CV events	Specific ARVs	No	Age >40 y, diabetes, HTN
VA [69]	36,766	R	1,207 CHD	ART or PI	No	Not evaluated

Figure 1.4a: Incidence of myocardial infarction relative to duration of HAART in the D:A:D Study [54]. A small increase in incident CVD is associated with duration of HAART

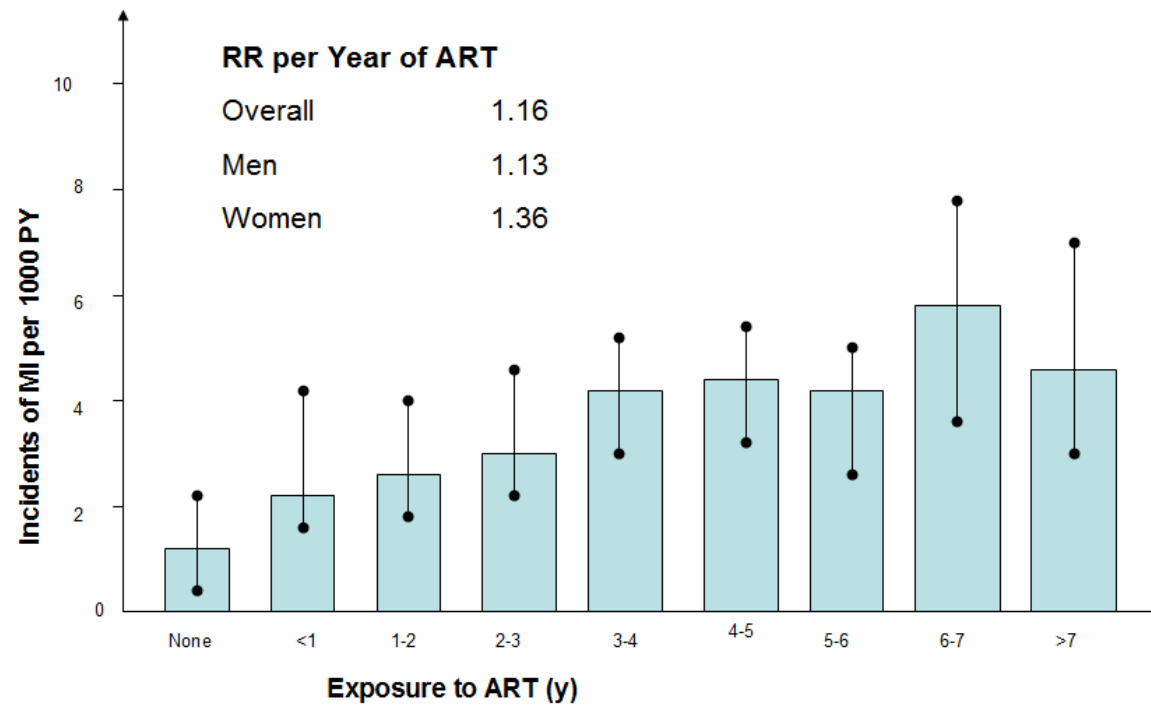




Figure 1.4 b: Influence of HAART and lipids on CVD events (adapted from D:A:D study [54])

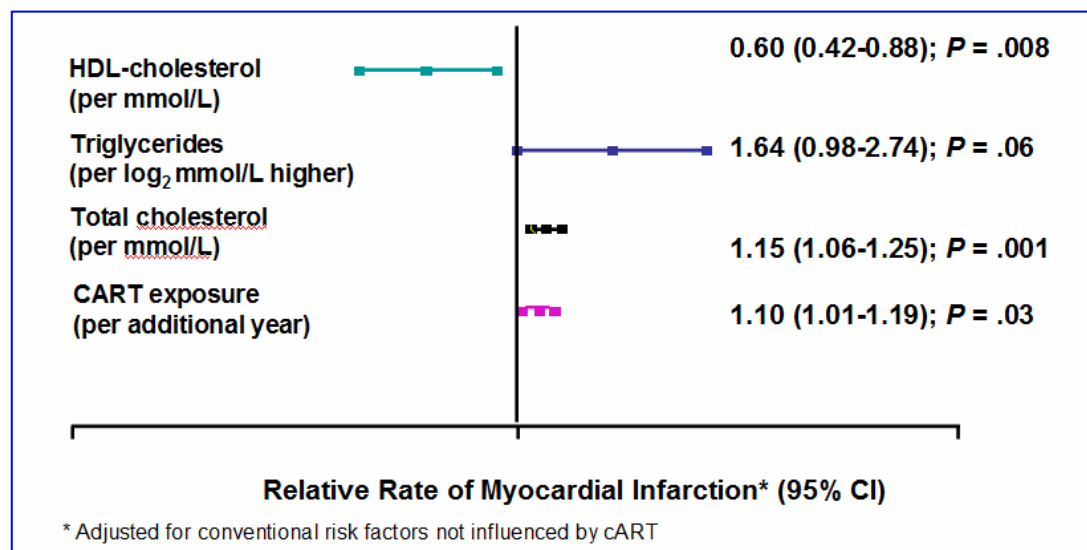
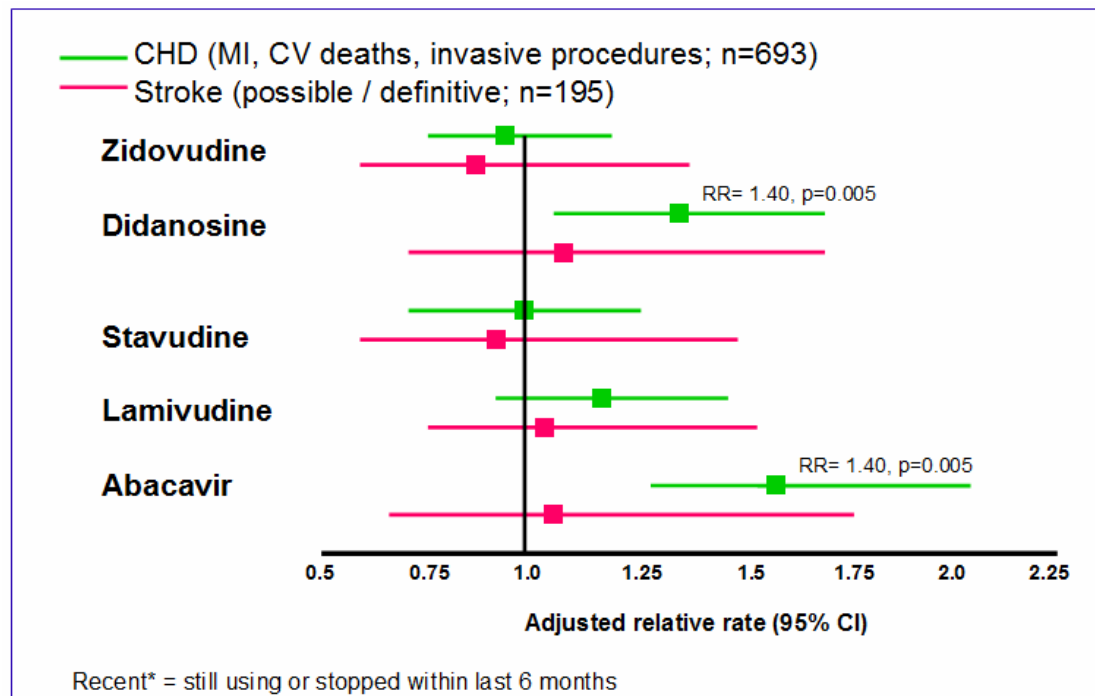


Figure 1.4c: Association of CVD with recent use of abacavir and didanosine (adapted from D:A:D study) [27, 59]



## **Section 2: Metabolic complications of the disease and treatment**

### **1.2.1. Methodology**

A Medline search from 1985 through March 2009 was performed using the key words "HIV," "antiretroviral therapy", "toxicities", "dyslipidaemia", "lipodystrophy", "insulin resistance", "adipocytokines", "cytokines", "lipoproteins", "apolipoprotein-B". Further search was performed combining key words "HIV and dyslipidaemia", "HIV and lipodystrophy", "HIV and insulin resistance", "HIV and adipocytokines", "HIV and cytokines", "HIV and lipoproteins" "HIV and apolipoprotein-B", "HIV and toxicities", "antiretroviral therapy and toxicities".

A total of 668 articles were found with the combination of "HIV and dyslipidaemia," and the search was limited to the English language, resulting in 568 articles. In the similar way "HIV and lipodystrophy", resulted 1587 articles, "HIV and adipocytokines" resulted 108 articles, "HIV and insulin resistance" resulted 752 articles and "HIV and apolipoprotein-b" resulted 24 articles. Other sources included peer reviewed abstracts from recent HIV-related conferences that presented data pertinent to metabolic and toxicology issues.

The articles included were those reported primary data on lipid abnormalities and related topics in patients with HIV infection. The search was further focused on articles dealing with pathogenesis of dyslipidaemia and lipodystrophy in HIV infection. In addition the bibliographies of eligible studies as well as those of relevant review articles were reviewed to identify additional studies not captured by the database searches.

Because this is a rapidly evolving field and, for many clinical questions, the evidence is limited to case series or small observational studies, the articles were initially not excluded on methodological grounds. Finally studies were selected based on their impact on our understanding of HIV infection and its treatment and complication. In general more emphasis was given on differentiating clearly between randomized controlled trials and observational studies.

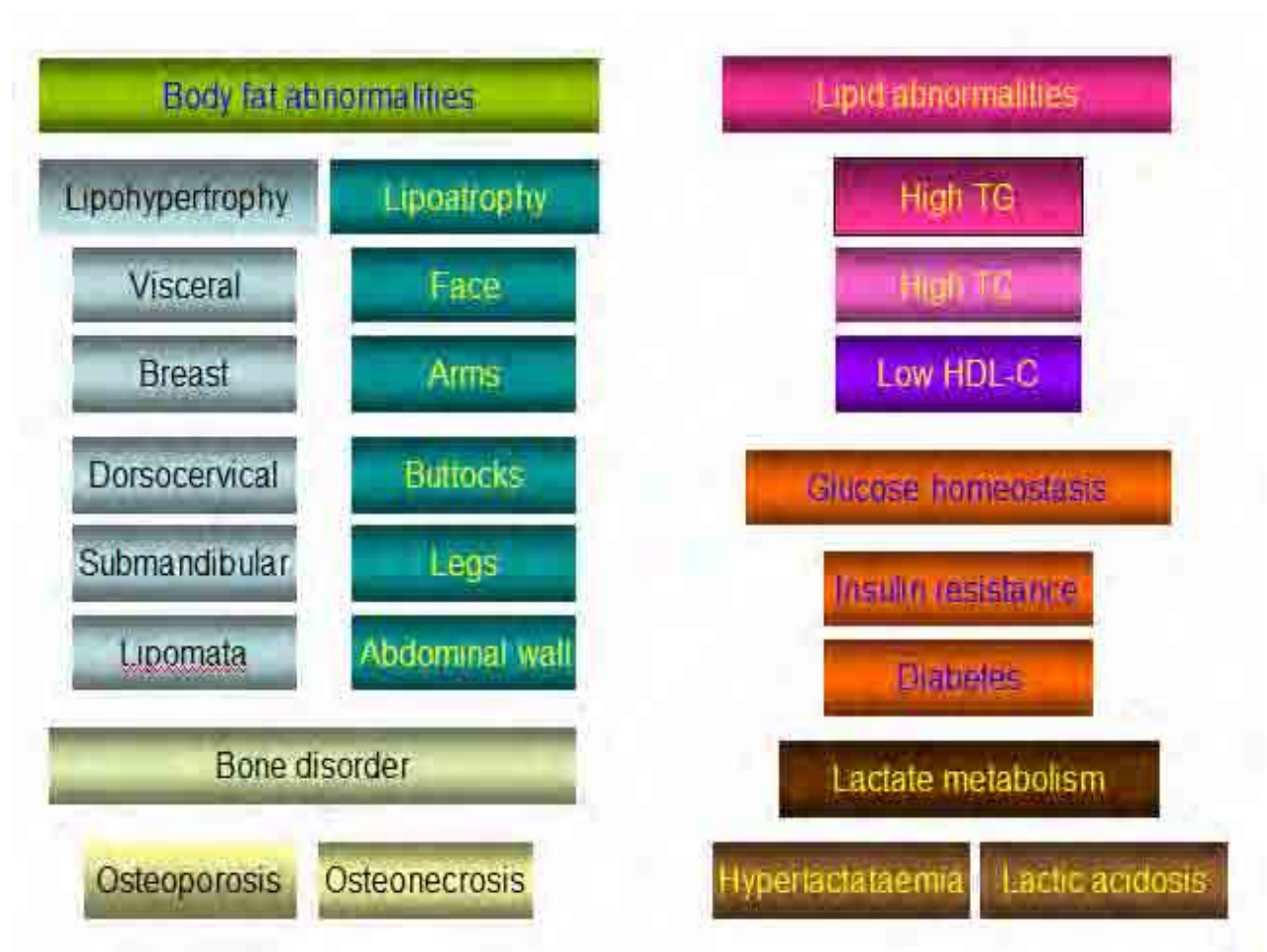
### **1.2.2. Overview** (figure 1.5)

The widespread use of different antiretroviral regimens has coincided with increasing reports of metabolic abnormalities including lipid disorders, impaired glucose metabolism and insulin resistance, lactic acidosis [73, 74] and osteopenia [75]. Distressing morphologic changes in body habitus associated with these metabolic abnormalities are characterized by accumulation of fat in the abdomen (visceral fat compartment) and in the dorsocervical area of the neck, breast enlargement as well as by the depletion of fat in the face, buttocks, and extremities [76]. A single pathogenic mechanism linking peripheral lipoatrophy and central lipohypertrophy may not exist. Increasing evidence suggests these disorders, have distinct pathologic pathways and can occur independently of each other [77]. However, the morphologic changes and the metabolic changes may have a close link between them.

The causes of the metabolic disturbances and morphologic changes related to ART are not understood completely. The etiology is likely to involve the effect of HIV per se as well as the direct and indirect effects of ART superimposed on individual characteristics such as genetic

predisposition, gender, and age. There are likely to be both drug class-specific as well as drug-specific differences in the tendency of antiretroviral medications to cause these effects [73]. Although some of the metabolic disturbances may be linked to one another, the interconnections among these metabolic abnormalities have yet to be elucidated.

Figure 1.5 Cluster of metabolic complications associated with HIV infection  
(Adapted from Wohl D *et al* Contemporary Views of Metabolic Complications and Cardiovascular Risk in HIV-Infected Patients  
<http://www.clinicaloptions.com/HIV/Resources/CME>)



### **1.2.3. Basic physiology of lipid metabolism**

#### **1.2.3.1. Plasma lipoproteins: structure, nomenclature and occurrence**

Plasma lipoproteins are complexes of lipids and proteins, called apolipoproteins. With the exception of free cholesterol, the lipoprotein lipids are of different lipid types that include cholesterylesters (CE), triglycerides (TG) and phospholipids [78]. The lipoproteins comprise a continuum of particles differing gradually in density, volume and in lipid and protein content. Various methods can isolate different relatively distinct subclasses and are operationally classified according to their densities and electrophoretic mobilities. In order of decreasing densities, they are high density lipoprotein (HDL), low density lipoprotein (LDL), intermediate density lipoprotein (IDL), very low density lipoprotein (VLDL), and chylomicron. The plasma lipoproteins can be altered in structure and composition by various types of hyperlipidaemia depending upon the content of triglyceride or cholesterol [79].

#### **1.2.3.2. Apolipoproteins**

Apolipoproteins (apoproteins) are a specialised group of proteins that associate with lipids and mediate several biochemical steps associated with plasma lipid metabolism. The apolipoproteins are designated by Roman letters and numerals (as example apo A-1 and apo C-2) [78, 80]. Apolipoproteins act as the vehicles for solubilisation and transport of lipids in the plasma compartment. Many of the apolipoproteins contain determinants that regulate several activities essential to normal lipid metabolism. Some apolipoproteins stimulate enzymes that degrade plasma lipids. Others contain the ligands that mediate the binding of

lipoproteins to cell surface receptors, binding is succeeded by the internalisation of all or part of a lipoprotein and the regulation of intracellular lipid synthesis [81].

#### **1.2.3.3. Intracellular lipid synthesis and lipoprotein assembly: very low density lipoproteins (VLDL)**

The major secreted lipoproteins are VLDL, assembled in the liver, and the chylomicron, derived from the intestine. Both lipoproteins undergo substantial remodelling in the plasma compartment, thus remnants or mature forms or circulating particles are found under fasting conditions [80, 82].

The assembly of lipoproteins containing apo-B is a complex process that occurs in the lumen of the endoplasmic reticulum (ER) [83, 84]. The VLDL secretion appears to be driven by the availability of plasma free fatty acids (FFA) that act as fuel for lipid synthesis. Before being secreted in the blood the VLDL particle acquire other proteins and undergo massive remodelling by plasma lipid transfer proteins and lipases [85].

#### **1.2.3.4. Lipase family**

Lipase family consists of different enzymes [85-90] which cause hydrolysis of glycerides present in different lipid fractions, resulting in formation of free fatty acid and different relatively smaller lipid fractions.

a. Lipoprotein lipase (LPL) is a protein secreted primarily by adipocytes and muscle cells into the circulation where it is bound to the luminal surface of capillary endothelial cells [86, 88]. In the presence of its cofactor, apo C-II [91-93], lipoprotein lipase hydrolyzes triglycerides present in chylomicrons and very low density lipoproteins to free fatty acids (FFA) which can be utilized as sources of energy or re-esterified for

storage. During the hydrolytic process LPL is intimately involved in the initial remodelling of the triglyceride-rich lipoprotein particles and thus plays a key role in both normal triglyceride and lipoprotein metabolism.

b. Hepatic lipase (HL) is synthesized in hepatocytes and transported to hepatic endothelial cells where it is bound by means of heparin sulphate [85, 94]. The major role of HL appears to be hydrolysis of triglycerides and phosphoglycerides of HDL and IDL with the help of apo-C II and also LDL.

It also acts sequentially to lipoprotein lipase on TG rich lipoproteins catalysing the hydrolysis of intermediate density lipoprotein glycerides to produce chylomicron remnants and low density lipoproteins.

c. Hormone sensitive lipase [95] is an intracellular enzyme. It hydrolyses adipose tissue store of triglycerides which is the major source of FFA in the fasting state.

d. Endothelial lipase (EL) [96-98] is synthesized by endothelial cells and is expressed in organs including liver, lung, kidney and placenta, but not in skeletal muscle. It has a considerable molecular homology with lipoprotein lipase (LPL) and hepatic lipase (HL). As a lipase, EL has primarily phospholipase A1 activity, playing a role in HDL metabolism. However, recent study has shown that EL have role in metabolism of apo-B containing lipoproteins [96].

#### **1.2.3.5. Lipoprotein transport (figure 1.6a, 1.6b, 1.6c)**

There are two major pathways of lipoprotein transport [78, 81, 99]. The exogenous pathway transports dietary lipids to the periphery and the liver and the endogenous pathway transports hepatic lipids to the periphery.



In the exogenous pathway [78], cholesterol and fatty acids after intestinal absorption, are re-esterified to form triglyceride (TG) and cholesteryl ester (CE) in intestinal mucosal cells. These lipids are then packaged together with apo-B 48, phospholipids, unesterified cholesterol and several apolipoproteins including apo-C and apo-E into nascent chylomicrons, secreted into the lacteals and transported via the thoracic duct into the blood. During transport in the circulation chylomicrons are degraded to smaller chylomicron remnants which are recognised by the apo-E receptors on hepatic parenchymal cells and rapidly removed from the blood stream[100].

In the endogenous pathway [78], triglycerides and cholesterol are transported to different tissues in the form of VLDL, IDL and LDL. The liver assembles and secretes apo-B containing lipoproteins, mainly VLDL. Fatty acids surplus to oxidative requirements in the liver are esterified to form triglyceride in a manner similar to chylomicron formation and are packed together with cholesterol, phospholipid apolipoprotein B-100 (apo-B), apolipoprotein-C (apo-C) and apolipoprotein-E (apo-E) and secreted into the blood as VLDL. During transport the VLDL undergoes delipidation and becomes smaller and denser resulting in the formation of IDL and subsequently LDL. In contrast to chylomicron remnants, VLDL remnants and IDL can either be taken up by LDL receptors on the liver or undergo further delipidation and lose apo-E to form LDL. The LDL is removed from the circulation by LDL receptors present in the liver and to a small extent by extra hepatic tissues including macrophages. The main structural protein that remains associated with VLDL, IDL and LDL is apo-B 100. Hence apoB-100 is a suitable marker for tracing the metabolic pathway of these

lipoprotein particles (figure 1.6b). This is the fundamental principle employed in studies of the metabolism of apoB-containing lipoproteins based on the use of stable isotopically labelled amino acid precursors [101-103].

Excess cholesterol from the periphery is transported back to the liver for excretion in the bile by a process called reverse cholesterol transport (figure 1.6 c). Both intestine and liver secrete individual apolipoproteins with a small amount of phospholipid. These cholesterol-poor proteins can absorb free cholesterol on the surface from different cell membranes and become nascent HDL and considered to be the initial step in reverse cholesterol transport. These particles can continue to adsorb more free cholesterol and with the help of lecithin-cholesterol acyltransferase (LCAT) esterifies free cholesterol into cholesteryl esters (CE); forming mature HDLs that can be selectively taken up by the liver via scavenger receptor class BI (SR-BI). Formation of CE increases the capacity of HDLs to accept more cholesterol, because the core of the molecules can accommodate more CE molecules. These larger sized cholesterol rich HDL transfer CE to other TG rich lipoproteins with the help of CETP resulting in formation of CE rich apo-B containing lipoproteins VLDL, IDL or LDL and TG rich HDL which then is further modified by hepatic lipase (HL) into smaller HDL and cleared from circulation [104]. The CE rich VLDL and chylomicrons can be either taken up by the liver or further hydrolysed to smaller IDL and LDL [105, 106].

Figure 1.6a: Lipid transport.

The exogenous and endogenous lipoprotein metabolic pathways [107] .  
(adapted from Fauci AS *et al*, Harrison's principles of internal medicine, 17th edition). LPL, lipoprotein lipase; FFA, free fatty acid; VLDL, very low density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor.

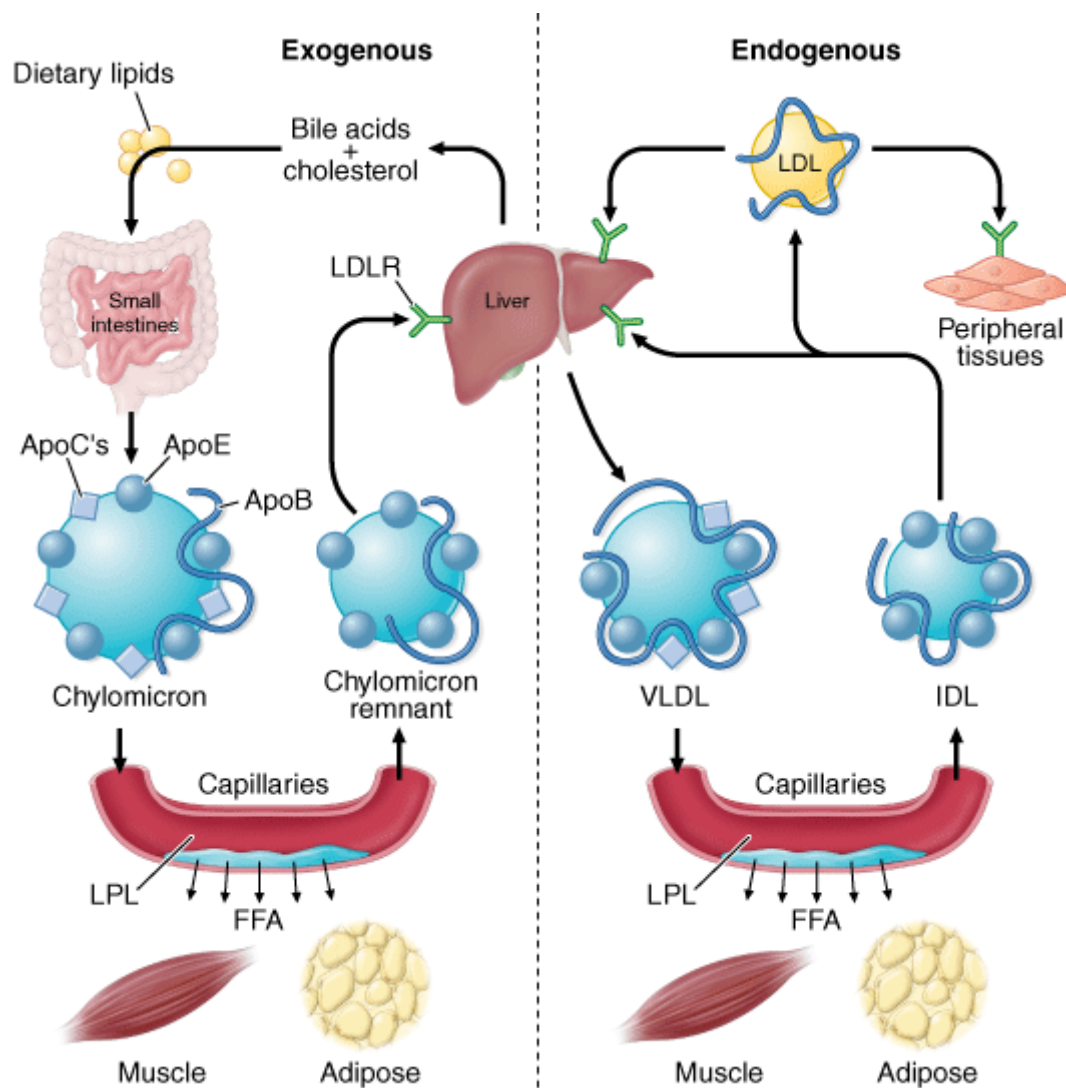


Figure 1.6b: Apo-B 100 in endogenous transport (adapted from Gibbons *et al* [108]).  $^{13}\text{C}$  Leucine: stable tracer isotope

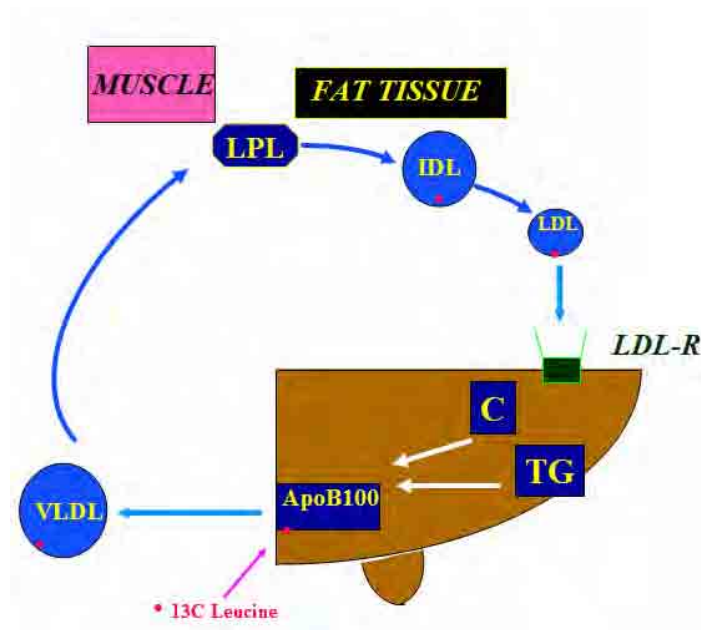
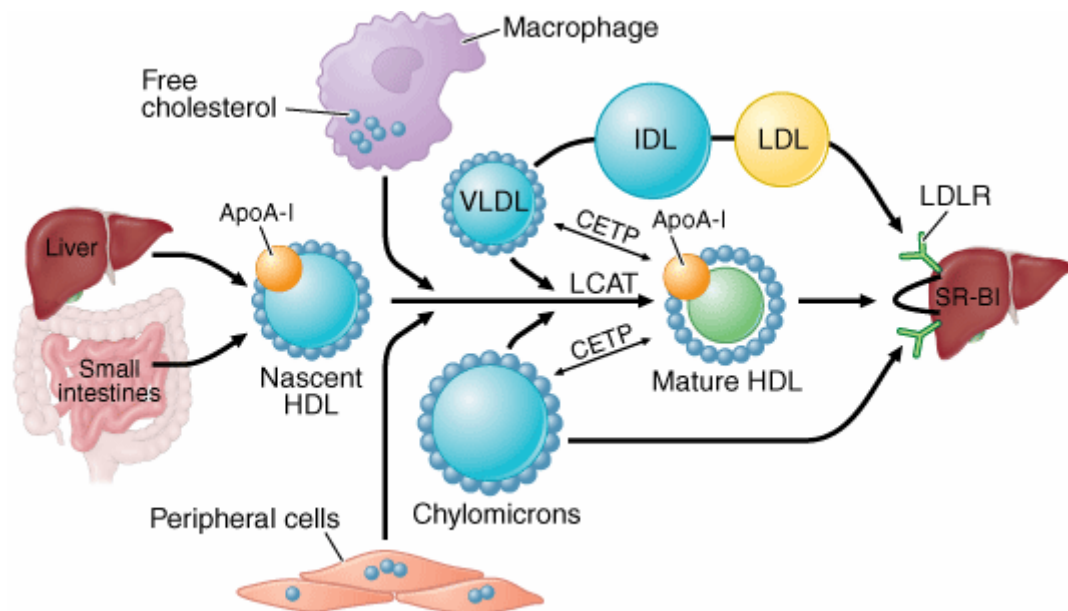


Figure 1.6c: Reverse cholesterol transport [107](adapted from Fauci AS *et al*, Harrison's principles of internal medicine, 17th edition). LCAT, lecithin-cholesterol acyltransferase; CETP, cholesteryl ester transfer protein; VLDL, very low density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; LDLR, low-density lipoprotein receptor; TG, triglyceride.



## Section 3: Lipid disorders in HIV infection

### 1.3.1. Overview and prevalence

The lipid disorders seen in individuals with HIV infection include elevated triglycerides (TG) and total cholesterol (TC), a decrease in high-density lipoprotein (HDL) cholesterol, and variable effects on low-density lipoprotein (LDL) cholesterol [55] (figure 1.7, 1.9). The exact mechanism is still not clear and the cause could be multifactorial. The individual contributions of HIV infection, specific antiretroviral agents, host genetics and changes in body composition, all should be considered (table 1.4) .

Figure 1.7: Pattern of dyslipidaemia (Total cholesterol > 6.2 mmol/l, Triglyceride >2.5 mmol/l and HDL-cholesterol <0.9mmol/l), in HIV infected adults according to current antiretroviral regimen, from D:A:D cohort [55].

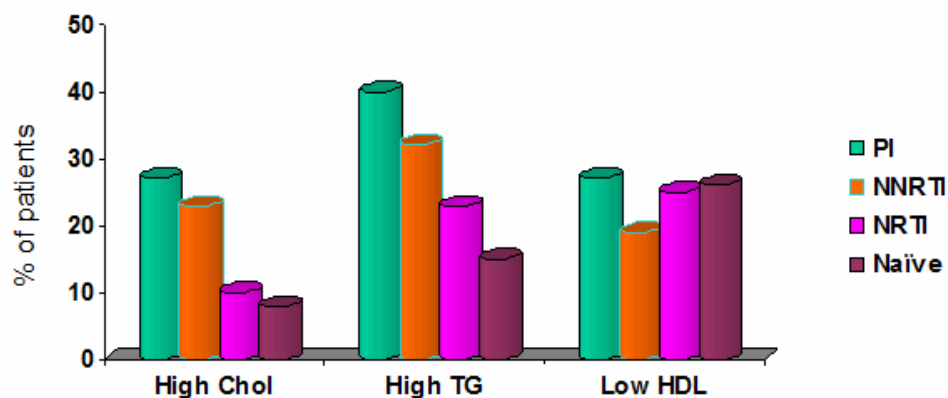


Table 1.4: Overview of studies on effect of HIV infection and HAART and different antiretrovirals (ARV) on lipid changes

TC: total cholesterol, TG: triglyceride, LDL: Low density lipoprotein, HDL: high density lipoprotein  
 d4T: stavudine, ddI: didanosine, AZT: zidovudine, 3TC: lamivudine, FTC: emtricitabine, ABC: abacavir, TDF: tenofovir,  
 CBV: Combivir (AZT + 3TC), TVD: truvada (TDF+FTC), TA: thymidine analogue, IDV: indinavir, LPV: lopinavir, RTV:  
 ritonavir, ATV: atazanavir, NFV: nelfinavir, SQV: saquinavir, FPV: fosamprenavir, TPV: tipranavir, DRV: daurunavir, r: small  
 dose (100mg) of ritonavir, NRTI: nucleoside reverse transcriptase inhibitor, NNRTI: non-nucleoside reverse transcriptase  
 inhibitor, EFV: efavirenz, NVP: nevirapine

## 1.4.1. Effect of HIV and HAART

Study (name)	Number	Design	Comparing arms	Changes in lipid
El-Sadr <i>et al</i> [73].	419	Retrospective	HIV infection and AIDS	Decrease in TC and HDL but increase in TG in advanced HIV infection
Grunfeld <i>et al</i> [109].	75	Cross-sectional	HIV infection and AIDS	Increase in TG
MACS[110]	50	Prospective	HIV and HAART	Decrease in TC, LDL and HDL after HIV infection. Increase in TC and LDL after HAART
DAD[55]	17852	Observational cohort	HAART	Increase in TG, TC, LDL and decrease of HDL
SMART[65]	5472	Observational cohort	Stopping HAART	Reduction in TC, TG and LDL, but not HDL

## 1.4.2. Effect of NRTI on lipid changes

## 1.4.2a. Studies switching to TDF or ABC

Switching to TDF				
Study name (Ref)	Number	Design	Comparing arms	Changes in lipids
Lipo-Rec sub-study (Llibre <i>et al</i> [70])	352	Prospective	48 week switch d4T to TDF	Decrease in TC, LDL, TG but not HDL in TVD arm
COMET (Schmid <i>et al</i> [111]).	402	Prospective	24 week switch CBV to TVD	Decrease in TC, LDL, TG and HDL In TVD arm
Gilead 903extension (Gallant <i>et al</i> [112]).	173	Randomized	144 week switch d4T to TDF	Decrease in TC, LDL, TG and HDL in TVD arm
RAVE (Moyle <i>et al</i> [113])	105	Randomized	48 week switch d4T/AZT to TDF [or ABC]	Decrease in TC, LDL, TG and HDL In TVD but not in ABC arm
SWEET (Moyle <i>et al</i> [111])	234	Randomized	CBV to TVD	Decrease in TC, LDL and TG in TVD arm
BICOMBO (Martinez <i>et al</i> [112])	333	Randomized	TA to TDF or ABC	More decrease in TC, TG, LDL and HDL in TVD arm
Switching to ABC				
RAVE (Moyle <i>et al</i> [113]).	105	Randomized	48 week switch d4T/AZT to ABC [or TDF]	Increase in TC,LDL,TG and HDL in ABC arm
MITOX ( Carr <i>et al</i> [116]).	111	Randomized	24 week data TA to ABC	Increase in TC and HDL in ABC arm
MITOX extension (Carr <i>et al</i> [117])	85	Randomized	104 week data TA to ABC	Increase in TC,LDL,TG and HDL in ABC arm

## 1.4.2. Effect of NRTI on lipid changes

## 1.4.2b. Non-switch studies

Study (Ref)	Number	Design	Comparing arms	Change in lipids
Gilead 903 (Gallant <i>et al</i> [112, 118]).	602	Randomized double blind prospective	d4T versus TDF in ART	TC, LDL and TG were higher in d4T group
Gilead 934 (Gallant <i>et al</i> [119])	487	Randomized	CBV + EFV versus TDF/FTC + EFV	TC, LDL higher and HDL lower in TDF group
CNA30024 (DeJesus <i>et al</i> [113]).	649	Randomized	AZT Vs. ABC	No difference
ABCDE (Podzamczar <i>et al</i> [114]).	237	Randomized	d4T versus ABC	More rise in TG in d4T, more rise in HDL in ABC arm
HEAT[115] Smith <i>et al</i>	564	Randomized double blind	TDF/FTC vs. ABC/3TC	More rise in TC and TG in ABC/3TC
ESS40002 (Kumar <i>et al</i> [116]).	254	Randomized	ABC/AZT/3TC vs. d4T/3TC/NFV	More increase in TC, LDL and TG in d4T/3TC/NFV
START II (Eron <i>et al</i> [90]).	205	Randomized	ddI/d4T/IDV vs. AZT/3TC/IDV	More rise in TG in ddI,/d4T/IDV
TOKEN (Das <i>et al</i> [117]).	195	Prospective	ABC/3TC vs. TDF/FTC	More rise in TC in ABC



Table 1.4.3: Effect of PI and NNRTI on lipid changes

Study name (Ref)	Number	Design	Comparing arms	Change in lipids
GEMINI (Walmsley <i>et al</i> [118])	337	Randomized	SAQ/r vs. LPV/r	More rise in TC, LDL and TG in LPV/r
ARTEMIS (DeJesus <i>et al</i> [92])	689	Randomized	DRV/r vs. LPV/r	More rise in TC, LDL and TG in LPV/r
ALERT (Smith <i>et al</i> [119])	106	Randomized	ATV/r vs. FPV/r	More rise in TG in FPV/r
CASTLE (Molina <i>et al</i> [120]).	883	Randomized	ATV/r vs. LPV/r	More rise in TC and TG in LPV/r
KLEAN (Eron <i>et al</i> [96]).	878	Randomized	LPV/r vs FPV/r	No difference
BMS 045[95] Johnson <i>et al</i> .	334	Randomized	ATV/r OR ATV/SQV vs LPV/r	Reduction of TC, TG in ATV/r or ATV/SQV
ATAZIP (Martinez <i>et al</i> [121]).	248	Randomized	ATV/r vs. LPV/r	Reduction of TC, TG in ATV/r
SWAN Gatell <i>et al</i> [122].	419	Prospective	Switch to ATV or ATV/r from other PI	Reduction of TC and TG in ATV or ATV/r
ACTG 5142 (Haubrich <i>et al</i> [123]).	757	Randomized	LPV/r vs. EFV	More rise in TG in LPV/r
613 substudy (Cameron <i>et al</i> [98]).	155	Randomized	LPV/r vs. EFV	More rise TC and TG in LPV/r
2NN (Van Leth <i>et al</i> [124]).	706	Randomized	NVP vs. EFV	Increase in HDL in NVP
Swiss HIV cohort (Young <i>et al</i> [125]).	1065	Prospective	PI vs. NNRTI	Decrease in TG and increase in HDL in NNRTI
Negredo <i>et al</i> [100].	34	Randomized	NVP vs. PI	Increase in HDL in NVP
Shafran <i>et al</i> [126].	20	Prospective	Immediate effect of RTV and LPV in healthy volunteers	Low dose RTV increased TC, TG, LDL and lowered HDL. Addition of LPV increased HDL

Table 1.4.4. Lipid metabolism in HIV infection

Study	Number	Design	Changes
Stein <i>et al</i> [101].	65	Cross-sectional	Delayed clearance of postprandial VLDL in HIV patients. Delay in clearance of IDL and LDL , but not VLDL in patients taking PIs
Rimland <i>et al</i> [127].	271	Cross-sectional	Higher CIII and TG in patients treated with PI or NNRTI
Ouguerram <i>et al</i> [128].	14	Cross-sectional Apo-B kinetic study	Increased production and delayed clearance of apo-B in HIV patients, more with lipodystrophy
Petit <i>et al</i> [104].	11	Cross-sectional Apo-B kinetic study	VLDL and IDL apo-B production rate was higher in PI group
Mauss <i>et al</i> [105]	187	Cross-sectional	High serum VLDL and increase in size of VLDL particles but not numbers
Reeds <i>et al</i> [101].	12	Cross-sectional Tracer kinetic study	Increased VLDL-TG secretion and delayed clearance in fasting and non-fasting state leads to increase in serum TG
Carpentier <i>et al</i> [129]	13	Prospective	Elevation VLDL early in the course of HAART caused by the combination of impaired VLDL clearance already present in HAART-naïve HIV patients and HAART-mediated increase in VLDL secretion
Schmitz <i>et al</i> [130].	11	Cross-sectional	Patient on HAART showed increased serum TG due to increased apo-B synthesis and diminished rate of VLDL transfer to IDL and LDL

### **1.3.2. Effect of HIV infection**

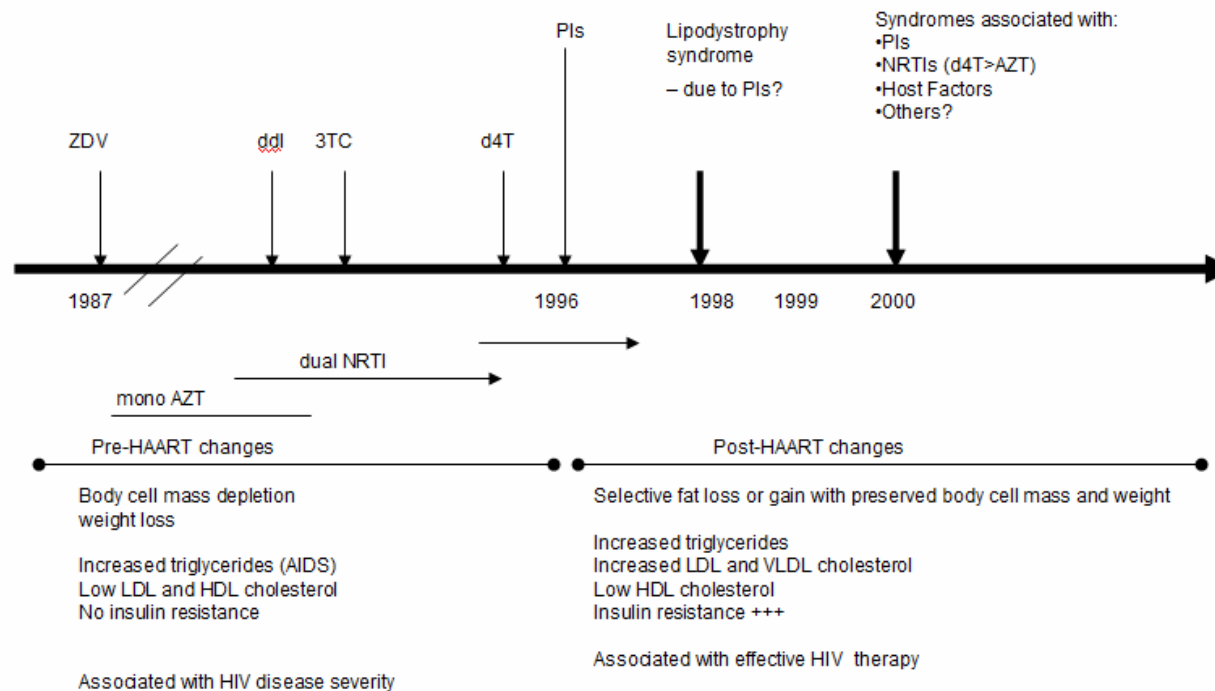
Abnormalities of lipid metabolism in HIV-infected patients were described before the use of HAART [109, 131-134] (figure 1.8). Increased serum triglyceride and reduced total cholesterol concentrations were observed in advanced HIV infection [73]. Patients with advanced HIV infection or with AIDS have also had lower levels of HDL-C and LDL-C, increased TG level and a predominance of small, dense LDL particles, compared with HIV negative individuals [73]. In the early 1990s a number of investigators described the lipid abnormalities associated with HIV infection. A consistent finding from these studies was that patients with advanced HIV infection or AIDS had high levels of circulating triglycerides and low levels of HDL cholesterol [109, 133]. El-Saadar *et al* [73] using data from HIV treatment naive patients have shown that the higher HIV RNA level was independently associated with lower LDL cholesterol and higher VLDL cholesterol and triglyceride levels, while a history of AIDS-defining events was associated with higher total cholesterol, VLDL cholesterol and triglyceride levels. The lipid changes were found to be restricted to patients with a history of *P. jiroveci* pneumonia (PCP) and were related to recent diagnosis of this condition. This data thus demonstrated that low CD4 lymphocyte count, high HIV RNA level and a history of AIDS-defining events, all markers of more advanced HIV disease, were all associated with higher concentrations of VLDL cholesterol and triglycerides.

Even among subjects with HIV infection and relatively preserved CD4+ cell counts, the levels of triglycerides appeared to be higher than among HIV-uninfected subjects and were thought to be due to increased levels of very low density lipoprotein (VLDL) cholesterol in the circulation[109].

These changes in triglycerides were accompanied by reduced levels of total and LDL cholesterol. Thus, in a study of 63 patients with HIV infection, Zangerle and colleagues [135] reported decreased levels of total cholesterol, HDL cholesterol, and LDL cholesterol in 3.2%, 48%, and 56% of the subjects respectively. Triglycerides were elevated in 32% of the patients in this cohort. The prevalence of lipid abnormalities was higher among those with lower CD4+ cell counts.

Figure 1.8 Timeline of development in antiretroviral treatment and 'lipodystrophy syndrome(s)' (adapted from Nolan) [77]

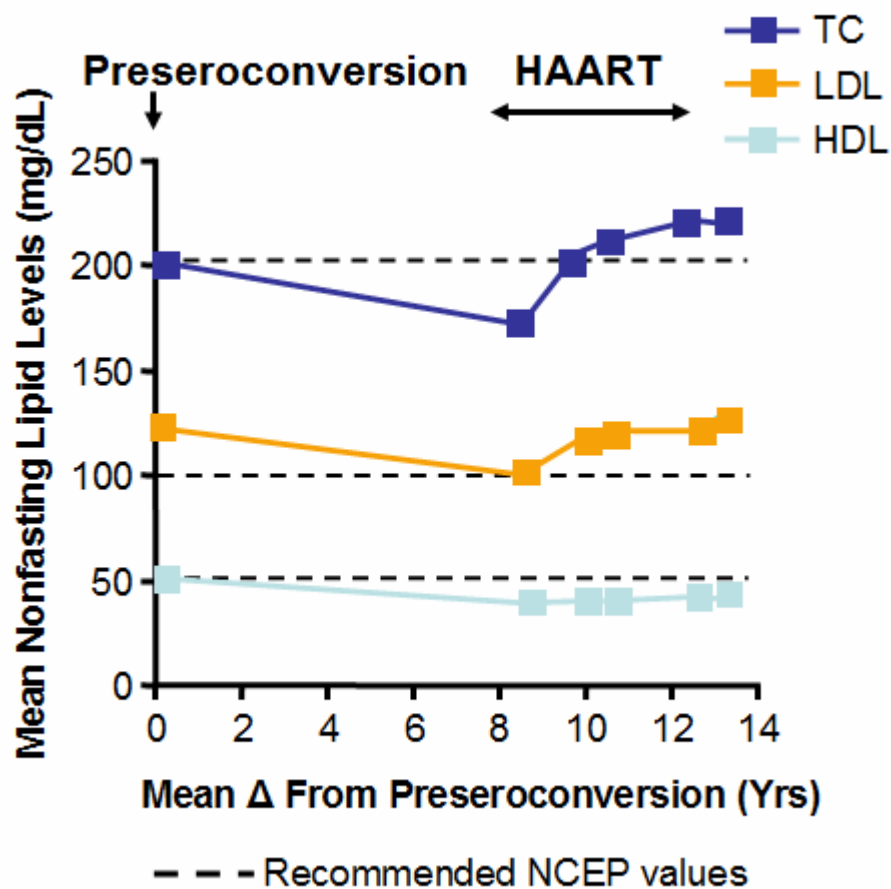
**AZT**= zidovudine; **3TC** = lamivudine; **d4T** = stavudine; **ddl** = didanosine; **HAART** = Highly active antiretroviral therapy; **HDL** = high density lipoprotein; **LDL** = low density lipoprotein; **NRTI** = nucleoside reverse transcriptase inhibitor; **PI** = Protease Inhibitor; **VLDL** = very low density lipoprotein;



A prospective analysis of HIV seroconverters (the Multicenter AIDS Cohort Study) has helped to illuminate the role of HIV infection in the pathogenesis of lipid disorders (figure 1.9). MACS investigators examined data on lipids among a subgroup of participants with known dates of seroconversion and stored samples, making it possible to compare their lipid levels before they became infected, after they seroconverted, and after they started antiretroviral therapy [110].

Figure 1.9: HIV acquisition associated with lipid reductions, with reversion on HAART (Multicenter AIDS Cohort Study) [110]

NCEP: National cholesterol education programme



After seroconversion, patients experienced reductions in total cholesterol, LDL cholesterol, and HDL cholesterol, compared with their pre-seroconversion levels [110]. When they started antiviral therapy, their total cholesterol, LDL cholesterol and triglyceride levels but not their HDL cholesterol levels promptly returned toward their pre-seroconversion levels (figure 1.8).

Of great interest was the observation that HDL cholesterol levels decreased at or soon after seroconversion, and remained low even after antiretroviral therapy was initiated.

The recently presented SMART study has shown that treatment interruption improved TC, LDL-C, TG but not HDL-C [65]. Whether this change is due to the withdrawal effect of HAART or the effect of viral replication is not clear.

### **1.3.3. Effect of Antiretrovirals**

#### **1.3.3.1. Protease inhibitors (PIs)**

Hyperlipidaemia associated with PI use is more common and more severe than what was observed before HAART was used. In patients who receive a PI-containing antiretroviral regimen, the prevalence of hyperlipidaemia ranges from 28% to 80% [136-138], and it includes hypertriglyceridaemia in the majority of cases (40–80%), followed by hypercholesterolaemia (10–50%) [44, 136, 139-144].

In a cohort of 212 HIV-positive patients who started a new PI-based antiretroviral regimen, the incidence of hypertriglyceridaemia and hypercholesterolaemia was 38.2% and 25%, respectively, after a 12

month follow-up [145]. Sixty-two (47%) of 133 PI recipients at one study had lipid abnormalities [146] that met the previous National Cholesterol Education Programme (NCEP), 1994 intervention criteria [147]. In the Swiss HIV Cohort [148], hypercholesterolemia and hypertriglyceridaemia were 1.7–2.3 times more common among individuals receiving PI-containing HAART regimen. Hypercholesterolemia (cholesterol level, >6.2mmol/l) and severe hypertriglyceridaemia (triglyceride level, >5mmol/dl) occurred in 60% and 75% of subjects, respectively, receiving PIs at one centre [149].

In the large survey of the **D**ata Collection on **A**dverse Events of Anti-HIV **D**rugs (D:A:D) study [55], hypercholesterolemia (defined as total cholesterol 6.2 mmol/L) was found in 27% of subjects receiving HAART including a PI, compared with 8% in treatment-naïve subjects (figure 1.7). Raised LDL-C (above 3.4mmol/L) in 58% of patients, compared to 31% in treatment naïve patients, triglyceride levels higher than 2.3 mmol/L were seen in 40%, compared 15% in naïve subjects, and low levels of HDL-C [0.9 mmol/L] were in 27%, compared to 26% in treatment naïve subjects. There was a much higher rise in TC, TG and LDL-C and lowering of HDL-C, when antivirals of three different classes were used (figure 1.9).

The effect of PIs on the lipoprotein fractions appear to be different [138, 150]. Hyperlipidaemia associated with PI-uses often includes the increase in the level of VLDLs [150, 151] and, to a lesser extent, IDLs [152]. Increased LDL-C levels have been reported in some studies but not others [153-155]. The HDL-C levels tends not to change or to increase, but inconsistent changes in small and large HDL particles have been described



as well [156, 157]. A small lowering of HDL-C has been observed with atazanavir which tend not to have any effect on the other lipid parameters [158]. Increased triglyceride concentrations, found in all lipoprotein fractions, were accompanied by rise in apo-B concentration. The changes in apo-B and apo-A in plasma with PI use did not always correspond with the rise of the different lipoprotein fractions. While lopinavir/r use showed significant rise in TG and TC with little change in HDL-C and rise in TC/HDL, the apo-B/ apo-A1 did not show significant changes [153].

There are very few systematic comparisons of the lipid effects of different PIs. Amongst them, lipid abnormalities tend to be most marked with ritonavir and lopinavir-ritonavir[159]. Amprenavir and nelfinavir tend to have intermediate effects, whereas indinavir and saquinavir tend to have the fewest effects. Amongst the newer PIs, atazanavir [160] and daurunavir [161] appears to have little effect on lipid concentrations, as determined on the basis of preliminary reports. Severity of lipid elevations particularly TC, TG and LDL-C are most likely to be dependent on the dose of ritonavir used as booster of the PI [160-162].

### **1.3.3.2. Nucleoside reverse-transcriptase inhibitors (NRTIs)**

Subjects in MITOX (**Mitochondrial Toxicity**) study have failed to show differences in nonfasting cholesterol and triglyceride levels associated with receipt of stavudine- compared with zidovudine-containing regimens [163]. However, in a prospective, randomized study, antiretroviral-naïve subjects who initiated therapy with stavudine-lamivudine-nelfinavir had significant increases in TC, LDL-C, and TG levels, compared with subjects receiving zidovudine-lamivudine-nelfinavir [116]. In another randomized

study elevations in nonfasting TG levels were more common in association with stavudine-didanosine-indinavir than with zidovudine-lamivudine-indinavir [164]. The NRTI tenofovir was associated with lesser increases in cholesterol and triglyceride levels than was stavudine [165] and zidovudine [166, 167]. Tenofovir containing regimens have better lipid profiles compared to AZT and d4T. Abacavir containing regimens are associated with rise in TC, LDL-C and TG along with rise HDL-C [168, 169]. Switch studies have shown better total cholesterol (TC), LDL cholesterol (LDL) and triglyceride (TG) but not HDL profile in the tenofovir group compared to the abacavir group [166, 168, 170]. Additional data are needed before any firm conclusions can be drawn regarding the relative tendencies of individual nucleoside analogues to alter lipid profiles.

#### **1.3.3.3. Nonnucleoside reverse-transcriptase inhibitors (NNRTIs)**

The NNRTIs cause alterations in the lipid profiles, although generally to a lesser degree than has been observed with PIs. In the D:A:D cohort (figure 1.7), raised cholesterol was seen in 23% receiving an NNRTI but no PI, and in 10% receiving NRTIs only [55]. Raised TGs were seen in 32% of NNRTI use compared to 15% of NRTI use only and low HDL-C was less common in 19% of NNRTI use compared to 25% in NRTI use [55, 171]. In the recently presented ACTG 5142 study [172], efavirenz containing regimen had similar rise of TC compared to ritonavir boosted lopinavir (LPV/r), but TG were significantly higher with LPV/r. Increases in HDL cholesterol were not different.

ACTG 5142 was a prospective, randomized, open-label trial evaluating the effects of an NRTI-sparing regimen on lipids [172]. Treatment-naïve subjects received one of three treatment regimens: the NRTI-sparing regimen lopinavir boosted with ritonavir (LPV/r) + efavirenz (EFV), LPV/r + two NRTIs, or EFV + two NRTIs. The NRTIs were selected before randomization from zidovudine, stavudine or tenofovir (each plus lamivudine). At week 96 of treatment, the differences in the change from baseline in lipoprotein levels between EFV and LPV/r when either was combined with NRTIs were minimal, although statistically greater increases in TG levels were observed with the PI. Increases in HDL-C were almost the same in these two groups. In contrast, the NRTI-sparing regimen of LPV/r + EFV increased lipids significantly more than either LPV/r or EFV + two NRTIs.

On the contrary NNRTI use, particularly nevirapine is associated with substantial increases in HDL-C levels to a degree not generally seen with PIs or NRTI [173-175]. In the 2NN study [176], the TC/HDL ratio improved significantly with nevirapine but remained unchanged with efavirenz. Efavirenz did show some improvement initially but the effect disappeared with time which was not the case with nevirapine.

Since pre-treatment TC usually remains lower in HIV patients, the rise from baseline after treatment could initially be correction of the initial defect. This needs to be taken into consideration when looking at the cohort studies.

#### **1.3.3.4. Effect of other agents**

The early data indicates that the recently licensed fusion inhibitors, CCR-5 inhibitors and integrase inhibitors do not appear to be associated with dyslipidaemia and hence considered to be lipid neutral [177, 178].

#### **1.3.4. Mechanism of Lipid disorders**

The mechanism of dyslipidaemia is not fully understood, but is probably multifactorial. Whether plasma lipid alterations are a direct consequence of antiretroviral therapy or reflect an HIV-associated metabolic defect that is exacerbated by the drug remains unclear. A recent study [179] has shown that HIV replication alone without any influence of antiretroviral drugs enhances production of free fatty acids, lipoproteins and many key proteins associated in lipid synthesis, transport and metabolism.

A key question is whether elevations in lipids are associated with increased production or decreased clearance apo-B containing lipoproteins. Several theories have been proposed to explain the mechanism of dyslipidaemia, particularly the role of PIs (table 1.5).

##### **1.3.4.1. Increased lipoprotein (hepatic cholesterol and triglyceride) synthesis**

##### **1.3.4.1.1. Cytoplasmic retinoic acid binding protein type- 1 (CRABP-1) and Lipoprotein receptor related protein (LRP)**

An early hypothesis developed by Carr and Cooper suggested that the PIs

might bind to several proteins involved in lipid and adipocyte metabolism after similarities between the HIV protease enzyme and regions of

Table 1.5: Possible Mechanisms for Dyslipidemia

<p><b>Increased lipoprotein (hepatic cholesterol and triglyceride) synthesis</b></p> <ul style="list-style-type: none"> <li>• Impaired CRABP-1 and inhibition of LRP</li> <li>• Decreased proteasome activity</li> <li>• Increased hepatocyte accumulation of SREBP-1c</li> <li>• Improved nutritional status and increased hepatic substrate delivery</li> </ul> <p><b>Impaired lipoprotein clearance</b></p> <ul style="list-style-type: none"> <li>• Downregulation of low density lipoprotein receptor expression</li> <li>• Inhibition of lipoprotein lipase</li> <li>• Impaired lipoprotein cell-surface interaction</li> <li>• Impaired CRABP-1</li> </ul> <p><b>Genetic Predisposition</b></p> <ul style="list-style-type: none"> <li>• Heterozygous or homozygous apolipoprotein E2 phenotype</li> <li>• Abnormal regulation of apolipoprotein C-III</li> </ul> <p><b>Effect of Cytokines</b></p> <ul style="list-style-type: none"> <li>• Tumour Necrosis Factor (TNF)-<math>\alpha</math></li> <li>• Interleukin-6</li> <li>• Interleukin-8</li> </ul> <p><b>Other</b></p> <p>CRABP-1: cytoplasmic retinoic acid binding protein ; LRP: low density lipoprotein receptor-related protein; SREBP-1: sterol regulatory element binding protein</p>
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cytoplasmic retinoic acid binding protein type-1 (CRABP-1) and lipoprotein receptor related protein were identified [180, 181]. However, to date direct inhibition of CRABP-1 and LRP inhibition as a causative mechanism for increased TG levels has not been demonstrated [76, 182]. Furthermore, the concept of a class based effect for PIs [183] has subsequently been shown to be outdated [76]. Specific agents have been implicated in the development of lipid abnormalities, but these effects do

not appear to be class specific with individual PIs having differential effects on both lipid and glucose metabolism [184].

#### **1.3.4.1.2. Decreased proteosomal activity: Impaired apo-B degradation**

Apo-B is a key protein involved in the metabolism of lipoproteins [78, 100]. Increased synthesis or decreased clearance of this protein can be associated with raised TG in blood. Liang *et al* [185] reported that ritonavir inhibited the normal proteasomal degradation of apolipoprotein B (apoB) in HepG2 cells leading to increased intracellular accumulation of apoB. Riddle *et al* [186] also found ritonavir to increase VLDL cholesterol production in mice when fed a high fat western diet, an effect which corresponded to higher levels of hepatic apoB accumulation.

#### **1.3.4.1.3. Sterol regulatory element binding protein-1 (SREBP-1)**

Some studies indicate that PIs may directly suppress adipocyte differentiation in vitro at an early step involving impaired sterol-regulatory-element-binding-protein-1 (SREBP-1) which actively promotes lipogenesis and adipocyte differentiation [187-189]. Therefore, an increase in SREBP-1 activity will increase both the size of the adipocyte as well as increasing production of VLDL cholesterol.

Riddle *et al* [186] found an increase in VLDL cholesterol production in mice when treated ritonavir. They found no effect of ritonavir on lipoprotein clearance from the circulation. These investigators observed a significantly higher level of hepatic apo-B secretion in the ritonavir-treated animals. This led them to hypothesize that the hypertriglyceridemic effects of ritonavir were due to an increase in hepatic lipoprotein production by the

inhibition of proteasome-mediated degradation of apo-B and SREBP-1 in the liver.

Caron *et al* [190] reported that indinavir in-vitro reduced the translocation of SREBP-1 from the endoplasmic reticulum to the nucleus in addition to abnormal phosphorylation of its active form [191]. Increased and altered form of SREBP-1 can activate lipogenic genes responsible for increased synthesis of fatty acids and lipids.

#### **1.3.4.1.5. Improved nutritional status and increased hepatic substrate delivery**

The nutritional status and fat content of the diet can increase apo-B synthesis. The activity of SREBP-1 in the liver is also affected by nutritional status and by levels of insulin as well as by other nuclear receptors. Several of the PIs appear to increase fasting insulin levels over time. Indirect effects of PI therapy on circulating insulin levels could further stimulate an increase in SREBP-1. The lipid effects of ritonavir described in the studies by Riddle *et al* [186], were greatly influenced by the fat content of the diet fed to the mice. While it is not likely that diet *a/one* will change the lipid abnormalities observed among patients, it may play an indirect role.

#### **1.3.4.2. Impaired lipoprotein clearance**

##### **1.3.4.2.1. Down regulation of low density lipoprotein receptor expression**

LDL receptor expression [192] may be impaired in HIV patients. In a small cross-sectional study Petit *et al* [193] showed that in HIV-infected subjects, LDL receptor expression was lower in those on antiviral treatment, particularly in those with lipodystrophy compared to those

without, and inversely proportional to total cholesterol and LDL blood levels.

#### **1.3.4.2.2. Inhibition of lipoprotein lipase**

The Lipoprotein lipase (LPL) family plays an important role in the clearance of lipoproteins and inhibition of LPL can increase the lipid levels [82, 85]. Lipoprotein lipase and hepatic lipase levels were negatively correlated with triglyceride levels, suggesting that reduced levels of these enzymes may contribute to the PI-associated hyperlipidaemia[80].

#### **1.4.4.2.3. Impaired lipoprotein cell-surface interaction**

The interaction of lipoproteins with specific receptors depends on the lipoprotein composition and structures. Smaller CE rich LDL may have less affinity for LDL hepatic receptors and can remain in the circulation for long. Similarly CE rich larger HDL particles has less affinity for hepatic receptors and can remain in the circulation for longer time, but their role in cardioprotective action is doubtful.

#### **1.4.4.2.4. Cytoplasmic retinoic acid binding protein type- 1 (CRABP-1)**

The interaction between PIs and CRABP-1 was thought to reduce the activity of 9-cis-retinoic acid, which was postulated to have downstream effects on fat cell differentiation [181]. The interaction between PIs and LRP would impair hepatic chylomicron uptake and triglyceride clearance. This hypothesis has not been confirmed, as pointed out in a [182] subsequent review by Mooser and Carr [182] . In a mouse model, inhibition of LRP in a manner similar to that proposed by Carr *et al* earlier [181] did not result in hyperlipidemia. In addition, more recent studies [194] found that mice treated with PIs did not exhibit decreased clearance



of triglycerides. Finally, data from healthy volunteers treated with ritonavir do not show inhibition of lipoprotein lipase.

#### **1.3.4.3. Genetic Predisposition**

Genetic predisposition may explain the variability between patients in respect of the lipid effects of PIs. The observation that some patients experience increases in triglycerides to over 10 mmol/l during therapy with ritonavir, while others sustain no changes, indicates a role for genetic predisposition.

##### **1.3.4.3.1. Apolipoprotein E2**

Apolipoprotein E2 (apo E2) is involved in the clearance of triglycerides [78]. HIV-negative people who are homozygous for the E2 phenotype are predisposed to develop a form of hyperlipidaemia similar to that observed with PI therapy. HIV-infected patients who are heterozygous or homozygous for the E2 genotype have been reported to have dramatic increases in cholesterol and triglyceride levels when receiving PI therapy [138]. However, the prevalence of the E2 phenotype does not explain all cases of hyperlipidaemia on PI therapy [136].

##### **1.3.4.3.1. Apolipoprotein C-III**

Apolipoprotein C-III (apo C-III) may also play a role in the hyperlipidaemia associated with PI therapy. Apo C-III is the major lipoprotein of hepatic VLDL, and specific polymorphisms in the apo C-III gene are associated with certain types of hypertriglyceridaemia in the general population [195]. Fauvel *et al* [196] examined the prevalence of specific apolipoprotein C-III polymorphisms on PI induced lipid increases following 3 months of treatment in sixty consecutively treatment naive

males. Carriers of the -455c variant had 30% lower HDL levels than those without the variant as well as increased levels of triglycerides [196]. These results in combination indicate that genetic factors can clearly interact with both HAART as well as the severity of metabolic changes; however, the design of all these studies was based on small non-ethnically diverse populations, and thus need to be confirmed in larger groups.

#### **1.3.4.4. Effect of cytokines**

Previous studies [197, 198] have shown that lipoprotein changes in patients with HIV-infection were related with humoral and cellular immune markers. Interferon (IFN)- $\alpha$ , beta2-microglobulin and tumor necrosis factor (TNF)- $\alpha$  were correlated positively with total and VLDL-triglycerides and negatively with HDL-cholesterol [199]. As an ongoing chronic infection, the inflammatory cytokines including TNF- $\alpha$ , IL-6 and interferon-  $\alpha$  may remain abnormal in different stages of HIV infection and may be responsible for the lipid abnormalities seen in patients with HIV infection [200].

The mechanism of dyslipidaemia with NRTI use appears to be different and most of the actions may be mediated indirectly through impaired adipogenesis. Increased lipolysis may generate excess FFA that in turn act as a fuel for increased synthesis of triglycerides. Increased fat loss can also cause lack of plasma adiponectin which may have role on lipid metabolism.

#### **1.3.4.5. Role of HIV**

Proteomic studies [179, 201] have shown effect of different viral proteins on lipid metabolism. Expression of HIV *Nef* protein that enhances HIV replication and infectivity can inhibit ATP binding cassette transporter (ABCA-1) dependent cholesterol efflux from macrophages [201]. While *Nef* transfected cells exhibit increased binding of Apo-A1, but internalisation of Apo-A1 was blocked, suggesting that *Nef* at plasma membrane prevents lipidation of Apo-A1 and thereby reduce serum HDL-cholesterol. Further study has shown that the *Nef protein* can enhance synthesis of several lipoproteins either by upregulating or down regulating several key proteins responsible for lipid metabolism [179]. However, their role in clinical studies has yet to establish.

### **Section 4: Abnormalities of Fat distribution**

#### **1.4.1. Overview**

HIV patients are at higher risk of development of fat redistribution and the condition was first described as lipodystrophy syndrome by Carr *et al* in 1998 [180]. Lipodystrophy syndrome is a common term in the literature traditionally used to describe several morphologic (fat atrophy and fat hypertrophy) and metabolic disturbances including dyslipidemia and insulin resistance found in patients with HIV infection. Increasing evidence suggests these disorders, though commonly clustering in a syndromal pattern, have distinct pathologic pathways and can occur independently of each other (table 1.6).

Table 1.6a: Over view of key studies describing fat redistribution (lipodystrophy), HIV and HAART

d4T: stavudine, ddI: didanosine, AZT: zidovudine, 3TC: lamivudine, FTC: emtricitabine, ABC: abacavir, TDF: tenofovir, CBV: Combivir (AZT + 3TC), TVD: truvada (TDF+FTC), TA: thymidine analogue, IDV: indinavir, LPV: lopinavir, RTV:ritonavir, ATV: atazanavir, NFV: nelfinavir, SQV: saquinavir, FPV: fosamprenavir, TPV: tipranavir, DRV: daurunavir, r: small dose (100mg) of ritonavir, NRTI: nucleoside reverse transcriptase inhibitor, NNRTI: non-nucleoside reverse transcriptase inhibitor, EFV: efavirenz, NVP: nevirapine

Study (Ref)	Number	Design (comparing arm)	Risk factor	
			Lipoatrophy	Lipohypertrophy
ACTG 5142 (Haubrich <i>et al</i> [202])	753	Randomized (TA/EFV or TA/LPV/r or EFV/LPV/r)	More in PI sparing arm	No difference in trunk fat, but more gain in peripheral fat in PI containing arm
613 sub-study (Cameron <i>et al</i> [203]).	155	Randomized (CBV/EFV vs. LPV/r monotherapy)	More in AZT/3TC/EFV	No difference
5005 sub study (Dube <i>et al</i> [204]).	157	Randomized (AZT/3TC vs. d4T/DDI with EFV or NFV)	More in d4T/ddI	No difference
Mallon <i>et al</i> [205].	40	Prospective observational (Effect of HAART)	d4T use	Increase in CD-4 count and drop in VL
ABCDE study (Podzamczar <i>et al</i> [114]).	57	Randomized (d4T vs. ABC)	More in d4T compared to ABC	No difference
Gilead 903 (Gallant <i>et al</i> [206]).	232	Randomized (d4T vs. TDF)	More in d4T compared to TDF	No difference
Gilead 934 (Gallant <i>et al</i> [207]).	93	Randomized (CBV vs. TVD)	More in AZT/3TC compared to TVD	No difference
MITOX extension (Carr <i>et al</i> [208]).	85	Prospective (TA switched to ABC)	Thymidine analogue use	

Table 1.6a (cont.): Over view of key studies describing fat redistribution (lipodystrophy), HIV and HAART

Study (Ref)	Number	Design (comparing arm)	Risk factor	
			Lipoatrophy	Lipohypertrophy
PIILR (Martin <i>et al</i> [209]).	45	Randomized (Switching from PI d4T and AZT)	d4T use	
Boyd <i>et al</i> [210].	60	Prospective (Switching from NRTI to PI and EFV)	NRTI use	Fat gain in IDV/RTV/EFV use
RAVE (Moyle <i>et al</i> [166]).	105	Randomized 48 week switch (d4T/AZT → TDF [or ABC])	d4T and AZT use	
SWEET (Moyle <i>et al</i> [111]).	234	Randomized (CBV to TVD)	AZT use	
Walmsley <i>et al</i> [211].	68	Prospective (Different PIs and NNRTIs)	No difference amongst different PI and NNRTI	No difference amongst different PI and NNRTI

Table 1.6b: Overview of key epidemiological studies describing fat redistribution (lipodystrophy), HIV and HAART

Study [Ref]	Number	Design (comparing arm)	Risk factor	
			Lipoatrophy	Lipohypertrophy
Miller <i>et al</i> [212].	1348	Cross-sectional	Older age, CDC stage, baseline viral load (VL), NRTI use, d4T, ddI, AZT, PI use	Older age, CDC stage baseline VL,
Lichtenstein <i>et al</i> [213].	1244	Prospective	White race, Low CD-4 count, low BMI	
Lichtenstein <i>et al</i> [214].	1077	Cross-sectional	White race, older age, low CD-4 count, d4T use, IDV use	Older age, BMI gain, duration treatment
Heath <i>et al</i> [215].	745	Cross-sectional	d4T use, AIDS diagnosis	d4T, PI use
Saves <i>et al</i> [216].	614	Cross-sectional	d4T use, male sex, older age	CD-4 increase, SQV, 3TC
Thiebaut <i>et al</i> [217].	581	Cross-sectional	Duration of treatment, male sex, older age	Higher BMI, Increased waist/hip ratio
Martinez <i>et al</i> [218].	494	Prospective	Duration of treatment, d4T use	Female, duration of treatment
Joly <i>et al</i> [219].	170	Prospective	d4T and ddI use, lower CD-4 count	Female, older age
Bogner <i>et al</i> [220].	115	Prospective	Older age, white race, duration of treatment, high baseline VL	Duration of treatment, PI use
Bonfanti <i>et al</i> [221].	1480	Observational cohort	Older age, duration of treatment, homosexuality	RTV use, female sex
Johnson <i>et al</i> [222].	55	Cross-sectional	Serum IL-6 and TNF- R2 and increased TNF-alfa secretion from adipose tissue	Serum IL-6 and TNF- R2 and increased TNF-alfa secretion from adipose tissue

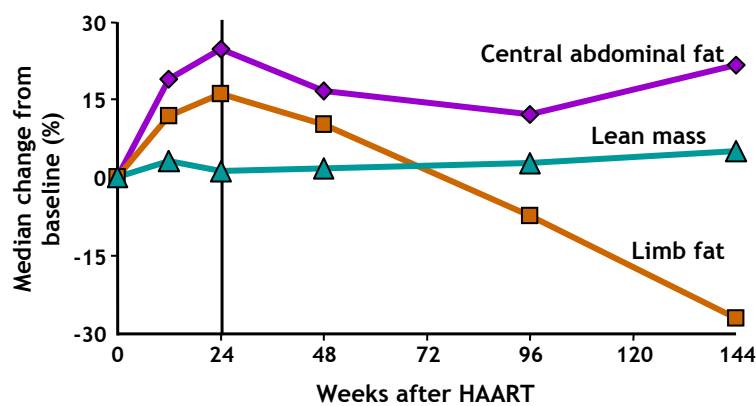
There are two major phenotypes described in HIV infected patients.

Lipohypertrophy is characterised by the presence of a hypertrophied pad of fat accumulated in various parts of the body. The anatomical sites of fat deposits are abdominal (visceral and abdominal wall), breast tissue, and head-neck region (dorsocervical, submandibular, trapezio-occipital, mastoid)[77].

Lipoatrophy is characterised by peripheral fat wasting with loss of subcutaneous tissue in the face, arms, legs, and buttocks, producing an emaciated appearance [223] and prominence of the superficial veins in these sites (pseudovenomegaly). Patients starting antiretroviral treatment for the first time may demonstrate initial increases in limb fat during the first few months of therapy, followed by a progressive decline during the ensuing years. In contrast, truncal fat increases initially and then remains stable during the ensuing years, resulting in relative central adiposity (figure 1.10). Changes in limb and central fat masses may become clinically evident in 20 to 35 percent of patients after approximately 12 to 24 months of combination antiretroviral therapy [205].

Fat accumulation or lipohypertrophy is more common with PI use and fat loss or lipoatrophy with thymidine analogues.

Figure 1.10: HAART induces initial fat gain followed by limb fat loss  
(Adapted from Mallon PWG *et al.* [205])



### 1.4.2. Prevalence

The prevalence of fat redistribution amongst HIV patients can vary from 11- 80% [220, 224-239]. One 5-year retrospective cohort study of patients with HIV infection who were treated with PIs demonstrated a 13% prevalence of lipodystrophy. In untreated patients with HIV infection, a 4% prevalence rate has been reported [149]. On the other hand, prospective cohort study demonstrated a 17% prevalence rate after an 18-month follow-up [240]. The variance in prevalence estimates probably arises from the use of different diagnostic criteria, differences in demographic factors, HIV treatment practices, disparities between patient and physician assessments and lack of a universally accepted definition of lipodystrophy or fat redistribution syndrome.



### **1.4.3. Definition of Lipodystrophy**

It has been a long debate between the different study groups and no consensus has been reached regarding a unifying definition of lipodystrophy. Definition of lipodystrophy by Lipodystrophy Case Definition Study Group was based on a case-control study of patients with or without features of fat redistribution [241]. A series of tests (table 1.5) provided an objective definition with sensitivity and specificity of less than 80%.

Table 1.7: HIV lipodystrophy case definition and scoring system[241]. The model has a sensitivity of 79% (95% CI, 70–85%) and specificity of 80% (95% CI, 71–87%). A total lipodystrophy score is derived by adding the relevant individual score for each parameter and then subtracting 43 (the constant). A final score for a given patient of  $\geq 0$  constitutes presence of lipodystrophy and a score  $< 0$  constitutes no lipodystrophy. CDC, Centres for Disease Control and Prevention; HDL, high-density lipoprotein; VAT, intra-abdominal adipose tissue (visceral fat); SAT, subcutaneous adipose tissue.

Parameter	Unit	OR	95% CI	p-value	Lipodystrophy score
<b>Gender</b>	Male	1.0		<0.001	0
	Female	9.33	3.86-22.52		22
<b>Age</b>	<40 years	1.0		0.008	0
	>40 years	2.02	1.20-3.40		7
<b>Duration of HIV</b>	<4 years	1.0		<0.001	0
	>4 years	3.11	1.69-5.71		11
<b>CDC stage</b>	A	1.0		0.361 0.043	0
	B	1.32	0.73-2.39		3
	C	1.92			7
<b>Waist/hip ratio</b>	<1.0	1.34	1.06-1.69	0.014	Multiply by 29
<b>HDL-cholesterol</b>	mmol/l	0.87	0.81-0.94	<0.001	Multiply by -14
<b>Anion gap</b>	mmol/l	1.01	1.04-1.16	0.001	Multiply by 1
<b>VAT/Sat ratio</b>	<0.45	1.0		0.613 0.416 0.007	0
	0.45-0.83	0.82	0.38-1.76		-2
	0.83-1.59	1.40	0.62-3.18		3
	>1.59	3.70	1.44-9.55		13
<b>Trunk/limb fat</b>	1.0	1.72	1.12-2.66	0.014	Multiply by 5
<b>Leg fat percent</b>	>21.4	1.0		0.559 0.051 0.001	-16
	14.5-21.4	1.27	0.57-2.87		-14
	8.8-14.5	2.32	1.00-5.40		-8
	<8.8	5.04	1.90-13.35		0

The other definition introduced by the FRAM study group (**Fat Redistribution And Metabolic change**) considered lipoatrophy (LA) as a distinguishing trait of HIV lipodystrophy [242]. It was initially thought that a decrease in peripheral fat occurred in tandem with an increase in central adiposity. However, preliminary data reported by Gripshover *et al* [243]

raised questions about this perception and suggest that a single pathogenic mechanism linking peripheral lipoatrophy and central lipohypertrophy may not exist. The FRAM group further showed no association between lipoatrophy and visceral adiposity [242]. Loss of fat in the periphery was however associated with loss of subcutaneous fat from the abdominal wall, suggesting that fat loss is a general phenomenon that occurs in the arms, legs, face and trunk and that central accumulation of fat is not necessarily associated. The FRAM study further showed that patients tend to lose more fat in the legs compared to arms, with relative sparing of fat in the trunk [244]. This further suggested that abnormal body shape is a relative rather than an absolute phenomenon. FRAM study however, included more Caucasian patients [242] and results could be different in black or other ethnic groups.

Currently peripheral lipoatrophy is described as localized fat wasting in the face, arms, legs, and buttocks; Central lipohypertrophy, is described as fat accumulation in the abdomen, breasts, or dorsocervical region (buffalo hump) [25]; Fat redistribution is described comprising both conditions either present alone or together. Lipoatrophy should be distinguished from HIV wasting, which is a generalized loss of body fat and lean body mass. A number of host factors (table 1.6) [221, 223, 245], in addition to type and duration of antiretroviral drug exposure, have been associated with fat distribution abnormalities. These include older age [212], baseline body mass index or change in body mass index [246], duration of HIV-1 infection [223], effectiveness of viral suppression, baseline degree of immunodeficiency and subsequent immune restoration with therapy, and white race [213, 214, 223, 247]. The risk of lipoatrophy is increased in

whites (5.4 odds ratio) compared with blacks [213]. Gender-based differences in presentation have been reported. Women are more likely to report fat accumulation in the abdomen and breasts, whereas men are more likely to describe fat depletion from the face and extremities [212]. These findings may reflect differences in baseline body composition. Although older age is a consistently reported risk factor, body fat changes occur naturally with aging and body fat distribution abnormalities have also been reported in HIV-infected children [248, 249].

#### **1.4.4. Effect of HIV**

Abnormalities of fat distribution has been reported in individuals with HIV infection who have never been treated with antiretrovirals [250-252]. It is not clear whether this is due to the direct effect of the virus or due to the effect of cytokines liberated by chronic infection [251, 252].

Several studies suggested a link between immune or inflammatory factors and fat redistribution. Pro-inflammatory cytokines are known to stimulate lipolysis and inhibit adipose tissue lipogenesis [74, 222, 253, 254]. Some risk factors for fat distribution apart from ART have been identified within cohort studies and potentially in prospective studies that include host and genetic factors. In the HIV Out-patient Study (HOPS), patients who had low CD-4 count that did not recover in the survey period of 23 months had a 10 fold increased risk of lipoatrophy independent of drug treatment [213]. Other Studies have found associations between the development of lipodystrophy and nadir CD4+ cell count as well as the rise in CD4+ cell count during HAART, independent of specific antiretroviral agents [213, 214]. Data from Western Australia [255, 256] showed doubling of the risk of LA in association with a polymorphism of TNF alpha gene.

#### **1.4.5. Effect of PI**

Several studies have now shown that PI use is associated with lipohypertrophy [215, 218]. The median time from the use of protease inhibitors to the development of lipodystrophy is 18 months [215, 218]. Prior reports had shown that ritonavir/saquinavir combinations had a stronger association with lipodystrophy than indinavir or nelfinavir [212, 221]. One study revealed that switching from other protease inhibitors to nelfinavir led to an improvement in lipodystrophy symptoms [257]; however, recent findings suggest that the incidence of lipodystrophy does not vary significantly across different protease inhibitors [25, 211, 258]. On the other hand other studies suggest that risk of lipoatrophy with ritonavir boosted PI is less compared to NNRTI or unboosted PI. The effects of ritonavir boosted lopinavir (LPV/r) with zidovudine and lamivudine (AZT/3TC) combination therapy followed by LPV/r monotherapy on lipoatrophy and lipohypertrophy were evaluated in a 96-week sub analysis of 613 study [203]. Treatment-naïve patients (n=155) were randomly assigned to treatment with LPV/r + AZT/3TC for up to 48 weeks, followed by LPV/r monotherapy or efavirenz (EFV) with AZT/3TC or LPV with AZT/3TC combination. Lipoatrophy and lipohypertrophy were defined as >20% limb fat loss and gain, respectively, on DEXA scans. Only 5% of patients receiving LPV/r monotherapy exhibited lipoatrophy compared with 34% in the EFV treatment arm ( $P<0.001$ ). Between LPV/r monotherapy and AZT/3TC/EFV combination group lipohypertrophy occurred in similar percentages of both groups (45% and 44% of the LPV/r and EFV groups, respectively;  $P<0.99$ ), and lipohypertrophy and lipoatrophy occurred together in 0% and 16% of the groups, respectively

( $P=0.002$ ). These results indicate that treatment with LPV/r is significantly associated with a sparing of peripheral lipoatrophy when compared with an NNRTI-containing regimen. However, there was no difference in peripheral fat loss between AZT/3TC/LPV/r arm and AZT/3TC/EFV arm, suggesting that lipoatrophy observed in this study was mostly due to AZT use in both arms. Further randomized control prospective study might give more light in to the effect of boosted PI on fat loss.

#### **1.4.6. Effect of NRTI**

Although several early cross-sectional studies suggested association of fat distribution abnormalities with protease inhibitor use, other studies provided clear evidence that these could occur in PI- naive subjects [259]. Specific roles for each class of drugs have not been defined, but an association between lipoatrophy and NRTIs have been shown in other studies [260, 261].

Several studies assessing lipoatrophy, the most common statistically significant risk factors were exposure to and duration of thymidine analogues, most commonly stavudine (d4T). A prospective, randomized, controlled trial (ACTG-384) after 80 weeks of follow up, demonstrated more rapid and severe fat loss in d4T-ddI arm compared to AZT-3TC [262]. Data from the mitochondrial toxicity (MITOX) study demonstrated that peripheral (limb) fat, as assessed by DEXA and CT scans, increased over long-term follow-up after switching from thymidine analogue-based therapy to abacavir-based therapy [163]. At week 104, patients who switched to abacavir showed a 1.26-kg mean increase in limb fat, relative to baseline. Multivariate analysis demonstrated that the increase in limb

fat was associated with less exposure to the thymidine analogues before the study [208]. The effect of thymidine analogues on lipoatrophy has been further exemplified in various other switch studies. Protease Inhibitor Induced Lipodystrophy Reversal Study (PIILR) after 120 weeks [209] revealed a statistically significant association with switching to thymidine analogue and limb fat mass reduction (d4T: 0.72 kg/y,  $P = 0.004$ ; zidovudine [AZT]: 0.29 kg/y,  $P = 0.019$ ). On the other hand, Boyd *et al* [210] reported preliminary results on the impact of replacing an NRTI regimen with an NRTI-sparing regimen (indinavir-ritonavir-efavirenz) in a 48-week observational study of 61 patients. DEXA scans showed statistically significant increases in limb fat after 96 weeks of the NRTI-sparing regimen. The recently presented RAVE (**R**andomized **A**bacavir versus **V**iread **E**valuation) study [166] and SWEET (**S**implification **W**ith **E**asier **E**mticitabine and **T**enofovir) study demonstrated improvement in fat gain after switching patients from d4T and or AZT containing regimen to tenofovir or abacavir containing regime [263].

### 1.4.7. Effect of NNRTI

Current data indicates that some NNRTIs may also be associated with lipoatrophy. In the recently presented ACTG 5142 study after 96 weeks of follow up [172], limb fat had increased from baseline, a median of 18% in the ritonavir boosted lopinavir (LPV/r) with efavirenz (EFV) group compared with a median gain of 9.8% in the LPV/r with two NRTIs group. On the other hand 32% of patients receiving EFV with two NRTIs had lipoatrophy, compared with only 17% in the LPV/r with two NRTIs group and 9% in the NRTI-sparing LPV/r with EFV group. Although lopinavir and efavirenz were randomized, the patients had been preselected with which

nucleoside they were going to use. So they either used stavudine (d4T), zidovudine (AZT), or tenofovir (TDF). As expected lipoatrophy was more common in patients exposed to stavudine (d4T) and the individuals who used tenofovir, no matter which arm they were in, had very little lipoatrophy. Hence the possibility of channelling bias could not be excluded from the results. However, fat wasting of the limb was still more likely when participants were also taking EFV along with d4T, compared with patients given LPV/r with d4T. The use of tenofovir (TDF) was associated with the lowest incidence of lipoatrophy. In a logistic regression model, taking EFV along with the NRTIs increased the odds of developing lipoatrophy by 2.7-fold. In contrast to lipoatrophy findings, trunk fat increased by 12%–16% and did not differ significantly across the treatment groups. The results of this study indicate that EFV exacerbates the lipotrophic effects of NRTIs and that thymidine analogues (AZT and d4T) produce more lipoatrophy of the extremities than TDF. However, EFV did not exert a demonstrable lipotrophic effect when combined with LPV/r, suggesting that either the lipotrophic effect is dependent on the presence of NRTIs or LPV/r has a protective effect against lipoatrophy. The ACTG 384 study [264], on the other hand had previously shown that patients randomly assigned to receive nelfinavir had a greater percentage loss of limb fat than those randomly assigned to the efavirenz arm; limb fat in the efavirenz arm also decreased, though to a lesser degree. The association of NNRTI with lipoatrophy could be due to the effect of nucleoside backbone and appears to be worse than ritonavir boosted PI. This could be the fact that ritonavir boosted PI have protective action against lipoatrophy but NNRTIs, particularly efavirenz may not have that



effect. Further prospective studies might be able to add more insight into the role of NNRTI on fat loss.

In essence, both lipoatrophy and lipohypertrophy and fat redistribution as a whole in individual patients could be the effect of combination of several factors including the combined effect of different antiretroviral drugs. Apart from thymidine analogues lipoatrophy has not been clearly associated with any other antiretrovirals. Fat gain in the early weeks of treatment is common with other antiretrovirals and fat gain as a whole is more common with regimens containing ritonavir boosted PIs.

#### **1.4.8. Assessment**

Different cross-sectional imaging techniques have been used for the assessment of fat redistribution. However, there is no gold standard method for measuring body fat. Computed tomography (CT) scanning provides information about abdominal subcutaneous and visceral fat, but it is associated with radiation exposure and should not be used clinically for this purpose [25].

The dual-energy x-ray absorptiometry (DEXA) scan and magnetic resonance imaging (MRI) may have merits but are not used in routine clinical practice mostly because of cost and limited availability [265]. A clinical case definition, based on physician and patient agreement [241], is of limited value for individual patient management, because of lack of specificity and is not recommended in routine use [25].

Anthropometric measurements such as the waist-to-hip ratio have been repeatedly shown to correlate with health outcomes [266-268]. The major advantages of anthropometry are its safety, portability, and low cost. The

lack of specificity of changes in the waist-to-hip ratio limits its use as a clinical tool. Although the measurements are standardized, they require considerable training and retraining for the results to be reproducible. In addition, only gross cut-offs are available for assigning subjects as normal or abnormal, and no published data allow translation of waist circumference to visceral adipose tissue (VAT). Also very little data are available for non-white other ethnic groups.

Bioelectrical impedance analysis (BIA) estimates whole body composition [266], though attempts have been made to modify the measurement for regional purposes. To date, methods to use BIA for regional body composition have not been validated against criterion methods, and thus cannot be recommended for this purpose.

Theoretically, ultrasound is a better technique than DEXA since it can accurately separate adipose and lean compartments and allow 3-dimensional measurements. The depth of specific adipose tissue compartments can be measured, including those on the face, an area that has not been measured with cross-sectional imaging to date. However, its application to HIV infection has been very limited.

#### **1.4.9 Mechanism of fat distribution or lipodystrophy**

Several pathogenic mechanisms and risk factors for fat redistribution in HIV have been postulated (table 1.8). The mechanism appears to be different for lipoatrophy and lipohypertrophy.

Table 1.8: Potential risk factors and mechanism for Fat distribution in HIV infection [189, 213, 221, 223, 269-275]

- Host Factors
  - Male Sex
  - White Ethnicity
  - Older Age
  - Baseline BMI
  - Duration of HIV Illness
  - Effectiveness of viral suppression
- Impairment of adipocyte function
  - Adipocyte apoptosis
  - Adipocyte differentiation
- Mitochondrial toxicity
  - Inhibition of DNA polymerase- $\gamma$
  - Decrease in DNA content
  - Mitochondrial dysfunction
- Genetic Predisposition
  - Haemochromatosis gene expression (HGE)
  - Association with DNA haplo type J

#### **1.4.9.1. Impairment of adipocyte function**

##### **1.4.9.1.1. Adipocyte apoptosis**

Lipoatrophic tissue from HIV patients showed increased expression of TNF- $\alpha$ , a cytokine known to induce apoptosis of adipocytes. A genetic case-control study [276] conducted in HIV-positive patients both with (n = 61) and without (n = 35) lipodystrophy found a significant difference between groups in the frequency of polymorphism -238 in the promoter region of the TNF- $\alpha$  gene, suggesting that the -238 polymorphism is a determinant in the development of HIV-related lipodystrophy.

Nolan *et al* [277], using fat biopsy samples from HIV patients including both treatment-naïve and treatment-experienced patients showed increased expression of several adipocyte-specific pro-inflammatory cytokines (IL-18, IL-6, TNF- $\alpha$ , IL-8, IL-12) and significant correlation with increased lipoatrophy. More macrophage infiltration, cytokine expression, and mtDNA depletion were noted with stavudine or zidovudine therapy but not following initiation of abacavir (tenofovir was not studied in this study). Switching from stavudine or zidovudine was associated with increased mtDNA content and reduced macrophage numbers and cytokine expression, although without improvement in body fat mass. Protease inhibitor therapy had no effect.

##### **1.4.9.1.2. Adipocyte differentiation**

Bastard *et al* [278] showed that adipocytes from patients with lipoatrophy treated with NRTIs and PIs have higher levels of sterol-regulatory-element-binding-protein-1 (SREBP1c, an adipocyte transcription factor) and higher expression of TNF- $\alpha$ , but the adipocytes were smaller and

tended to cluster, suggesting an impairment of differentiation despite the increased amount of SREBP1c protein. In other study [279] using 3T3 human preadipocytes, efavirenz induced a strong inhibition of the SREBP-1c-dependent lipogenic pathway that might contribute to adipose tissue atrophy and could explain increased lipoatrophy seen in patients in EFV arm in ACTG 5142 study.

#### **1.4.9.1.3. Mitochondrial toxicity** (figure 1.11)

Inhibition of mitochondrial DNA polymerase is associated with nucleoside analogues and has been hypothesized to play a role in fat redistribution. Initial studies in lipodystrophic patients implicated a reduction in mitochondrial DNA [280] caused by interference of nucleoside analogues with mitochondrial DNA polymerase [281] and which was correlated to the clinical degree of lipoatrophy. Studies performed in vivo [167, 282, 283] and in vitro [284, 285] suggest a hierarchical effect of nucleoside analogues on mitochondrial DNA (mtDNA) with the dideoxy compounds (didanosine and stavudine) having a severe detrimental effect and the cytidine analogues (lamivudine, emtricitabine) the least. Zidovudine (AZT) and abacavir (ABC) are intermediate and tenofovir (TDF) appears to have little effect on adipocyte mtDNA [283]. Substitution of stavudine (d4T) with either AZT or ABC increases mtDNA in adipocytes [286]. It should be noted that many of the published studies have been performed on peripheral blood mononuclear cells (PBMC) which may behave differently from subcutaneous fat [287, 288].

Abnormalities in mitochondrial-encoded respiratory complex genes and disturbances in cellular metabolism can also be shown in the absence of demonstrable reduction in mtDNA both in HIV-infected treatment-naïve

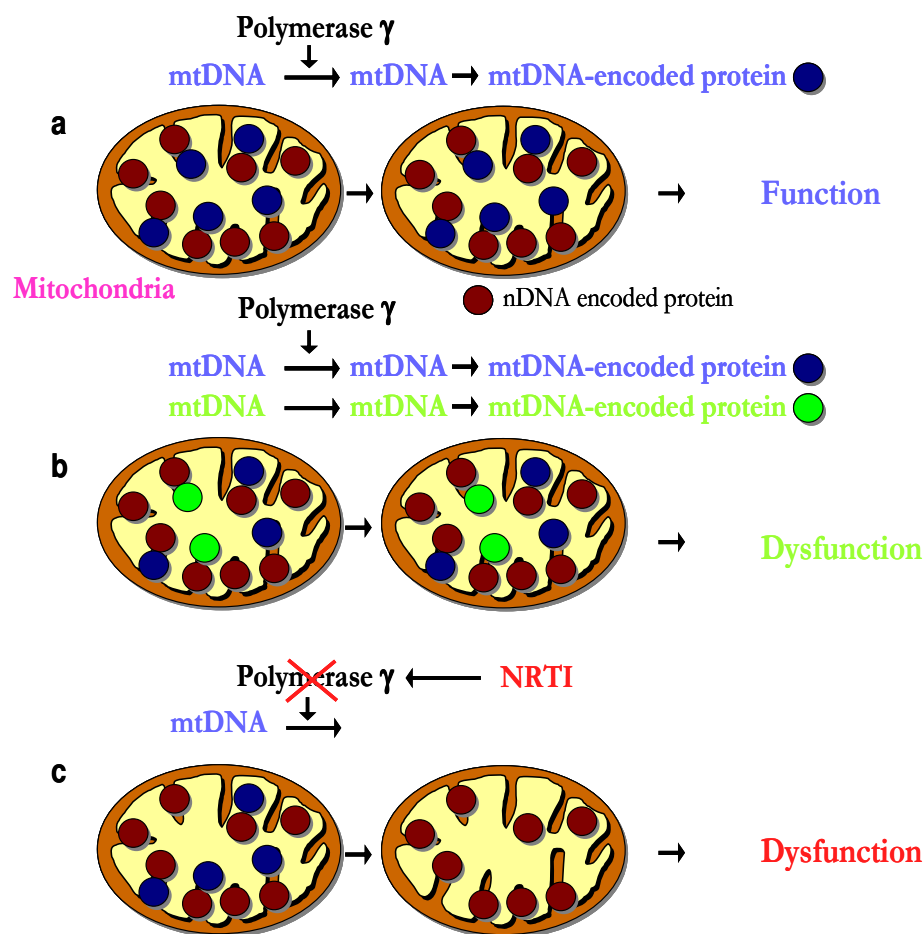
patients [289] and in patients with lipodystrophy [290] and may occur before demonstrable changes in mtDNA in both HIV-infected patients [291] and HIV negative subjects [292]. Adipocyte maturation and differentiation is a complex process consisting of sequential activation of master transcription factors leading to expression of genes that determine adipocyte specific metabolic function [293, 294]. A number of transcription factors and co-activators have been shown to be reduced in lipodystrophic subjects compared to those without body fat changes [294]. While d4T causes lipoatrophy through depletion of adipocyte mtDNA depletion [295], AZT produced less, or no, mtDNA loss [283, 288, 296] and the effect may be predominantly through other mechanisms such as interference with both nuclear and mitochondrial-encoded mitochondrial respiratory gene expression and oxidative function in the adipocyte and muscle and other tissues [292]. Mitochondrial DNA depletion has also been shown to correlate poorly with respiratory chain activity [297]. Even at low rates of respiratory impairment, increase in reactive oxygen species (ROS) can occur [298] and may be important in the early crucial events in HIV related lipodystrophy [187].

Lower mitochondrial DNA content per adipocyte from subcutaneous biopsies was correlated with severity of lipoatrophy and DNA depletion was more common in d4T group compared to AZT group. However, some studies have shown decrease in mtDNA content but not mitochondrial count or mitochondrial deletion, suggesting that a mitochondrial dysfunction is the result of reduced DNA content rather than DNA deletions. Similarly, Walker *et al* [281], found that mean mitochondrial DNA content was lower in buttock fat biopsies from NRTI-treated patients

than in the non-NRTI group, and patients with lipoatrophy had a lower mitochondrial DNA content than patients without lipoatrophy. Cherry *et al* [299], on the other hand have shown an increase of mitochondrial DNA (copies per adipocyte) 48 weeks after switching from a d4T-containing regimen to abacavir- or AZT-based therapy along with some improvement in arm, leg, and trunk fat.

In an extensive study, Nolan *et al* [300] found mtDNA content to be reduced by 85% in patients treated with either zidovudine or stavudine, thereby exceeding the threshold for cell function to become impaired (Nolan *et al.* 2003). In another study Nolan *et al* also identified morphological differences within the biopsies consistent with other syndromes of mitochondrial depletion [76]. The effect of stavudine was consistently greater than that of zidovudine confirming the studies reported previously [277].

Figure 1.11: Mitochondrial toxicity. In the normal situation (a) mitochondrial DNA (mtDNA) encodes for proteins (blue circles) in the respiratory chain, situated at the inner mitochondrial membrane. Most respiratory enzymes are encoded by nuclear DNA (nDNA) (red circles). Replication of mtDNA is regulated by the enzyme DNA polymerase  $\gamma$ . In inherited mitochondrial diseases (b), parts of mtDNA have been mutated or even deleted (green), which results in altered mtDNA-encoded proteins (green circles), leading to mitochondrial dysfunction. During NRTI treatment (c), DNA polymerase  $\gamma$  will be inhibited, leading to mitochondrial depletion [280].



#### 1.4.9.1.4. Genetic predisposition

Mitochondrial haplotypes defined by pattern of single nucleotide polymorphism in mitochondrial genes and mutation in haemochromatosis gene (HFE) may have effect on fat distribution[301]. HIV-infected patients with a common mutation in the hemochromatosis gene (HFE), in combination with mitochondrial DNA haplogroup J, may be protected from



the development of lipoatrophy associated with antiretroviral therapy. In a sub study, AIDS Clinical Trials Group [301] assessed relationships between HFE gene variants, mitochondrial haplogroups, and peripheral lipoatrophy during ART in 96 ART-naive individuals randomized to didanosine-stavudine or zidovudine-lamivudine, combined with efavirenz, nelfinavir, or both. Patients with the HFE 187C>G polymorphism were less apt to develop lipoatrophy during ART. This association was independent of other factors including age, CD4 lymphocyte count, and specific antiretroviral therapy received [301]. In contrast TNF- $\alpha$  gene -238G/A promoter polymorphism were associated with a more rapid onset of lipodystrophy [276, 302] and in a sub analysis of ACTG 5202 study, patients with mitochondrial haplotype-I were found to have a tendency ( $p=0.07$ ) to more peripheral fat loss compared to non-I haplotypes.

#### **1.4.9.1.5. Refutation of the Mitochondrial Toxicity hypothesis**

Supporting evidence for NRTIs as a pathogenic factor in lipoatrophy remains limited and has been refuted [303, 304]. Some studies failed to detect sufficient mtDNA inhibition to induce mitochondrial dysfunction [305, 306]. Other studies raised question about the validity of threshold in vivo [306, 307]. Arnaudo *et al* found myopathy with zidovudine use at only 22% of mtDNA depletion [308]. In addition, patients with less mitochondrial content or impairment were still found to be symptomatic. Molecular analysis of mitochondrial depletion in non-HIV inherited diseases was unable to substantiate an in-vivo threshold sufficient for mtDNA depletion and mitochondrial dysfunction [309, 310].

In summary, the underlying mechanisms and specific site(s) of dysregulation accounting for the morphologic abnormalities have not been identified. NRTIs possibly affect mitochondrial maturation and replication, PIs affect preadipocyte differentiation and HIV may have effects on both directly or via cytokines.

## **Section 5: Insulin Resistance and Diabetes**

### **1.5.1. Overview**

Before the use of highly active antiretroviral therapy (HAART), blood glucose abnormalities were infrequently seen in people with HIV. But in June 1997, soon after protease inhibitors (PIs) came into widespread clinical use, the U.S. Food and Drug Administration (FDA) issued a health advisory warning of an association between PIs and hyperglycaemias and diabetes mellitus [311]. Since then, there have been continued reports of insulin resistance and diabetes in people using HAART (table 1.9).

### **1.5.2. Prevalence**

Different studies have yielded widely varying estimates of the prevalence of impaired glucose metabolism in people on HAART. The prevalence of frank diabetes mellitus (DM) in people with HIV is relatively low, with studies reporting rates from 0.5% to 15% [312-315]. Impaired glucose tolerance is considerably more common, affecting an estimated 15-25%, and some studies suggest that some degree of insulin resistance may occur in one-half of patients taking PIs [149, 316, 317].

Table 1.9: Overview of key studies describing insulin resistance and diabetes in HIV infection

Study (Ref)	Number	Design (comparing arm)	Risk factor
DAD (de Wit <i>et al</i> [318]).	33,389	Observational cohort	Thymidine analogues
MACS [319]	1288	Prospective	Cumulative use of NRTI
Lo <i>et al</i> [320].	95	Cross-sectional	Cumulative NRTI use
Visnegarwala <i>et al</i> [321].	1389	Cross-sectional	HCV infection, family history of diabetes, BMI, age above 50
Yoon <i>et al</i> [322].	147	Case-control	BMI, family history and raised liver enzyme (ALT)
Women's Interagency HIV study (Tien <i>et al</i> [323]).	2088	Prospective	Duration of NRTI (d4T), family history, BMI, HCV infection, menopause
Mehta <i>et al</i> [324].	1230	Retrospective	PI use and HCV infection
Mulligan <i>et al</i> [325].	29	Retrospective	PI use
Blumer <i>et al</i> [326].	20	Randomized (AZT/3TC/LPV/r vs. LPV/r/NVP)	AZT/3TC use
Carr <i>et al</i> [327].	140	Randomized (TVD/LPV/r vs. TVD/TPV/r)	No difference in LPV/r vs. TPV/r
Noor <i>et al</i> [328].	26	Randomized cross-over (LPV/r vs. ATV/r)	Increase in insulin resistance in LPV/r
Noor <i>et al</i> [329].	30	Randomized double blind cross over study (ATV vs. LPV/r)	Increase in insulin resistance in LPV/r
Noor <i>et al</i> [330].	6	Randomized double blind cross over study (Single dose of IDV use)	Immediate increase in insulin resistance after IDV use
Brar <i>et al</i> [331].	9151	Cross-sectional General population vs. HIV treatment naïve patients	No difference
Bedimo <i>et al</i> [332].	19,424	Cross-sectional	HCV co-infection
Shikuma <i>et al</i> .	838	Cross-sectional	HCV co-infection

### 1.5.3. Effect of HIV

Several risk factors have been proposed as an underlying factor responsible for insulin resistance in HIV infection (table 1.10). Although hyperinsulinemia was described in HIV-infected patients in the pre-HAART era, HIV infection by itself has not been conclusively implicated as a cause of insulin resistance. Potential mechanisms by which HIV infection could induce insulin resistance are not confirmed.

Table 1.10: Potential risk factors and mechanism for Insulin resistance in HIV infection [320, 321, 333-350]

<b>Risk Factors</b> <ul style="list-style-type: none"><li>• Peripheral lipoatrophy</li><li>• Increased liver / muscle fat</li><li>• Low testosterone</li><li>• Oxidant stress</li><li>• HCV infection</li><li>• Protease Inhibitors</li></ul>
<b>Reduced Glucose uptake</b> <ul style="list-style-type: none"><li>• Defect in GLUT-4</li><li>• Defect in SREBP</li></ul>
<b>Pancreatic beta cell dysfunction</b>
<b>Defect in adipocytokine</b> <ul style="list-style-type: none"><li>• Low adiponectin</li><li>• Increased TNF-<math>\alpha</math>, IL-6, IL8</li></ul>

Prevalence of insulin resistance in HIV patients does not appear to be higher in treatment naïve cases. Whether the virus itself has any direct role on glucose metabolism needs more investigation. In a cross-sectional

survey, Brar *et al* [331] (CPCRA clinical trial) compared factors associated with prevalent diabetes mellitus among HIV-infected antiretroviral-naive individuals versus HIV negative individuals in the National Health and Nutritional Examination Survey cohort. The prevalence of DM in the CPCRA clinical trials versus the NHANES was 3.3% versus 4.8%. The data did not suggest an increased prevalence of DM in ART-naive HIV-infected patients. The virus as such or the stage of the disease (HIV RNA level and CD-4 count) has not been found to have specific effects on the prevalence of diabetes mellitus in another study [322].

In contrast, during a 4-year follow-up period in the Multicenter AIDS Cohort Study (MACS), 24 (10%) of 229 HIV-infected subjects receiving HAART developed DM compared with 10 (3%) of 361 HIV-seronegative men. After adjustment for BMI and age, this difference represents a greater than 4-fold increase in the risk of incident DM among HIV-infected subjects receiving HAART [314].

The recently presented result from D:A:D study [318] showed a lower incidence of new-onset diabetes mellitus than in the Multicenter AIDS Cohort Study (5.72 vs. 4.7 per 1,000 PYFU) [314]. This difference could be related to different size and demographic compositions of both cohorts as the MACS involved white males exclusively, who were likely to be exposed to the typical North American diet and who were older and had higher BMI than the D:A:D participants.

Tien *et al* [351] recently reported a relative risk of incident self-reported DM of 2.0 (95% CI, 1.0- 4.1) when HIV-infected women receiving a PI were compared with an HIV-seronegative subgroup prospectively followed

in the Women's Interagency HIV Study. The higher crude rate of incident DM in the HIV-infected, HAART-exposed group in the MACS compared with the Women's Interagency HIV Study (4.7 vs 2.8 [95% CI, 1.6-4.1] cases per 100 person-years) may reflect a more sensitive case ascertainment method in the MAC cohort [314].

Antiretroviral medications likely play a causative or permissive role in the pathogenesis of hyperglycemia in HIV-infected patients. In MACS cohort [314] only ritonavir use was significantly associated with an increased risk of a combined end point of DM or hyperglycemia.

HIV-related non drug factors may be important in the development of metabolic abnormalities in HIV-infected patients. Severity of HIV disease, as estimated by the nadir CD4 cell count, has been associated with increased risk of lipoatrophy, and or lipodystrophy. In the MACS cohort, HIV-infected men with lower nadir CD4 cell counts had an increased risk of incident glucose abnormalities compared with those with higher nadir CD4 cell counts. The possibility that confounding factors, such as more diabetogenic antiretroviral regimens in the more severely ill patients, contributed to this finding cannot be excluded. In contrast, higher CD4 lymphocyte count was associated with lower insulin concentrations, and less evidence of insulin resistance. However, there was no association between HIV RNA level or history of prior AIDS diagnoses and measures of glucose homeostasis [73].

#### **1.5.4. Effect of Hepatitis C Virus (HCV)**

Co-infection with the hepatitis C virus (HCV) increases the risk of blood glucose abnormalities [344, 352, 353]. Studies have shown that people

with chronic HCV infection are more likely to develop insulin resistance and type-2 diabetes [321, 324, 354-356]. A study from Johns Hopkins University in Baltimore found that people with HCV were four times more likely to develop type-2 diabetes than HCV negative people. The same study also found that HIV/HCV-co-infected patients were five times more likely to develop hyperglycaemia than those with HIV alone [324]. Similarly, recent study from USA [357] (Shikuma C *et al.* Po:931) and data from D:A:D group [358] and Veteran Affair (VA) group [359] found an increased association of insulin resistance and diabetes in HCV/ HIV coinfecting patients.

Although it is not clear how chronic hepatitis promotes blood sugar abnormalities, it is believed that liver damage affects the metabolism of glycogen and the production of glucose [344, 345]. Hepatic steatosis, iron deposition in the liver, and progressive HCV-induced liver damage may induce insulin resistance and predispose to DM. Increased hepatic fat content has been strongly correlated with elevated fasting insulin levels and is associated with impaired glucose tolerance in HIV-infected individuals. Chung *et al* from USA [360] reported that elevated alanine transaminase (ALT) liver enzyme levels independently can predict insulin resistance in HIV-positive individuals with lipodystrophy whether or not they were coinfecting with HCV. None of the studies so far has found any association with hepatitis B virus (HBV) infection.

### **1.5.5. Effect of Protease Inhibitors (PI)**

The use of PI, particularly indinavir appears to be more directly related to disorders of glucose metabolism than to other metabolic complications

such as body fat gain or loss [361, 362]. A study from Germany [150] reported that 46% of PI recipients had impaired glucose tolerance and 13% had diabetes, compared with 24% and none, respectively, among PI-naïve subjects. Another study from Munich [317] reported that 61% PI-treated subjects had reduced insulin sensitivity, which was seen in none of the treatment-naïve HIV controls.

A study from USA [325] reported development of insulin resistance (IR) within an average of 3.4 months after starting treatment with PI; the patients did not have any body shape changes. The group treated with only NRTIs did not experience similar changes.

Among the PIs, indinavir has been most strongly associated with impaired glucose metabolism [330, 362]. Dube *et al* [363] detected signs of IR in patients with HIV within eight weeks of starting indinavir. Mustafa Noor *et al* [330] from San Francisco found that insulin resistance without elevated lipid levels or body shape changes, developed within four weeks after starting indinavir in HIV-negative volunteers. In one of their studies, glucose disposal (uptake of glucose by cells) was reduced after a single dose of the drug, suggesting that indinavir itself directly triggers insulin resistance [364].

Although most PIs are associated with significant glucose intolerance, saquinavir has relatively little effect, and atazanavir has no discernable effect [329]. Another study using boosted lopinavir and tipranavir with a backbone of tenofovir and lamivudine did not show any evidence of insulin resistance at 48 weeks therapy, but was present when used with AZT



[327]. Hence insulin resistance could be related more to thymidine analogues than to PIs.

#### **1.5.6. Effect of NRTI**

Use of thymidine analogues is increasingly associated with increase in insulin resistance estimated by homeostasis assessment (HOMA) model.

The recently published Women Interagency HIV Study has shown an association of increased risk of diabetes with cumulative exposure of NRTIs [323]. Another study using boosted lopinavir and tipranavir with a backbone of tenofovir and lamivudine did not show any evidence of insulin resistance at 48 weeks therapy [327], but was present when used with AZT [326]. Hence insulin resistance could be related more to thymidine analogues than to PIs. However, Goebel *et al* [317] detected evidence of insulin resistance in 27% of HIV-positive people treated with NRTIs, although the rate in those receiving PIs was twice as high.

NRTIs especially d4T and AZT (thymidine analogues) may indirectly contribute to glucose abnormalities by causing peripheral fat loss.

However, in the recently presented D:A:D study the incidence of diabetes increased with cumulative exposure to HAART, an association that remained significant after adjustment for potential risk factors for diabetes [318]. The strongest relationship with diabetes was exposure to stavudine; exposures to zidovudine and didanosine were also associated with an increased risk of diabetes. Although lipodystrophy was significantly associated with diabetes, adjustment for this did not modify the relationship between HAART and diabetes.

### **1.5.7. Effect of NNRTI**

Studies have not implicated non-nucleoside reverse transcriptase inhibitors (NNRTIs) in blood glucose abnormalities, although they have been linked with other metabolic manifestations in some studies.

Similarly other agents including fusion inhibitors and integrase inhibitors have not any influence on glucose metabolism.

### **1.5.8. Mechanism of Insulin resistance and Diabetes**

It is not clear exactly how PIs affect glucose metabolism, but research points to a variety of possible mechanisms including, decreased uptake of glucose by peripheral cells, decreased insulin production by beta cells in the pancreas, and increased glucose production by the liver.

#### **1.5.8.1. Studies *in vitro***

##### **1.5.8.1.1. Reduced glucose uptake by peripheral cells** (figure 1.12)

Several laboratory, animal, and clinical studies suggest that PIs may directly interfere with the transport of glucose into cells. An insulin-sensitive glucose transport protein called GLUT-4 plays a key role in transporting glucose into fat and muscle cells [342, 343, 362, 365]. The insulin signalling pathway within both skeletal and adipose tissue is mediated by a series of phosphorylation cascades which can be either phosphatidylinositol 3-kinase (PI-3) dependent or independent. Within the PI-3 dependent pathway, activation of Akt is an important link in the translocation of GLUT-4 to the cell membrane [366]. It involves the intracellular translocation of GLUT-4 to the cell surface enabling the facilitated diffusion of glucose (figure 1.12). PI inhibits the glucose uptake

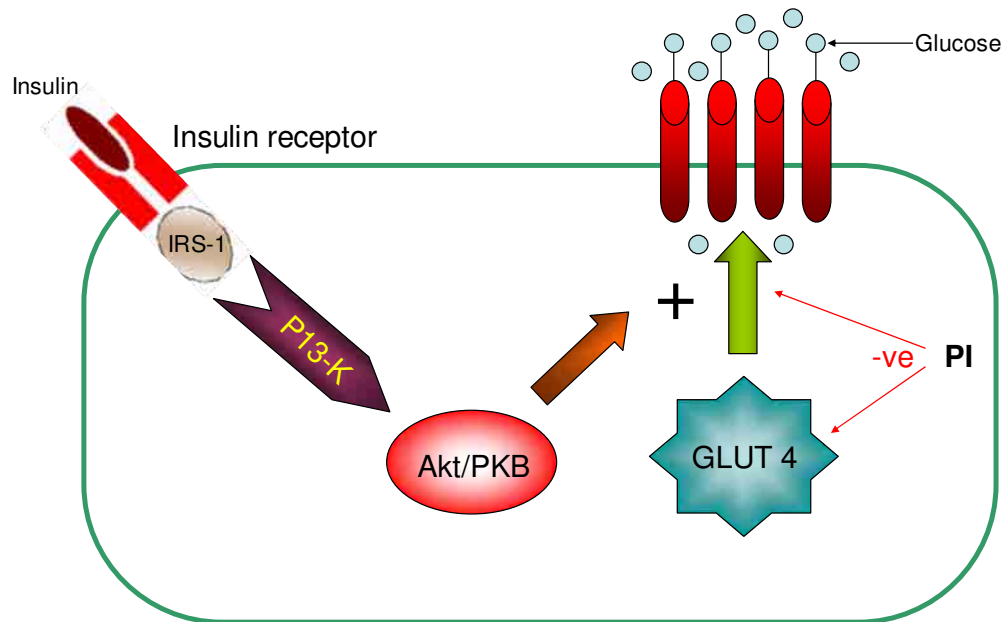
through GLUT-4. PIs down regulates the phosphatidyle inositol-3 (PI 3) kinase cascade and also impair the intrinsic activity of GLUT-4, leading to reduced glucose uptake by cells [367]. In laboratory studies using 3T3-L1 adipocytes, Haruhiko *et al* [368] found that indinavir and nelfinavir reduced glucose uptake by inhibiting GLUT-4 activity. This inhibition occurred within minutes, and was reversed when indinavir was removed.

Caron *et al* [190] proposed another mechanism involving the sterol regulatory element binding protein (SREBP), a key fat cell messenger that triggers stem cells to differentiate into adipocytes. PIs including indinavir, nelfinavir, and amprenavir inhibited the production and activity of SREBP and also stimulated increased production of peroxisome proliferating activation factor gamma (PPAR-gamma), which promotes cellular glucose uptake in the presence of insulin.

**Figure 1.12: The entrance of glucose into cells via GLUT-4**

**translocation.** PIs block GLUT-4 initiated glucose uptake in fat and muscle cells.

IRS-1: Insulin Receptor Substrate-1; P1 3-kinase: phosphatidylinositol 3 kinase; Akt/PKB: protein kinase B.



**1.5.8.1.2. Beta cell dysfunction**

Alternatively, the changes seen in glucose disposal may occur without GLUT-4 blockade. In HIV-negative individuals, impaired glucose metabolism is often characterised by up-regulated insulin secretion rates (ISR) relative to impaired peripheral insulin sensitivity. Furthermore, insulin resistance within the  $\beta$ -cell itself may cause up-regulated insulin secretion. Using the hyperglycaemic clamp, Woerle *et al* [350] showed impairment of pancreatic  $\beta$ -cell function following 12 weeks of PI therapy, presumably by preferential impairment of first phase insulin secretion

[350, 369]. Similar results have also been reported in heavily treated NRTI and PI patients with Lipodystrophy [370].

A related protein, GLUT-2, allows beta cells in the pancreas to take up glucose to monitor blood sugar levels and regulate insulin release. Joseph Koster J *et al* from Washington University [371], found that indinavir in doses similar to those used in humans and other PIs at higher concentrations inhibit the activity of GLUT-2, thus reducing glucose uptake by beta cells.

#### **1.5.8.2. Studies *in vivo***

Using the hyperinsulinemic euglycaemic clamp technique, Marc van der Valk *et al* [372] found that hepatic glucose production was 47% higher in the PI recipients than in HIV-negative control subjects. In addition, insulin induced suppression of glucose production was less in the PI group than in controls. Similarly, M Noor *et al* [330] found that hepatic glucose production in healthy volunteers (both gluconeogenesis and glycogenolysis ) increased within four weeks of starting indinavir. Recently Noor *et al* [329] using hyperinsulinemic euglycaemic clamp technique has shown that HIV patients taking lopinavir had increased IR, as evidenced by increased glucose production and reduced rate of glucose disposal. The effect was not found in atazanavir users. However, Jackson *et al* [373] using same technique in HIV patients did not find any difference in IR between atazanavir and saquinavir, after 4 weeks of therapy.

### **1.5.9 Insulin Resistance and Fat distribution**

Much remains to be learned about the relationship between blood glucose abnormalities and fat redistribution and lipid abnormalities in HIV patients. It is not clear whether all of these result from a common pathogenic mechanism.

Several studies have shown that among patients taking PIs, insulin resistance was more common in those with body shape changes, either abdominal obesity or peripheral fat loss. Carr *et al* [374] further reported that patients with "buffalo hump" (accumulation of fat at the back of the neck) were at higher risk for insulin resistance and diabetes, although other studies have yielded conflicting results [244, 269, 374, 375].

Similarly, Hadigan *et al* from Boston [376] found that among HIV-positive people in the Framingham Offspring Study (a large study of cardiovascular risk), those with body fat changes were more likely to have impaired glucose tolerance (IGT) and frank diabetes mellitus (DM). The IGT was much higher than DM (32% and 9% respectively). In another study, [377] insulin levels were most elevated in HIV-positive women with abdominal fat accumulation, independent of PI use. The same study group also reported insulin resistance in men with AIDS-related wasting syndrome who were treated with NRTIs but not PIs, and noted that reduced lean body mass and increased abdominal fat were the primary predictors of hyperinsulinemia [319].

Peripheral fat loss leads to increased insulin resistance [378]. Vigouroux *et al* [379] found that among study participants receiving PIs, 11 out of 14 (79%) with severe facial wasting had either insulin resistance or

diabetes, compared with just four out of 20 (20%) without facial fat loss. In this study, elevated triglycerides were also more common in the group with facial wasting and insulin resistance was greatest in those with fat loss. Similarly, Andersen *et al* [369] from Copenhagen reported that loss of limb fat was the strongest predictor of insulin resistance and decreased insulin production, independent of the type of antiretroviral therapy used.

Other studies indicate that blood glucose abnormalities are not directly caused by body fat changes or dyslipidaemia. Mulligan *et al* [325], for example, found that blood glucose abnormalities developed just a few months after people began taking PIs, well before body shape changes occurred. Saint-Marc *et al* [380] reported that when individuals with peripheral fat loss switched from d4T (which is strongly associated with lipoatrophy) to either abacavir or AZT, they experienced increased subcutaneous fat but no improvement in insulin resistance. In contrast substitution d4T with abacavir (ABC) in ABCDE study [114] showed improvement in insulin resistance in the ABC group.

There are not yet enough data to establish the exact relationship between blood glucose abnormalities, fat redistribution and lipid abnormalities in HIV patients. It is not clear whether all of these results from a common pathogenic mechanism, but a link between all these changes are possible. The fat cells may play an important role.

## **Section 6: Fat cells as secretory organs: adipocytokines**

### **1.6.1. Overview**

It is now recognized that adipose tissue produces multiple bioactive peptides, termed adipokines or adipocytokines, which not only influence adipocyte function but also affect more than one metabolic pathway through the bloodstream [381-385]. So far, many adipokines have been identified (figure 1.13). They all integrate in a communication network with other tissues and organs such as the skeletal muscle, adrenal cortex, brain and sympathetic nervous system and participate in appetite and energy balance, immunity, insulin sensitivity, angiogenesis, blood pressure, lipid metabolism and haemostasis [384]. Role of different adipocytokines, proinflammatory and inflammatory cytokines in lipid disorders, fat redistribution and insulin resistance in HIV infection have been described in different studies (table 1.11).



Table 1.11: Overview of key studies describing role of adiponectin and other cytokines in HIV infection

PAI: plasminogen activator inhibitor-1; sTNFR1: soluble tumour necrosis factor-alpha receptor 1

Study	Number	Design	Changes
Reeds <i>et al</i> [386]	30	Cross-sectional	Low adiponectin and high IL-6 correlated with insulin resistance
Chaparro <i>et al</i> [253].	34	Cross-sectional	PI exposure reduces adiponectin gene expression
Addy <i>et al</i> [387].	112	Cross-sectional	Low adiponectin correlated with raised TG, insulin resistance and visceral fat, but negatively with peripheral fat loss and low HDL
Carr <i>et al</i> [327],	140	Prospective	Adiponectin increased more with higher dose of RTV
Kosmiski <i>et al</i> [388]	1429	Cross-sectional	Adiponectin positively correlated with leg fat, but not arm fat
Jones <i>et al</i> [389]. 2006	64	Cross-sectional	Adiponectin correlated with peripheral fat loss and more common in patients receiving d4T and AZT
Vigouroux <i>et al</i> [390]. 2003	131	Cross-sectional	Insulin sensitivity correlated with adiponectin and negatively with leptin and IL-6
Dzwonek <i>et al</i> [391].	104	Prospective	No association of serum leptin with lipodystrophy
Verkauskiene <i>et al</i> [392].	130	Cross-sectional	Lypodystrophy associated with serum adiponectin but not with leptin
Wunder <i>et al</i> [393].	97	Case-control	No association of lipoatrophy with serum leptin levels
He <i>et al</i> [394].	36	Cross-sectional	Visceral fat gain associated with increased plasma TNF-alfa, sTNFR1 and PAI-1

### **1.6.2. Adiponectin**

Adiponectin is almost exclusively expressed in white adipose tissue [395]. Adiponectin sensitises peripheral tissues to insulin [396, 397]. Adiponectin appears to be the only adipose-specific protein known to date that is negatively regulated in obesity. In a group of normal weight and obese women plasma adiponectin was negatively correlated not only with the body mass index and body fat mass, but also with fasting insulin concentration and calculated insulin resistance [398].

Adiponectin activates adenosine monophosphate kinase (AMPK) in skeletal muscles and the liver, increasing fatty-acid oxidation and reducing hepatic glucose production [399]. Two receptors for adiponectin, Adipo R1 and Adipo R2 have been cloned and are expressed predominantly in muscles and liver [400].

In a large number of non diabetic women with dyslipidaemia, Matsubara and others [401] have shown that plasma adiponectin is negatively correlated with serum triglyceride, atherogenic index, apo-B or apo-E and positively correlated with serum HDL cholesterol or apo-A1 levels. This data suggests that existence of a negative feedback mechanism between adipose mass and production of adiponectin in humans is possible.

Adiponectin also has antiatherogenic properties, as shown *in vitro* by its inhibition of monocyte adhesion to endothelial cells and macrophage transformation to foam cells [402, 403]. High plasma adiponectin was associated with reduction of risk of myocardial infarction (MI) in men [404].

A relationship between low circulating levels of adiponectin and lipodystrophy in HIV patients has been described [387, 405]. Use of

stavudine was associated with low adiponectin level in plasma, and increased insulin resistance compared to abacavir [114]. But a relationship of adiponectin with lipid metabolism in HIV patients has not been delineated, especially in the absence lipoatrophy.

Figure 1.13: The adipocyte as a secretary organ [406]

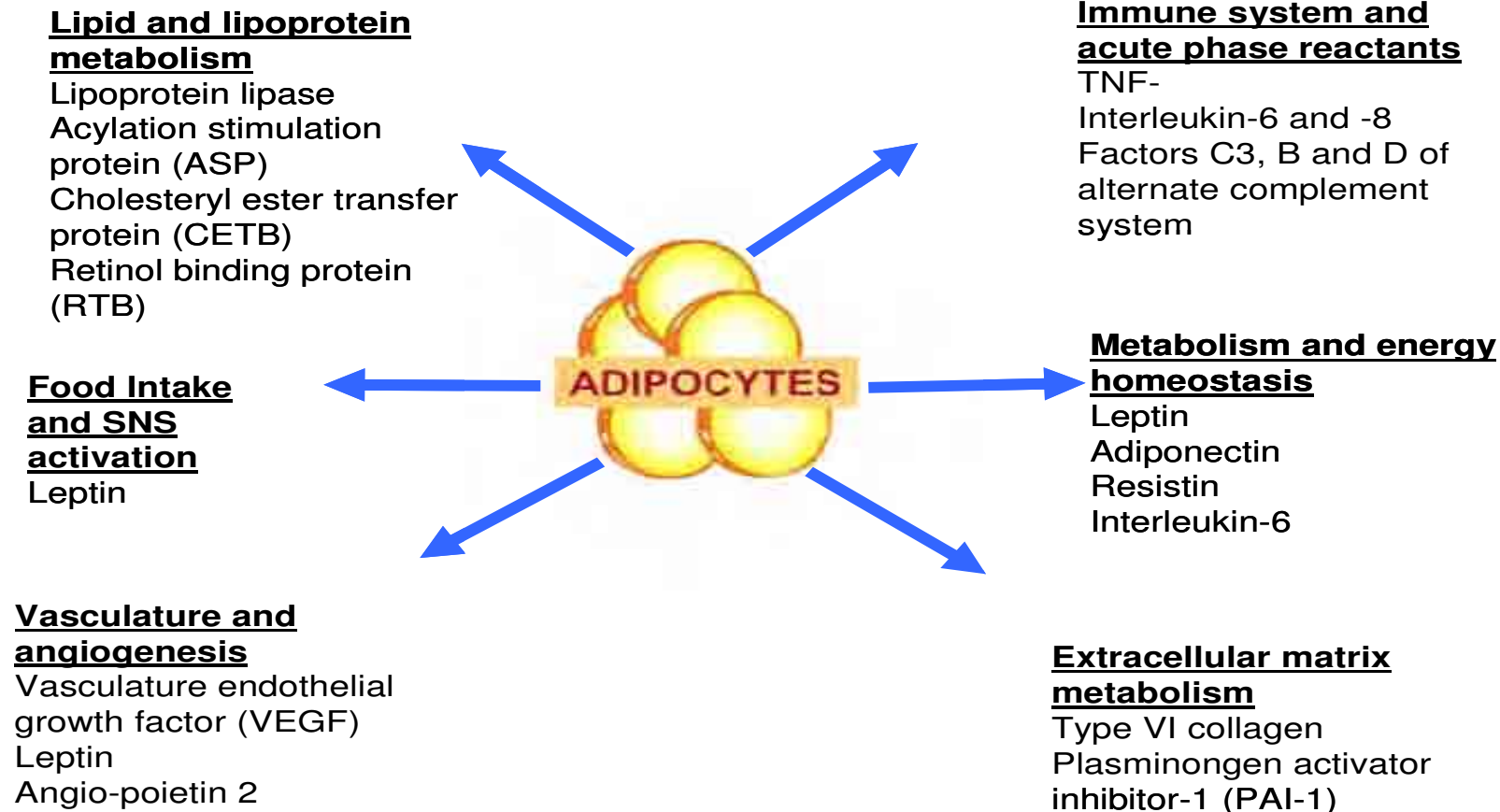
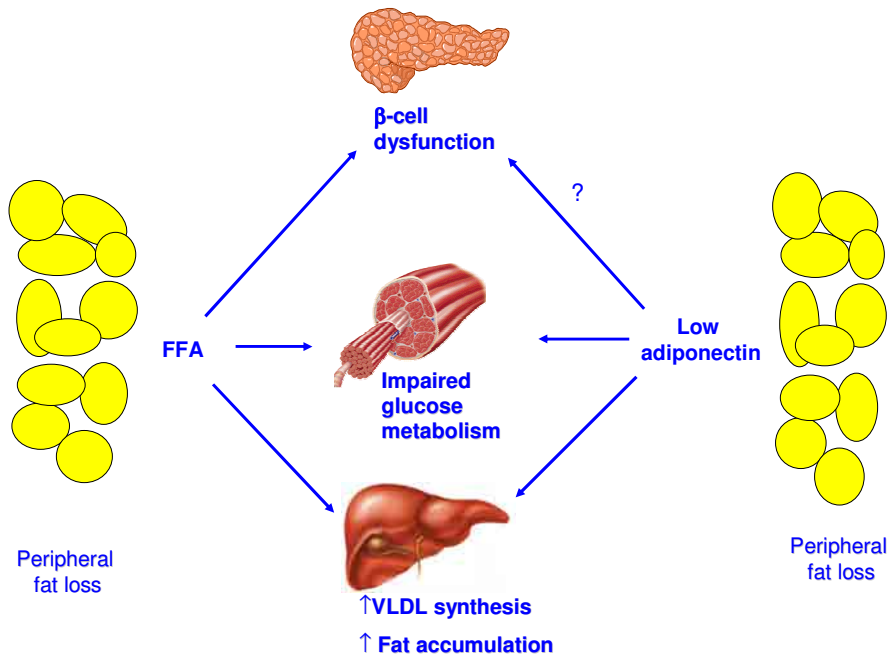


Figure 1.14: Peripheral fat loss leads to increased insulin resistance (adapted from Van Wijk *et al* [378]).



### 1.6.3. Leptin

Leptin is perhaps the most intensively studied adipocytokine with reported effects on food intake [407, 408], energy expenditure, and lipid metabolism, particularly in rodents [409]. In humans, adipocytes secrete leptin in direct proportion to the amount of adipose mass, and this secretion is greater in subcutaneous tissue relative to visceral adipose stores [410]. There is strong evidence showing that the dominant action of leptin is to act as a 'starvation signal'. Leptin declines rapidly during fasting. In patients with lipodystrophy and leptin deficiency, leptin replacement therapy improved glycemic control and decreased triglyceride levels [411, 412].

### **1.11.3. Tumour necrosis factor (TNF)- $\alpha$**

TNF- $\alpha$  is a cytokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction. It is produced mainly by macrophages, but they are produced also by a broad variety of other cell types including adipose tissue [413]. TNF- $\alpha$  has been shown by a number of authors to be over expressed in patients with lipodystrophy [250, 252], as well as correlating with markers of insulin resistance and hyperlipidemia [250] in patients with subcutaneous peripheral wasting. Patients who stop taking PIs have been shown to have a significant fall in circulating plasma TNF- $\alpha$ , perhaps indicating a pathogenic role for this class of antiretrovirals [414]. Similarly, both animal and human studies have indicated that TNF- $\alpha$  can inhibit adipocyte differentiation[415], modulate lipid metabolism [416] and enhance plasminogen activator inhibitor levels [417, 418], suggesting important effects in patients with lipodystrophy [419] and insulin resistance.

Two cell surface TNF- $\alpha$  receptors were described in humans, TNF-R1 and TNF-R2. Soluble forms of those receptors (sTNF-R1 and sTNF-R2) are present in plasma and it is supposed that their concentrations might reflect local action of TNF- $\alpha$  in tissues [420, 421]. Soluble tumour necrosis factor receptors (sTNF-Rs) play a role as modulators of the biological function of TNF- $\alpha$  in an agonist/antagonist pattern. It is proposed that soluble TNF receptors, particularly sTNF-R2 might serve as the best predictor of local TNF- $\alpha$  system activity [421]. In various pathologic states the production and release of sTNF-Rs may mediate host response and

determine the course and outcome of disease by interacting with TNF- $\alpha$  and competing with cell surface receptors. The determination of sTNF-Rs in body fluids such as plasma or serum has been showing high accuracy in the follow-up and prognosis of various diseases. Endogenous sTNF-Rs concentrations appear to reflect the activation state of the TNF- $\alpha$  /TNF receptor system [422].

### **1.6.4. Interleukin-6 (IL-6)**

Interleukin-6 (IL-6) is reported to have multiple effects ranging from inflammation to host defence and tissue injury [423]. It is secreted by adipose tissue and many other cell types, including immune cells, fibroblasts, endothelial cells and skeletal muscle and only about 10% of the total IL-6 appears to be produced exclusively by fat cells. Secretion of IL-6 varies from different sites, omental fat producing threefold more IL-6 than subcutaneous adipose tissue [424]. Plasma IL-6 concentrations correlate positively with human obesity and insulin resistance, and high IL-6 levels are predictive of type 2 diabetes. IL-6 is related to insulin resistance in patients with high-grade inflammation. An association with increased incidence of CVD has been observed in HIV patients when antiretroviral treatment was interrupted [66]. A positive correlation was found with viral replication and IL-6 level [66]. Whether IL-6 plays any definite role in lipid metabolism in HIV patients has not been established.

### **1.6.5. Interleukin-8 (IL- 8)**

Interleukin-8 is produced and released from human subcutaneous and visceral adipose tissue, and subcutaneous adipose tissue obtained from

HIV-infected people with fat redistribution and insulin resistance [425]. It is a member of the chemokine super family [426], enhancing vascular smooth muscle cell proliferation, monocyte adhesion to endothelial cells, and is believed to have pro-atherogenic properties[427]. Oxidized low-density lipoprotein, which is well described in the atherosclerotic process, stimulates the release of IL-8 from macrophages in the atherosclerotic lesion[428]. IL-8 has, besides its implications for atherosclerosis, mostly been known for its association with different inflammatory processes. Higher serum IL-8 levels was found in HIV patients with impaired glucose tolerance [429], and this is consistent with findings in type 2 diabetes mellitus [430], obesity and non-alcoholic fatty liver disease [431]. It is possible that the chronic inflammatory state in HIV-infection can be associated with elevated fasting serum level of IL-8. Higher serum IL-8 may also reflect increased production and release from the larger adipose depots and may have influence on lipid metabolism along with its effect on insulin resistance. The level of IL-8 in blood might change with HAART and the effect in lipid metabolism and insulin resistance might change as well.

### **1.6.6. Other adipose secretory proteins**

A new adipocytokine termed resistin was identified in 2001 and was found to play an important link between insulin resistance and obesity in rodent models[432]. In humans, however, studies investigating the link between resistin and metabolic disease are conflicting. Resistin in human is produced mainly by macrophages rather than adipocytes and has shown to have strong association with inflammation and inflammatory states[433, 434]. Patients with HIV infection, resistin levels decreased after use of rosiglitazone, but correlation between resistin level and insulin



resistance or inflammatory markers were found[435]. However, further study failed to show any significant difference between mean resistin levels of HIV subjects with and without lipodystrophy[436].

Plasminogen activator inhibitor (PAI-1) is another adipocytokine produced mostly by the adipocyte. PAI-1 is a key factor in the regulation of fibrinolysis [437]. Both visceral and subcutaneous adipose tissue produce PAI-1, but data are controversial as to whether visceral adipose tissue produces more, similar amounts of, or less [438-440] PAI-1 than subcutaneous adipose tissue.

## **Chapter 2**

### **Study Rationale**

Antiretroviral treatment of HIV infection is associated with disturbances in body fat distribution[180, 224, 441], dyslipidaemia [150, 180, 224] , insulin resistance [150] and diabetes [314, 318]. The exact mechanisms of these changes have not been fully understood. At least three different processes may contribute: the effect of HIV infection, the effects of some antiretroviral drugs and the chronic effects of antiretroviral therapy on regional fat distribution.

HIV infection itself has been reported to increase triglyceride levels [109, 132], impair triglyceride metabolism and lipoprotein lipase activity [132]. HIV-infected patients also exhibit reduced plasma cholesterol, low density lipoprotein (LDL) cholesterol and apolipoprotein-B 100 [442]. Additionally they have reduced high density lipoprotein (HDL) and apo-A1 associated with this lipoprotein [132], and higher total cholesterol/HDL cholesterol ratio [109]. The changes may be due to direct effect of the virus or may be driven by different cytokines generated by the infection.

Treatment with HIV protease inhibitors for very short periods causes hypertriglyceridaemia and impaired insulin sensitivity [363] even in HIV negative subjects [194]. Antiretrovirals of nucleoside reverse transcriptase inhibitor (NRTI) group cause dyslipidaemia as well [55]. The non-nucleoside reverse transcriptase (NNRTI) affect lipid metabolism [55] but the changes are different from others.

The fat redistribution, which only becomes apparent after several months on treatment, is associated with dyslipidaemia and insulin resistance [264,

443] though dyslipidaemia may occur in the absence of obvious lipodystrophy [326]. Both the nucleoside reverse transcriptase inhibitor (NRTI) and the protease inhibitor (PI) component of highly active antiretroviral therapy (HAART) contribute in an as yet undefined way to the lipid abnormalities and body fat distribution. The non-nucleoside reverse transcriptase inhibitor (NNRTI) component of HAART may also contribute to the dyslipidaemia [55, 172] though its relation to the lipodystrophy is unclear.

Fat redistribution with loss or accumulation of excess fat in different regions can be associated with changes in adipocytokines. The adipocytokines also play key roles in glucose and fat metabolism.

### **Hypothesis**

Lipoprotein metabolism takes place in a number of physiological compartments and serum levels are a function of two opposing processes of either synthesis or catabolism.

We postulate that lipoprotein catabolism and possibly synthesis may differ in patients receiving PIs compared to those taking NNRTIs and perhaps also between different NRTIs.

We also postulate that loss of peripheral fat in HIV patients would result in decreased plasma adipocytokines, in particular adiponectin, which would be associated with changes in VLDL, IDL and LDL apolipoprotein-B (apo-B) kinetics.

**Our key aim is :**

1. To investigate the relationship of apo-B metabolism with dyslipidaemia.
2. To investigate the relationship between apo-B metabolism and fat distribution.
3. To investigate the relationship of insulin resistance with fat distribution and lipid changes.
4. To investigate the relationship of adiponectin and other cytokines with apo-B metabolism, lipid changes, insulin resistance and body fat distribution.

## CHAPTER 3

### **Apolipoprotein-B Kinetics in HIV infection**

#### **3.1. Introduction**

With the advent of highly active antiretroviral therapy (HAART) the spectrum of HIV illness has changed remarkably. There has been dramatic improvement in the survival rate amongst HIV patients [36]. However, metabolic abnormalities including dyslipidaemia in the form of high triglycerides (TG), high total cholesterol (TC), low density lipoprotein (LDL) -cholesterol and low high density lipoprotein (HDL) -cholesterol have been observed more frequently [55, 180]. The mechanisms responsible for the changes in different lipoprotein fractions in HIV infection and with different treatment regimes are not clear.

Dyslipidaemia in HIV patients can directly stem from the viral infection. Several studies in early 1990s [109, 132] have shown dyslipidaemia in treatment naïve HIV patients. Hypertriglyceridaemia due to increased very low density lipoproteins (VLDL) has been reported in bacterial, viral and other infections [444-446]. Decrease in plasma cholesterol levels have also been reported during infection ([447, 448]4-8). Infections other than HIV can increase plasma triglyceride (TG) levels by decreasing the clearance of circulating lipoproteins, a process thought to be the result of reduced lipoprotein lipase (LPL), or by stimulating hepatic lipid synthesis through increases in either hepatic fatty acid synthesis or reesterification of fatty acids derived from lipolysis [131]. Patients with HIV infection also exhibited reduced plasma cholesterol, low density lipoprotein (LDL)

cholesterol, apolipoprotein-B 100 (apo-B), reduced high density lipoprotein (HDL) and apolipoprotein-A1 (apo-A1) [109]. The Multicenter AIDS Cohort Study (MACS) found that lipids were in the normal range in men prior to infection but TC, LDL-C, and HDL-C declined on seroconversion [110].

Lipid disorders are more common with antiretrovirals especially with PI use. Treatment with ritonavir for very short periods causes hypertriglyceridaemia [363]. Both nucleoside reverse transcriptase inhibitor (NRTI), particularly thymidine analogues and the protease inhibitor (PI) component of highly active antiretroviral therapy (HAART) contribute to the lipid abnormalities and both drugs can cause abnormalities of body fat distribution. The underlying mechanism is yet to be clearly defined. The non-nucleoside reverse transcriptase inhibitor (NNRTI) component of HAART may also cause lipid abnormalities though its relation to body fat redistribution is not clear.

Kinetic studies [103, 449] performed to explore VLDL-TG metabolism in type 2 diabetes mellitus patients, have demonstrated an increased VLDL-TG synthesis and decreased VLDL-TG fractional catabolic rate. Both contributed to high plasma TG level in type-2 diabetic patients. However, conflicting results have been described about metabolism of apo-B containing lipoproteins; either increased synthesis or decreased catabolism or both have been described in different studies in HIV patients [128, 130, 152, 450]. Increased synthesis of apo-B containing lipoproteins has been described in a small number of HIV patients who have significant dyslipidaemia [130]. In five patients with ART induced high TG and

moderate TC level, a stable isotope tracer kinetic analysis revealed reduced transfer rates of VLDL in to denser lipoproteins suggesting a lower activity of lipoprotein lipase delipidation activity. The same study in addition showed increased synthesis of apo-B. The rate of apo-B transfer from more TG rich larger VLDL (VLDL 1) to smaller VLDL (VLDL 2) was also reduced. It was noteworthy that all the five patients had significant insulin resistance. The discrepancies observed between different results concerning VLDL apo-B metabolism in HIV patients may be due to differences in patient characteristics and the small numbers of the study population [130]. Very little information has been reported on IDL apo-B and particularly LDL apo-B metabolism, while LDL apo-B plays an important role in lipid metabolism and atherogenesis.

This study investigated the effect of established antiretroviral therapy, for at least one year (1-6 years), on very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and low density lipoprotein (LDL) kinetics. We performed a cross sectional stable isotope tracer kinetic study in a large number of subjects including HIV-negative controls, HIV-infected patients naïve to antiretroviral therapy and patients taking HAART including PI, or the NNRTI efavirenz or nevirapine.

### **3.2. Objective**

To explore whether the changes in lipid metabolism in HIV patients is related to modification of synthesis or catabolism or both in different apo-B containing lipoprotein fractions.

### **3.3. Subjects and Methods**

#### **3.3.1. Subjects**

Subjects were 67 individuals which included 41 cases and 26 controls (table 3.1). Cases were HIV patients recruited from the HIV clinic in University Hospital Birmingham at Selly Oak hospital.

#### **3.3.2. Cases.**

Cases were HIV infected patients who were taking two nucleoside analogues plus either a protease inhibitor (PI, n=15), or the non-nucleoside reverse transcriptase inhibitors nevirapine (NVP, n=11), or efavirenz (EFV, n=14) for between 1 – 6 years. Patients on PI were taking nelfinavir (n= 6) lopinavir/ritonavir (n=3), ritonavir alone (n=2) and with indinavir (n= 2) or saquinavir (n=1) or indinavir alone (n=1). The most common nucleoside analogues were zidovudine (AZT) and lamivudine (3TC) used in 28 patients (66.7%, table 3.2). Twenty nine (69%) patients were taking AZT containing and 10 (24%) were taking stavudine (d4T) containing regimens. Seven patients (16.7%) had previously taken another antiretroviral regimen for a median of 17.2 months (range 5-44) but had been on the current treatment for a median of 42.9 months (range 14-71) at the time of the investigation.

#### **3.3.3. Controls**

Controls were fifteen HIV infected patients naive to antiretroviral therapy (TN) and twelve presumed HIV-negative individuals who were mostly from medical students or healthcare professionals in attachment or working in



the University Hospital Birmingham. A negative HIV test within the last three months was required if history revealed a risk of HIV acquisition. Two subjects were identified who subsequently had the HIV antibody test and the results were negative. In the others an HIV antibody test was not done as unlinked anonymous HIV tests in patients attending sexually transmitted disease (STD) clinic in Birmingham between 1994-2001 showed HIV rates ranging from 0.04 - 0.25% in those without identifiable risk factors and there was no identifiable trend. The results were similar to those obtained nationally, excluding London and Scotland, in 2002 of 0.2 % in UK born heterosexual females [451].

#### **3.3.4. Medical history**

The following history was obtained: history of cardiovascular disease, history of past medical illness and diabetes in first degree relatives, smoking, alcohol intake, use of other medication. All patients had a thyroid function test performed.

#### **3.3.5. Exclusion criteria**

The exclusion criteria were fasting glucose more than 6.0 mmol/liter, hypothyroidism, serum creatinine more than 150 mmol/liter, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), gamma glutamyle transferase (GGT) more than five times upper limit of normal, clinical anaemia or haemoglobin less than 10 gm% in females and 12 gm% in males, and more than 10% loss in body weight in the last 6 months and history of AIDS defining illness in last 3 months, use of glucocorticoids, or any other drugs that are known to affect lipid metabolism. A minimum of 6 weeks of wash out period was allowed in

case any patient was on lipid lowering treatment and patients were advised to restart lipid lowering treatment the day after the study was performed.

### **3.4. Methods and Materials**

#### **3.4.1. Study design**

This is a cross-sectional case control, collaborative study between the Department of HIV Medicine, University Hospital Birmingham and Department of Diabetes and Endocrinology, St Thomas Hospital, London. The patients and all other study participants were recruited by Dr Satyajit Das (SD) and Dr Mohsen Shahmanesh (MS) and consent was obtained by SD on the day of the test. The Study protocol was written jointly by SD and MS. Samples were collected by SD and the nursing staffs of Welcome Research Facility, University Hospital Birmingham (UHB). The laboratory work was performed in the biochemistry laboratory, St Thomas Hospital, London, under the supervision of Dr Margo Umpleby. All the data were collected and analysed by SD and final analysis was reviewed by MS and Mr peter Nightingale, statistician from Welcome Research facility, UHB.

#### **3.4.2. Ethics committee approval and patient consent**

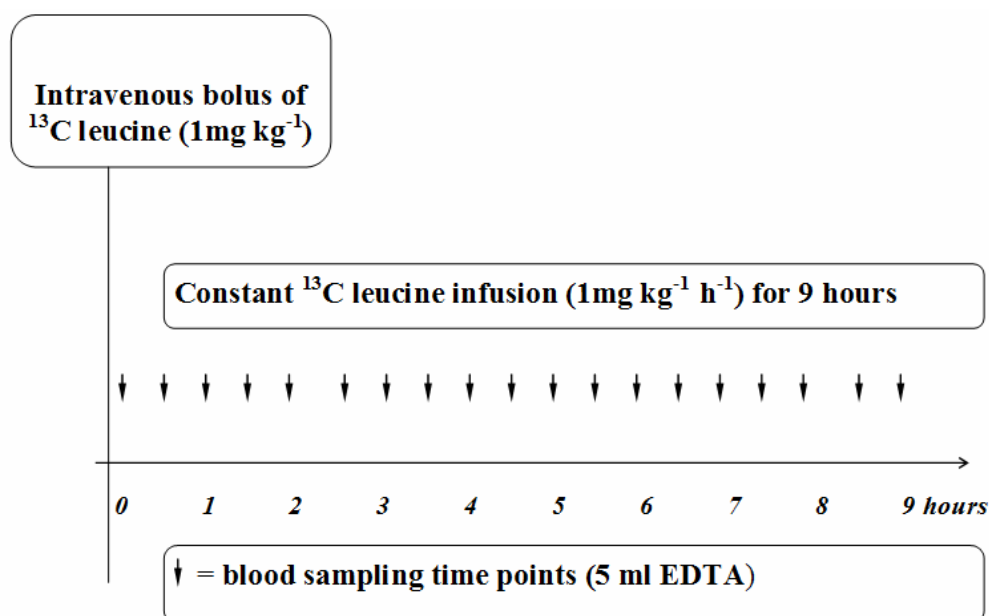
Ethical approval was obtained from South Birmingham Local Research Ethics Committee. All subjects have been given written information about the study and all subjects gave written informed consent before taking part into the study.

### **3.4.3. Statistical analyses**

Initial comparison between the four groups was by one-way ANOVA or Kruskal-Wallis followed by Bonferroni's or Dunn's multiple comparison test (SPSS 10.0.7 for Windows; SPSS Inc, Chicago, IL, USA). Fisher's exact test was used for categorical data between groups, and associations were analyzed by Spearman's rank correlation test. A stepwise linear regression model examined the effect of variables on VLDL and IDL and LDL metabolism. Variables entered were age, sex, ethnicity, smoking, family history of diabetes, family history of cardiovascular disease, alcohol intake, peripheral fat/ LBM, trunk fat/LBM, glucose, pre-treatment and current CD-4, pre-treatment HIV RNA copies amongst HIV positive groups, baeline HOMA, and FFA. In a separate model, only patients on treatment were included. Assuming a 50% difference between any of the patient groups and the controls the study has an 80% power to detect a significant difference at the 5% level if 14 patients are recruited for each group[130].

**3.4.4. Study protocol** (figure 3.1):

Figure 3.1: Clinical protocol for sample collection in the Wellcome trust, Queen Elizabeth Hospital, Birmingham, UK



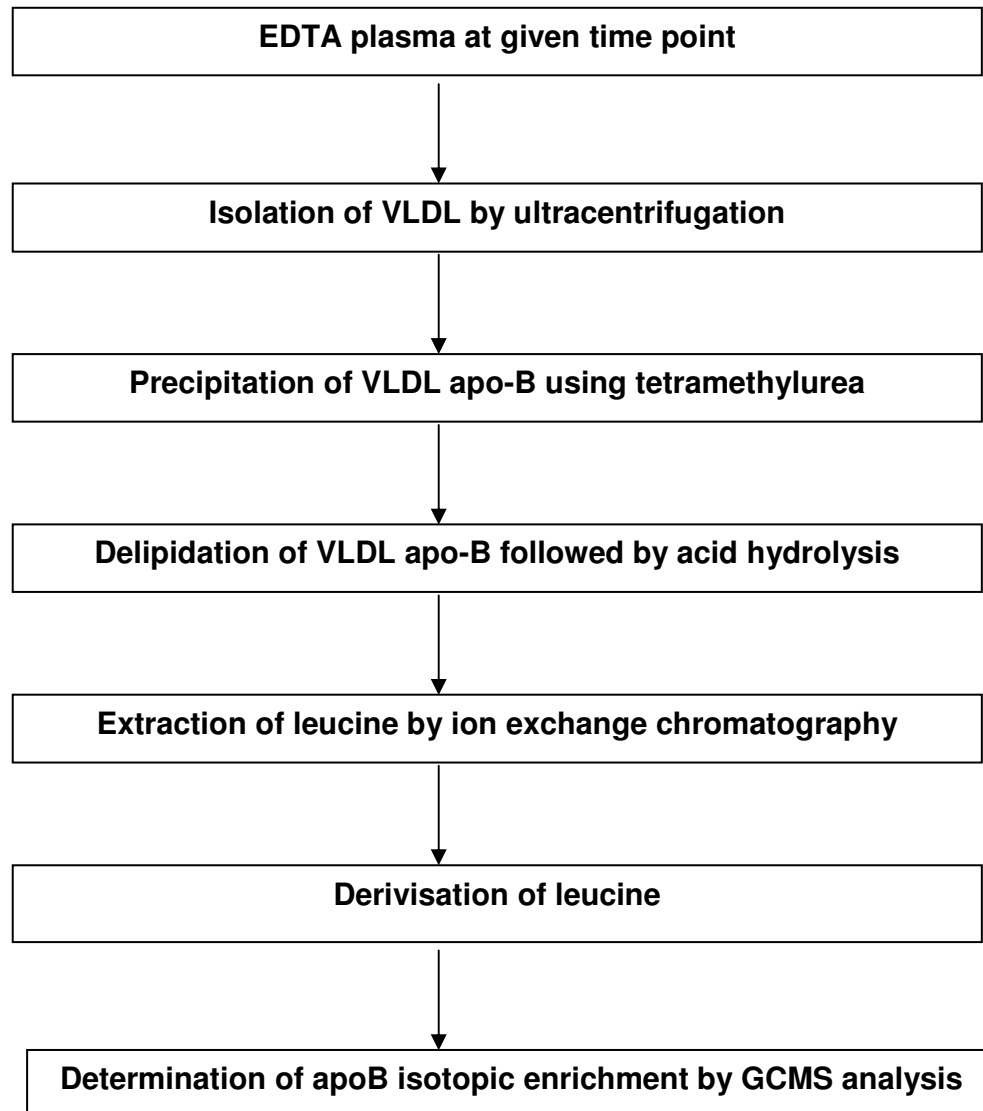
After an overnight fast, subjects were admitted to the Wellcome Trust Clinical Research Facility, Queen Elizabeth Hospital, Birmingham. An intravenous line was placed in the antecubital vein of each arm; one line was used for administration of the isotopic solution and the other for drawing blood samples. An infusion of  $^{13}\text{C}$  leucine ( $^{13}\text{C}$  enrichment 99% purity, Cambridge Isotope Laboratory, MA) was administered as a primed ( $1 \text{ mg/kg}$ ) constant infusion ( $1\text{mg/kg h}^{-1}$ ) for 9 hours. At the beginning of the study, 10ml plasma was collected for measurement of total cholesterol (TC), triglycerides (TG), high density lipoprotein-cholesterol (HDL-C), free fatty acids (FFA) and glucose. Five ml of ethylenediamine tetraacetate (EDTA) plasma samples were collected for apoB-100 enrichment determination at minus 0.5, 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6,

7, 8 and 9 hours. Two ml lithium heparin samples were also taken at baseline and after 3, 5, 7 and 9 hours to determine  $^{13}\text{C}$  enrichment of  $\alpha$ -ketoisocaproate ( $\alpha$ -KIC), the deamination product of leucine, which provides a measure of intracellular leucine enrichment. In addition, 5ml EDTA plasma was collected for measurement of very low density lipoprotein (VLDL), low density lipoprotein (LDL) and intermediate density lipoprotein (IDL) apolipoprotein-B (apo-B) concentrations and VLDL-TG, VLDL-C, IDL-TG, IDL-C, LDL-TG and LDL-C concentrations at 0,3,6, and 9 hours. Samples were sent by courier, (kept at 4°C for no more than 24 hours), to St Thomas' Hospital, London, UK.

#### **3.4.5. Experimental protocol**

The laboratory analysis was performed in the biochemistry laboratory, St Thomas' Hospital, London, UK. The laboratory was blinded as to patient categories. A summary of the experimental steps has been shown in the figure 3.2.

Figure 3.2: Summary of analytical steps (GCMS: gas chromatography and mass spectrometry)



#### **3.4.5.1. Isolation and measurement of isotopic enrichment of apolipoprotein-B**

VLDL and IDL were separated by sequential floatation ultracentrifugation (Beckman Coulter Optima LE80-K ultracentrifuge, High Wycombe, UK). Plasma (overlaid with sodium chloride density solution, density = 1.006 kg/L) was centrifuged at 37,000 rpm for 16 h in a 50.4 Ti Beckman rotor to obtain VLDL. The VLDL fraction was isolated by tube slicing and the density of the remaining supernatant was raised to 1.019 kg/L. This was centrifuged at 37,000 rpm for 18 h to isolate IDL. The LDL fraction was isolated by adjusting plasma density to 1.063 g/ml and spinning for another 20 hours on a Beckman Coulter Optima LE80-K ultracentrifuge (High Wycombe, UK). The apolipoprotein B-100 was isolated by preparative sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE): the delipidated apo-B containing material was solubilized in 0.05 mmol/L Tris buffer (pH 8.6) containing 3% SDS, 3% mercaptoethanol and 10% glycerol and applied to a 3-mm thick vertical slab gel (3% acrylamide). After staining with Coomassie blue R-250, apo-B was cut from the gel and hydrolysed in 6M HCL for 24 hour at 115°C . Samples were then centrifuged to remove polyacrylamide. Supernatants were lyophilized in a Speed Vac (Sevant Instrument, Farmingdale, NY). Lyophilized samples were dissolved in 50% acetic acid, applied on AC-50 W-X8 200-400-mesh cation exchange column (Bio-rad, Richmond, CA), and amino acids were recovered by elution with 4 M NH<sub>4</sub>OH and lyophilized.

Leucine enrichment of VLDL, IDL and LDL apoB-100 was measured by gas chromatography-mass spectrometry (GC-MS, Hewlett Packard 5890 series II-5917A MSD, Bracknell, UK) in electron impact ionization mode, monitoring ions 302 and 303 representing the [m-57] natural abundance and [m-57]+1 enriched fragments of the bis (tert-butyldimethylsilyl) derivative. Plasma  $\alpha$ -KIC  $^{13}\text{C}$  enrichment was measured as the quinoxalinol-trimethylsilyl derivative by monitoring of fragment ions at m/z 259 and 260.

### **3.4.5.2. Quantification of apo-B and other anylates.**

The apoB-100 VLDL, IDL and LDL concentrations were determined by an in-house specific sandwich Elisa using a polyclonal antibody against apo-B (The Binding Site Ltd, Birmingham, UK) as a capture antibody and a biotinylated anti apoB-100, 4G3 antibody (Ottawa Heart Institute, Ottawa, Canada) as a detection antibody. Plasma total, VLDL, IDL and LDL cholesterol and triglyceride were measured enzymatically (ABX Diagnostics, Shefford, UK). HDL-C was separated by precipitation of apo-B-containing lipoproteins with dextran sulfate/magnesium chloride and measured enzymatically (Boehringer Mannheim, Mannheim, Germany) after magnesium phosphotungstate precipitations for HDL-C.

Enzymatic methods were used to measure serum TC, LDL-C and TG (ABX Diagnostics, Shefford, UK) and HDL-C (Roche Diagnostics Ltd, Lewes, UK). Serum FFA was measured enzymatically (Wako Chemicals, Neuss, Germany) and Lp(a) with an immunoturbidimetric method (Diasorin Ltd., Wokingham, UK) using a Cobas Fara II analyzer (Roche Diagnostics). Serum insulin was measured by ELISA (Mercodia, Uppsala,



Sweden) and glucose concentrations using a glucose analyzer (Roche Diagnostics).

#### **3.4.5.3. Calculation of VLDL, IDL and LDL apo-B secretion and clearance rates:**

A multi-compartmental model was used to calculate VLDL, IDL and LDL fractional catabolic rate (FCR pools/day). The fractional catabolic rate (FCR)[103] and absolute secretion rate (ASR)[452] of each lipoprotein were estimated using a multicompartmental model with an intrahepatic delay function as previously described by Duvillard *et al* [103] using Simulation Analysis and Modelling (SAAM II software, SAAM Institute, Seattle, WA). The precursor compartment for the incorporation of  $^{13}\text{C}$  leucine into VLDL (forcing function) is the steady state tracer: tracee ratio of  $\alpha\text{KIC}$ . The absolute secretion (production) rate (ASR mg/kg/day) was then calculated from the FCR and apo B pool size.

Patients were in a steady state in the study as shown by the constant VLDL, IDL and LDL apoB concentration (data not shown here). In this case, the fractional secretion rate (FSR) equals the fractional catabolic rate (FCR). The VLDL, IDL and LDL apoB ASR (mg/kg/day) were calculated from the product of the fractional secretion rate (pools/d) and the pool size (mg) divided by body weight. The VLDL or IDL or LDL pool size was calculated from the product of the mean VLDL or IDL or LDL apoB concentration (mean concentration of apoB in four pooled samples) and the plasma volume.

Plasma volume was calculated using the formula of Pearson *et al* [453].

As the experiment was performed in the steady state, fractional synthetic rate equalled fractional catabolic rate [454-456].

Because of problems with the sample analysis, it was not possible to model IDL data from one subject in the TN group, two subjects in the PI group, and five subjects in the NNRTI group.

### **3.5. Results**

#### **3.5.1. Patient Characteristics (table 3.1)**

Most of the subjects were male (n=50) and the distribution was similar in each group, except the HIV negative control group (table 3.1). Forty-one were homosexual and two were in the HIV-negative control group. Most of the subjects (n=49) were of white ethnic origin. The median age of the subjects was 36 (21-67) years. There was no significant difference between groups with regard to age and ethnicity and gender. Daily alcohol intake in HIV negative group was higher but not significant statistically.

Smoking history was similar between all the groups.

Family histories of ischemic heart disease and strokes were present in 17 subjects but this was not different between groups (table 3.1). A family history of diabetes was present in 6 subjects and was higher in the treatment naïve group compared to HIV negative controls (table 3.1).

Table 3.1: Baseline characteristics (mean+/-SD) of subjects (n= 67)

	Control (n=12)	Cases (n=55)		
		TN (n=15)	PI (n=15)	NNRTI (n=25)
Male sex	5	11	13	21
Homosexual	2	7	11	21
White ethnicity	10	7	13	19
Age (year)	32.8+/-12.2	37.9+/-11	43.6+/-9.3	38.4+/-10.8
BMI Kg/m2	23.0+/-3.6	24.1+/-2.7	24.4+/-3.6	23.1+/-3.1
Family history of CVD	4	3	5	5
Family history of Diabetes	0*	3*	2	1
Smokers	3	5	6	12
Daily alcohol use units/wk	12.3+/-12.9	8.4+/-11	8.8+/-8.7	7.7+/-8.4

\*Controls vs. TN (Fisher's exact test,  $P = 0.012$ ).

### 3.5.2. Treatment History (table 3.2)

The median duration of treatment was 40.5 (12-71) months (table 3.2), and was lower in the NNRTI group than the PI group (31 vs. 49,  $p < 0.001$ ). There was no history of treatment interruption or change of treatment regimen in any of the patients. Four patients who were on lipid lowering agents for dyslipidaemia stopped taking the drugs 6 weeks before they took part in to this study.

The commonest nucleoside backbones used were zidovudine (AZT) and lamivudine (n=27), stavudine (d4T) and lamivudine (3TC) in 6 patients, stavudine (d4T) and didanosine (ddI) in 3 patients and other drugs or combinations used were abacavir (ABC) and lamivudine in 2 patients. Eleven patients were on nevirapine (NVP) and 14 were on efavirenz (EFV) containing regimen. Fifteen patients were on PIs of which 8 were with ritonavir (RTV) either as booster (100mg twice daily) or therapeutic dose (600mg twice daily). The other PIs were nelfinavir (n=4), indinavir (n=3), lopinavir (n=6). None of the patients was on atazanavir or saquinavir.

Table 3.2: Treatment history with HAART (Mean+/-SD)

	Control (n=12)	Cases (n=55)		
		TN (n=15)	PI (n=15)	NNRTI (n=25)
Duration of treatment (months)	-	-	*48.7+/-14.7	*30.9+/-2.9
Nucleoside backbone				
AZT/3TC	-	-	8	19
d4T/3TC	-	-	2	4
d4T/ddI	-	-	3	1
Other	-	-	2	1

AZT=Zidovudine, 3TC= Lamivudine, d4T= Stavudine, ddI=Didanosine

\*PI vs NNRTI ( $p=0.001$ )

**3.5.3. Laboratory Results (table 3.3)**

Liver function tests (LFT) were within normal limits in all the subjects.

Serum alkaline phosphatase was lower in the HIV negative controls but not statistically significant from the other groups. Serum AST, GGT and haemoglobin was similar in all the groups.

None of the subjects had hepatitis C or was a carrier for hepatitis B virus.

Table 3.3: Mean (+/-SD) baseline laboratory Results

	Control (n=12)	Cases (n=55)			p-value between groups
		TN (n=15)	PI (n=15)	NNRTI (n=25)	
Haemoglobin gms%	13.0 (1.7)	13.8 (1.8)	15.0 (1.2)	14.8 (1.4)	NS
AST (mmol/liter)	22 (2.0)	25 (7.0)	24 (6.0)	26 (13.0)	NS
Alk Phos (mmol/liter)	128 (27.0)	179 (68.0)	177 (49.0)	193 (33.0)	NS
GGT mmol/l	28 (23.0)	34 (26.0)	55 (52.0)	44 (25.0)	NS

**3.5.4. Viral load and CD-4 counts (table 3.4)**

Current CD4 counts of the patients were not different between HIV groups (table 3.4), nor were current or pre-treatment viral loads or pre-treatment CD4 counts among the treatment groups. Two PI-treated patients on HAART had a detectable viral load (800 and 1100 copies/ml) at the time of the study. The remaining patients had viral loads below the detection limit of the assay (less than 50 copies/ml).

**Table 3.4: Viral load (VL) and CD-4 counts (mean+/-SD)**

	Control (n=12)	Cases (n=55)		
		TN (n=15)	PI (n=15)	NNRTI (n=25)
Log Pre-treatment VL (copies/ml)	-	4.49+/-0.76	5.12 +/- 0.54	4.83 +/- 0.74
Current VL (copies/ml)	-	Log (5.01+/- 5.1)	Log (2.1+/- 2.5)	Less than 50
Pre-treatment CD-4 count (cells/dl)	NA	367+/-222	125+/-122	175+/-128
Current CD-4 count (cells/dl)	NA	367+/-222	435 +/- 187	469+/- 265

**3.5.5. Lipid Profiles** (Table 3.5)

Only five patients had baseline fasting plasma cholesterol levels above 6.0 mmol/liter (three PI and two NNRTI), and 13 had fasting plasma triglyceride levels over 2.3 mmol/liter (two TN, four PI, and seven NNRTI). Nine patients (six on PI and three on NNRTI) had calculated LDL-cholesterol levels above 3.0mmol/l. Severe hyperlipidaemia (cholesterol > 7 mmol/liter and /or triglyceride > 5 mmol/liter) was observed in only two patients and both of them were on PI.

In the PI group, total cholesterol (table 3.5) and VLDL-cholesterol (table 3.6) was significantly higher than the TN group ( $P < 0.03$ ), and plasma triglyceride (table 3.5,  $p < 0.01$ ), VLDL-TG (table 3.6,  $p < 0.01$ ), IDL triglyceride (table 3.9,  $p < 0.002$ ), were significantly higher than the control group.

Plasma IDL triglyceride was also greater in the TN group than the control group ( $P < 0.05$ , table 3.9). Plasma HDL cholesterol was lower in TN, PI,

and NNRTI group compared with the control group ( $P < 0.01$ , table 3.5).

There was no difference in the lipid profile in the nevirapine- and efavirenz-treated patients in the NNRTI group (table 3.7).

Plasma level of FFA was similar in all the groups (table 3.5), but was higher ( $p < 0.003$ ) in antiretroviral-treated patients with dyslipidemia (cholesterol  $\geq 6$  mmol/liter or triglyceride  $\geq 2.3$  mmol/liter,  $n=13$ ) compared to those without dyslipidaemia ( $n=54$ ).

Table 3.5: Baseline lipids and body composition [median (interquartile range)]

	Control (n=12)	Cases (n=55)			p-value between groups
		TN (n=15)	PI (n=15)	NNRTI (n=25)	
Total cholesterol (mmol/l)	4.6 (4.1–5.2)	4.3 (3.4 –5.1)	<sup>1</sup> 5.6 (4.6–6.0)	4.9 (4.1–5.6)	0.03
Total Triglyceride (mmol/l)	1.0 (0.7–1.5)	1.1 (0.9 –1.6)	1.8 (1.3–3.4) <sup>2</sup>	1.5 (0.8 –2.4)	0.02
LDL cholesterol (mmol/l)	1.7 (1.4–2.4)	1.8 (1.4–2.2)	2.5 (1.8–3.1)	2.2 (1.7–2.7)	NS
HDL cholesterol (mmol/l)	1.9 (1.6 –2.2) <sup>3</sup>	1.1 (0.9 –1.3)	1.1 (1.0 –1.5)	1.2 (1.1–1.4)	0.01
FFA (mmol/l)	0.46 (0.3–0.7)	0.49 (0.2– 0.6)	0.47 (0.3–0.6)	0.42 (0.2– 0.6)	NS
Plasma volume (l)	2.4 (2.2–2.9)	2.9 (2.6–3.2)	3.0 (2.7–3.2)	2.8 (2.7–3.1)	NS

<sup>1</sup>p<0.02 between TN vs. PI, <sup>2</sup>p<0.03 between TN & control vs. PI

<sup>3</sup>p<0.01 between control vs. TN, PI and NNRTI



### **3.5.6. Apo-B kinetics (table 3.6-3.14)**

#### **3.5.6.1. VLDL kinetics (table 3.6-3.8)**

##### **3.5.6.1.1. Between the different groups (table 3.6)**

There was no significant difference in VLDL apoB ASR. The VLDL FCR was higher in the HIV-negative control compared to HIV treatment naïve ( $p < 0.04$ ) group. In the PI and NNRTI groups, VLDL apo-B FCR were lower ( $P < 0.001$ ) and VLDL residence time were higher than the control group ( $P < 0.01$ ) but were not different from the HIV treatment naïve patients. The VLDL apoB pool size was higher in the PI group compared to all the other groups ( $p < 0.001$ ). The VLDL apoB kinetics, in the HIV treatment naïve patients otherwise showed a trend in the same direction as the antiretroviral treated groups. VLDL-TG was higher in the PI group ( $p < 0.01$ ), but VLDL-cholesterol, VLDL apoB/TG and VLDL apoB/cholesterol were not different between groups.

Table 3.6: VLDL kinetics [median interquartile range (IQR)]

	Control (n=12)	Cases (n=55)			p-value between groups
		TN (n=15)	PI (n=15)	NNRTI (n=25)	
VLDL ASR (mg/kg/d)	7.7 (3.0 – 10.7)	6.3 (3.1– 10.6)	6.4 (3.7– 11.2)	5.1 (3.0– 7.7)	NS
VLDL FCR (pools/d)	12.7 (10.2– 15.9)	8.3 (4.8 – 14.1) <sup>a</sup>	6.1 (4.3– 8.5)	6.3 (3.4– 8.5)	0.001
Pool size mg	51.7(13.5– 73.9)	57.7 (23.8– 86.1)	90.0 (49.5– 149.5) <sup>b</sup>	65.7 (29.8– 118.8)	0.001
Residence time hours	1.9 (1.5– 2.3)	2.9 (1.7– 4.9)	3.9(2.8– 5.6) <sup>c</sup>	3.8(2.8– 7.0)	0.01
VLDL cholesterol mmol/l	0.17 (0.06– 0.17)	0.15 (0.07–0.3)	0.4 (0.12– 0.7) <sup>d</sup>	0.2 (0.1– 0.5)	0.03
VLDL- triglyceride (mmol/l)	0.5 (0.2– 0.9)	0.4 (0.2– 1.4)	1.1 (0.7– 1.9) <sup>e</sup>	0.7 (0.4– 1.6)	0.01
VLDL cholesterol/apoB ratio	3.3 (2.4– 4.1)	4.4 (2.8– 4.9)	3.2 (2.9 –5.6)	4.2 (3.2– 5.5)	NS
VLDL triglyceride/apoB ratio	39.5 (17.0 –59.1)	29.4(19.2– 34.9)	31.7 (21.1– 59.5)	30.7 (27.1– 45.1)	NS

<sup>a</sup>p<0.04 between HIV-ve control vs. TN, <sup>b</sup>p<0.001 between HIV-ve control vs. PI and NNRTI, <sup>c</sup>p<0.01 between HIV-ve control vs. PI and NNRTI, <sup>d</sup>p< 0.03 between TN vs. PI, <sup>e</sup>p<0.01 between HIV-ve control & TN vs. PI

**3.5.6.1.2. In the NNRTI group: Nevirapine vs. Efavirenz** (table 3.7)

There was no difference in VLDL apoB kinetics between the nevirapine- and efavirenz-treated patients in the NNRTI group.

Table 3.7: VLDL kinetics [median (IQR)] in patients on antiretroviral regimens containing nevirapine and those receiving efavirenz.

	Nevirapine N=11	Efavirenz N=14	P value
VLDL ASR mg/kg/day	5.1 (2.9-8.7)	5.2 (2.8-6.08)	NS
VLDL FCR pools/day	6.3 (5.1-8.2)	6.05 (3.09-10.4)	NS
VLDL residence time hours	3.8 (2.9-4.6)	4.04 (2.3-7.8)	NS
VLDL apoB pool size mg	80.05 (21.6-122.5)	59.3 (29.9-102.5)	NS
VLDL cholesterol mmol/l	0.2 (0.1-0.5)	0.22 (0.13-0.6)	NS
VLDL cholesterol/apoB ratio	4.1 (3.6-5.0)	4.0 (2.6-5.6)	NS
VLDL triglyceride mmol/l	0.78 (0.3-1.7)	0.77 (0.5-1.9)	NS
VLDL triglyceride/apoB ratio	35.7 (25.4-40.4)	30.8 (27.5-59.4)	NS

**3.5.6.1.3. In the PI group: ritonavir vs. non-ritonavir group** (table 3.8)

Patients on ritonavir-containing regimens had a lower VLDL apoB FCR [median, 4.6 pools/d; IQ range, (2.6 –7.0);  $P < 0.05$ ] and increased residence time [5.2 h (3.4–9.2);  $P < 0.05$ ] compared to non-ritonavir-containing PI regimens [8.4 pools/d (6.1–9.4) and 2.86 h (2.5– 4.0), respectively]. Antiretroviral-treated patients with dyslipidemia (cholesterol  $\geq 6.0$  mmol/liter or triglyceride  $\geq 2.3$  mmol/liter, n=11) had lower VLDL FCR ( $P < 0.001$ ), larger VLDL apoB pool size ( $P < 0.003$ ), increased VLDL residence time ( $P < 0.001$ ) compared to the other subjects without significant dyslipidaemia (cholesterol  $< 6$  mmol/l and or triglyceride  $< 2.3$  mmol/l, n=29).

Table 3.8. VLDL kinetics [median (IQR)] in patients on antiretroviral regimens containing the protease inhibitor ritonavir and those receiving other protease inhibitors.

	Ritonavir N=8	Non-Ritonavir N=7	P value
VLDL ASR mg/kg/day	6.51 (3.08-10.3)	6.44 (3.7-13.9)	NS
VLDL FCR pools/day	4.6 (2.6-7.0)	8.48 (6.0-9.4)	0.05
VLDL residence time hour	5.2 (3.4-9.2)	2.8 (2.4-3.9)	0.05
VLDL apoB pool size mg	110.8 (56.7-214.9)	53.0 (35.9-127.7)	NS
VLDL cholesterol mmol/l	0.48 (0.4-0.9)	0.25 (0.1-0.3)	NS
VLDL cholesterol/apoB ratio	4.3 (3.0-9.0)	3.0 (2.5-5.4)	NS
VLDL triglyceride mmol/l	1.4 (0.9-2.5)	0.8 (0.3-1.1)	NS
VLDL triglyceride/apoB ratio	34.3 (19.5-58.1)	26.6 (19.1-59.5)	NS

### 3.5.6.2. IDL kinetics (table 3.9-3.11)

#### 3.5.6.2.1. Between the groups (table 3.9)

In the PI and NNRTI groups, IDL apo-B FCR were lower ( $P < 0.002$ ) and IDL residence time were higher than the control group ( $P < 0.002$ ) but were not different from the HIV treatment naive patients. There was no significant difference in IDL apoB ASR between groups, although IDL apoB pool size was higher in the PI-treated patients compared with the HIV-negative control subjects ( $P < 0.03$ ). The IDL apoB kinetics in the HIV treatment naive patients showed a trend in the same direction as the antiretroviral treated groups. IDL- TG was lower in the HIV-negative controls (0.002), but IDL-cholesterol, IDL apoB/TG and IDL apoB/cholesterol were not different between groups.

Table 3.9: IDL kinetics (median [IQR])

	Control (n=12)	Cases (n=55)			p-value between groups
		TN (n=15)	PI (n=15)	NNRTI (n=25)	
IDL ASR (mg/kg/day)	3.9 (2.5- 7.7)	2.7 (1.7- 5.4)	3.1 (1.0- 6.4)	1.7 (0.7- 3.3)	NS
IDL FCR (pools/day)	9.1 (5.9- 11.6) <sup>a</sup>	4.1 (2.7- 11.4)	2.9 (1.0 -7.2)	2.8 (1.8 - 5.2)	0.002
Pool size mg	28.3 (23.6- 50.6) <sup>b</sup>	44.4 (31.0- 66.5)	52.7 (42.6 - 96.7)	36.2 (23.3- 70.8)	0.03
Residence time hours	2.7 (2.1- 4.1) <sup>c</sup>	5.8 (2.1- 9.0)	8.2 (3.4 -23.1)	8.5 (4.7- 13.3)	0.002
IDL cholesterol mmol/l	0.06 (0.05- 0.09)	0.09 (0.06- 0.14)	0.12 (0.09- 0.21)	0.12 (0.06- 0.17)	NS
IDL-triglyceride (mmol/l)	0.06 (0.04- 0.08) <sup>d</sup>	0.19 (0.06- 0.22)	0.19 (0.10- 0.24)	0.09 (0.04- 0.24)	0.002
IDL cholesterol/apoB ratio	2.5 (1.4 -3.6) 2.6	2.6 (1.7- 3.5)	2.7 (2.3- 2.9)	3.2 (2.4- 4.5)	NS
IDL triglyceride/apoB ratio	4.5 (2.4 -7.5)	9.2 (3.9 -11.5)	6.3 (4.1- 9.6)	6.5 (3.7- 7.9)	NS

<sup>a</sup>p<0.002 between HIV-ve control vs. PI and NNRTI

<sup>b</sup>p<0.03 between HIV-ve control vs. PI.

<sup>c</sup>p<0.002 between HIV-ve control vs. PI and NNRTI

<sup>d</sup>p<0.002 between HIV-ve control vs. PI and p<0.01 between HIV-ve control vs. TN

### 3.5.6.2.2. In the NNRTI group: nevirapine vs. efavirenz (table 3.10)

There was no difference in IDL apoB kinetics between the nevirapine- and efavirenz-treated patients in the NNRTI group.

Table 3.10: IDL kinetics [median (IQR)] in patients on antiretroviral regimens containing nevirapine and those receiving efavirenz.

	Nevirapine N=11	Efavirenz N=14	P value
IDL ASR mg/kg/day	1.02 (0.5-2.9)	1.9 (1.2-3.3)	NS
IDL FCR pools/day	2.67 (1.6-5.1)	3.33 (1.8-5.3)	NS
IDL residence time hour	9.0 (5.0-15.4)	7.2 (4.5-13.3)	NS
IDL apoB pool size mg	32.5 (28.1-58.4)	33.2 (22.4-74.4)	NS
IDL cholesterol mmol/l	0.1 (0.07-0.15)	0.11 (0.06-0.1)	NS
IDL cholesterol/apoB ratio	2.5 (2.0-4.6)	3.1 (2.6-3.7)	NS
IDL triglyceride mmol/l	0.12 (0.05-0.23)	0.17 (0.07-0.2)	NS
IDL triglyceride/apoB ratio	6.6 (3.5-9.3)	5.4 (4.0-7.4)	NS

### 3.5.6.2.3. In the PI group: ritonavir vs. non-ritonavir (table 3.11)

Patients on ritonavir-containing regimens had a lower IDL apoB FCR ( $P < 0.001$ ), increased residence time [5.21 h (3.43–9.2);  $P < 0.001$ ] and increased pool size ( $p < 0.01$ ) compared with non-ritonavir-containing PI regimens.

Antiretroviral-treated patients with dyslipidaemia (total cholesterol  $\geq 6.0$  mmol/liter or triglyceride  $\geq 2.3$  mmol/liter,  $n=11$ ) had larger IDL apoB pool size ( $P < 0.003$ ), but the IDL residence time was not different compared to the other subjects without significant dyslipidaemia (cholesterol  $< 6$  mmol/l and or triglyceride  $< 2.3$  mmol/l,  $n=29$ ).

Table 3.11: IDL kinetics [median (IQR)] in patients on antiretroviral regimens containing the protease inhibitor ritonavir and those receiving other protease inhibitors.

	Ritonavir N=8	Non-Ritonavir N=7	P value
IDL ASR mg/kg/day	2.8 (1.4-22.9)	3.4 (0.8-4.8)	NS
IDL FCR pools/day	0.12 (0.06-0.2)	0.2 (0.04-0.3)	0.001
IDL residence time hour	8.35 (3.7-16.0)	3.8 (3.1-23.2)	0.001
IDL apoB pool size mg	74.6 (38.7-198.2)	50.2 (36.9-65.7)	0.01
IDL cholesterol mmol/l	0.1 (0.08-0.5)	0.1 (0.09-0.1)	NS
IDL cholesterol/apoB ratio	2.6 (2.4-2.8)	2.2 (1.61-3.3)	NS
IDL triglyceride mmol/l	0.2 (0.07-0.3)	0.2 (0.1-0.2)	NS
IDL triglyceride/apoB ratio	4.5 (3.7-6.4)	10.4 (7.5-12.5)	NS

### 3.5.6.3. LDL kinetics (table 3.12-3.14)

#### 3.5.6.3.1. Between the groups (table 3.12)

LDL-cholesterol was not different between groups (table 3.5). LDL apoB ASR was higher in the HIV-negative control ( $p<0.002$ ) and LDL FCR were lower ( $p<0.002$ ) and LDL residence time higher ( $p<.001$ , in the PI and NNRTI groups than in the control group ( $p<0.01$ ). In the treatment naïve group LDL apoB ASR was lower ( $p<0.05$ ) and there was a trend for LDL apoB FCR to be lower and LDL residence time to be higher ( $p=0.07$ ) than the control subjects. The PI group exhibited a higher LDL apoB pool size ( $p<0.01$ ) compared to controls. LDL- TG, LDL apoB/TG and LDL apoB/cholesterol were not different between groups (table 3.12).

Table 3.12: LDL kinetics (median [IQR])

	Control (n=12)	Cases (n=55)			+p- value between groups
		TN (n=15)	PI (n=15)	NNRTI (n=25)	
LDL ASR (mg/kg/day)	9.0 (7.3-9.6) <sup>a</sup>	5.54 (3.7-7.0)	5.12 (4.2- 6.8)	6.24 (3.7- 7.5)	0.002
LDL FCR (pools/d)	0.48 (0.3-0.5) <sup>b</sup>	0.2 (0.2- 0.3)	0.2 (0.1- 0.3)	0.27 (0.1- 0.4)	0.002
Pool size mg	44.0 (37.0-58.0) <sup>c</sup>	49(36- 57)	71(46-77)	50.0 (43- 70)	0.002
Residence time hours	2.08 (1.8-2.9) <sup>d</sup>	3.71(2.7- 4.0)	4.11(2.8- 10.5)	3.6 (2.3- 6.0)	0.01
LDL cholesterol mmol/l	1.7 (1.4-2.4)	1.8 (1.4- 2.2)	2.5 (1.8- 3.1)	2.2 (1.7- 2.7)	NS
LDL-triglyceride (mmol/l)	0.14 (0.09-0.18)	0.14 (0.12- 0.18)	0.20 (0.13- 0.23)	0.14 (0.10- 0.17)	NS
LDL cholesterol/apoB ratio	1.5 (1.49-1.6)	1.5 (1.4- 1.5)	1.5 (1.4- 1.6)	1.5 (1.4- 1.6)	NS
LDL triglyceride/apoB ratio	0.2 (0.1-0.3)	0.3 (0.2- 0.3)	0.3 (0.2- 0.35)	0.2 (0.19- 0.27)	NS
Oxidised LDL	108.9 (86.9-117.1)	105.9 (95.3- 119.8)	97.2 (72.3- 112.3)	91.2 (72.3- 112.3)	NS

<sup>a</sup>p<0.002 between HIV-ve vs. TN, p<0.002, PI, p<0.001, NNRTI, p<0.006

<sup>b</sup>p<0.002 between HIV-ve vs. TN, PI and NNRTI,

<sup>c</sup>HIV-ve vs. PI, p<0.002. <sup>d</sup>p<0.01 between HIV-ve vs. TN,PI,NNRTI



**3.5.6.3.2. In the NNRTI group: nevirapine vs. efavirenz (table 3.13)**

There was no difference in LDL apoB kinetics between the nevirapine- and efavirenz-treated patients in the NNRTI group.

Table 3.13: LDL kinetics [median (IQR)] in patients on antiretroviral regimens containing nevirapine and those receiving efavirenz.

	Nevirapine N=11	Efavirenz N=14	P value
LDL ASR mg/kg/day	5.63 (3.1-7.6)	6.28 (4.8-7.5)	NS
LDL FCR pools/day	0.26 (0.1-0.4)	0.28 (0.2-0.4)	NS
LDL residence time hour	3.85 (2.4-7.1)	3.48 (2.3-5.1)	NS
LDL apoB pool size mg	5.8 (5.0-6.7)	5.2 (4.3-7.2)	NS
LDL cholesterol mmol/l	2.6 (2.0-2.73)	1.96 (1.5-2.8)	NS
LDL cholesterol/apoB ratio	1.6 (1.5-1.7)	1.5 (1.4-1.5)	NS
LDL triglyceride mmol/l	0.15 (0.12-0.18)	0.14 (0.09-0.1)	NS
LDL triglyceride/apoB ratio	0.27 (0.2-0.27)	0.2 (0.19-0.2)	NS

**3.5.6.3.3 In the PI group: ritonavir vs. non-ritonavir (table 3.14)**

In the PI group patients on ritonavir-containing regimens had a lower LDL apoB ASR ( $p=0.009$ ) and a trend to a lower LDL apoB FCR and increased residence time compared to non-ritonavir containing PI regimens ( $p=0.054$ ).

Antiretroviral-treated patients with LDL-cholesterol  $\geq 3.0$  mmol/liter ( $n=11$ ) had lower LDL-FCR ( $p<0.02$ ), increased LDL apoB pool size ( $P < 0.001$ ), increased LDL-TG ( $p<0.001$ ) compared to LDL-cholesterol to those with LDL- cholesterol  $<3.0$ mmol/l ( $n=29$ ). The LDL ASR and LDL-residence time was not different.

Table 3.14: LDL kinetics [median (IQR)] in patients on antiretroviral regimens containing the protease inhibitor ritonavir and those receiving other protease inhibitors.

	Ritonavir N=8	Non-Ritonavir N=7	P value
LDL ASR mg/kg/day	4.56 (2.5-5.1)	6.87 (5.2-8.47)	0.009
LDL FCR pools/day	0.15 (0.09-0.2)	0.27 (0.2-0.42)	0.054
LDL residence time hour	6.86 (3.9-11.6)	3.76 (2.3-4.11)	0.054
LDL apoB pool size mg	74.0 (49.0-104.0)	61.0 (44.0-73.0)	NS
LDL cholesterol mmol/l	2.95 (1.8-4.0)	2.10 (1.5-3.06)	NS
LDL cholesterol/apoB ratio	1.59 (1.4-1.7)	1.45 (1.3-1.64)	NS
LDL triglyceride mmol/l	0.22 (0.1-0.4)	0.16 (0.1-0.21)	NS
LDL triglyceride/apoB ratio	0.29 (0.2-0.3)	0.29 (0.2-0.30)	NS

### 3.6 Discussion

This study has investigated VLDL, IDL and LDL apo-B metabolism in HIV-patients naïve to antiretroviral therapy, HIV-patients taking HAART including PI, or the NNRTI efavirenz or nevirapine and compared these with HIV negative controls.

These studies have shown a significant reduction in VLDL, IDL and LDL apoB FCR with longer residence time in the three lipoprotein compartments in all the HIV positive groups compared to HIV negative controls. The HIV positive treatment groups had mild dyslipidaemia. The treatment naïve group and the HIV-negative control group did not have any difference in serum TG and TC. Compared to the control subjects PI-treated patients exhibited raised serum triglyceride, LDL cholesterol and IDL cholesterol that was not exhibited by NVP or EFV treatment groups; this was more marked in patients taking ritonavir containing PI regimen.

The severity of dyslipidaemia in the patients in our study was less marked than in many previous studies of this kind [130, 152, 457]. Only four

patients were on lipid lowering treatment who had stopped treatment 6 weeks before this study.

Unlike previous studies we were unable to show a significant decrease in LDL cholesterol in the treatment naïve patients [135] or an increase in LDL cholesterol concentration with PI and NNRTI treatment compared to the control subjects [325, 458]. This was particularly surprising in the treated patients and may again reflect the exclusion of patients who were on lipid lowering drugs. Since LDL cholesterol concentration is determined by the LDL ASR and LDL FCR, a normal LDL-cholesterol concentration will result if there is a decrease in both LDL ASR and FCR as found in all HIV patient groups in our study [78]. The HDL cholesterol was reduced in all the HIV positive patient groups and this was similar to what was described previously [109, 132]. Nevirapine increases HDL-C, but efavirenz does not [176]. In this study there was no difference in the HDL-C level between nevirapine and efavirenz group.

The particle size of different lipoproteins is important. Observational and epidemiological studies suggest that subjects having a predominance of small, dense particles have increased risk of cardiovascular disease (CVD)[459]. Further studies have shown that the number, not the size, of LDL- cholesterol particles predicts CVD risk [460, 461].

The VLDL, IDL and LDL-cholesterol and triglyceride/apoB ratios were not different between the controls and HIV treatment groups suggesting that there was no change in VLDL, IDL and LDL particle size in contrast with a study in patients on PI which reported an increased VLDL triglyceride/apoB ratio [462].

A decrease in LDL apo-B FCR results in increase of LDL apo-B residence time. Previously it has been shown that LDL apo-B residence time, measured by stable isotope techniques is positively related to surrogate markers of LDL apo-B oxidation in healthy subjects and patients with familial defective apo-B 100 [463]. In this study oxidised LDL apo-B was not different between groups and did not correlate with LDL apo-B FCR.

The oxidation of LDL is regulated by a complex set of reactions, mainly stimulated by the presence of free radicals in the endothelium. Nitric oxide down-regulates this oxidation process catalyzed by L-arginine. The activity of L-arginine is inhibited by asymmetric dimethylarginine (ADMA) which is a metabolic by-product of continual protein modification processes in the cytoplasm of all human cells [464]. In this study, L-arginine activity was measured but not the ADMA activity which may have influence on the result. There is also another possibility that the difference in results of LDL apo-B oxidation may be due to the different methods used for measuring LDL apo-B oxidation. The previous study measured the oxidation of LDL apo-B poline and arginine residue to gamma glutamyl semialdehyde [465], where as in our study LDL-apo-B oxidation was measured by an ELISA with an antibody to malondialdehyde-LDL.

Our study demonstrates that the mild dyslipidaemia in the treatment groups is due to a decrease in VLDL, IDL and LDL apoB FCR. In all the HIV positive groups the VLDL and IDL TG was higher but LDL TG was not.

In the presence of increased TG rich lipoproteins in the plasma and normal levels of activity of the plasma cholesteryl ester transfer protein (CETP), TG from VLDL can be exchanged for cholesteryl esters (CE) in HDL and

LDL [106, 466]. The VLDL particles give up TG molecules, donating it to the HDL and LDL, in return for more of the cholesteryl ester molecules from HDL and LDL.

There are a number of consequences of elevated TG content in LDL and HDL. Both TG rich LDL and HDL become substrate for hepatic lipase (HL) which removes much of the TG by hydrolysis making both LDL and HDL smaller in size. The TG rich LDL contains a structurally altered apo-B whose interaction with LDL receptors are impaired [467]. A cholesterol-rich VLDL remnant particle is either taken up by hepatic receptors or further delipidated to cholesterol rich LDL particles and a triglyceride-rich cholesterol-depleted HDL particle. The TG-rich HDL particle can undergo further modification including hydrolysis of its triglyceride, probably by hepatic lipase, which leads to the dissociation of the structurally important protein apo A-I [468-470]. The free apo A-I in plasma is cleared more rapidly than apo A-I associated with HDL particles. One of the sites of clearance is the kidney [471]. In this situation, HDL cholesterol is reduced, and the amount of circulating apo A-I and therefore the number of HDL particles is also reduced [470]. The prolonged residence time of apo-B containing lipoproteins will allow exchange of triglyceride for cholesterol esters with HDL through cholesterol ester transfer protein (CETP) pathway, which would increase clearance providing a mechanism for the reduced HDL-cholesterol seen in both treatment naïve patients and those on antiretroviral therapy.

The changes in VLDL secretion and clearance rate have not been found to be uniform in different studies. In a prospective study Carpentier *et al* [129] showed that secretion and clearance of VLDL apo-B were both

impaired in HIV patients and treatment with HAART increased the secretion but the clearance remaining unchanged. In that study the elevation of circulating VLDL early in the course of HAART was caused by the combination of impaired VLDL clearance already present in HAART-naïve HIV- patients and HAART-mediated increase in VLDL secretion. In that study, there was a significant reduction of VLDL FCR in HIV-positive individuals that was not changed after use of HAART. These changes occurred concomitantly with an elevation of plasma free fatty acids before significant change in body composition. In contrast, Reeds *et al* [457] showed that HIV patients who had dyslipidaemia, the rate of hepatic VLDL-TG secretion during fed and fasted conditions were much greater, and clearance of VLDL-TG from plasma was slightly lower, compared to healthy men who did not have dyslipidaemia. Therefore, both increased VLDL-TG secretion into plasma and decreased plasma VLDL-TG clearance contribute to hypertriglyceridaemia. In the present study there appeared to be no difference in VLDL and IDL apo-B secretion rate, but the clearance rate of both were diminished as was the cases in previous studies which found a correlation of increase in serum FFA and VLDL apo-B secretion rate. However, serum FFA levels, in our study were not different between any of the groups.

There was no significant difference in VLDL and IDL ASR between any groups. This contrasts with a previous study which found an increase in VLDL ASR in PI treated patients, although all of whom had marked abnormalities in serum lipids and the samples were taken in a fed state [130, 152]. The same study also reported reduced rates of VLDL transfer into denser lipoproteins implying a lower rate of lipoprotein lipase-

mediated delipidation. The decrease in LDL ASR in our study may be due to a reduction in the direct hepatic secretion of LDL and/or reduced production of LDL formed as a result of remodelling of IDL and VLDL. The reduction in VLDL and IDL apoB FCR in the HIV patient groups might have also been played a contributory factor.

At the time our study was done there was only one study looking at the effect of NNRTI on lipoprotein kinetics [152]. The VLDL and IDL apo B kinetics in six PI treated patients were compared with five patients treated with nevirapine in the fed state. No comparison was made with control subjects or HIV treatment naïve patients. Contrary to our study, the VLDL and IDL apoB secretion rate were higher in the PI treated patients. This difference may be due to small numbers tested or the fed state of the subjects.

Apart from apo-B pool size, the apo-B kinetics was not different in the PI and NNRTI group from the TN group in this study. However, when the patients in the PI group were subdivided into those treated with ritonavir and those treated with other PIs, it was shown that the abnormal apo-B kinetics was exacerbated by ritonavir treatment. Apo-B FCR in the ritonavir group was extremely low, particularly in VLDL and IDL compartment. The LDL FCR was reduced but not to that extent. The LDL ASR was reduced as well. This was similar to levels reported in patients with familial defective apolipoprotein B-100 in which binding of LDL to the LDL receptor is impaired [472, 473]. LDL cholesterol was not significantly increased by ritonavir since there was a decrease in LDL ASR along with some decrease LDL FCR.

This study together with the previous studies suggests [130, 152, 457] that a primary abnormality in HAART treated patients is a decrease in apo-B clearance which leads to mild dyslipidaemia whereas severe dyslipidaemia is the result of both a decrease in apo-B clearance and an increase in VLDL ASR.

In conclusion this study suggests that in patients with mild dyslipidaemia reduced apo apoB FCR and consequently an increased apo-B residence time may be a primary abnormality in lipoprotein kinetics in HIV infection. The same abnormalities were also found in patients treated with either a protease inhibitor, or NNRTI containing HAART regimens, with more marked changes in apo-B kinetics in the patients treated with the PI, ritonavir.



## **CHAPTER 4**

### **Relation of Fat Redistribution, insulin resistance and apolipoprotein-B kinetics**

#### **4.1. Introduction**

Abnormalities in body fat distribution have been reported in 40 to 50 percent of ambulatory HIV-infected patients and the proportion is greater in those receiving combination antiretroviral therapies [212, 214, 228]. Prevalence rates vary widely, from 11 to 83 percent, in cross-sectional studies [224, 225]. A systematic study that attempted to provide a unifying definition suggested that peripheral fat wasting was associated with abdominal visceral adiposity, dyslipidaemia and insulin resistance and the condition was called lipodystrophy syndrome [180]. The vast majority of patients were on PI at that time and the condition was thought to be related to PI use. However, further studies revealed that lipodystrophy is a separate phenomenon and mostly associated with use of thymidine analogues and lipohypertrophy with PI use [77].

Adipose tissue demonstrates extreme plasticity in its capacity to vary both in volume and cell number. The adipocyte cells have the capacity to increase its diameter 20 fold and volume by 1000 fold when required to store extra energy in the form fat vacuoles [474]. An increase in adipose mass or size may be the result of both hypertrophy of existing adipocytes, increased adipocyte differentiation from preadipocytes from the stromal vascular fraction, or an impairment of apoptosis. In contrast a decrease in adipose mass or size may be the result of atrophy of existing adipocytes

or defect in adipocyte differentiation and that may lead to impairment of several metabolic functions.

#### **4.2. Regional Differences in adipocyte function**

Several studies with obese individuals have clearly indicated that excess adiposity is linked to metabolic abnormalities [474-477]. Excess intra-abdominal, particularly visceral rather than peripheral or subcutaneous fat is more strongly associated with diabetes, hypertension and cardiovascular disease, indicating wider metabolic differences within the different adipose tissue regions [478]. Intra-abdominal fat can be further divided into an intraperitoneal depot (omental [0.5-3 kg] and mesenteric [0.5-2 kg]) and retroperitoneal, i.e. perirenal fat (0.5-2 kg) [479](147). Omental and mesenteric fat depots are also referred to as visceral fat since their venous drainage is mainly through the portal vein. However, subcutaneous fat is the largest abdominal fat depot with an estimated weight of 1-20 kg [479]. Of the whole body adipose tissue mass, subcutaneous fat constitutes at least 80% in both lean and obese subjects [478]. In addition, isotopic tracer studies have shown that the TG synthesis, breakdown and lipid turnover as a whole is substantially higher in the visceral in contrast to the subcutaneous depot [480].

#### **4.3. Fat distribution and Dyslipidaemia**

Dyslipidaemia associated with PI therapy is characterised by high levels of triglyceride rich lipoproteins [363] with increased abdominal adiposity, but with thymidine analogues the dyslipidaemia is associated more with lipoatrophy [208]. Lipohypertrophy is more common with PI use [481]

and lipoatrophy with thymidine analogues [482] and a role of NNRTI has become debatable since ACTG 5142 [172] showed an association of lipoatrophy with use of efavirenz as described in chapter 1. Both lipoatrophy and lypohypertrophy can be associated with dyslipidaemia and insulin resistance [483, 484]. While switching from thymidine analogues to non-thymidine analogues are associated with improvement in lipoatrophy and dyslipidaemia [166], lypohypertrophy does not improve with switching of PIs to non-PI regimens [209, 485]. However, dyslipidaemia improves in switching PI regimen to a non-PI regimen [144] or switching to newer PIs like atazanavir [486].

While lipoatrophy is less common in patients on nucleoside sparing NNRTI and ritonavir boosted PI regimen, lipid disturbance in the form of raised TC and TG are more common in the same regimen [172, 487].

#### **4.4. Fat distribution, Dyslipidaemia and Insulin resistance**

There is strong evidence from *in vivo* [329, 488] and *in vitro* [336, 368] studies that PI therapy is associated with insulin resistance. A single dose of indinavir in HIV negative volunteers has been associated with significant reduction of glucose disposal [330]. Hence PI induced insulin resistance can develop rapidly and is not necessarily secondary to body fat changes. However, the same may not happen in HIV patients. Noor *et al* [329] showed significant differences in rate of glucose disposal in HIV negative patients when using atazanavir and lopinavir with ritonavir. A recent study by Jackson *et al* [489] did not show any difference when atazanavir was compared with saquinavir in HIV patients.

The recently published Women Interagency HIV Study [323] has shown an association of increased risk of diabetes with cumulative exposure of NRTIs. Another study using boosted lopinavir and tipranavir with a backbone of tenofovir and lamivudine did not show any evidence of insulin resistance at 48 weeks therapy [327], but was present when used with AZT [327]. Hence insulin resistance could be related more to thymidine analogues than to PIs.

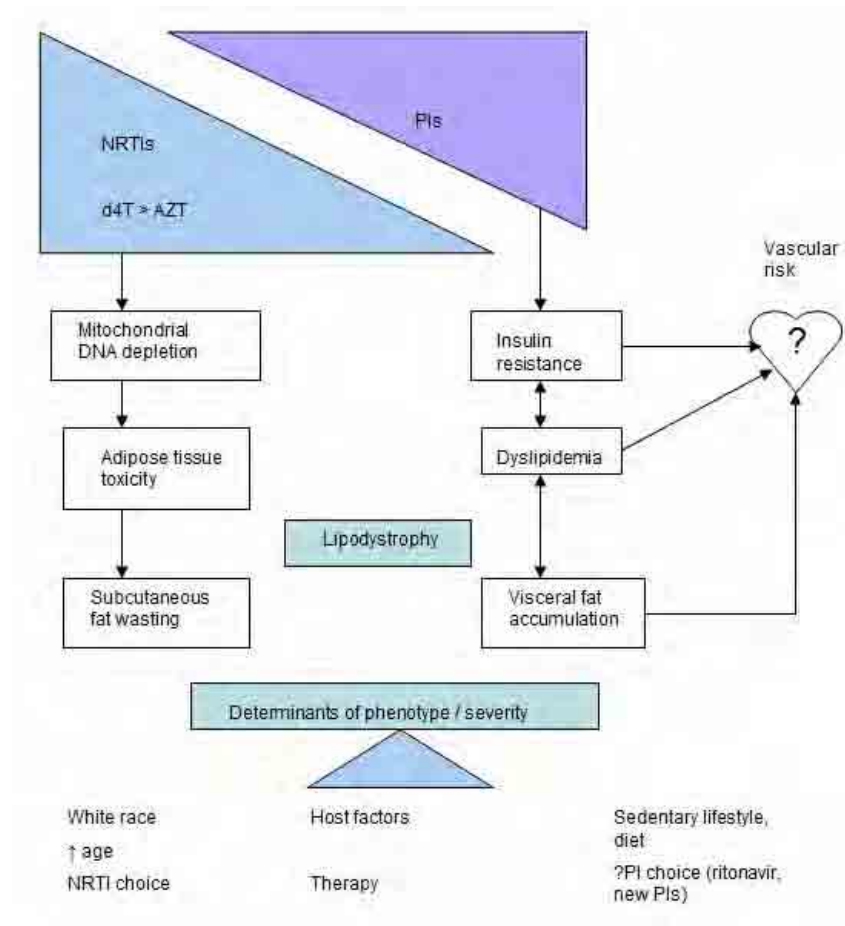
In the recently published D:A:D study the incidence of diabetes increased with cumulative exposure to HAART. The strongest relationship with diabetes was exposure to thymidine analogue including stavudine and then zidovudine. Time-updated measurements of total cholesterol, HDL cholesterol, and triglycerides were all associated with diabetes. Adjusting for each of these variables separately reduced the relationship between HAART and diabetes slightly. Although lipodystrophy was significantly associated with diabetes, adjustment for this did not modify the relationship between HAART and diabetes.

### **4.5. Adipocentric model** (figure 4.1)

The available evidence implicates both PIs and NRTIs in the pathogenesis of lipodystrophy and dyslipidaemia and insulin resistance. In an attempt to bring together the mixed findings of both mitochondrial dysfunction in relation to NRTI therapy and PI related insulin resistance plus impaired maturation of adipocytes an 'adipocentric' model of lipodystrophy has been proposed [76]. The clinical appearance of this syndrome is purported to be related to the dominance of the drug class, whereby greater NRTI toxicity would lead to lipoatrophy, whilst the combination of

PI induced insulin resistance as well as NRTI toxicity would lead to a mixed morphological appearance. The divergent effects upon the adipocyte by the different classes of drugs could therefore combine to produce a greater risk of firstly developing lipodystrophy as well as dictating the severity of this condition, a suggestion that has been supported by clinical findings [490, 491].

**Fig. 4.1:** A multifactorial Adipocentric model of lipodystrophy syndrome(s); a collection of abnormalities with partially overlapping risk factors (PI and NRTI) adapted from Nolan *et al*, 2001[76]. **d4T** = stavudine; **NRTI** – nucleoside reverse transcriptase inhibitor; **PI** = protease inhibitor; **AZT** = zidovudine



The exact mechanisms of these changes have yet to be determined but may be related to the effect of HIV infection, the adverse effects of some antiretroviral drugs and the long term consequence of antiretroviral therapy on regional fat distribution and insulin resistance. A recent study of treated and untreated HIV patients with and without lipodystrophy showed that lipodystrophy in HIV was associated with a lower expression of LDL receptors [193].

It was hypothesised that the reported increase in LDL cholesterol in PI and NNRTI treated patients may be due to a decrease in LDL clearance and that this may be related to changes in body fat distribution.

The study in the previous chapter has shown that patients treated with protease inhibitors or non-nucleoside reverse transcriptase inhibitors with mild dyslipidaemia have a decreased clearance of apo-B containing lipoproteins. This study will describe the fat distribution of the same subjects and investigate the relation of the apo-B kinetics with fat distribution and insulin resistance.

#### **4.6. Objective.**

To find out the relation between fat distribution, insulin resistance and apo-B kinetics in HIV patients.

#### **4.7. Subjects and Methods**

##### **4.7.1. Subjects**

The subjects were same as described in the previous chapter. One patient on treatment with the PI, nelfinavir was unable to attend for the dexa scan.

Exclusion criteria were the same as used for apo-B kinetics.

##### **4.7.2. Experimental design:**

A cross-sectional case control study.

#### **4.7.3. Ethics committee approval and patient consent**

Ethical approval was obtained from South Birmingham Local Research Ethics Committee and all subjects have been given written information about the study and all subjects gave written informed consent before taking part into the study.

#### **4.7.4. Study protocol**

Following the day of admission in the Wellcome Trust Clinical Research Facility, Queen Elizabeth Hospital, Birmingham, the subjects attended the Department of Nuclear Medicine in the same Hospital either on the same day or any other day within seven days of the admission for the measurement of their body fat distribution.

#### **4.7.5. Measurement of body fat distribution**

Body fat distribution was measured by whole-body dual-energy x-ray absorptiometry (DEXA) scan within seven days of the leucine infusion. The trunk was defined as the region including the chest, abdomen and pelvis, excluding head and neck. The proximal limit of the leg regions were placed through the hip joints at an angle of approximately 45 degree and for the arm regions vertically through the shoulder joints. The area between horizontal lines at the level of xiphoid process and the iliac crest was denoted abdominal region.

Peripheral fat mass was defined as the sum of arm and leg masses. The precision of DEXA scan in this department was 3% for total fat mass, 4% for trunk fat mass and 5% for extremity fat mass. The DEXA scan was



performed and reported in random order and the operator who performed the analysis were not aware of any treatment or any history details of the subjects. The inter-observer error was eliminated by having a single investigator to perform all measurements. The coefficient of variation of repeated skinfold measurements by the same trained observer is approximately only 5%, but it rises to 10-20% between different observers [492].

A total of 57 subjects were scanned using a Hologic QDR 4500A (version 11.2:3, Hologic Inc., Waltham, MA) and 9 subjects using a Lunar DPX-L (version 1.3 g, GE Medical Systems, Waukesha, WI). Results are presented as leg fat, peripheral fat (arm plus leg), or trunk fat (Kg), divided by the lean body mass (bone plus muscles) Kg.

### **4.7.6. Insulin Resistance**

The fasting glucose and fasting insulin concentration were utilised to estimate insulin resistance. The fasting insulin concentration is dependent on fasting glucose level [493]. Fasting glucose and insulin levels are determined by a simple feedback loop between the liver and the beta cells of pancreas. Decreased insulin levels allow increased gluconeogenesis, until plasma glucose level rises to a level that stimulates an optimal insulin response from beta cells.

Homeostasis model assessment (HOMA) is a mathematical model of the glucose and insulin interaction that enables an interpretation of the feedback loop [494]. This model was based on the assumption that normal weighed healthy subject aged less than 35 years have insulin resistance of 1 and beta cell function of 100%. The model estimates a unique combination of beta cell function and insulin sensitivity which

produced the given glucose and insulin values. The main advantage of HOMA is that it is a simple method that can estimate relative beta cell function and insulin resistance (IR).

The accuracy and precision of the estimate have been determined by comparison with independent measures of insulin resistance and beta-cell function using hyperglycaemic and euglycaemic clamps and an intravenous glucose tolerance test. As a method for assessing insulin resistance, HOMA correlates well with the euglycaemic clamp ( $r=0.8$ ,  $p<0.0001$ ), but its estimate of beta cell function appears to be less accurate, when compared with hyperglycaemic clamp ( $r=0.6$ ,  $p<0.01$ ) and with the estimate from the intravenous glucose tolerance test ( $r = 0.6$ ,  $p < 0.05$ ). There was no correlation with any aspect of insulin-receptor binding. The coefficient of variation is high, about 30% for both insulin resistance and beta cell function [494].

The formula represents an approximation to the HOMA where  $HOMA_{IR}$  stays for Insulin Resistance and  $HOMA_{secr}$  for beta cell function.

We used the  $HOMA_{IR}$  for calculation of Insulin Resistance (IR) :

$$\text{fasting plasma Insulin} \times \text{fasting plasma Glucose} / 22.5$$

(Units: insulin in U/ml and fasting plasma glucose in mmol/L)

### 4.7.7. Statistical analyses

Initial comparison between the four groups was by one-way ANOVA or Kruskal-Wallis followed by Bonferroni's or Dunn's multiple comparison tests (SPSS 10.0.7 for Windows; SPSS Inc, Chicago, IL, USA). Fisher's exact test was used for categorical data between groups, and associations

were analyzed by Spearman's rank correlation test. A stepwise linear regression model examined the effect of variables on VLDL and IDL and LDL metabolism. Variables entered were age, sex, ethnicity, smoking, family history of diabetes, family history of cardiovascular disease, alcohol intake, peripheral fat/ LBM, trunk fat/LBM, glucose, HOMA, and FFA. Pre-treatment CD-4 count, HIV RNA copies and current CD-4 counts were included when HIV positive groups were analysed separately and in a separate model, only patients on antiretroviral treatment and their duration of treatment were included.

## **4.8. Results**

### **4.8.1. Patient Characteristics, treatment history and lab results**

were similar to what has already been described in the previous chapter (table 3.1-3.3).

### **4.8.2. Viral load and CD-4 counts** (table 3.4)

Current CD4 counts of the patients were not different between HIV positive groups, nor were current or pre-treatment viral loads or pre-treatment CD-4 counts among the treatment groups. Two PI-treated patients on HAART had a detectable viral load (800 and 1100 copies/ml). The remaining patients had viral loads below the detection limit of the assay (less than 50 copies/ml).

### **4.8.3. Fat distribution** (table 4.1- 4.3)

Only four patients had clinical evidence of lipodystrophy, one in the PI group and three in the NNRTI group. Three of them had both lipoatrophy

and visceral adiposity and one had lipoatrophy only. All those four patients were on thymidine analogue based nucleoside backbones.

A lipodystrophy score was not applied on the patients because not all the information was available to perform the scoring and besides the scoring has not been validated in clinical practice because of lack of specificity (as discussed in chapter 1).

DEXA results were available in 66 subjects (table 3.1). Peripheral fat/lean body mass (LBM) and leg fat/LBM were significantly reduced in the PI and NNRTI groups compared with the control group ( $P < 0.05$ ). Lean body mass, total fat (Kg) and trunk fat (Kg) were similar in all the groups. Total peripheral fat ( $p < 0.01$ ), arm fat ( $p < 0.01$ ), leg fat ( $p < 0.01$ ), peripheral fat/LBM ( $p < 0.01$ ), leg fat/LBM ( $p < 0.001$ ) were lower in the PI and NNRTI group, but not different between the two control groups.

Table 4.1: Median (IQR) Body fat distribution by DEXA scan of all subjects (n=64)

	Control (n=12)	Cases (n=54)			p-value between groups
		TN (n=15)	PI (n=14)	NNRTI (n=25)	
Total fat Kg	17.4 (11.3-20.3)	16.3 (10.4-17.8)	14.7 (9.7-17.2)	11.5 (8.0-16.2)	NS
Trunk fat Kg	7.9 (4.3-9.2)	7.3 (5.9-9.1)	9.0 (4.9-10.3)	6.5 (4.4-9.4)	NS
Total peripheral fat Kg	9.4 (6.3-11.0) <sup>a</sup>	8.0 (5.1-9.2)	5.5 (3.7-8.2)	4.3 (2.8-6.6)	0.01
Lean body mass Kg	40.6 (33.8-53.7)	47.5 (45.0-59.2)	54.8 (45.6-61.5)	53.0 (45.5-56.7)	NS
Arm fat Kg	1.9 (1.3-2.5) <sup>b</sup>	1.6 (1.4-2.2)	1.5 (1.1-2.0)	0.9 (0.7-1.7)	0.01
Leg fat Kg	7.5 (5.0-8.0) <sup>c</sup>	5.9 (3.6-7.1)	4.0 (2.5-6.0)	3.4 (2.1-5.1)	0.01
Trunk fat/peripheral fat ratio	0.8 (0.6-1.0)	1.0 (0.6-1.2)	1.6 (0.9-2.0)	1.3 (1.0-1.9)	NS
Trunk fat (kg)/LBM (Kg)	0.16 (0.11-0.21)	0.16 (0.09-0.19)	0.14 (0.11-0.19)	0.14 (0.08-0.20)	NS
Limb fat (Kg)/LBM(kg)	0.2(0.1-0.3) <sup>d</sup>	0.1 (0.09-0.21)	0.09 (0.07-0.15)	0.08 (0.06-0.14)	0.001
Leg fat (Kg)/LBM(Kg)	0.05 (0.03-0.05) <sup>e</sup>	0.04 (0.02-0.05)	0.03 (0.02-0.04)	0.02 (0.01-0.04)	0.001

<sup>a</sup>p-value between control vs. PI& NNRTI and TN vs. PI & NNRTI<sup>b</sup>p-value between control vs. PI& NNRTI<sup>c</sup>p-value between control vs. PI& NNRTI<sup>d</sup>p-value between control vs. PI& NNRTI<sup>e</sup>p-value between control vs. PI& NNRTI

Distribution of fat was not different between AZT and d4T users (table 4.2).

Table 4.2: Median (IQR) Body fat distribution by DEXA scan amongst thymidine analogue user (d4t vs. AZT)

	AZT (n=27)	d4T (n=10)	p-value
Total fat (Kg)	14.0 (10.0-20.0)	10.2 (8.0-13.7)	NS
Trunk fat (Kg)	8.2 (5.7-11.6)	6.3 (4.4-8.8)	NS
Total peripheral fat (arm + leg) Kg	5.4 (4.0-7.8)	3.7 (2.7-6.2)	NS
Lean body mass = bone plus muscle (Kg)	55.3 (47.8-61.8)	50.7 (40.2-55.7)	NS
Arm fat (Kg)	1.5 (0.8-1.9)	1.0 (0.7-1.6)	NS
Leg fat (Kg)	4.0 (3.0-6.1)	2.6 (1.9-4.5)	NS
Total fat (Kg)/lean body mass (Kg)	0.2 (0.16-0.34)	0.20 (0.1-0.3)	NS
Limb fat Kg/Lean body mass Kg	0.03 (0.01-0.04)	0.02 (0.01-0.04)	NS

Amongst the group using PI, fat distribution was similar between ritonavir user and non-user (table 4.3).

There was no significant difference in regional body fat changes in patients with significant dyslipidaemia (cholesterol  $\geq 6$  mmol/l and or triglyceride  $\geq 2.3$  mmol/l, n=13) compared to the other subjects without significant dyslipidaemia (cholesterol  $< 6$  mmol/l and or triglyceride  $< 2.3$  mmol/l, n=53).

Table 4.3: Median (IQR) Body fat distribution by DEXA scan amongst ritonavir as PI booster or non -ritonavir user

	Ritonavir as PI or booster (n=8)	Non-ritonavir PI (n=7)	p-value
Total fat (Kg)	14.8 (9.1-19.0)	13.0 (9.1-19.1)	NS
Trunk fat (Kg)	9.1 (5.5-10.0)	7.3 (3.6-13.7)	NS
Total peripheral fat (arm + leg) Kg	5.2 (3.1-8.8)	5.5 (4.2-7.2)	NS
Lean body mass = bone plus muscle (Kg)	53.7 (42.3-58.7)	58.1 (44.4-63.3)	NS
Arm fat (Kg)	1.7 (0.9-2.0)	1.4 (1.0-1.9)	NS
Leg fat (Kg)	3.5 (2.1-6.7)	4.0 (2.9-5.6)	NS
Total fat (Kg)/ lean body mass (Kg)	0.24 (0.16-0.33)	0.26 (0.19-0.33)	NS
Limb fat Kg/Lean body mass Kg	0.08 (0.06-0.16)	0.10 (0.07-0.15)	NS

**4.8.4. Insulin Resistance [HOMA<sub>IR</sub>] (table 4.4, 4.5)**

Median fasting blood sugar was 5.1 (range: 3.9-6.1) mmol/l, and was not different amongst the groups (two subjects having fasting blood sugar 6.0 and 6.1mmol/l, one with PI and one with NNRTI).

Fasting plasma insulin level was higher in PI group compared to HIV-negative controls ( $p < 0.01$ ). Compared to HIV-negative controls, HOMA<sub>IR</sub> was higher in PI group but not in others ( $p < 0.01$ ). There was no difference in HOMA<sub>IR</sub> amongst the HIV positive groups (table 4.4).

Table 4.4: Baseline plasma glucose, insulin and HOMA<sub>IR</sub> [median (interquartile range)]

	Control (n=12)	Cases (n=66)			p- value between n groups
		TN (n=14)	PI (n=15)	NNRTI (n=25)	
Glucose mmol/l	4.9 (4.7– 5.2)	4.9 (4.7– 5.0)	5.2 (4.9 – 5.4)	5.1 (4.5–5.5)	NS
Insulin (pmol/l)	30.0 (20.0– 40.3)	37.5 (28.1– 56.2)	42.3 (31.1– 114.2)	37.0 (18.7– 64.8)	NS
HOMA <sub>IR</sub>	0.9 (0.6 – 1.3)	1.3 (0.9 – 1.9)	1.4 (1.0 – 3.3) <sup>1</sup>	1.3 (0.5–2.0)	0.01

<sup>1</sup>P <0.01 Between PI and control

HOMA<sub>IR</sub> was not different amongst the HIV patients who had significant dyslipidaemia (cholesterol  $\geq 6$  mmol/l and or triglyceride  $\geq 2.3$  mmol/l, n=14) compared to the other subjects without significant dyslipidaemia (cholesterol <6 mmol/l and or triglyceride <2.3 mmol/l, n=40).

However, in the antiretroviral-treated patients with dyslipidemia (cholesterol  $\geq 6$  mmol/liter or triglyceride  $\geq 2.3$  mmol/liter, n=11) had lower VLDL FCR ( $P < 0.001$ ), larger VLDL apoB pool size ( $P < 0.003$ ), increased VLDL residence time ( $P < 0.001$ ), increased trunk fat/BMI ( $P < 0.04$ ), HOMA ( $P < 0.04$ ), and FFA ( $P < 0.003$ ) compared with those without dyslipidemia (n=28).

#### 4.8.5. Correlation of fat distribution, HOMA<sub>IR</sub> and apo-B kinetics

Duration of treatment was negatively correlated with total fat/LBM ( $r=0.4$ ,  $p < 0.001$ ) and leg fat/LBM (0.54,  $p < 0.0001$ ), but not with trunk fat / LBM (table 4.5).



Table 4.5: Correlations between duration of treatment and fat distribution

		Total fat (g)/ lean mass (g)	Limb fat (g) / lean mass (g)	Trunk fat (g) / lean mass (g)
Total duration of treatment	Correlation Coefficient (r)	-.40(**)	-.54(**)	-.19
	Sig. (2-tailed)	.001	.000	.116

\*\* Correlation is significant at the 0.01 level (2-tailed).

LBM: lean body mass

Peripheral fat/LBM was positively correlated with trunk fat/LBM

( $r=0.6, p<0.0001$ , table 4.6). Peripheral fat/LBM was positively correlated with VLDL apoB FCR ( $r=0.38, p<0.002$ ), IDL apo-B FCR ( $r=0.4, p<0.002$ ) and LDL apo-b FCR ( $r=0.3, p<0.01$ , table 4.6, figure 4.2, 4.3 and 4.4).

HOMA<sub>IR</sub> correlated with trunk fat/LBM ( $r=0.3, p=0.01$ ) but not with limb fat/LBM (table 4.6).

HOMA<sub>IR</sub> correlated positively with serum TG ( $r=0.37, p<0.002$ , table 4.7) and negatively with HDL-cholesterol ( $r=0.4, p<0.001$ , table 4.7).

HOMA<sub>IR</sub> correlated negatively with VLDL apo-B FCR ( $r=0.29, p<0.01$ , table 4.8), IDL apo-B FCR ( $r=0.29, p<0.02$ , table 4.9) and LDL apo-B FCR ( $r=0.33, p<0.008$ , table 4.10) and LDL ASR ( $r=0.32, p<0.008$ , table 4.10) respectively.

HOMA<sub>IR</sub> correlated positively with VLDL apo-B pool size, VLDL residence time, VLDL-TG, VLDL-C ( $r=0.36, 0.3, 0.42, 0.44, p<0.003, 0.01, 0.0001, 0.0001$ , respectively, table 4.7), and IDL residence time ( $r=0.3, p<0.02$ , table 4.9) and also with LDL pool size and residence time ( $r=0.31, 0.33, p<0.01, 0.007$ , table 4.10 respectively) but not with IDL-TG and IDL-C (table 4.9) and LDL-TG, LDL-C (table 4.10).

Table 4.6: Correlations between HOMA, apo-B FCR and fat distribution

		HOMA (insulin.glu cose/22.5)	VLDL FCR pools/day	IDL FCR/day	LDL FCR pools/day	Trunk fat (g) / lean body mass (g)	Peripheral Fat / lean body mass
HOMA (insulin.glu cose/22.5)	Correlation Coefficient (r)		-.290(*)	-.299(*)	-.334(**)	.385(**)	.013
	Sig. (2-tailed)		.018	.022	.008	.002	.921
VLDL FCR pools/day	Correlation Coefficient (r)	-.290(*)		.746(**)	.700(**)	-.078	.382(**)
	Sig. (2-tailed)	.018		.000	.000	.536	.002
IDL FCR/day	Correlation Coefficient (r)	-.299(*)	.746(**)		.557(**)	-.143	.405(**)
	Sig. (2-tailed)	.022	.000		.000	.283	.002
LDL FCR pools/day	Correlation Coefficient (r)	-.334(**)	.700(**)	.557(**)		-.111	.309(*)
	Sig. (2-tailed)	.008	.000	.000		.385	.014
Trunk fat (g) / lean body mass (g)	Correlation Coefficient (r)	.385(**)	-.078	-.143	-.111		.665(**)
	Sig. (2-tailed)	.002	.536	.283	.385		.000
Peripheral fat / lean body mass	Correlation Coefficient (r)	.013	.382(**)	.405(**)	.309(*)	.665(**)	
	Sig. (2-tailed)	.921	.002	.002	.014	.000	

\* Correlation is significant at the 0.05 level (2-tailed). \*\* Correlation is significant at the 0.01 level (2-tailed).

Table 4.7: Correlations between HOMA<sub>IR</sub>, lipids and FFA

		total cholesterol (mmol/L)	HDL cholesterol	total triglyceride	Free fatty acids mmol/l	LDL cholesterol mmol/l
HOMA <sub>IR</sub> (insulin.glucose /22.5)	Correlation Coefficient (r)	.193	-.413(**)	.373(**)	-.086	.128
	Sig. (2-tailed)	.120	.001	.002	.495	.316

\*\* Correlation is significant at the 0.01 level (2-tailed). \* Correlation is significant at the 0.05 level (2-tailed).

Table 4.8: Correlations of HOMA<sub>IR</sub> and VLDL fractions

		VLDL FCR (pools/day)	VLDL ASR (mg/kg/ day)	ApoB pool size mg	VLDL chol (mmol/l)	VLDL triglyceride (mmol/l)	VLDL residence time (h)
HOMA <sub>IR</sub> (insulin.glucose /22.5)	Correlation Coefficient (r)	-.290(*)	.070	.365(**)	.442(**)	.428(**)	.3(*)
	Sig. (2-tailed)	.018	.576	.003	.000	.000	.018

\*\* Correlation is significant at the 0.01 level (2-tailed). \* Correlation is significant at the 0.05 level (2-tailed).

Table 4.9: Correlations of HOMA<sub>IR</sub> and IDL fractions

		IDL FCR pools/day	IDL ASR (mg/kg/ day)	IDL Apo-B pool size (mg)	IDL cholesterol (mmol/l)	IDL triglyceride (mmol/l)	IDL residence time (h)
HOMA <sub>IR</sub> (insulin.glucose /22.5)	Correlation Coefficient (r)	-.299(*)	-.233	.135	.154	.044	.304(*)
	Sig. (2-tailed)	.022	.079	.279	.218	.725	.020

\*\* Correlation is significant at the 0.01 level (2-tailed). \* Correlation is significant at the 0.05 level (2-tailed).

Table 4.10: Correlations of HOMA<sub>IR</sub> and LDL fractions

		LDL FCR pools/day	LDL ASR	LDL apoB g	LDL cholesterol g/l	LDL triglyceride mmol/l	LDL residence time (h)
HOMA <sub>IR</sub> (insulin.glucose /22.5)	Correlation Coefficient (r)	-.334(**)	-.329(**)	.314(*)	.127	.028	.335(**)
	Sig. (2-tailed)	.008	.008	.012	.323	.827	.007

\*\* Correlation is significant at the 0.01 level (2-tailed). \* Correlation is significant at the 0.05 level (2-tailed).

Figure 4.2: Correlation between peripheral fat and VLDL apo-B FCR

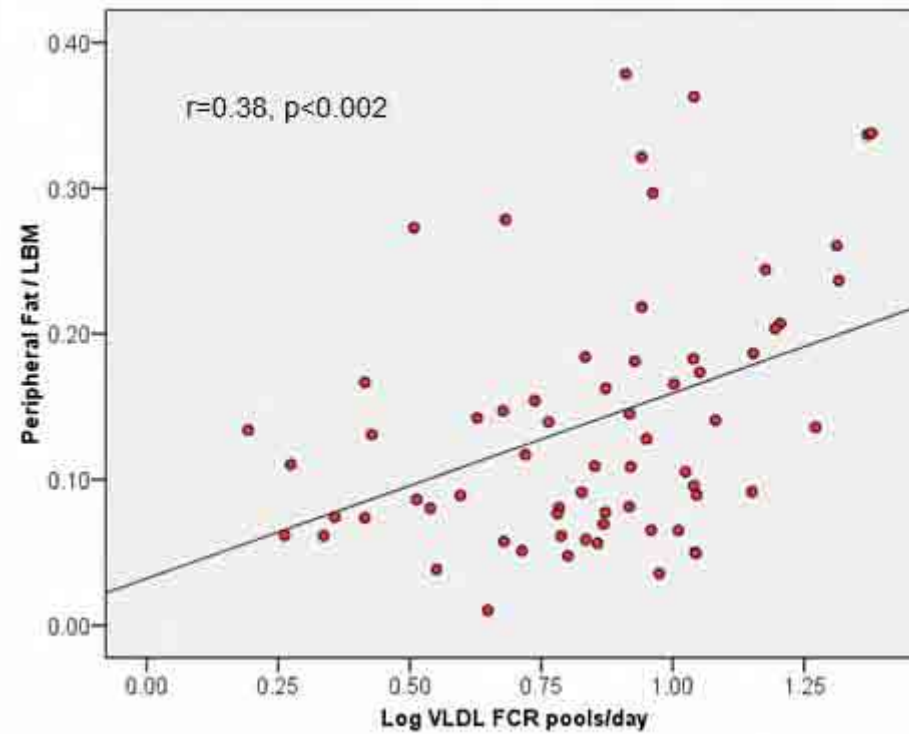


Figure 4.3: Correlation between peripheral fat and IDL apo-B FCR

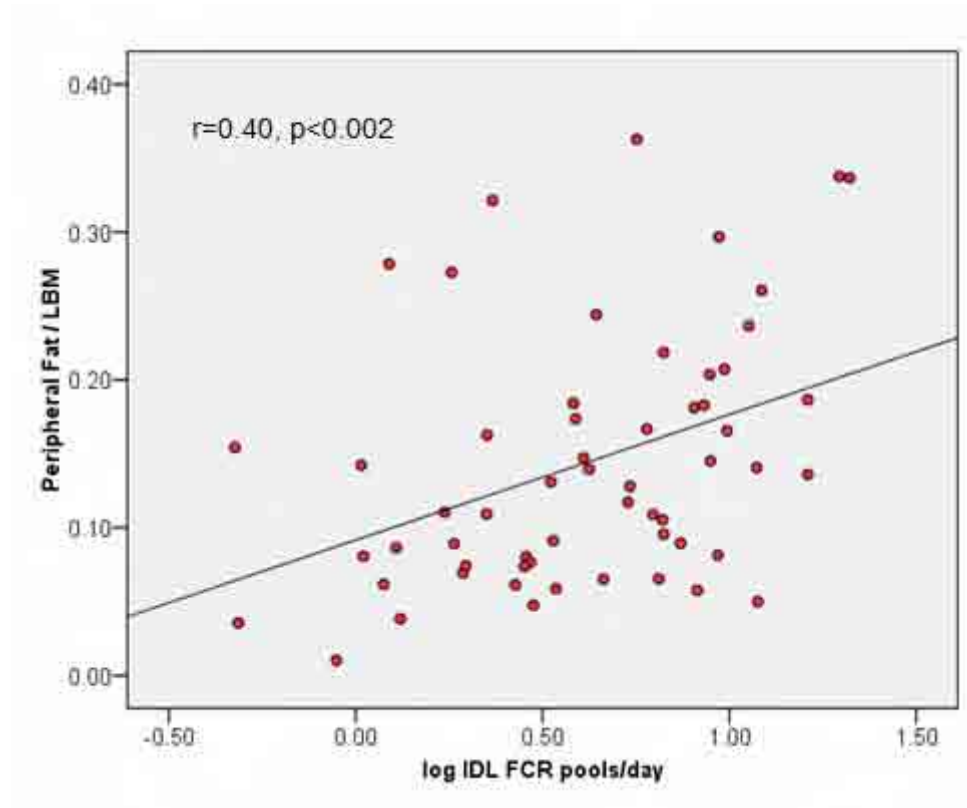
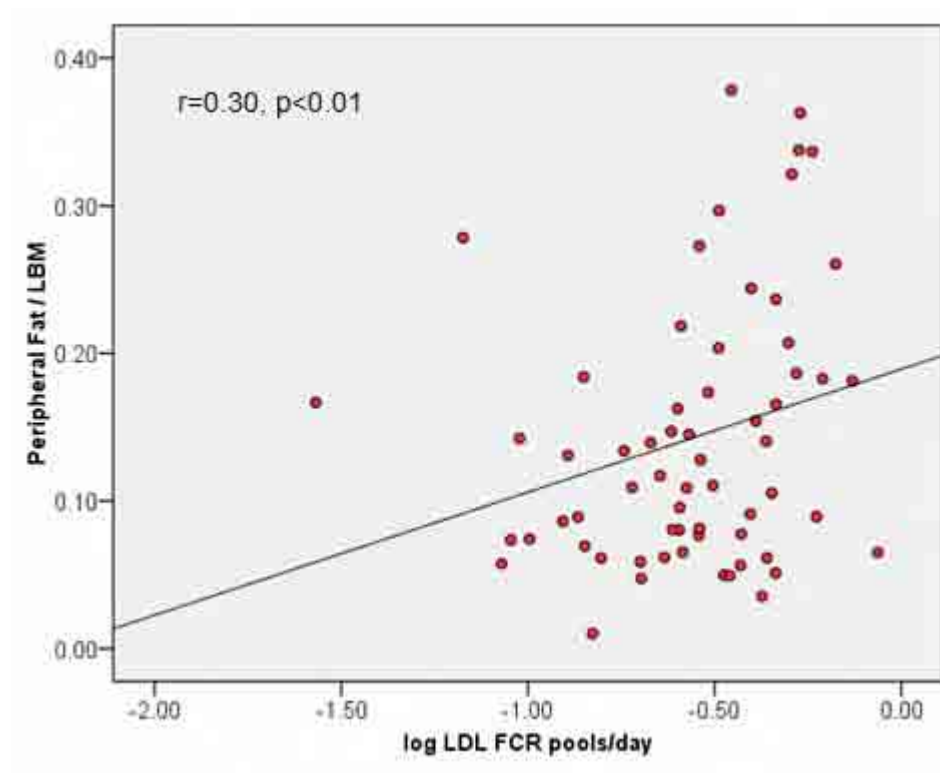


Figure 4.4: Correlation between peripheral fat and LDL apo-B FCR



#### **4.8.6. Linear regression analysis**

In a linear regression model HOMA predicted VLDL apoB residence time ( $P < 0.005$ ), VLDL cholesterol ( $P < 0.006$ ), VLDL triglyceride ( $P < 0.008$ ), LDL residence time (0.01) and FCR (0.01) and inversely predicted VLDL apoB FCR ( $P < 0.005$ ) and IDL ASR ( $P < 0.02$ ) and LDL ASR ( $p = 0.005$ ). Peripheral fat predicted VLDL apoB FCR ( $P < 0.001$ ) and IDL apoB FCR ( $P < 0.001$ ) but inversely predicted IDL cholesterol ( $P < 0.001$ ). Trunk fat predicted VLDL apoB pool size ( $P < 0.006$ ), VLDL cholesterol ( $P < 0.001$ ), and IDL residence time ( $P < 0.001$ ), LDL apoB pool size ( $p < 0.019$ ), negatively IDL apoB FCR ( $P < 0.001$ ) and LDL FCR ( $p = 0.016$ ). FFA concentration predicted LDL apoB pool size ( $p = 0.012$ ), LDL cholesterol ( $p = 0.044$ ) and LDL TG ( $p < 0.001$ ).



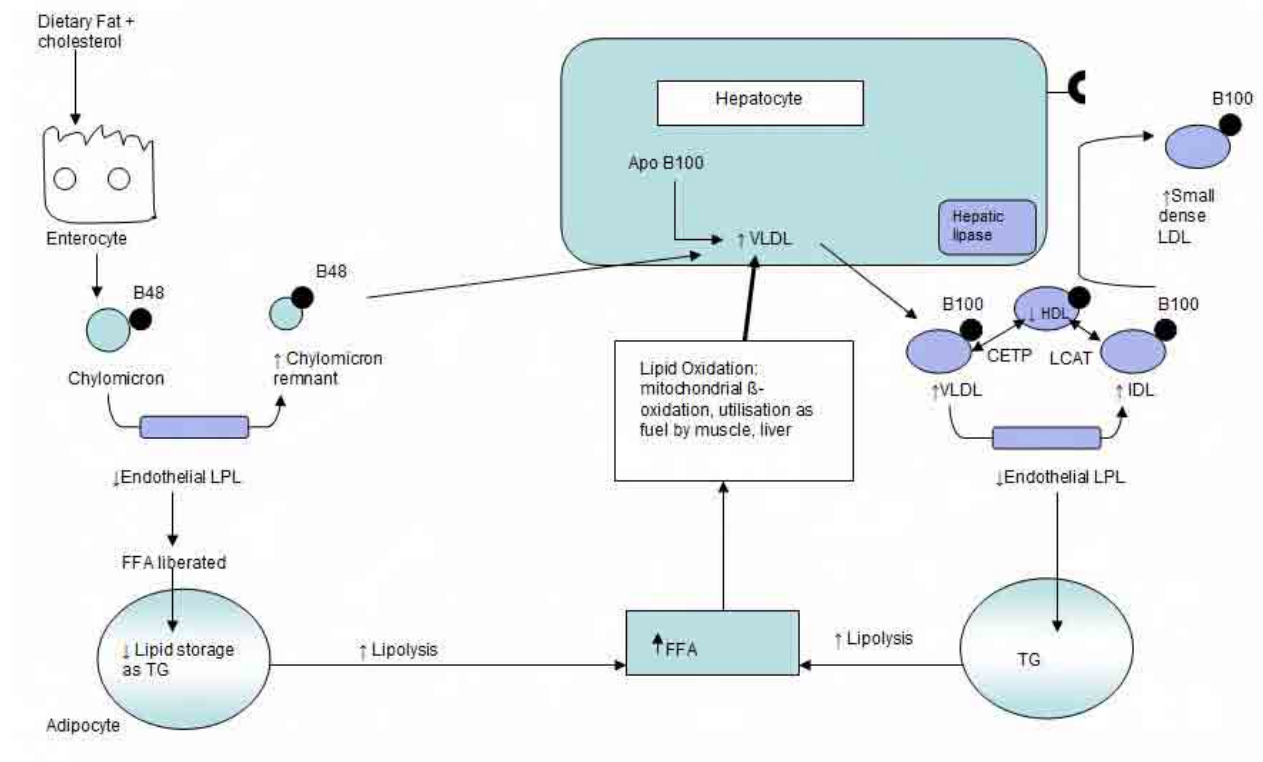
#### **4.8. Discussion**

This study has shown that HIV patients on treatment had more loss of peripheral fat compared to treatment naïve HIV patients and HIV negative controls and this was associated with impaired apo-B clearance rate, but not with insulin resistance. Insulin resistance was associated with increased trunk fat and increased VLDL and LDL-apo-B pool size, but not with IDL pool size. Patients on longer duration of treatment had more loss of peripheral fat, but their insulin resistance was not different from the other patients. Amongst the group using PI, fat distribution was similar between ritonavir user and non-user. Distribution of fat was not different between AZT and d4T users. However, this is likely to be the effect of sample size as such a difference has been previously well established[495, 496].

Insulin resistance decreases delipidation of lipoproteins [497] derived from liver or from gut (chylomicrons) through lipoprotein lipase which is produced and secreted by adipocytes under the influence of insulin [498]. Increased FFA liberated from lipolysis, and increased chylomicron remnants can stimulate more synthesis of hepatic VLDL [499-501]. Increased VLDL production leads to increased production of LDL that is then cleared by LDL receptors. However, altered TG enriched VLDL produces TG rich LDL which then leads to formation of smaller dense LDL that is less effectively cleared by the LDL receptors and is more susceptible to oxidative modification (figure 4.4). Alternatively TG rich VLDL can also produce IDL [502] with the help of endothelial lipase [97, 502, 503] (figure 4.4). The TG rich IDL can either be taken up by liver receptors or transformed further to TG rich LDL.

The larger apo-B containing lipoproteins (VLDL) rich in triglyceride are metabolised by sequential delipidation through a transient IDL to cholesterol-rich LDL. Several components contribute to the regulation of this process, including the lipolytic enzymes lipoprotein lipase and hepatic lipase, apolipoproteins CII, CIII and E, and the apolipoprotein B/E or LDL receptor [504]. Clearance of IDL to LDL is dependent on plasma hepatic lipase activity [504-506]. Lipoprotein lipase acts primarily on large VLDL. Hepatic lipase on the other hand seems to be critical for the conversion of smaller particles. In our study  $HOMA_{IR}$  correlated with VLDL and LDL-apo-B pool size but not with IDL-apoB pool size. While LPA activity is reduced in insulin resistance, the activity of hepatic lipase increases [505]. This could explain the lack of association of insulin resistance with IDL apo-B pool size. Insulin resistance has influence on the activities of lipase systems. Influence of other factors including adipocytokines is possible.

Fig. 4.5: Insulin resistance and lipid metabolism: Insulin resistance lowers delipidation of different lipoproteins through LPL system resulting increased production of TG rich VLDL-C. These are cleared either by IDL pathway or by LDL pathway exchanging TG load with cholesterol with HDL-C (adapted from Nolan *et al*[76]. **Apo B100** = apolipoprotein B100; **B48** = apolipoprotein B48; **CETP** – cholesterol ester transfer protein; **FFA** = free fatty acid; **HDL** = high density lipoprotein; **IDL** = intermediate density lipoprotein



In contrast to many previous studies, this study did not show a significant change in insulin resistance in any of the treatment group.

However, in a regression model insulin resistance predicted VLDL apo-B FCR, apo-B concentration, and cholesterol and triglyceride content, possibly through the control of insulin on lipoprotein lipase activity [507]. Central obesity and increased visceral fat is strongly associated with insulin resistance [508]. The failure to find a significant change in insulin resistance between groups in the present study may be explained by the lack of a significant change in trunk fat and also could be due to small sample size as a correlation between trunk fat and HOMA became evident when the subjects were combined. Interestingly trunk fat, FFA and HOMA were significantly higher in patients with dyslipidaemia (cholesterol  $\geq 6$  mmol/l and or triglyceride  $\geq 2.3$  mmol/l) compared to those without. The association between visceral fat and insulin resistance and dyslipidaemia is well-documented [475].

Several studies have shown that PI induced insulin resistance and TG rich dyslipidaemia have a common association with abnormal fatty acid metabolism. The increased production of FFA from breakdown of fat or decreased clearance of FFA from the plasma by adipocytes and muscles acts as a fuel for the synthesis of increased VLDL in the liver [509].

Increased FFA in the plasma can also act as a fuel for increased gluconeogenesis in the liver [510], impair the uptake of glucose in the muscle or fat and can induce beta cell dysfunction in the pancreas (discussed in chapter 1, figure 1.14) [378]. This association was not present in our study, but an association was found only in patients who had severe dyslipidaemia. The patients who had severe dyslipidaemia had

higher HOMA and higher FFA, along with decreased apo-B FCR and increased apo-B pool size and increased trunk fat. It is possible that in HIV patients insulin resistance may not have any direct influence on apo-B kinetics and fat distribution in the absence of significant dyslipidaemia.

Although insulin resistance in HIV infection can develop earlier in the absence of lipodystrophy [326], factors other than insulin resistance may play an important role in the impairment of apo-B kinetics and may have association with peripheral fat loss.

Our data suggests that in patients with mild dyslipidaemia reduced VLDL, IDL and LDL-apoB FCR may be a primary abnormality in lipoprotein kinetics in HIV infection, possibly in consequence to the peripheral fat loss, which is further exacerbated by treatment with either a protease inhibitor, or nevirapine or efavirenz-containing regimen. The association of VLDL, IDL and LDL apoB FCR with regional fat distribution suggests that the mechanism for decrease in this parameter may be related to a decrease in lipoprotein lipase and or a possible change in hepatic lipase activity. Interestingly the patients with significant dyslipidaemia in our study did not have any difference in the peripheral fat distribution, but their HOMA and FFA were higher along with decreased apo-B FCR, and increased trunk fat.

Insulin resistance has been described more with PI use and visceral adiposity. Association with peripheral fat loss has not been well described till recently when a few studies pointed to the influence of thymidine analogues [318, 323, 326], but the mechanisms have yet to be defined. A role of adiponectin has been supported by some other studies [387, 511]. In diabetic patients insulin resistance plays an important role in VLDL apo-

B clearance [103, 129, 512, 513]. The absence of association of insulin resistance or HOMA with VLDL apo-B FCR in our study possibly indicates the role of some other factors in the clearance of apo-B in HIV patients. In healthy subjects adiponectin has been found to increase the insulin sensitivity in different tissues including liver and skeletal muscles [514]. The inter relationship of adiponectin, other cytokines, apo-B kinetics and body fat distribution will be investigated in the next chapter.

## CHAPTER 5

### Adipocytokines and Dyslipidaemia in HIV patients

#### 5.1. Introduction

Adipose tissue is a hormonally active system [515] involved in the control of intermediary metabolism and not merely a storehouse of excess energy [385]. The term adipocytokines has been used to refer to a series of adipocyte-derived biologically active molecules which may influence the function as well as the structural integrity of other tissues. It has now become clear that the adipocytes release free fatty acids and several cytokines and hormones, including tumour necrosis factor alpha (TNF- $\alpha$ ), interleukins, plasminogen activator inhibitor type 1, leptin, adiponectin and resistin. These secretory products have paracrine and endocrine actions and allow the adipocyte to have an important role in the regulation of metabolism, including insulin resistance and to participate in the atherogenic processes [381].

##### 5.1.1. Adiponectin

The most abundant protein within the adipocyte is adiponectin [396]. There seems to be a clear relationship between adiponectin and fat mass in humans [516]. Adiponectin is the only adipose-specific protein known to date that is negatively regulated in obesity [396]. The relationships between adiponectin and serum lipid concentrations have recently been studied [517-520]. Plasma adiponectin has been found to be correlated negatively with serum triglyceride and positively with serum HDL cholesterol in female non diabetic subjects [401].

The mechanisms responsible for the control of the production of adiponectin have not been determined so far. The only hormone implicated in the regulation of adiponectin expression has been insulin [521]. But the mechanism regulating the decreased adiponectin concentration in insulin resistance remains obscure. TNF- $\alpha$  is one of the candidate molecules responsible for causing insulin resistance [522]. The expression and secretion of adiponectin from adipocytes are significantly reduced by TNF- $\alpha$  [523-525] and therefore increased TNF-  $\alpha$  might be partially responsible for the decreased adiponectin production in obesity or in insulin resistance.

### **5.1.2. Adiponectin, Dyslipidaemia and Lipodystrophy**

Several authors have investigated the potential role of adiponectin in the pathogenesis of PI associated lipodystrophy [347, 387, 425, 526].

Patients with HIV associated lipodystrophy have a reduction in both mRNA and plasma concentrations of adiponectin which correlate with markers of insulin resistance and lipolysis [387]. However, it is difficult to assess whether this is the effect or cause of the lipodystrophy and whether the change in adiponectin is due to HIV or the drugs used in the HAART regimen. In particular, the extensive use of PIs within these studies has led to suggestions that this class is responsible for the reductions in adiponectin witnessed in patients with lipodystrophy [527, 528]. Using a murine cell line Xu *et al* [529], demonstrated that indinavir (IDV), nelfinavir (NFV) and ritonavir (RTV) could decrease adiponectin mRNA and adiponectin secretion, with RTV having the most profound effect. A few recent studies on the other hand have suggested that NRTI use rather PI



use has greater effect on the reduction of plasma adiponectin in HIV patients [387, 390, 526].

Furthermore the lack of prospective studies makes it difficult to assess whether hypoadiponectaemia is simply a reflection of reduced adipocyte size rather than a specific drug induced effect. In support of that, patients with non-HIV congenital lipodystrophy have lower amounts of plasma adiponectin than patients with only partial lipodystrophy [530].

### **5.1.3. Leptin, Dyslipidaemia and Lipodystrophy**

Leptin is synthesized and released from fat cells in response to changes in body fat mass [531]. Leptin reduces intracellular lipid levels in skeletal muscle, liver and pancreatic beta cells, and thereby improves insulin sensitivity [409]. These changes are partially mediated by central sympathetic activation of adrenergic receptors [532]. Leptin deficiency is a rare monogenic disorder and thus confined to only a few patients, but that in simple obesity leptin resistance prevails [533]. Thus leptin treatment does not appear to be a suitable option for obesity or lipodystrophy in the general population [534]. In patients with lipodystrophy and leptin deficiency, leptin replacement therapy improved glycaemic control and decreased triglyceride levels, but no change in lipodystrophy. However, the role of leptin in HIV infection and its response to HAART has not been established [411].

### **5.1.4. Tumour Necrosis Factor (TNF)- $\alpha$**

TNF- $\alpha$ , is a multipotential cytokine. It has been proposed as a molecular link between obesity and insulin resistance [535]. Studies in obese rats with blockade or neutralization of TNF- $\alpha$  have shown improvement in insulin resistance [418, 420]. In humans TNF- $\alpha$  is synthesized and

secreted by different tissues including adipocytes and stroma of vascular cells [536, 537]. Adipose tissue TNF- $\alpha$  mRNA correlates with body mass index, percentage of body fat and hyperinsulinaemia [538]. Several mechanisms could account for the effect of TNF- $\alpha$  on obesity-related insulin resistance that include increased release of FFA by adipocytes, reduced adiponectin synthesis and impaired insulin signalling.

The role of TNF-  $\alpha$  in HIV can be complex as plasma levels can vary with different stages of infection. TNF- $\alpha$  has been shown by a number of authors to be over expressed in patients with lipodystrophy [250, 252], as well as correlating with markers of insulin resistance and hyperlipidaemia [250] in patients with subcutaneous peripheral wasting. Both animal and human studies have indicated that TNF- $\alpha$  can inhibit adipocyte differentiation [539, 540], 2000), modulate lipid metabolism [416] and enhance plasminogen activator inhibitor levels [541]; effects that are typically evident in patients with lipodystrophy [419].

In humans two cell surface TNF- $\alpha$  receptors, TNF-R1 and TNF-R2 have been described. Soluble forms of those receptors (sTNF-R1 and sTNF-R2) are present in plasma and their concentrations might reflect local action of TNF- $\alpha$  in different tissues [420, 421]. Mynarcik *et al* [250] found relationship between the degree of insulin resistance and sTNFR2 levels and suggested an inflammatory stimulus is contributing to the development of HIV-associated lipodystrophy. Levels of sTNFR2 were elevated in all HIV-infected subjects, but they were significantly higher in those with lipodystrophy than without, and sTNFR2 levels strongly correlated with the reduction in insulin sensitivity.

#### **5.1.5. Interleukin-6 (IL-6)**

IL-6 is a circulating cytokine with multiple effects ranging from inflammation to host defence and tissue injury. It is secreted by adipose tissue and many other cell types [540, 542, 543]. There is a positive correlations between plasma IL-6 concentrations with human obesity and insulin resistance [544], and high IL-6 levels are predictive of type 2 diabetes and myocardial infarction [545-547]. In the recently presented SMARTY study IL-6 was associated with high plasma HIV RNA level and also associated with increased risk CVD. The role of IL-6 in fat distribution and dyslipidaemia in HIV patients, have not been established.

#### **5.1.6. Interleukin-8 (IL-8)**

It is possible that the chronic inflammatory state associated with HIV infection, and reflected as elevated fasting serum IL-8 levels, may contribute to abnormalities in glucose metabolism. IL-8 has possible effect on atherogenesis [426, 548]. IL-8 is produced and released from human subcutaneous and visceral adipose tissue [549] and subcutaneous adipose tissue obtained from HIV-infected individuals with fat redistribution and insulin resistance [425]. Serum IL-8 levels are increased in diabetic patients, and IL-8 has been suggested to be involved in the pathogenesis of atherosclerosis. Enhanced mRNA levels of TNF- $\alpha$ , IL-6, and IL-8 and low adiponectin were found in subcutaneous adipose tissue of patients who have HIV associated lipodystrophy [425]. Increased adipocytokine expression might play a role for the reduced levels of adiponectin found in HIV associated LD patients.

### **5.1.7. Other cytokines**

A host of other cytokines [540, 550, 551] have been proposed to have effects on lipid and insulin metabolism and this can vary with the disposition of fat distribution while the fat cells of different region have different actions.

Resistin was found to have influence on glucose metabolism possibly through insulin resistance [432] [552]. But subsequent studies have conflicting results and various authors have failed to associate the adipocyte as being a key secretor of resistin [434].

Cytokines including IL-1 $\alpha$  and  $\beta$ , IL-12 have been found to have effects on lipid metabolism and insulin resistance [553]. These cytokines have effects on the secretion of other cytokines including adiponectin.

Macrophage related involution of fat cells have been described in HIV negative individuals [554]. Adipose tissue from patients with HIV-related lipodystrophy presents a state of chronic inflammation. Altered expression of cytokines or adipokines and macrophage infiltration could be involved in patients' insulin resistance and lipodystrophy. Increased chemokine or cytokine production by adipocytes and macrophages could be involved in macrophage recruitment and participate in lipodystrophy and insulin resistance [553, 555, 556].

As discussed in the previous chapter the available evidence implicates both PIs and NRTIs in the pathogenesis of lipodystrophy. The adipocentric model of lipodystrophy proposed by Nolan, attempted to bring together the mixed findings of both NRTI therapy related mitochondrial dysfunction

and PI related insulin resistance plus impairment of adipocyte maturation [76]. The divergent effects upon the adipocyte by the different classes of drugs and the virus might be responsible for secretion of different cytokines that could be the mediator of different metabolic disturbances seen in HIV patients.

In the previous chapters we demonstrated in HIV patients, a significant reduction in VLDL, IDL and LDL apoB fractional catabolic rate [6, 7] which was inversely related to peripheral fat, without any significant difference in insulin resistance. The underlying cause for the altered lipid metabolism and consequent dyslipidaemia may be due to loss of peripheral fat but the mechanism is not understood.

In this study it was hypothesised that loss of peripheral fat in HIV patients would result in decreased plasma adipocytokines, in particular adiponectin, which would be associated with changes in VLDL, IDL and LDL apolipoprotein-B (apo-B) kinetics.

## **5.2. Objective**

To find out the relationship between plasma adipocytokines and apo-B kinetics and fat distribution in HIV patients

## **5.3. Subjects and Methods**

### **5.3.1. Subjects**

The subjects were same as described in the previous chapter. One patient on treatment with PI, nelfinavir was unable to attend for the dexa scan.

Exclusion criteria were the same as used for apo-B kinetics.

### **5.2.2. Experimental design:**

A cross-sectional case control study.

### **5.2.3. Ethics committee approval and patient consent**

Ethical approval was obtained from South Birmingham Local Research Ethics Committee and all subjects have been given written information about the study and all subjects gave written informed consent before taking part into the study.

### **5.2.4. Study protocol**

The fasting plasma samples were taken at the same time as other samples were taken for apo-B kinetics.

#### **5.2.5. Adiponectin and Leptin assay**

Plasma adiponectin and plasma leptin were measured by radioimmunoassay assay using Linco Research, St Louis, MO, USA . The LINCO Research, Inc. adiponectin RIA assay utilizes <sup>125</sup>I-labeled murine adiponectin and a multispecies adiponectin rabbit antiserum to determine the level of adiponectin in serum, plasma or tissue culture media by the double antibody/PEG technique. The adiponectin standards were prepared using recombinant mouse adiponectin and were used to determine the circulating levels of adiponectin in mouse or rat serum/plasma samples. Human adiponectin sensitivity is at 1ng/ml when using a 100micro lit sample size. Assay range is 1-200 ng /ml. Specificity is <0.01% for human C1q and 400% for mouse adiponectin. Interassay % CV (variance) is 9.25 on a sample concentration of 1.5 ug/l with intra-assay CV (variance) of 3.59%.

#### **5.2.6. Cytokines assay**

The following cytokines were measured in fasting blood sample: TNF- $\alpha$  including TNF-soluble receptor 1 and TNF-soluble receptor-2, interleukin-1 beta (IL-1b), IL-6, IL8, IL-10 and IL-12p70. We used the Becton Dickinson Human Inflammation Cytometric Bead Array to measure the cytokines on a dual-laser FACS Calibur flow cytometer. The detection limit of the assay was 3 pg/ml.

#### **5.2.7. Statistical analyses**

Initial comparison between the five groups was by one-way ANOVA or Kruskal-Wallis followed by Bonferroni's or Dunn's multiple comparison test

(SPSS 10.0.7 for Windows; SPSS Inc, Chicago, IL, USA). Fisher's exact test was used for categorical data between groups, and associations were analyzed by Spearman's rank correlation test.

A stepwise linear regression model examined the effect of variables on VLDL and IDL and LDL metabolism. Variables entered were age, sex, ethnicity, smoking, family history of diabetes, family history of cardiovascular disease, alcohol intake, peripheral fat/ LBM, trunk fat/LBM, glucose, HOMA, FFA, cytokines including adiponectin, leptin, TNF- $\alpha$ , TNFR-1, TNFR-2, IL-8. Pre-treatment and current CD-4 count and pretreatment HIV RNA copies were included when compared between the HIV positive groups. In a separate model, only patients on treatment were included. All the variables entered in to the multivariate model were log transformed.

### **5.3. Results**

#### **5.3.1. Patient Characteristics, treatment history and lab results**

were similar to what has already been described in the previous chapter (table 3.1-3.3)

**5.3.2. Viral load and CD-4 counts** were similar to what has already been described in the previous chapter. has been described in the previous chapter (table 3.4).



### **5.3.3. Adipocytokines**

#### **5.3.3.1. Adiponectin** (table 5.1)

Adiponectin was lower in all HIV patients, including TN patients compared to controls ( $p < 0.003$ ) but was not different between the three HIV groups.

Adiponectin was lower ( $p < 0.01$ ) in patients with dyslipidaemia (cholesterol  $\geq 6$  mmol/l and or triglyceride  $\geq 2.3$  mmol/l) compared to those without dyslipidaemia (3.3  $\mu\text{g/ml}$  [2.4-5.1] ( $n=14$ ) v 5.4  $\mu\text{g/ml}$  [4.7-7.9],  $n=41$ ).

#### **5.3.3.2. Leptin** (table 5.1)

Leptin levels were lower in HIV positive groups ( $p < 0.006$ ).

Amongst the HIV positive groups leptin level was lower in NNRTI group compared to the others ( $p < 0.05$ ).

There was no significant difference in leptin level in patients with significant dyslipidaemia (cholesterol  $\geq 6$  mmol/l and or triglyceride  $\geq 2.3$  mmol/l,  $n=14$ ) compared to those without dyslipidaemia ( $n=41$ ).

#### **5.3.3.3. TNF- $\alpha$ and other cytokines** (table 5.1)

Serum TNF- $\alpha$  was lower in HIV-ve controls ( $< 0.0001$ ) compared to HIV positive groups. Among the HIV+ve groups TNF- $\alpha$  was higher in the treatment naïve group (0.01) but there was no difference between the PI and NNRTI group.

Serum TNF-R1 was lower in TN ( $p < 0.03$ ) compared to other groups, but was not different amongst HIV-ve control, PI and NNRTI group. TNF-R2 was higher in PI compared to all other groups ( $p < 0.01$ ) and was lower in TN ( $p < 0.05$ ) compared to HIV-ve control ( $p < 0.05$ ) and NNRTI ( $p < 0.01$ ).

Serum IL-8 was higher ( $p < 0.01$ ) in TN and PI group, but not in NNRTI and HIV-negative controls.

IL-1 was lower in all HIV positive groups ( $p < 0.01$ ) compared to HIV-ve controls, but was not different between the HIV positive groups.

IL-1 $\beta$ , IL-6, IL-10 and IL-12p70 were below the detection limit of the assay for most samples and hence were excluded from statistical analysis.

Table 5.1: Median (IQR) adipocytokines and other cytokines

	Control (n=12)	Cases (n=55)			p-value between groups
		TN (n=15)	PI (n=15)	NNRTI (n=25)	
Adiponectin µg/ml	<sup>a</sup> 9.7 (6.9-13.3)	5.4 (4.7-8.5)	5.0 (3.3-6.4)	5.0 (3.1-6.7)	0.003
Leptin ng/ml	<sup>b</sup> 8.7 (3.4-13.5)	4.4 (2.5-7.5)	4.1 (3.3-6.2)	<sup>c</sup> 2.1(1.1-4.9)	0.006
TNF- α pg/ml	<sup>d</sup> 0.75 (0.5-0.9)*	<sup>e</sup> 3.7 (2.6-4.2)	1.5 (1.3-4.7)	1.1 (1.0-2.1)	0.0001
TNF soluble receptor I pg/ml	752.1 (720.3-1012.2)	<sup>f</sup> 431.7 (312.7-758.9)	936.6 (723.2-1132.1)	873.9 (569.7-1033.7)	0.03
TNF soluble receptor II pg/ml	2544.0 (2133.7-3086.9)	<sup>g</sup> 75.0 (37.9-5324.0)	<sup>h</sup> 8830.9 (818.8-9446.5)	5551.9 (2736.0-8343.6)	0.015
IL-1 α pg/ml	<sup>i</sup> 34.1 (8.7-00)d	14.7 (5.3-00)	18.7 (17.4-00)	14.7 (7.9-33.6)	0.01
IL-1 beta pg/ml	-	-	-	-	-
IL-6 pg/ml	-	-	-	-	-
IL-8 pg/ml	<sup>j</sup> 4.0 (3.3-4.2)	6.0 (4.2-7.5)	5.8 (4.7-6.4)	4.9 (3.9-7.6)	0.015
IL-10 pg/ml	0.4 (0.3-0.6)	0.5 (0.4-0.6)	0.4 (0.4-0.5)	0.4 (0.3-0.5)	NS
IL-12 pg/ml	-	18 (0-88.0)	2.5 (0-5.7)	3.0 (0-16.2)	-

<sup>a</sup>p<0.003 between HIV-ve control vs. TN, PI & NNRTI;

<sup>b</sup>p<0.006 between HIV-ve control vs. TN, PI & NNRTI; <sup>c</sup>p<0.05 between NNRTI vs. TN & PI

<sup>d</sup>p<0.0001 between HIV-ve controls vs. HIV positive groups; <sup>e</sup>p<0.01 between TN vs. PI and NNRTI group.

<sup>f</sup>P<0.03 between TN vs. control, PI & NNRTI <sup>g</sup>p<0.01 between PI vs. HIV-ve control, TN & NNRTI and <sup>h</sup>p<0.001 between TN vs. HIV-ve control. <sup>i</sup>P<0.01 between HIV-ve control vs. TN, PI & NNRTI; <sup>j</sup>p<0.01 between PI vs. HIV-ve control & NNRTI and TN VS. HIV-ve control & NNRTI

**5.3.4. Relation between apo-B kinetics, body fat distribution, HOMA<sub>IR</sub> and adipocytokines** (table 5.2- 5.8)

There was a negative correlation of adiponectin with HOMA<sub>IR</sub> ( $r=0.4$ ,  $p<0.0001$ ) and positive correlation with limb fat/LBM ( $r=0.6$ ,  $p<0.0001$ , figure 5.1 and table 5.2) and with total fat/LBM ( $r=0.4$ ,  $p<0.003$ , figure 5.2 and table 5.2). Leptin correlated with total fat/LBM ( $r=0.87$ ,  $p<0.0001$ ) and limb fat/LBM ( $r=0.79$ ,  $p<0.0001$ , table 5.2).

Adiponectin correlated positively with serum HDL-cholesterol ( $r=0.6$ ,  $p<0.0001$ , table 5.3), VLDL, IDL and LDL apo-B FCR ( $r=0.5$ ,  $0.5$ ,  $0.4$ ,  $p<0.0001$ ,  $0.0001$  and  $0.004$  respectively, table 5.4 and figure 5.3, 5.4, 5.6 respectively) and negatively with serum TG [ $(r=0.53, p<0.0001)$  table 5.3], VLDL, IDL and LDL apo-B pool size ( $r=0.46$ ,  $0.3$ ,  $0.42$ ,  $p<0.001$ ,  $0.01$  and  $0.001$ , table 5.5, 5.6 and 5.7 respectively), VLDL TG, VLDL-C, VLDL residence time ( $r=0.48$ ,  $0.5$ ,  $0.46$   $p<0.0001$ , table 5.5), IDL-C, IDL residence time ( $r=0.44$ ,  $0.5$ ,  $p<0.0001$  table 5.6), LDL-C, LDL-RT ( $r=0.25$ ,  $0.36$ ,  $p<0.04$ ,  $0.004$  respectively, table 5.7).

Adiponectin correlated positively with leptin ( $r=0.26$ ,  $p<0.01$ ), negatively with IL-8 [ $(r=0.3$ ,  $p<0.01$ , table 5.2) and TNF-R1 ( $r=0.3$ ,  $p<0.03$ , table 5.8).

Table 5.2: Correlations between adiponectin, HOMA, leptin, IL-8 and fat distribution (Spearman)

		Serum adiponectin (ug/ml)	HOMA <sub>IR</sub> (insulin.glucose /22.5)	Leptin (ng/ml)	IL-8 (pg/ml)	Total fat / lean mass	Limb fat / lean mass
Serum adiponectin (ug/ml)	Correlation Coefficient (r)		-.43(**)	.26(*)	-.30(*)	.40(**)	.60(**)
	Sig. (2-tailed)		.000	.032	.017	.003	.000
HOMA <sub>IR</sub> (insulin.glucose/22. 5)	Correlation Coefficient (r)	-.43(**)		.293(*)	-.079	.199	.013
	Sig. (2-tailed)	.000		.017	.545	.112	.921
Leptin (ng/ml)	Correlation Coefficient (r)	.26(*)	.29(*)		-.328(**)	.874(**)	.787(**)
	Sig. (2-tailed)	.032	.017		.009	.000	.000
IL-8 (pg/ml)	Correlation Coefficient (r)	-.30(*)	-.07	-.328(**)		-.323(*)	-.398(**)
	Sig. (2-tailed)	.017	.54	.009		.011	.001
Total fat / lean mass	Correlation Coefficient (r)	.40(**)	.2	.874(**)	-.323(*)		.913(**)
	Sig. (2-tailed)	.003	.112	.000	.011		.000
Limb fat / lean mass	Correlation Coefficient (r)	.60(**)	.013	.787(**)	-.398(**)	.913(**)	
	Sig. (2-tailed)	.000	.921	.000	.001	.000	

\*\* Correlation is significant at the 0.01 level (2-tailed). \* Correlation is significant at the 0.05 level (2-tailed).

Table 5.3: Correlations between adiponectin, HOMA<sub>IR</sub>, lipids and FFA

		Total cholesterol (mmol/L)	HDL cholesterol	Total triglyceride	Free fatty acids mmol/l	LDL cholesterol mmol/l
Serum adiponectin ug/ml	Correlation Coefficient (r)	-.251(*)	.60(**)	-.532(**)	.093	-.255(*)
	Sig. (2-tailed)	.043	.000	.000	.458	.044
HOMA <sub>IR</sub> (insulin.glucose /22.5)	Correlation Coefficient (r)	.193	-.413(**)	.373(**)	-.086	.128
	Sig. (2-tailed)	.120	.001	.002	.495	.316

\*\* Correlation is significant at the 0.01 level (2-tailed). \* Correlation is significant at the 0.05 level (2-tailed).

Table 5.4: Correlations between adiponectin, HOMA<sub>IR</sub>, apo-B FCR and fat distribution

		Serum adiponectin (ug/ml)	HOMA <sub>IR</sub> (insulin.glucose /22.5)	VLDL FCR (pools/day)	IDL FCR (pools/day)	LDL FCR (pools/day)
Serum adiponectin (ug/ml)	Correlation Coefficient(r)		-.432(**)	.50(**)	.50(**)	.40(**)
	Sig. (2-tailed)		.000	.000	.000	.004
HOMA <sub>IR</sub> (insulin.glucose /22.5)	Correlation Coefficient(r)	-.432(**)		-.290(*)	-.299(*)	-.334(**)
	Sig. (2-tailed)	.000		.018	.022	.008
VLDL FCR (pools/day)	Correlation Coefficient(r)	.50(**)	-.290(*)		.746(**)	.700(**)
	Sig. (2-tailed)	.000	.018		.000	.000
IDL FCR (pools /day)	Correlation Coefficient(r)	.50(**)	-.299(*)	.746(**)		.557(**)
	Sig. (2-tailed)	.000	.022	.000		.000
LDL FCR (pools/day)	Correlation Coefficient(r)	.40(**)	-.334(**)	.700(**)	.557(**)	
	Sig. (2-tailed)	.004	.008	.000	.000	

\*\* Correlation is significant at the 0.01 level (2-tailed). \* Correlation is significant at the 0.05 level (2-tailed).

Table 5.5: Correlations of adiponectin, HOMA<sub>IR</sub> and VLDL fractions

		VLDL FCR (pools/day)	VLDL ASR (mg/kg/day)	ApoB pool size mg	VLDL chol (mmol/l)	VLDL triglyceride (mmol/l)	VLDL residence time (h)
Serum adiponectin (ug/ml)	Correlation Coefficient (r)	.465(**)	-.182	-.461(**)	-.500(**)	-.484(**)	-.464(**)
	Sig. (2-tailed)	.000	.143	.000	.000	.000	.000
HOMA <sub>IR</sub> (insulin.glucose /22.5)	Correlation Coefficient (r)	-.290(*)	.070	.365(**)	.442(**)	.428(**)	.290(*)
	Sig. (2-tailed)	.018	.576	.003	.000	.000	.018

\*\* Correlation is significant at the 0.01 level (2-tailed). \* Correlation is significant at the 0.05 level (2-tailed).

Table 5.6: Correlations of adiponectin, HOMA<sub>IR</sub> and IDL fractions

		IDL FCR pools/day	IDL ASR (mg/kg/ day)	IDL Apo-B pool size (mg)	IDL cholesterol (mmol/l)	IDL triglyceride (mmol/l)	IDL residence time (h)
Serum adiponectin (ug/ml)	Correlation Coefficient (r)	.499(**)	.345(**)	-.307(*)	-.441(**)	-.154	-.500(**)
	Sig. (2-tailed)	.000	.008	.012	.000	.216	.000
HOMA <sub>IR</sub> (insulin.glucose /22.5)	Correlation Coefficient (r)	-.299(*)	-.233	.135	.154	.044	.304(*)
	Sig. (2-tailed)	.022	.079	.279	.218	.725	.020

\*\* Correlation is significant at the 0.01 level (2-tailed). \* Correlation is significant at the 0.05 level (2-tailed).



Table 5.7: Correlations of adiponectin, HOMA<sub>IR</sub> and LDL fractions

		LDL FCR pools/day	LDL ASR (mg/kg/ day)	LDL apoB g	LDL cholesterol g/l	LDL triglyceride mmol/l	LDL residence time (h)
Serum adiponectin ug/ml	Correlation Coefficient (r)	.361(**)	.225	-.426(**)	-.250(*)	-.205	-.361(**)
	Sig. (2-tailed)	.004	.076	.001	.048	.107	.004
HOMA <sub>IR</sub> (insulin.glucose /22.5)	Correlation Coefficient (r)	-.334(**)	-.329(**)	.314(*)	.127	.028	.335(**)
	Sig. (2-tailed)	.008	.008	.012	.323	.827	.007

\*\* Correlation is significant at the 0.01 level (2-tailed). \* Correlation is significant at the 0.05 level (2-tailed).

Table 5.8: Spearman's Correlations between adipocytokines and HOMA

		TNF- $\alpha$ (pg/ml)	TNF soluble receptor I (pg/ml)	TNF soluble receptor II (pg/ml)	IL-8 (pg/ml)	Leptin (ng/ml)
Serum adiponectin ug/ml	Correlation Coefficient (r)	-.159	-.271(*)	-.063	-.306(*)	.264(*)
	Sig. (2-tailed)	.207	.035	.653	.017	.032
HOMA <sub>IR</sub> (insulin.glucose /22.5)	Correlation Coefficient (r)	.043	.062	.043	-.079	.293(*)
	Sig. (2-tailed)	.734	.636	.759	.545	.017

\*\* Correlation is significant at the 0.01 level (2-tailed). \* Correlation is significant at the 0.05 level (2-tailed).

Figure 5.1: Correlation of adiponectin with limb fat / LBM (LBM: lean body mass)

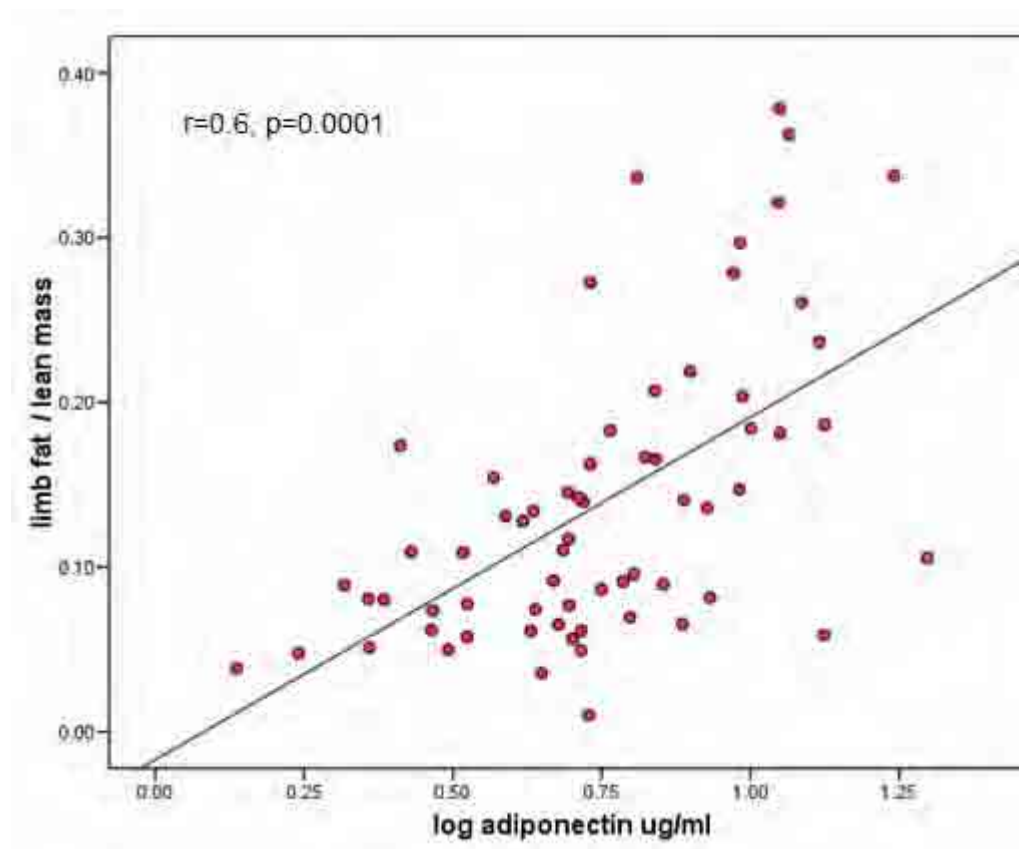


Figure 5.3: Correlation of adiponectin with VLDL apo-B FCR

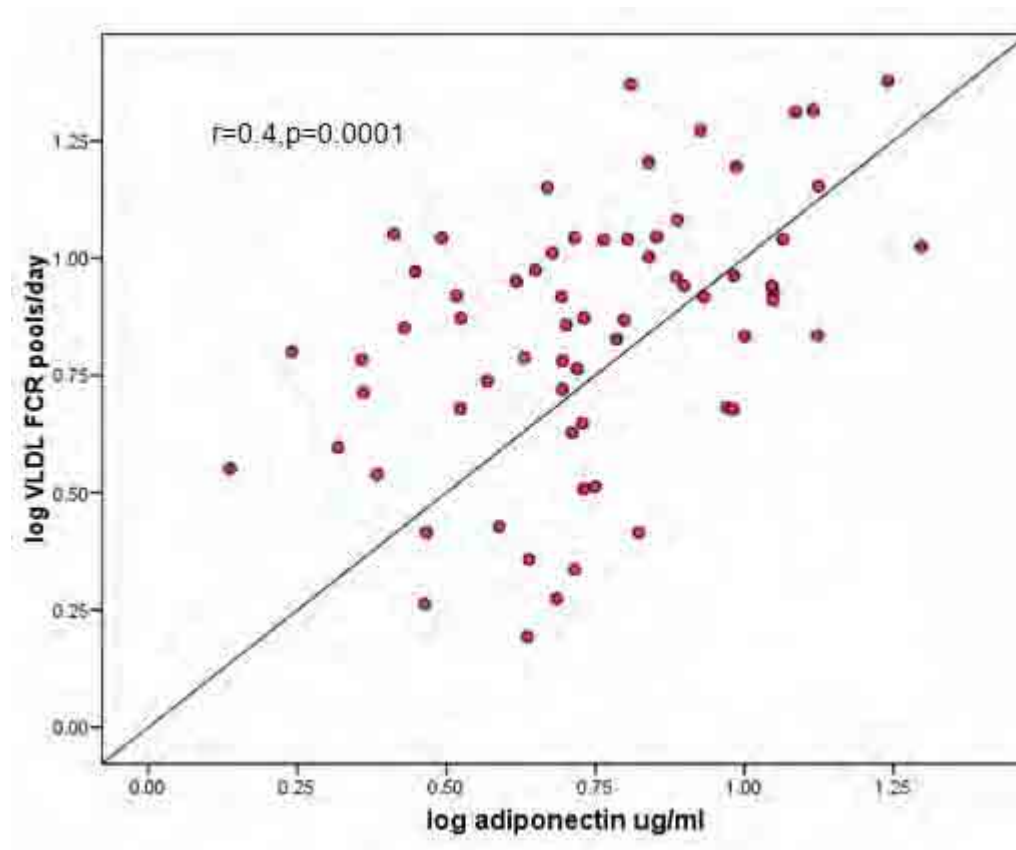


Figure 5.4: Correlation of adiponectin with IDL apo-B FCR

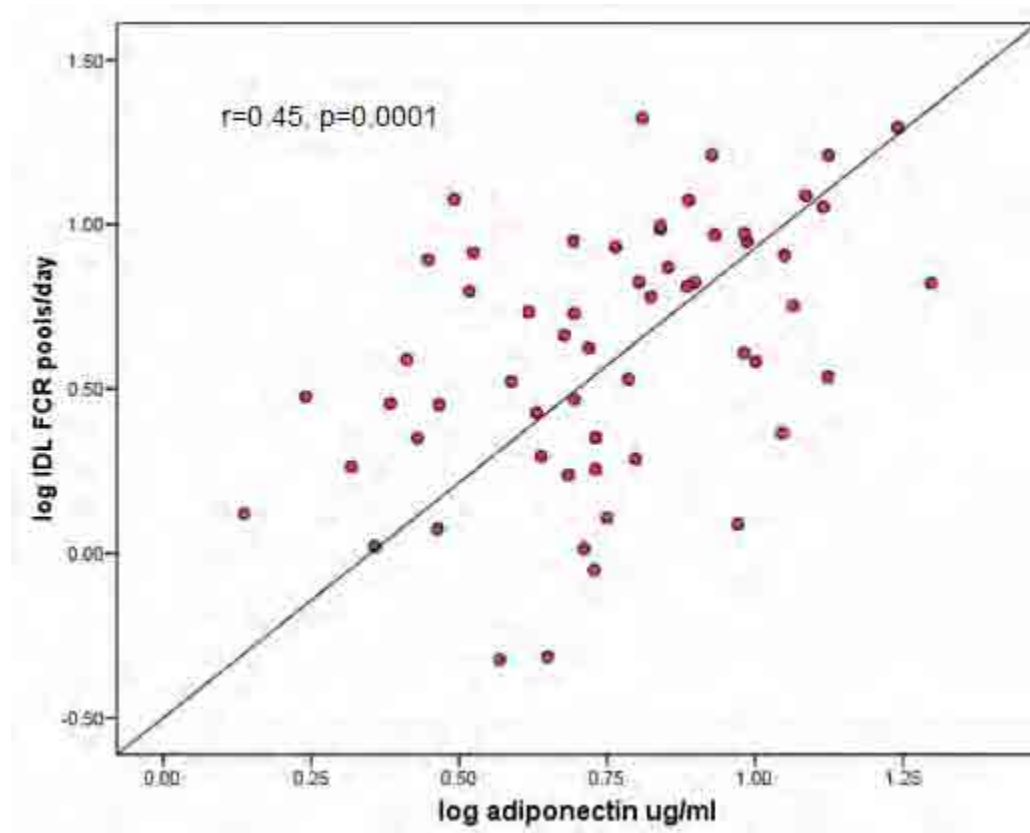
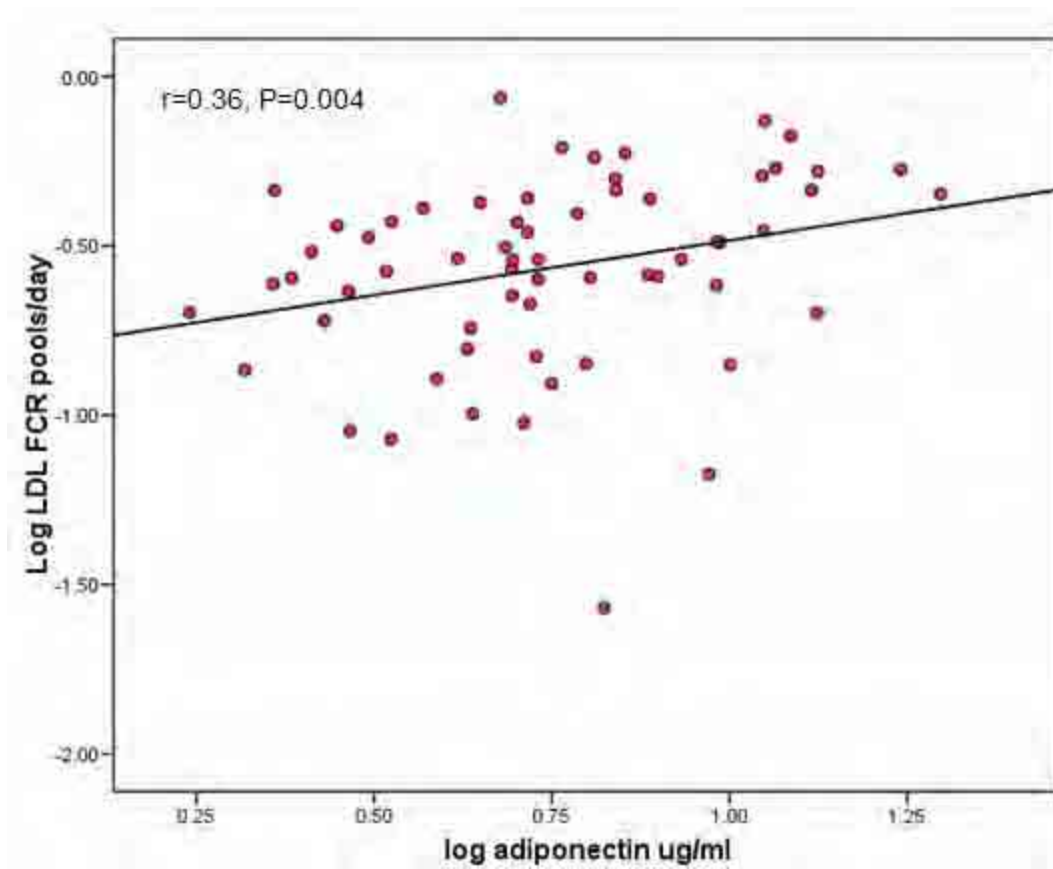


Figure 5.5: Correlation of adiponectin with LDL apo-B FCR



### **5.3.5. Linear regression analysis**

In a linear regression model adiponectin predicted VLDL, IDL and LDL apo-B FCR ( $p=0.01$ ,  $p<0.001$ ,  $p=0.02$  respectively) and HDL cholesterol ( $p<0.001$ ) and negatively predicted VLDL triglyceride ( $p=0.004$ ), IDL apo-B pool size ( $p=0.04$ ), LDL apoB pool size ( $p=0.01$ ) and total triglyceride ( $p<0.001$ ).  $HOMA_{IR}$  predicted VLDL apoB concentration ( $p=0.02$ ), VLDL cholesterol ( $p=0.002$ ) and LDL apoB pool size ( $p=0.04$ ) and negatively predicted LDL ASR ( $p=0.02$ ). Leptin predicted VLDL triglyceride ( $p=0.002$ ), IDL apo-B pool size ( $p=0.004$ ), and LDL cholesterol ( $p=0.02$ ).  $TNF-\alpha$  negatively predicted LDL ASR ( $p=0.01$ ) and HDL cholesterol ( $p=0.002$ ). IL-8 predicted VLDL triglyceride ( $p=0.042$ ), VLDL apo-B pool size ( $p=0.02$ ) and LDL triglyceride ( $p<0.001$ ). The IL-8,  $TNF-\alpha$  and the soluble receptors  $TNF-R1$  and  $TNF-R2$  did not independently predict any of the outcomes tested.

## 5.4. Discussion

This study has shown that compared to HIV negative controls, HIV infected patients taking antiretroviral medication with PI or NNRTI containing regimens, as well as treatment naïve patients, show a significant reduction in plasma adiponectin level. There was a correlation between adiponectin and limb fat loss and also with VLDL, IDL and LDL apo-B FCR. Although adiponectin levels correlated inversely with insulin resistance, measured by  $HOMA_{IR}$ , in a linear regression model which included  $HOMA_{IR}$ , adiponectin was an independent predictor of VLDL, IDL and LDL FCR and HDL cholesterol.

The decrease in adiponectin in HIV patients in the present study is in agreement with previous studies in HIV patients with lipodystrophy [387] although to our knowledge reduced adiponectin levels have not been previously reported in HIV treatment-naïve patients without lipoatrophy. It has been suggested that low adiponectin levels may lead to insulin resistance and the insulin resistance may cause the abnormalities in lipid metabolism in HIV lipodystrophy [387]. However we were unable to demonstrate a significant change in insulin resistance, as measured by  $HOMA_{IR}$ , in any of the treatment groups. Moreover, although  $HOMA_{IR}$  correlated with VLDL FCR, the effect was less striking than adiponectin and disappeared in the regression model when adiponectin was added. Leptin levels, which correlated closely with limb and total fat/LBM, did not correlate with VLDL, IDL or LDL apoB FCR. Adiponectin levels were significantly lower in the dyslipidaemic patients than patients with normal lipid levels, whereas  $HOMA_{IR}$  and leptin levels were not different. Our

results suggest a role for adiponectin in VLDL, IDL and LDL metabolism which could be independent of insulin action.

In this study peripheral fat loss was associated with low plasma adiponectin level. Atrophic adipose tissue contains poorly developed adipocytes with marked alteration in gene expression including adiponectin [290, 557]. Lihn *et al* [425] investigated the implications of cytokines for adiponectin levels by determining circulating levels of TNF- $\alpha$ , IL-6 and IL-8 as well as gene expression of these cytokines in lipodystrophic adipose tissue. Patients with lipodystrophy exhibited reduced plasma adiponectin levels and correspondingly, adiponectin mRNA levels in adipose tissue were reduced by more than 50%. The patients were insulin resistant, and a positive correlation was found between plasma adiponectin and insulin sensitivity and percent limb fat. Adipose tissue mRNA of TNF- $\alpha$ , IL-6 and IL-8 was increased in lipodystrophic subjects and both adipose tissue TNF- $\alpha$  mRNA and plasma TNF- $\alpha$  were negatively correlated to plasma adiponectin. In our study, TNF- $\alpha$  and IL-8 had negative correlation with adiponectin confirming previous studies [425, 558, 559]. Both TNF- $\alpha$  and IL-8 were higher in HIV treatment naïve patients but returned to control levels, reflecting the reduced immune activation after initiation of antiviral therapy. We were not able to comment on IL-6 as it was undetectable in most of the samples. This could be due to the assay we have used for the estimation of IL-6.

Cross-sectional studies [555, 560] revealed correlation of increased cytokine expression with current use of antiretroviral therapy, greater severity of lipoatrophy, and a greater degree of macrophage infiltration in



adipose tissue. More macrophage infiltration, cytokine expression, and mtDNA depletion were noted with stavudine or zidovudine therapy but not following initiation of abacavir [561]. No significant effect of protease inhibitor therapy was detected. Switching from stavudine or zidovudine was associated with increased mtDNA content and reduced macrophage numbers and cytokine expression, although without improvement in body fat mass. The inflammatory cytokines produced by macrophages, which are likely to be present to phagocytose dead or dying adipocytes, may exacerbate lipoatrophy[561].

Since VLDL, IDL and LDL apo-B fractional clearance rate correlated with limb fat this suggests that the primary abnormality may be lipoatrophy. It is likely that lipoatrophy leads to reduced gene expression of adiponectin. Reduced plasma adiponectin may lead to reduced clearance of VLDL, IDL and LDL apo-B by inhibiting lipoprotein lipase. Lipoprotein lipase mRNA has also been shown to be lower in HIV patients with lipodystrophy compared to patients without lipodystrophy [290] suggesting regulation may be at the level of transcription. Using real time PCR from fat biopsies Boothby *et al* [562] showed decrease in 11 $\beta$ -HSD1 mRNA and LPL gene expression in HIV patients but increase after ARV treatment alongside markers of adipocyte differentiation, lipid and glucose metabolism. The subjects did not have any lipodystrophy.

The association of LDL apoB FCR with adiponectin may also indicate a role for adiponectin in the control of LDL receptor number. By increasing the number of LDL receptors, adiponectin can increase LDL uptake by the liver and can cause increased clearance of LDL apo-B from the circulation.

Adiponectin has been found to have a positive correlation with serum HDL level [387, 563, 564]. Low adiponectin can be associated with increased hepatic lipase (HL) activity which causes breakdown of TG-rich HDL in to smaller HDL with rapid clearance from circulation. Low adiponectin in our study was associated with delayed clearance of apo-B containing lipoproteins including VLDL, IDL and LDL apo-B with a prolonged residence time resulting in low HDL level. This suggests again that adiponectin may have an important role in HDL metabolism independent of insulin resistance.

In summary we were able to demonstrate that loss of peripheral fat in HIV patients resulted in decreased plasma adiponectin and leptin but not other measured adipocytokines. Decreased adiponectin but not leptin was associated with changes in VLDL, IDL and LDL apo-B kinetics. The effect of adiponectin on apo-B kinetics was independent of insulin resistance and other cytokines.

## Chapter 6

### Concluding Discussion

#### 6.1. Overall Summary

This study, to our knowledge the largest of its kind, has investigated the apo-B metabolism in different lipoprotein components including VLDL, IDL and LDL and further investigated the relation between apo-B metabolism, regional body fat distribution, insulin resistance and adipocytokines in HIV infected-treatment naïve patients and those on established triple antiviral treatment and compared these with HIV negative controls.

In the current study, four groups of subjects were included. The cases included HIV positive treatment naïve and HIV positive treatment groups which again was divided in to PI and NNRTI group. The NNRTI group was divided further in to nevirapine and efavirenz group. The controls were HIV negative individuals. This gave the opportunity of comparing the results in different groups and also gave the opportunity of observing the effect of HIV and antiretroviral drugs on different metabolic functions including fat distribution. This is important, since a chronic HIV infection itself induces expression of inflammatory cytokines [131, 132]. It has been shown that human preadipocytes express CD4, CXCR4 and CCR5 receptors, which are necessary for entry of HIV into inflammatory host cells viruses [22, 23]. Adipocytes at least *in vitro*, can actually express viral proteins [565]. Therefore the mere presence of HIV may influence gene expression in adipocytes and influence on fat distribution and metabolism as well. When results were compared between HIV-infected

groups and two different HAART-treated groups, these potential interferences have been avoided.

The subjects were matched for age, ethnicity, BMI, and also for gender with the exception of the control group where number of female subjects was higher. However, the results of the study did not change when gender was included as a covariate in the analyses.

We have shown that HIV infected patients taking antiretroviral medication with PI or NNRTI containing regimens, with mild dyslipidaemia, show a significant reduction in VLDL, IDL and LDL apoB FCR and consequently a longer residence time compared to HIV negative controls. Moreover these studies have shown that patients on antiretroviral treatment had a significantly lower peripheral fat content than HIV negative and HIV positive treatment naïve controls. In all subjects there was a positive correlation between VLDL, IDL and LDL apoB FCR and peripheral fat. While treatment naïve patients were not significantly different from the control subjects in terms of serum triglyceride and total cholesterol, the apoB kinetics, or regional fat distribution they exhibited, had a trend in the same direction as the antiretroviral treated patients in almost all the variables measured. There were no significant differences between PI and NVP or EFV groups when compared to one another. However, compared to the control subjects PI-treated patients exhibited raised serum triglyceride, VLDL-TG and IDL TG and IDL-cholesterol but not exhibited by NVP or EFV treatment groups.

This study has further shown that HIV infected patients taking antiretroviral medication with PI or NNRTI containing regimens, as well as treatment naïve patients, had a significant reduction in adiponectin

compared to HIV negative controls. There was a strong correlation between adiponectin and limb fat loss and also with VLDL, IDL and LDL apo-B FCR and serum HDL-cholesterol.

## **6.2. Fat distribution**

The change in fat distribution which follows antiretroviral treatment is a complex interrelation between peripheral fat loss and visceral fat accumulation [76, 205, 566, 567]. Some host related risk factors for fat distribution apart from antiretroviral therapy have been identified in different studies [221, 223, 245]. In this study we did not find any effect of host related factors including gender, age, ethnicity, body mass index when they were used as covariates.

There is also evidence that HIV infection itself may cause some subcutaneous fat loss [250-252]. The baseline viral load or viral suppression, baseline CD-4 count or change of CD-4 count after treatment did not have any effect on our result. Pro-inflammatory cytokines including TNF- $\alpha$  may have effect on fat loss [277]. In our study the cytokines including TNF- $\alpha$ , IL-8 did not have any correlation with fat loss in the periphery or fat accumulation in the trunk. Our study showed peripheral fat was reduced to a similar extent in all the HIV treatment groups including patients on NNRTI-containing antiretroviral regimens despite different time exposure to anti-retroviral therapy. Although PI use has been shown to contribute to the central fat accumulation, results from our study did not show any difference in trunk fat between different groups. The fat loss due to antiretroviral therapy is thought to be mainly the result of the NRTI backbone of HAART regimens through their interference with mitochondrial DNA polymerase-gamma[213, 223, 568].

In the ACTG 5142 study [172] patients on NNRTI arm had more peripheral fat loss and PI had less. Further analysis of the same study [202] however, showed that the fat loss was predicted by thymidine analogues, baseline CD 4 count and probably not by NNRTIs. In another study [203] compared to ritonavir boosted PI monotherapy, lipoatrophy was significantly higher in the efavirenz, zidovudine and lamivudine combination treatment arm. However, the increased lipoatrophy could be due to the effect of zidovudine in the efavirenz arm. On the other hand the ritonavir boosted PI may have protective effect on fat loss [327]. In relation to body fat changes the interaction of NRTIs and PIs and recently NNRTIs is considered to be the dominant predictor of subcutaneous fat loss and visceral fat accumulation [569]. Nolan *et al* [76] suggested that interaction of different classes of antiretrovirals have more effect in fat distribution than any single class. Most of the patients in our study were on thymidine analogue as nucleoside backbone and the absence of changes in fat distribution in the treatment group might be a combined effect of the thymidine and non-thymidine analogues with PI and NNRTI.

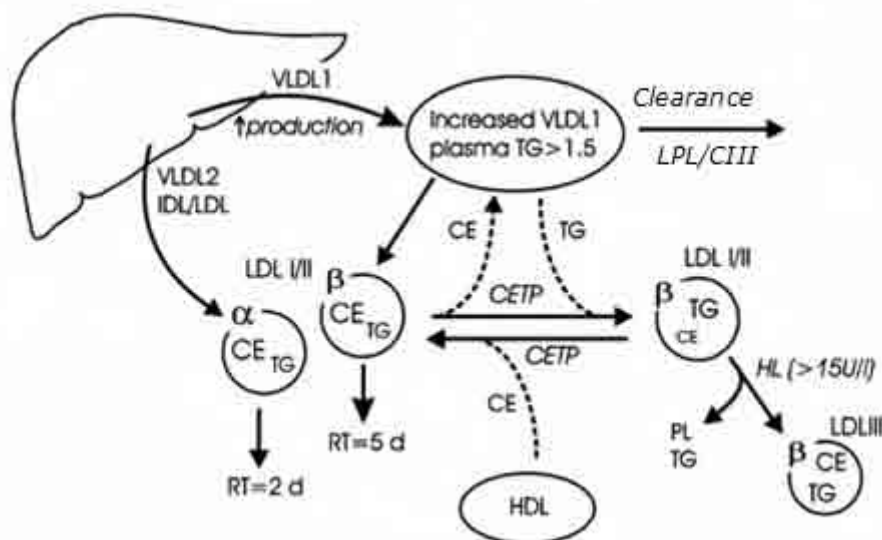
### **6.3. Dyslipidaemia**

The dyslipidaemia in the patients in our study was less marked than in many previous studies, many of which had been performed in patients taking PI-containing medication. Efavirenz containing HAART regimens are also reported to raise serum cholesterol and triglyceride levels while NVP containing regimens appear to have a better lipid profile than PI and EFV [176]. In this study PI-containing regimens found to have a greater dyslipidaemic effect compared to NNRTI in particular causing an increase

in serum total TG, and TG containing apo-b lipoproteins including VLDL and IDL -TG and LDL-TG concentration. The effect becomes more obvious in ritonavir containing PI regimens.

The nature of the apo-B containing particle secreted from the liver is related to the plasma FFA and triglyceride level. At low levels of plasma triglyceride (<1.0 mmol/l) a substantial portion (up to 50%) of apoB is released as VLDL of smaller size, also known as VLDL2 and directly as IDL or LDL [102] but at high normal levels of TG more than 90% of apoB is secreted in the form of TG rich VLDL, also known as VLDL1.

Figure 6.1: Two metabolically distinct pools in LDL ( $\alpha$  and  $\beta$ ) arise from different sources (adapted from Packard *et al* [570]). PL: phospholipid; CE: cholesteryl ester; TG: triglyceride; HE: hepatic lipase; LPL: lipoprotein lipase; CETP: cholesteryl ester transfer protein; RT: residence time; CIII: apolipoprotein CIII.



Kinetic evidence suggests that there are two distinct metabolic LDL pool in plasma ( $\alpha$  and  $\beta$ ) and the two pools arise from different sources. The two LDL species have substantially differing residence times (RT) in the circulation [570]). Pool  $\alpha$  is the major species detected by

multicompartmental modelling of subjects with low normal plasma triglyceride levels and is rapidly cleared by receptors. The LDL with the kinetic properties of longlived pool  $\beta$  has been shown to be the product of larger VLDL (VLDL1) delipidation [570]. Hence generation of LDL of smaller particle size is favoured when plasma triglyceride is high and hepatic lipase (HL) is in the normal range. When plasma TG levels are high larger VLDL (VLDL 1) are the main species and they are catabolised either to smaller VLDL (or VLDL 2), IDL and LDL by the action of LPL and the pathway leads to the formation of TG rich LDL (pool B, figure 6.1). The TG rich LDL becomes smaller by the action of HL taking longer time in the clearance, leaving with time an increasing proportion of long-lived pool  $\beta$ , a species with a relatively low affinity for receptors [571].

In our study PI-containing regimens had higher serum total TG including VLDL-TG, and IDL -TG and LDL-TG concentration compared to NNRTI. However, the LDL-C concentration was similar and also the particle size of different apo-B containing lipoproteins was similar. The particle size was different in another study [462]. The apo-b containing lipoprotein particle size can be measured directly by other techniques that could give a more accurate estimation than the indirect method we have used in our study. Using nuclear magnetic resonance (NMR) method Riddler *et al* [572] measured the size of LDL particles and has shown that the LDL particle size can be different in HIV patients compared to HIV negative patients and patients on ART have more smaller LDL particles.

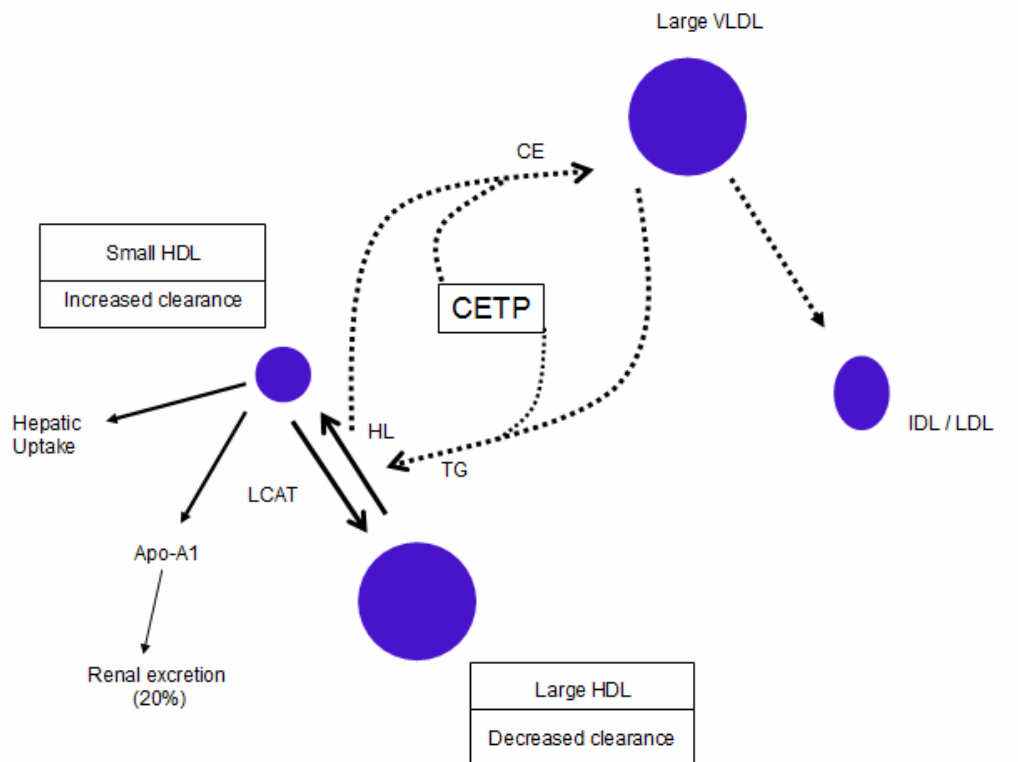
The dyslipidaemic effect was more marked in patients using ritonavir containing PI regimen with more delay in clearance of VLDL, IDL and LDL-apo-B and increase in VLDL, IDI and LDL apo-B pool size. This has already



been described in other studies [130]. Delayed clearance of apo-B or prolonged residence time exposes the apo-B for oxidation [463], but in our study the oxidised LDL-apoB was similar in all the groups. This again could be different assay we have used in our study.

This study, demonstrates that the mild dyslipidaemia in the treatment groups is due to a decrease in VLDL, IDL and LDL apo-B FCR. The resulting increased residence time will allow increased exchange of triglyceride from apoB containing lipoproteins with HDL and LDL cholesterol (figure 1.6b, 6.2). This could explain the reduced HDL cholesterol seen in all our HIV groups.

Figure 6.2: HDL metabolism through cholesterol ester transfer protein (CETP) exchange [104]. CE: cholesteryl ester; HL: hepatic lipase; LCAT: lecithine cholesterol acyle transferase



#### 6.4. Relation of Fat Distribution and Dyslipidaemia

Association of metabolic abnormalities with excess adiposity has been demonstrated in several previous studies [474-477]. Excess intra-abdominal, particularly visceral rather than peripheral or subcutaneous fat is more strongly associated with diabetes, hypertension and cardiovascular disease, indicating wider metabolic differences within the different adipose tissue regions. In our study the decrease in VLDL, IDL and LDL apoB FCR was strongly associated with peripheral fat loss. In a

regression model, regional fat changes including the peripheral fat losses predicted VLDL, IDL and LDL apoB FCR and VLDL, IDL and LDL cholesterol concentrations. These findings suggest that there may be common mechanisms shared between the regional fat redistribution and the metabolism of apoB-containing lipoproteins. This may be related to an interference of antiretroviral treatment with lipoprotein lipase which is or may be closely linked to the lipodystrophy through yet undefined mechanism. This is supported by the finding in another study [290] that lipoprotein lipase mRNA was lower in HIV patients with lipodystrophy compared to patients with no lipodystrophy. HIV infection itself has also been reported to reduce lipoprotein lipase and impair hepatic lipase activity [132]. The trend towards a reduction in VLDL, IDL and LDL apoB FCR and in crease in VLDL, IDL and LDL residence time and a significant increase in VLDL and IDL triglyceride in HIV treatment naive patients compared to controls would be in keeping with these reports. While short term exposure of healthy volunteers to PI has no effect on lipoprotein lipase activity it has been shown to reduce hepatic lipase [194]. Longer treatment with PI-containing HAART regimens is reported to result in reduced lipoprotein lipase activity [573]. PI treatment and lipodystrophy has been previously shown to be associated with insulin resistance [154]. This too may result in impairment in lipoprotein lipase activity. However, the activity of hepatic lipase could be altered rather than diminished as it has reciprocal relationship with lipoprotein lipase activity as discussed earlier in this chapter.

### **6.5. Insulin Resistance**

Prevalence of insulin resistance in HIV patients is higher compared to age and sex matched non-HIV population [312]. Insulin resistance is higher in patients on PIs [488]. We were unable to demonstrate a significant change in insulin resistance as measured by HOMA<sub>IR</sub> in any of the treatment groups. However, in a regression model insulin sensitivity predicted VLDL apoB FCR, while insulin resistance predicted apo-B pool size, cholesterol and triglyceride content and IDL ASR. This could possibly be mediated by the action of insulin on lipoprotein lipase activity. Central obesity and increased visceral fat is strongly associated with insulin resistance [574-576]. The failure to find a significant change in insulin resistance between groups in the present study may be explained by the lack of a significant change in trunk fat or the relatively small numbers in our study. However, when the groups were combined there was a highly significant negative correlation between trunk fat and HOMA. In the present study the visceral fat was not measured separately from trunk fat. Visceral adiposity is a better predictor of insulin resistance than trunk fat and subcutaneous adipose tissue in the abdominal wall may not have any association with insulin resistance. Interestingly trunk fat, VLDL apoB FCR and apoB concentration, FFA, and HOMA were significantly higher in patients with dyslipidaemia (cholesterol  $\geq 6$  mmol/l or triglyceride  $\geq 2.3$  mmol/l) compared to those without. The association between visceral fat and insulin resistance and dyslipidaemia is well-documented [475]. Dyslipidaemia is associated with insulin resistance and trunk fat accumulation. However, mild dyslipidaemia in the absence of significant insulin resistance may not be affected by trunk fat accumulation.

The LDL concentration was not increased in the treatment arm or not decreased in the treatment naïve arm or decreased compared to control subjects. This is dissimilar to many other studies and could be due to the fact that we have excluded patients who were unable to stop lipid lowering treatment at the time of the study. However, the variation in LDL concentration is not as common as in the other lipid parameters in HIV patients. The LDL cholesterol concentration is determined by the synthesis and clearance of LDL-cholesterol and a decrease in both LDL ASR and LDL FCR will result a normal concentration as we found in our study in all HIV patient groups.

The decrease in LDL apoB ASR and FCR was greater in the PI group, particularly in patients treated with ritonavir. This has already been well documented in previous studies which have shown ritonavir to have a greater lipodystrophic and dyslipidaemic effect than other PIs [577]. In our study, the LDL kinetics were not different in the PI and NNRTI group compared to treatment naïve (TN) group. But when the patients in the PI group were subdivided into those treated with ritonavir and those treated with other PIs, the abnormal LDL kinetics were exacerbated in the ritonavir treatment arm. The low level of LDL apo-b FCR in ritonavir treated arm may be due to defective binding of LDL to the LDL receptor [473] or reduction of LDL receptor number in the patient group.

However, a study of treated and untreated HIV patients with and without lipodystrophy showed that HIV-lipodystrophy was associated with a lower expression of LDL receptors and was not related to PI treatment [193].

Hence other mechanisms may also play a role. While hepatic lipase contributes to the remodelling of apo-B containing lipoproteins it also participates with surface proteoglycans and LDL receptor like protein (LRP) as a ligand for the hepatic uptake of apo-B containing remnant lipoproteins and LDL[578]. Decreased hepatic lipase activity may thus contribute to the decrease in LDL FCR. It has also been suggested that protease inhibitors may inhibit LRP since the catalytic region of HIV-1 protease, to which protease inhibitors bind, has approximately 60% homology to regions within LRP [181], but this theory has been subsequently challenged and probably not tenable any more. Since LDL FCR was not different in the PI treated patients from NNRTI or the treatment naïve group this is unlikely to be the major mechanism for the decrease in LDL FCR seen in the present study.

The LDL receptor is regulated by the cholesterol content of cells but is also under hormonal control both directly and indirectly by altering cholesterol content and metabolism. There is evidence that insulin up regulates the LDL receptor [579]. A previous study [580] has shown that the expression of LDL receptors is reduced in patients with type 2 diabetes with poor metabolic control and expression is increased after insulin treatment [581]. This provides an explanation for the decrease in LDL FCR observed in poorly controlled type 2 diabetic subjects and its normalisation in insulin-treated patients [580]. Although steady state insulin resistance measured by  $HOMA_{IR}$  was not significantly different between groups in the present study,  $HOMA_{IR}$  values within each group were very variable. The negative correlation between  $HOMA_{IR}$  and LDL FCR in the current study

suggests that insulin resistance, which may down regulate LDL receptors, may be one mechanism for the observed decrease in LDL FCR.

### **6.6. Adipocytokines**

Association of different cytokines with insulin resistance in HIV patients has already been described in previous studies. Some studies have shown association of low adiponectin with lipodystrophy in HIV patients [233, 387, 405, 582, 583], but to our knowledge, low diponectin in the absence of lipoatrophy in treatment naïve HIV patients have not been described. However, treatment naïve patients may have fat accumulation in the liver [584] which can cause low adiponectin and we were not unable to comment on fat accumulation in the different visceral compartments. In this study we were able to show that low adiponectin had strong correlation with peripheral fat loss and with clearance of apo-B containing lipoproteins independent of insulin resistance. Like adiponectin, leptin was low in HIV patients and had correlation with fat loss and insulin resistance, but not apo-B clearance rates. Adiponectin levels were significantly lower in subjects with dyslipidaemia compared to those whose with normal lipid levels, whereas leptin level and levels of other measured cytokines and HOMA<sub>IR</sub> were not different.

Adiponectin gene expression can be impaired in atrophic adipose tissue [425]. In our study the strong correlation of VLDL, IDL and LDL apoB fractional catabolic rate (FCR) with limb fat suggests that the primary abnormality could be lipoatrophy that leads to reduction of adiponectin gene expression resulting low adiponectin level in plasma. Previous studies have shown an association of decreased lipoprotein lipase (LPL)

activity with low plasma adiponectin. The association was independent of systemic inflammation and insulin resistance, suggesting that LPL may represent a link between low adiponectin levels and dyslipidemia [520, 585]. The relation of adiponectin with hepatic lipase is different and a significant inverse association between adiponectin and plasma hepatic lipase (HL) activity has been demonstrated in other study [520]. Lipases regulate the flow of the lipoprotein particles through the VLDL-IDL-LDL delipidation cascade. Large VLDL are converted to LDL by LPL and hepatic lipase (HL) operate at the opposite end of the pathway to transform IDL to LDL whereas both LPL and HL enzymes participate in the delipidation of smaller VLDL [586]. LPL and HL also facilitate receptor mediated uptake of TG rich lipoproteins [587]. Low adiponectin level may help HL to act on IDL and LDL and also their uptake by hepatic receptor [587-589]. The normal level of LDL in our study in all HIV patient groups inspite of having significant impairment of catabolism could be explained by the improved activity of hepatic lipase (HL) which is inversely related to adiponectin level.

Reduced plasma adiponectin may lead to reduced clearance of VLDL, IDL and LDL apo-B by inhibiting lipoprotein lipase. Lipoprotein lipase mRNA has also been shown to be lower in HIV patients with lipodystrophy compared to patients without lipodystrophy suggesting regulation may be at the level of transcription [290].

The association of LDL apoB FCR with adiponectin may indicate a role for adiponectin in the control of LDL receptor number. In contrast low



adiponectin level may increase the activity of hepatic lipase and help increased uptake of LDL by the available receptors.

All the patient groups in our study had low HDL-cholesterol (HDL). The metabolism of HDL-cholesterol is complex. The catabolism of HDL varies with its apolipoprotein composition and also the triglyceride (TG) or cholesterol ester (CE) content. The liver and the intestine produce nascent HDLs. Free cholesterol is acquired from macrophages and other peripheral cells and esterified by lecithin-cholesterol acyltransferase (LCAT), forming mature HDLs that can be selectively taken up by the liver via scavenger receptor class BI (SR-BI). Alternatively, HDL cholesteryl ester can be transferred by cholesteryl ester transfer protein (CETP) from HDLs to VLDLs and chylomicrons, which can then be taken up by the liver [505, 570].

The half life of TG rich HDL are shorter as this is broken down to smaller HDL and the individual apolipoproteins like A1 can catabolised to amino acids in the liver or can be excreted through kidney [590].

Low plasma adiponectin may lead to increased hepatic lipase activity [520] which may cause increased breakdown of TG rich HDL into smaller HDL which is cleared from circulation rapidly [591]. The TG rich apo-B containing will allow exchange of triglyceride for cholesterol esters with HDL, which would increase clearance providing a mechanism for the reduced HDL-cholesterol seen in both treatment naïve patients and those on antiretroviral therapy and may explain the strong correlation of adiponectin with HDL-cholesterol.

Other mechanism can be responsible for low HDL in HIV patients including patients in our study. Role of ATP-binding cassette transporter A1

(ABCA1) has been shown in patients with low HDL. Free cholesterol from the arterial wall and cell membrane are transferred to apo-A1 by ABCA1. The viral protein (nef gene protein [7]) impairs ABCA1-dependent cholesterol efflux from human macrophages ABC-A1 seems to play an important [201].

### **6.7. Limitations of the study**

The sample size may have impact on the outcome of different results. However, this was the largest study of this kind where apo-B kinetics were compared with subjects different groups.

As opposed to longitudinal or prospective study this was a cross-sectional study and the results have to be looked with some caution.

The control group had more females compared to other groups. The fat distribution in females may have over emphasized the difference in peripheral fat distribution compared to HIV positive groups. Only two subjects in the control arm had HIV test , others did not and this could be regarded as a limitation.

Body composition was measured using DEXA scan which can measure total truncal fat, but not separate the two depots. In contrast MRI and CT imaging allow depot-specific quantification of abdominal subcutaneous vs. intra-abdominal fat [492, 592]. Abdominal subcutaneous and intra-abdominal fat depots are most commonly measured using a single CT or MRI scan at the level of L4-L5.

Lack of MRI and CT imaging due to limited funding should be considered a limitation of the current study, since CT and MRI would have provided an additional measure of fat mass in the different visceral compartment,

even though a good correlation has been shown between the fat mass measured in the different regions including the total fat mass in the abdomen.

The use of different machines for DEXA scan may have some influence on the results as well. The use of Hologic and Lunar DEXA scan in different patient groups might have added to some heterogeneity of the result as the precision of the two different scans are not same. However, using the data separately did not show any effect on the outcome of the results. Fifty five subjects used the same machine and the results did not have any difference with the overall result.

We were unable to measure the other apolipoproteins, particularly apo-CI, CII and CIII which may have effect on the results. Our study did not look at the reverse cholesterol transport which may have some effect on the result as well. We measured HDL-cholesterol, but not the different fractions of HDL or the apolipoproteins namely apo-A1.

We measured adiponectin whole molecule and the effect of different fractions or dimmers and trimmers have different actions or the whole molecular assay may not reveal the function of adiponectin as a whole.

However, this study in spite of these limitations describes some important findings which may have some important impact on the understanding of the mechanism of dyslipidaemia and fat distribution in HIV patients with all these limitations

## **6.8 Conclusion**

In conclusion our data suggests that in patients with mild dyslipidaemia reduced VLDL, IDL and LDL apoB FCR and consequently an increased residence time may be a primary abnormality in lipoprotein kinetics in HIV infection, possibly consequent to body fat redistribution. Similar abnormalities were found in patients treated with either a protease inhibitor, or NNRTI containing HAART regimens. The association of apoB FCR with regional fat distribution suggests a common mechanism for lipodystrophy and abnormal VLDL, IDL and LDL metabolism. The latter may be due to a decrease in hepatic lipase, down regulation of the LDL receptor or LDL related receptor or a combination of all these. The increased residence time will lead to increased LDL oxidation and atherogenesis thus contributing to the increased risk for CHD reported in these patients.

## Chapter 7

### FUTURE DIRECTIONS

In our study we have shown that antiretroviral treatment significantly reduced VLDL, IDL and LDL apoB FCR compared to HIV negative controls. This suggests an impairment of lipoprotein lipase and hepatic lipase by antiretroviral therapy. This appears to be an accentuation of the trend seen in treatment naïve HIV-infected patients.

Cytokines may also affect lipid metabolism [131, 132, 200, 593]. TNF- $\alpha$  secreted by fat cells, has important local regulatory effects on the adipocyte [594, 595]. Interferon- $\alpha$  has also been shown to down regulate the activities of hepatic and lipoprotein lipase and cholesterol ester transfer proteins[131].

The exact role of adiponectin and other adipocyte secretory products remains to be determined, but is likely to be important. This study complements others highlighting the important role played by the adipocyte in the metabolic abnormalities in HIV infection. Previously Nolan proposed an adipocentric model [76] to explain both the lipodystrophy and metabolic abnormalities seen in antiretroviral therapy where the most powerful determinant is peripheral fat wasting, and where NRTI-induced mitochondrial toxicity interacts with PI-induced insulin resistance and impaired adipocyte maturation.

Lamivudine (3TC), emtricitabine (FTC) and the nucleotide analogue tenofovir have a less toxic effect on mtDNA, produce less dyslipidaemia [167, 170, 596] and would be expected to result in less lipodystrophy

than the thymidine analogues d4T and AZT. Similarly abacavir (ABC) causes less lipodystrophy [163, 208] and FTC may have less effect on DNA polymerase- $\gamma$  than 3TC. Thus if the lipodystrophy is an important mechanism underlying the metabolic abnormalities of HIV which is accelerated and accentuated by some ART regimens then ART regimens with the least lipodystrophic effect would be expected to cause the least long term disturbance in lipid and glucose metabolism.

A recent study [179] has shown that HIV replication alone without any influence of antiretroviral drugs enhances production of free fatty acids, low density lipoproteins and many key proteins associated in lipid synthesis, transport and metabolism. Further studies with these proteins may now shed light on how some of these proteins may be useful for early diagnosis of individuals who might be at high risk for developing lipid related disorders.

Longitudinal studies have shown that peripheral fat loss is progressive in the first two years but slows thereafter in a non linear fashion [205]. Further studies should be done to find out the changes in mitochondrial DNA and nuclear DNA ratio, mtDNA oxidative phosphorylation enzyme activity, mitochondrial uncoupling proteins (UCP) and genes involved in lipid and glucose metabolism in subcutaneous fat in patients taking AZT containing regimen compared to tenofovir or abacavir containing regimen.

Finally, the majority of studies in dyslipidaemia have examined patients with long treatment histories, making it difficult to attribute causation of metabolic abnormalities to individual drugs. It is therefore necessary for

future studies to be conducted prospectively in treatment naive patients, which will aid in the understanding of the timeframe of the development of this syndrome, as well as providing *in-vivo* support for the work conducted *in-vitro*. Further work needs to examine the role that genetic factors play in the development of dyslipidaemia using large and ethnically diverse populations.

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### **Publications arising from this thesis**

#### **Original peer reviewed journals**

1. Adiponectin levels are reduced in HIV infection and after antiretroviral treatment and correlate with apolipoprotein B clearance. Das S, Shahmanesh M, Umpleby M et al. *Diabetologia* 2006; 49:538-42
2. Antiretroviral treatment reduces very-low-density lipoprotein and intermediate-density lipoprotein apolipoprotein B fractional catabolic rate in human immunodeficiency virus-infected patients with mild dyslipidemia. Das S, Shahmanesh M, Umpleby M et al. *J Clin Endocrinol Metab.* 2005 Feb;90(2):755-60.
3. Low density lipoprotein apolipoprotein B metabolism in treatment-naïve HIV patients and patients on antiretroviral therapy. Das S, Shahmanesh M, Umpleby M et al. *Antiviral Therapy* 2005; 10:663-70

#### **Conference abstracts**

1. Pre-treatment alterations in lipoprotein metabolism predict changes in blood lipids after antiretroviral therapy. *Antiviral Therapy* 2009,14(suppl.2):A56 (presented in 10<sup>th</sup> international workshop on Adverse drug reactions and lipodystrophy, Philadelphia)
2. HIV infection results in body fat redistribution. Boothby M, Das S, Shahmanesh M et al. 11<sup>th</sup> Annual conference of the BHIVA with the BASHH, 20-23<sup>rd</sup> April, 2005, Dublin, UK; Abst: P46:26
3. Insulin resistance and apolipoprotein B (Apo-B) kinetics: a comparison of protease inhibitor, efavirenz or nevirapine containing antiretroviral regimens. Das S, Umpleby M,

## Publications

Shahmanesh M et al. 10-14<sup>th</sup> February, 2004 (presented at Sanfrancisco, USA, Abstract: 704

4. HIV infection increases VLDL apolipoprotein-B secretion and treatment with protease inhibitor, efavirenz and nevirapine decrease the clearance. Das S, Stolinski M, Jefferson W, Jackson N, Gilleran G, O'Connor M, Cramb R, Umpleby M., Shahmanesh M. 5<sup>th</sup> international workshop on Adverse drug reactions and lipodystrophy, Paris, 8-11 July, 2003, Antiviral Therapy 2003;8:L6
5. The relationship between fat redistribution and metabolic abnormalities in HIV infected adults. MSSVD / IUSTI joint Europe Spring Meeting , 12-14<sup>th</sup> June, 2003, Leeds, UK
6. The relationship between fat distribution and VLDL and IDL metabolism in HIV infected adults. Das S, Umpleby M, Shahmanesh M et al, 5<sup>th</sup> international workshop on Adverse drug reactions and lipodystrophy, Paris, 8-11 July, 2003
7. Mechanism of Dyslipidaemia in HIV patients (further study), Das S, Jackson N, Umplebey M, Gilleran G, Shahmanesh M et al. 10th Conference of Retroviruses and Opportunistic Infection, 10-14<sup>th</sup> February, 2003 at Boston, USA, Abstract: 753
8. Mechanism of dyslipidaemia in HIV infected adults, Das S, Jackson N, Umplebey M, Gilleran G, Shahmanesh M et al. Sixth International Congress on Drug Therapy in HIV Infection, 17-21 November, 2003, Glasgow, UK