Lipodystrophy and Cardiovascular Disease Risk in HIV Positive Patients Taking Antiretroviral Therapy

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of

Doctor in Medicine.

Jane Edith Mallewa

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Declaration

This work has not previously been accepted in substance for any degree and is not being currently submitted in candidature for any degree.

Signed.....

Jane Edith Mallewa **1 November 2011**

This thesis is the result of my own investigation, except where otherwise stated. Other sources are acknowledged and bibliography is appended.

Signed.....

Jane Edith Mallewa **1 November 2011**

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loan, and for the title and abstract to be made available to outside organisations.

Signed.....

Jane Edith Mallewa **1 November 2011**

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Abbreviations

3TC	Lamivudine
ABC	Abacavir
AMPK	Adenosine monophosphate-activated protein kinase
APV	Amprenavir
ART	Antiretroviral therapy
ARVs	Antiretroviral drugs
ATV	Atazanavir
AZT	Zidovudine
BHIVA	British HIV Association
CVD	Cardiovascular disease
D:A:D	Data Collection on Adverse Events of Anti-HIV Drugs
D4T	Stavudine
DDI	Didanosine
DHHS	Department of Health and Human Services
DRV	Darunavir
EACS	European AIDS Clinical Society
FPV	Fosamprenavir
FTC	Emtricitabine
GH	Growth hormone
GLUT-4	Glucose transporter-4
HAART	Highly active antiretroviral therapy
HDL-C	High density lipoprotein cholesterol
IDV	Indinavir
IHD	Ischaemic heart disease
IL-6	Interleukin-6
JBS	Joint British Societies
LA	lipoatrophy
LD	Lipodystrophy
LDL-C	Low density lipoprotein cholesterol
LPV	Lopinavir
NCEP	National Cholesterol Education Program
NFV	Nelfinavir
NHS	National Health Service
NRTI	Nucleoside reverse transcriptase inhibitor
PI	Protease inhibitor
RTV	Ritonavir
SAT	Subcutaneous adipose tissue
SQV	Saquinavir
TC	Total cholesterol
TDF	Tenofovir
TGs	Triglycerides
TNF-α	Tumour necrosis factor-α
VAT	Visceral adipose tissue

Dedication

This work would not have been possible had in not been for the tireless support from my husband, Dr Macpherson Mallewa. I dedicate this to him. The joy and richness my two children (Sungeni Michelle and Wenafwi Bright) bring into my life saw me through the difficult times when I was carrying out this work. I also dedicate this to them. Lastly but not least I thank my father and mother for being supportive and always rejoicing in my successes.

Abstract

Over the years, HIV infection has become a manageable disease with marked reduction in mortality from AIDS. However, major comorbidity due to the virus itselt and its treatments has become important. This includes development of lipodystrophy (LD) and cardiovascular disease (CVD). Up to 40% of HIV positive patients on antiretroviral therapy develop LD. CVD now accounts for up to 10% of deaths in these patients, with combination antiretroviral therapy being independently associated with a 26% relative increase in the rate of myocardial infarction per year of exposure during the first four to six years of use.

The aims of this thesis were to investigate the factors known to predispose to the development of LD and CVD namely blood lipids, insulin resistance and cytokine derangements such as tumour necrosis factor-α, interleukin-6, adiponectin, leptin, resistin and visfatin; to assess the levels of pre-existing conditions known to increase the risk factors of CVD; the actions taken by physicians in managing these conditions and to summarise the possible interactions between drugs used to treat dyslipidaemia and protease inhibitors. A population of 64 HIV-positive patients on antiretroviral therapy was studied over a 12-month period. Three groups were evaluated: those taking a thymidine based regimen (group 1), those taking a non-thymidine based regimen (group 2) and those who switched from a thymidine to a non-thymidine based regimen (group 3). Two audits were carried out involving the review of a total of 179 case-notes. A systematic review was conducted to summmarise the drug-drug interactions between protease inhibitors and statins, which are commonly used to treat dyslipidaemia.

In the longitudinal studies, the patients were virologically and immunologically controlled. Antiretroviral drugs such as thymidine analogues (stavudine and zidovudine) and protease inhibitors such as ritonavir boosted lopinavir were still being commonly used at the time the studies were conducted. LD was common, both as perceived by the patient or diagnosed and documented in the case-notes by clinicians. Pre-existing risk factors of CVD were common, with high smoking rates (up to 59%), high calculated CV risk over 10 years or pre-existing CVD (up to 15%), high lipids (up to 61%), high glucose levels (up to 13%), high blood pressure (up to 11.5%) and family history of premature ischaemic heart disease (up to 21%). Patients on abacavir showed a worsening of their calculated CV risk over 12 months. Glucose tolerance and insulin resistance were not significantly different between the three groups. TNF-α levels were higher in all groups (4.6-6.3pg/ml) than has been documented in HIV negative individuals (1.8pg/ml). IL-6 levels increased over the study period in group 3 and group 1 (2.6 pg/mL and 0.3 pg/mL respectively) and did not change in group 2. Adiponectin levels declined by 32% in group 1 and 22% group 3; leptin levels declined by 29% in group 1

while resistin levels increased by 37% in group 1 and 57% in group 3. Visfatin was undetectable in all patients.

The audits showed that in typical HIV clinics, CVD risk factors are common. In North Manchester, smoking rates amongst patients were up to 60%, dyslipidaemia was found in up to 61% of patients. Up to 21% of patients were at high risk (>20%) of developing CVD over a period of 10 years. Patients on abacavir were nearly twice as likely to have a moderate risk (10-20%) of developing CVD than those that were not on the drug (31% vs. 15.8% respectively) and also more likely to be at high risk of developing CVD than those not on the drug (33% vs. 20% respectively). Patients were on the whole appropriately investigated and managed to correct the abnormalities identified. However, in some instances, documentation was not complete, with up to 21% of case-notes having incomplete information so that CV risk percentage could not be calculated; which may have underestimated the true level of CV risk in this population. Additionally, some patients that required high levels of interventions according to existing guidelines were not managed accordingly.

The systematic review demonstrated that the pharmacokinetic changes of statins when concommittantly administered with PIs mostly depend on the extent to which the statin is metabolised by the cytochrome (CYP) P450 enzyme system. Simvastatin, being extensively metabolised by this system, rises to very high concentrations in the presence of PIs (AUC,3000%; Cmax, 500%), while atorvastatin, which is less extensively metabolised by CYP450, shows moderate increases (AUC,70%; Cmax, 122%). Rosuvastatin and pravastatin are metabolised by non-CYP pathways and therefore not expected to have any interactions with PIs. However, rosuvastatin levels rise (AUC, 200%; Cmax, 600%) while pravastatin levels drop (AUC and Cmax, 40-50%) when coadministered with some PIs. These unexpected changes could be due to interaction at the level of efflux drug transporters in the intestine and liver such as organic anion transporting polypeptides (e.g. OATP1B1), P-glycoprotein, multidrug resistance-associated protein (MRP2) and breast cancer resistance protein (BCRP). These interactions could result in reduction of the efficacy of statins, or predispose to toxicity which has resulted in deaths.

In conclusion, LD and CVD are common in HIV patients. Understanding the underlying mechanisms will help the development of management strategies for these conditions. Physicians treating these patients need to be aware of guidelines for treating these conditions, be vigilant for drug-drug interactions due to the polypharmacy that is necessary in these patients, and adopt a multidisplinary approach to manage these complex patients.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 HIV AND AIDS

1.1.1 History and epidemiology

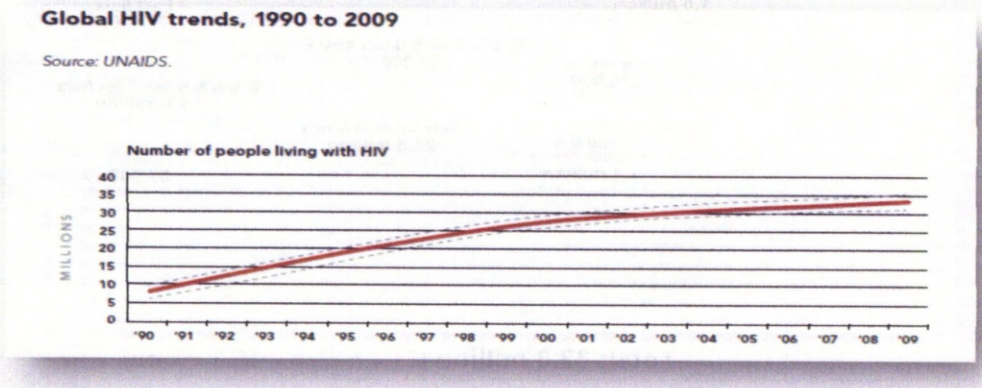
The acquired immunodeficiency syndrome (AIDS) was first described in 1981 in gay men in California in the USA who presented with opportunistic infections such as *Pneumocystis jiroveci* pneumonia or PCP (previously called *Pneumocystis carinnii)* (Gottlieb *et al.* 1981). The Centres for communicable Disease Control established a case definition of AIDS in 1982 (CDC 1982) and the virus was identified in 1983 (Barre-Sinoussi *et al.* 1983). Since 1981, the virus has rapidly spread through most countries of the world reaching pandemic proportions, with the brunt of the disease being borne by countries in Sub-Saharan Africa. The pandemic is thought to have peaked in 1999 and appears to have reached a plateau in 2007 (figure 1-1 Panel a-b). There are now fewer new infections, a decline of 19% since 1999. This is thought to be because of increased provision of antiretroviral therapy (ART) and change in behaviour including increased condom use resulting in reduction in transmission of the disease (Montaner *et al.*; Shelton *et al.* 2006; UNAIDS 2010). There are now 33.3 million people living with the disease as of 2010; and two thirds of cases being in Sub-Saharan countries (figure 1-2) (Shelton *et al.* 2006; UNAIDS 2010).

The advent of highly active antiretroviral therapy (HAART) has turned HIV infection from an almost uniformly fatal disease to a manageable chronic illness. Indeed deaths from HIV or AIDS are declining due to the scale up of

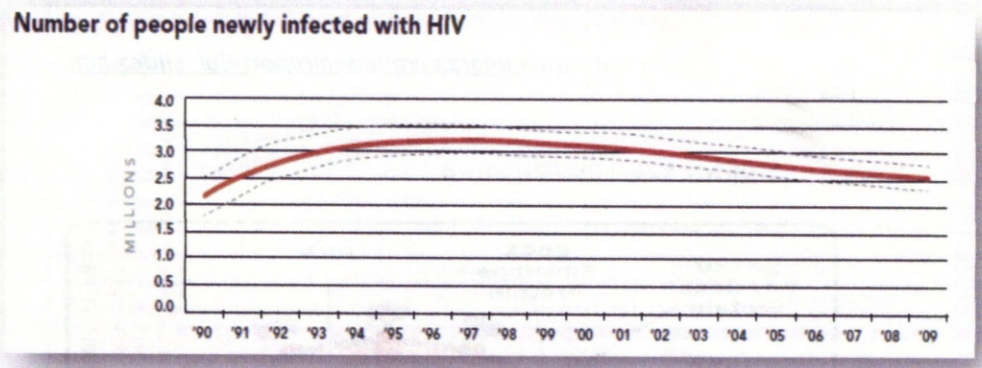
the provision of ART, particularly in developing countries (figure 1-1 Panel c) (UNAIDS 2007; UNAIDS 2010).

Figure 1-1: Epidemiology of HIV/AIDS

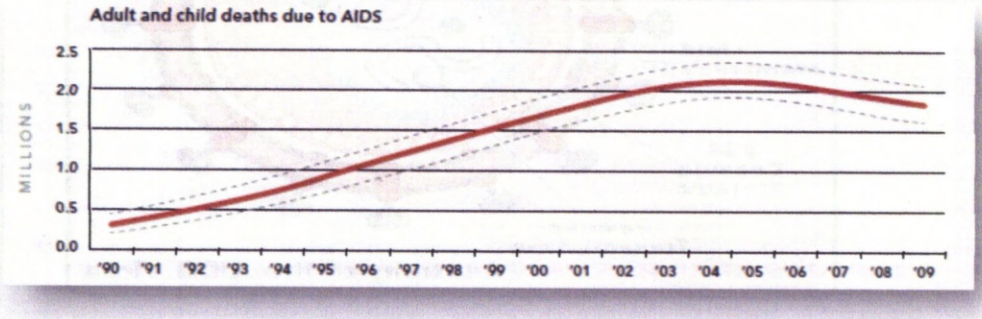
Panel (a)



Panel (b)

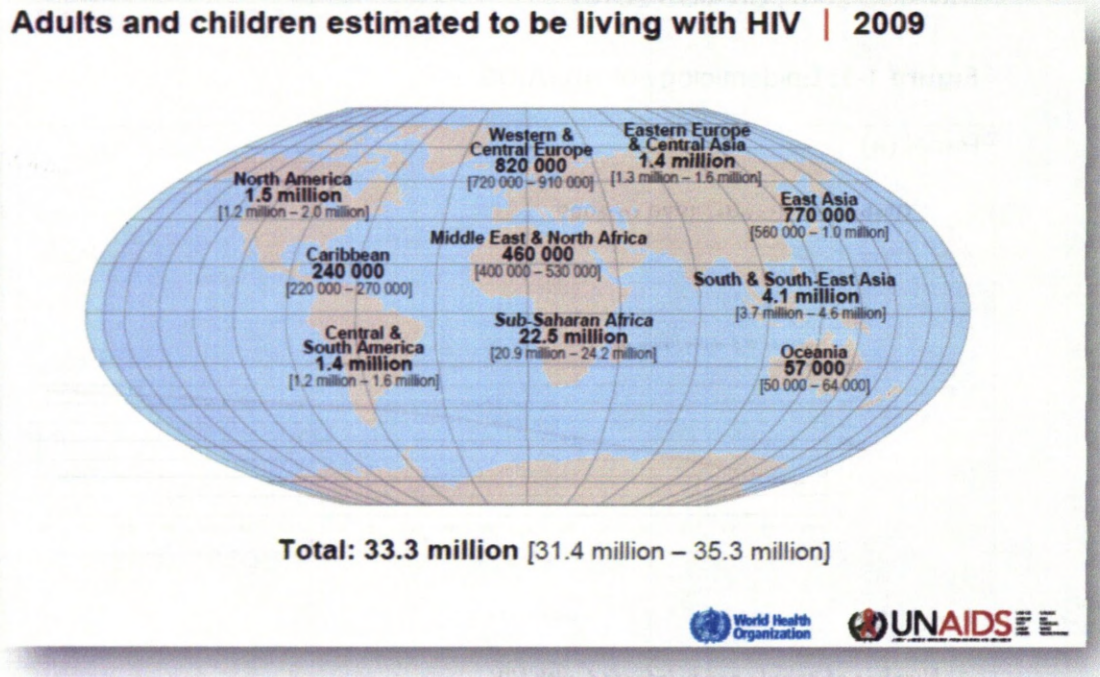


Panel (c)



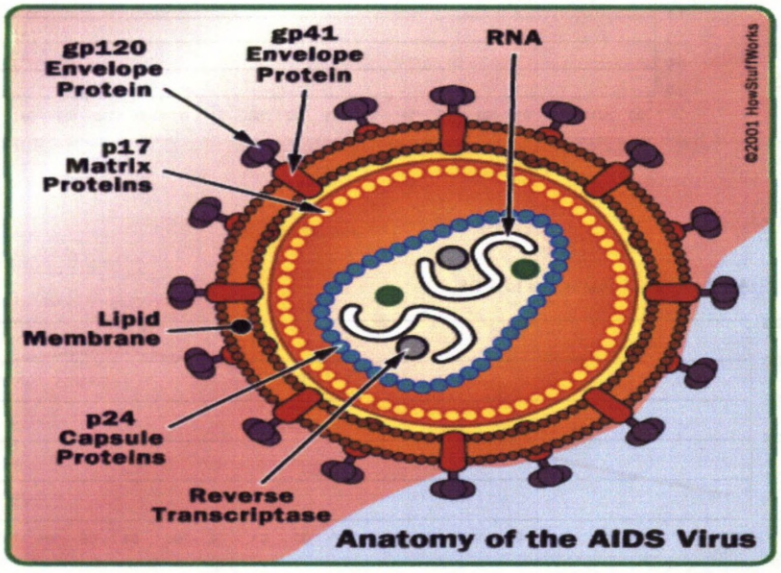
Panel a-c taken from: www.unaids.org/globalreport/Epi_slides.htm

Figure 1-2: HIV prevalence by region



Taken from: www.unaids.org/globalreport/Epi_slides.htm

Figure 1-3: HIV Structure



Adapted from http://static.howstuffworks.com/gif/aids-hiv-anatomy.gif

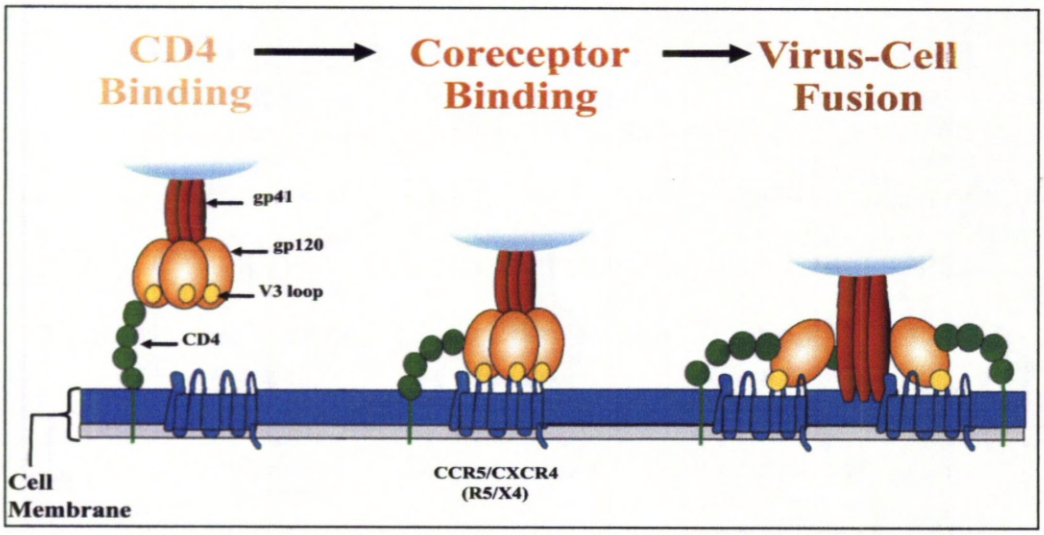
1.1.2 Pathogenesis of HIV Infection

HIV is an RNA virus (Figure 1-3), a member of the human lentivirus family which has a propensity to infect cells of the immune system (Haase 1986; Fauci 1988; Haase *et al.* 1990; Levy 1993). There are two types of HIV: HIV 1 is widespread throughout the world and is more virulent; and HIV 2, which causes a less severe disease and is confined to West Africa. Co-infection with both viruses does occur and is common in West Africa (Whittle *et al.* 1994).

HIV is acquired via sexual intercourse or parenterally via intravenous drug use or blood products. HIV gains entry into the host cell via attachment of its surface glycoprotein (gp120) to the CD4 receptor on cells bearing this receptor namely the helper T lymphocytes, follicular dendritic cells (in lymph nodes), monocytes, macrophages and antigen presenting cells such as dendritic cells (in blood and mucous membranes) and langerhans cells (in skin) (Pope *et al.* 1994). The virus also uses chemokine coreceptors namely CCR5 and CXCR4 to attach to the target cells (Figure 1-4) (Ray and Doms 2006; Sierra *et al.* 2007). Within the cell cytoplasm the virus uses its reverse transcriptase to transcribe its RNA into double stranded DNA which is then integrated into the host chromosomes using viral integrase enzyme within the host cell nucleus (Figure 1-5). Once the provirus DNA has integrated, the cell machinery is then used to produce viral polyproteins. Viral protease enzyme cleaves the large Gag-Pol polyprotein to yield definitive components of the virus which are then assembled and the mature virus buds out of the host cell (Figure 1-6) (Gotte *et al.* 1999; Van Maele and Debyser 2005; Vandegraaff and Engelman 2007;

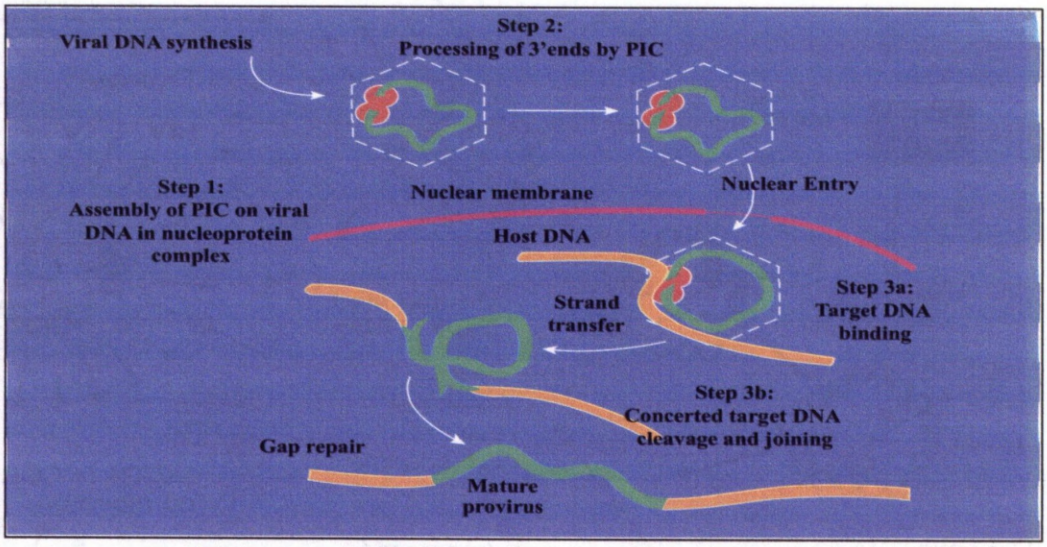
Basu *et al.* 2008; Delelis *et al.* 2008). The virus initially multiplies within the mucosal membrane, e.g. the genital tract or rectum, spreads to and multiplies within regional lymph nodes followed by systemic spread within 6-10 days (Miller *et al.* 2005; Morrow *et al.* 2007) (Figure 1-7).

Figure 1-4: HIV entry



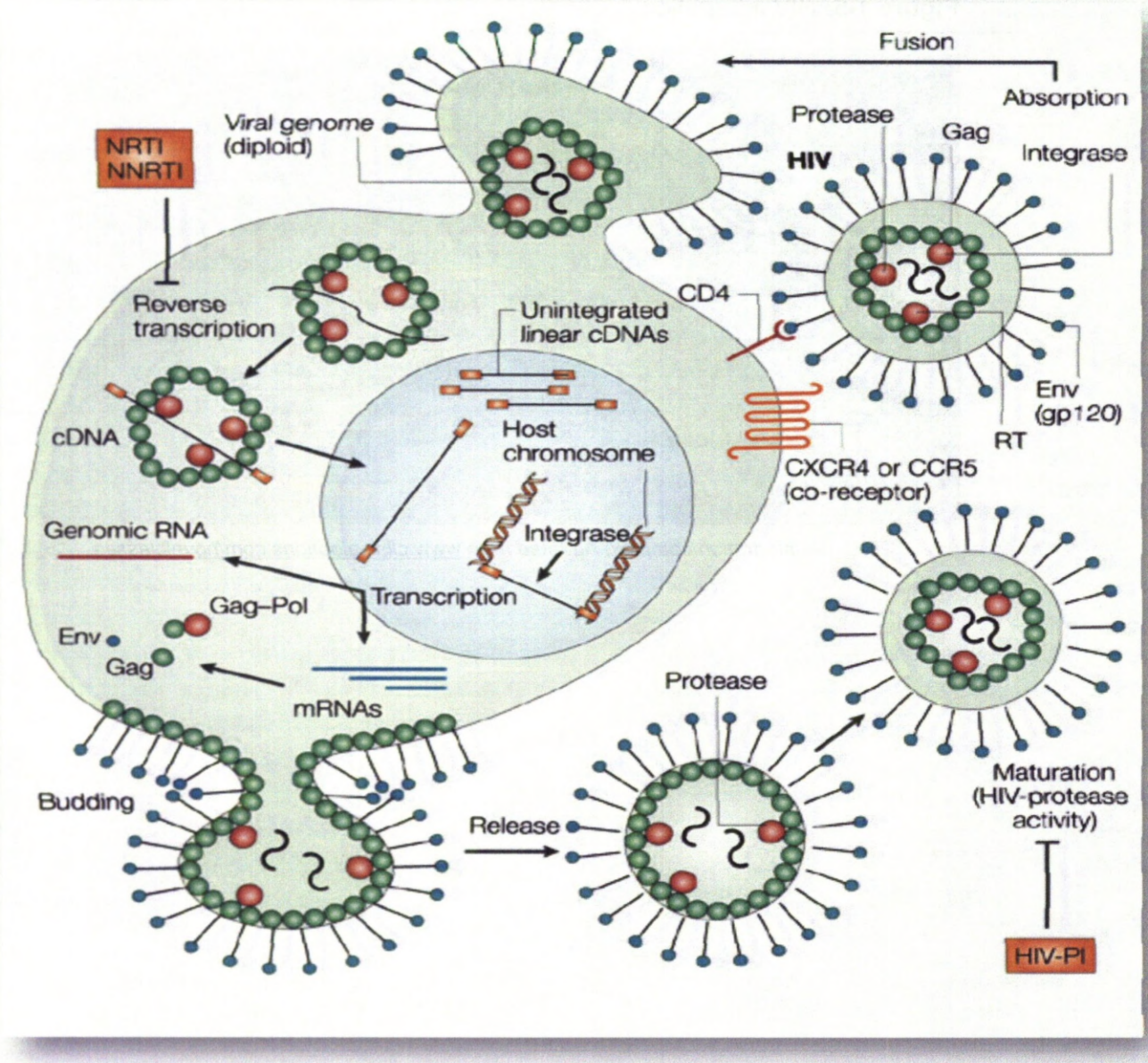
Adapted from www.clinicaloptions.com/novelclasses

Figure 1-5: HIV Integration



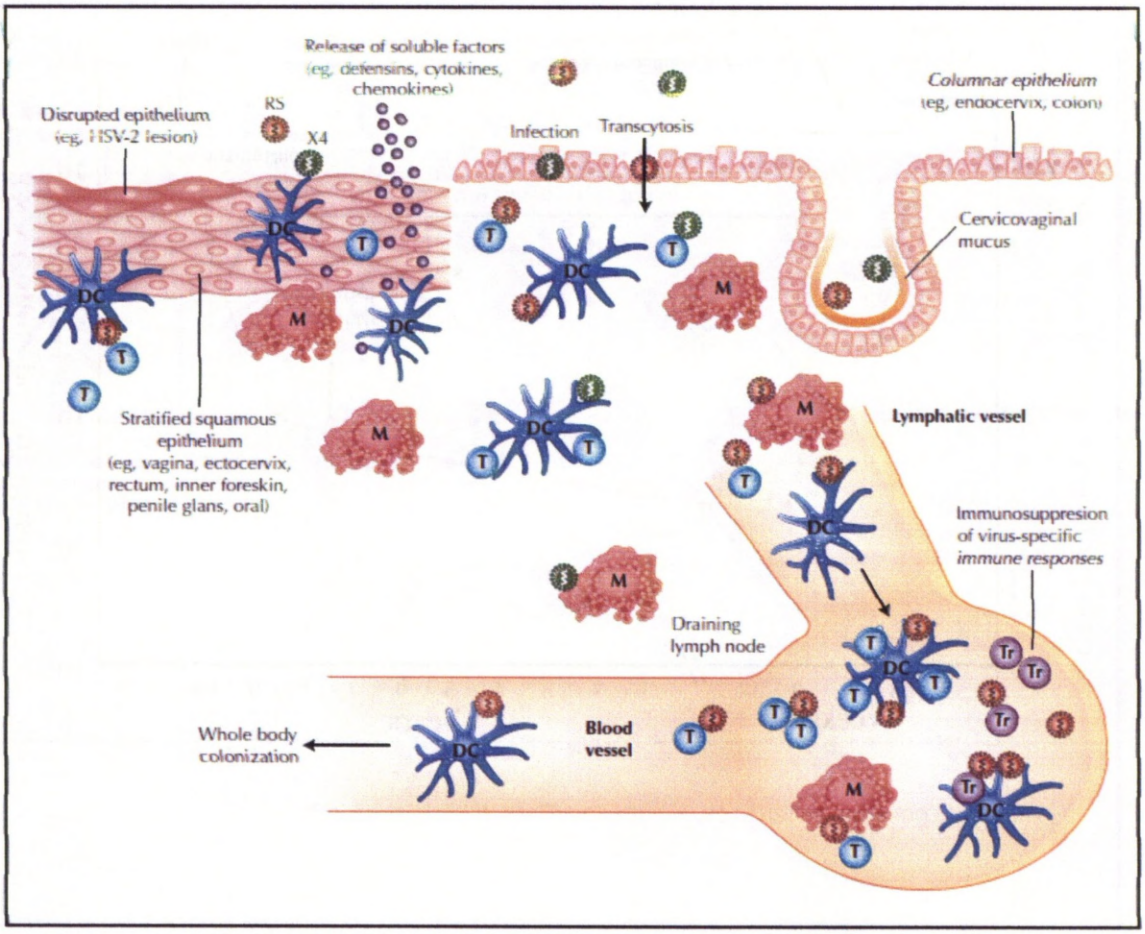
PIC: preintegration complex; Adapted from www.clinicaloptions.com/novelclasses

Figure 1-6: HIV lifecycle



RT: reverse transcriptase
Adapted from http://www.nature.com/nrc/journal/v4/n11/images/nrc1479-i1.jpg

Figure 1-7: Mucosal transmission of HIV

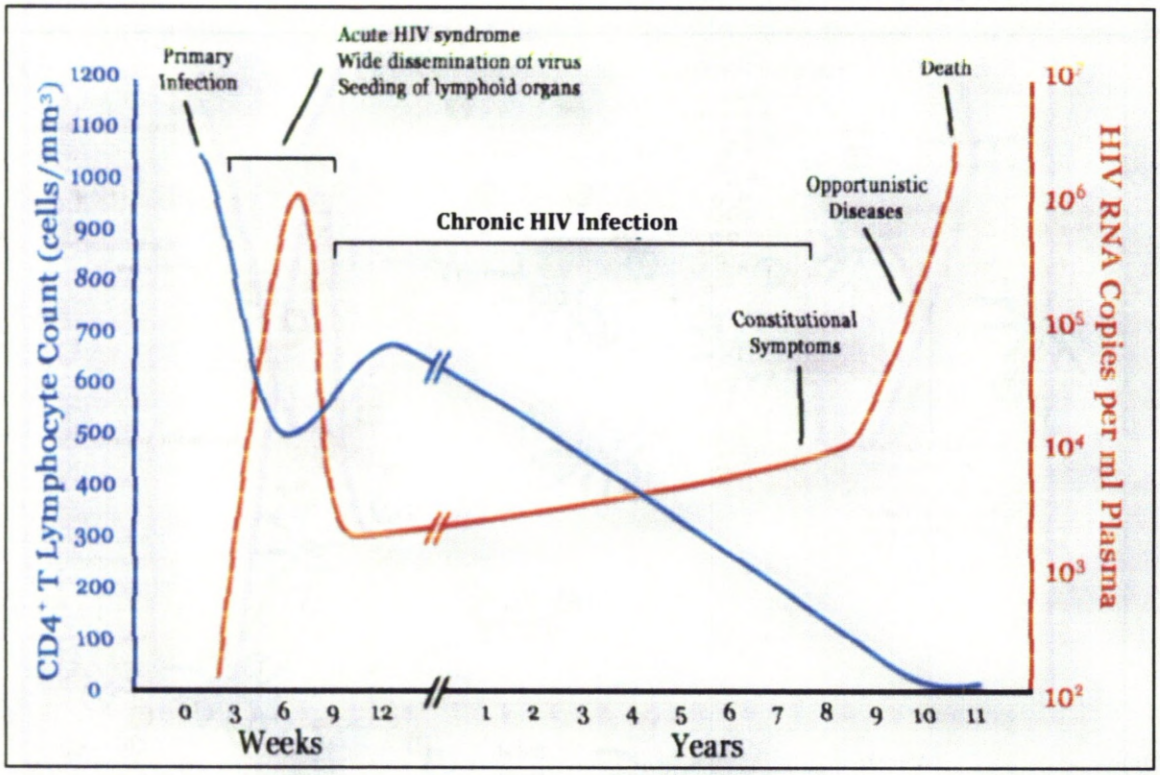


Cervicovaginal mucus helps entrap the virus. Soluble factors may help prevent infection. Disrupted epithelium allows direct access to target cells: T cells (T), dendritic cells (DC), and macrophages (M). Virus may directly infect or transcytose the epithelium. Resident Langerhans cells may entrap and traffic virus to the subepithelial compartment. Virus bearing DCs can migrate to lymph nodes and transmit virus to permissive T cells leading to virus dissemination. Activation of Tregs (Tr) may limit virus-specific immune responses. Although both R5 and X4 HIV possibly cross the epithelial barrier there seems to be preferential amplification and dissemination of R5 HIV. HSV-2: Herpes simplex virus-2. Picture taken from Morrow et al (Morrow et al. 2007)

1.1.3 Natural course of HIV disease

Figure 1-8 depicts the natural course of HIV infection in the absence of treatment.

Figure 1-8: HIV time line



Adapted from Clinical Medicine, Kumar and Clark, 5th Edition (2002), p134

1.1.3.1 Primary HIV infection or seroconversion illness

This occurs within days or weeks after the initial infection and patients that develop this are likely to be fast progressors than those that don't (Lindback *et al.* 1994; Vanhems *et al.* 1998). It can manifest as:

- "Glandular fever-like" syndrome: fever, pharyngitis, lymphadenopathy and malaise.
- Dermatological: erythematous maculopapular rash, urticaria, and mouth ulceration.
- Gastrointestinal: nausea, diarrhoea, and candidiasis.

- Neurological: headache, meningoencephalitis, peripheral neuropathy, and Guillain-Barré syndrome (Tindall *et al.* 1988; Schacker *et al.* 1996).

1.1.3.2 Chronic HIV infection

With continuing destruction of CD4+ effector cells, significant immunosuppression eventually develops over years and the patients succumb to opportunistic infections and malignancies such as Kaposi's sarcoma and lymphomas amongst others resulting in death. Chemotherapy with antiretroviral drugs dramatically changes the outlook of this disease, with restoration of immune function and suppression of viral replication.

1.1.4 Treatment of HIV infection

Treatment guidelines for the management of HIV have been established. Treatment guidelines recommend that patients be administered a combination of at least two to three active drugs from at least two different classes, and that the goal of therapy for all patients should be to control virus replication, as measured by a plasma viral load below the quantification limit of current available assays, i.e. 50 or 40 HIV RNA copies/mL (Gazzard 2008; DHHS 2009; EACS 2009; Thompson *et al.* 2010). This aims to minimise the development of mutations in the virus. Starting treatment depends on the stage of disease.

1.1.4.1 Primary HIV infection (PHI)

The aims of treating PHI are:

(1) Preservation of specific anti-HIV immune responses that would otherwise be lost, and which are associated with long-term nonprogression in untreated individuals.

(2) Reduction in morbidity associated with high viraemia and CD4 depletion during acute infection.

(3) Reduction in the risk of onward transmission of HIV.

Situations in which treatment may be beneficial include

- neurological involvement
- any AIDS-defining illness
- a CD4 cell count persistently below 200 cells/mL (i.e. for 3 months or more).

However, the benefits of treating this stage, often using a finite course of ART are not well established, with only short term improvements in immunological markers, viral load and CD4 lymphocyte count (Hecht *et al.* 2006; Fidler *et al.* 2007). More recently this approach was shown to produce some benefit with modest delay in progression to requiring therapy in those offered treatment compared to those deferring treatment (Hogan C 2010). However, new treatment guidelines recommending earlier treatment initiation and complications associated with treatment interruption in the SMART study (increased opportunistic diseases and death in those interrupting treatment) means this approach may not be applicable (Lundgren *et al.* 2008a; DHHS 2009).

1.1.4.2 Chronic HIV infection

It is recommended that treatment is started when the CD4 count falls below 350cells/mm^3. This is based on the fact that studies have shown increased risk of both death and disease progression as well as mortality from non-AIDS illnesses (e.g. malignancies and CVD) associated with lower CD4 cell counts, though there is no specific clear threshold at which risk increases (Opravil *et al.* 2002; El-Sadr *et al.* 2006; Phillips *et al.* 2007). Instances where treatment may start early when the CD4 count is above 350cells/mm^3 include

- AIDS diagnosis (e.g. Kaposi's sarcoma); any HIV-related comorbidity
- Hepatitis B infection, where treatment of hepatitis B is indicated
- Hepatitis C infection in some cases, where treatment for hepatitis is deferred
- Low CD4 percentage (e.g. 14%, where PCP prophylaxis would be indicated)
- Established CVD or a very high risk of cardiovascular events (e.g. Framingham risk of CVD >20% over 10 years) (Gazzard 2008).

1.1.4.3 Special situations requiring ART

WHO recommends that pregnant women with confirmed HIV serostatus initiate ART for their own health if the CD4 cell count ≤350 cells/mm3, irrespective of WHO clinical staging; or if in WHO clinical stage 3 or 4, irrespective of CD4 cell count. Provision of HAART to pregnant women not needing ART for their own health is recommended for the prevention of transmission of the virus to the baby perinatally starting from 14 weeks of pregnancy or as soon as possible when women present later in pregnancy or in labour or delivery until one week

after all exposure to breast milk has ended. Additionally, WHO recommends that infants (breast-fed or otherwise) born to HIV-infected women receiving ART for their own health should receive ART in the form of monotherapy with either NVP or AZT for 4 to 6 weeks and those born to mothers taking ART prophylaxis for prevention of mother to child transmission (PMTCT) receive daily NVP from birth until one week after all exposure to breast milk has ended (WHO 2009). ART reduces the transmission from mother to child from an average of 26% to 1% (Lyall *et al.* 2001).

Post-exposure prophylaxis is ART given to prevent HIV transmission after sexual intercourse or occupational exposure to bodily fluids e.g. needlestick injury. It is reserved for exposures that are associated with a credible possibility of HIV transmission, usually considered to be at least a 0.1% risk of transmission from a source patient who is known to be HIV-positive or a source patient whose serologic status is unknown but who is at high risk for HIV infection (Landovitz and Currier 2009). Prophylaxis with zidovudine alone has been shown to reduce the risk of seroconversion after occupational exposure by 81% (Cardo *et al.* 1997). Current PEP guidelines recommend PI based HAART for 4 weeks started within a few hours up to a maximum of 48 hours of exposure (Tsai *et al.* 1998; EACS 2009).

Preventing acquisition of HIV in HIV negative people has received much attention lately. Early treatment of HIV positive partners in serodiscordant couples regardless of CD4 count levels has been shown to be effective in

preventing transmission of HIV to the HIV negative partner, showing a 96% reduction in transmissions (Cohen *et al.* 2011). Pre-exposure prophylaxis with ART in HIV negative people at high risk of acquisition of HIV has also recently been studied, with two studies showing effectiveness in this intervention. Grant *et al* in the iPrEx trial showed a 42% reduction in transmission of HIV with daily oral truvada (tenofovir and emtricitabine) *vs.* placebo in HIV negative men having having sex with men (MSM) and transgender women taking, but this was very much dependent on the level of adherence to treatment (Grant *et al.* 2010). Intravaginal tenofovir (1%) gel applied before and after sexual in women has shown an overall 39% reduction (54% reduction with high gel adherence) in acquisition of HIV infection compared to placebo (Q.A. Karim *et al.* 2010). However a study currently undergoing closure has reported interim results revealing equal numbers of infection in the treatment arm (TDF and FTC) and placebo groups (FHI 2011). Based on the MSM study, the US Centres for Disease Control and Prevention has issued interim guidance for clinicians to support daily oral truvada in high risk MSM (CDC 2011). The British HIV Association (BHIVA) and British Association for Sexual Health and HIV (BASHH) have jointly recommended that at the present time pre-exposure prophylaxis be offered in the context of clinical research studies (Fidler 2011).

1.1.4.4 Monitoring of treatment

CD4 cell count and plasma viral load are used as surrogate markers of success of treatment with ART. Reduction in viral load leads to a rise in peripheral blood CD4 cell count, with greater rises being seen in those with greater and more sustained viral suppression. However the CD4 cell count is a

better indicator of the immediate risk of AIDS-defining diseases than the viral load in those on ART (Staszewski *et al.* 1999; Ledergerber *et al.* 2004; Gazzard 2008).

1.2 ANTIRETROVIRAL DRUGS

1.2.1 ART classes

The first antiretroviral drug to be licensed for use against HIV was zidovudine (AZT) in 1987 (Ezzell 1987). Since then, there are now many licensed antiretroviral drugs which attack the virus at different levels of its replication cycle. The need to develop new drugs is driven by several factors, namely to overcome the effects of resistance mutations in the virus, to improve the toxicity profiles of the drugs and to reduce the pill burden which would impact on compliance with treatment. Table 1-1 lists the ART drugs currently licensed for use in the UK.

Nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs, NtRTIs) are analogues of natural nucleosides and so act as alternative substrates for DNA polymerases. NRTIs are inactive as administered, requiring anabolic phosphorylation within target cells to achieve their antiretroviral effects. All NRTIs are converted to nucleoside triphosphates, which serve as the active metabolites (the NtRTI, TDF, only requires conversion to the diphosphate form) (Piliero 2004). Because NRTIs lack a hydroxyl group in the 3′-position (required for the addition of the next nucleotide onto the primer) their

incorporation causes termination of the growing DNA strand (Cherry and Wesselingh 2003).

Table 1-1: Antiretroviral drugs licensed in the UK

Reverse transcriptase inhibitors			**Protease inhibitors**	**Entry inhibitors**		**Integrase inhibitors**
Nucleoside	**Nucleotide**	**Non-nucleoside**		**Fusion inhibitors**	**CCR5 inhibitors**	
Zidovudine	Tenofovir	Efavirenz	Ritonavir	Efunvirtide	Maraviroc	Raltegravir
Stavudine		Nevirapine	Lopinavir			
Lamuvudine		Etravirine	Saquinavir			
Emtricitabine			Indinavir			
Abacavir			Atazanavir			
Didanosine			Fosamprenavir			
			Tipranavir			
			Darunavir			
			Nelfinavir			

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are polycyclic compounds that show chemical diversity. They inhibit RT by binding to a site near its catalytic site. The interaction with RT induces conformational changes that impact the catalytic activities of the enzyme. NNRTs are active against HIV 1 only, and not other retroviruses like HIV 2 (Sluis-Cremer *et al.* 2004; de Bethune 2009).

Protease inhibitors (PIs) work at the level of viral maturation by blocking the cleaving of the Gag-Pol polyprotein by viral protease enzyme (Dau and Holodniy 2009).

There are a number of new drugs that have been approved in recent years targeting the virus at different levels of its life cycle. Entry inhibitors include the fusion inhibitor efunvirtide (T20) which bind to the viral transmembrane fusion peptide gp41 preventing the viral membrane from fusing with the host cell membrane; and CCR5 co-receptor antagonists which block the binding of the virus to the CCR5 receptor, blocking cell entry. Maraviroc has recently been approved in this class while vicriviroc's development was halted in 2010. Integrase inhibitors are a new class of antiretrovirals that inhibit HIV proviral DNA integration into the host genome. Raltegravir is a new drug that inhibits the strand transfer in the integration process. Elvitegravir is in advanced clinical trials (Pommier *et al.* 2005; Dau and Holodniy 2009).

1.2.2 Adverse effects of antiretroviral drugs

The use of a combination of drugs in HAART means that adverse effects are common. The adverse effects may be due to the individual drugs. Adverse effects of HAART are considerable and may be severe, having both short and long term impact on outcome of the disease. Adverse effects can occur early at start of therapy, consisting of side effects that are common to most drugs in clinical use such as gastrointestinal effects (nausea, vomiting and abdominal pain) and headache. Liver toxicity and skin rashes can occur with nevirapine (NVP) particularly in the first 6 weeks of therapy which can occasionally lead to life threatening hepatitis in 3% of patients and toxic epidermal necrolysis or Steven-Johnson syndrome in 0.3% of patients (Pollard *et al.* 1998; Popovic *et al.* 2009). Neuropsychiatric problems are the commonest early adverse effects

of efavirenz (EFV), manifesting as vivid dreams and/or nightmares, sleep and mood disturbance, drowsiness, dizziness and disorientation. These are mostly mild but may be severe enough to warrant discontinuation of the drug and drug switch (Boly *et al.* 2006; BSM 2011). EFV may also be teratogenic; there have been six retrospective reports of neural tube defects in mothers taking efavirenz in the first trimester; and in a prospectively reported ART Pregnancy registry, one of 14 of 501 live births (first trimester exposure) was a neural tube defect as of July 2009. However, the relative risk of efavirenz use in early pregnancy remains uncertain (BSM 2011). Treatment guidelines recommend that women of childbearing potential be warned about becoming pregnant whilst on efavirenz and wherever possible EFV should be avoided in women who may contemplate pregnancy (Gazzard 2008; EACS 2009).

Late adverse effects of HAART have become the subject of interest lately due to their impact on the success of HAART, adherence and non-AIDS related mortality such as from cardiovascular disease (CVD). The long term effects caused by the three classes of drugs, i.e. the NRTIs, the NNRTIs and PIs, which form part of HAART in regimens in common use at present, are discussed. The long term effects include lypodystrophy, hyperlipidaemia, insulin resistance with resultant glucose intolerance or diabetes and cardiovascular disease.

1.2.2.1 HIV lipodystrophy

Lipodystrophy (LD) refers to body fat redistribution. It comprises peripheral lipoatrophy (LA), localized fat accumulation or lipohypertrophy (visceral,

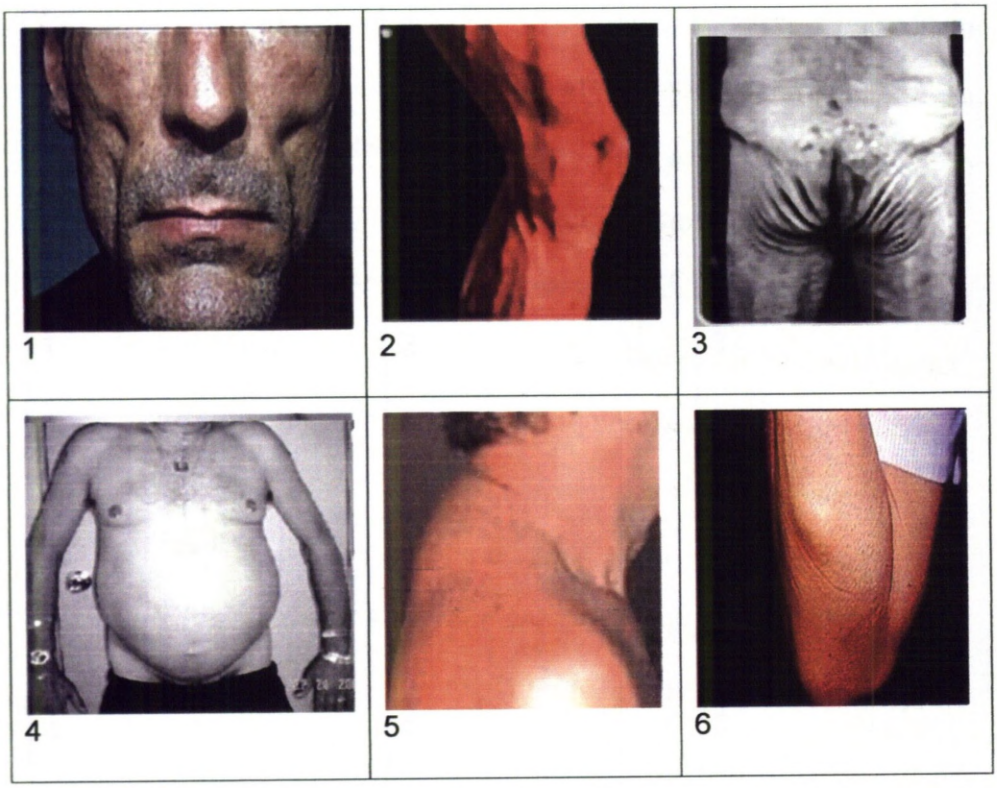
breasts, back of neck and lipomata). The LD syndrome is a combination of these morphological changes and hyperlipidaemia, insulin resistance and hyperglycaemia (Chen *et al.* 2002). The prevalence rate of LD in patients on highly active antiretroviral therapy (HAART) is reported to be up to 40% (Chen *et al.* 2002; Kinlaw and Marsh 2004). The prevalence of the condition increases with time on therapy, and changes usually become clinically apparent only after several months (Veny *et al.* 1998). The frequency of LD, not unexpectedly varies with the drugs used. Nucleoside reverse transcriptase inhibitors (NRTIs), particularly thymidine analogues (AZT and D4T), have been associated with extremity fat loss (Moyle *et al.* 2006), while protease inhibitors (PIs) have been associated with localized accumulation of fat (Behrens *et al.* 1999). Since the drugs are often used together as part of highly active antiretroviral therapy (HAART), clinical data suggest that they act synergistically in causing LD. The virus itself is thought to contribute to development of LD, though changes continue to worsen despite virological and immunological control in patients that are followed up after starting ART (Mallon *et al.* 2003). The body changes of LD are distressing and can be stigmatizing for sufferers (Figure 1-9).

The mechanism of LD is complex. In studies involving HIV patients, lipodystrophic patients show morphological alterations of the adipose tissue with abnormal expression of adipocytokines (such as adiponectin) and pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α) and interleukin 6 (IL6), as well as adipocyte transcription factors (Lagathu *et al.* 2004). Patients have elevated circulating levels, and increased tissue mRNA

expression, of TNF-α and IL-6 (Lihn *et al.* 2003; Vigouroux *et al.* 2003; Jan *et al.* 2004). Lipoatrophic fat from patients has also been shown to have reduced adipogenic transcription factors such as sterol regulatory element-binding protein 1c (SREBP-1c), CAAT enhancer-binding protein-a (C/EBP-a) and peroxisome proliferator activated receptor-gamma (PPAR-γ), which are all involved in adipocyte differentiation (Kannisto *et al.* 2003; Jan *et al.* 2004; Jones *et al.* 2005c). Additionally, lipodystrophic HIV patients also have reduced mRNA expression of adiponectin and leptin, and decreased levels of these proteins (Vigouroux *et al.* 2003; Jan *et al.* 2004; Lagathu *et al.* 2005). Taken together, all these changes probably account for the increased apoptosis, and decreased differentiation, of adipocytes seen in these patients, which ultimately lead to lipoatrophy (Jan *et al.* 2004).

The metabolic effects of the LD syndrome namely the dyslipidaemia, glucose intolerance and insulin resistance are traditional risk factors for cardiovascular disease in the general population (Stamler *et al.* 1986; Wilson *et al.* 1998).

Figure 1-9: Various Appearances of HIV Lipodystrophy



1. Facial lipoatrophy; 2. Lipoatrophy of leg; 3. Lipoatrophy of buttocks; 4. Increased abdominal girth secondary to visceral adiposity; 5. Buffalo hump; 6. Lipoma

1.2.2.2 Mitochondrial toxicity

Mitochondrial toxicity occurs through inhibition of mitochondrial DNA (MtDNA) polymerase-γ leading to hyperlactataemia and organ-based toxicities involving the liver, pancreas, peripheral nerves and skeletal muscle in particular (Walker 2003). Walker *et al* (2001) have also shown a link between peripheral lipoatrophy and mitochondrial toxicity in that patients with lipoatrophy had more pronounced mitochondrial depletion in biopsies from subcutaneous lipoatrophic tissues. The degree of mitochondrial toxicity depends on the drugs used, with in vitro studies using HepG2 human hepatoma cells showing the worst effects with zalcitabine (DDC, which is no longer in clinical use), DDI and

D4T in reducing order. TDF and 3TC show minimal or no mitochondrial toxicity. AZT, FTC and ABC impair cell proliferation and increase lactate and lipid production but show no mitochondrial depletion. Combinations of the drugs show that they act synergistically to cause mitochondrial toxicity, with pyrimidine combinations (such as DDC and D4T) being the worst offenders (Squires 2001; Walker *et al.* 2002; Walker 2003; Venhoff *et al.* 2007). The level of affinity of the active metabolites of the drugs for mtDNA polymerase-γ is thought to underlie these differences (Squires 2001).

1.2.2.3 Insulin resistance

Insulin resistance occurs when there is a reduction in the ability of circulating insulin to induce uptake of glucose into cells. Insulin stimulates its cell surface receptor, which sets up a phosphorylation cascade, firstly of insulin receptor substrate-1 (IRS-1) which in turn initiates a number of further phosphorylation reactions. These eventually result in translocation of the glucose transporter 4 (GLUT4), from the cytosol to the cell surface where it facilitates glucose entry into the cell (Samaras *et al.* 2007).

Insulin resistance underlies many of the metabolic diseases including obesity, type II diabetes, atherosclerotic heart disease and hyperlipidaemia (Reaven 1988; Samaras *et al.* 2007). Dysfunction of adipose tissue may be involved in the pathogenesis of insulin resistance. The adipocyte has been shown to be actively involved in energy homeostasis by secreting hormones or proteins that have collectively been termed adipocytokines (such as adiponectin, leptin, resistin, visfatin and vaspin) (Fasshauer and Paschke 2003; Rajala and

Scherer 2003; Fukuhara *et al.* 2005). Adipocytes also secrete pro-inflammatory cytokines such as TNF-α and IL-6 (Rajala and Scherer 2003; Lagathu *et al.* 2004). These cytokines are known to affect glucose and lipid metabolism and may also alter body habitus; derangements in the levels of these cytokines result in insulin resistance (Fasshauer and Paschke 2003; Rajala and Scherer 2003; Lihn *et al.* 2005). Obese and type II diabetic patients have raised levels of TNF-α, IL-6 and leptin; they also show reduced levels of adiponectin while levels of resistin have been variable (Lagathu *et al.* 2004). Altered levels of adipocytokines and pro-inflammatory markers have also been demonstrated in *in vitro* (in murine and human adipocyte cell lines) and *in vivo* studies with the use of NRTIs (particularly AZT and D4T) and PIs in HIV patients (Janneh *et al.* 2003; Jones *et al.* 2005b; Lagathu *et al.* 2005; Kim *et al.* 2006; Noor *et al.* 2006). PIs led to the following changes in these studies:

- A reduction in lipid accumulation in adipocytes.
- Increase in adipocyte apoptosis leading to a reduction in cell numbers.
- Induction of insulin resistance by:
    - Inhibition of insulin-stimulated glucose uptake via inhibition of the glucose transporter (GLUT-4).
    - Induction of IL-6 and TNF-α.
    - Reduction in gene expression and secretion of adiponectin.
    - Increased lipolysis.

The ability of PIs to induce these changes varies between the individual drugs, with LPV, RTV, SQV and NFV being the worst offenders (Janneh *et al.* 2003; Jones *et al.* 2005b). The PI ATV has a much reduced effect (Kim *et al.* 2006; Noor *et al.* 2006). IDV did not have much effect on cell viability or lipogenesis

but inhibited glucose uptake to a greater extent than the other PIs (Janneh *et al.* 2003; Jones *et al.* 2005b). With regard to NRTIs, Jones et al. and Janneh et al. showed in murine adipocyte cell lines (3T3-L1 and 3T3-F442A) that AZTand D4T were not cytotoxic, did not affect adipogenesis or induce lipolysis and did not up-regulate the expression of IL-6 and TNF-α. However, Lagathu *et al.* working with the same murine cell lines found that AZT and D4T raised levels of IL-6 and TNF-α (Lagathu *et al.* 2005). These two thymidine analogues also reduced the levels of adiponectin, which could contribute to insulin resistance (Lagathu *et al.* 2004; Jones *et al.* 2005b). Pacenti et al. also found that AZT and 3TC, either alone or in combination, did not significantly alter the viability and adipogenesis of 3T3-L1 cells (Pacenti *et al.* 2006). The differences in findings in these studies may be due to differences reasons. For example, drug concentrations used were different; Janneh *et al* and Jones *et al* used 20μM of PIs while Lagathu *et al* used 10μM, while for NRTIs, Janneh *et al* used 1μM, Jones *et al* used 20μM and Lagathu *et al* used 10μM. Sources of the drugs, cell lines and assays used such as for ELISA tests were sourced from different suppliers with attendant different methodologies. Additionally, different methods were used to determine cell viability and adipocyte function were different.

Large cohort studies in HIV patients on ART have shown development of insulin resistance in these patients with resultant glucose intolerance or diabetes (Brown *et al.* 2005; Rosso *et al.* 2007; Tien *et al.* 2008).

1.2.2.4 Dyslipidaemia

Lipid disorders are common in HIV-infected patients. Deranged lipids have been demonstrated in HIV positive patients who are treatment-naive with low total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) and these effects are thought to be due to the virus itself, with factors such as low CD4 counts, high viral loads, age and race playing a part (Riddler *et al.* 2003; El-Sadr *et al.* 2005). Upon starting ART, HIV infected patients have been observed to have increases in TC and LDL-C with little change in HDL-C (Riddler *et al.* 2003; El-Sadr *et al.* 2005). Additionally, compared to their HIV uninfected counterparts, patients on ART have been shown to have higher triglycerides and lower HDL-C as well as to have an atherogenic lipid profile similar to that found in obese and type 2 diabetic patients (Riddler *et al.* 2008). HAART has also been associated with the development of dyslipidaemia. PIs have mostly been shown to have this effect, with the development of triglyceridaemia and hypercholesterolaemia over time (Carr *et al.* 1998; Jones *et al.* 2005a). NRTIs have also been shown to cause dyslipidaemia. This depends on the type of NRTI, with thymidines especially D4T being most implicated. TDF and ABC are associated with a more lipid friendly profile and indeed switching from a thymidine NRTI to TDF and ABC based regimens results in improvement in lipid levels (Gallant *et al.* 2004; Jones *et al.* 2005a; Vigano *et al.* 2005; Moyle *et al.* 2006; Pozniak *et al.* 2006). The NNRTI NVP shows little effect on lipids while EFV has been shown to have a lipid profile comparable to RTV boosted LPV (Behrens *et al.* 1999; Heath *et al.* 2001; Jones *et al.* 2005a; Pirmohamed 2009). The mechanisms of

dyslipidaemia caused by ART are many and include mitochondrial toxicity, increase in lipolysis and adipocyte apoptosis as well as reduction in lipid accumulation in adipocytes as described above. Other mechanisms thought to underlie this effect are adipose gene expression derangements and proteasome defects leading to increased serum lipids:

Adipose gene expression derangements

Whole genome expression analysis using microarrays have provided interesting insights into the derangements that occur in the genes involved in adipocyte differentiation and lipid metabolism on exposure to antiretrovirals (Pacenti *et al.* 2006). An *in vitro* study with 3T3-L1 cells showed that PIs (IDV, SQV and LPV) induced or up-regulated genes that inhibit adipocyte differentiation, and down-regulated the expression of master adipogenic transcription factors as well as several genes mainly involved in lipid metabolism (Fukuhara *et al.* 2005). These are summarized in Table 1.2. The same study showed that NRTIs (AZT, 3TC, D4T and DDC) showed a milder effect on these genes though they were found to induce a gene, Aebp-1 (adipocyte enhancer-binding protein-1), the over-expression of which could inhibit adipogenesis (Fukuhara *et al.* 2005). The PI suppression of adipogenesis and lipogenesis genes has been replicated *in vivo* in the subcutaneous adipose tissue (SAT) from lipodystrophic HIV-infected patients (Bastard *et al.* 2002; Kannisto *et al.* 2003; Gougeon *et al.* 2004; Jan *et al.* 2004).

Proteasome dysfunction

Dysfunction of proteasome activity in cells has been implicated in the pathogenesis of HIV LD. Proteasomes are multi-subunit protease complexes engaged in the turnover of short-lived proteins that regulate a variety of cellular processes including signal transduction, stress response, transcription control, chromosomal segregation, DNA repair and cell cycle progression (Kruger *et al.* 2001). Proteasome activity coordinates protein handling in the endoplasmic reticulum (ER) by removing misfolded proteins; disruption of this process leads to an ER stress response (Travers *et al.* 2000; Parker *et al.* 2005). An in vitro study by Parker et al. [working with a human hepatoma cell line (HepG2) and 3T3-L1 mouse cells] found that PIs inhibited proteasome chemotryptic, and to a lesser extent tryptic, activity (Parker *et al.* 2005). The ER stress response induced by the PIs differed between the hepatocytes and the adipocytes as outlined in Table 1.3.

Table 1-2: Protease inhibitor effects on adipose tissue genes

Genes inhibiting adipocyte differentiation	Master adipogenic transcription factors	Genes involved in lipid metabolism
↑ wingless related MMTV integration site 10a (*Wnt10a*)	↓ sterol regulatory element-binding protein 1c (*SREBP-1c*)	↓ fat specific gene 27 (*Fsp27*)
↑ Δ-6 fatty acid desaturase (*Fad2*)[a]	↓ CAAT enhancer-binding protein-α (*C/EBP-α*)	↓ leptin (*Lep*)
	↓ peroxisome proliferator activated receptor-γ (*PPAR-γ*)	↓ adipsin (*Adn*)
		↓ adiponectin (*Adipoq*)
		↓ inducible 6-phosphofructose-2-kinase (*Pfkfb 3*)
		↓ *CD36/FAT + S100A8*[b]

↑:induced or up-regulated; ↓: inhibited or down-regulated.
[a]*Fad2 has also been associated with insulin resistance.*
[b]*CD36/FAT and S100A8 interact to facilitate cellular uptake of fatty acids.*

Table 1-3: Protease inhibitor-induced endoplasmic reticulum (ER) stress responses

Cell type/ER stress response	Effect
Hepatocytes (HepG2)	
↑adipogenic and lipogenic transcription factors (*C/EBP, PPAR-γ, SREBP-1c*), ↑mRNA of enzymes of fatty acid/cholesterol biosynthesis (e.g. acetyl CoA carboxylase, fatty acyl CoA ligase, fatty acid synthase, HMGCoA reductase)	Increased production of triglycerides and to a lesser extent, cholesterol; little effect on glucose transport via GLUT-4[a]
Adipocytes (3T3-L1)	
Inhibition of GLUT-4, ↓adiponectin (an insulin sensitizer)	Inhibition of glucose uptake

↑:up-regulation or increase in levels; ↓:down-regulation or decrease in levels.
[a]*HepG2 cells express GLUT-3 and GLUT-1 (which are not sensitive to PIs), rather than GLUT-4 (Parker et al. 2005).*

1.2.2.5 Cytokines in HIV patients on ART

Derangements in proinflammatory cytokines and adipose tissue derived cytokines (adipocytokines) have been extensively studied and are thought to underlie most of the adverse effects of ART. The effects of some of the individual pro-inflammatory cytokines and adipocytokines can be summarized as follows:

1.2.2.5.1 Tumour necrosis factor-α

TNF-α is a pro-inflammatory cytokine. The level of TNF-α in adipocytes is increased in obesity, type II diabetes and HIV patients with LD (Lihn *et al.* 2003; Ruan and Lodish 2003; Vigouroux *et al.* 2003; Jan *et al.* 2004). With regard to obesity, overexpression of TNF-α is higher in omental, compared with subcutaneous fat.(Cao *et al.* 2008) Visceral fat comprises omental and mesenteric fat and has been linked in epidemiological studies to the development of insulin resistance, dyslipidaemia, type 2 diabetes and cardiovascular disease (Haslam and James 2005; Cao *et al.* 2008). Visceral

fat accumulation can be a feature of HIV LD (Chen *et al.* 2002). TNF-α may be the link between visceral obesity and development of these complications (Cao *et al.* 2008). TNF-α mediates insulin resistance via reduction of insulin receptor kinase activity. It induces lipolysis and down-regulates insulin receptor substrate (IRS)-1 and the insulin-sensitive glucose transporter (GLUT)-4. It may act in an autocrine manner thereby profoundly altering adipose tissue biology (Fasshauer and Paschke 2003). It also induces apoptosis, which might underlie the lipoatrophy caused by nucleoside reverse transcriptase inhibitors (NRTIs) (Lagathu *et al.* 2004). The secretion of TNF-α is, at least partly, under genetic control. The TNF-α gene contains numerous polymorphisms and exhibits a complex haplotype structure. Many of these polymorphisms are thought to be functionally important, including the promoter region polymorphisms at positions -308 and -238, which have been the most widely studied. Maher et al. showed an increased frequency of the -238 G-A transition in HIV LD patients compared with those without LD. The patients were on both protease inhibitors (PIs) and NRTIs. This effect was thought to be attributable to the PIs, although the authors acknowledged that the NRTIs might also have contributed (Maher *et al.* 2002). This study, taken together with the replication of the findings in an Australian cohort (Nolan *et al.* 2003), suggests that TNF-α -238 promoter region gene polymorphism, which is functionally active, increases the risk of developing antiretroviral-related LD (Lindegaard *et al.* 2004). The metabolic effects of HIV LD namely hyperlipidaemia, insulin resistance and hyperglycaemia are traditional risk factors of CVD.

1.2.2.5.2 Interleukin-6

IL-6 is a pro-inflammatory protein; systemic IL-6 is derived from adipose tissue (30%), macrophages and smooth muscle cells in vascular endothelium and mediates insulin resistance (Fasshauer and Paschke 2003). IL-6 levels are raised in obesity and insulin resistance states, and independently predict future development of type II diabetes and cardiovascular disease (Pickup *et al.* 1997; Plutzky 2001; Pradhan *et al.* 2001; Cesari *et al.* 2003). IL-6 stimulates or induces hepatic gluconeogenesis with resultant hyperglycaemia and compensatory hyperinsulinaemia. It also induces hepatic triglyceride secretion. In murine adipocytes and hepatocytes, IL-6 directly impairs insulin signaling with decreased activation of IRS-1 and phosphatidylinositol 3-kinase (Fasshauer and Paschke 2003). Studies show that IL-6 levels are significantly higher in HIV infected patients on ART compared to healthy controls with HIV patients having levels that are twice those in HIV uninfected individuals (Jan *et al.* 2004; Ross *et al.* 2009). The virus itself contributes to raised levels of IL-6 as demonstrated in the SMART study in those that interrupted therapy with resultant detectable viral loads (Kuller *et al.* 2008). The type of ART drugs the patient is on appears to have an effect on IL-6 levels with *in vitro* studies using both murine and human adipocytes showing increased expression and secretion of this cytokine in response to both PIs (such as LPV, NFV and RTV) and NRTIs (AZT and D4T) (Lagathu *et al.* 2004; Vernochet *et al.* 2005).

1.2.2.5.3 C-reactive protein (CRP)

CRP is an acute phase reactant produced in the liver which is marker of low grade inflammation and has been implicated in the pathogenesis of atherosclerosis in humans (Blake and Ridker 2002). Its secretion is

predominantly stimulated by IL-6 (Pepys and Hirschfield 2003). High levels of this protein have been found be a predictor of coronary heart disease, congestive heart failure and ischaemic stroke in healthy subjects (Tracy *et al.* 1997; Koenig *et al.* 1999; Ridker *et al.* 2002; Cesari *et al.* 2003; Pai *et al.* 2004). In HIV patients, CRP levels are higher than in the general population. In a study by Noursadeghi et al (Noursadeghi and Miller 2005) the median CRP was 5.9mg/dL (range 0.5–108.6) in HIV positive patients. The CRP level is <3mg/dL in the general population (Pepys and Hirschfield 2003). This reflects the fact that there is a state of chronic inflammation in people with HIV; as such these patients will be expected to be at higher risk of cardiovascular disease.

1.2.2.5.4 Adiponectin

Adiponectin is a 30kDa cytokine synthesized and secreted exclusively by adipose tissue (Lindegaard *et al.* 2004). It is a potent insulin sensitizer acting in adipose tissue, muscle and liver. It has also been shown to have anti-atherogenic, anti-inflammatory (Ouchi *et al.* 2003; Hara *et al.* 2005), antiangiogenic and anti-tumour functions (Brakenhielm *et al.* 2004). The level of adiponectin is reduced in obesity, particularly in visceral obesity, and in insulin resistant states (Weyer *et al.* 2001; Lindegaard *et al.* 2004). It has been shown from epidemiological studies that low levels of adiponectin are an independent predictor for developing type 2 diabetes (Daimon *et al.* 2003; Spranger *et al.* 2003), and myocardial infarction (Laughlin *et al.* 2007; Rathmann *et al.* 2007). Adiponectin profoundly influences endogenous insulin sensitivity: it reduces plasma glucose concentration (by reducing liver output of glucose), increases fatty acid oxidation in muscle and inhibits hepatic

gluconeogenesis through its activation of 5'-AMP-activated protein kinase (AMPK) (Fasshauer and Paschke 2003; Rajala and Scherer 2003; Tsuchida *et al.* 2005). Adiponectin knockout mice have impaired insulin sensitivity providing further evidence of its important role in the metabolic diseases that afflict a huge proportion of the global population (Kubota *et al.* 2002; Maeda *et al.* 2002). Adiponectin acts via the adiponectin receptor 1 (AdipoR1) in skeletal muscle and adiponectin receptor 2 (AdipoR2) in the liver (Kinlaw and Marsh 2004). An additional receptor for hexameric and high molecular-weight adiponectin is T-cadherin (Kralisch *et al.* 2007). HIV-associated LD is associated with low plasma adiponectin levels and low expression of adiponectin in adipose tissue (Vigouroux *et al.* 2003; Leszczyszyn-Pynka *et al.* 2005; Lihn *et al.* 2005) particularly after administration of PIs (via inhibition of adipogenesis), but has also been seen with D4T and AZT (Lindegaard *et al.* 2004; Jones *et al.* 2005c). However, it is thought that the low levels seen with NRTIs may be due to a reduction in adipose tissue mass (seen in peripheral lipoatrophy) rather than a direct effect of the drugs (Lindegaard *et al.* 2004; Jones *et al.* 2005c). Consistent with this, *in vitro* studies have shown that adiponectin gene expression is down-regulated by PIs rather than by NRTIs (Parker *et al.* 2005; Pacenti *et al.* 2006). However, it is important to bear in mind that feedback loops operate with regard to cytokine expression and levels with *in vitro* studies in mouse and human adipocytes, showing that the levels of adiponectin can also be reduced by the pro-inflammatory cytokines TNF-a and IL-6 (Kappes and Loffler 2000; Maeda *et al.* 2001; Fasshauer *et al.* 2002; Fasshauer and Paschke 2003).

1.2.2.5.5 Resistin

There is conflicting evidence as to whether resistin plays a major role in obesity and insulin resistance (Kralisch *et al.* 2007). It has been suggested that resistin impairs glucose tolerance by inducing severe hepatic but not peripheral insulin resistance. However, conflicting data from other studies suggest that it may not have a major role in glucose homeostasis. For instance, while some studies have shown high levels of resistin in obesity, others have shown low levels (Fasshauer and Paschke 2003). Data on the effects of ARVs on resistin are scanty. A study in HIV patients showed that single nucleotide mutations in the resistin gene (C-T transition in the second intron of the gene) were associated with the development of adverse metabolic changes on HAART. This was further investigated by the same group where they showed that patients on HAART (in this study, patients were either on D4T and DDI or AZT and 3TC NRTI backbone) with this mutation developed significantly elevated lipids and insulin resistance and also experienced significant body composition changes, particularly limb fat loss, and all the patients were on a thymidine NRTI (Ranade *et al.* 2008). A study by Kamin et al found that levels of resistin were higher in HIV positive patients (both male and female) than those in HIV negative individuals (Kamin *et al.* 2005).

1.2.2.5.6 Leptin

Leptin is mostly expressed in adipose tissue and is found both in the circulation and cerebrospinal fluid (Rajala and Scherer 2003). It acts centrally to increase energy expenditure, inhibit appetite and weight gain by decreasing appetite-enhancing and increasing appetite-inhibiting peptide expression in the arcuate nucleus of the hypothalamus. It also has peripheral effects on skeletal

muscle, liver, pancreas, adipose tissue and other cell types where it acts via 5'-AMP-activated protein kinase to decrease anabolic pathways (such as glucose, lipid and protein synthesis) and increase catabolic pathways (glucose and lipid utilization) (Rajala and Scherer 2003). Its levels are raised in obesity (Fasshauer and Paschke 2003). Obesity is a known risk factor for cardiovascular disease. Hubert *et al* and Lieb *et al* found that leptin levels were strongly correlated with body mass index (BMI) and high circulating levels were associated with a greater risk of congestive heart failure (CHF) and CVD, though this risk was attenuated upon adjustment for BMI (Hubert *et al.* 1983; Lieb *et al.* 2009). Leptin levels have also been found to positively correlate with BMI in HIV patients on HAART, although plasma levels of leptin did not differ between patients on or off treatment (Leszczyszyn-Pynka *et al.* 2005; Wunder *et al.* 2005). However, *in vitro* studies have demonstrated that leptin levels increase when human adipocyte stem cells are exposed to the PIs RTV, LPV and APV (Vernochet *et al.* 2005).

1.2.2.5.7 Visfatin

This is a visceral fat adipocytokine that was previously identified as pre-B cell-colony-enhancing factor. It exerts insulin mimetic effects in cultured cells and in mice, leading to a lowering of plasma glucose levels, triglyceride accumulation in preadipocytes in fat depots and accelerated triglyceride synthesis from glucose. It binds to and activates the insulin receptor, at a site different from where insulin binds. Visfatin levels are high in obese people, particularly in those with increased visceral adiposity (Fukuhara *et al.* 2005; Haider *et al.* 2006). A study found that visfatin levels increased by about 7-fold in HIV-

positive patients who were on HAART for 1 year compared with HIV-negative individuals, although other parameters of glucose metabolism and body fat mass were unchanged (Schindler *et al.* 2006).

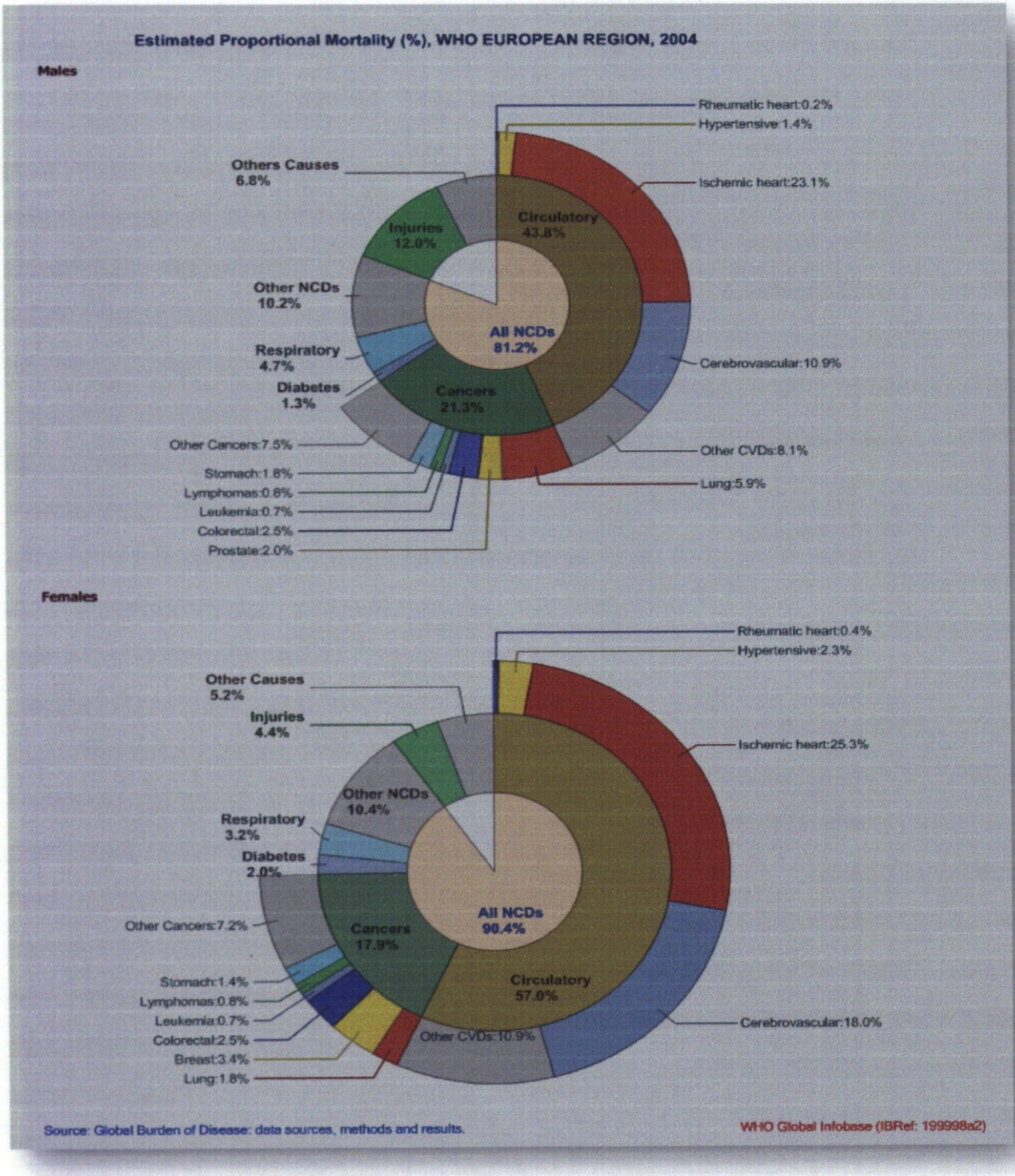
1.2.2.6 Cardiovascular disease (CVD)

1.2.2.6.1 CVD in the general population

Atherosclerosis underlies CVD. The initial insult is vascular endothelial dysfunction which is caused by chronic exposure to various stressors such as oxidative stress (including the hyperglycaemic state of diabetes), modified low-density lipoprotein and stress from disturbed blood flow (such as in hypertension). These stresses result in impairment of nitric oxide (NO) production (a powerful vasodilator and inhibitor of platelet aggregation and thrombosis) and chronic inflammation (Antoniades *et al.* 2004; Hartge *et al.* 2007). Raised levels of pro-inflammatory cytokines (e.g. IL-6, TNF-α and CRP), myeloperoxidase (a leucocyte enzyme), plasminogen-activator inhibitor-1 (PAI-1), adhesion molecules (e.g. soluble vascular cellular adhesion molecule-1 or sVCAM-1 and soluble intercellular adhesion molecule-1 or sICAM-1), monocyte chemoattractant protein (MCP-1) as well as increased carotid intima-media thickness (IMT) have been demonstrated, all of which contribute to atherogenesis. These markers are independent predictors of cardiovascular events (Ridker *et al.* 2000; Zhang *et al.* 2001; Brennan *et al.* 2003; Cesari *et al.* 2003; Antoniades *et al.* 2004; Pai *et al.* 2004; Hartge *et al.* 2007); (Bots *et al.* 1999; Espeland *et al.* 2005; Ogata *et al.* 2005).

Large population studies have demonstrated certain conditions to be associated with increased risk of developing CVD. These include obesity, dyslipidaemia, insulin resistance and diabetes mellitus, hypertension, smoking, age and family history of myocardial infarction and angina pectoris (Hubert *et al.* 1983; Stamler *et al.* 1986; Hulley 1988; Neaton and Wentworth 1992; Austin *et al.* 1998; Wilson *et al.* 1998). CVD remains the most important cause of mortality and morbidity in the developed world, accounting for up to 57% of all cause mortality in Europe (Figure 1-10). Indeed many preventive and treatment programs in countries most affected by CVD have been instituted to counter these conditions which include life style modifications (smoking cessation, optimal diet, exercise and weight reduction) as well as drug treatments for dyslipidaemia, hypertension, diabetes and antiplatelet therapy for those at high risk of developing CVD as determined by the Framingham algorithms (NCEP 2002; JBS2 2005; Graham *et al.* 2007; NICE-TA94 2009).

Figure 1-10: All cause mortality in WHO Europe region in 2004



NCD: non-communicable disease; CVD: cardiovascular disease; adapted from https://apps.who.int/infobase/Mortality.aspx

1.2.2.6.2 CVD in HIV patients on ART

With the control of HIV infection and prolongation of life, patients will experience diseases of aging including CVD. Apart from the known risk factors of CVD namely age, obesity, sex, smoking, family history of ischaemic heart disease or inherited dyslipidaemias, ART places patients at risk of developing modifiable risk factors such as dyslipidaemia, insulin resistance with glucose intolerance or frank diabetes and hypertension. Additionally, smoking rates tend to be higher in HIV patients than in the general population (Friis-Moller *et al.* 2003(a); Ross *et al.* 2009). As such CVD may occur at even younger age in this population. Indeed it has become apparent in recent years that patients with HIV and on treatment are at excess risk of myocardial infarctions, and that CVD now accounts for up to 10% of all deaths in these patients. Of particular concern is the fact that drugs such as ABC and DDI have been shown to be especially associated with this risk (Sabin *et al.* 2008).

HIV itself is associated with a chronic inflammatory state which predisposes to atherosclerosis with increased risk of CVD. Studies in HIV patients not on ART show increased pro-inflammatory markers (such as TNF-α, IL-6), markers of vascular endothelial activation, carotid intima-media thickness and platelet activation which are all markers of inflammation and predispose to atherosclerosis and CVD (Laughlin *et al.* 2007; Rathmann *et al.* 2007; Triant *et al.* 2007; Kaplan *et al.* 2008; Francisci *et al.* 2009; Ross *et al.* 2009). Additionally, ART interuption with attendant detectable viraemia has been associated with increased pro-inflammatory markers, implicating the virus in the pathogenesis of the chronic inflammatory state (Kuller *et al.* 2008).

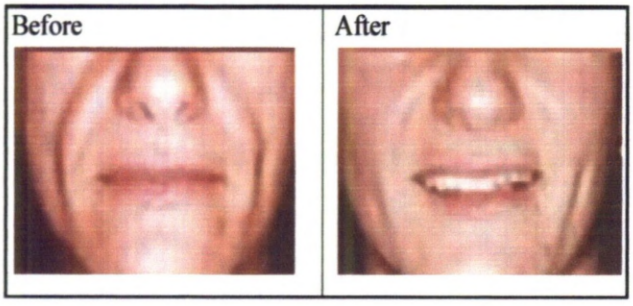
1.3 MANAGEMENT OF COMPLICATIONS OF ART

Management of the long-term effects of ART is fraught with problems due to interactions that can result between the individual drugs within HAART and the drugs being used to counter the adverse effects. This is because of overlapping toxicities or common metabolising pathways. Reversibility of some long-term effects may also be slow if not impossible.

1.3.1 Reconstructive therapy for facial LA

This involves injection into the face of either biodegradable fillers such as poly-L-lactic acid and hyaluronic acid (temporary measure, requiring re-treatment) or permanent fillers such as bio-alcamid (with the attendant risk of foreign body reaction or granuloma formation) (Moyle *et al.* 2004; Jones *et al.* 2005c; Moyle *et al.* 2006). This option is not possible for LA involving the limbs or buttocks. Figure 1-11 show the cosmetic results of facial injections with poly-L-lactic acid.

Figure 1-11: Before and After Poly-L-lactic acid injections



1.3.2 Modifications of Antiretroviral Treatment

The main option has been to switch from drugs known to cause the LD syndrome, particularly switching from thymidine analogues (AZT and D4T) to

TDF or ABC in LA (Martin *et al.* 2004; Moyle 2004; Moyle *et al.* 2006; EACS 2009), or switching from PIs associated with hyperlipidaemia to a PI-sparing option or lipid friendly PIs. The morphological changes are not always completely reversible and can take a long time to resolve after switching regimens and alternative options might be restricted because of intolerance, co-morbidity, drug interactions, or viral resistance. Hence, a significant minority of patients may need to remain on potentially lipodystrophic regimens. Structured treatment interruption is no longer an option in light of new data on increase in opportunistic disease, non-AIDS illness and deaths in those that interrupted treatment (El-Sadr *et al.* 2006); there is also the risk that in the long term treatment interruption could result in virological rebound with risk of progression of the illness (Arjona *et al.* 2006). Current guidelines do not recommend treatment interruption as an option for treating LD (EACS 2009).

1.3.3 Cytokines, adipocyte transcription factors and nuclear receptors

Some of the studies looking at the use of cytokines such as leptin and PPAR-γ agonists in HIV LD are summarized in Table 1-4.

1.3.3.1 Cytokines

Leptin therapy has been used successfully in cases of congenital and acquired non-HIV-related LD with resultant improvements in glycaemic control, hypertriglyceridaemia and a decrease in fatty infiltration of the liver (Oral *et al.* 2002; Tsiodras and Mantzoros 2006). In HIV LD, leptin administration improves lipid profiles, insulin resistance and fat accumulation and is well

tolerated, but the studies were small (Brown *et al.* 2005; Lee *et al.* 2006; Mulligan 2007). More recent studies have confirmed most of these findings, except with respect to peripheral fat mass, which did not change (Brennan *et al.* 2009; Magkos *et al.* 2011). Adiponectin as well as its receptors AdipoR1 and AdipoR2 are attractive future targets for drug development (Kralisch *et al.* 2007). Studies with TNF-α antagonists designed to improve insulin sensitivity have not been promising in humans with obesity and type II diabetes (Ofei *et al.* 1996). Use of TNF-α antagonists such as infliximab in Crohn's disease and rheumatoid arthritis does not improve insulin sensitivity (Kralisch *et al.* 2007). However, use of TNF-α antagonists has not been studied in HIV-treatment-associated LD since there is a risk of activation of opportunistic infections such as tuberculosis.

Table 1-4: Study summaries of adipocytokine and PPAR ligand use in HIV LD

Research studies	Patient population	Treatments and duration	Results
Leptin			
(Brown *et al.* 2005) - *in vitro* study	ritonavir-treated mice	leptin administration or polyunsaturated fatty acid (PUFA) diet	leptin use showed: – reversal of raised TC – ↓ in RTV-induced interscapular fat – improved liver steatosis
(Lee *et al.* 2006) -randomized, placebo-controlled, double-blinded, crossover study	7 men with HAART-induced LA, low leptin and hypertriglyceridaemia	recombinant human leptin 0.04 mg/kg daily or placebo for 2 months	leptin improved insulin resistance, HDL, truncal fat mass
(Mulligan 2007) -open-label, proof-of-principle study	8 men with HIV-induced LA, low leptin, insulin resistance	recombinant methionyl human leptin for 6 months (0.01 mg/kg twice daily for 3 months, followed by 0.03 mg/kg twice daily for 3 months)	– 30%↓ in visceral fat (*P* = 0.001) – ↓ in TC, LDL-C, non-HDL-C, TGs – improved hepatic insulin sensitivity – ↓ in lipolysis
(Brennan *et al.* 2009) - randomized, placebo-controlled double-blinded, crossover study	7 men with HAART-induced LA, low leptin (<3ng/mL)	recombinant methionyl human leptin for 3 months (0.04 mg/kg/day in 2 divided doses) or placebo	Leptin (> placebo): –↓fasted serum insulin –No change in truncal or peripheral fat mass –↓HOMA-IR 30% –↓ in visceral fat (*P* = 0.001) –attenuation of postprandial glycaemia
(Magkos *et al.* 2011) - randomized, placebo-controlled double-blinded, proof-of-principle study	9 men with HAART-induced LA, low leptin (<4ng/mL)	Patients on pioglitazone 30mg daily received either recombinant methionyl human leptin for 3 months (0.04 mg/kg once daily) n=5 or placebo n=4	Leptin (> placebo): –↓fasted serum insulin –No change in truncal or peripheral fat mass –↓HOMA-IR 30% –↓ in visceral fat (*P* = 0.001) –attenuation of postprandial glycaemia

↑: increase; ↓: decrease/reduction; HAART: highly active antiretroviral therapy; RTV: ritonavir; bd: twice daily; od: once daily; TC: total cholesterol; TGs: triglycerides; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; d4T: stavudine; po: orally. TH: thiamidine

Table 1-4: Study summaries of adipocytokine and PPAR ligand use in HIV LD (continued)

Research studies	Patient population	Treatments and duration	Results
Thiazolidinediones			
(Carr *et al.* 2004) -randomized, double-blind, placebo-controlled study	108 patients with HIV LA	rosiglitazone 4 mg bd po or placebo for 48 weeks	rosiglitazone: – improved insulin sensitivity – no improvement of LA – ↑ in TC, TGs
(Slama *et al.* 2008) -randomized, double-blind, placebo-controlled study	130 HIV patients with LA	pioglitazone 30 mg od or placebo for 48 weeks	pioglitazone group: – ↑ in limb fat (patients not on d4T) – ↑ in HDL-C – no change in other lipid fractions
(Calmy *et al.* 2003) -open-label, prospective study	11 HIV patients (all on d4T)	pioglitazone 30 mg od × 3 months, then 45 mg od × 3 months	– ↑ in body fat mass (total and leg) – no change to lipids
(Barb *et al.* 2005) -2 × 2 factorial, randomized, double-blinded, placebo-controlled study	14 HIV patients with HAART-induced metabolic side effects	pioglitazone 30–45 mg od po versus fenofibrate 200 mg od for 12 months	pioglitazone (but not finofibrate) improved insulin resistance, blood pressure and lipid profile
(Tungsiripat *et al.* 2010) -randomized, double-blinded, placebo-controlled study	71 HIV patients with LA on TH sparing regimens >24weeks	Rosiglitazone (4mg od x 4weeks then bd) vs. placebo for 48 weeks	Rosiglitazone (> Placebo): – ↑ in limb fat – ↑ in TC – ↓in insulin resistance
(Schindler *et al.* 2009) -randomized, double blind, placebo controlled parallel group study	40 HIV patients on HAART	Rosiglitazone (4mg od) vs. placebo for 6 months	Rosiglitazone (> Placebo): – no change in body fat distribution – ↑ in TC/LDL-C – ↓ in peripheral insulin resistance

↑: increase; ↓: decrease/reduction; HAART: highly active antiretroviral therapy; RTV: ritonavir; bd: twice daily; od: once daily; TC: total cholesterol; TGs: triglycerides; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; d4T: stavudine; po: orally. TH: thiamidine

1.3.3.2 Thiazolidinediones

Thiazolidinediones or glitazones (such as rosiglitazone and pioglitazone) are ligands for the transcription factor PPAR-γ and are used in type II diabetes to improve insulin sensitivity (Haider *et al.* 2007). Use of rosiglitazone in HIV LD appears not to be a viable option with its inability to improve peripheral lipoatrophy as well as its adverse effects on lipids (Carr *et al.* 2004) (Schindler *et al.* 2009), particularly in light of recent data on increased risk of cardiovascular events in type 2 diabetes patients with use of rosiglitazone (Lipscombe *et al.* 2007; Nissen and Wolski 2007). However, a recent study demonstrated increases in limb fat with rosiglitazone compared to placebo in patients that were on a non-thymidine regimen for more than 6months (Tungsiripat *et al.* 2010). Since most ART naive patients will be starting on a non-thymidine regimen, LD will be minimised, the CVD risks with rosiglitazone would outweigh the benefits gained in terms of fat redistribution. Pioglitazone may have a place in the treatment of HIV LD in that it may increase limb fat though its effects on lipids are conflicting, with some studies showing no change and others showing improvement (Calmy *et al.* 2003; Barb *et al.* 2005; Slama *et al.* 2008).

1.3.4 Other drug options

Various drugs have been studied in the treatment of HIV LD. These include, among others, growth hormone (GH) and GH secretagogues, metformin, statins and uridine supplementation. Table 1-5 summarizes the results of studies looking at the use of these drugs in HIV LD patients.

Table 1-5: Summary of studies of other drugs in HIV LD

Research studies	Patient population	Treatments and duration	Results
Recombinant GH			
(Luzi *et al.* 2005) -randomized, placebo-controlled, double-blinded, crossover study	30 HIV patients with fat redistribution and metabolic derangement	rGH 0.2 IU/kg per week SC versus placebo ×6 months	rGH group: – ↓ in truncal fat (P = 0.048) – ↓ in limb fat (P = 0.0248) – ↓ in TC, TGs (non-significant)
(Moyle *et al.* 2001) -open-label study	8 men with HIV-associated fat accumulation	GH 3 mg/day SC × 6 months	– ↓ in total body fat (P = 0.05); primarily in the trunk region – ↑ in lean body mass (P = 0.03)
(Honda *et al.* 2007) -prospective, open-label study	25 HIV-1 patients with moderate to severe facial lipoatrophy	rGH 5 mg alternate days SC × 6 months; further 1 month follow-up	– ↑ soft tissue thickness at the level of zygomatics sustained at month 12 (P = 0.021)
GHRH analogues			
(Falutz *et al.* 2007) -multicentre, randomized, placebo-controlled study	412 patients with HIV with abdominal fat accumulation	Tesamorelin 2 mg od SC (or placebo) ×26 weeks	Tesamorelin group: – ↓ VAT by 15.2% in the Tesamorelin group (versus ↑ by 5.0% in the placebo group) – ↓ TC, TGs, TC:HDL-C ratio – ↑ HDL-C
(Koutkia *et al.* 2004a) -randomized, double-blind, placebo-controlled study	31 HIV-infected men with lipodystrophy	Geref or GHRH 1–29 1 mg bd SC or placebo ×12 weeks	Geref group: – ↑ lean body mass with GHRH (P = 0.04) – trunk fat (P = 0.03) – improved ratio of trunk:lower extremity fat (P = 0.005) – levels of glucose, insulin and lipids unchanged
Statins			
(Mallon *et al.* 2006) -randomized, placebo-controlled study	33 HIV-positive men ↑ TC (>6.5 mmol/L) on PI-based HAART	pravastatin (40 mg each night) or placebo ×12 weeks	pravastatin group: – tendency to ↓ in TC – no change in TGs – ↑ SAT (↑ limb fat, P < 0.04; ↑ abdominal subcutaneous fat, P = 0.02)
Uridine			
(Ross *et al.* 2009) -pilot study (safety and effectiveness)	16 patients with lipoatrophy on stavudine-containing ART	NucleomaxX 36 g TDS every other day ×16 weeks	–improvement in LA scores –no changes in body mass index, lactate, lipids, insulin –no changes in fat and PBMC mtDNA levels
(Sutinen *et al.* 2007) -randomized, double-blinded, placebo-controlled study	20 patients with HAART-associated LA	dietary uridine supplement (36 g TDS for 10 consecutive days/month) or placebo, × 3 months	uridine group: – ↑ total limb fat (P < 0.001) – ↑ intra-abdominal fat (P < 0.05) – ↑ total body fat (P < 0.001)

Table 1-5: Summary of studies of other drugs in HIV LD (Continued)

Research studies	Patient population	Treatments and duration	Results
Metformin			
(Hadigan *et al.* 2000; Hadigan *et al.* 2002) -randomized, double-blinded, placebo-controlled, pilot study followed by a 6 month open-label continuation phase	26 HIV, non-diabetic patients with fat redistribution and abnormal OGTT, hyperinsulinaemia, or both	metformin 500 mg bd po or placebo ×3 months	metformin group: **pilot phase** – ↓ insulin resistance (↓ insulin area under curve by 20%) (P = 0.01) – ↓ weight (P = 0.005) – ↓ diastolic blood pressure (P = 0.009) – VAT:SAT ratio unchanged **continuation phase** – sustained ↓ in insulin levels, weight circumference – ↓ t-PA and PAI-1
(Saint-Marc and Touraine 1999) -randomized, controlled study	29 HIV patients with central adiposity and hyperinsulinaemia on PI-based HAART	metformin 850 mg TDS po or no treatment (control group) ×2 months	metformin group: – ↓ basal plasma glucose, insulin, C-peptide – ↓ triglycerides by 30.1% – ↓ VAT, total adipose tissue and waist-to-hip ratio (increase of these in control group)
(Mulligan 2006) -randomized, controlled study	105 HIV patients with ↑ waist-to-hip ratio and hyperinsulinaemia	metformin (500–1000 mg bd) or rosiglitazone (4 mg od) or combined versus placebo	– no significant changes in VAT, SAT or total extremity fat among the groups (↑ in lower extremity fat with rosiglitazone)
(Kohli 2008) -randomized, double-blinded, placebo-controlled study	48 HIV patients with lipodystrophy (↑ abdominal girth and waist-hip ratio) and normal glucose tolerance	metformin 1500 mg daily or placebo × 24 weeks	metformin group: – ↓ appendicular fat mass compared with placebo (P = 0.03) – no change in VAT and lipids
(Martinez *et al.* 2004) -randomized, blinded, placebo-controlled study	108 HIV patients on PI-based HAART with abdominal obesity and ↑ TGs	metformin 850 mg, gemfibrozil 600 mg or placebo bd ×1 year	in both groups: – loss of total and regional fat in all three groups (less with gemfibrozil) – no significant change in waist-to-hip ratio

↑: increase; ↓: decrease/reduction; HAART: highly active antiretroviral therapy; TDS: three times daily; bd, twice daily; od: once daily; TC: total cholesterol; TGs: triglycerides; HDL-C: high-density lipoprotein cholesterol; GHRH: growth hormone releasing hormone; OGTT: oral glucose tolerance test; VAT: visceral adipose tissue; SAT: subcutaneous adipose tissue; mtDNA: mitochondrial DNA; PBMC: peripheral blood mononuclear cells; t-PA: tissue plasminogen activator; PAI-1: plasminogen activator inhibitor type 1; SC: subcutaneously; po, orally.

[a] *NucleomaxX (dietary supplement containing uridine).*

1.3.4.1 GH therapy

GH deficiency in HIV-negative patients is associated with increased cardiovascular mortality (Sesmilo *et al.* 2000). Effects of recombinant GH (rGH) therapy have been studied in patients with adult GH deficiency, obesity and HIV wasting. In these patients, GH replacement increases lean body mass; reduces fat mass with reductions in abdominal subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT); and increases energy expenditure, exercise performance and cardiac function. There may be a tendency for lipids to decrease (Johannsson *et al.* 1997; Schambelan *et al.* 2002; Tai *et al.* 2002; Yin and Glesby 2005). GH therapy has, however, been thought to increase the risk of cancers, though extensive studies of the outcome of GH replacement in childhood cancer survivors showed no evidence of an excess of *de novo* cancers (Jenkins *et al.* 2005).

In HIV LD, patients have markedly reduced GH levels in association with excess visceral adiposity (Rietschel *et al.* 2001; Koutkia *et al.* 2004b). Impaired GH secretary patterns in these patients are influenced by gender, race and fat redistribution (increased waist–hip ratio) (Koutkia *et al.* 2006). GH therapy in HIV LD increases lean body mass; decreases trunk or visceral fat and limb fat; and improves facial lipoatrophy with little or no effects on lipid profiles. The most common adverse effects of GH therapy are fluid retention, arthralgia, muscle pains, carpal tunnel syndrome and a decrease in insulin sensitivity. These effects return to baseline levels after treatment with GH stops (Moyle *et al.* 2001; Luzi *et al.* 2005; Honda *et al.* 2007). The long-term effects of GH therapy on visceral fat, limb fat, insulin sensitivity, lipid profiles and the risk of

carcinogenesis are not known. The possible worsening of insulin resistance may expose patients to an increased risk of cardiovascular disease, and the risk of further limb fat loss may be intolerable to patients.

Other studies have looked at use of GH secretagogues such as GHRH (growth hormone releasing hormone) analogues to induce a more physiological release of GH to avoid the side effects associated with treatment with GH (Koutkia *et al.* 2004a; Falutz *et al.* 2005). Compared with placebo, GHRH analogues (Geref or GHRH 1–29 and Tesamorelin or TH9507) significantly increase levels of IGF-1, increase lean body mass, decrease visceral and trunk fat and improve the ratio of visceral to lower extremity fat. There are no significant changes in levels of glucose and insulin. Adverse effects associated with GH excess are not seen (Koutkia *et al.* 2004a; Falutz *et al.* 2005; Falutz *et al.* 2007). The effects on lipids vary, with some studies finding no significant changes (Koutkia *et al.* 2004a) and others (Falutz *et al.* 2007) finding significant reductions in triglyceride and cholesterol to HDL ratio. Use of GHRH analogues appears to require long-term treatment for continued benefit, as patients who stop treatment regain their VAT (Falutz *et al.* 2007).

Both GH and GHRH analogues have to be given by injection, which might not be acceptable to some patients and, additionally, the cost of both drugs is prohibitive.

1.3.4.2 Metformin therapy

Metformin is a biguanide oral anti-diabetic agent, which is widely used in type 2 diabetes, particularly in patients with obesity. It acts by reducing endogenous

glucose production by the liver and by sensitizing the liver and peripheral tissues to insulin by acting partly through the activation of AMPK (Hundal and Inzucchi 2003; Zou *et al.* 2004; Natali and Ferrannini 2006). Metformin has been shown to reduce cardiovascular risk in patients with type 2 diabetes through its reduction of lipids and markers of lipid peroxidation and platelet activation (isoprostanes and thromboxanes), and increases tissue antioxidants (vitamins A and E), which leads to a reduction in atherosclerosis (UKPDS34 (1998; Formoso *et al.* 2008). Metformin therefore represents an interesting therapeutic option in HIV LD, in which fat redistribution and insulin resistance are prominent features (Hadigan *et al.* 2000). Indeed, studies do show that metformin may have a place in the management of HIV LD with reductions in VAT, total adipose fat and waist-to-hip ratio (Saint-Marc and Touraine 1999; Hadigan *et al.* 2002). However, some studies show no change in waist-to-hip ratio and, rather worryingly, further loss in limb fat (Martinez *et al.* 2004; Mulligan 2006; Kohli 2008).

1.3.4.3 Statins

Statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) are used in the treatment of hyperlipidaemia and established cardiovascular disease in the general population. They have anti-inflammatory, anti-thrombotic, as well as inhibitory effects on T-cell proliferation and endothelial effects that contribute to their overall beneficial effects on reducing mortality from cardiovascular disease in both healthy people and diabetics (Egashira *et al.* 1994; Rosenson and Tangney 1998; Rosenson *et al.* 1999; Sommeijer *et al.* 2004; Brugaletta *et al.* 2006). The effects of statins on insulin resistance are contradictory with some studies showing beneficial, neutral or worsening

effects (Sonmez *et al.* 2003; Gannage-Yared *et al.* 2005). The use of statins to treat the hyperlipidaemia associated with HAART has been extensively studied, showing efficacy in lowering total and LDL cholesterol and triglycerides (Calza *et al.* 2003; Benesic *et al.* 2004). However, few studies exist that have looked at whether statins have beneficial effects on the physical aspects of HIV LD. A study by Mallon (Mallon *et al.* 2006) showed that apart from lowering lipids, pravastatin also increased subcutaneous fat and limb fat in patients when compared with placebo. There was no significant effect on visceral fat, trunk fat and body mass index.

The anti-inflammatory, anti-thrombotic and endothelial effects of statins make them an interesting therapy in untreated HIV patients to counteract the chronic inflammatory state induced by uncontrolled viraemia which would reduce risk of CVD. In vitro studies have also shown that statins may have anti-HIV effects (Maziere *et al.* 1994; Giguere and Tremblay 2004). A combination of these reasons would possibly mean that ART naive patients treated with statins could delay the start of ART, thereby avoiding the metabolic and physical effects of ART. In vivo studies in HIV patients do show that statins have anti-inflammatory effects but unfortunately show no effect on HIV-1 RNA levels (Negredo *et al.* 2006; Ganesan *et al.* 2011). Obviously studies into the use of statins to counter chronic inflammation induced by HIV need to continue.

1.3.4.4 Uridine

In vitro studies show that the pyrimidine precursor uridine reverses the mitochondrial toxicity induced by pyrimidines such as DDC and D4T with restoration of cell proliferation. Uridine also reverses the cell depletion and

lactic acidosis seen with AZT and 3TC combination. It, however, does not reverse similar effects caused by DDI (a purine analogue) (Walker 2003). Use of uridine in HIV patients shows that it may be of benefit, with the improvement of lipoatrophy, and is well tolerated (Sutinen *et al.* 2007; Ross *et al.* 2009).

1.3.5 Management of frank diabetes, dyslipidaemia, hypertension and CVD

The management of patients that develop diabetes, dyslipidaemia, hypertension, coronary heart disease and cerebrovascular accidents follow the guidelines established for the HIV negative general population (NCEP 2002; Schambelan *et al.* 2002; JBS2 2005; Gazzard 2008; EACS 2009; NICE-TA94 2009).

1.4 AIMS OF THE THESIS

1. To explore the markers of cardiovascular disease and HIV lipodystrophy in HIV patients on ART, comparing changes over 12 months of these markers in patients:

   -continuing on a thymidine regimen

   -continuing on a non-thymidine regimen

   -switching from a thymidine to a non-thymidine regimen.

   Markers explored include lipid profiles; glucose and insulin sensitivity; pro-inflammatory markers (IL-6 and TNF-α); adipocytokines (adiponectin, leptin, resistin and visfatine); plasma viscosity and markers of disease control (CD4 count and HIV viral load).

2. To explore the levels of traditional risk factors of CVD in HIV patients coming from a background population at high risk of CVD and to evaluate the attending physicians' ability to assess and manage appropriately conditions known to increase risk of CVD according to available guidelines.
3. To summarise interactions between drugs used to treat HAART induced dyslipidaemia and ART drugs, as an example of difficulties and complexicities faced by attending physicians treating a population of patients required to be on polypharmacy to achieve disease control.

1.5 Hypothesis

LD, lipid, glucose and insulin levels and markers of inflammation will be adversely affected over time in patients continuing on a thymidine regimen; with favourable profiles in those on a non-thymidine regimen while improvement should occur in the profiles in those switching from a thymidine to a non-thymidine regimen. Derangements in these measurements would place patients at risk of CVD.

CHAPTER TWO

CARDIOVASCULAR RISK, LIPID AND INSULIN SENSITIVITY PROFILES WITH TYPE OF ANTIRETROVIRAL DRUG IN HIV POSITIVE PATIENTS

2.1 INTRODUCTION

Morbidity and mortality from cardiovascular disease (CVD) are becoming an increasing problem in HIV positive patients taking antiretroviral therapy (ART) and the incidence of myocardial infarction increases with increasing exposure to combination ART. (Law *et al.* 2003; Friis-Moller *et al.* 2003(a); Friis-Moller *et al.* 2003(b); Triant *et al.* 2007; Sabin *et al.* 2008) This is because ART causes or accelerates derangements in levels of lipids, glucose and insulin resistance (Carr *et al.* 1998; Chen *et al.* 2002) which are traditional risk factors for CVD. Studies have shown an increased risk of myocardial infarction (MI) with the use of specific ART drugs. The protease inhibitors class has been most associated with increased CVD risk (Henry *et al.* 1998; Holmberg *et al.* 2002; Klein *et al.* 2002; Mary-Krause *et al.* 2003). Additionally, Durand et al showed that any exposure to abacavir (ABC), didanosine (DDI), stavudine (D4T), zalcitabine (DDC), lopinavir (LPV) and ritonavir (RTV) is associated with increase in MI risk and that recent exposure to ABC, D4T, LPV, RTV are also associated with increased risk (Durand M 2009) (Durand *et al.* 2011). The D:A:D study demonstrated that recent, but not cumulative, use of ABC or DDI was associated with an increased rate of MI, but found no association between the rate of MI and cumulative or recent use of zidovudine (AZT), D4T, or lamivudine (3TC) (Sabin *et al.* 2008). A lot of controversy surrounds ABC with regard to its association with risk of CVD, with some studies demonstrating excess risk while others showing no such association (summarised in Table 2-1). It is postulated that ABC increases risk of CVD events by adversely affecting markers known to increase the risk of CVD such as raising lipids (LDL and total cholesterol) (Martinez *et al.* 2010), reducing endothelial function (Hsue *et al.* 2009b) increasing markers of chronic inflammation such as high

sensitivity C-reactive protein and interleukin-6 (SMART/INSIGH/D:A:D 2008) and increasing platelet activation induced by the abacavir metabolite carbovir triphosphate (Baum *et al.* 2011). However, others studies have shown no adverse change in such markers (Martinez *et al.* 2010) (McComsey 2009; Palella *et al.* 2010). The controversy around ABC will probably continue.

Despite these contradictions, CVD disease is becoming important in an aging HIV population and is now believed to account for 10% of deaths among HIV-infected individuals (Lewden *et al.* 2007; Sabin *et al.* 2008). Apart from the side effects of ART, HIV infection per se regardless of virological or immunological control is associated with a state of chronic inflammatory and endothelial activation which predispose to atherosclerosis (as measured by an increase in carotid intima-media thickness (IMT) compared to HIV negative controls) and therefore an increased risk of CVD (Hsue *et al.* 2009a; Ross *et al.* 2009).

Identification and management of risk factors for CVD in HIV patients is therefore a major concern for HIV physicians, and issues of which drugs to start or maintain patients on to minimise risk have become all important. Indeed, organisations focusing on HIV care have issued guidelines on the management of CVD in HIV positive patients. These include European AIDS Clinical Society (EACS) and the International AIDS Society–USA Panel (IAS) (Schambelan *et al.* 2002; Lundgren *et al.* 2008b). The EACS 2008 guidelines have recommended that all HIV-infected persons should be screened for history of metabolic disease, dyslipidaemia, diabetes mellitus, hypertension and alteration of body composition at regular intervals and that the composite

Table 2-1: Summary of Clinical Trial and Cohort Analyses of Abacavir Use and increased CVD Risk

Study	Design	CV Events	Effect of ABC?
(Lundgren 2009) D:A:D Study (N = 33347)	Observational cohort	Prospective, predefined	Yes
(Lang *et al.* 2010) (N = 1173)	Case control study	MI retrospectively validated	Yes 1st yr of exposure
(SMART/INSIGH/D:A:D 2008) (N = 2752)	RCT, observational analyses	Prospective, predefined	Yes
(Martin *et al.* 2009) (STEAL study) (N = 357)	RCT	Prospective	Yes
(Durand *et al.* 2011) (N=7053)	Observational cohort, nested case–control	Retrospective database search for AMI	Yes
(Cutrell *et al.* 2008) (GSK analysis) (N = 14174)	54 RCTs	Retrospective database search	No
(Ribaudo *et al.* 2011) (ALLRT ACTG A5001) (N = 3205)	5 RCTs	Retrospective by 2 independent reviewers	No
(Bedimo *et al.* 2011) (N=19424)	Observational cohort	Retrospective database search for AMI and CVA	No

CVD: cardiovascular disease; CVA: cardiovascular accident; AMI: acute myocardial infarction; RCT: randomised controlled trial; FHDH, French Hospital Database on HIV; MI, myocardial infarction; RCT, randomized controlled trial.

of cardiovascular risk factors for individual patients should be summarized by the calculation of the 10-year absolute risk of contracting ischaemic heart disease (IHD) using the Framingham equations. This approach allows stratification into three risk categories: low risk (<10% 10-year risk of IHD), medium risk (10–20% 10-year risk of IHD) and high risk (>20% 10-year risk of IHD). As a matter of course all such individuals should be encouraged to initiate lifestyle changes namely; a healthy diet, increase physical activity and stop smoking. The guidelines also recommend drug treatment for LDL

cholesterol, hypertension and blood sugar in individuals at high risk of CVD. The IAS guidelines pre-date the body of evidence that examines risk factors for CVD in HIV patients but essentially the guidelines were addressing the lipid abnormalities that ART induces. Additionally, guidelines on starting and switching ART are published which emphasize the need to look for evidence of CVD before starting or switching such ART as well as annual reviews of CVD status. Drugs that are less likely to increase the CVD risk are recommended for use (Gazzard 2008; Lundgren *et al.* 2008b). This emphasizes the importance of CVD in the HIV positive population.

In the UK in general, CVD is an important health problem. In England, death rates from all circulatory diseases stood at 125 per 100,000 of population in 1999. This was higher than most European Union countries whose membership predates 2004. For example, the CVD death rate for France in 1999 was 50 per 100,000. This is because there are highly modifiable risk factors in England; for instance, there are 10 million smokers (22% of the population) while 22% of men and 23% of women are obese. Twenty percent of coronary heart disease (CHD) deaths are linked to smoking in men and 17% in women. Deaths from CVD in the UK have since been reduced by 40% (to 83.8 deaths per 100,000 population) by the year 2006 through government initiatives to reduce deaths from CHD, but even with this impressive achievement, CHD kills more than 110,000 people in England every year, making it the biggest killer (DOH-UK 1999; DOH-UK 2004; DOH-UK 2007).

HIV patients in the UK will have the same non-modifiable traditional risk factors for CVD namely age, sex, race, family history of premature coronary heart disease and familial dyslipidaemia as the general public. However, HIV patients may be at higher risk of developing CVD than HIV non-infected people due to the adverse effects of ART drugs, having higher rates of modifiable risk factors such as smoking (Friis-Moller *et al.* 2003(a); Ross *et al.* 2009), hypertension, diabetes, dyslipidemia (Triant *et al.* 2007) and having the virus itself which has been shown to be an independent risk for atherosclerosis that is similar in magnitude to traditional CVD risk factors such as smoking and advancing age (per decade of life) (Grunfeld *et al.* 2009; Palella and Phair 2011). Indeed it has been shown that HIV infected patients whether on ART or not have a higher risk of developing ischaemic heart disease than HIV non-infected individuals (Obel *et al.* 2007; Triant *et al.* 2007); (Durand *et al.* 2011). As such the vulnerability to development of CVD in HIV patients on ART needs further investigation. This study addressed the issue of the prevalence and type of risk factors for CVD in HIV patients attending the North Manchester General Hospital Infectious Diseases unit.

2.2 METHODOLOGY

2.2.1 The Research Site

Recruitment and follow-up of patients were done at the North Manchester General Hospital Infectious Diseases unit. This is a tertiary infectious diseases unit with 3 wards with a total of 41 beds. The unit has a large number of HIV positive patients totalling ~1500 who attend dedicated daily HIV outpatient clinics. The unit has a large research unit, which is involved in multi-centre

research trials. The hospital is within Crumpsall, an area located to the north of the city of Manchester in the Northwest of England. It has a population of approximately 15,000 people which is very diverse comprising 73% white and 27% non-white which are mostly Asian (MCC 2009).

2.2.2 The Studies

Patients were recruited into two studies:

STUDY 1: Effect of antiretroviral therapy containing the Nucleoside and Nucleotide reverse transcriptase inhibitors Zidovudine, Stavudine, Didanosine, or Tenofovir on lipid profiles, adipocytokine levels and insulin resistance in HIV-positive patients;

STUDY 2: Effects of switching a thymidine nucleoside reverse transcriptase inhibitor (zidovudine or stavudine), or didanosine), on lipid profiles, adipocytokines and insulin resistance in HIV positive patients.

Some challenges were encountered during recruitment into the two studies. Recruitment into some arms of the studies was slow, particularly patients maintained on D4T or DDI as most of the patients were being switched to non-thymidine, non-DDI regimens in accordance to guidelines at that time (BHIVA 2006). For the same reasons, only small numbers could be swiched from DDI regimens. Amendments were done accordingly so that the drugs studied were AZT, D4T, TDF and ABC within HAART. A total of 64 participants were recruited into the following groups:

Group 1: A total of 20 patients remaining on a thymidine (18 on AZT and 2 on D4T)

Group 2: A total of 27 patients remaining on a non-thymidine regimen (17 on TDF/FTC, 7 on ABC/3TC and 3 on TDF/ABC).

Group 3: A total of 17 patients switching from a thymidine to a non-thymidine regimen (ABC/3TC, TDF/FTC or TDF/ABC). 14 switched from AZT and 3 switched from D4T.

A post-hoc power calculation was done using total cholesterol (TC) based on the number of patients in groups 1 and 2 based on total cholesterol (TC). This is based on the larger Study 934 which reported at 48 weeks an increase in total cholesterol that was greater in patients continuing on AZT containing regimen than those continuing on a TDF based regimen (mean change 0.91mmol/L vs. 0.54mmol/L respectively, $P<0.001$; mean difference 0.37mmol/L) (Gallant *et al.* 2006). Our study had 24% power to detect the same mean difference. We would have needed a sample size of 115 participants in each group to detect this mean difference (0.37mmol/L) in TC between group 1 and 2, using an estimate of standard deviation of 1, at the 5% significance level to give the study an 80% power. TC was chosen as it has been shown in large population studies to be a marker of CVD (Unknown 1984; Stamler *et al.* 1986; Assmann *et al.* 1998; Austin *et al.* 1998; Wilson *et al.* 1998).

2.2.2.1 Objectives

To investigate the effects of remaining on a thymidine (AZT or D4T) regimen, non-thymidine regimen (containing TDF and/or ABC) or switching from a thyimidine to a non-thymidine regimen as part of HAART on lipid profiles (total cholesterol, low density lipoprotein cholesterol, high density lipoprotein cholesterol and triglycerides), proinflammatory cytokines (TNF-α and IL-6),

adipocytokines (adiponectin, leptin, resistin and Visfatin) and insulin resistance in HIV positive patients.

2.2.2.2 Eligibility Criteria

The level of CD4 count or viral load did not form part of the eligibility criteria. While most research inclusion criteria are very narrow, all adults regardless of age were included, with the exclusions mainly being diabetes, occurrence of an opportunistic infection within the preceding 3 months and serious illicit drug use or existing poor adherence, the latter two because such patients would be unlikely to complete follow-up in the study. This was done to give an idea as to the level of CVD in a typical large HIV unit in order to inform HIV clinicians on the management of patients, taking into consideration published guidelines. The participants were recruited if they were HIV infected, above 18 years of age, able to give written consent to join the study, on their treatment consistently for at least 12 months, there was no anticipated change in ARV treatment for 3 months and they were able to commit to one year of follow-up in the study.

2.2.2.3 Study Conduct

The study protocols, patient information sheets and consent forms were approved by Oldham Local Research Ethics Committee.

2.2.2.3.1 Patient Selection

Patients due to attend consultant clinics for routine follow-up, had their notes pre-screened for inclusion and exclusion criteria. An invitation to participate was entered in the patients' notes asking the physician to discuss their potential inclusion in the study. If the patient agreed, the study chief investigator was then informed. The patient was given the study patient

information sheet and consent form to take and read at home for at least 24 hours to conform to good clinical practice requirements for human based research projects. A convenient date and time was agreed with the patient for them to attend for baseline investigations.

2.2.2.3.2 Study Procedures

On the day the patients enrolled into the study, written signed informed consent was obtained. In Study 2, patients due to switch their antiretroviral regimen were seen for study enrolment on the day they were due to change their regimen. Patients self-assessed for evidence of lipodystrophy (LD) using a validated questionnaire from the LD case definition study (Carr *et al.* 2003) (Appendix 1) and the investigator checked for antiretroviral drug adherence (Study 2) using a validated adherence questionnaire (Appendix 2) from the GEEMA study (Knobel *et al.* 2002). Anthropometric assessment namely weights and heights were done. Blood pressure was determined using an automated sphygmomanometer. Forty (40) millilitres (mLs) of blood (following an overnight fasting of at least 9 hours) were obtained from each participant at base-line:

1. 30mls of the blood was used to determine fasted total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides (TGs), glucose, haemoglobin (Hb), liver and renal function, plasma viscosity and C-reactive protein (CRP) and these were performed at the North Manchester General Hospital (NMGH) laboratory as part of the routine blood tests that patients on HAART undergo during their routine 3-monthly clinic visits. The NMGH laboratory uses the Benson Automated Plasma Viscometer BV 200 to

measure plasma viscosity, which is determined by the concentration of plasma proteins present.The major influence on plasma viscosity is exerted by fibrinogen and immunoglobulins. Plasma viscosity changes when protein fractions are altered, such as in general illness, infection, inflammation and malignancy and all result in an increase in plasma viscosity. Samples are collected in EDTA blood bottles and are centrifuged to obtain plasma before they are analysed, and are therefore platelet-poor. The principle of the test is based on the time for an aliquot (50µL) of fluid to travel along a uniform capillary, in a fixed vacuum and temperature, relative to calibration fluid of a known viscosity.

2. 10 mLs of blood were used to determine fasted insulin and cytokine levels. The insulin and cytokine analysis was performed by Millipore using Millipore's Cytokine Profiler™ according to their standard procedures (Millipore 2008). This service has been used previously by other investigators (de Jager *et al.* 2003; de Jager *et al.* 2005; Liu *et al.* 2009). The detection limit for insulin was 50.9 pg/mL.

A description of the cytokines investigated is detailed in the next chapter (Chapter 3, Section 3.2, Methodology, page 101).

The body mass index (BMI) was calculated from weight and height using the BMI calculator (NHL-BMI 2008) which uses weight in kilograms divided by the square of height in meters. The cardiovascular (CV) risk over 10 year period was calculated using the CV risk calculator (Payne 2005) based on Joint British Societies and British National Formulary format with age, sex, systolic blood pressure, smoking status, total cholesterol and HDL cholesterol as

variables. The Homeostasis Model Assessment (HOMA) was used to estimate β-cell function (HOMA-%B) and insulin sensitivity (HOMA-%S) as a percentage of a normal reference population, and insulin resistance (HOMA-IR) using the HOMA Calculator v2.2.2 (DTU 2004) which is based on the formula: fasting glucose level (mg/dL) multiplied by the fasting insulin level (mU/mL) and divided by 22.5 (Matthews *et al.* 1985; Hadigan *et al.* 2000). Determination of insulin resistance using HOMA is an acceptable method because the estimate of insulin resistance obtained by HOMA correlates with estimates obtained by use of the euglycaemic hyperinsulinaemic clamp (R_s=0.88, P<0.0001) and the hyperglycaemic clamp (R_s=0.69, P< 0.01). Additionally, the estimate of deficient β-cell function obtained by HOMA correlates with that derived using the hyperglycaemic clamp and with the estimate from the intravenous glucose tolerance test (R_s= 0.61, P< 0.01), as demonstrated by Matthews *et al* (Matthews *et al.* 1985; Hadigan *et al.* 2000).

All the blood tests, weight and blood pressure were repeated at three further visits at 3, 6 and 12 months (with a window period of plus or minus 1 week for each visit) as part of the longitudinal follow-up of the patients to see the trends in the levels of the lipids, glucose, CRP, plasma viscosity, liver and renal function. Cytokine and insulin levels were determined at baseline, visit 3 (6 months) and visit 4 (12 months). When stable on treatment, patients are usually routinely seen every 3 months; therefore, the study visits were timed to coincide with these time points. Table 2-1 summarises the study visits and procedures.

Table 2-2: Study Visit Schedule

Study visits and procedures				
Procedure	**Visit 1 (Enrolment)**	**Visit 2 (3 months)**	**Visit 3 (6 months)**	**Visit 4 (12 months)**
Consent	X			
Eligibility check	X			
Medical history	X			
LD questionnaire	X	X	X	X
Adherence questionnaire*	X	X	X	X
Physical examination	X	X	X	X
Blood draw (40mL)	X	X	X	X

*LD: lipodystrophy; *Study 2*

2.2.3 Statistical Analysis

The independent sample T-Test was used to compare the mean change between the three groups between 12 months and baseline for blood pressure, weights, body mass index, TC, HDL-C, LDL-C, TGs, TC-HDL–C ratio and plasma viscosity (SPSS v. 16, SPSS Inc. Chicago, USA). The independent sample T-Test was also used to compare the subjective lipodystrophy perception mean severity scores for various body regions between the groups between 6 months and baseline. The mean changes of all parameters were approximately normally distributed on testing using histograms. Descriptive analysis was used to compare CV risk over time between the groups. Fisher's exact test was used to compare adherence levels between the three groups (STATA, Texas, USA, http://www.stata.com.).

2.3 RESULTS

Results for cytokines, plasma viscosity, CRP and CD4 count are described in the next chapter.

2.3.1 Baseline Characteristics

Most patients were male (78.1%). The median age was 42.5 years (29-73). Forty six (71.9%) patients were white and 17 (26.6%) were black African. Forty two patients (65.6%) acquired HIV through men having sex with men; 15 (23.4%) acquired HIV via heterosexual means; and 7 patients (10.9%) acquired HIV via other means (blood products, needle stick injury or source was unknown). Twenty five (39.1%) of the patients were current smokers with average smoking pack years of 22.2 (0.45-75.3) and 17 (26.6%) were ex-smokers. Forty four (68.8%) of the patients took alcohol with an average alcohol intake of 10.7 units (range 1-60) per week. Three (15%) of the patients in Group 1, 5 (18.5%) in Group 2 and 3 (17.6%) in Group 3 had systolic BP $\geq$ 140mmHg. For Group 3 patients, 18/20 (90%) switched drugs because of toxicity and 2/20 (10%) switched due to virological rebound. More patients in Group 3 had a diagnosis of LD in their case notes (65%) compared to Group 1 (25%) and Group 2 (48%). This was expected because Group 3 patients were switching treatment mostly because of toxicity which included LD. Table 2-3 presents the baseline characteristics of the study population classified according to groups.

2.3.2 Antiretroviral Drugs Used

Figure 2-1 presents frequency data for the use of NNRTIs and PIs according to group. Most patients were on a HAART regimen that included an NNRTI.

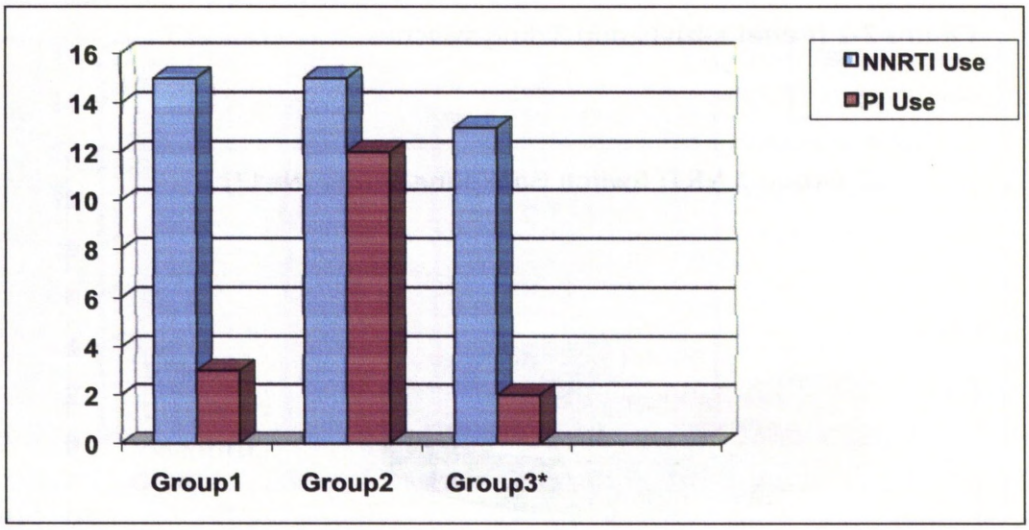
EFV was the most frequently prescribed NNRTI, but in Group 2 the use of PIs such as FPV and ATV was higher.

Table 2-3: Baseline Characteristics for all patients

	Group 1 (n=20)	Group 2 (n=27)	Group 3 (n=17)
Age (years)-Median (range)	41.5 (29-73)	41.0 (31-63)	41.0 (34-56)
Duration of current ART (Months)–Median (range)	35 (13-72)	13 (3-89)	49 (7-86)
Duration all ART (years)- Median (range)	4.3 (1.2-12)	6.3 (0.8-16)	5.8 (0.7-11)
Previous stavudine exposure*	4 (20%)	13 (48%)	3 (18%)
Previous protease inhibitor exposure*	7 (35%)	17 (63%)	8 (47%)
Previous AIDS defining illness*	5 (25%)	8 (30%)	5 (29%)
Viral Load ≤ 40 copies/ml*	17 (85%)	26 (96%)	13 (76%)
CVD Risk Factors*:			
Smokers	7 (35%)	13 (48%)	5 (29%)
hypertension/dyslipidaemia/CVA/CHD	0	4 (15%)	1 (6%)
Family history of CVD and dyslipidaemia	5 (25%)	7 (26%)	1(6%)
Existing LA/LD diagnosis and/or on examination*	5 (25%)	13 (48%)	11 (65%)
Systolic BP-median (range)	121 (100-152)	121 (98-188)	116 (98-149)
Diastolic BP-median (range)	82 (58-105)	80 (65-105)	80 (66-107)
Weight (kg)-median (range)	68 (48-88)	72 (58-118)	73 (57-89.1)
BMI-median (range)	23.7 (18.7-28.7)	23.5 (19-47.3)	24.0 (19.9-28.7)
CVD risk:			
Median CV Risk % (range)	4.3 (0.3-25)	6.2 (0.2-22.5)	3.9 (0.5-17.5)
CV Risk>20%*	2 (10%)	2 (7%)	0
CV Risk 10-20%*	3 (15%)	5 (19%)	2 (12%)
CV Risk <10%*	15 (75%)	20 (74%)	15 (88%)

Group1: patients on a thymidine regimen; Group 2: patients on a non-thymidine regimen; Group 3: patients who switched from a thymidine to a non-thymidine regimen; LA: lipoatrophy; LD: lipodystrophy; BP: blood pressure; CVD: cardiovascular disease; CHD: coronary heart disease

Figure 2-1: NNRTI and PI usage

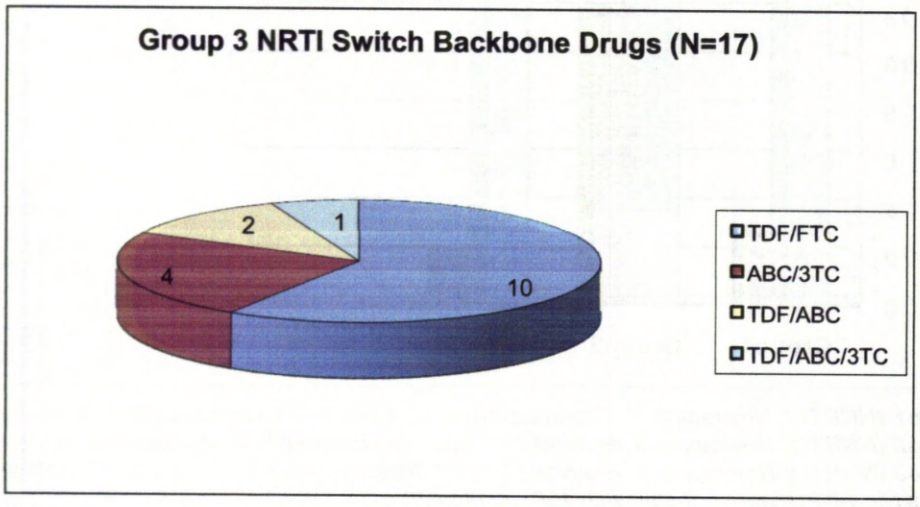


*Group1 (NNRTIs: Nevirapine 5, Efavirenz 10; PIs: Kaletra 1, fosamprenavir 1, amprenavir 1); Group2 (NNRTIs: Nevirapine 9, Efavirenz 6; PIs: fosamprenavir 4, atazanavir 6, double PI 2); Group3 (NNRTIs: Nevirapine 5, Efavirenz 8; PIs: fosamprenavir 1, Kaletra 1); *2 patients were on Trizivir; All PIs were boosted with 100 mg ritonavir*

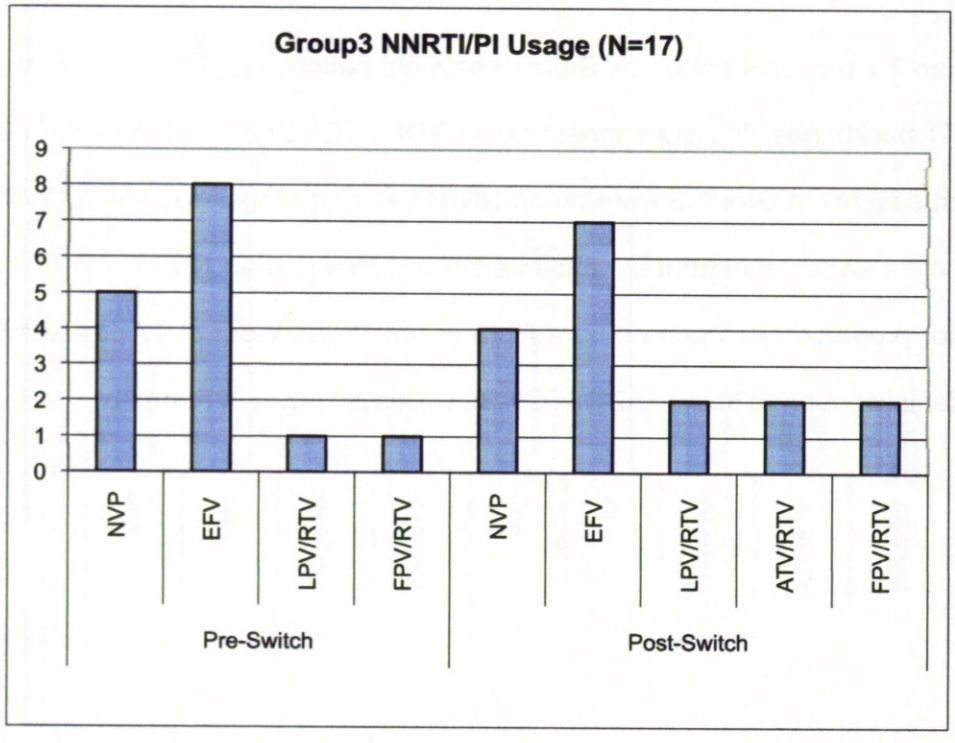
Figure 2-2 presents the drugs Group 3 patients switched to. Panel (a) shows the NRTI backbones, the commonest being TDF/FTC (58.8%). Before the switch most patients in Group 3 were on an NNRTI as part of their regimen (13 of 17). After the switch, though most patients still remained on an NNRTI (11 of 17), a larger proportion had gone onto a PI (6 out of 17 vs. 2 out of 17 pre-switch), 2 patients went on to trizivir (AZT/3TC/ABC). This is shown in Panel (b).

Figure 2-2 (Panel a-b): Group 3 drug switches

Panel (a)



Panel (b)



TDF: tenofovir
ABC: abacavir
3TC: lamivudine

LPV/RTV: *boosted lopinavir*
ATV/RTV: boosted atazanavir
FPV/RTV: boosted fosamprenavir

NVP: Nevirapine
EFV: Efavirenz

2.3.3 Perception of Lipodystrophy

All patients filled out an LD questionnaire at each visit. However, enough data were available for the first 3 visits of the studies, that is, up to 6 months of follow-up. This was due to staffing problems towards the last part of the studies precluded getting data to 12 months; the analysis for the first 6 months was discussed and agreed upon by the study supervisors and the funder and sponsor (NMGH Research and Development Department). Table 2-4 (Panel a-b) summarises the patient perceptions of LD in various parts of the body at baseline, 3 and 6 months.

More patients in group 3 reported fat loss in various parts of the body at the start of the study before they switched their medication than in the other groups, though the differences were not statistically significant expect with respect to the buttocks which was worse in this group compared to group 1 (mean severity scores 2.3 and 1.4 respectively, P=0.03). At 6 months, fewer patients in group 3 reported fat loss in the same areas. The proportion of patients reporting fat loss remained approximately similar between baseline and 6 months in groups 1 and 2. Few patients in all the groups reported fat gain in the face over the 6 month period. In group 3, patients reported more fat gain at the waist (64%) at baseline than in the other groups (33% in group 1 and 42% in group 2), though again the differences between the groups were not statistically siginificant. At 6 months, fewer patients in group 3 reported fat gain at the waist (25%) while there was no change in patients reporting this in the other 2 groups.

Prominence of veins was reported by most patients in all groups and the numbers increased over time, even in patients in group 3 who had switched from a thymidine to a non-thymidine based regimen (58% at baseline vs. 67% at 6 months) and those in Group 2 who had remained on a non-thymidine (42% at baseline vs. 46% at 6 months). However, the worsening in the prominence of veins was not statistically different between baseline and 6 months within each of the groups.

More patients in group 2 reported lipomata at base line than the other 2 groups, though the difference was not statistically significant. Interestingly, the numbers did not change at 6 months in the groups, indicating that no new lesions occurred in the rest of the patients.

Table 2-4 (Panel a-c): Patient Lipodystrophy Perception
Panel (a): Baseline visit (visit 1)

	Group 1		Group 2		Group 3	
	n(%)	Mean severity score	n(%)	Mean severity score	n(%)	Mean severity score
No Fat abnormality	5(33)		5(26)		1(9)	
Lipoatrophy						
Face	8(55)	1.5	6(32)	1.7	8(73)	1.6
Neck	4(27)	1.3	3(16)	1.3	3(27)	1.5
Arms	8(55)	1.8	8(42)	1.5	7(64)	1.4
Breasts	3(20)	1.5	1(5)	1.0	1(9)	1.0
Waist	4(27)	1.3	1(5)	1.0	0	0.0
Buttocks	9(60)	1.4	11(73)	1.7	6(55)	2.3
Legs	5(33)	1.6	10(53)	1.7	7(64)	2.0
Prominent veins	6(40)	1.3	11(73)	1.5	7(64)	2.0
Fat Accumulation						
Face	1(7)	1.0	4(21)	1.0	3(27)	1.5
Neck	0	0.0	1(5)	3.0	0	0.0
Arms	0	0.0	0	0.0	0	0.0
Breasts	4(27)	1.5	6(32)	1.3	1(9)	2.0
Waist	5(33)	1.6	8(42)	1.9	7(64)	1.6
Buttocks	1(7)	2.0	1(5)	1.0	4(36)	1.3
Legs	1(7)	1.0	1(5)	2.0	1(9)	1.0
Lipomata	2(13)		5(26)		1(9)	

Group 1: patients continuing on a thymidine regimen; Group 2: patients continuing on a non-thymidine regimen; Group 3: patients switching from a thymidine to a non-thymidine regimen
There was no statistically significant difference in the mean severity scores for all areas between the groups except for lipoatrophy of the buttocks which was more severe in Group 3 (mean score 2.3) compared to Group 1 (mean score 1.4) P=0.045.

Table 2-4: Patient Lipodystrophy Perception (continued)
Panel (b): 6 month visit (visit 2)

	Group 1		Group 2		Group 3	
	n(%)	Mean severity score	n(%)	Mean severity score	n(%)	Mean severity score
No Fat abnormality	5(42)		2(13)		6(50)	
Lipoatrophy						
Face	4(33)	1.5	6(40)	2.2	1(8)	2.0
Neck	1(8)	1.0	4(27)	1.5	0	0.0
Arms	4(33)	2.0	7(47)	1.4	2(16)	1.0
Breasts	1(8)	2.0	0	0.0	0	0.0
Waist	2(16)	1.0	2(13)	1.0	0	0.0
Buttocks	4(33)	1.7	5(33)	2.0	0	0.0
Legs	2(16)	2.0	9(60)	2.0	3(25)	1.7
Prominent veins	3 (25)	0.0	5 (33)	0.0	5(42)	2.0
Fat Accumulation						
Face	0	0.0	1(7)	2.0	3(25)	1.0
Neck	0	0.0	0	0.0	1(8)	1.0
Arms	0	0.0	1(7)	1.0	0	0.0
Breasts	1(8)	2.0	2(13)	2.0	3(25)	1.5
Waist	4(33)	2.0	6(40)	1.7	2(16)	2.0
Buttocks	1(8)	2.0	2(13)	2.0	0	0.0
Legs	0	0.0	0	0.0	0	0.0
Lipomata	2(16)		5(33)		1(8)	

Group 1: patients continuing on a thymidine regimen; Group 2: patients continuing on a non-thymidine regimen; Group 3: patients switching from a thymidine to a non-thymidine regimen.
There was no statistically significant difference in the mean severity scores for all areas between the groups.

2.3.4 Adherence to Antiretroviral therapy

Patients in Study 2 filled out a treatment adherence questionnaire at each visit. Data available are up to the 6 months assessment point. The adherence data are summarised in Table 2-5. The majority of the patients were adherent with treatment. At baseline, however, more patients in the thymidine switch arm forgot to take their medication at some time (76.9%), had missed a dose or two in the preceding 1 week (23.1%) and had missed at least a day of treatment in the preceding 3 months (53.9%) compared to patients on a non-thymidine regimen (35%, 17.6% and 35.3% respectively). Fewer patients in the thymidine switch arm were 95-100% compliant in the week preceding their baseline visit compared to those that were on a non-thymidine regimen (76.9% vs. 82.4%). Additionally, fewer thymidine switch patients were assessed overall as being adherent with treatment (84.6%) compared to the non-thymidine patients (94.1%) at baseline. However, all these differences were not statistically significant.

Over the course of the study, adherence to treatment improved in both groups. In the thymidine switch group, 92% were 95-100% compliant with treatment in the week preceding their third visit compared to 77% at baseline, though this was not statistically significant (P=0.6). In the non-thymidine group 94% were 95-100% compliant in the week preceding their third visit compared to 82% respectively at baseline, though again this change was not statistically significant.

Table 2-5: Adherence to antiretroviral therapy

	Baseline (Visit 1)			3 months (Visit 2)			6 months (Visit 3)		
	NTH N=17 n (%)	TH N=13 n (%)	*P* value	NTH N=17 n (%)	TH N=13 n (%)	*P* value	NTH N=17 n (%)	TH N=13 n (%)	*P* value
Missed doses preceding weekend:									
	2 (12)	2 (15)	1.00	1 (6)	2 (15)	0.57	0	0	NA
Missed doses previous 1 week:									
0	14 (82)	10 (77)	1.00	15 (88)	12 (92)	1.00	16 (94)	12 (92)	1.00
1-2	3 (18)	3 (23)	1.00	3 (18)	1 (8)	0.61	1 (6)	1 (8)	1.00
>3	0	0	NA	0	0	NA	0	0	NA
Missed days previous 3 months:									
0	11 (65)	6 (46)	0.46	11 (65)	8 (62)	1.00	13 (77)	11 (85)	0.67
1-2	4 (24)	5 (39)	0.44	5 (29)	4 (31)	1.00	4 (24)	2 (15)	0.67
≥3	2 (12)	2 (15)	1.00	1 (6)	1 (8)	1.00	0	0	NA
% Compliance preceding week									
95-100%	14 (82)	10 (77)	1.00	15 (88)	12 (92)	1.00	16 (94)	12 (92)	1.00
85-94%	3 (18)	3 (23)	1.00	2 (12)	1 (8)	1.00	1 (6)	1 (8)	1.00
<85%	0	0	NA	0	0	NA	0	0	NA
Overall number of Patients adherent:									
	16 (94)	11(85)	0.57	16 (94)	12 (92)	1.00	17 (100)	13 (100)	NA

NTH: Patients continuing on a non-thymidine regimen; TH: Patients switching from a thymidine regimen; P value using Fisher's Exact Test; NA: not applicable

2.3.5 Lipids, glucose, insulin, HOMA, blood pressure, weights, BMI, haemoglobin (Hb), alanine aminotransferase (ALT), creatinine and calculated CVD risk percentage

Figure 2-3 (Panel A-D) shows the group means for each time point for TC, LDL-C, HDL-C, TGs, TC:HDL-C ratio, glucose, insulin, HOMA, blood pressure, weight, BMI, Hb, ALT and creatinine.

There was no change in the means for the three groups over the 12 month follow-up period for parameters such as TC, HDL-C, LDL-C, TGs, glucose, systolic blood pressure, ALT and creatinine. The TC:HDL-C ratio increased by 1.2 from 6.6 to 7.8 in Group 1 and by 1.4 from 7.7 to 9.1 in Group 2. The Hb increased from 13.4 to 14.2g/dl in Group 3 and did not change in the other 2 groups. The ratio did not change much in Group 3 (5.2 and 5.6). There was no change in the BMI within each group over 12 months. However, group 2 had a BMI which was 1.5Kg/M^2 higher than the other groups throughout the study period though this was not statistically different.

Figure 2-3: Panel (a-d): Trends in group means for Lipids, glucose, insulin, HOMA, blood pressure, weight, BMI, haemoglobin, ALT and creatinine over time

Panel (a): Lipids

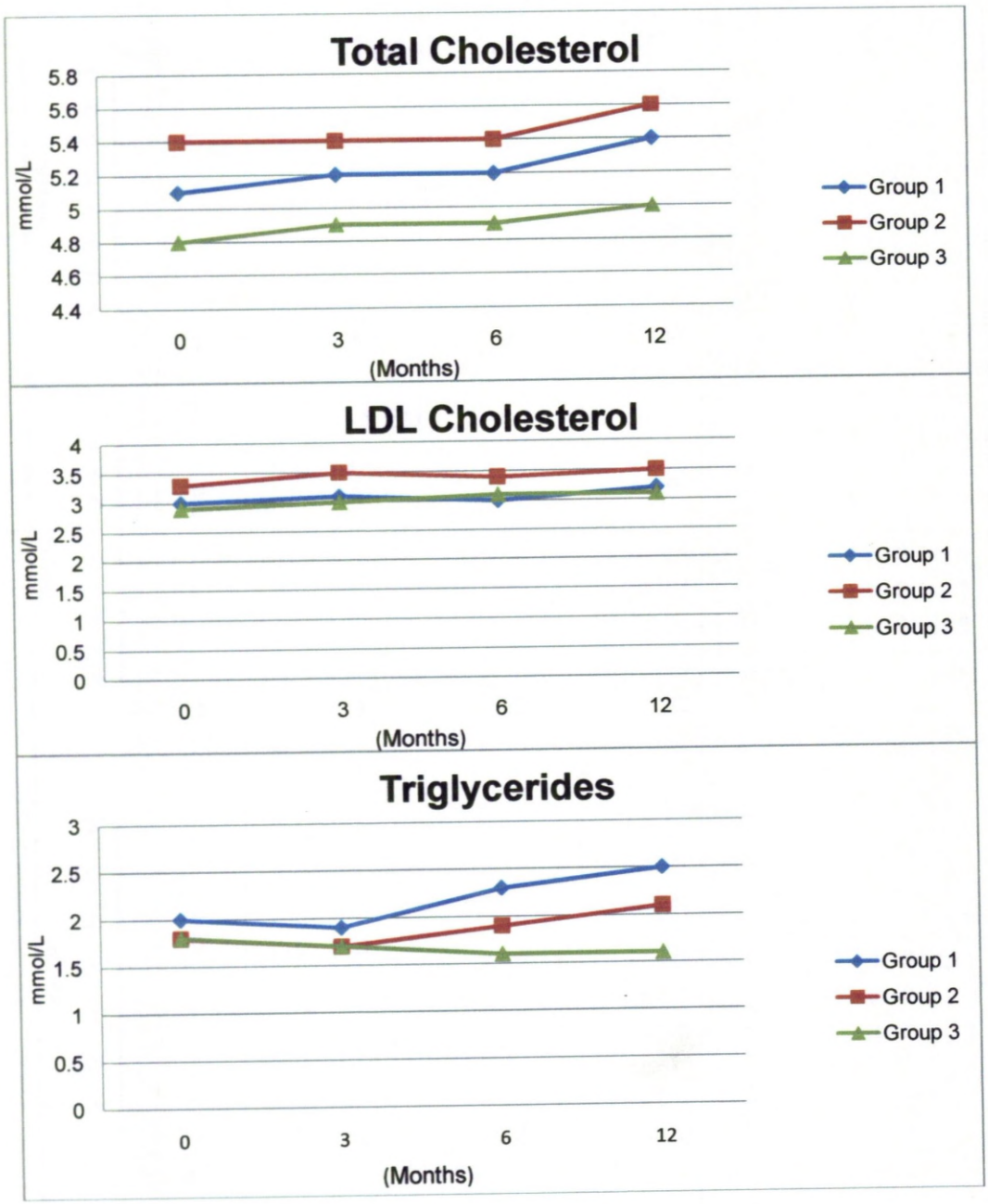
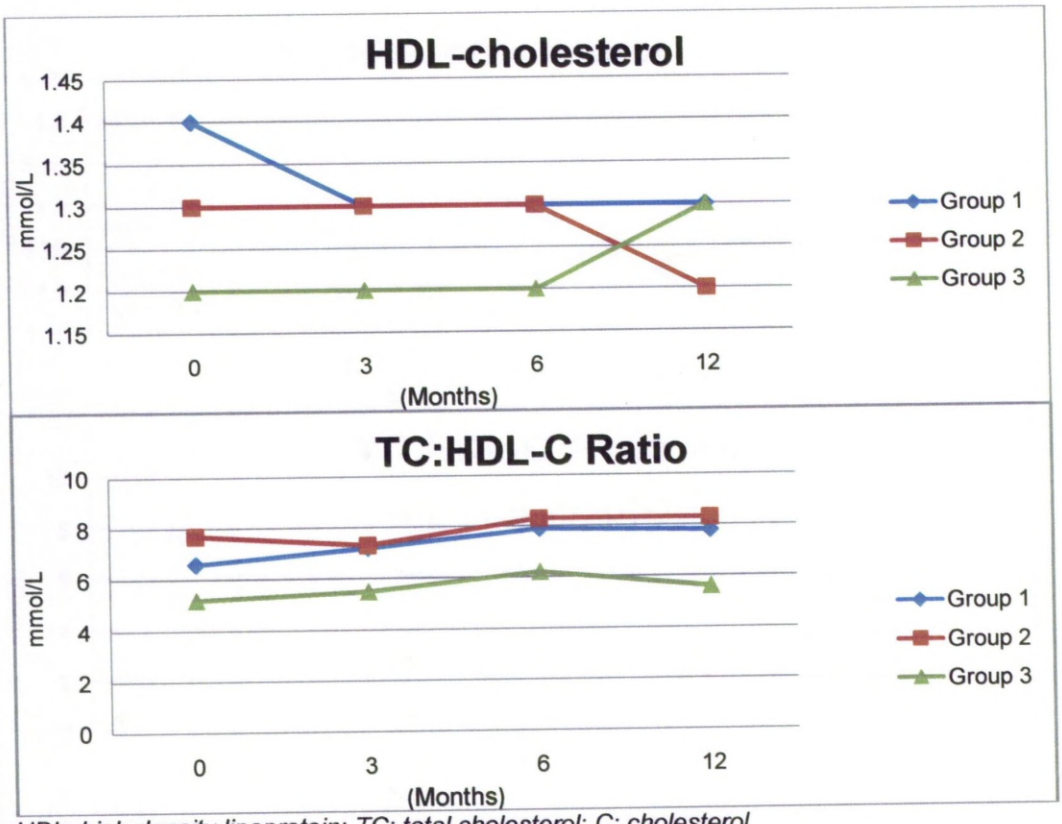


Figure 2-3: continued

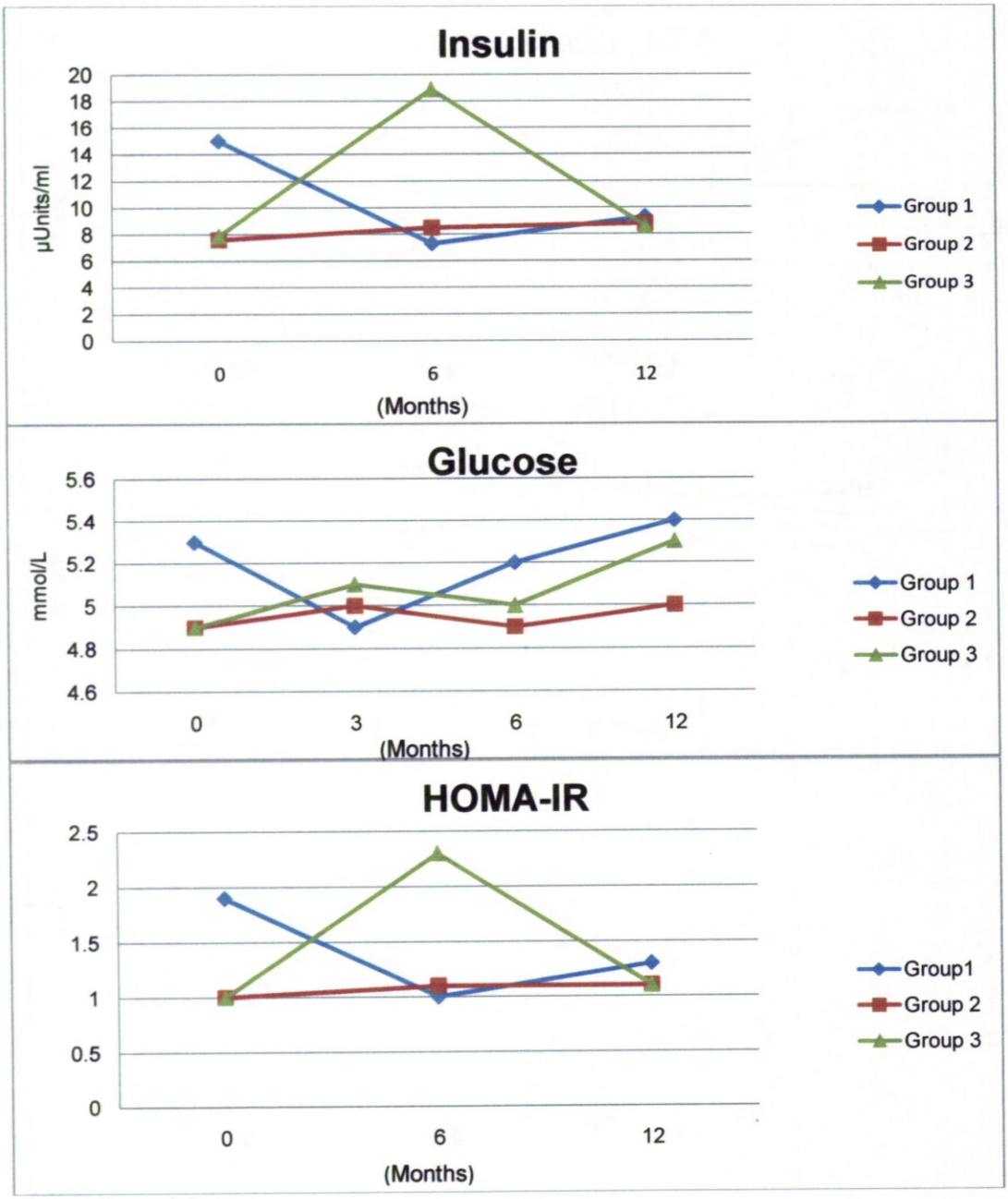
Panel (a): Lipids continued



HDL: high density lipoprotein; TC: total cholesterol; C: cholesterol

Figure 2-3: continued

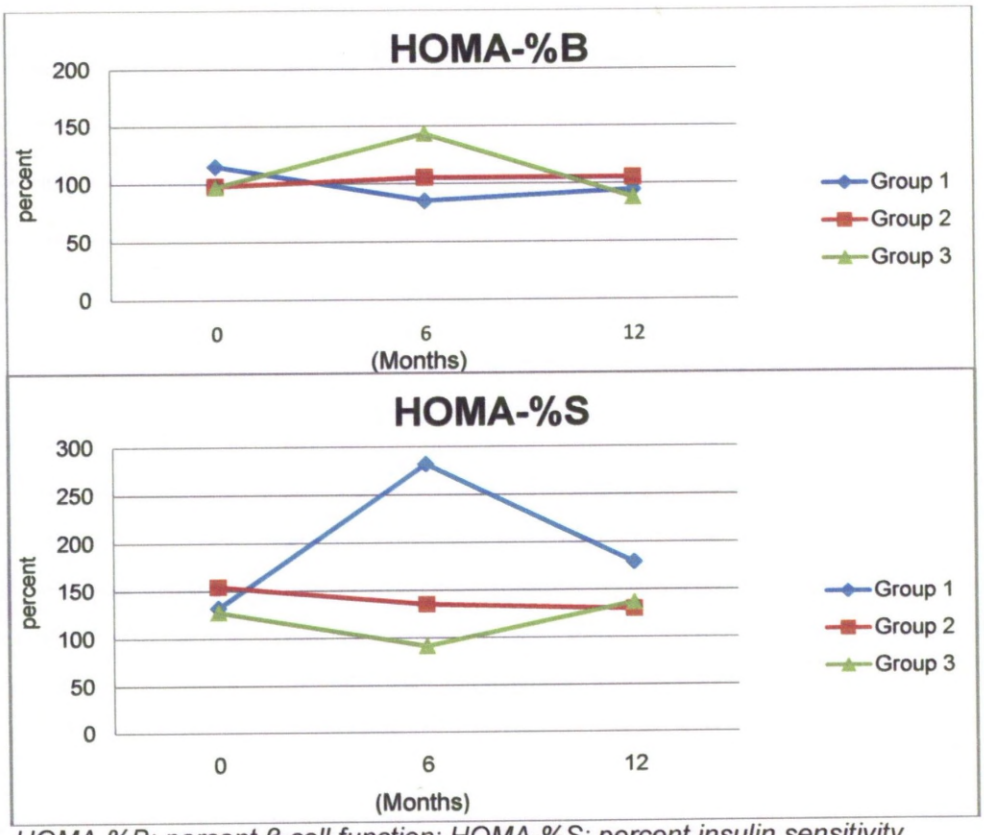
Panel (b): Glucose and Insulin sensitivity



IR: insulin resistance

Figure 2-3: continued

Panel (b): Glucose and Insulin sensitivity continued



HOMA-%B: percent β-cell function; HOMA-%S: percent insulin sensitivity

Figure 2-3: continued

Panel (c): Systolic Blood Pressure and Anthropometrics

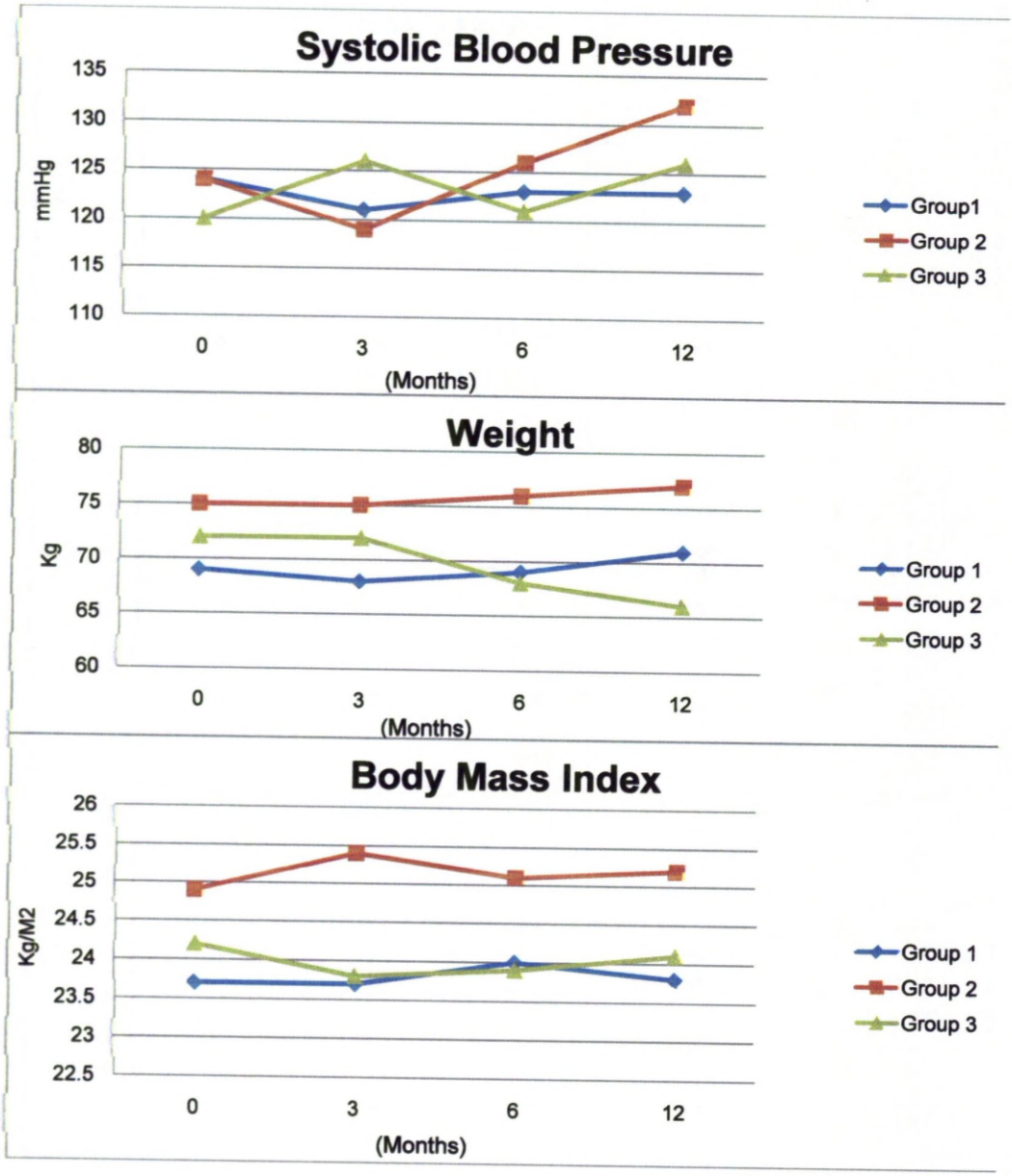
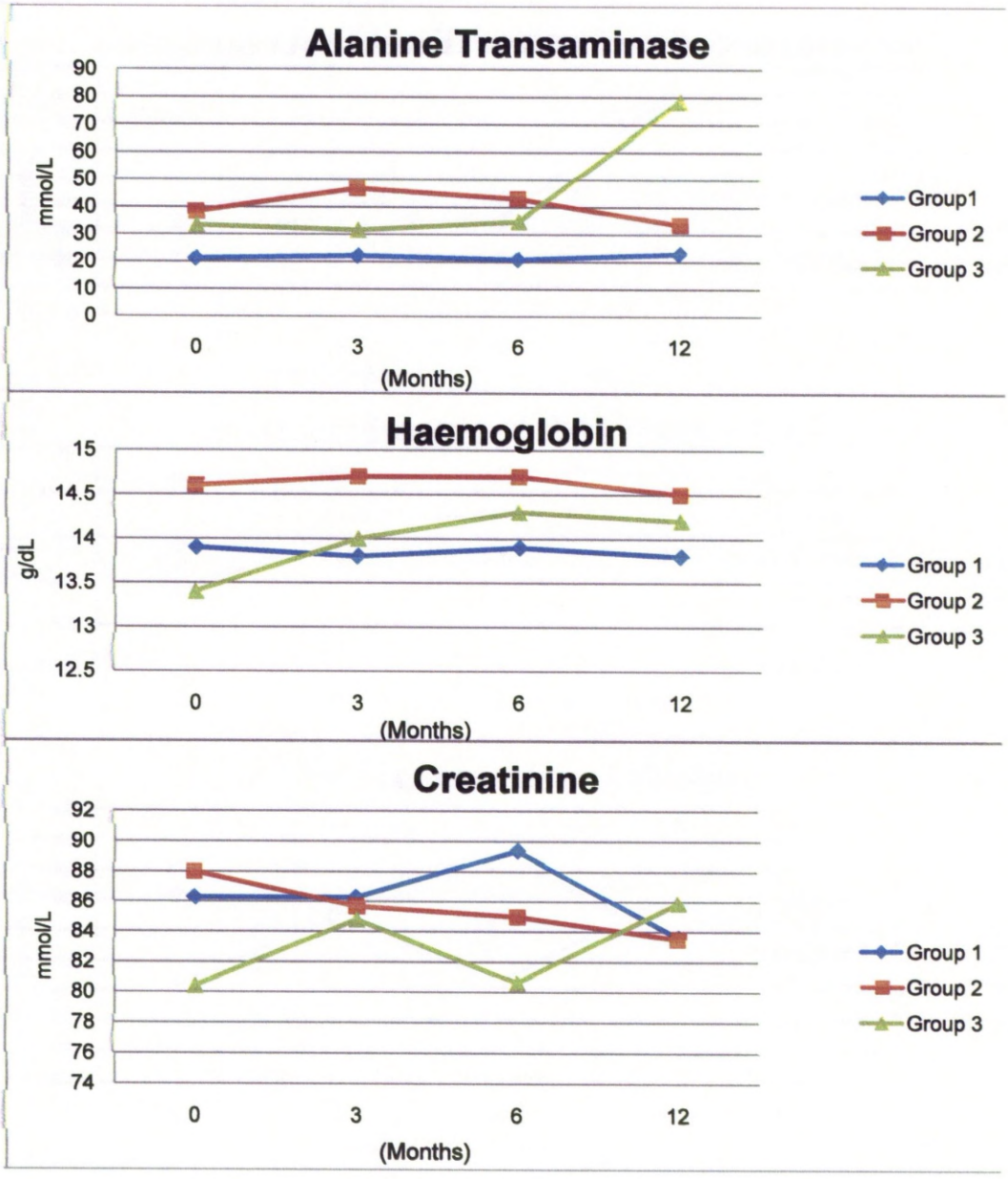


Figure 2-3: continued

Panel (d): Haemoglobin, Renal and Liver function



The mean insulin levels, β-cell function (HOMA-%β), insulin sensitivity (HOMA-%S) and insulin resistance (HOMA-IR) did not change much in Group 2 between baseline and 12 months. In Group 1, the mean insulin level was highest at baseline compared to levels at 6 and 12 months. The HOMA-IR was similarly highest at baseline while the HOMA-%S was lowest at at the same timepoint. The HOMA-%β remained the same throughout the 12 month period. In Group 3, the mean insulin level was highest at 6 months. Similarly, the HOMA-IR and HOMA-%β were highest while the HOMA-%S was lowest at the same time point. It is important to note that 2 patients each in Group 1 (at the baseline visit) and Group 3 (at the 6 month visit) had isolated high mean levels of insulin compared to the rest of their visits. Probably these patients had not fasted before blood samples were taken, which could explain the findings in these two groups.

In this cohort, most patients had mean insulin levels <15μU/ml, with only a small proportion having levels >15μU/ml (4 patients in Group 1 (20%); 1 in Group 2 (3.7%) and 2 in Group 3 (11.8%)).

The systolic and diastolic BP remained relatively normal in all the groups, though there was a reduction of 8mmHg in the diastolic blood pressure from baseline in Group 1 (86 at baseline *vs.* 78 at 12 months).

The calculated CV risk remained the same at 12 months compared to baseline in Group 3 patients with most patients having a CV risk of <10% (87% at 12 months *vs.* 88% at baseline) with no patients having a risk of >20%. In Group

1 and 2, the majority of the patients had a CV risk of <10% (75% and 74% respectively) at baseline. However, at 12 months, fewer patients remained in this low risk category, particularly in Group 2 (67% in Group 1 and 52% in Group 2), with more patients having a moderate CV risk of 10-20% than at baseline (25% *vs.* 15% in Group 1 and 33% *vs.* 19% in Group 2). At 12months, the number of patients with a CV risk of >20% did not change much compared to baseline (8% *vs.* 10% in Group 1 and 10% *vs.* 7% in Group 2).

Table 2-6 (Panel a-c) shows the mean change over the 12 months follow-up period, and the mean differences between the groups for TC, LDL-C, HDL-C, TGs, TC:HDL-C ratio, glucose, insulin, HOMA, blood pressure, weight, BMI, Hb, ALT and creatinine. There were no significant differences in the mean change between the groups in all the parameters except for Hb which had significantly increased in Group 3 compared to both Group 2 (P=0.02) and Group 1 (P=0.049).

Table 2-6 (Panel a-c): Mean Change in parameters and Pairwise comparisons of change between groups
Panel (a): Lipids

Variable	Group	Mean Difference Between 12 Months and baseline (SD)	Pairwise comparisons		*P* value
			Groups	Mean difference	
Total Cholesterol (mmol/L)	1	0.28 (0.7)	1 *vs.* 2	0.07	0.81
	2	0.21 (1.1)	1 *vs.* 3	0.07	0.72
	3	0.21 (0.6)	2 *vs.* 3	0.002	0.99
HDL cholesterol (mmol/L)	1	-0.71 (0.2)	1 *vs.* 2	-0.01	0.99
	2	-0.70 (0.3)	1 *vs.* 3	-0.11	0.39
	3	0.04 (0.5)	2 *vs.* 3	-0.11	0.36
Low Density Lipoprotein (mmol/L)	1	0.22 (0.60)	1 *vs.* 2	0.11	0.63
	2	0.11 (0.71)	1 *vs.* 3	-0.01	0.97
	3	0.2 (0.7)	2 *vs.* 3	0.12	0.59
Triglycerides (mmol/L)	1	0.42 (0.99)	1 *vs.* 2	0.04	0.93
	2	0.38 (1.61)	1 *vs.* 3	0.59	0.07
	3	-0.2 (0.8)	2 *vs.* 3	0.55	0.21
TC:HDL cholesterol ratio	1	0.60 (0.8)	1 *vs.* 2	0.37	0.23
	2	0.23 (1.1)	1 *vs.* 3	0.59	0.11
	3	0.01 (1.3)	2 *vs.* 3	-0.21	0.55

Group 1: patients on a thymidine regimen; group 2: patients on a non-thymidine regimen; group 3: patients that switched from a thymidine to a non-thymidine regimen; SD: standard deviation

Table 2-6: Mean Change in parameters and Pairwise comparisons of change between groups continued
Panel (b): Glucose and Insulin sensitivity

Variable	Group	Mean Difference Between 12 Months and baseline (SD)	Pairwise comparisons		*P* value
			Groups	Mean difference	
Glucose (mmol/L)	1	0.05 (1.59)	1 vs. 2	-0.05	0.88
	2	0.10 (0.62)	1 vs. 3	-0.3	0.45
	3	0.4 (0.7)	2 vs. 3	-0.3	0.19
Insulin (pmol/L)*	1	-57.3(187.4)	1 vs. 2	-67.6	0.11
	2	10.2 (40.5)	1 vs. 3	-64.6	0.20
	3	7.3 (38.6)	2 vs. 3	2.9	0.83
HOMA-%B	1	-28.4 (95.2)	1 vs. 2	-38.7	0.15
	2	10.3 (56.7)	1 vs. 3	-21.6	0.43
	3	-6.8 (38.2)	2 vs. 3	17.1	0.31
HOMA-%S	1	23.1 (61.0)	1 vs. 2	54.3	0.23
	2	-31.2 (139.1)	1 vs. 3	17.8	0.71
	3	5.2 (146.1)	2 vs. 3	-36.5	0.45
HOMA-IR	1	-1.0 (3.4)	1 vs. 2	-1.2	0.11
	2	0.2 (3.4)	1 vs. 3	-1.2	0.20
	3	0.2 (0.7)	2 vs. 3	0.0	0.99

**Insulin levels in brackets are converted levels in µUnits/ml; Group 1: patients on a thymidine regimen; group 2: patients on a non-thymidine regimen; group 3: patients that switched from a thymidine to a non-thymidine regimen; SD: standard deviation*

Table 2-6 continued: Mean Change in parameters and Pairwise comparisons of change between groups continued
Panel (c): Systolic blood pressure, anthropometrics, routine blood tests and CD4 count

Variable	Group	Mean Difference Between 12 Months and baseline (SD)	Pairwise comparisons		*P* value
			Groups	Mean difference	
Systolic BP (mmHg)	1	-1.5 (13.3)	1 vs. 2	-9.2	0.09
	2	7.7 (16.4)	1 vs. 3	-9.7	0.08
	3	8.3 (14.7)	2 vs. 3	-0.6	0.92
Weight (kg)	1	2.4 (7.1)	1 vs. 2	1.0	0.57
	2	1.4 (2.7)	1 vs. 3	5.6	0.27
	3	-3.2 (14.4)	2 vs. 3	4.6	0.16
Body Mass Index	1	0.2 (1.5)	1 vs. 2	-0.3	0.43
	2	0.5 (1.0)	1 vs. 3	0.1	0.84
	3	0.04 (1.1)	2 vs. 3	-0.4	0.22
ALT	1	2.9 (8.5)	1 vs. 2	7.3	0.19
	2	-4.4 (21.2)	1 vs. 3	-42.1	0.25
	3	44.9 (147.5)	2 vs. 3	-49.4	0.09
Haemoglobin	1	-0.04 (0.8)	1 vs. 2	0.1	0.71
	2	-0.1 (0.9)	1 vs. 3	-0.9	0.049
	3	0.8 (1.6)	2 vs. 3	-0.9	0.02
Creatinine	1	-0.5 (9.6)	1 vs. 2	4.2	0.38
	2	-4.7 (17.4)	1 vs. 3	-6.0	0.15
	3	5.5 (13.5)	2 vs. 3	-10.2	0.05

HOMA: homeostatic model assessment (-%B=β-cell function, -%S= insulin sensitivity, -IR= insulin resistance); ALT: alanine aminotransferase; TC: total cholesterol; HDL: high density lipoprotein; Group 1: patients on a thymidine regimen; group 2: patients on a non-thymidine regimen; group 3: patients that switched from a thymidine to a non-thymidine regimen; SD: standard deviation

2.4 DISCUSSION

Thymidine analogues (AZT and D4T) and DDI have been associated with the development of lipoatrophy and dyslipidaemia through a combination of mitochondrial toxicity, induction of insulin resistance and altered lipogenesis. TDF and ABC have been shown not to cause these effects to a great extent (Jones *et al.* 2005a). It was anticipated that patients who remained on a thymidine based regimen for 12 months would develop or experience worsening of lipoatrophy, dyslipidaemia, insulin resistance and an adverse calculated CV risk. In comparison, those that were and continued on a non-thymidine/non-DDI regimen would have better profiles at baseline and demonstrate continuing improvements in these parameters. Additionally, those patients that were switched from a thymidine to a non-thymidine/non-DDI regimen would demonstrate improvements in these profiles at 12 months compared to baseline levels. This was the rationale for chosing the 3 groups reported in these studies.

The baseline characteristics were similar between the groups as has been found in similar studies (Moyle *et al* 2006, Gallant *et al* 2004). Smoking rates were higher in this HIV population than in the general population in England (39.1% *vs.* 25%). High smoking rates have been seen in other studies of HIV positive patients (Friis-Moller *et al.* 2003(a); Ross *et al.* 2009). The patients were generally virologically well controlled though less so in Group 3, which was expected, as part of the inclusion criteria in this group was that the patients were switching regimens due to virological rebound.

Overall for all the groups, patients were at low risk (≤10%) of cardiovascular disease over 10 years, but 10% and 7% of patients in Group 1 and 2 respectively had a CV risk of ≥20%. Patients in Group 2 had apparent worsening of their CV risk, having more patients move from a low risk of <10% at baseline to a moderate risk of 10-20% at 12 months. It is important to note that more patients in Group 2 had previous exposure to D4T and PIs than the other groups. Some studies have shown an increased risk of CVD with exposure to D4T and that PI use is associated with thicker carotid IMT (a marker of atherosclerosis (Stein *et al.* 2008; Hsue *et al.* 2009a). Group 2 also had more patients who had the traditional CVD risk factors namely smoking, pre-existing history of hypertension, dyslipidaemia, cardiovascular disease, ischaemic heart disease, family history of premature heart disease and familial dyslipidaemia. This could therefore explain the worsening of the calculated CV risk in Group 2 patients over one year follow up period.

LD changes were subjectively reported by the patients. The changes of fat loss, fat gain and prominence of veins were mostly being reported as mild or moderate in severity, with only a few patients reporting severe changes. At 6 months, fewer patients who switched from a thymidine to a non-thymidine based regimen reported obvious fat loss than at baseline though they did not see obvious fat gain in various parts of the body. The fat gain which may not be discernible to the patient could have been demonstrated by DEXA or CT scan. Others have demonstrated fat gain by DEXA scanning in patients who switched from a thymidine NRTI to either tenofovir or abacavir at 48 weeks

(Moyle *et al.* 2006). Patients remaining on a thymidine based regimen mostly reported fat loss which has been replicated in other studies (Heath *et al.* 2001; Gallant *et al.* 2004; Shlay *et al.* 2005; Shlay *et al.* 2008; Shlay *et al.* 2009). Patients who remained on a non-thymidine regimen variably reported fat loss and fat gain with no consistent pattern over the 6 month period. We expected this group to mostly report peripheral fat gain as has been shown by other researchers such as Gallant or Moyle *et al* (Moyle *et al.* 2006). There are limitations to the assessment of LD in this study. We reported LD results up to 6 months (24 weeks) of follow up which may have been an insufficient period of time for LD changes to have become overt. We could have seen similar changes if we had reported LD changes at 48 weeks as was the case in the other studies. The subjective nature of the LD reporting by questionnaire without clinician verification may have over or underreported LD changes, however, this method of assessment has been shown to have a reported 98% concordance between patient self-assessment and clinical examination findings (Carr *et al.* 1998).

Most patients in this cohort were adherent to treatment. However, poor adherence was mostly at baseline in patients that were on a thymidine based regimen who were about to switch ART drugs compared to those that were continuing on a non-thymidine based regimen. Adherence did improve markedly by 6 months of follow-up. These differences at baseline could be due to adverse effects (particularly lipoatrophy) of the thymidine-based regimens influencing patient adherence before they switched drugs, with improvement of

adherence on being on non-thymidine drug regimen. The phenomenon of poor adherence because of fear of side effects of ART is well documented (Lenert *et al.* 2002; Nachega *et al.* 2009). Indeed, the thymidine switch patients reported less lipoatrophy at 6 months, showing possible improvement in perception of their body image and therefore willingness to adhere to treatment. However, adherence levels could also have improved because patients knew they would be filling out an adherence questionnaire at each visit.

With regards to lipids, large population studies have shown a link between the development of new-onset and recurrent CHD events in men and women and high TC, LDL-C, TG levels and low levels of HDL-C (Unknown 1984; Stamler *et al.* 1986; Assmann *et al.* 1998; Austin *et al.* 1998; Wilson *et al.* 1998). In HIV patients, raised TC and TGs have also been shown to be associated with an increased risk of myocardial infarction (Friis-Moller *et al.* 2003(b)). The lipids (TC, LDL-C, HDL-C and TGs) in this cohort did not change over time in each group and there was no significant difference in these parameters over the 12 month period between the groups. The mean TC in all the groups was <6.5mmol/L (The US National Cholesterol Education Program III 2002 guidelines define as high TC ≥ 6.5mmol/L). However, the mean TC:HDL-C ratio was high in Group 1 (7.4) and Group 2 (8.1) and did increase in these two groups over the 12 month period while it remained stable in Group 3 (TC:HDL-C ratio greater than 6.5 mmol/L is defined as high by the US National Cholesterol Education Program III guidelines). Most studies in HIV patients on ART show a favourable lipid profile in patients on a non-thymidine containing

regimen than those on a thymidine containing regimen. Pozniac *et al* showed a rise in TC and LDL-C at 96 weeks which was significantly lower in patients on TDF/3TC/EFV combination compared to those on AZT/3TC/EFV (25 mg/dL *vs.* 38 mg/dL respectively, *P*=0.001), however, the change in fasting TGs was not significantly different between the two groups (Pozniak *et al.* 2006). Gallant *et al* also demonstrated increases in TC, LDL-C, HDL-C and TGs which were significantly attenuated in patients using a TDF containing regimen compared to those on a D4T regimen over 144 weeks (Gallant *et al.* 2004). Jones *et al* showed that patients on a TDF regimen did not develop hypercholesterolaemia (defined as >6.5 mmol/L [254 mg/dL]), while those on a D4T or PI containing regimen (particularly nelfinavir) developed hypercholesterolaemia in a shorter period of time. They also showed that NNRTI containing regimens had a favourable lipid profile (Jones *et al.* 2005a). Additionally, studies show improvement of lipid levels in patients who switch from a thymidine to a non-thymidine based regimen. Viganò–Alessandra *et al* demonstrated in a paediatric population switching from D4T to a TDF based regimen improvements in TC, TC:HDL-C ratio and TG levels in the TDF group over 48 weeks (Vigano *et al.* 2005). In addition, Moyle *et al* reported in a UK based population that switching from either AZT or D4T to either TDF or ABC showed modest improvements in TC, LDL-C and TGs in the TDF group but were unchanged with ABC over a 48 week follow up period (Moyle *et al.* 2006).

In the present study, it was expected that patients in Group 1 who remained on a thymidine based regimen would demonstrate worsening in lipid profiles over

time. It was also expected that the components of the lipid profile would improve in patients in Group 2 who remained on a non-thymidine regimen; and for those in Group 3 who switched to a non-thymidine regimen. An adverse lipid profile at start of the study when the patients were adherent to a thymidine regimen was not observed, and the expected improvement in lipid profile over time after the patients switched to a TDF or ABC based regimen was absent. Failure to detect such changes may be due to several factors. Firstly, use of lipid lowering drugs such as statins obviously lowers lipid levels and we did not exclude patients on lipid lowering drugs. However, very few patients (2) were co-prescribed such drugs. Secondly, most of the patients in all the groups were on an NNRTI particularly EFV rather than PIs (particularly older ones such as nelfinavir and indinavir which could contribute to unfavourable lipid profiles). Thirdly, 44% of patients in Group 2 were on a PI; however, usage of kaletra was low, with most patients being on atazanavir (known to be lipid friendly) (Sax and Kumar 2004) which could explain the lack of adverse lipid profile changes. Fourthly, use of D4T was low (20%) in Group 1, this drug being the most implicated in dyslipidaemia as detailed above. Fifthly, most patients (48%) in Group 2 had previous D4T exposure while 63% in the same group and 47% in Group 3 had previous PI exposure, probably the older PIs. The long-term effects of these may not have adequately reversed for us to observe favourable lipid profiles, as previous exposure to PIs has been associated with elevated TC and TGs (Heath *et al.* 2001).

Group 2 had a higher BMI than the other two groups which was expected as they were on regimens not expected to cause fat loss. It was expected that the

BMI in group 3 would improve after switching from a thymidine, compared to those that remained on a thymidine regimen, because these patients would be expected to regain some of the fat lost while they were on a thymidine as has been shown in other studies (Carr *et al.* 2002; Moyle *et al.* 2006). This could be because the duration of follow-up was too short for improvements to occur or the patients may have been on the thymidine drug for too long so that fat loss was possibly irreversible. In this study, the median time on a thymidine before the switch was 48 months (range 7-86) (4 years, range 0.6-7.3) (Carr *et al.* 2002; Moyle *et al.* 2006).

NRTIs and PIs have been implicated both in *in vitro* and *in vivo* studies to cause the development of glucose intolerance and insulin resistance via adverse effects on adipose tissue differentiation, insulin-stimulated glucose uptake and induction of lipolysis, the worst offending drugs being the thymidine NRTIs (D4T and AZT) and some PIs (lopinavir, ritonavir, saquinavir, indinavir and nelfinavir) but not others such as atazanavir (Janneh *et al.* 2003; Jones *et al.* 2005b; Lagathu *et al.* 2005; Kim *et al.* 2006; Noor *et al.* 2006; Pacenti *et al.* 2006). Indeed many studies have investigated glucose intolerance and insulin resistance in HIV patients on HAART with patients developing hyperglycaemia, hyperinsulinaemia and insulin resistance with cumulative exposure to NRTIs and PIs but not NNRTIs (Hadigan *et al.* 1999; Hadigan *et al.* 2000; Brown *et al.* 2005; Tien *et al.* 2008). Glucose homeostasis remained normal throughout the study period. This did not worsen in the group that remained on a thymidine nor improve in the group that switched from a thymidine to a nonthymidine based regimen. The mean glucose level for all the groups was below 6.0

mmol/L; β-cell function was comparable to that of a normal population (being 98-110%) in all the groups; and group mean insulin levels were below 15μU/ml (levels >15μU/ml have been considered to signify hyperinsulinaemia in studies) (Brown *et al.* 2005). In my studies, use of the more offending drugs such as D4T was low (2/20 patients in Group 1 remained on D4T, 3/27 patients in Group 3 switched from D4T) and none of the patients were on the older PIs such as IDV, SQV and NFV as such a favourable glucose profile could be expected. Additionally, even though previous use of D4T and older PIs was prevalent, their effects on glucose homeostasis may have reversed by the time this study was conducted.

Renal toxicities have been reported with adherence to TDF, present data would indicate maintenance of normal renal function in all groups despite high level of TDF use in treatment groups 2 and 3. This is in keeping with renal function results reported by others (Moyle *et al.* 2006; Pozniak *et al.* 2006). ALT as a surrogate marker of liver function showed the liver function was not different between the groups. Haemoglobin significantly improved in Group 3 at 12 months compared to the other groups. This is likely because most patients in this group switched from AZT-based regimen, a drug known to cause anaemia which is reversible on stopping the drug (GSK 2010).

2.5 CONCLUSIONS

In this study, lipid levels, insulin sensitivity and weights remained normal over a one year period and did not differ between patients that remained on a thymidine or non-thymidine or those that switched from a thymidine to a non-thymidine based regimen. This cohort had high levels of pre-existing traditional risk factors of CVD, though most patients had a low or moderate risk of developing CVD over 10 years.

The study population was diverse and the exclusion criteria were minimal. The results were meant to reflect the situation in real clinic settings. The patients were generally adherent to their treatment. The results show that pre-existing risk factors for CVD are prevalent in HIV patients on HAART. We have also shown that the relationship between the development of dyslipidaemia, deranged glucose homeostasis, abnormalities of blood pressure or changes in body habitus and the level of cardiovascular risk is not straight forward. Use of newer NRTIs (e.g. TDF and ABC) is affecting patient perception on body habitus changes particularly in those that switch from a thymidine to nonthymidine NRTI noting subjective improvements in lipodystrophy over time. Caution must be exercised in that these are subjective rather than objective assessments, since the present data do not statistically support this view. Additionally, use of the newer NRTIs and PIs (atazanavir) may be having a favourable effect on lipid profiles and glucose metabolism. Several limitations exist with the current work; the numbers of patients enrolled in each study was

small as such we cannot draw firm conclusions on the effects of individual drugs on lipid profiles, glucose homeostasis and CV risk.

CHAPTER THREE

ANTIRETROVIRAL DRUGS AND BIOMARKERS OF CARDIOVASCULAR DISEASE AND INFLAMMATION IN HIV PATIENTS

3.1 INTRODUCTION

In the general population, inflammation has been shown to play a role in development of cardiovascular disease (CVD) (Ross 1999; Hansson 2005). Inflammatory cytokines such as interleukin 6 (IL-6), tumour necrosis-α (TNF-α), C-reactive protein (CRP), myeloperoxidase, markers of vascular endothelial activation and carotid intimal-media thickness (IMT) have all been shown to predict the development of myocardial infarction, stroke and diabetes mellitus (Bots *et al.* 1999; Ridker *et al.* 2000; Zhang *et al.* 2001; Brennan *et al.* 2003; Cesari *et al.* 2003; Pai *et al.* 2004; Espeland *et al.* 2005; Ogata *et al.* 2005).

Apart from the inflammatory markers, adipose tissue derived cytokines (adipocytokines) such as leptin, adiponectin, resistin and visfatin are involved in energy homeostasis (lipid, glucose and insulin metabolism) and have also been implicated in the pathogenesis of the metabolic derangements found in obesity, diabetes mellitus and cardiovascular disease (Hubert *et al.* 1983; Fasshauer and Paschke 2003; Rajala and Scherer 2003; Lieb *et al.* 2009).

HIV infection is a chronic inflammatory disease in which derangements of inflammatory cytokines, adipocytokines as well increased carotid IMT have been demonstrated, showing that HIV infected patients are at risk of accelerated atherosclerosis and therefore increased risk of myocardial infarctions (Laughlin *et al.* 2007; Rathmann *et al.* 2007; Triant *et al.* 2007; Ross *et al.* 2009). Indeed, HIV patients have been shown to be at higher risk of

developing ischaemic heart disease by virtue of having the virus or being on antiretroviral therapy compared to HIV negative people (Obel *et al.* 2007; Triant *et al.* 2007). With this in mind, we investigated the serum levels of adipocytokines namely adiponectin, leptin, resistin and visfatin; the proinflammatory cytokines TNF-α and IL-6; and CRP over a one year follow-up period in patients on antiretroviral therapy, specifically to look at any trends in cytokine levels depending on the type of drugs the patients were on. We also looked at disease control by looking at the trends in CD4 count and HIV viral load. Additionally, we also looked at plasma viscosity, particularly as there have been case reports in the literature of the hyperviscosity syndrome occurring in HIV infected patients which predisposes to a thrombotic state (Garderet *et al.* 2004).

3.2 METHODOLOGY

The research site, the study protocols, ethics approvals, study conduct and procedures are described in the foregoing Chapter 2 (Methodology, Section 2.2, pages 61-65). To summarize, 2 studies were conducted from which the following comparison groups were derived:

1. Group 1: A total of 20 patients remaining on a Thymidine based highly active antiretroviral therapy (HAART) regimen (18 on AZT and 2 on D4T)

2. Group 2: A total of 27 patients remaining on a non-thymidine regimen (17 on tenofovir and emtricitabine (TDF/FTC), 7 on abacavir and lamivudine (ABC/3TC) and 3 on TDF/ABC).

3. Group 3: A total of 17 patients switching from a thymidine to a non-thymidine non-DDI (ABC/3TC, TDF/FTC or TDF/ABC) regimen. 14 switched from AZT and 3 switched from D4T.

Blood for cytokine level determination was taken after an overnight fast at baseline (day 0), 6 months and 12 months. After venesection, the blood was immediately placed on ice and allowed to stand for 20 minutes. It was then centrifuged to obtain serum. The serum was aliquoted and stored in a -80°C freezer. The cytokine levels (TNF-α, IL-6, adiponectin, leptin and resistin) were determined using Millipore CytokineProfiler™ service (Millipore 2008). The detection limits were: adiponectin 145.4pg/mL, resistin 6.7pg/mL, IL-6 1.6pg/mL, Leptin 85.4 pg/mL and TNF-α 0.14pg/mL. Visfatin levels were determined by ELISA (human Visfatin, AdipoGen, Axxora UK LTD) and the detection limit was 30pg/mL. Blood for determination of CD4 cell count, CRP and plasma viscosity was analyzed at the North Manchester General Hospital laboratory as part of the routine blood tests that patients on HAART undergo during their routine 3-monthly clinic visits (see Chapter 2, Section 2.2.2.3.2, Study Procedures, page 64).

3.2.1 Statistical analysis

The independent sample T-Test was used to compare the mean change between the three groups between 12 months and baseline for the variables TNF-α, IL-6, leptin, adiponectin, resistin, plasma viscosity and CD4 cell count

(SPSS v. 16, SPSS Inc. Chicago, USA). The mean changes of all parameters were approximately normally distributed on testing using histograms. Median CRP levels were determined for the groups and the Mann-Whitney U test was used to test for significance in the difference in the medians between the groups. The Fisher exact test (STATA, Texas, USA, http://www.stata.com.) was used to assess the significance of any difference in virological control between baseline and 12 months for each group.

3.3 RESULTS

Baseline characteristics were as described in the foregoing chapter 2 (Section 2.3.1, page 68 and Table 2-3, page 69). Table 3-1 shows the mean change over the 12 months follow-up period in the parameters TNF-α, IL-6, leptin, adiponectin, resistin, plasma viscosity and CD4 cell count. Figure 3-1 (Panel A-C) shows the trends in group means over 12 months for the same parameters.

3.3.1 Proinflammatory Cytokines and CRP

TNF-α level did not change between baseline and 12 month in groups 1 and 3. There was an increase of 0.6 pg/mL in the TNF-α level in group 2 by 12 months. There was no statistically significant difference in the group mean change at 12 months from baseline between the groups. At 12 months, IL-6 level increased by 0.3 pg/mL in group 1 and by 2.6 pg/mL in group 3. The level did not change in group 2. There was no statistically significant difference in the group mean change at 12 months from baseline between the groups.

Table 3-1: Mean Change in parameters and Pairwise comparisons of change between groups

Variable	Group	Mean of Delta value For 12 Months – baseline (SD)	Pairwise comparisons		*P* value
			Groups	Mean difference	
Tumour necrosis factor-α (pg/ml)	1	0.3(1.9)	1 *vs.* 2	-0.18	0.81
	2	0.4(2.1)	1 *vs.* 3	-0.01	0.98
	3	0.3(2.9)	2 *vs.* 3	0.2	0.84
Interleukin 6 (pg/ml)	1	0.3(0.9)	1 *vs.* 2	0.3	0.62
	2	0.002(2.0)	1 *vs.* 3	-2.3	0.60
	3	2.6(14.3)	2 *vs.* 3	-2.6	0.40
Leptin (ng/ml)	1	-4.8(23.2)	1 *vs.* 2	-4.4	0.40
	2	-0.3(6.0)	1 *vs.* 3	-5.4	0.39
	3	-0.6(4.6)	2 *vs.* 3	-0.9	0.60
Adiponectin (pg/ml)	1	-3.5 (6.2)	1 *vs.* 2	-3.5	0.26
	2	-0.01(9.3)	1 *vs.* 3	0.18	0.97
	3	-3.7(13.1)	2 *vs.* 3	3.7	0.32
Resistin (ng/ml)	1	7.9(34.9)	1 *vs.* 2	8.1	0.32
	2	-0.2(12.2)	1 *vs.* 3	1.5	0.89
	3	6.4(21.3)	2 *vs.* 3	-6.6	0.24
CD4 count (cells/mm^3)	1	79(107)	1 *vs.* 2	52	0.27
	2	27(173)	1 *vs.* 3	-51	0.45
	3	130(253)	2 *vs.* 3	-103	0.15
CD4 %	1	-1.0(7.7)	1 *vs.* 2	0.3	0.91
	2	-1.3(7.2)	1 *vs.* 3	-2.1	0.51
	3	1.1(9.8)	2 *vs.* 3	-2.3	0.41
Plasma viscosity (mPa)	1	4.4(13.4)	1 *vs.* 2	-4.2	0.51
	2	8.6(25.7)	1 *vs.* 3	6.2	0.14
	3	-1.8(11.0)	2 *vs.* 3	10.4	0.12

**Group 1: patients on a thymidine regimen; group 2: patients on a non-thymidine regimen; group 3: patients that switched from a thymidine to a non-thymidine regimen; *overall group mean over the 12 month period; SD: standard deviation*

Figure 3-1 Panel (a-c): Group means for Tumour necrosis factor-α, Interleukin 6, Leptin, Adiponectin, Leptin, resistin, CD4 cell count, CD4 cell percentage and Plasma viscosity at each time point.

Panel (a): Tumour necrosis factor-α, Interleukin 6 and Adiponectin

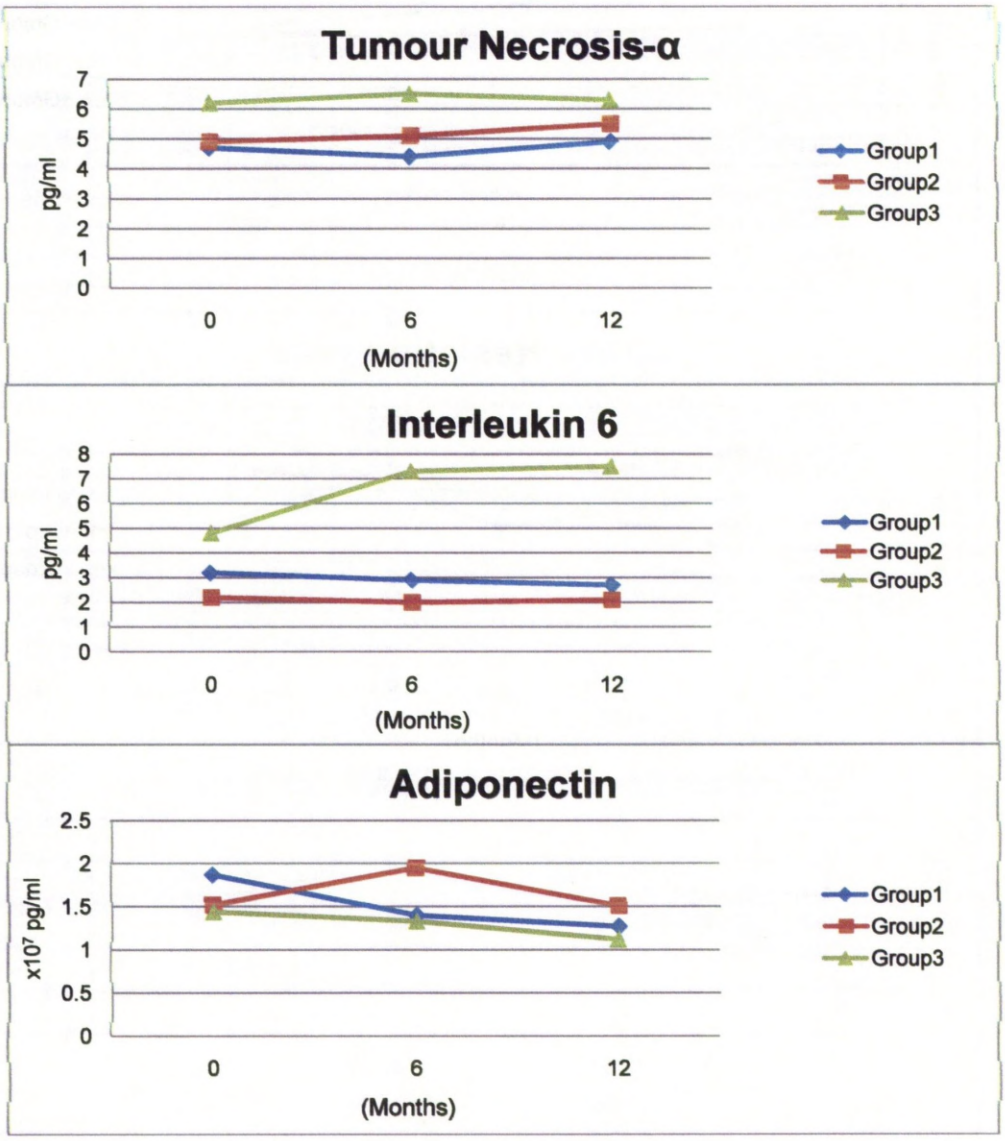


Figure 3-1: Panel (b): Leptin and Resistin (continued)

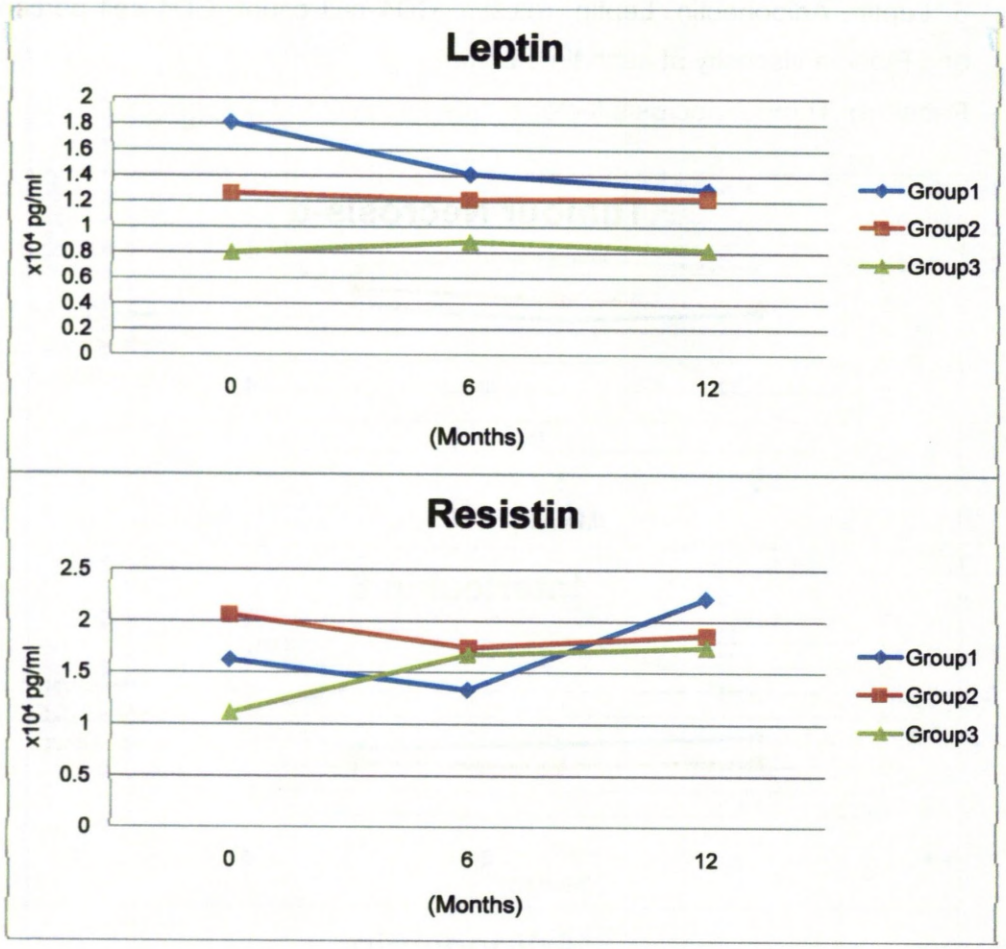
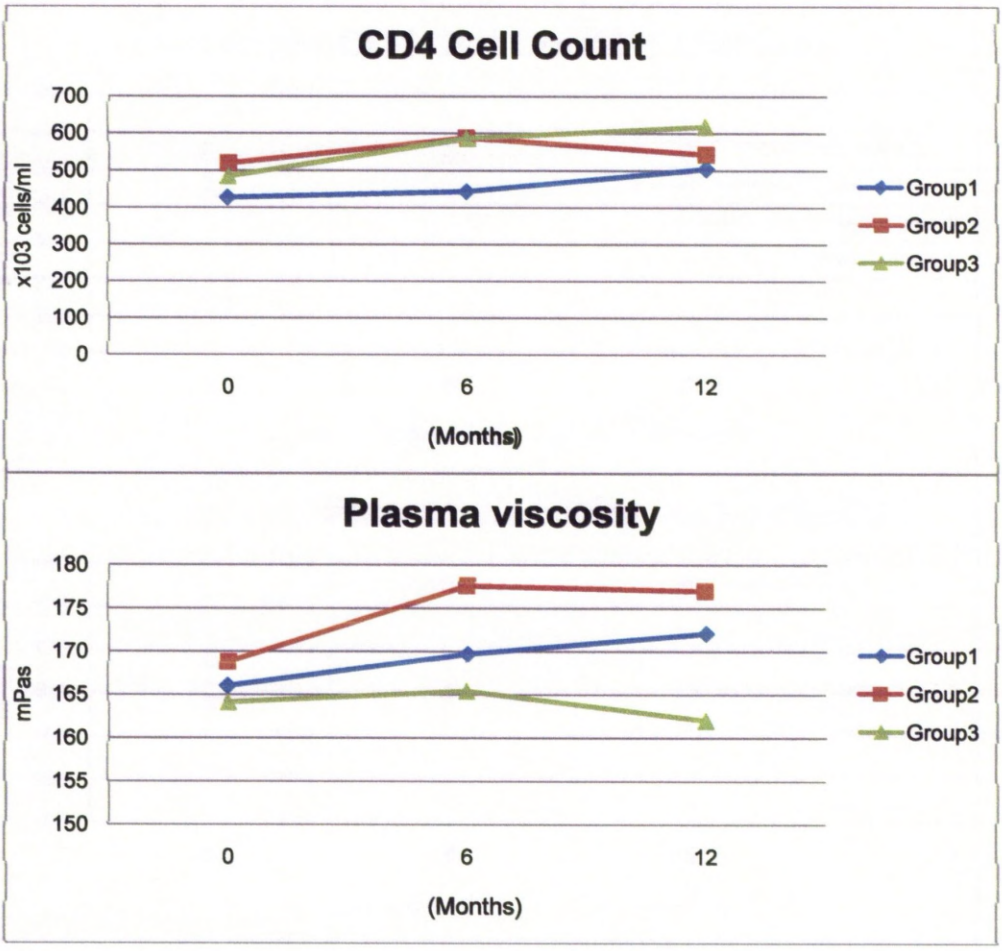


Figure 3-1: Panel (c): CD4 cell count and Plasma viscosity (continued)



The median CRP in group 1 was 2.9 (1.0-37.8), in group 2 was 2.6 (1.0-27.0) and in group 3 was 1.8 (1.0-13.0). There was no significant difference between the 3 groups. Patients with a CRP>3 were 13 (72%) in group 1, 11 (44%) in group 2 and 7 (44%) in group 3, with *P*=0.11 for group 1 *vs.* group 2 and *P*=0.16 for group 1 *vs.* group 3. In group 1, 4 (22%) patients had CRP>5.9 while this was 7 (28%) and 4 (25%) patients in group 2 and 3 respectively.

3.3.2 Adipocytokines

Levels of leptin decreased by 4.8 ng/mL at 12 months in group 1; there was little change in group 2 and 3. There was no statistically significant difference in the group mean change at 12 months from baseline between the groups.

Adiponectin levels decreased by 3.5 µg/mL in group 1 and by 3.7 µg/mL in group 3 by 12 months. In group 2, the level of adiponectin increased by 4.3 µg/mL by 6 months but returned to baseline levels by 12 months. There was no statistically significant difference in the group mean change at 12 months from baseline between groups. Resistin levels increased by 7.9 ng/ml in group 1 and by 6.4 ng/mL in group 3 by 12 months. The level dropped by 0.2 ng/mL in group 2. There was no statistically significant difference in the group mean change at 12 months from baseline between the groups. Visfatin levels are not reported as all samples had levels below the detection limit of the assay used, despite our attempts to optimize the assay which included reducing the number of wash cycles.

3.3.3 Immune status, virological control and plasma viscosity

At baseline, the median CD4 count was 425cells/mm^3 (229-816) in group 1; 496cells/mm^3 (176-1302) in group 2 and 512cells/mm^3 (99-1128) in group 3. The median CD4 percentage of the total lymphocyte count was 27% (17-53) in group 1; 27% (9-48) in group 2 and 28.5 (12-48) in group 3. None of the patients in Group 1 had a CD4 count <200 cells/mL^3. However, 4 (14.8%) patients in Group 2 and 3 (17.6%) patients in Group 3 had a CD4 count < 200 cells/mL^3. The group median CD4 cell levels remained above 400 cells/mm^3 in

all the groups between baseline and 12 months. The CD4 cell percentage remained reasonably good as well, being between 25-29% throughout the study period. The CD4 count increased by 79 cells/mm^3 in Group 1, 130 cells/mm^3 in Group 3 and 27 in Group 2 over 12 months. There was no statistically significant difference in the group mean change at 12 months from baseline between the groups.

The majority of the patients had an undetectable viral load of ≤ 40 copies/mL of blood (87.5%, 88% and 82% in Group 1, 2 and 3 respectively) at 12 months. This is comparable to base line levels (85%, 96% and 76% for Group 1, 2 and 3 respectively) with P=0.7, 0.7 and 0.4 for group 1, 2 and 3 respectively. Most patients who had detectable viral load could have been due to minor viral bleeps. Two patients each in Group 2 and 3 had clear-cut virological failure with levels between 500 and 509887 copies/mL. One of these patients had adherence level of 85-94% with some missed doses over the preceding week and 3 months. Although the other 3 patients' overrall adherence was 95-100%, they all had missed one or more doses over the preceding 3 months. While the patients would have had a resistance test done, this data was not recorded in this study.

There was not much change in the plasma viscosity over time within each group and there was no statistically significant difference in the group mean change at 12 months from baseline between the three groups. With regard to patients on a non-ABC/non-DDI regimen, there was a median increase at 12 months from baseline of 5mPAs in the plasma viscosity while this was

1.5mPAs for those on an ABC regimen and this difference was not statistically significance (P=0.06).

3.4 DISCUSSION

This longitudinal study was designed to investigate the levels of proinflammatory cytokines TNF-α and IL-6; adipocytokines namely adiponectin, leptin, resistin and visfatin; the nonspecific marker of inflammation CRP; immune status specifically looking at the CD4 count and viral load as well as to explore plasma viscosity and to relate these parameters to the NRTI backbone the patients were taking.

The patients were immunologically and virologically well controlled throughout the study period. There were increases in the CD4 counts in groups 1 and 3. The increase in CD4 cells was greater in patients that switched from a thymidine to a nonthymidine regimen (group 3) compared to those remaining on a thymidine (group 1) (130 vs. 79 cells/mm^3) which is in agreement with other studies such as Pozniak *et al* who demonstrated a significantly greater increase in CD4 count (270 vs. 237 cells/mm3; P= 0.036) in patients on a TDF regimen compared to those on an AZT regimen (Pozniak *et al.* 2006). With respect to virological control, there was no difference between the 3 groups as demonstrated by others who have studied patients on AZT or D4T based regimens compared to TDF or ABC containing regimens (Gallant *et al.* 2004). However, virological and immunological control do not appear to have an effect on overall risk of development of atherosclerosis and hence CVD (Hsue *et al.* 2009a; Ross *et al.* 2009).

The plasma viscosity did not increase markedly in all the groups, though those patients on a non-ABC/non-DDI regimen had a higher increase in the plasma viscosity than those on an ABC regimen but this was not statistically significant. The lack of major changes in plasma viscosity with the use of the various drugs in this study could be a reflection of the fact that the hyperviscosity syndrome in HIV infection was described in those with untreated disease with end-stage AIDS and is believed to be due to dysregulation and polyclonal activation of B-lymphocytes (Jin *et al.* 2000), and therefore these derangements could be brought under control with virological control due to treatment of the disease.

Although the median CRP was <3 in all the groups, which is the level quoted for the general population (Pepys and Hirschfield 2003), a quarter of patients in each group had a CRP>5.9, the median level found in HIV patients in another study (Noursadeghi and Miller 2005). These patients could potentially be at risk of CVD (Tracy *et al.* 1997; Koenig *et al.* 1999; Ridker *et al.* 2002; Cesari *et al.* 2003; Pai *et al.* 2004).

High levels of TNF-α and IL-6 have been shown to predict the development of CVD in the general population. High levels of these cytokines have been demonstrated in HIV infected people (Ross *et al.* 2009) which would mean that these patients would be at high risk of CVD. Indeed studies have shown increased risk of CVD in HIV patients by virtue of having the virus and being

on ART (Friis-Moller *et al.* 2003(b); Obel *et al.* 2007; Triant *et al.* 2007). Both *in vitro* and *in vivo* studies have demonstrated high levels of these cytokines in association with type or class of drugs within ART. In these studies, incubating differentiating adipocytes with PIs particularly indinavir, nelfinavir, ritonavir and lopinavir as well as AZT and d4T resulted in increased expression of the proinflammatory cytokines (Lagathu *et al.* 2004; Jones *et al.* 2005b). High circulating levels and increased mRNA expression in adipose tissue of TNF-α and IL-6 have been demonstrated in HIV patients with lipodystrophy (LD) compared with those that did not have LD (Lihn *et al.* 2003), showing that patients with LD are at increased risk of CVD.

The mean serum levels for the three groups for TNF-α levels in this study were similar to those found by Ross et al (2009) in HIV infected people (4.6-6.3pg/ml in this study vs. 4.0pg/mL in Ross's study). This is higher than the levels found in HIV negative people (1.8pg/mL in the study by Ross et al). This study confirms that the levels of this cytokine are raised in HIV infected people, which would put them at increased risk of developing CVD. There was no statistically significant difference between patients that remained on a thymidine or a non-thymidine and those switching from a thymidine to a non-thymidine regimen and the levels of TNF-α did not rise over the one year follow-up period.

With regard to IL-6, the highest levels were in the switch group (group 3, mean: 6.6 pg/mL) which is similar to the findings by Ross et al (6 pg/mL) and the

levels increased over the one year follow-up in this group. Patients continuing on a thymidine (group 1) or a non-thymidine (group 2) had levels that were similar to those in HIV uninfected people in the study by Ross et al (i.e. 2.8pg/mL *vs.* 3.0pg/mL in group 1 and 2.2pg/mL in group 2 in this study). However, there was no statistically significant difference in the group mean change at 12 months from baseline between the groups. It was expected that IL-6 levels would be higher in patients on a thymidine compared to those on a non-thymidine regimen and that levels would improve in patients that switched from a thymidine to a non-thymidine (group 3), the thymidine drugs having been shown to cause increased expression of this cytokine by others (Lagathu *et al.* 2004; Jones *et al.* 2005b; Lagathu *et al.* 2007). The unexpected high level of IL-6 in group 3 could be explained by the fact that 3 patients had very high levels of this cytokine. In 2 patients, IL-6 levels were high at baseline and at 6 months which improved at 12 months (for patient 1: 18.9, 23.7 and 3.6pg/mL respectively; for patient 2: 24.25, 33.75 and 12.5pg/mL respectively), both had switched to TDF, however, one of the patients had been on ABC with AZT and 3TC for 51 months before they switched. Both patients had TNF-α levels that were similar to the other patients within the group. The third patient (with levels of 2.27, 8.76 and 52.08pg/mL at baseline, 6 and 12 months respectively), had switched to ABC, and had TNF-α levels above 4pg/ml at all the visits (7.59, 13.7 and 12.1pg/mL respectively). One patient switched drugs for virological failure and the other two for LD. Neither of these 3 patients had intercurrent illness at any of the visits to explain the high levels of IL-6. Within group 3, 7 patients switched to ABC and they had median change of 0.17pg/mL at 12 months from baseline; 10 patients switched to TDF and these

had a median change of 0.9pg/mL, the difference between the two was not statistically significant (P=0.96). It is possible that the increase in IL-6 in group 3 may be driven by ABC use, as has been shown in the SMART study (Lundgren 2008) but the numbers in this study are low for firm conclusions to be drawn.

Both *in vitro* and *in vivo* studies have shown reduced circulating levels and expression of mRNA in the adipose tissue of adiponectin and leptin in HIV patients on ART particularly in lipodystrophic patients (Lihn *et al.* 2003; Jan *et al.* 2004; Lagathu *et al.* 2007). Reduced levels of adiponectin and leptin could be explained by the reduction in adipose tissue mass in these patients (Sweeney *et al.* 2007). As is the case with the proinflammatory cytokines, PIs and NRTIs have been shown to reduce the levels of these adipocykines. In the study by Lagathu *et al*, thymidines NRTIs (AZT and D4T) (ABC) and PIs (lopinavir, nelfinavir and indinavir) reduced the release of adiponectin and leptin from differentiated human adipocytes; ABC and some PIs (ATV and APV) had no effect (Lagathu *et al.* 2007). Eighty percent of patients were on D4T in addition to other NRTIs and PIs in the study by Jan *et al* in which fat from HIV patients had reduced adiponectin and leptin mRNA, further showing the adverse effects of thymidines (Jan *et al.* 2004). High resistin levels have been demonstrated in HIV positive patients compared to HIV negative individuals (Kamin *et al.* 2005). Additionally, patients with a single nucleotide polymorphism in the resistin gene on thymidine NRTIs, DDI and 3TC have been shown to develop physical changes of LD as well as dyslipidaemia and insulin resistance (Ranade *et al.* 2008).

In this study, adiponectin levels declined at 12 months from baseline levels in patients remaining on a thymidine (by 3.5µg/mL) which is in agreement with the studies described. After an initial rise in adiponectin levels at 6 months, levels returned to baseline values in patients that remained on a non-thymidine regimen, it was expected that this group would have a favourable adiponectin profile. Adiponectin levels declined in those switching from a thymidine to a nonthymidine regimen (by 3.7µg/mL); the expectation here was that there would be an adverse adiponectin profile which would improve upon switching to a nonthymidine. With regard to leptin, although the mean change from baseline were not different between the three groups, the levels declined in those patients remaining on a thymidine regimen (by 4.8ng/mL) in agreement with other studies and remained the same over the 12 month period in the other 2 groups. Similarly with resistin, although there were no statistically significant differences in the mean change between the three groups in the level of this cytokine at 12 months, levels did increase in those continuing on a thymidine regimen (by 7.9ng/mL), which is in agreement with previous studies described showing that this group of drugs has an adverse effect on adipose tissue derived cytokines. The increase (by 6.4ng/mL) in resistin in the group that switched from a thymidine to a nonthymidine regimen was unexpected, as the nonthymidine drugs should have more friendly effects on adipose tissue function. This probably shows that even the nonthymidines (TDF or ABC) may have a negative effect on adipose tissue or the effects of the preceding thymidine NRTIs had not completely reversed. Additionally, for those unexpected cytokine levels at different time points (e.g. in the case of

adiponectin), this may indicate that biomarker changes are dynamic as such the trend in levels may be demonstrated better with longer follow up.

Schindler *et al* clearly demonstrated high levels of visfatin in HIV patients on HAART compared to HIV negative controls (Schindler *et al.* 2006). This study was unable to demonstrate detectable levels of visfatin in all the patients. This could have been due to sample handling or problems with the assay used. To characterize the effects of various HIV drugs on this relatively new cytokine and its effects on CVD is still of great interest and as such merits further work.

There has been a lot of new data showing increased risk of CVD with current or recent use of ABC (Sabin *et al.* 2008). However, Martinez *et al* have recently shown that ABC does not affect cardiovascular biomarkers of inflammation, endothelial dysfunction, insulin resistance, or hypercoagulability in virologically suppressed HIV-infected patients. There was no difference at 48 weeks between patients on ABC and TDF based regimens in the serum levels of high-sensitivity CRP, monocyte chemoattractant protein-1 (MCP-1), osteoprotegerin, IL-6, IL-10, TNF-α, ICAM-1, VCAM-1, selectin E and P, adiponectin, insulin, and D-dimer (Martinez *et al.* 2010). This is in agreement with the *in vitro* study by Lagathu *et al* (2007) which found that ABC did not increase the release from human adipocytes of IL-6 and TNF-α or decrease the release of adiponectin and leptin. In our study, 41% of patients who switched ART went on to an ABC based regimen and 37% patients in Group 2 were on an ABC regimen, which shows that there was substantial use of this

drug. In this study, all the mean levels of markers under study (with the exception of resistin and adiponectin which showed adverse change in those that switched from a thymidine to a non-thymidine regimen) did not differ between the various drug regimens, including those that switched drugs, which shows that ABC did not add adversely to CVD biomarkers. This is in agreement with the findings of the study by Martinez *et al.* The adverse change in resistin and adiponectin cannot directly be attributed to use of ABC, particularly as the findings with regard to the other biomarkers in this study do not clearly support that ABC had an adverse effect on CVD biomarkers.

3.5 CONCLUSIONS

We set out to investigate cytokine levels in a typical large HIV unit. Cytokine derangements have been implicated in the pathogenesis of the metabolic syndrome, diabetes and cardiovascular disease. HIV positive patients are living longer and therefore the prevalence of cardiovascular disease and morbidity and mortality from this disease will increase. Investigating underlying cytokine derangements will provide a platform for investigating treatments to ameliorate these problems. This study has shown that thymidine NRTIs cause derangements in cytokines that have been shown to predict the development of CVD. ABC use did not clearly cause adverse changes in these cytokines. However, the numbers of patients in this study are small and we did not have a control group therefore firm conclusions cannot be drawn. Differences in findings within this study from other studies can be due to differences in methodology and populations. However, the accumulated data on the adverse

effects of thymidines has lead to the World Health Organization recently recommending that the thymidine D4T be removed from national ART programs (WHO 2009).

Clearly, research into risk factors of CVD posed by HIV drugs needs to continue, and treatments explored so that the success of treating HIV and turning it into a chronic manageable disease is not marred by development of disabling or fatal CVD.

CHAPTER FOUR

AUDITS OF CARDIOVASCULAR DISEASE MANAGEMENT IN HIV CLINICS IN MANCHESTER

4.1 INTRODUCTION

The HIV population is living longer with the aid of antiretroviral therapy (ART) and as such will experience diseases of aging such as CVD since they also will have the same risk factors as the general population (Friis-Moller *et al.* 2003(b)). These include smoking, dyslipidaemia, increased age, male sex, obesity, diabetes, hypertension and a family history of premature heart disease which increase the risk of CVD (Stamler *et al.* 1986; Wilson *et al.* 1998). Indeed HIV patients may have enhanced risks. Studies have shown increased rates of smoking of up to 51% in HIV patients (Friis-Moller *et al.* 2003(a); Ross *et al.* 2009) compared for example with a smoking rate of 25% in England amongst adults (NHS 2006). Usage of ART drugs particularly nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors (PIs) cause increases in total cholesterol (TC), LDL cholesterol (LDL-C), triglycerides (TGs), glucose intolerance and insulin resistance (Carr *et al.* 1998; Behrens *et al.* 1999; Jones *et al.* 2005a). There has been a lot of controversy on risk associated with particular ART drugs, especially abacavir (ABC). The D:A:D study (Sabin *et al.* 2008; Worm *et al.*) and Durand *et al* showed an increased risk of myocardial infarction (MI) with recent use or any exposure to ABC and didanosine (DDI) while Bedimo *et al* did not demonstrate this effect (Bedimo R 2009; Durand M 2009). Regardless of this conflicting information, combination ART has been shown to be independently associated with a 26 percent relative increase in the rate of myocardial infarction per year of exposure during the first four to six years of use and that cardiovascular disease now contributes to more than 10% of deaths in HIV-infected patients (Law *et al.* 2003; Friis-Moller *et al.* 2003(a); Friis-Moller *et al.* 2003(b); Sabin *et al.* 2008).

HIV physicians therefore face the challenge of monitoring, identifying, treating and or referring patients who develop risk factors of CVD on ARVs.

In the North West of England, there were 6363 cases of HIV infection by the end of 2007. Of these, 5212 accessed treatment and care from statutory treatment centres. An audit represents a valuable tool to assess the way HIV physicians identify and manage patients who develop metabolic complications of ARVs that would put them at risk of CVD. The Joint British Societies (JBS2) Guidelines on Prevention of Cardiovascular Disease in Clinical Practice can be used as a standard for such an audit (JBS2 2005). The Joint British Societies comprise the British Cardiac Society, the British Hypertension Society, Diabetes UK, HEART UK, the Primary Care Cardiovascular Society and the Stroke Association. The JBS2 guidelines are aimed at improving the management of individuals with risk factors for the development of CVD in the general population. The target population for management of these risk factors have been defined as:

- Patients with any form of established atherosclerotic CVD;
- Patients with diabetes (type 1 or 2);
- Asymptomatic individuals without established CVD who have >20% estimated risk of developing atherosclerotic CVD over 10 years;
- People with elevated single risk factors
    - Blood pressure >160mmHg systolic or >100mmHg diastolic, or lesser degrees of blood pressure elevation with target organ

damage, including left ventricular hypertrophy, grade 3 or 4 hypertensive retinopathy, raised creatinine, micro/macro-albuminuria or proteinuria;

- Ratio of total to high density lipoprotein cholesterol (TC:HDL-C) >6mmol/l;
- Familial dyslipidaemias, such as familial hypercholesterolaemia or familial combined hyperlipidaemia.

- Patients with a family history of premature CVD (CVD event in a male first degree relative before age 55 or a female first degree relative before age 65 years) should have their CVD risk assessed and treated accordingly.

The guidelines suggest that patients require professional lifestyle and risk factor management to achieve the defined targets in the following categories:

- Lifestyle changes:
  - Smoking cessation;
  - Healthier food choices;
  - Increase aerobic physical activity; and
  - Achieve optimal weight and weight distribution

- Optimal Blood Pressure (BP):
  - Systolic BP <140 mmHg and diastolic BP <85 mmHg in high risk people (<130 mm Hg systolic and <80 mmHg diastolic in patients

with established atherosclerotic disease, diabetes or chronic renal failure)

- Optimal Lipids:
    - TC <4.0 mmol/l or a 25% reduction;
    - LDL-C <2.0 mmol/l or a 30% reduction in LDL-C
- Blood Glucose
    - Optimal fasting glucose <6.0mmol/l (patients with levels between 6.1-6.9 on 2 occasions would require an oral glucose tolerance test, while those with levels >7mmol/l on 2 occasions are considered diabetic)
- Cardioprotective drugs in selected people:
    - Antithrombotic therapy (aspirin or clopidogrel)
    - All people over the age of 50 years who have a total CVD risk > 20%
    - All people with established coronary, peripheral or cerebrovascular atherosclerotic disease
- Blood pressure lowering therapy (such as β-blockers, angiotensin converting enzyme inhibitors, calcium channel blockers and diuretics alone or in combination)
    - In patients with established atherosclerotic disease whose blood pressures are not below the optimum or patients with severe high blood pressure as a single risk factor.

- Lipid lowering therapy (Statins plus other lipid lowering drugs (LLAs))
    - People with established atherosclerotic disease
    - People with high total risk of developing CVD >20%
    - Diabetics aged 40years or more
    - Diabetics aged less than 40years with microvascular disease, poorly controlled diabetes, TC >6mmol/l, on treatment for hypertension, the metabolic syndrome and a family history of premature CVD in a first-degree relative.

Use of statins is also recommended in these groups of patients by the National Institute of Health and Clinical Excellence (NICE-TA94 2009).

Other than the optimal standards of care which should be achieved for high risk people, the JBS has less stringent audit standards which are considered to be the minimum standard of care for high-risk people though it is recommended that wherever possible, the optimal treatment targets should be achieved. The audit standards include achieving a blood pressure of < 150 mm Hg systolic and <90 mmHg diastolic; TC of <5.0 mmol/l (or a 25% reduction in total cholesterol) and LDL-C of <3.0 mmol/l (or a 30% reduction in LDL-C).

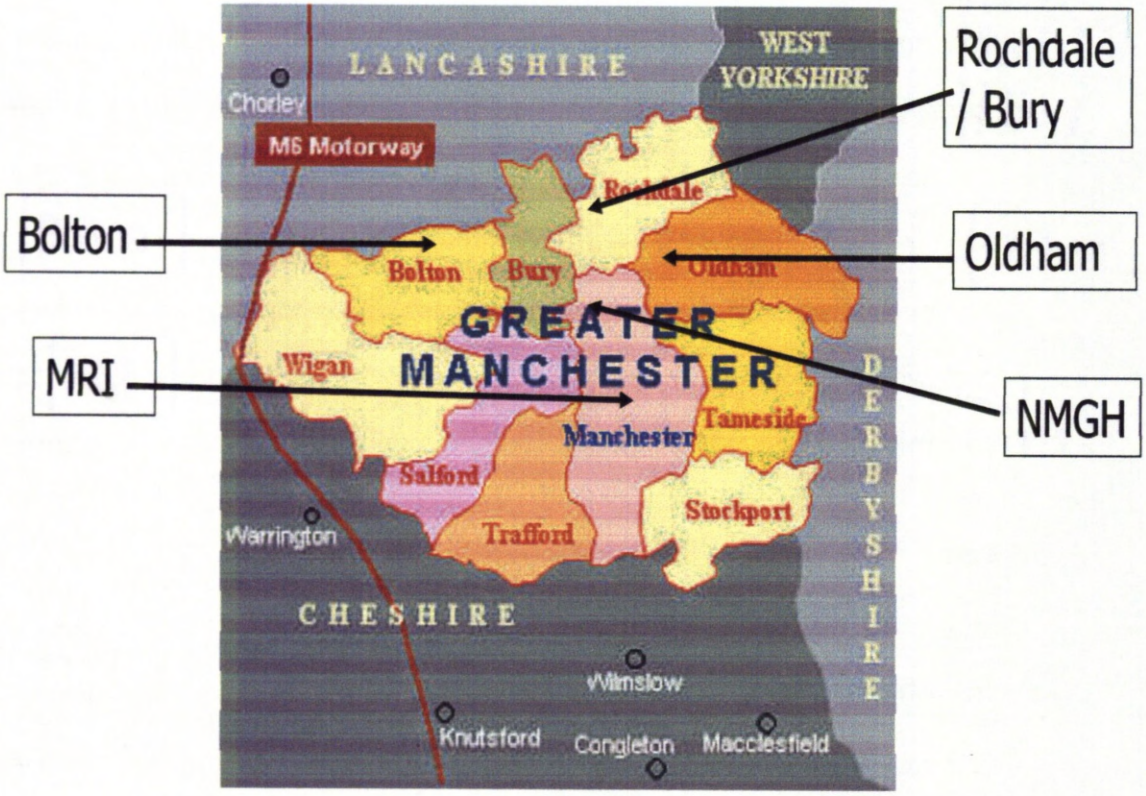
Two audits were undertaken in HIV positive patients in the North West. The first audit was conducted at North Manchester General Hospital (NMGH) -

Audit 1, and the second at 5 clinics in the Greater Manchester area (namely NMGH, Bolton, Oldham, Manchester Royal Infirmary [MRI] and Rochdale/Bury) - **Audit 2**. In these audits, we compared our patient management with the JBS2 audit standards rather than optimal standards of care. The JBS2 optimal standards are more strigent and require lower levels to be achieved for the CVD risk factors while the audit standards are less stringent, requiring levels that are higher than the optimal standards to be achieved. Since we were carrying out these audits for the first time, it was felt that we should compare our practice against the audit standards, with a view to comparing against the optimal standards upon re-auditing in the future.

4.2 Aims and Objectives

To determine if the HIV treatment clinics in the Greater Manchester area (Figure 4-1) appropriately identify CVD risk factors in HIV patients on HAART and institute corrective measures or treatments according to JBS2 guidelines.

Figure 4-1: Greater Manchester HIV treatment clinics



MRI: Manchester Royal Infirmary; NMGH: North Manchester General Hospital

4.3 Methodology

The audits were retrospective HIV patient case note reviews looking at record keeping, identification and institution of corrective measures for cardiovascular risk factors. Case notes reviewed were those retrieved by medical records in readiness for consultant clinics.

4.3.1 Data collection

In Audit 1, patients were those attending clinic from January 2006 to January 2007 at the NMGH, which has 1500 HIV patients registered for care. One hundred and one (101, 7%) case notes were reviewed for this audit.

Consecutive casenotes were selected from lists of patients attending each of the consultants' clinics. Up to five lipid (for TC, LDL, HDL-C, TGs and TC:HDL ratio) and glucose results documented in the case notes or on the hospital computer chemistry results system over the one year period were recorded. All patients had at least 1 reading. The medians for each lipid fraction and glucose over the year were calculated per patient.

In audit 2, the case notes of 78 patients under the care of 16 HIV consultants working within 5 clinics in Manchester (as listed above) were reviewed, each consultant reviewing approximately 5 case notes. The clinics involved were either Infectious Diseases or Genito-Urinary Medicine, and in total they had just over 2500 HIV patients registered for care. Data from April 2005 – April 2006 were recorded. The highest recorded TC level and any HDL-C level or the highest recorded TC:HDL-C ratio in the year of interest were used in the audit. The glucose was recorded as either euglycaemic or raised.

In both audits, the highest blood pressure measurement recorded when the patient was noted to be well was used. The lipid and glucose levels were mostly random, rather than fasted. Data were recorded on predesigned data collection forms, each of the 2 audits having their own form. Data collection forms were designed in line with the wishes of the participating consultant physicians, agreed and used across all sites. The medians for each lipid fraction, glucose, blood pressure (systolic and diastolic) and duration of treatment were calculated; the Mann-Whitney U test was used to check for

statistical significance of the differences in the above parameters for the different drug regimens (SPSS v. 16, SPSS Inc. Chicago, USA). Appendix 3 and 4 show the data collection forms for Audit 1 and 2 respectively.

CVD risk calculation was done using the University of Edinburgh cardiovascular risk calculator and chart v2.0 (Payne 2005). Variables entered were age, systolic blood pressure, smoking status, TC and HDL-C. A percentage risk over 10 years was obtained for all patients except those with established diabetes and ischaemic heart disease, who should be considered high risk and treated accordingly.

4.3.2 Lifestyle advice recorded

For life style advice and risk factor management, the following documentation was sought in the case notes:

Smoking cessation – if the physician documented having counselled the patient to stop smoking; if the patient was advised to access general practitioner (GP)-led smoking cessation clinics; or the patient was prescribed nicotine patches or gum.

Diet - if the physician documented having informed the patient to change their diet or any documentation of nutritional advice or a referral to a dietician.

Weight reduction and exercise - if the physician documented having informed the patient to reduce weight either via diet or increase in exercise, or referral to a fitness unit.

Alcohol moderation - if the physician documented having informed the patient to reduce their alcohol intake, or if the patient was advised to seek community based alcohol misuse treatment centres or if the patient was referred to a hospital based alcohol misuse team.

4.3.3 Additional analysis

\With the new controversy over increased risk of myocardial infarction with ABC and DDI, analyses have been undertaken to look at CV risks for patients on combination ART that includes these 2 drugs.

4.4 RESULTS

The results of the two audits are presented separately because the audits were undertaken at different times, the design was different and separate databases were set up. This was because more complete lipid and glucose profiles were collected in audit 1 (5 for each lipid fraction and glucose) while the highest TC and TC:HDL-C ratio in the year were recorded and, additionally, glucose levels were recorded as "euglycaemic", "raised" or "borderline" in audit 2.

4.4.1 Audit 1

4.4.1.1 Demographics

Eighty-three patients out of 101 were male and the median age was 41 years (range: 21-71 years). Sixty eight percent of patients were Caucasian, 13% were Black African, 1% were Asian and in 12%, the ethnicity was not recorded or was unknown.

4.4.1.2 Antiretroviral drug usage:

Table 4-1 shows the antiretroviral therapy (ART) drugs used at the time of the audit. Zidovudine with lamivudine (AZT/3TC) was the most commonly used nucleoside reverse transcriptase inhibitor (NRTI) backbone (27/101, 26.7%), tenofovir (TDF) with 3TC or emtricitabine (FTC) being the second commonest NRTI backbone (24/101, 23.8%). There was increasing use of lipid friendly protease inhibitors (PIs) such as atazanavir (ATV) rather than boosted lopinavir (LPV/RTV) (15% vs. 10% respectively). Most patients were on a non-NRTI (NNRTI) rather than on a PI in keeping with guidance at the time (Gazzard *et al.* 2006). A third of the patients were on ABC based regimen while only a small proportion (11%) were on a DDI based regimen.

Patients on a non-thymidine (NTH) based regimen had been on their current regimen for shorter duration of time than those on a thymidine (TH) based regimen (P=0.04) though the total duration on ARVs was not significantly different. Additionally, the NTH group had more frequently been exposed to PIs (P=0.009) as well as being triple-class experienced than those on a TH based regimen (see Table 4-4). Nearly three quarters of the patients on ABC and DDI regimens had previous PI exposure (72 and 73% respectively) while a smaller proportion (39%) of those on a non-ABC/non-DDI regimen had previous PI exposure (ABC *vs.* non-ABC/non-DDI: P=0.003; DDI *vs.* non-ABC/non-DDI P=0.04). Additionally, nearly 50% of the patients in the ABC and DDI group were triple class experienced while this was only 19% in the non-ABC/non-DDI group (ABC *vs.* non-ABC/non-DDI: P=0.005; DDI *vs.* non-

ABC/non-DDI *P*=0.06). However, the duration of the current regimen or total duration on all ART was similar between the 3 groups (see table 4-5).

Table 4-1: Antiretroviral drugs used (Audit 1)

Group	Drug	No. of Patients	% of total (101)
Thymidine (45, 44.5%)	AZT based double or triple NRTI backbone	37	38.4
	D4T based double or triple NRTI backbone	8	8.1
Non-thymidine (56, 55.4%)	TDF/FTC or 3TC	24	10.1
	ABC/3TC or FTC	9	8.1
	DDI combinations[α]	10	10.1
	TDF/ABC	11	10.1
	Other NRTI combinations	2	2.0
ABC (non-DDI) based (29, 28.7%)	ABC/3TC or FTC	9	8.9
	ABC/TDF	11	10.1
	ABC/D4T +/- other NRTI	4	4.0
	Other NRTI combination	5	4.9
DDI (non-ABC) based (11, 10.9%)	Various double/triple NRTI combinations	11	10.9
Protease Inhibitors (38, 37.6%)	Ritonavir boosted Lopinavir	10	10.1
	Atazanavir	15	15.2
	Fosamprenavir	11	11.1
	Double-boosted PIs	2	2.0
NNRTIs (60, 59.4%)	Efavirenz	30	30.3
	Nevirapine	30	30.3

[α] *In combination with TDF, 3TC or ABC; NNRTI: non- nucleoside reverse transcriptase inhibitor*

4.4.1.3 Cardiovascular Risk FactorsPre-existing Risk Factors for CVD

Table 4-2 shows the risk factors that were documented in the case notes for the time period of the audit for all the patients. There were high levels of smoking and family history of premature ischaemic heart disease (IHD). There were low levels of pre-existing diabetes, hypertension or IHD, though it should be noted that frequently, case notes did not have a record of whether a patient did or did not have a risk factor for CVD (99.1% of case notes for pre-existing IHD, 45.5% for family history of CVD, 16.8% for smoking status, and 9.9% for

hypertension). It was not possible to ascertain from the case notes whether the patients did not have these risk factors or that the clinicians omitted to ask about them or failed to document appropriately. Table 4-3 presents risk factors according to NRTI backbone. The highest rates of smoking were in patients taking DDI (63.6%) and ABC (58.6%) compared to those on a non-ABC/non-DDI regimen (47.4%) or those on a TH (48.9%) and NTH regimen (53.6%). More patients on DDI also had a family history of premature CVD compared to patients on the other regimens. Diabetes and hypertension were uncommon in all the drug groups.

Table 4-2: Recorded cardiovascular disease risk factors (Audit 1)

Risk Factor	Yes (%)	No (%)
Smoker	52 (51.5)	36 (31.7)
FH of CVD	21 (20.8)	34 (33.7)
Diabetes	7 (7)	88 (87.1)
Hypertension	7 (7)	84 (83.1)
Pre-existing IHD	1 (0.9)	

FH of CVD: family history of premature cardiovascular disease;
IHD: ischaemic heart disease

Table 4-3: Cardiovascular disease risk factors and NRTI backbone (Audit 1)

	Thymidine regimen n=45	Non-Thymidine regimen n=56	ABC (non-DDI) regimen n=29	DDI (non-ABC) regimen; non-ABC n=11	Non-ABC, non-DDI regimen n=57
CV Risk:					
>20 %	3 (6.7%)	4 (7.1%)	2 (6.9%)	1 (9%)	4 (7%)
10-20%	9 (20%)	12 (21.4%)	9 (31%)	2 (18%)	9 (15.8%)
<10%	27 (60%)	32 (57.1%)	16 (55.2%)	6 (54.5%)	34 (59.6%)
Smokers:					
Current	22 (48.9%)	30 (53.6%)	17 (58.6%)	7 (63.6%)	27 (47.4%)
Exsmokers	9 (20%)	8 (14.3%)	4 (13.8%)	1 (9%)	11 (19.3%)
Diabetes	2 (4.4%)	5 (8.9%)	2 (6.9%)	1 (9%)	4 (7%)
FHx of CVD	8 (17.8%)	13 (23.2%)	5 (17.2%)	5 (45.4%)	10 (17.5%)
Hypertension	2 (4.4%)	5 (8.9%)	1 (3.4%)	1 (9%)	4 (7%)

4.4.1.3.2 Lipids

The median TC was 4.8mmol/l (3.0-7.9) and median LDL-C was 2.6 mmol/l (1.3-5.2), both of which were below the JBS2 audit standards. Forty five (44.5%) patients had a median TC >5.0mmol/l, 27(60%) of these patients also had an LDL-C >3.0mmol/l. Twenty nine (28.7%) patients had LDL-C >3.0mmol/l alone. The total number of patients identified with raised lipids (either TC or TGs or both) at some point in the 1 year period of the case note review was 58 (57.4%). Twenty (34.5%) patients out of 58 had raised TC alone, 9 (15.5%) patients had raised TGs alone and 29 (50%) patients had raised TC and TGs. Twenty seven (45.5%) had raised LDL-C. The median (and range) of lipid fractions over a 1 year period, as well as the duration of treatment and previous PI use with type of NRTI backbone and PI or NNRTI use, are shown in Tables 4-4 to 4-6. There were no differences in the lipid

fractions between TH *vs.* NTH, ABC or DDI *vs.* nonABC/nonDDI regimen. Patients on a PI-based ARV regimen had lower levels of TC and LDL-C than those on an NNRTI (P=0.03 and 0.007 respectively); however, there was no significant difference between the two groups with respect to TGs.

4.4.1.3.3 Blood Pressure and Blood glucose

Median systolic blood pressure for the 101 patients was 124mmHg (range 96-170) which was below the JBS2 audit standard. Of the 101 patients, 95 had BP recorded while 6 case notes (6.3%) did not. Of the 95 patients with a BP record, 6 (6.3%) had a systolic blood pressure greater than 150mmHg. Notably, 7 patients (7%) had pre-existing hypertension and 5 of these were already on treatment, however, only 2 of these 7 patients had a recorded systolic BP <150mmHg. There was no difference in the systolic blood pressure between TH *vs.* NTH, ABC or DDI *vs.* nonABC/nonDDI regimens and PI *vs.* NNRTI users.

Of the 101 patients, 99 (98%) had blood glucose results in the case notes while 2 (2%) had no record over the whole year of interest. The median blood glucose for the 99 patients was 4.9mmol/l (3.9-10.2). Thirteen patients (13.1%) had a blood glucose level >6.1mmol/L; of these, 7 (7%) were already diabetic. There were no differences in the blood glucose levels between TH *vs.* NTH, ABC or DDI *vs.* nonABC/nonDDI regimens and PI *vs.* NNRTI users (Tables 4-4 to 4-6).

Table 4-4: Lipids, Blood pressure (BP), glucose levels and CV risk– thymidine and non-thymidine NRTI backbone (Audit 1)

	Thymidine NRTI use; n=45	Non-thymidine NRTI use; n=56	2-tailed P value (Mann-Whitney U test)
Age (median, range)	41 (29-71)	41 (21-69)	0.80
Previous PI exposure (n, %)*	18 (40%)	37 (66.1%)	0.009
Triple-Class drug exposure (n, %)*	10 (22%)	22 (39%)	0.07
Duration current drugs (months)- (median, range)	10 (4-159)	13 (1-89)	0.04
Duration all ART (months)- (median, range)	60 (6-192)	70.5 (11-214)	0.28
TC(mmol/l) - (median, range)	4.9 (2.0-7.0)	4.7 (3.1-7.9)	0.61
LDL(mmol/l) - (median, range)	2.5 (1.4-5.2)	2.6 (1.3-5.1)	0.56
TG(mmol/l) - (median, range)	2.0 (0.7-6.3)	1.6 (0.6-5.2)	0.12
HDL-C(mmol/l) - (median, range)	1.2 (0.5-4.2)	1.2 (0.4-2.8)	0.51
TC:HDL-C - (median, range)	4.5 (2.0-8.0)	4.0 (2.0-13.5)	0.47
BP systolic (mmHg) - (median, range)	129 (105-160)	123 (96-170)	0.12
Blood Glucose (mmol/l) - (median, range)	4.9 (3.9-8.7)	4.9 (4.0-10.2)	0.62
CV risk (%) - (median, range)	6.8 (0.2-20.3)	6.0 (0.2-28.3)	0.58

**Pearson's Chi Square test used for these variables*

Table 4-5: Lipids, Blood pressure, glucose levels and CV Risk – Abacavir and didanosine NRTI backbones (Audit 1)

NRTI backbone	ABC (non-DDI) based; n=29	DDI based (non-ABC); n=11	Non-ABC/ non-DDI regimen; n=57	2-tailed *P* value (Mann-Whitney U test)	
				ABC *vs.* Non-ABC/ non-DDI	DDI *vs.* Non-ABC/ non-DDI
Age – (median, range)	46 (26-55)	41 (36-69)	41 (29-71)	0.14	0.36
Previous PI exposure - (n, %)*	21 (72%)	8 (73%)	22 (39%)	0.003	0.04
Triple-Class drug exposure (n, %)*	14 (48%)	5 (45%)	11 (19%)	0.005	0.06
Duration current drugs (months) – (median, range)	19 (1.5-62)	17 (7-49)	21 (1.0-84)	0.29	0.32
Duration all ART (months) – (median, range)	76 (13-214)	85 (14-150)	57 (6-144)	0.05	0.10
TC(mmol/l) – (median, range)	4.7 (3.0-7.9)	5.3 (3.9-7.0)	4.8 (3.1-7.8)	0.81	0.60
LDL(mmol/l) – (median, range)	2.5 (1.3-5.2)	3.0 (1.9-4.7)	2.6 (1.5-5.1)	0.71	0.28
TG(mmol/l) – (median, range)	1.8 (0.8-5.4)	2.2 (0.7-5.7)	1.6 (0.6-6.3)	0.57	0.56
TC:HDL-C ratio- (median, range)	4.0 (2.0-13.5)	4.5 (2.0-7.0)	4.3 (2.0-7.0)	0.87	0.41
BP systolic (mmHg) – (median, range)	123 (105-158)	124 (100-170)	125 (96-170)	0.75	0.92
Blood Glucose(mmol/l) - median, range	5.1 (4.0-6.6)	4.7 (4.0-9.9)	4.9 (3.9-10.2)	0.35	0.36
CV risk (%) – (median, range)	8.2 (0.6-24.4)	6.1 (2.2-20.9)	6.5 (0.2-29.3)	0.22	0.32

**Pearson's Chi Square test used for these variables*

Table 4-6: Lipid and blood pressure levels with PI or NNRTI use (Audit 1)

	Current PI use; n=38	Current NNRTI use; n=62	2-tailed P value (Mann-Whitney U test)
TC – (median, range)	4.5 (3.0-7.9)	4.8 (3.1-7.8)	0.03
LDL(mmol/l) – (median, range)	2.2 (1.3-5.2)	2.6 (1.5-5.1)	0.007
TG(mmol/l) – (median, range)	1.8 (0.6-5.7)	1.6 (0.6-6.3)	0.41
TC:HDL-C ratio – (median, range)	4.0 (2.0-13.5)	4.3 (2.0-7.0)	0.71
BP systolic (mmHg) – (median, range)	123 (96-149)	125 (96-170)	0.29
Blood Glucose (mmol/l) – (median, range)	4.8 (4.0-6.6)	4.9 (3.9-10.2)	0.07
CV risk (%) – (median, range)	6.5 (0.2-24.4)	6.5 (0.2-29.3	0.99

4.4.1.3.4 Cardiovascular risk calculation

The CV risk was not calculated in 8 (7.9%) patients due to pre-existing CVD (7 with diabetes, 1 with IHD). Six (5.9%) did not have enough data to calculate the CV risk %. Eighty seven (86%) patients had enough data to calculate the CV risk. The median CV risk was 6.5% (0.2-29.3%). Overall, most patients (80, 79.2%) were at low to moderate risk of CVD with a calculated CV risk of <20%. These patients however did have some isolated CVD risk factors, as shown in Table 4-7, and frequently, they had 2 or more of these risk factors namely: raised lipids, smoking, family history of CVD, hypertension, high recorded BP or blood glucose. Seven (8%) patients had a high calculated CV risk of >20%.

Table 4-7: Cardiovascular Risk < 20% and associated Risk factors, n=80 (Audit 1)

Risk Factor	Number of patients (%)
Smoking	43 (54)
High Lipids	41 (51)
Having ≥ 2 risks (raised lipids, smoking, FH of CVD, hypertension, high recorded BP or blood glucose)	22 (27)

Fifteen out of the 101 patients (14.8%) were identified to be at high risk of CVD in the following categories:

- Calculated CV risk >20% over 10 years 7 (7.4%)
- Diabetes 7 (7.4%)
- Established ischaemic heart disease 1 (0.9%)

Other associated risks identified in the 15 high-risk patients are shown in table 4-8 below which shows high prevalence of risk factors particularly smoking (67%) and high lipids (80%). All 7 patients with a high calculated CV risk had 2 or more risk factors for CVD (namely raised lipids, smoking, family history of CVD, hypertension, high recorded BP or blood glucose).

Table 4-8: Associated Risks in High Risk Patients (Audit 1)

Risk Factor	Number (%)
Smoking	10 (67%)
Raised Lipids	12 (80%)
Hypertension	3 (20%)
FH of CVD	5 (33%)

FH of CVD: family history of cardiovascular disease

4.4.1.4 Management of cardiovascular risks:

Table 4-9 shows the management aspects that were documented in the case notes of patients identified to be at low to moderate risk of CVD (<20%). Interventions in the form of life style advice such as smoking cessation or dietary advice were offered to about a third of patients with high lipids (31.8%) or those who smoked (36.6%). A small proportion (4, 9.1%) were started on lipid lowering therapy namely statins (such as pravastatin or atorvastatin) or a fibrate (most commonly fenofibrate).There were only small numbers of patients with hypertension (2.5%) and raised glucose levels (8.8%) in this group, 2 patients who had hyperglycaemia received smoking cessation advice, and no action was taken to address hypertension.

Table 4-10 shows the management aspects that were documented in the case notes of patients identified to be at high risk of CVD (>20%). Most patients were likely to have received some advice regarding life style such as dietary or smoking cessation, though this was not done in all patients. Importantly, some patients did receive multiple interventions. Most of the patients with diabetes and those with dyslipidaemia were on lipid lowering therapy. Antiplatelet drugs

as such aspirin were not uniformly used in this high-risk group, with 3 of the 15 being on aspirin.

Table 4-9: Management of risk factors in patients with cardiovascular risk <20% (n=80) (Audit 1)

	Total	Action taken (In patients with risk)	Type of action taken	
			Life style advice	Drug treatment or Referral
Smokers	41 (51%)	15 (36.6%)	Smoking cessation (15)	0
↑Glucose	7 (8.8%)	2 (28.6%)	Smoking cessation (2)	0
↑ Lipids	44 (55%)	14 (31.8%)	Dietary advice (9) Smoking cessation (7) Alcohol moderation (1)	Statins (3) Statin and fibrate (1)
↑systolic BP(>150 mmHg)	2 (2.5%)	0		

↑(Increased or raised); BP: blood pressure

4.4.2 Audit 2

4.4.2.1 Demographics

Three out of the 5 units self-reported that they followed the JBS guidelines, the other 2 following clinical judgement. The median age of patients was 39.5 years (range 24-70). Males comprised 69%; 58% were Caucasian and 33% were Black African. Ethnicity was not recorded in 6%.

Table 4-10: Management of risk factors in patients with calculated cardiovascular risk >20% or pre-existing cardiovascular disease (n=15) (Audit 1)

	Total (%)	Action taken (in patients with risk)	Type of action taken	
			Life style advice	Drug treatment or Referral
Smokers	10 (67)	5 (50%)	Smoking cessation (5)	0
↑ Glucose (including Diabetics)	8 (53)	7 (88%)	Dietary advice (3)	Statins (4) Statins+fibrate(2) Fish oil (1) Antidiabetics (4) Aspirin (1) Anti-hypertensives (2)
↑ Lipids (No Diabetes)	7 (47)	5 (71%)	Dietary advice (4)	Statins (2) Statin+fibrate (1) Aspirin (2)
↑ BP	2 (13)	1 (50%)		Antihypertensives (1)

↑:(Increased or raised; BP: blood pressure

4.4.2.2 Antiretroviral drug usage

Most patients were treatment experienced. Median duration on treatment was 4.5 years (range 1-16). Table 4-11 shows the type of NRTI backbone and the concomitant PI or NNRTI patients were on. Most patients were on NTH NRTIs TDF or ABC in combination with each other or with FTC or 3TC (59%), with fewer being on TH NRTIs (17 were on AZT and 3 on D4T as part of a 2 or more nucleoside backbone). Thirty (38%) patients were on an ABC based regimen, while 6 (8%) patients were on a combination that included DDI. Nine (12%) patients were on a triple or more nucleoside backbone. Most patients (57.7%) were on an NNRTI, particularly EFV, compared to protease inhibitors (37.2%). Boosted LPV was the most commonly used PI in this population. As was the case in audit 1, patients in this audit who were on a NTH based regimen had been on their current regimen for shorter duration, they had been on ART for a longer duration and had been more frequently exposed to PIs as

well as being triple-class experienced than those on a TH based regimen (Table 4-12), however, most of these differences were not statistically significant.

Table 4-11: Antiretroviral Drugs Used (Audit 2)

	Drug	No. of Patients	% of total (78)
NRTIs	Zidovudine/lamivudine	14	17.9
	Tenofovir/emtricitabine or lamivudine	22	28.2
	Abacavir/lamivudine	16	20.5
	Tenofovir/Abacavir	8	10.3
	Other combinations	7	9
	Triple nucleoside combinations	9	11.5
Protease Inhibitors[α]	Lopinavir*	14	17.9
	Atazanavir*	5	6.4
	Fosamprenavir*	6	7.7
	Saquinavir*	2	2.6
	Double-boosted PIs	2	2.6
NNRTIs	Efavirenz	32	41
	Nevirapine	13	16.7

**ritonavir boosted, [α]one patient was on unboosted atazanavir.*

4.4.2.3 Cardiovascular disease Risk FactorsPre-existing Risk Factors for CVD

Forty six (59%) patients were smokers. Fifteen (19%) of the patients had a history of pre-existing CVD (hypertension, ischaemic heart disease, cerebrovascular accident, diabetes or dyslipidaemia). Nineteen percent had a family history of premature CVD. Eight percent were hyperglycaemic and 8% had impaired glucose tolerance. CVD risk factors were not documented in a

proportion of case notes as follows: smoking status (19%), family history of CVD (40%) and previous CVD (17%).

4.4.2.3.2 Blood Pressure, lipids and Blood sugar

Tables 4-12 to 4-14 present the lipid and blood pressure levels with drug regimens. The median systolic BP for the whole sample was 126mmHg (91-210). Most patients had normal blood pressure. Nine patients (11.5%) had a systolic BP of ≥ 150mmHg. Of these, 5 were on a TH based regimen (25% of this group) and 4 were on a NTH (7.1%). Twenty one patients out of 30 on ABC had recorded BPs and of these 3 (14%) had a systolic BP>150mmHg. One of 6 (17%) patients on DDI and 5 of 57 (9%) patients on non-ABC/non-DDI regimen had a systolic BP>150mmHg. Four (20%) patients on PIs and 5 (15%) on NNRTI had systolic BP>150mmHg. The systolic blood pressure level was not significantly different between the TH *vs.* NTH groups, PI *vs.* NNRTI groups, ABC *vs.* Non-ABC/non-DDI groups and DDI *vs.* Non-ABC/non-DDI groups.

Median TC was 5.2mmol/l (2.3-8.7), which is above the JBS2 audit standard (<5.0mmol/l). The median TC:HDL-C ratio was 4.3 (1.5-7.1). Seventy one patients had a recorded TC level and 43(61%) had TC >5.0mmol/l. Of the 20 patients who were on a TH based regimen, 19 had cholesterol levels recorded, of these 14 (74%) had high total cholesterol levels (≥ 5.0mmol/l). Fifty six patients were on a NTH regimen and of these, 51 had TC levels recorded with 22 patients (48%) having high total cholesterol levels of ≥ 5.0mmol/l. The TC was > 5.0mmol/l in 14 (67%) of patients on ABC, 1 (17%) on DDI, 26 (46%) on non-ABC/non-DDI, 13(52%) on PIs and 29(67%) on NNRTIs. However, there

was no statistically significant difference in the TC levels between the TH *vs.* NTH groups, PI *vs.* NNRTI groups, ABC *vs.* Non-ABC/non-DDI groups and DDI *vs.* Non-ABC/non-DDI groups.

Most patients (>75%) were recorded as euglycaemic on all the drug regimens. However, patients on an ABC or DDI regimen were more likely to be euglycaemic than those on a non-ABC/non-DDI regimen (P=0.005 and 0.03 respectively).

Table 4-12: Lipid, blood pressure (BP), glucose levels and CV risk – thymidine and non-thymidine NRTI backbone (Audit 2)

NRTI backbone	Thymidine NRTI use; n=20	Non-thymidine NRTI use; n=56	2-tailed P value (Mann-Whitney U test)
Age (median, range)	40.5 (24-54)	39.0 (24-70)	0.41
Previous PI exposure*	3 (15%)	19 (33.9%)	0.11
Triple-class drug exposure*	2 (10%)	13 (23%)	0.20
Duration current drugs (months) – median (range)	36 (4-72)	11.5 (1-68)	0.04
Duration all ART (years) – median (range)	4 (1-12)	4.7 (1-16)	0.97
TC (mmol/l)	5.3 (2.8-7.8)	5.1 (2.3-8.7)	0.67
TC:HDL-C ratio–median (range)	4.1 (2.0-7.0)	4.4 (2.4-7.0)	0.84
BP systolic (mmHg)-median (range)	131 (91-210)	122 (97-170)	0.24
CV Risk (%)-median, range	8.3 (0.4-22.8)	5.5 (0.1-37.7)	0.26
Blood Glucose – n(%)*:			
Euglycaemic	16 (80)	46 (82)	0.83
Raised glucose	4 (20)	10 (18)	0.83

**Pearson's Chi Square test used for these variables*

Table 4-13: Lipids, blood pressure, glucose levels and CV Risk – Abacavir and didanosine NRTI backbones (Audit 2)

NRTI backbone	A Abacavir (non-DDI) based; n=30	B DDI based (non-ABC); n=6	C Non-ABC, non-DDI regimen; n=57	2-tailed P value (Mann-Whitney U test)	
				A *vs.* C	B *vs.* C
Age - median (range)	37.5 (24-59)	43.5 (34-53)	42 (24-70)	0.04	0.85
Previous PI exposure*	10 (33%)	1 (17%)	8 (14)	0.04	0.86
Previous triple-Class drug exposure*	7 (23%)	1 (1%)	6 (11)	0.11	0.65
Duration current drugs (months) - median (range)	10 (1-48)	36 (13-36)	17 (2-72)	0.18	0.89
Duration all ART (months) - median (range)	5 (1-16)	4.7 (3.0-9.0)	4 (1-15)	0.40	0.97
TC(mmol/l) - median (range)	5.1 (2.3-8.7)	4.8 (4.7-7.4)	5.2 (2.8-7.7)	0.96	0.18
TC:HDL-C - median (range)	4.1 (2.6-7.0)	4.9 (2.4-7.0)	4.4 (2.0-7.0)	0.61	0.97
BP systolic (mmHg) - median (range)	122 (109-170)	125 (109-165)	126 (91-210)	0.78	0.71
CV Risk (%) - median, range	8.7 (0.3-37.7)	4.8 (1.6-6.9)	6.0 (0.1-22.8)	0.90	0.66
Blood Glucose – n (%)*:					
Euglycaemic	25 (83)	6 (100)	30 (53)	0.005	0.03
Raised glucose	5 (17)	0	6 (11)	0.41	0.40

**Pearson's Chi square test used for these variables*

Table 4-14: Lipid and blood pressure levels with Protease inhibitor or Non-nucleoside reverse transcriptase inhibitor use (Audit 2)

	Current PI use; n=29	Current NNRTI use; n=44	2-tailed P value (Mann-Whitney U test)
Age - median, range	39 (24-59)	40.5 (24-70)	0.72
TC(mmol/l) - median, range	5.1 (2.3-8.7)	5.2 (2.8-7.7)	0.67
TC:HDL-C ratio - median, range	4.9 (1.0-7.1)	4.1 (2.0-7.0)	0.41
BP systolic(mmHg) - median, range	125 (106-170)	126 (91-210)	0.66
CV Risk (%)- median, range	5.4 (0.1-37.7)	6.5 (0.4-22.8)	0.10
Blood glucose – n(%)*:			
Euglycaemia	26 (90)	34 (77)	0.18
Raised glucose	3 (10)	10 (23)	0.18

**Pearson's Chi Square test used for these variables*

4.4.2.3.3 Cardiovascular risk calculation

A CV risk percent was not calculated in 16(21%) patients due to incomplete data and 11 patients (14%) due to age being less than 30 (the cut-off for the CV calculator v2.0) and considered at low risk. Fifteen patients (19%) were already classified as having pre-existing CVD. Therefore 36 of 78 (46%) patients had CV risk calculated. The median CV risk for the whole sample was 6.5% (0.1-37.7). Thirty three patients (42%) had a low to medium calculated CV risk of <20% and 3 patients (4%) had a high CV risk of ≥20%. All patients with a high calculated CV risk had 1 or more other factors such as high recorded lipids or blood glucose, smoking or family history of premature CVD. Fifteen patients (45%) with a low to medium CV risk had one or more isolated risk factor (smoking, family history of premature CVD or high recorded TC). Of

the 3 patients with a high calculated CV risk, 1 was on ABC and 2 were on an NTH regimen.

For patients on a TH (n=14), 7(50%), 6(43%) and 1(7%) had a CV risk of <10%, 10-20% and >20% respectively. This was 24(70%), 6(18%) and 4(12%) for patients on an NTH regimen; 8(53%), 5(33%) and 2(13%) for patients on ABC regimen; 18(60%), 7(20%) and 3(6.7%) for patients on a Non-ABC, non-DDI regimen.

4.4.2.3.4 Management of cardiovascular Risks

Units not following the JBS2 guidance (total number of patients 26) instituted corrective measures in nearly all patients with identified risk factors (5/5 smokers, 4/4 hypertensive, 5/6 dyslipidaemic and 4/4 overweight patients). Measures included counselling, referral to other specialties, medication, advice or change of HAART.

Tables 2-15 and 2-16 show the management of patients in units that followed JBS2 guidelines (total number of patients 52). In general, for patients at low risk of CVD (<20% over 10 years, n=19), some intervention was instituted for patients with identified risk. For patients with multiple risk factors, several interventions were instituted in the same patient, or for a single risk such as high lipids, the patient received dietary advice, drugs or was referred. The interventions were however not across the board for patients with identified risk factors.

In high risk patients (n=14: calculated CV risk n=3 and pre-existing CVD n=11), interventions were also instituted but not uniformly across all the patients and

crucially, only one patient in this category was started on aspirin. As was the case in low/moderate risk, patients with multiple or single risk factors received several interventions for each risk factor including being commenced on drugs/drug switches or being referred.

Table 4-15: Management of cardiovascular risk factors in patients with cardiovascular risk <20% (n=19) (Audit 2)

	Total	Action taken (In patients with risk)	Type of action	
			Life style advice	Drug treatment or Referral
Smokers	6 (43%)	4 (67%)	Smoking cessation (4)	
↑Glucose	5 (23%)	4 (80%)	Dietary advice (2) Weight reduction (1) Exercise (2) Alcohol moderation (3)	Referred to GP (2) HAART changed and statins started (2)
↑ Lipids	12 (63%)	8 (67%)	Dietary advice (5) Exercise (3) Alcohol moderation (4) Smoking cessation (2)	HAART changed and statins started (2) Referred to a GP (3)
↑ BP	3 (16%)	1 (33%)	Diet (1) Weight reduction/exercise (1)	Referred to GP (1)

*BP: blood pressure; *statins included pravastatin and simvastatin*

Table 4-16: Management of risk factors in patients with calculated cardiovascular risk >20% or Pre-existing cardiovascular disease (n=14*) (Audit 2)

	Total	Action taken (In patients with risk)	Type of action	
			Life style advice	Drug treatment or Referral
Smokers	8 (57%)	3 (38%)	Smoking cessation (3)	Stop smoking clinic referral (1)
↑Glucose	6 (43%)	3 (50%)	Dietary advice (3) Weight reduction (3) Alcohol reduction (2)	Referred to GP (3)/lipid specialist (1) Statins started (3) Aspirin and ACEI started (1)
↑ Lipids	9 (64%)	5 (56%)	Dietary advice (2) Smoking cessation Exercise (2) Weight reduction (2) Alcohol reduction (2)	Statin started (4) HAART changed (1) Referred to GP (2)/dietician(1)
↑BP	3 (21%)	3 (100)%	Diet/weight reduction/exercise/alcohol moderation (1)	Statin started(1) Referred to GP (1)

**calculated CV risk n=3, pre-existing CVD, n=11, ↑(Increased or raised); BP: blood pressure*

4.5 Discussion

ART and HIV infection place patients at particular risk of CVD. Large cohort studies have demonstrated increased rates of CVD in HIV patients (Friis-Moller *et al.* 2003(b)). Apart from the traditional CVD risk factors of sex, age, obesity and family history of premature heart disease which apply in this population just like in the general population, HIV patients have been shown to have higher rates of some modifiable risk factors. Smoking rates are higher in HIV patients than in the general population. The study by Ross *et al* showed a smoking rate of 38% in HIV patients compared to 10% in their non-infected counterparts (Ross *et al.* 2009). Smoking rates as high as 51-59% have been documented in the HIV infected population (Friis-Moller *et al.* 2003(b); Pere *et*

al. 2008). Hyperlipidaemia as an adverse effect of ART has been extensively reported. Development of hyperlipidaemia in patients on ART is worse in patients on thymidine NRTIs (particularly D4T) and is less pronounced in patients on non-thymidine NRTIs. PIs, particularly the older generation ones such as lopinavir (LPV), RTV, SQV, NFV and IDV as well as the newer PI fosamprenavir (FPV) have been shown to cause increase in TC and TGs, while the new PIs ATV and DRV showed smaller increases in these parameters. Additionally, EFV has an adverse lipid profile comparable to that caused by PIs such as RTV boosted LPV and FPV (Behrens *et al.* 1999; Heath *et al.* 2001; Jones *et al.* 2005a; Pirmohamed 2009). Development of insulin resistance leading to glucose intolerance or frank diabetes has also been seen in HIV patients on ART. Older generation PIs and thymidine NRTIs have been implicated, with the newer PI ATV and newer NRTIs TDF and ABC having little or no effect (Shlay *et al.* 2007; Tien *et al.* 2008).

We assessed via audits if the HIV physicians within clinics in the Greater Manchester area evaluated patients for risk factors of CVD and whether corrective measures were instituted for identified risk factors, the JBS2 guidelines being used as the standard of care. Whether clinics followed the JBS2 guidelines or used clinical judgement, on the whole most patients did have some clinical assessment (e.g. smoking history, BP check and medical history) or had biochemical evaluation of lipids and glucose which were documented in the case notes. However, the documentation was incomplete in some notes with no record found regarding some risk factors (For Audit1:

previous CVD 99.1%; family history of CVD 45.5%; smoking status16.8%; and for Audit 2: previous CVD: 17%; family history of CVD 40%; smoking status 19%). As such, a higher proportion of patients may be at unidentified higher risk of CVD and would not be appropriately treated.

Our cohort of patients had high smoking rates (up to 60%) comparable to those found by Pere *et al* and Friis-Moller *et al.* This was also higher than the smoking rates of 2004 in England (25%) and in the Northwest of England (28%), which was the second highest smoking rate by region in England as reported by the NHS National Statistics (NHS 2006).

High lipids were also fairly common (seen in 44.5% of patients in audit 1 and 61% in audit 2) but it is important to note that patients did not always fast overnight before having their blood taken for lipid levels though a large study found a less than 5% difference in lipid fraction concentrations between the fasted and nonfasted state (Mora *et al.* 2008). The TC was higher in patients from audit 2 compared to those in audit 1 (5.2 vs. 4.8mmol/l respectively). However, the TC level which was used in audit 2 was the highest that was recorded in the case notes during the year of interest. This could have resulted in more patients being classified as having high TC though they may have had normal levels during most of the year. The median TC, TGs and LDL-C in audit 1 were more representative as they were derived from several readings over the one year period. Compared to other studies, we found no difference in the levels of the various lipid fractions between patients on a TH and NTH; an ABC

and non-ABC/non-DDI or a DDI and non-ABC/non-DDI based regimens. We however demonstrated in audit 1 (but not in audit 2) significantly higher TC and LDL-C in patients on an NNRTI compared to patients on a PI (*P*=0.03 and 0.007 respectively). This might have been due to the fact that the NNRTI EFV was commonly used (30.3% of all the patients) and this has been shown to have an adverse lipid profile (Behrens *et al.* 1999; Heath *et al.* 2001; Jones *et al.* 2005a; Pirmohamed 2009). Additionally, there was increasing use of the lipid friendly PI ATV. Our results could have been influenced by the duration of the current regimens, previous treatment experience and type of PI drugs used. It is important to note also that patients on an NTH regimen in audit 1 were likely to have been on their current drugs for a longer duration (P=0.04), more triple class experienced (but this did not reach statistical significance at *P*=0.07), to have been exposed to PIs (*P*=0.009) and to have been on all ART for a longer duration than patients on a TH based regimen (but this was not statistically significant), reflecting the fact that patients on an NTH regimen may have undergone drug switches from less lipid or CVD friendly drugs, whose effects could have not reversed yet, thus explaining the lack of the expected favourable lipid profile in this group.

The majority of our patients were at low calculated risk of CVD over 10 years. However a large proportion of these had either single or multiple risk factors for CVD namely smoking, hypertension, and family history of CVD or dyslipidaemia (43.5% in Audit 1 and 36% in Audit 2). We did find quite a good proportion of patients who had either a high calculated risk of developing CVD

within 10 years or had pre-existing CVD (15% in Audit 1 and 23% in Audit 2) though it should be noted that patients with a history of hypertension and dyslipidaemia were included within the group classified as having pre-existing CVD in Audit 2 (due to the design of the data collection form). As such the rate of patients at high risk of CVD may have been overestimated in this audit. Any planned re-auditing should classify hypertension and dyslipidaemia as risk factors to allow appropriate calculation of CV risk (as was the case in Audit 1), which would in turn prevent the risk of overestimation of numbers of patient in the high risk category. TH drugs particularly D4T have been reported to cause hyperlipidaemia as reported, but large cohort studies have shown no excess risk to developing MIs with the use of AZT and D4T (Sabin *et al.* 2008). We found similar CV risk percent categories between patients on a TH compared to an NTH regimen.

There is conflicting information regarding ABC and its effect on myocardial infarction (MI). Some studies show increase in risk of MI (Sabin *et al.* 2008; Durand M 2009) while others show no increased risk (Bedimo R 2009). ABC has been thought to have a favourable effect on lipid profiles (Moyle *et al.* 2006), though a recent study by Hill *et al* has shown ABC use (like AZT and D4T use) to cause elevations in TC, TGs and LDL-C compared to TDF (Hill *et al.* 2009). These reports were not available when the audits were done but a re-analysis of the data reveals no difference in the lipid fractions between patients on ABC and non-ABC regimens. Other parameters such as BP or blood glucose were similarly not different. However, in Audit 1, patients on

ABC were twice as likely to have a moderate CV risk (10-20%) than those on a non-ABC/non-DDI regimen (31% vs. 15.8% respectively). This was also replicated in Audit 2, with 33% patients on ABC having a moderate risk while this was 20% in those on a non-ABC/non-DDI regimen. Additionally, twice as many patients in audit 2 on ABC (13%) had a high CV risk of >20% compared to those on a non-ABC/non-DDI regimen (6.7%). This would give the impression that patients on ABC in this cohort are at higher CV risk than those who are not on this drug which would be in agreement with studies described previously. However, it should be noted that patients on ABC in these audits had high smoking rates, were more likely to be heavily treatment experienced, have previous PI exposure and be on ART for longer duration than those not on the drug, which may have influenced the results. Additionally, the numbers are too small to make firm conclusions. GKS Trial ABC prescription biais

The number of patients on DDI regimens was too small for any meaningful conclusions regarding CVD risk to be made.

With respect to management of risk factors, the clinics instituted some corrective measures in most patients with specific risk factors. However, all patients at high risk of CVD require intensive risk factor management to correct identified risks through life style changes, drug treatment or referral for specialist care. The clinics did not institute these measures in all the patients identified to be at high risk, for instance, not all patients received life style advice and the use of antiplatelet therapy (such as aspirin) was very

inadequate with 3 of 15 patients in audit 1 and none of the 11 patients in audit 2 being on these drugs. Referrals for specialist treatment were at best patchy, which means patients would not be managed appropriately. A national British HIV Association (BHIVA) audit on CVD management in HIV patients involving 137 clinical centres in the UK which was carried out in 2006 had similar results with clinics mostly able to carry out routine lab tests for lipids and glucose and identifying CV risks appropriately but access to lipid specialists, smoking cessation services and exercise classes was poor (BHIVA 2006).

Obviously there are a number of limitations with these audits. The design of data collection forms were different in the two audits as such combining the data to get unified conclusions was difficult. Documentation by clinicians was not always complete as such missing data could have led to over or underestimation of the impact of a risk factor. The highest record in the case notes of some parameters such as BP or TC were recorded which may have led to overestimation of the CV risk percentage, taking an average of readings over the one year of interest could have given more accurate results. But on the whole, these audits were designed to provide an idea as to whether clinicians acted on abnormal results. The data collection forms were also meant to be easy to fill, which was a requirement by the participating consultants due to time constraints. As such the quality of the information collected may not have been the best to give an idea of the true level of CV risk in this population.

The results of these audits were presented in the joint meetings of the involved HIV clinics. In order to improve care with regard to CVD and other conditions in HIV patients that rely on diligent documentation, I was tasked to design proformas: the first one to be completed at start of ART and the second one annually thereafter and at each drug regimen change (appendix 5 and 6). These proformas have recently been adopted for use by the NMGH HIV unit. A re-audit of CVD risk management has been undertaken at the MRI to assess any change in managent practices following these audits.

4.6 Conclusion

Long-term survival of HIV patients on ARV drugs presents problems of diseases associated with an aging population such as cardiovascular disease. ART drugs and HIV infection are associated with risk factors of CVD. Identification and treatment of these risk factors is the modern day challenge of managing HIV patients. Units treating HIV patients could improve management of patients by:

1. Adopting a formal system to identify and manage CVD risk factors. Formal guidelines which are used in the general population and can equally apply to HIV patients include the JBS ones which we used as our standards in these audits; the World Health Organisation guidelines for Prevention of Cardiovascular Disease which take into account geographical diversity in the level of CVD risk (WHO 2007); and the National Cholesterol Education

Program (NCEP) guidelines for management of high cholesterol and CVD (Adult Treatment Panel III) (NCEP 2002). It is uncertain whether these guidelines that apply to an HIV negative population can be directly applied to HIV patients on ART, particularly as this population may be at higher risk for CVD than the general population because of the adverse effects of ART drugs on lipid, glucose and insulin sensitivity profiles as well as the chronic inflammatory state due to the virus itself. The European AIDS Clinical Society (EACS) guidelines on the prevention and management of metabolic diseases in HIV infected patients have been developed to address the issue of management of CVD risks in this particular group of people. These guidelines use the same targets in terms of lipids, glucose and blood pressure as the guidelines for the general population but go further to tackle other metabolic complications such as lactic acidosis, renal and bone disease (Lundgren *et al.* 2008b). The D:A:D group has also developed risk equations specific to HIV infected people taking into account the ART drugs the patient takes such as indinavir, lopinar and abacavir in addition to the parameters used in the Framingham equations namely age, sex, systolic blood pressure, smoking status, family history of CVD, diabetes, total cholesterol and HDL cholesterol. This model has been shown to estimate more accurately the CVD outcomes in HIV patients than the Framingham score (Friis-Moller *et al.* 2010). A calculator based on the D:A:D risk equations has been developed and will be more appropriate to use in HIV patients for the estimation of 10 year CVD risk (CHIP 2010).

2. Developing proformas to aid recording of CVD risk factors and action taken. These could be used at the start of ART and revisited at regular intervals such as annually or at change of treatment regimen and filed in patient case notes.

3. Establishing clear referral routes (Dietician, lipid or cardiology clinics, General Practitioner, lipodystrophy clinic) for further specialist management.

CHAPTER FIVE

MANAGEMENT OF DYSLIPIDAEMIA IN HIV PATIENTS: A SYSTEMATIC REVIEW OF DRUG-DRUG INTERACTIONS WITH CONCOMITANT USE OF PROTEASE INHIBITORS AND STATINS

5.1 BACKGROUND

Dyslipidaemia is a common adverse effect of treatment of HIV infection with highly active antiretroviral therapy (HAART) and the virus itself (Behrens *et al.* 1999; Carr *et al.* 1999). The dyslipidaemia associated with HAART is characterised by hypertriglyceridaemia, hypercholesterolaemia and decreased high-density lipoprotein cholesterol (HDL-C) (Martinez *et al.* 2004). Protease inhibitors (PIs) have most strongly been shown to cause dyslipidaemia (Behrens *et al.* 1999; Periard *et al.* 1999; Manfredi and Chiodo 2001). However, nucleoside reverse transcriptase inhibitors (NRTIs) have also been implicated in the pathogenesis of dyslipidaemia, particularly stavudine (D4T) (Jones *et al.* 2005a). Additionally, the non-nucleoside reverse transcriptase inhibitor (NNRTI) efavirenz has also been shown to have an adverse lipid profile (Behrens *et al.* 1999; Heath *et al.* 2001; Jones *et al.* 2005a; Pirmohamed 2009).

In the general population, dyslipidaemia is a risk factor for cardiovascular disease (CVD) (Stamler *et al.* 1986; Assmann *et al.* 1998; Austin *et al.* 1998; Wilson *et al.* 1998). Many guidelines are in use for the management of the disorder, which include lifestyle changes (such as sensible diet, regular exercise) and lipid lowering drugs (NCEP 2002; JBS2 2005). It is now understood that HAART associated dyslipidaemia in HIV patients needs to be managed along the same lines as in the general population. Indeed, guidelines have been developed for the management of this problem in HIV patients (Lundgren *et al.* 2008b). Management includes lifestyle changes as above,

drug switches to PI-sparing regimens or to lipid-friendly antiretroviral (ARV) drugs and drug treatment with lipid lowering drugs.

Drug treatment of hypercholesterolaemia includes 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) and/or ezetimibe. Fibric acid derivatives (fibrates) with or without Omega 3 acid ester drugs or nicotinic acid derivatives are used to treat hypertriglyceridaemia. Statins and fibrates can be combined in the case of mixed dyslipidaemias. The recommended statins for use in the setting of HAART dyslipidaemia are pravastatin and fluvastatin with caution being emphasised with the use of atorvastatin and rosuvastatin (Schambelan *et al.* 2002; Gazzard 2008; Lundgren *et al.* 2008b).

Drug treatment of HAART associated dyslipidaemia can be complicated by serious interactions between statins and PIs due to the nature of the metabolism of these drugs. The interactions can be at the level of absorption, distribution, metabolism or excretion. These interactions mostly occur at the level of the intestine and liver (Dresser *et al.* 2000). Statins and PIs are either substrates, inhibitors, inducers or a combination of these of the Cytochrome P450 (CYP450) mixed function oxidases which are a family of enzymes responsible for the majority of oxidative biotransformation of xenobiotics and endogenous compounds. The most important CYPP450 isoform is CYP3A4, which is responsible for the metabolism of about 50-60% of all drugs in current use. Other isoforms that have been demonstrated to be important for drug metabolism include CYP2D6, 2C9, 2C19, 1A2, 2E1, 2B6 and 2A6 (Dresser *et*

al. 2000; Fichtenbaum and Gerber 2002; Khoo *et al.* 2005). Other than the CYP450 system, several cell membrane transporters are involved in the disposition of endogenous organic compounds (such as bile salts, steroid conjugates, thyroid hormones) and numerous drugs including statins and PIs. The organic anion transporting polypeptides (OATPs) are expressed in many tissues and are involved in the sodium-dependent transport of a diverse range of compounds. OATP1B1 (or OATP-C, OATP2) is expressed in the liver at the basolateral (sinusoidal) membrane of hepatocytes and is involved in the hepatic clearance of statins. Bioavailability of statins is also affected by efflux transporters located in the apical membrane of intestinal enterocytes, proximal tubules of kidneys and canalicular membrane of hepatocytes. These include multidrug resistance-protein 1 (MDR1 or P-glycoprotein), multidrug resistance-associated protein (MRP2), breast cancer resistance protein (BCRP) and bile salt export pump (BSEP). These efflux transporters decrease drug absorption into the portal circulation and increase drug elimination into bile and urine. Alterations in the function of all these transporters can decrease statin elimination. Variations in genes encoding these transporters (such as *SLCO1B1* gene encoding OATP1B1) can also result in alterations in function of the transporters (Dresser *et al.* 2000; Shitara and Sugiyama 2006; Ho *et al.* 2007; Kivisto and Niemi 2007; Consortium 2008; Neuvonen *et al.* 2008). PIs have been shown to be substrates and inhibitors of these transporters as such interactions with statins could occur at this level (Williams and Feely 2002; Pal and Mitra 2006; Neuvonen *et al.* 2008).

With regard to statins, simvastatin and lovastatin are lipophilic lactone prodrugs that undergo extensive first-pass metabolism by CYP3A4/5 and to a lesser extend CYP2C8 in the intestinal wall and liver to their active acid forms and several other active and inactive metabolites. Being lipophilic, they are also extensively protein bound (>95%). Their bioavailability is therefore low (≤5%) (Fichtenbaum and Gerber 2002; Williams and Feely 2002; Neuvonen *et al.* 2008). Atorvastatin is also metabolised by CYP3A4 though less extensively than simvastatin and has a bioavailability of 12%. It is also extensively protein bound (>98%). Fluvastatin is metabolised by CYP2C9 and to a lesser extent by CYP3A4 and CYP2D6. It has a better bioavailability (30%) and is even more extensively protein bound (>99%) (Fichtenbaum and Gerber 2002; Williams and Feely 2002; Neuvonen *et al.* 2008). Pravastatin and rosuvastatin are excreted largely unchanged into urine and bile. About 10% of rosuvastatin is metabolized, mainly by CYP2C9 while pravastatin is partially degraded in the stomach and partially metabolized by non-CYP enzymes (mostly glucuronidation). Being hydrophilic, pravastatin is less protein bound (50%) and has a bioavailability of 18% (Yee and Fong 1998; Fichtenbaum and Gerber 2002; Williams and Feely 2002; Martin *et al.* 2003; Consortium 2008; Frishman and Horn 2008; Neuvonen *et al.* 2008).

HIV PIs (APV, DRV, FPV, IDV, NFV, RTV, LPV, SQV and tipranavir (TPV)) are both substrates and potent inhibitors of CYP3A4, with RTV being the most potent inhibitor followed by IDV, NFV, APV and SQV in decreasing order of potency (Inaba *et al.* 1997; Fichtenbaum and Gerber 2002; Granfors *et al.*

2006; Vourvahis and Kashuba 2007; Back *et al.* 2008; Frishman and Horn 2008). RTV and NFV also induce the CYP2C9, 2C19, 1A2 and 2E1 isoforms as well as some of the enzymes involved in conjugation (Fichtenbaum and Gerber 2002). Additionally, PIs may interact with efflux drug transporters in the intestine and liver. RTV and SQV have been shown to be substrates and inhibitors of P-glycoprotein and MRP2. Interactions with other substrates of these drug transporters, e.g. statins, would therefore be possible (Gutmann *et al.* 1999; Kis *et al.* 2009).

With increasing polypharmacy in HIV patients and the metabolic effects of HAART, drug interactions are inevitable. Concomitant use of statins for HAART-associated dyslipidaemia and boosted or unboosted PI regimens can result in increased oral bioavailability of the parent statin drug and/or its active metabolites leading to toxicity or can lead to reduced statin levels resulting in attenuation of the lipid lowering effect. Toxicity resulting from increased exposure to statins includes gastrointestinal symptoms such as nausea and vomiting, elevated liver function enzymes (LFTs) and benign myalgias which occur in 0.5-2.5% of patients (Williams and Feely 2002), although these are mostly mild (Baldini *et al.* 2000; Moyle *et al.* 2001; Doser *et al.* 2002; Palacios *et al.* 2002; Aberg *et al.* 2005). However, significant interactions leading to clinically significant conditions have been reported. These include myopathy (characterised by a 10-fold elevation in creatine kinase with muscle pain or weakness) in up to 0.3% of patients which can rarely progress to rhabdomyolysis, a life-threatening condition which is characterised by massive

muscle necrosis, hyperkalaemia and severe myoglobinuria, which may precipitate oliguric renal failure and can result in death (Williams and Feely 2002). Most cases of these have involved the concomitant use of simvastatin and PIs (Aboulafia and Johnston 2000; Hare *et al.* 2002; Schmidt *et al.* 2007; Mikhail *et al.* 2009). Indeed this led to the withdrawal of cerivastatin from clinical use (Charatan 2001). Of the currently available statins, simvastatin and lovastatin pose the most concern in PI-treated patients because of their extensive metabolism by the CYP3A4 isoform, and are therefore contraindicated in PI-treated patients. Lower doses and caution are emphasized with use of atorvastatin. Pravastatin and fluvastatin do not utilise CYP3A4 for their metabolism and are therefore the statins of choice for the treatment of dyslipidaemia in PI-treated patients. Starting at low doses is recommended for atorvastatin and rosuvastatin (Baldini *et al.* 2000; Fichtenbaum and Gerber 2002; Schambelan *et al.* 2002; Martinez *et al.* 2004; Gazzard 2008; Lundgren *et al.* 2008b)

5.2 OBJECTIVES

The aim of this systematic review was to look at the evidence in the literature of the pharmacokinetic interactions between statins and protease inhibitors. The clinical significance of such interactions was analysed. A previous systematic review undertaken in 2007 reviewed the available evidence concerning the treatment of dyslipidaemias in the setting of HIV infection and HAART, with regard particularly to the effects of interventions on lipid levels as well as on efficacy and safety (McGoldrick and Leen 2007). The current review

summarises the interactions between the two groups of drugs and relates these to the nature, severity and frequency of adverse effects.

It was anticipated that there would be a scarcity of data on this topic. As such, randomised controlled studies, retrospective/prospective cohort and/or case-control studies were all included in the review.

The outcome measures included:

Drug levels of PIs and statins (when concomitantly used) in HIV positive and negative patients.

The nature, frequency and severity of the adverse effects encountered.

5.3 METHODS

The review included all studies that recruited HIV positive patients that were: a) established on a PI-based regimen and started on statins, or b) established on the statins and then commenced on a PI-based regimen. Studies in HIV negative people administered a statin and a PI to study the pharmacokinetics; safety and tolerability of concomitant use of these drugs were also included. Table 5-1 shows the inclusion and exclusion criteria used to identify relevant records.

Table 5-1: Inclusion and Exclusion Criteria

Inclusion criteria:
a. Studies in the English language
b. Studies looking at pharmacokinetic interactions between statins and PIs
c. Results show or list pharmacokinetic effects and clinical side effects encountered
d. Studies that report adverse effects associated with combining statins and PIs
e. Studies published after 1996
Exclusion Criteria:
a. Studies that do not address the pharmacokinetic and/or adverse effects of concomitant use of statins and protease inhibitors.
b. Studies not relevant to purpose of systematic review
c. Studies published in a language other than English

5.3.1 Search Strategy

Several databases were searched and the search terms used were as follows:

- PubMed: (HIV OR AIDS) AND (HAART OR Antiretroviral* OR Protease inhibit*) AND (Statin* OR HMG-CoA Reductase Inhibit*);
- SCOPUS: ALL("HIV" OR "AIDS") AND (HAART OR Protease inhibitors) AND (Dyslipidemia OR Hyperlipidemia) AND (Statins OR HMG-CoA Reductase Inhibitor) AND (Interaction OR Pharmacokinetics) AND (Adverse effects);
- ISI Web of Knowledge: (Statin*) AND (Protease inhibit*) AND (interact*) refined by (HIV);
- The Cochrane Central Register of Controlled Trials abstract databases: (HIV OR AIDS) AND (Dyslipid* OR Hypercholesterol* OR Hyperlipid* OR Hypertriglycerid*) AND (Protease inhibit* OR PI*) AND (Statin* OR

HMG-CoA Reductase Inhibit*) AND (Interact* OR pharmacokinetic* OR PK) and

- The grey literature (SIGLE) database (www.opensigle.inist.fr) for unpublished data: ((Dyslipid* OR Hyperlipid*) AND (Statin* OR HMG-CoA Reductase Inhibit*) AND (protease inhibit*) AND (interact* OR "adverse effect" OR pharmacokinetic*)).

Additionally, reference lists of all primary studies identified were also scrutinised for further papers of potential interest. The titles of studies and their corresponding abstracts were combined as well as the lists of references and any duplicates removed. The titles and abstracts were then reviewed and irrelevant reports removed. The full text of the remaining studies were then retrieved and reviewed to see if they fulfilled the inclusion criteria. Only those studies that reported pharmacokinetics, drug levels or listed adverse effects and their frequencies when statins and PIs were concomitantly used were included. For all the databases, the search was limited to studies published after 1996, when protease inhibitors came into clinical use.

5.3.2 Data Extraction

Data were extracted in accordance with the methods set out in the Cochrane Handbook for Systematic Reviews of Interventions. A data collection form was prepared and used to record data which included study design, objectives, study period, study duration, sample size, HIV status of subjects, mean or

median age of subjects, the drugs being investigated, pharmacokinetic (PK) changes (maximum concentration, Cmax; minimum concentration, Cmin; and area under the concentration curve, AUC) or drug concentration changes (increase or decrease in plasma drug level), adverse effects and their frequencies. Outcome data was collected in the format that it was reported or transformed as necessary at a later stage.

5.3.3 Statistics

Descriptive analysis was used to summarise the nature of interactions between statins and protease inhibitors. The proportion of patients with adverse effects and the nature as well as the severity grading of those side effects was summarised.

5.4 RESULTS

Figure 5.1 summarises the systematic review process. Thirteen studies were included in the analysis. Ten of these studies reported PK percentage or fold changes of the drugs which included area under the concentration curve (AUC), maximum concentration (Cmax), minimum concentration (Cmin) or change in drug concentration. These studies also reported the adverse effects encountered as well as their frequency. Three studies reported only on the safety of concomitant use of statins and PIs, listing the nature and frequency of these adverse effects. The PK studies involved sequential administration of the study drugs. In some studies, the statin was initiated in patients already

established on a PI-based ART. PK studies were mostly conducted in HIV negative participants, the studies were of shorter duration and participants were younger. In contrast, safety and efficacy studies were mostly conducted in HIV positive participants who were older and the study durations were longer.

Tables 5-2 to 5-4 show the summaries of the studies included in this review. Table 5-2 shows the study details including the main author, study design and objectives, the year the study was conducted or the study period, the sample size, the subjects' HIV status and their median or mean age. Table 5-3 shows the drug concentration changes encountered with combinations of the PI and statin drugs, specifically summarising the drug PK changes (AUC, Cmax, and Cmin) or plasma drug level change (the drug whose PK was under study is indicated in bold). Table 5-4 summarises the nature of the adverse effects encountered including their frequencies, total events in each study as well as any discontinuations and their nature.

Figure 5-1: Systematic literature review process

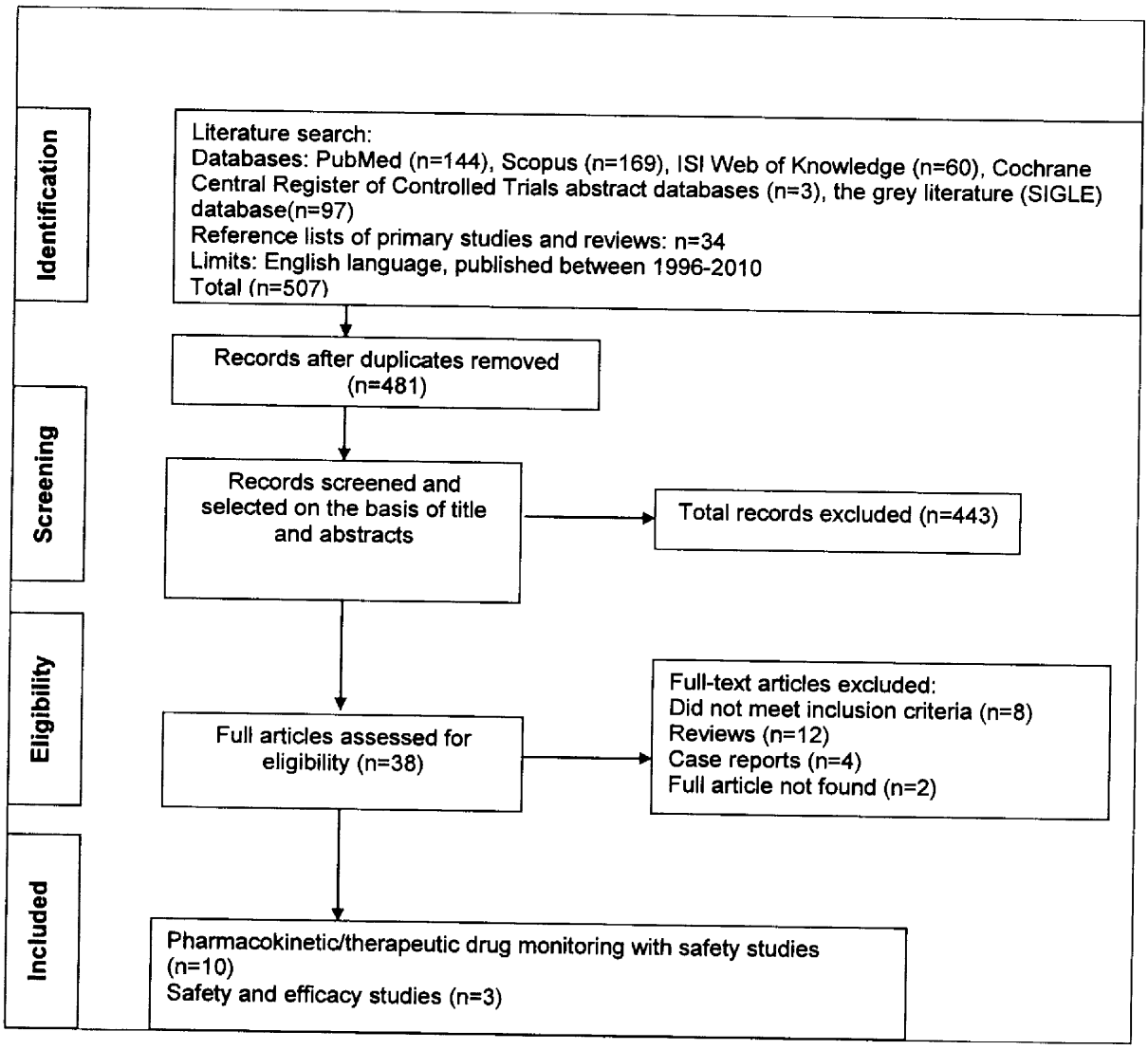


Table 5-2: Study Summaries: Study details

Study	Study Design	Study objectives	Study period/year	Study duration	Sample size	Subjects	Age (Median/Mean)
Fichtenbaum *et al* 2002	Randomised open label	PK drug interaction	July-Oct 1999	18 days	67	HIV negative	32
Kiser *et al* 2008	open-label, single arm	PK drug interaction	not specified	24 days	15	HIV negative	27
Aberg *et al* 2006	open label, multiple dose	PK drug interaction	Aug-Dec 2001	16 days	14	HIV negative	29
		Safety					
Hsyu *et al* 2001	open label, multiple dose	PK drug interaction	not specified	28 days	32	HIV negative	(Atorva)31.5
		Safety					(Simva)38.3
Busti *et al* 2008	prospective, single dose	PK drug interaction	not specified	28 days	6	HIV negative	28
	cross over	Safety					
van der Lee *et al* . 2007	open label, multiple dose	PK drug interaction	May2004-July2005	12 weeks	22	HIV positive	48
		Efficacy and safety				PI-based ART	
Benesic *et al.* 2004	Prospective, open label	therapeutic drug monitoring	not specified	72 weeks	8	HIV positive	40.4
		Efficacy and safety					

Table 5-2: Study Summaries: Study details continued

Study	Study Design	Study objectives	Study period/year	Study duration	Sample size	Subjects	Age (Median/Mean)
Aslangul *et al* 2010	Randomised, open label	Efficacy and safety	Oct2005-Jan2005	45 days	83	HIV positive	47
		therapeutic drug monitoring					
Moyle *et al* 2002	Randomised, open label	PI drug exposure	not specified	24 weeks	14	HIV positive	not specified
Calza *et al* 2003	Randomised, open label,	Efficacy and safety	not specified	12 months	37	HIV positive	44.2
	Prospective						
Calza *et al* 2005	prospective, pilot	Efficacy and safety	1 June-31 July 2004	24 weeks	16	HIV positive	44.2
Calza *et al* 2008	open-label, randomized, prospective	Efficacy and safety	Jan-Dec 2006	12 months	94	HIV positive	Rosuva (36.3)
							Prava (37.4)
							Atorva (38.1)
Hoody *et al* 2007	open label, 3-phase	PK drug interaction	not specified	24 days	15	HIV negative	27
		Efficacy and safety					

Table 5-3: Study Summaries: Drug concentration changes

Study	Study Drugs	PK Changes						Plasma Drug level change
		AUC % or Fold change (nX)	90% CI	P value	Cmax % or fold change (nX) or (Cmin)	90% CI	P value	
Fichtenbaum *et al* 2002	**Prava** (vs. RTV/SQVsgc) n=14	↓50%		0.005	↓1.6X		0.09	
	Simva (vs. RTV/SQVsgc) n=14	↑3059%		<0.001	↑30X		<0.001	
	Atorva (vs. RTV/SQVsgc) n=14	↑79%		<0.001	↑2.5X		<0.001	
	NFV (vs. Prava) n=14	↔		0.58	↔		0.76	
Kiser *et al* 2008*	**Rosuva** (vs. LPV/RTV)	↑2.1X	1.7-2.6X	<0.0001	↑4.7X	3.4-6.4	0.0001	
	LPV (vs. Rosuva)	↔1.1X	1.0-1.2	0.054	↔1.1X	0.9-1.2	0.36	
	RTV (vs. Rosuva)	↔1.1X	0.9-1.3	0.27	↔1.1X	0.9-1.3	0.52	
Aberg *et al* 2006*	**Prava** (vs. NFV)	↓46.5%	0.46-0.65	0.0004	↓40.1%	0.4-0.7	0.0023	
Hsyu *et al* 2001	**Atorva** (vs NFV)	↑74%			↑122%			
	Simva (vs NFV)	↑507%			↑517%			
	NFV (vs. atorva or Simva)	↔			↔			
Busti *et al** 2008	**Rosuva** (vs.ATV/RTV)	↑213%		0.002	↑600%		0.001	
	Rosuva (vs. FPV/RTV)+	↔		0.86	↔		0.76	
van der Lee *et al.* 2007	**Rosuva** (vs. LPV/RTV)				(Cmin)↑1.6x		not given	
	LPV (vs.rosuva)				(Cmin)↔		0.44	
	RTV (vs.rosuva)				(Cmin)↔		0.26	
Benesic *et al.* 2004	**IDV** (vs. Fluva)							↔
	IDV (vs. Prava)							↔

**Studies looked at AUC time concentration curve(AUC_{0-t}); +the Rosuva lactone metabolite AUC_{0-24} was increased; LFTs=liver function tests, CPK=creatine phosphokinase; GIT=gastrointestinal symptoms; prava=pravastatin; atorva=atorvastatin; rosuva=rosuvastatin; simva=simvastatin; RTV=ritonavir; ATV=atazanavir; LPV=lopinavir; APV=amprenavir; FPV=fosamprenavir; NFV=nelfinavir; SQV=saquinavir; IDV=indinavir; ↑=raised or increased; ↓=reduced or decreased; ↔=no significant change; CI=confidence interval*

Table 5-3: Study Summaries: Drug concentration changes continued

Study	Study Drugs	PK Changes						Plasma Drug level change
		AUC % or Fold change-nX	90% CI	P value	Cmax % or fold change nX or (Cmin)	90% CI	P value	
Aslangul *et al* 2010	**ATV/APV/LPV** (vs. prava/Rosuva)				(Cmin)↔			
	Prava/Rosuva (vs. ATV/APV/LPV)				(Cmin)↔			
Moyle *et al* 2002	**IDV** (vs. prava) n=5							↔
	RTV (vs. prava) n=8							↔
	SQV (vs. prava) n=6							↔
Calza *et al* 2003	Prava + PIs[1] n=19	adverse effects only reported, see adverse section of table						
	Fluva + PIs[1] n= 18							
Calza *et al* 2005	Rosuva + PIs[2] n=16	adverse effects only reported, see adverse section of table						
Calza *et al* 2008	**Rosuva** + PIs[3] n=26	adverse effects only reported, see adverse section of table						
	Prava + PIs[3] n=31							
	Atorva + PIs[3] n=28							
Hoody *et al* 2007	**Rosuva** (vs. LPV/RTV)	↑ 2.1X	1.7-2.6X	<0.0001	↑ 4.7X	3.4-6.4X	<0.0001	
	LPV/RTV (vs. Rosuva)	↔			↔			

[1]Total participants were 106 (69 took fibrates), PIs included RTV, IDV, NFV and double PI; [2]PIs used included: LPV-RTV/NFV/SQV-RTV/IDV-RTV; [3]PIs used included: LPV/RTV [44], FPV/RTV[19], ATV/RTV[11], NFV[11], SQV/RTV[8], TPV/RTV[1]; prava=pravastatin; atorva=atorvastatin; rosuva=rosuvastatin; simva=simvastatin; RTV=ritonavir; ATV=atazanavir; LPV=lopinavir; APV=amprenavir; FPV=fosamprenavir; NFV=nelfinavir; SQV=saquinavir; IDV=indinavir; ↑=raised or increased; ↓reduced or decreased; ↔ no significant change

Table 5-4: Study summaries: Adverse effect summary

Study	Study Drugs	Adverse Effects				Discontinuations
		Severity	Total events n (%)	Type	Total events for study	
Fichtenbaum *et al* 2002	Prava + RTV/SQVsgc n=14	≥grade 2 Lab	2 (14)	↑CPK/LFTs	25% (14% clinical, 11% lab)	11 (protocol defined toxicities)^
		≥grade 2 clinical	1 (7)	GIT		
	Atorva + RTV/SQVsgc n=14	≥grade 2 Lab	1 (7)	↑TGs		
		≥grade 2 clinical	1 (7)	Other		
	Simva + RTV/SQVsgc n=14	≥grade 2 Lab	2 (14)	↑TGs		
		≥grade 2 clinical	4 (29)	GIT/other		
	NFV + Prava n=14	≥grade 2 Lab	1 (7)	↑TGs		
		≥grade 2 clinical	2 (14)	GIT		
Kiser *et al* 2008	Rosuva alone n=20	mild-clinical	5 events	myalgia/other	cannot calculate	2 (severe neutropenia, rash)
		mild/moderate-Lab	6 events	↑CPK/↑bilirubin/↓Hb		
	LPV/RTV alone n=19	mild/moderate – clinical	25 events	GIT(18)/myalgia(1)/liver*/other		
		mild/moderate-Lab	11 events	↑bilirubin/↑CPK/neutropenia/other		
		severe-Lab	1 event	Neutropenia		
	Rosuva + LPV/RTV n=17	mild/moderate – clinical	28 events	GIT(15), myalgia(3)/liver*/other		
		mild/moderate-Lab	13 events	↑CPK(2)/↑LFTs(9)/other		
		Life threatening-Lab	1 event	↑CPK		
	all 3 phases	Lab - severity not reported	"commonly reported"	↑total bilirubin		
Aberg *et al* 2006	Prava + NFV n=18	grade 2 –clinical	3(21]	GIT/skin eruption	35%	4 (protocol defined toxicities)[$]
		grade 2/3 –Lab	2(14)	↑ALT and AST/↑LDL		

*↑=raised or increased; *liver toxicity with nausea, vomiting, anorexia and jaundice; ^GIT symptoms during PI only phase (10), no measurable drug (1); [$]grade 2 ↑bilirubin, grade2 rash, grade2 ↑CPK, noncompliance; prava=pravastatin; atorva=atorvastatin; rosuva=rosuvastatin; simva=simvastatin; RTV=ritonavir; LPV=lopinavir; APV=amprenavir; NFV=nelfinavir; SQV=saquinavir; LFTs=liver function tests; CPK=creatine phosphokinase; GIT=gastrointestinal symptoms; ALT=alanine aminotransferase; AST=aspartate aminotransferase; Hb=haemoglobin; TG=triglycerides;*

Table 5-4: Study summaries: Adverse effect summary continued

Study	Study Drugs	Adverse Effects				Discontinuations
		Severity	Total events n (%)	Type	Total events for study	
Hsyu *et al* 2001	NFV + atorva or Simva n=32	severity not reported	17 (53)	GIT	59%	1(rash)
		grade 3	1 (3)	Rash		
		grade 3	1 (3)	migraine headache		
Busti *et al* 2008	Rosuva + ATV/RTV then FPV/RTV n=6	mild -clinical	67%	jaundice (on ATV/RTV phase)	67%	None
van der Lee *et al* 2007	Rosuva + LPV/RTV n=22	mild -clinical	9 (41)	GIT/myalgia or cramp/other	55%	None
		mild/moderate -Lab	3 (14)	↑CPK		
Benesic *et al* 2004	IDV + Fluva or Prava n=25			no LFT elevation		None
				no serious side effects		
Aslangul *et al* 2010	ATV/APV/LPV + prava/Rosuva n=83			no renal or hepatic events		None
				no muscular events		
Moyle *et al* 2002	Prava + IDV/RTV/SQV n=19			no severe/serious adverse events		5 (personal reasons)
				no change in lab parameters		

prava=pravastatin; atorva=atorvastatin; rosuva=rosuvastatin; simva=simvastatin; Fluva=fluvastatin; RTV=ritonavir; ATV=atazanavir; LPV=lopinavir; APV=amprenavir; FPV=fosamprenavir; NFV=nelfinavir; SQV=saquinavir; IDV=indinavir; LFTs=liver function tests; CPK=creatine phosphokinase; GIT=gastrointestinal symptoms; ALT=alanine aminotransferase; AST=aspartate aminotransferase; Hb=haemoglobin; TG=triglycerides;

Table 5-4: Study summaries: Adverse effect summary continued

Study	Study Drugs	Adverse Effects				Discontinuations
		Severity	Total events n (%)	Type	Total events for study	
Calza et al 2003	Prava/Fluva + PIs[1] n= 37	mild - clinical	2 (5.4)	GIT, no myalgia/myositis	5.4%	none
				no ↑CPK/ALT/AST		
Calza *et al* 2005	Rosuva + PIs[2] n=16	mild - clinical	2 (12)	GIT	12%	none
				no myalgia/myositis		
				no lab events		
Calza *et al* 2008	Rosuva + PIs[3] n=26	mild-clinical	8 (41)	GIT, no myalgia/myositis	14.1%	9[4]
				no ↑CPK/ALT		
	Prava + PI[3] n=31	mild-clinical	5 (16)	GIT, no myalgia/myositis		
				no ↑CPK/ALT		
	Atorva + PIs[3]n=28	mild-clinical	4 (14)	GIT, no myalgia/myositis		
				no ↑CPK/ALT		
Hoody *et al* 2007	Rosuva alone phase	lab-none specific to phase			cannot calculate	1 (neutropaenia)
	LPV/RTV alone phase	grade 4 – lab	1 (5)	Neutropenia		
	Rosuva + LPV/RTV phase	grade 1 – lab	1 (5)	↑LFT		
		grade 4 - lab	1 (5)	↑CPK		
	all 3 phases (n=20 for all phases)	Unspecified – clinical	"common"	GIT		
		Unspecified – lab	"most common"	↑bilirubin		
			5 (25)	↑CPK(3)/↓Hb(2)		

[1]Pis included RTV, IDV, NFV and double PI; [2]PIs used included: LPV-RTV/NFV/SQV-RTV/IDV-RTV;[3]PIs used included: LPV/RTV [44], FPV/RTV[19], ATV/RTV[11], NFV[11], SQV/RTV[8], TPV/RTV[1]; [4]low adherence, non-serious adverse events, loss to follow-up, persistent GIT symptoms; LFTs=liver function tests; CPK=creatine phosphokinase; GIT=gastrointestinal symptoms; ALT=alanine aminotransferase; AST=aspartate aminotransferase; Hb=haemoglobin; RTV=ritonavir; ATV=atazanavir; LPV=lopinavir; APV=amprenavir; FPV=fosamprenavir; NFV=nelfinavir; SQV=saquinavir; IDV=indinavir;TPV=tipranavir; prava=pravastatin; atorva=atorvastatin; rosuva=rosuvastatin; simva=simvastatin; ↑=raised or increased; ↓reduced or decreased; ↔=no significant change

Three studies evaluated the PK of pravastatin, the AUC and Cmax were reduced significantly by 40-50% by RTV boosted SQV (Fichtenbaum *et al.* 2002) or NFV (Aberg *et al.* 2006). The third study evaluated the Cmin of pravastatin, and this remained unchanged when used with ATV, APV or lopinavir (LPV) (Fichtenbaum *et al.* 2002; Aberg *et al.* 2006; Aslangul *et al.* 2010). Five studies evaluated the PK of rosuvastatin 3 of these studies showed that there was more than 200% and nearly 600% increase in the AUC and Cmax, respectively, when co-administered with RTV-boosted LPV or ATV while RTV-boosted FPV did not affect these parameters (Hoody 2007; Busti *et al.* 2008; Kiser *et al.* 2008). Similar findings have been demonstrated in a recent study co-administering rosuvastatin with darunavir (DRV) which resulted in increases in the Cmax and AUC of rosuvastatin of 140% and 48% respectively (Samineni *et al.* 2011). Two studies looked at Cmin of rosuvastatin. One of these studies demonstrated an increase of rosuvastatin Cmin of 1.6 times with co-administration with RTV-boosted LPV (van der Lee *et al.* 2007) while the other showed no significant change in this parameter with ATV, APV or LPV (Fichtenbaum *et al.* 2002; Aberg *et al.* 2006; Aslangul *et al.* 2010). Two studies that evaluated simvastatin PK levels demonstrated profound elevations in AUC and Cmax (500-3059% and greater than 500% increase, respectively) with co-administration with RTV/SQV or NFV. Atorvastatin levels were evaluated in the same 2 studies which showed a greater than 70% increase in AUC and 122% increase in Cmax with co-administration of RTV/SQV or NFV (Hsyu *et al.* 2001; Fichtenbaum *et al.* 2002). This is in agreement with data presented at a conference by Carr et al

who demonstrated moderate increases in the Cmax and AUC of atorvastatin (4.7 and 5.9 fold, $P<0.02$) when coadministered with RTV boosted LPV (previously known as ABT138) (Carr 2000) and the study by Hoetelmans *et al* which showed increases in atorvastatin AUC, when atorvastatin 10mg was administered with RTV boosted DRV, to levels similar to atorvastatin 40mg dose (Hoetelmans 2004). No studies were found evaluating PK effects of co-administration of lovastatin and PIs.

Seven of the 13 studies evaluated various PI drug levels (AUC, Cmax, Cmin, plasma drug levels) with co-administration with all the statins currently in clinical use and all found no significant change in the PI levels (Hsyu *et al.* 2001; Fichtenbaum *et al.* 2002; Benesic *et al.* 2004; Hoody 2007; van der Lee *et al.* 2007; Kiser *et al.* 2008; Aslangul *et al.* 2010).

Of the adverse effects encountered in the 13 studies included in this review, gastrointestinal (GIT) adverse effects (AEs) were the commonest, being reported in 9 of the 12 studies and included mostly nausea, vomiting, diarrhoea and abdominal pain. Myalgia was reported in 2 studies and this was less frequent than the GIT symptoms, being graded as mild to moderate. No episodes of rhabdomyolysis were reported. Various other clinical adverse effects were reported but were less common and included rashes, headache and were mostly mild or moderate in severity. Kiser et al reported a case each of liver toxicity with nausea, vomiting, anorexia and jaundice in the LPV/RTV alone and rosuvastatin with LPV/RTV arms which were mild and did not

necessitate withdrawal from the trial (Kiser *et al.* 2008). Two thirds of patients in the study by Busti *et al* developed mild jaundice, which is expected with ATV use (Busti *et al.* 2008). Laboratory adverse events were also commonly encountered, the commonest being raised creatinine phosphokinase (CPK), which was reported as mostly mild. However, 6 participants from 3 studies had CPK values that were high and described as life threatening in some cases but discontinuations did not mostly occur because of this. One participant had CPK levels of 17 times the upper limit of normal (ULN, 195u/l) (Kiser *et al.* 2008); one had levels 4 times the ULN (ULN 120u/l) and 3 reported to have had levels of 363-676u/l (van der Lee *et al.* 2007); and one had 16.9 times ULN (ULN, 195u/l) (Hoody 2007). One study reported grade 2 or higher rise in CPK though the level was not defined (Fichtenbaum *et al.* 2002). However, 1 patient in the study by Aberg *et al* discontinued from participation due to grade 2 rise in CPK (levels not defined) and was not included in the PK analysis (Aberg *et al.* 2006). The next commonly encountered laboratory adverse effect was elevated liver enzymes which were mostly mild to moderate in severity and did not result in discontinuations. Rises in CPK and LFTs occurred with all statins and were not confined to simvastatin use only. Neutropaenia was reported in 2 participants in 2 studies and in both cases resulted in discontinuation from study participation (Hoody 2007; Kiser *et al.* 2008).

5.5 DISCUSSION

Concomitant use of PIs and statins in HIV positive patients with dyslipidaemia is common and will increase as an aging HIV population develops

cardiovascular problems requiring pharmacotherapy. Interactions between the PIs and statins are to be expected. As previously stated, guidelines recommend that certain statins (such as pravastatin, rosuvastatin and fluvastatin) can be used relatively safely in PI induced dyslipidaemia. Caution is to be exercised with the use of others statins (atorvastatin) while others are to be avoided altogether (simvastatin and lovastatin). These recommendations are mostly based on the knowledge of the drugs' metabolism by the CYP450 enzyme system, with the aim of minimising the chances of adverse interactions.

The results of some of studies in this review clearly demonstrate that differences in interactions between the individual statins and PIs are a result of the differences in the metabolism pathways used by the drugs as well as the extent to which a drug is metabolised by a particular enzyme, such that the levels of a statin that is extensively metabolised by the main CYP isoforms (3A4) rise to dangerous proportions when combined with PIs, which are potent inhibitors of CYP3A4. This phenomenon is clearly demonstrated in the study by Hsyu et al (Hsyu *et al.* 2001), where nelfinavir use with atorvastatin resulted in an increase in the AUC and Cmax of atorvastatin by 74% and 122%, respectively. This is consistent with the fact that atorvastatin is metabolised by CYP3A4 to a lesser extent. However, in the same study, nelfinavir resulted in a massive increase in the AUC and Cmax of simvastatin (505% and 517% respectively) while Fitchtenbaum et al demonstrated a rise in AUC of 3059% and a 30 fold rise in Cmax of simvastatin. Both studies thus clearly

demonstrate that simvastatin metabolism will be affected greatly when used with potent inhibitors of CYP3A4.

Rosuvastatin and pravastatin, being largely excreted unchanged, are ideal statins to use concomitantly with PIs, with little or no clinically relevant interactions expected. However, PK studies have shown unexpected changes in these statins. The unexpected increased levels (Cmax and AUC) of rosuvastatin might be attributable to a change in bioavailability mediated by changes in drug transporters at the level of the intestine and liver. Rosuvastatin is a substrate for OATP1B1 and BCRP and these transporters play a role in the distribution and elimination of this drug (Hsiang *et al.* 1999; McTaggart 2003; Simonson *et al.* 2004; Zhang *et al.* 2006; Kiser *et al.* 2008). LPV and/or RTV may possibly be inhibiting rosuvastatin uptake by BCRP and OATP1B1 in the intestine and the liver (Kiser *et al.* 2008); indeed ritonavir has been shown to inhibit BCRP and OATP1B1 (Gupta *et al.* 2004; Hirano *et al.* 2006; Neuvonen *et al.* 2008). ATV and APV (the active moiety of FPV) have been shown in an *in vitro* study to be inhibitors of BCRP and ATV was the more potent inhibitor than APV (Weiss *et al.* 2007; Busti *et al.* 2008). CYP450 interactions are unlikely to play a major role in these unexpected findings because previous studies have shown no relevant drug-drug interactions with rosuvastatin involving CYP2C9, CYP2C19 or CYP3A4 (Cooper *et al.* 2002; Cooper *et al.* 2003a; Cooper *et al.* 2003b; van der Lee *et al.* 2007). The conflicting rosuvastatin Cmin results (increased in one study and unchanged in another) could be due to differences in methodology although where the Cmin

remained unchanged could reflect what is expected about this statin, i.e, there should be no significant interactions with PIs.

The three studies summarised in this review that evaluated the PK of pravastatin showed unexpected reduction in the AUC and Cmax in the presence of RTV boosted SQV or NFV while the Cmin remained unchanged in the presence of ATV, APV and LPV (Fichtenbaum *et al.* 2002; Aberg *et al.* 2006; Aslangul *et al.* 2010). This is in contrast with data from 2 studies presented at conferences which showed a 30% increase in Cmax and AUC of pravastatin with co-administration with RTV boosted LPV though this was not statistically significant (Carr 2000) and increases in AUC of 81% and Cmax of 63% when pravastatin was coadministered with RTV boosted DRV in healthy volunteers (Sekar 2007). These variable findings could be due to several factors. Non-CYP3A4 oxidation and glucuronidation are involved in the metabolism of pravastatin, and RTV is believed to be an inducer of glucuronidation, which could explain the reduced level of pravastatin (Kim *et al.* 1998; Wang *et al.* 2001; Williams and Feely 2002; Aberg *et al.* 2006; Pal and Mitra 2006). Additionally pravastatin is a substrate of the drug transporters OATP1B1 and P-glycoprotein which are involved in its bioavailability and elimination. PIs are also substrates and inhibitors of these drug transporters, as such, complex interactions at this level could explain the reduction or increase in plasma levels of pravastatin with concomitant use with some PIs (Kim *et al.* 1998; Wang *et al.* 2001; Williams and Feely 2002; Aberg *et al.* 2006; Pal and Mitra 2006). The reduction in levels of pravastatin may have

clinical implications in that it may result in reduction in lipid lowering efficacy. Indeed studies have shown an attenuation of lipid lowering effects of statins with smaller proportions of HIV patients on HAART reaching targeted reductions in the various lipid fractions compared to HIV negative individuals (Silverberg *et al.* 2009).

With regard to atorvastatin, the findings of moderately raised levels in the presence of PIs is consistent with the known PK profile of this statin, that is, it is metabolised less extensively by CYP3A4 as such its levels would not be increased to the same extent as, for example, simvastatin.

All the studies that evaluated the PK of PIs when co-administered with statins found no significant changes in AUC, Cmax and Cmin of the PIs (Hsyu *et al.* 2001; Fichtenbaum *et al.* 2002; Moyle *et al.* 2002; Benesic *et al.* 2004; Hoody 2007; van der Lee *et al.* 2007; Kiser *et al.* 2008; Aslangul *et al.* 2010). The lack of effect of statins on PI drug levels is consistent with what is known about statin metabolism, in that they are substrates rather than inhibitors or inducers of CYP450 enzymes (Fichtenbaum and Gerber 2002).

Clinical and laboratory adverse effects were commonly reported in the studies included in this review. The most commonly reported were GI effects such as nausea, vomiting, diarrhoea and abdominal pain. These were mostly mild to moderate in severity and did not result in discontinuation from the studies. It is important to note that GI AEs could be attributable to the expected adverse effects of the individual PIs and statins, rather than as a result of the combined

use as detailed in the package inserts of some of the drugs in this review (Bristol-Myers-Squibb 2007; Pfizer 2009; Abbott 2010; Bristol-Myers-Squibb 2010; GlaxoSmithKline 2010; Merck 2010; Merck(a) 2010). Muscle problems reported were mostly mild or moderate and did not progress to rhabdomyolysis. CPK increases were also mostly not clinically significant, and these were not exclusively reported with simvastatin use but rather with the rest of the statins. These findings are in agreement with the results of most of the efficacy and safety studies in which few AEs were reported with concomitant use of PIs and statins (Baldini *et al.* 2000; Moyle *et al.* 2001; Doser *et al.* 2002; Palacios *et al.* 2002; Aberg *et al.* 2005). However, a case of life threatening rise in CPK and 2 cases of liver toxicity reported in this review highlight the dangers posed by these drugs which although uncommon, could lead to serious consequences as has been reported by Aboulafia *et al*, Schmidt et al, Hare *et* al and Mikhail *et al* (Aboulafia and Johnston 2000; Hare *et al.* 2002; Schmidt *et al.* 2007; Mikhail *et al.* 2009).

The risk or likelihood of an adverse outcome in an individual on PIs and statins depends on other factors apart from the known metabolising pathways. These include individual variation in the intestinal and hepatic expression of CYP isoenzymes and efflux transporters, concomitant use of other drugs with inhibitory or inducing effects which would have additive or synergistic effects leading to marked changes in PK parameters of the affected substrate drug, use of alcohol (a substrate and inducer of some CYP isoenzymes) as well as genetic polymorphisms and racial differences in the genes coding for the efflux

transporters such as *SLCO1B1* gene (encoding OATP1B1) (Sweeney and Bromilow 2006; Ho *et al.* 2007; Kivisto and Niemi 2007; Schmidt *et al.* 2007; Neuvonen *et al.* 2008)

There are several limitations identified with regard to studies analysed in this review. Firstly, sample sizes were mostly small and the duration of the studies was short. Secondly, in most studies, there was lack of a randomized control group and lack of information on concomitant medication. Thirdly, diet, geographical and racial differences could influence drug handling by the intestines and liver; however, these were not reported in the studies. The different populations in the studies, each with their own population coefficient of variation with regards to the results would also affect the generalizability of the results. Most studies did not report on smoking status or intake of alcohol, both of which have effects on the CYP450 enzyme system (Sweeney and Bromilow 2006). Fourthly, the methodology, drug doses and dosing schedule as well as analytical methods were different in the studies. And lastly, data were extracted from the publications of the studies which are included in this review but the authors were not contacted for their primary data. These would affect the generalizability of the results and largely precluded the undertaking of a meta-analysis as there was the need to review data on concommittant use of a number of statins and PIs in current clinical use in HIV patients.

5.6 CONCLUSIONS

Polypharmacy in HIV patients is common due to the complex nature of the disease. Physicians caring for HIV patients would need to be vigilant when prescribing drugs in this population on complex polypharmacy because of the potential for harmful interactions. To avoid pharmacokinetic alterations, they would need to have information about the metabolizing enzymes and transporters involved in the pharmacokinetics of all manner of drugs used in this population. This information may not be readily available within the clinic setting of many physicians; particularly as most would have time constraints. Access within the clinics to national formularies detailing possible interactions between various drugs would help physicians to avoid potentially dangerous combinations of drugs. Additionally, an internet based program such as the one provided by the University of Liverpool (http://www.hiv-druginteractions.org/) allows clinicians to quickly check for possible harmful interactions before prescribing drugs. However, many clinicians working in resource poor settings may not have internet access or comprehensive national formularies may not exist and additionally, access to alternative safe drugs to treat many of the complications of HIV and its treatments may be limited by financial constraints. As such, most HIV patients on polypharmacy will be at risk of potentially harmful drug-drug interactions. The harms that can result are demonstrated by the many case reports that have been published showing how interactions, for example, between statins, particularly simvastatin, and PIs have caused severe rhabdomyolysis resulting in acute renal failure which in some cases caused patient deaths. Complex and unpredictable interactions can occur between different statins and PIs.

Increased levels can lead to increased risk of adverse effects while decreased levels can lead to attenuation of the lipid lowering effects of the statins.

On the whole, the choice of statins for use in HIV patients taking PI-based regimens as recommended by current guidelines are consistent with the known interactions demonstrated by this systematic review. However, while the studies did not find any unexpected or unsual results due to some unknown interactions; some contradictory findings which cannot be explained easily could be due to many reasons such as the ones detailed i.e. multiple pathways for metabolism, drug doses, diet, race amongst others. Research needs to continue particularly in drug development to identify new drugs using different pathways of metabolism from those used by PIs or other antiretroviral drugs, but also to explore the effects of race, geographical differences or diet to see if these have significant impact on drug interactions. This could then be used to formulate guidelines tailored to different population groups in-order to maximise the benefits of concommittant use of these drugs while minimising the risk of dangerous interactions.

CHAPTER SIX

CONCLUSIONS

HAART has dramatically changed the management and outlook of HIV infection, with the disease moving from a nearly always fatal outcome to a chronic manageable disease. However, this success comes at a price, with the development of unwanted adverse effects mostly due to the drugs. These can be early or late after commencing the drugs. The late adverse effects have gained much interest in recent years, particularly the metabolic and morphological effects as they have now been shown to increase the risk of development of CVD (Friis-Moller *et al.* 2003(b); Friis-Moller *et al.* 2007; Pere *et al.* 2008; Sabin *et al.* 2008; Worm *et al.* 2010). Many studies have demonstrated the occurrence of dyslipidaemia, insulin resistance, glucose intolerance or frank diabetes, increased inflammatory state and fat redistribution with use of ART drugs, with some drugs or class of drugs having a higher association with these adverse effects than others (Behrens *et al.* 1999; Carr *et al.* 1999; Heath *et al.* 2001; Jones *et al.* 2005a; Moyle *et al.* 2006; Friis-Moller *et al.* 2007; Pere *et al.* 2008). Some life style risk factors such as smoking are more frequent in the HIV infected populations than in the general population (Friis-Moller *et al.* 2003(a); Pere *et al.* 2008). The findings of the studies and audits in this thesis confirm some of these effects, especially with particular ART drugs used within HAART. Some of our findings were however unexpected.

We have shown that thymidine NRTIs AZT and D4T are associated with fat loss which is reported less upon switching to non-thymidine drugs. Our patients did not report fat gain after switching per se but a DEXA scan or a CT scan may have characterised this better as was demonstrated by others

(Gallant *et al.* 2004; Moyle *et al.* 2006; Shlay *et al.* 2008; Shlay *et al.* 2009). Patients who were on a non-thymidine regimen from baseline to the end of the study variably reported fat loss and fat gain, with no discernible pattern, which probably means fat loss was not a prominent problem. Notably, more patients in this group had previous exposure to D4T and older PIs such as IDV, SQV and NFV; as such the expectation was that this group would mostly be reporting fat gain. This was not reported with any consistency even at the individual patient level, which could be due to many reasons including the fact that the patients may not have detected any changes, failed to report any changes or no changes were occurring.

The lipids in our cohort were unchanged over the 12 month follow-up period and were not different between those that continued on a thymidine regimen, those that remained on a non-thymidine regimen and those that switched from a thymidine to a non-thymidine regimen. An adverse lipid profile on a thymidine regimen which improves on switching drugs was not observed, which is in contrast to the findings in other switch studies (Gallant *et al.* 2004; Moyle *et al.* 2006; Pozniak *et al.* 2006). Of interest is the finding of a high TC:HDL-C ratio in patients remaining on a non-thymidine regimen, which was even higher than that in patients on a thymidine regimen. This worsened over the duration of the studies. Though the total cholesterol was within normal range in this group, this could reflect a gradual reduction in levels of the HDL-C denominator, and the worsening TC:HDL-C ratio could explain the fact that a significant proportion of patients in this group who initially had a low calculated CV risk (<10%) at baseline later on in the study moved to the moderate risk bracket (10-20%). Obviously other factors were at play in the non-thymidine

regimen group (which should have had a better lipid profile) such as previous exposure to drugs with a worse effect on lipids. It should be noted that 10 of the 27 patients in this group were on ABC. Whether this could explain the worsening CV risk percentage, with what has already been reported about ABC being associated with increased CVD risk is difficult to say because the numbers in our studies are small, though it should be pointed out that more recent data now suggests that ABC does not increase CVD risk (Bedimo R 2009; Durand M 2009).

There was no perturbation in glucose homeostasis in our studies. The glucose levels, β-cell function and insulin levels remained unchanged at 12 months compared to baseline and were comparable to levels in the general population. There was also no difference between the thymidine, non-thymidine or switch groups. Others have demonstrated insulin resistance and glucose intolerance development with use of NRTIs and PIs (Hadigan *et al.* 1999; Hadigan *et al.* 2000; Brown *et al.* 2005; Tien *et al.* 2008). The small sample sizes in our studies could explain these differences.

We have demonstrated high levels of the proinflammatory cytokine TNF-α in our cohort similar to that found by others (Ross *et al.* 2009), further showing that serum levels of this cytokine are higher in patients with HIV infection than in HIV negative people. We did not demonstrate significant differences in TNF-α levels between the drugs used. We can only summise that having HIV infection per se causes a rise in the levels of this cytokine regardless of drugs used or that the differences with other studies was due to differences in

methodology. Our findings regarding IL-6 were unexpected, with levels at the end of the study in those on a thymidine regimen being lower than those in patients who switched to a non-thymidine regimen and the levels being comparable to those found in HIV uninfected people in the study by Ross et al (Hsue *et al.* 2009a; Ross *et al.* 2009). This is in contrast to the findings of studies which demonstrated higher levels of this cytokine induced by thymidine analogues (Lagathu *et al.* 2004; Jones *et al.* 2005b; Lagathu *et al.* 2007). We cannot draw firm conclusions from these results regarding IL-6, as our results may have been skewed by unexpected and unexplained high levels of this cytokine in a couple of patients. Our finding of declining levels of adiponectin in patients on a thymidine is in agreement with what is known about the effects of this group of drugs on this cytokine (Lindegaard *et al.* 2004; Jones *et al.* 2005c). However, the lack of improvement in the levels of this cytokine upon switching from a thymidine to a non-thymidine regimen in this study could be due to the fact that these patients switched mostly because of LD; as such, they may not have gained enough adipose tissue mass to cause an increase in this cytokine. Reduced adipose tissue mass has been shown to contribute to reduced levels of adiponectin by Sweeney et al (2007). On the whole, most of the unexpected findings in the levels of the cytokines in this study could be due to the fact that patients who had been on non-thymidine drugs such as TDF may also have had previous exposure to older PIs, thymidines and DDI, whose negative effects may not have reversed completely.

Our patients maintained normal renal function. Renal toxicity was not observed at all even though usage of TDF was common. However, we did not check for urine protein and additionally serum phosphate testing became routine when

the studies were already significantly underway. Urine protein and low serum phosphate are the earliest features of TDF renal toxicity before overt derangement of renal function tests. However, our findings are in agreement with the findings of others (Moyle *et al.* 2006; Pozniak *et al.* 2006).

Our audits conducted in large HIV treatment centres in the Northwest of England showed that physicians treating HIV patients face the complexity of treating the infection, but must now also be vigilant to the occurrence of potentially serious long term effects. We were able to show that various risk factors of CVD are common in patients with HIV and on ART. These include higher rates of smoking than in the local population which is in agreement with the findings of others (Friis-Moller *et al.* 2003(a); Pere *et al.* 2008). High rates of raised or high lipids were also found though the levels did not differ significantly between the drugs used except with the use of NNRTIs in which the lipids were higher than those in patients on a PI. Additionally, patients had CVD risk factors either occurring singly or in combination namely smoking, hypertension and family history of pre-existing CVD or dyslipidaemia. The majority of our patients had a low risk of developing CVD over 10 years (<10% risk). However, a good proportion (15% and 23% in Audit 1 and 2 respectively) were at high risk of CVD (CV risk>20%) and therefore warranted intensive therapy. Of particular interest is our finding in both audits that patients on ABC were nearly twice more likely to have a moderate or high risk of CVD than patients not on this drug. Though our numbers are small, they are in agreement with the larger studies previously described which reported increased CVD risk with ABC use (Lundgren 2008; Sabin *et al.* 2008), but as previously stated, recent evidence has thrown into doubt this association

(Bedimo R 2009; Durand M 2009). The physicians in our audit on the whole clinically assessed and instituted biochemical tests to identify various side-effects or existing medical conditions associated with development of CVD and instituted the management of the conditions identified. In some instances, these actions were not complete, i.e. not all patients had complete documentation, received lifestyle advice, drug treatment or were referred on for further management by appropriate specialties. These omissions were most likely due to lack of awareness of existing guidelines for management of these conditions, or lack of availability of proformas to standardize documentation so that physicians could be alerted if abnormalities were picked up which needed attention. Similar findings by the nationwide British HIV Association audit as detailed previously shows that physicians treating HIV need to be particularly aware of the need to actively look for and manage appropriately risk factors of CVD in this population (BHIVA 2006).

Adding to the complexity of treating HIV infection are drug-drug interactions. The concommittant use of statins and PIs can result in such interactions. However, the data summarised in the systematic review in this thesis shows that most interactions are predictable with the current knowledge of metabolising pathways of these two groups of drugs. Unexpected derangements in the PK levels of the statins could actually be explained by other known pathways of drug disposition such as drug transporter changes, and other factors such as race or diet could also be playing a role. Indeed the systematic review findings support the current guidelines' choice of safe statins to use in HIV patients on PIs.

There are several limitations in this thesis that would affect the generalisation of our results. The sample sizes in our longitudinal studies were small and we studied a number of drugs which makes the drawing of firm conclusions from our lipid, glucose, insulin and cytokine results from such small numbers difficult. The two audits in the thesis had different designs, making it difficult to combine and analyse the data as one entity, thereby weakening the impact of the results. The studies included in the systematic review were mostly small, the methodology, drug doses and populations were different and did not include randomised control groups which probably contributed to the variable results. But on the whole, some of our results were in agreement with findings of published work looking at CVD, its markers and risk factors as well as the management of some of the risk factors.

There's a lot of data now on pathophysiology of HIV LD and CVD. Research needs to focus on new ARV drug design, aiming at drugs that will not cause these problems. However, due to issues of tolerability and resistance mutations, some patients will remain on drugs that will cause the physical and metabolic effects of LD and ultimately CVD. As such research also needs to focus on development of drugs that will ameriorate the physical effects of LD or the treatable risk factors of CVD, but at the same time ensuring that the cost and pill burden of such drugs are kept to the minimum and that ease of administration is taken into account. Development and regular updating of web-based programs will be important to allow physicians to assess the likelihood of drug-drug interactions during clinics.

In conclusion, this work shows that lipodystrophy and CVD risk factors are common in HIV patients on ART. Underlying cytokine derangements are not easy to reproduce in different studies but a chronic inflammatory state and adipose tissue dysfunction in these patients are evident and need further studies to help find therapies to stop progression to CVD. Dyslipidaemia is one of the most well known adverse effects of ART and its treatment may be complicated by serious toxicities as a result of drug-drug interactions with PIs. Finally, physicians treating HIV patients need to be aware of current guidelines for treating metabolic complications of ART and also need to be aware of referral routes for further management of these complications because the management of HIV patients who develop these problems needs a multidisplinary approach.

References

(1998) Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet.* **352**, 854-65.

Abbott (2010) Norvia (RTV) Summary of product characteristics. http://www.rxabbott.com/pdf/norpi2a.pdf. Accessed 6/09/2010.

Aberg, J. A., S. L. Rosenkranz, C. J. Fichtenbaum, B. L. Alston, S. W. Brobst, Y. Segal and J. G. Gerber (2006) Pharmacokinetic interaction between nelfinavir and pravastatin in HIV-seronegative volunteers: ACTG Study A5108. *Aids.* **20**, 725-9.

Aberg, J. A., R. A. Zackin, S. W. Brobst, S. R. Evans, B. L. Alston, W. K. Henry, M. J. Glesby, F. J. Torriani, Y. Yang, S. I. Owens and C. J. Fichtenbaum (2005) A randomized trial of the efficacy and safety of fenofibrate versus pravastatin in HIV-infected subjects with lipid abnormalities: AIDS Clinical Trials Group Study 5087. *AIDS Res Hum Retroviruses.* **21**, 757-67.

Aboulafia, D. M. and R. Johnston (2000) Simvastatin-induced rhabdomyolysis in an HIV-infected patient with coronary artery disease. *AIDS Patient Care STDS.* **14**, 13-8.

Antoniades, C., D. Tousoulis, C. Tountas, C. Tentolouris, M. Toutouza, C. Vasiliadou, C. Tsioufis, P. Toutouzas and C. Stefanadis (2004) Vascular endothelium and inflammatory process, in patients with combined Type

2 diabetes mellitus and coronary atherosclerosis: the effects of vitamin C. *Diabet Med.* **21**, 552-8.

Arjona, M. M., R. Perez-Cano, R. Garcia-Juarez, A. Martin-Aspas, C. F. del Alamo and J. A. Giron-Gonzalez (2006) Structured intermittent interruption of chronic HIV infection treatment with highly active antiretroviral therapy: effects on leptin and TNF-alpha. *AIDS Res Hum Retroviruses.* **22**, 307-14.

Aslangul, E., L. Assoumou, R. Bittar, M. A. Valantin, O. Kalmykova, G. Peytavin, M. H. Fievet, F. Boccara, D. Bonnefont-Rousselot, J. C. Melchior, P. Giral and D. Costagliola (2010) Rosuvastatin versus pravastatin in dyslipidemic HIV-1-infected patients receiving protease inhibitors: a randomized trial. *Aids.* **24**, 77-83.

Assmann, G., H. Schulte, H. Funke and A. von Eckardstein (1998) The emergence of triglycerides as a significant independent risk factor in coronary artery disease. *Eur Heart J.* **19 Suppl M**, M8-14.

Austin, M. A., J. E. Hokanson and K. L. Edwards (1998) Hypertriglyceridemia as a cardiovascular risk factor. *Am J Cardiol.* **81**, 7B-12B.

Back, D., V. Sekar and R. M. Hoetelmans (2008) Darunavir: pharmacokinetics and drug interactions. *Antivir Ther.* **13**, 1-13.

Baldini, F., S. Di Giambenedetto, A. Cingolani, R. Murri, A. Ammassari and A. De Luca (2000) Efficacy and tolerability of pravastatin for the treatment of HIV-1 protease inhibitor-associated hyperlipidaemia: a pilot study. *Aids.* **14**, 1660-2.

Barb, D., S. G. Wadhwa, J. Kratzsch, A. Gavrila, J. L. Chan, C. J. Williams, A. W. Karchmer and C. S. Mantzoros (2005) Circulating resistin levels are not associated with fat redistribution, insulin resistance, or metabolic profile in patients with the highly active antiretroviral therapy-induced metabolic syndrome. *J Clin Endocrinol Metab.* **90**, 5324-8.

Barre-Sinoussi, F., J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Vezinet-Brun, C. Rouzioux, W. Rozenbaum and L. Montagnier (1983) Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science.* **220**, 868-71.

Bastard, J. P., M. Caron, H. Vidal, V. Jan, M. Auclair, C. Vigouroux, J. Luboinski, M. Laville, M. Maachi, P. M. Girard, W. Rozenbaum, P. Levan and J. Capeau (2002) Association between altered expression of adipogenic factor SREBP1 in lipoatrophic adipose tissue from HIV-1-infected patients and abnormal adipocyte differentiation and insulin resistance. *Lancet.* **359**, 1026-31.

Basu, V. P., M. Song, L. Gao, S. T. Rigby, M. N. Hanson and R. A. Bambara (2008) Strand transfer events during HIV-1 reverse transcription. *Virus Res.* **134**, 19-38.

Baum, P. D., P. M. Sullam, C. A. Stoddart and J. M. McCune (2011) Abacavir increases platelet reactivity via competitive inhibition of soluble guanylyl cyclase. *AIDS.*

Bedimo R, A. W., et al. (2009) "Abacavir use and risk of acute myocardial infarction and cerebrovascular disease in the HAART era". Abstract

MOAB202. http://www.iasociety.org/Default.aspx?pageId=11&abstractId=20072147 1. Accessed 4/01/2010.

Bedimo, R. J., A. O. Westfall, H. Drechsler, G. Vidiella and P. Tebas (2011) Abacavir use and risk of acute myocardial infarction and cerebrovascular events in the highly active antiretroviral therapy era. *Clin Infect Dis.* **53**, 84-91.

Behrens, G., A. Dejam, H. Schmidt, H. J. Balks, G. Brabant, T. Korner, M. Stoll and R. E. Schmidt (1999) Impaired glucose tolerance, beta cell function and lipid metabolism in HIV patients under treatment with protease inhibitors. *Aids.* **13**, F63-70.

Benesic, A., M. Zilly, F. Kluge, B. Weissbrich, R. Winzer, H. Klinker and P. Langmann (2004) Lipid lowering therapy with fluvastatin and pravastatin in patients with HIV infection and antiretroviral therapy: comparison of efficacy and interaction with indinavir. *Infection.* **32**, 229-33.

BHIVA (2006) Overview of standards project survey of management of cardiovascular risk, preliminary findings of mortality audit. http://www.bhiva.org/documents/ClinicalAudit/FindingsandReports/followupfinal.ppt. Accessed 1/07/2009.

Blake, G. J. and P. M. Ridker (2002) Inflammatory bio-markers and cardiovascular risk prediction. *J Intern Med.* **252**, 283-94.

Boly, L., V. Cafaro and T. Dyner (2006) Depressive symptoms predict increased incidence of neuropsychiatric side effects in patients treated with efavirenz. *J Acquir Immune Defic Syndr.* **42**, 514-5.

Bots, M. L., A. W. Hoes, A. Hofman, J. C. Witteman and D. E. Grobbee (1999) Cross-sectionally assessed carotid intima-media thickness relates to long-term risk of stroke, coronary heart disease and death as estimated by available risk functions. *J Intern Med.* **245**, 269-76.

Brakenhielm, E., N. Veitonmaki, R. Cao, S. Kihara, Y. Matsuzawa, B. Zhivotovsky, T. Funahashi and Y. Cao (2004) Adiponectin-induced antiangiogenesis and antitumor activity involve caspase-mediated endothelial cell apoptosis. *Proc Natl Acad Sci U S A.* **101**, 2476-81.

Brennan, A. M., J. H. Lee, S. Tsiodras, J. L. Chan, J. Doweiko, S. N. Chimienti, S. G. Wadhwa, A. W. Karchmer and C. S. Mantzoros (2009) r-metHuLeptin improves highly active antiretroviral therapy-induced lipoatrophy and the metabolic syndrome, but not through altering circulating IGF and IGF-binding protein levels: observational and interventional studies in humans. *Eur J Endocrinol.* **160**, 173-6.

Brennan, M. L., M. S. Penn, F. Van Lente, V. Nambi, M. H. Shishehbor, R. J. Aviles, M. Goormastic, M. L. Pepoy, E. S. McErlean, E. J. Topol, S. E. Nissen and S. L. Hazen (2003) Prognostic value of myeloperoxidase in patients with chest pain. *N Engl J Med.* **349**, 1595-604.

Bristol-Myers-Squibb (2007) Pravachol (pravastatin). Summary of product characteristics. http://packageinserts.bms.com/pi/pi_pravachol.pdf. Accessed 6-09-2010.

Bristol-Myers-Squibb (2010) Reyataz (ATV) Summary of product characteristics. http://packageinserts.bms.com/pi/pi_reyataz.pdf. Accessed 6-09-2010.

Brown, T. T., X. Li, S. R. Cole, L. A. Kingsley, F. J. Palella, S. A. Riddler, J. S. Chmiel, B. R. Visscher, J. B. Margolick and A. S. Dobs (2005) Cumulative exposure to nucleoside analogue reverse transcriptase inhibitors is associated with insulin resistance markers in the Multicenter AIDS Cohort Study. *Aids.* **19**, 1375-83.

Brugaletta, S., L. M. Biasucci, M. Pinnelli, G. Biondi-Zoccai, G. Di Giannuario, G. Trotta, G. Liuzzo and F. Crea (2006) Novel anti-inflammatory effect of statins: reduction of CD4+CD28null T lymphocyte frequency in patients with unstable angina. *Heart.* **92**, 249-50.

BSM (2011) Sustiva. http://packageinserts.bms.com/pi/pi_sustiva.pdf. Accessed 18/10/2011.

Busti, A. J., A. M. Bain, R. G. Hall, 2nd, R. G. Bedimo, R. D. Leff, C. Meek and R. Mehvar (2008) Effects of atazanavir/ritonavir or fosamprenavir/ritonavir on the pharmacokinetics of rosuvastatin. *J Cardiovasc Pharmacol.* **51**, 605-10.

Calmy, A., B. Hirschel, D. Hans, V. L. Karsegard and C. A. Meier (2003) Glitazones in lipodystrophy syndrome induced by highly active antiretroviral therapy. *AIDS.* **17**, 770-2.

Calza, L., R. Manfredi and F. Chiodo (2003) Statins and fibrates for the treatment of hyperlipidaemia in HIV-infected patients receiving HAART. *AIDS.* **17**, 851-9.

Cao, Y. L., C. Z. Hu, X. Meng, D. F. Wang and J. Zhang (2008) Expression of TNF-alpha protein in omental and subcutaneous adipose tissue in obesity. *Diabetes Res Clin Pract.* **79**, 214-9.

Cardo, D. M., D. H. Culver, C. A. Ciesielski, P. U. Srivastava, R. Marcus, D. Abiteboul, J. Heptonstall, G. Ippolito, F. Lot, P. S. McKibben and D. M. Bell (1997) A case-control study of HIV seroconversion in health care workers after percutaneous exposure. Centers for Disease Control and Prevention Needlestick Surveillance Group. *N Engl J Med.* **337**, 1485-90.

Carr, A., S. Emery, M. Law, R. Puls, J. D. Lundgren and W. G. Powderly (2003) An objective case definition of lipodystrophy in HIV-infected adults: a case-control study. *Lancet.* **361**, 726-35.

Carr, A., K. Samaras, S. Burton, M. Law, J. Freund, D. J. Chisholm and D. A. Cooper (1998) A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. *Aids.* **12**, F51-8.

Carr, A., K. Samaras, A. Thorisdottir, G. R. Kaufmann, D. J. Chisholm and D. A. Cooper (1999) Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor-associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study. *Lancet.* **353**, 2093-9.

Carr, A., C. Workman, D. Carey, G. Rogers, A. Martin, D. Baker, H. Wand, M. Law, K. Samaras, S. Emery and D. A. Cooper (2004) No effect of rosiglitazone for treatment of HIV-1 lipoatrophy: randomised, double-blind, placebo-controlled trial. *Lancet.* **363**, 429-38.

Carr, A., C. Workman, D. E. Smith, J. Hoy, J. Hudson, N. Doong, A. Martin, J. Amin, J. Freund, M. Law and D. A. Cooper (2002) Abacavir substitution

for nucleoside analogs in patients with HIV lipoatrophy: a randomized trial. *JAMA*. **288**, 207-15.

Carr, R. A. (2000) Concomitant administration of ABT-378/ritonavir results in a clinically important pharmacokinetic (PK) interaction with atorvastatin but not pravastatin. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy. 17-20 September 2000, Toronto, Canada. Abstract 1644. http://gateway.nlm.nih.gov/MeetingAbstracts/ma?f=102247106.html. Accessed 03/01/2011.

CDC (1982) Update on acquired immune deficiency syndrome (AIDS)--United States. *MMWR Morb Mortal Wkly Rep*. **31**, 507-8, 513-4.

CDC (2011) Interim Guidance on HIV Pre-Exposure Prophylaxis for Men Who Have Sex with Men. http://www.cdc.gov/nchhstp/newsroom/PrEPMSMGuidanceGraphic.html . Accessed 17/10/2011.

Cesari, M., B. W. Penninx, A. B. Newman, S. B. Kritchevsky, B. J. Nicklas, K. Sutton-Tyrrell, S. M. Rubin, J. Ding, E. M. Simonsick, T. B. Harris and M. Pahor (2003) Inflammatory markers and onset of cardiovascular events: results from the Health ABC study. *Circulation*. **108**, 2317-22.

Charatan, F. (2001) Bayer decides to withdraw cholesterol lowering drug. *Bmj*. **323**, 359.

Chen, D., A. Misra and A. Garg (2002) Clinical review 153: Lipodystrophy in human immunodeficiency virus-infected patients. *J Clin Endocrinol Metab*. **87**, 4845-56.

Cherry, C. L. and S. L. Wesselingh (2003) Nucleoside analogues and HIV: the combined cost to mitochondria. *J Antimicrob Chemother.* **51**, 1091-3.

CHIP (2010) DAD 5 Year Estimated Risk calculator. http://www.chip.dk/TOOLS/DADRiskEquations/tabid/437/Default.aspx. Accessed 18/10/2011.

Cohen, M. S., Y. Q. Chen, M. McCauley, T. Gamble, M. C. Hosseinipour, N. Kumarasamy, J. G. Hakim, J. Kumwenda, B. Grinsztejn, J. H. Pilotto, S. V. Godbole, S. Mehendale, S. Chariyalertsak, B. R. Santos, K. H. Mayer, I. F. Hoffman, S. H. Eshleman, E. Piwowar-Manning, L. Wang, J. Makhema, L. A. Mills, G. de Bruyn, I. Sanne, J. Eron, J. Gallant, D. Havlir, S. Swindells, H. Ribaudo, V. Elharrar, D. Burns, T. E. Taha, K. Nielsen-Saines, D. Celentano, M. Essex and T. R. Fleming (2011) Prevention of HIV-1 infection with early antiretroviral therapy. *N Engl J Med.* **365**, 493-505.

Consortium, T. I. T. (2008) FDA Critical Path Transporter Workshop, 2–3 October 2008 Bethesda, MD http://www.nature.com/clpt/journal/v87/n1/pdf/clpt2009236a.pdf. Accessed 01/04/2011.

Cooper, K. J., P. D. Martin, A. L. Dane, M. J. Warwick, A. Raza and D. W. Schneck (2003a) The effect of erythromycin on the pharmacokinetics of rosuvastatin. *Eur J Clin Pharmacol.* **59**, 51-6.

Cooper, K. J., P. D. Martin, A. L. Dane, M. J. Warwick, A. Raza and D. W. Schneck (2003b) Lack of effect of ketoconazole on the

pharmacokinetics of rosuvastatin in healthy subjects. *Br J Clin Pharmacol.* **55**, 94-9.

Cooper, K. J., P. D. Martin, A. L. Dane, M. J. Warwick, D. W. Schneck and M. V. Cantarini (2002) The effect of fluconazole on the pharmacokinetics of rosuvastatin. *Eur J Clin Pharmacol.* **58**, 527-31.

Cutrell, A., C. Brothers, J. Yeo, J. Hernandez and D. Lapierre (2008) Abacavir and the potential risk of myocardial infarction. *Lancet.* **371**, 1413.

Daimon, M., T. Oizumi, T. Saitoh, W. Kameda, A. Hirata, H. Yamaguchi, H. Ohnuma, M. Igarashi, M. Tominaga and T. Kato (2003) Decreased serum levels of adiponectin are a risk factor for the progression to type 2 diabetes in the Japanese Population: the Funagata study. *Diabetes Care.* **26**, 2015-20.

Dau, B. and M. Holodniy (2009) Novel targets for antiretroviral therapy: clinical progress to date. *Drugs.* **69**, 31-50.

de Bethune, M. P. (2009) Non-nucleoside reverse transcriptase inhibitors (NNRTIs), their discovery, development, and use in the treatment of HIV-1 infection: a review of the last 20 years (1989-2009). *Antiviral Res.* **85**, 75-90.

de Jager, W., B. J. Prakken, J. W. Bijlsma, W. Kuis and G. T. Rijkers (2005) Improved multiplex immunoassay performance in human plasma and synovial fluid following removal of interfering heterophilic antibodies. *J Immunol Methods.* **300**, 124-35.

de Jager, W., H. te Velthuis, B. J. Prakken, W. Kuis and G. T. Rijkers (2003) Simultaneous detection of 15 human cytokines in a single sample of stimulated peripheral blood mononuclear cells. *Clin Diagn Lab Immunol.* **10**, 133-9.

Delelis, O., K. Carayon, A. Saib, E. Deprez and J. F. Mouscadet (2008) Integrase and integration: biochemical activities of HIV-1 integrase. *Retrovirology.* **5**, 114.

DHHS (2009) Panel on Antiretroviral Guidelines for Adults and Adolescents - Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf. Accessed 5 January 2011.

DOH-UK (1999) Saving Lives: Our Healthier Nation (1999). UK Department of Health. http://www.dh.gov.uk/en/Healthcare/Coronaryheartdisease/Nationalserviceframework. Accessed 18/12/2009.

DOH-UK (2004) Winning the War on Heart Disease, Progress report 2004. UK Department of Health. http://www.dh.gov.uk/en/Healthcare/Coronaryheartdisease/Nationalserviceframework. Accessed 18/12/2009.

DOH-UK (2007) The Coronary Heart Disease National Service Framework, Building for the future, Progress report for 2007. UK Department of Health.

http://www.dh.gov.uk/en/Healthcare/Coronaryheartdisease/Nationalserviceframework. Accessed 18/12/2009.

Doser, N., S. Kubli, A. Telenti, C. Marzolini, J. P. Chave, F. Feihl, T. Buclin, A. Pannatier, R. Darioli, P. Nicod, B. Waeber and V. Mooser (2002) Efficacy and safety of fluvastatin in hyperlipidemic protease inhibitor-treated HIV-infected patients. *Aids.* **16**, 1982-3.

Dresser, G. K., J. D. Spence and D. G. Bailey (2000) Pharmacokinetic-pharmacodynamic consequences and clinical relevance of cytochrome P450 3A4 inhibition. *Clin Pharmacokinet.* **38**, 41-57.

DTU (2004) University of Oxford HOMA calculator-Diabetes Trials Unit (DTU). http://www.dtu.ox.ac.uk/homacalculator/download.php. (Accessed 01/09/2009). **Version 2.2.2**.

Durand M, O. S., et al. (2009) "Relation between use of nucleoside reverse transcriptase inhibitors (NRTI) and risk of myocardial infarction (MI): a nested case control study using Quebec's public health insurance database (QPHID)". Abstract TUPEB175. http://www.iasociety.org/Default.aspx?pageId=11&abstractId=20072865. (Accessed 4/01/2010). .

Durand, M., O. Sheehy, J. G. Baril, J. Lelorier and C. L. Tremblay (2011) Association between HIV infection, antiretroviral therapy, and risk of acute myocardial infarction: a cohort and nested case-control study using Quebec's public health insurance database. *J Acquir Immune Defic Syndr.* **57**, 245-53.

EACS (2009) Clinical Management and Treatment of HIV Infected Adults In Europe (V5). http://www.europeanaidsclinicalsociety.org/guidelinespdf/EACS-EuroGuidelines2009FullVersion.pdf. Accessed 31/12/2010.

Egashira, K., Y. Hirooka, H. Kai, M. Sugimachi, S. Suzuki, T. Inou and A. Takeshita (1994) Reduction in serum cholesterol with pravastatin improves endothelium-dependent coronary vasomotion in patients with hypercholesterolemia. *Circulation.* **89**, 2519-24.

El-Sadr, W. M., J. D. Lundgren, J. D. Neaton, F. Gordin, D. Abrams, R. C. Arduino, A. Babiker, W. Burman, N. Clumeck, C. J. Cohen, D. Cohn, D. Cooper, J. Darbyshire, S. Emery, G. Fatkenheuer, B. Gazzard, B. Grund, J. Hoy, K. Klingman, M. Losso, N. Markowitz, J. Neuhaus, A. Phillips and C. Rappoport (2006) CD4+ count-guided interruption of antiretroviral treatment. *N Engl J Med.* **355**, 2283-96.

El-Sadr, W. M., C. M. Mullin, A. Carr, C. Gibert, C. Rappoport, F. Visnegarwala, C. Grunfeld and S. S. Raghavan (2005) Effects of HIV disease on lipid, glucose and insulin levels: results from a large antiretroviral-naive cohort. *HIV Med.* **6**, 114-21.

Espeland, M. A., H. O'Leary D, J. G. Terry, T. Morgan, G. Evans and H. Mudra (2005) Carotid intimal-media thickness as a surrogate for cardiovascular disease events in trials of HMG-CoA reductase inhibitors. *Curr Control Trials Cardiovasc Med.* **6**, 3.

Ezzell, C. (1987) AZT given the green light for clinical treatment of AIDS. *Nature.* **326**, 430.

Falutz, J., S. Allas, K. Blot, D. Potvin, D. Kotler, M. Somero, D. Berger, S. Brown, G. Richmond, J. Fessel, R. Turner and S. Grinspoon (2007) Metabolic effects of a growth hormone-releasing factor in patients with HIV. *N Engl J Med.* **357**, 2359-70.

Falutz, J., S. Allas, D. Kotler, M. Thompson, P. Koutkia, J. Albu, B. Trottier, J. P. Routy, P. Cote, T. Abribat and S. Grinspoon (2005) A placebo-controlled, dose-ranging study of a growth hormone releasing factor in HIV-infected patients with abdominal fat accumulation. *AIDS.* **19**, 1279-87.

Fasshauer, M., J. Klein, S. Neumann, M. Eszlinger and R. Paschke (2002) Hormonal regulation of adiponectin gene expression in 3T3-L1 adipocytes. *Biochem Biophys Res Commun.* **290**, 1084-9.

Fasshauer, M. and R. Paschke (2003) Regulation of adipocytokines and insulin resistance. *Diabetologia.* **46**, 1594-603.

Fauci, A. S. (1988) The human immunodeficiency virus: infectivity and mechanisms of pathogenesis. *Science.* **239**, 617-22.

FHI (2011) FHI Statement on FEM-PrEP. www.fhi.org/en/AboutFHI/Media/Releases/FEM-PrEP_statement041811.htm. Accessed 17/10/2011.

Fichtenbaum, C. J. and J. G. Gerber (2002) Interactions between antiretroviral drugs and drugs used for the therapy of the metabolic complications encountered during HIV infection. *Clin Pharmacokinet.* **41**, 1195-211.

Fichtenbaum, C. J., J. G. Gerber, S. L. Rosenkranz, Y. Segal, J. A. Aberg, T. Blaschke, B. Alston, F. Fang, B. Kosel and F. Aweeka (2002) Pharmacokinetic interactions between protease inhibitors and statins in HIV seronegative volunteers: ACTG Study A5047. *Aids.* **16**, 569-77.

Fidler, S. (2011) BHIVA and BASHH Position Statement on PrEP in the UK. www.bashh.org/guidelines. Accessed 17/10/2011.

Fidler, S., J. Fox, G. Touloumi, N. Pantazis, K. Porter, A. Babiker and J. Weber (2007) Slower CD4 cell decline following cessation of a 3 month course of HAART in primary HIV infection: findings from an observational cohort. *AIDS.* **21**, 1283-91.

Formoso, G., E. A. De Filippis, N. Michetti, P. Di Fulvio, A. Pandolfi, T. Bucciarelli, G. Ciabattoni, A. Nicolucci, G. Davi and A. Consoli (2008) Decreased in vivo oxidative stress and decreased platelet activation following metformin treatment in newly diagnosed type 2 diabetic subjects. *Diabetes Metab Res Rev.* **24**, 231-7.

Francisci, D., S. Giannini, F. Baldelli, M. Leone, B. Belfiori, G. Guglielmini, L. Malincarne and P. Gresele (2009) HIV type 1 infection, and not short-term HAART, induces endothelial dysfunction. *AIDS.* **23**, 589-96.

Friis-Moller, N., P. Reiss, C. A. Sabin, R. Weber, A. Monforte, W. El-Sadr, R. Thiebaut, S. De Wit, O. Kirk, E. Fontas, M. G. Law, A. Phillips and J. D. Lundgren (2007) Class of antiretroviral drugs and the risk of myocardial infarction. *N Engl J Med.* **356**, 1723-35.

Friis-Moller, N., C. A. Sabin, R. Weber, A. d'Arminio Monforte, W. M. El-Sadr, P. Reiss, R. Thiebaut, L. Morfeldt, S. De Wit, C. Pradier, G. Calvo, M.

G. Law, O. Kirk, A. N. Phillips and J. D. Lundgren (2003(b)) Combination antiretroviral therapy and the risk of myocardial infarction. *N Engl J Med.* **349**, 1993-2003.

Friis-Moller, N., R. Thiebaut, P. Reiss, R. Weber, A. D. Monforte, S. De Wit, W. El-Sadr, E. Fontas, S. Worm, O. Kirk, A. Phillips, C. A. Sabin, J. D. Lundgren and M. G. Law (2010) Predicting the risk of cardiovascular disease in HIV-infected patients: the data collection on adverse effects of anti-HIV drugs study. *Eur J Cardiovasc Prev Rehabil.* **17**, 491-501.

Friis-Moller, N., R. Weber, P. Reiss, R. Thiebaut, O. Kirk, A. d'Arminio Monforte, C. Pradier, L. Morfeldt, S. Mateu, M. Law, W. El-Sadr, S. De Wit, C. A. Sabin, A. N. Phillips and J. D. Lundgren (2003(a)) Cardiovascular disease risk factors in HIV patients--association with antiretroviral therapy. Results from the DAD study. *Aids.* **17**, 1179-93.

Frishman, W. H. and J. Horn (2008) Statin-drug interactions: not a class effect. *Cardiol Rev.* **16**, 205-12.

Fukuhara, A., M. Matsuda, M. Nishizawa, K. Segawa, M. Tanaka, K. Kishimoto, Y. Matsuki, M. Murakami, T. Ichisaka, H. Murakami, E. Watanabe, T. Takagi, M. Akiyoshi, T. Ohtsubo, S. Kihara, S. Yamashita, M. Makishima, T. Funahashi, S. Yamanaka, R. Hiramatsu, Y. Matsuzawa and I. Shimomura (2005) Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. *Science.* **307**, 426-30.

Gallant, J. E., E. DeJesus, J. R. Arribas, A. L. Pozniak, B. Gazzard, R. E. Campo, B. Lu, D. McColl, S. Chuck, J. Enejosa, J. J. Toole and A. K.

Cheng (2006) Tenofovir DF, emtricitabine, and efavirenz vs. zidovudine, lamivudine, and efavirenz for HIV. *N Engl J Med.* **354**, 251-60.

Gallant, J. E., S. Staszewski, A. L. Pozniak, E. DeJesus, J. M. Suleiman, M. D. Miller, D. F. Coakley, B. Lu, J. J. Toole and A. K. Cheng (2004) Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial. *Jama.* **292**, 191-201.

Ganesan, A., N. Crum-Cianflone, J. Higgins, J. Qin, C. Rehm, J. Metcalf, C. Brandt, J. Vita, C. F. Decker, P. Sklar, M. Bavaro, S. Tasker, D. Follmann and F. Maldarelli (2011) High dose atorvastatin decreases cellular markers of immune activation without affecting HIV-1 RNA levels: results of a double-blind randomized placebo controlled clinical trial. *J Infect Dis.* **203**, 756-64.

Gannage-Yared, M. H., R. R. Azar, M. Amm-Azar, S. Khalife, M. Germanos-Haddad, R. Neemtallah and G. Halaby (2005) Pravastatin does not affect insulin sensitivity and adipocytokines levels in healthy nondiabetic patients. *Metabolism.* **54**, 947-51.

Garderet, L., B. Fabiani, K. Lacombe, P. M. Girard, J. F. Flejou and N. C. Gorin (2004) Hyperviscosity syndrome in an HIV-1-positive patient. *Am J Med.* **117**, 891-3.

Gazzard, B., A. J. Bernard, M. Boffito, D. Churchill, S. Edwards, N. Fisher, A. M. Geretti, M. Johnson, C. Leen, B. Peters, A. Pozniak, J. Ross, J. Walsh, E. Wilkins and M. Youle (2006) British HIV Association (BHIVA)

guidelines for the treatment of HIV-infected adults with antiretroviral therapy (2006). *HIV Med.* **7**, 487-503.

Gazzard, B. G. (2008) British HIV Association Guidelines for the treatment of HIV-1-infected adults with antiretroviral therapy 2008. *HIV Med.* **9**, 563-608.

Giguere, J. F. and M. J. Tremblay (2004) Statin compounds reduce human immunodeficiency virus type 1 replication by preventing the interaction between virion-associated host intercellular adhesion molecule 1 and its natural cell surface ligand LFA-1. *J Virol.* **78**, 12062-5.

GlaxoSmithKline (2010) Lexiva (FPV). Summary of product characteristics. http://us.gsk.com/products/assets/us_lexiva.pdf. Accessed 6-09-2010.

Gotte, M., X. Li and M. A. Wainberg (1999) HIV-1 reverse transcription: a brief overview focused on structure-function relationships among molecules involved in initiation of the reaction. *Arch Biochem Biophys.* **365**, 199-210.

Gottlieb, M. S., R. Schroff, H. M. Schanker, J. D. Weisman, P. T. Fan, R. A. Wolf and A. Saxon (1981) Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. *N Engl J Med.* **305**, 1425-31.

Gougeon, M. L., L. Penicaud, B. Fromenty, P. Leclercq, J. P. Viard and J. Capeau (2004) Adipocytes targets and actors in the pathogenesis of HIV-associated lipodystrophy and metabolic alterations. *Antivir Ther.* **9**, 161-77.

Graham, I., D. Atar, K. Borch-Johnsen, G. Boysen, G. Burell, R. Cifkova, J. Dallongeville, G. De Backer, S. Ebrahim, B. Gjelsvik, C. Herrmann-Lingen, A. Hoes, S. Humphries, M. Knapton, J. Perk, S. G. Priori, K. Pyorala, Z. Reiner, L. Ruilope, S. Sans-Menendez, W. S. Op Reimer, P. Weissberg, D. Wood, J. Yarnell, J. L. Zamorano, E. Walma, T. Fitzgerald, M. T. Cooney, A. Dudina, A. Vahanian, J. Camm, R. De Caterina, V. Dean, K. Dickstein, C. Funck-Brentano, G. Filippatos, I. Hellemans, S. D. Kristensen, K. McGregor, U. Sechtem, S. Silber, M. Tendera, P. Widimsky, A. Altiner, E. Bonora, P. N. Durrington, R. Fagard, S. Giampaoli, H. Hemingway, J. Hakansson, S. E. Kjeldsen, L. Larsen, G. Mancia, A. J. Manolis, K. Orth-Gomer, T. Pedersen, M. Rayner, L. Ryden, M. Sammut, N. Schneiderman, A. F. Stalenhoef, L. Tokgozoglu, O. Wiklund and A. Zampelas (2007) European guidelines on cardiovascular disease prevention in clinical practice: full text. Fourth Joint Task Force of the European Society of Cardiology and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of nine societies and by invited experts). *Eur J Cardiovasc Prev Rehabil.* **14 Suppl 2**, S1-113.

Granfors, M. T., J. S. Wang, L. I. Kajosaari, J. Laitila, P. J. Neuvonen and J. T. Backman (2006) Differential inhibition of cytochrome P450 3A4, 3A5 and 3A7 by five human immunodeficiency virus (HIV) protease inhibitors in vitro. *Basic Clin Pharmacol Toxicol.* **98**, 79-85.

Grant, R. M., J. R. Lama, P. L. Anderson, V. McMahan, A. Y. Liu, L. Vargas, P. Goicochea, M. Casapia, J. V. Guanira-Carranza, M. E. Ramirez-Cardich, O. Montoya-Herrera, T. Fernandez, V. G. Veloso, S. P.

Buchbinder, S. Chariyalertsak, M. Schechter, L. G. Bekker, K. H. Mayer, E. G. Kallas, K. R. Amico, K. Mulligan, L. R. Bushman, R. J. Hance, C. Ganoza, P. Defechereux, B. Postle, F. Wang, J. J. McConnell, J. H. Zheng, J. Lee, J. F. Rooney, H. S. Jaffe, A. I. Martinez, D. N. Burns and D. V. Glidden (2010) Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. *N Engl J Med.* **363**, 2587-99.

Grunfeld, C., J. A. Delaney, C. Wanke, J. S. Currier, R. Scherzer, M. L. Biggs, P. C. Tien, M. G. Shlipak, S. Sidney, J. F. Polak, D. O'Leary, P. Bacchetti and R. A. Kronmal (2009) Preclinical atherosclerosis due to HIV infection: carotid intima-medial thickness measurements from the FRAM study. *AIDS.* **23**, 1841-9.

GSK (2010) Combivir. http://us.gsk.com/products/assets/us_combivir.pdf. Accessed 18/10/2011.

Gupta, A., Y. Zhang, J. D. Unadkat and Q. Mao (2004) HIV protease inhibitors are inhibitors but not substrates of the human breast cancer resistance protein (BCRP/ABCG2). *J Pharmacol Exp Ther.* **310**, 334-41.

Gutmann, H., G. Fricker, M. Torok, S. Michael, C. Beglinger and J. Drewe (1999) Evidence for different ABC-transporters in Caco-2 cells modulating drug uptake. *Pharm Res.* **16**, 402-7.

Haase, A. T. (1986) Pathogenesis of lentivirus infections. *Nature.* **322**, 130-6.

Haase, A. T., E. F. Retzel and K. A. Staskus (1990) Amplification and detection of lentiviral DNA inside cells. *Proc Natl Acad Sci U S A.* **87**, 4971-5.

Hadigan, C., C. Corcoran, T. Stanley, S. Piecuch, A. Klibanski and S. Grinspoon (2000) Fasting hyperinsulinemia in human immunodeficiency virus-infected men: relationship to body composition, gonadal function, and protease inhibitor use. *J Clin Endocrinol Metab.* **85**, 35-41.

Hadigan, C., K. Miller, C. Corcoran, E. Anderson, N. Basgoz and S. Grinspoon (1999) Fasting hyperinsulinemia and changes in regional body composition in human immunodeficiency virus-infected women. *J Clin Endocrinol Metab.* **84**, 1932-7.

Hadigan, C., J. Rabe and S. Grinspoon (2002) Sustained benefits of metformin therapy on markers of cardiovascular risk in human immunodeficiency virus-infected patients with fat redistribution and insulin resistance. *J Clin Endocrinol Metab.* **87**, 4611-5.

Haider, D. G., A. Handisurya, A. Storka, E. Vojtassakova, A. Luger, G. Pacini, A. Tura, M. Wolzt and A. Kautzky-Willer (2007) Visfatin response to glucose is reduced in women with gestational diabetes mellitus. *Diabetes Care.* **30**, 1889-91.

Haider, D. G., K. Schindler, G. Schaller, G. Prager, M. Wolzt and B. Ludvik (2006) Increased plasma visfatin concentrations in morbidly obese subjects are reduced after gastric banding. *J Clin Endocrinol Metab.* **91**, 1578-81.

Hansson, G. K. (2005) Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med.* **352**, 1685-95.

Hara, K., T. Yamauchi and T. Kadowaki (2005) Adiponectin: an adipokine linking adipocytes and type 2 diabetes in humans. *Curr Diab Rep.* **5**, 136-40.

Hare, C. B., M. P. Vu, C. Grunfeld and H. W. Lampiris (2002) Simvastatin-nelfinavir interaction implicated in rhabdomyolysis and death. *Clin Infect Dis.* **35**, e111-2.

Hartge, M. M., T. Unger and U. Kintscher (2007) The endothelium and vascular inflammation in diabetes. *Diab Vasc Dis Res.* **4**, 84-8.

Haslam, D. W. and W. P. James (2005) Obesity. *Lancet.* **366**, 1197-209.

Heath, K. V., R. S. Hogg, K. J. Chan, M. Harris, V. Montessori, M. V. O'Shaughnessy and J. S. Montanera (2001) Lipodystrophy-associated morphological, cholesterol and triglyceride abnormalities in a population-based HIV/AIDS treatment database. *AIDS.* **15**, 231-9.

Hecht, F. M., L. Wang, A. Collier, S. Little, M. Markowitz, J. Margolick, J. M. Kilby, E. Daar, B. Conway and S. Holte (2006) A multicenter observational study of the potential benefits of initiating combination antiretroviral therapy during acute HIV infection. *J Infect Dis.* **194**, 725-33.

Henry, K., H. Melroe, J. Huebsch, J. Hermundson, C. Levine, L. Swensen and J. Daley (1998) Severe premature coronary artery disease with protease inhibitors. *Lancet.* **351**, 1328.

Hill, A., W. Sawyer and B. Gazzard (2009) Effects of first-line use of nucleoside analogues, efavirenz, and ritonavir-boosted protease inhibitors on lipid levels. *HIV Clin Trials.* **10**, 1-12.

Hirano, M., K. Maeda, Y. Shitara and Y. Sugiyama (2006) Drug-drug interaction between pitavastatin and various drugs via OATP1B1. *Drug Metab Dispos.* **34**, 1229-36.

Ho, R. H., L. Choi, W. Lee, G. Mayo, U. I. Schwarz, R. G. Tirona, D. G. Bailey, C. Michael Stein and R. B. Kim (2007) Effect of drug transporter genotypes on pravastatin disposition in European- and African-American participants. *Pharmacogenet Genomics.* **17**, 647-56.

Hoetelmans, R. (2004) The effect of TMC-114, a potent next-generation HIV protease inhibitor, with low-dose ritonavir on atorvastatin pharmacokinetics. 41st ICAAC, Washington, 2004. Abstract H-865.

Hogan C, V. D., E Daar, X Sun, C Del Rio, S Fiscus, T Frazier, B Hare, M Markowitz, S Little (2010) A Finite Course of ART during Early HIV-1 Infection Modestly Delays Need for Subsequent ART Initiation: ACTG A5217, the SETPOINT Study. Abstract 134. http://retroconference.org/2010/Abstracts/38406.htm accessed 17/10/2011.

Holmberg, S. D., A. C. Moorman, J. M. Williamson, T. C. Tong, D. J. Ward, K. C. Wood, A. E. Greenberg and R. S. Janssen (2002) Protease inhibitors and cardiovascular outcomes in patients with HIV-1. *Lancet.* **360**, 1747-8.

Honda, M., A. Yogi, N. Ishizuka, I. Genka, H. Gatanaga, K. Teruya, N. Tachikawa, Y. Kikuchi and S. Oka (2007) Effectiveness of subcutaneous growth hormone in HIV-1 patients with moderate to severe facial lipoatrophy. *Intern Med.* **46**, 359-62.

Hoody, D. W. (2007) "Drug-Drug Interaction Between Lopinavir/Ritonavir and Rosuvastatin." 14thConference on Retroviruses and Opportunistic Infections, February 25-28, 2007, Los Angeles, CA. Poster no. 564. http://www.retroconference.org/2007/PDFs/564.pdf Assessed 6/09/2010.

Hsiang, B., Y. Zhu, Z. Wang, Y. Wu, V. Sasseville, W. P. Yang and T. G. Kirchgessner (1999) A novel human hepatic organic anion transporting polypeptide (OATP2). Identification of a liver-specific human organic anion transporting polypeptide and identification of rat and human hydroxymethylglutaryl-CoA reductase inhibitor transporters. *J Biol Chem.* **274**, 37161-8.

Hsue, P. Y., P. W. Hunt, A. Schnell, S. C. Kalapus, R. Hoh, P. Ganz, J. N. Martin and S. G. Deeks (2009a) Role of viral replication, antiretroviral therapy, and immunodeficiency in HIV-associated atherosclerosis. *Aids.* **23**, 1059-67.

Hsue, P. Y., P. W. Hunt, Y. Wu, A. Schnell, J. E. Ho, H. Hatano, Y. Xie, J. N. Martin, P. Ganz and S. G. Deeks (2009b) Association of abacavir and impaired endothelial function in treated and suppressed HIV-infected patients. *AIDS.* **23**, 2021-7.

Hsyu, P. H., M. D. Schultz-Smith, J. H. Lillibridge, R. H. Lewis and B. M. Kerr (2001) Pharmacokinetic interactions between nelfinavir and 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors atorvastatin and simvastatin. *Antimicrob Agents Chemother.* **45**, 3445-50.

Hubert, H. B., M. Feinleib, P. M. McNamara and W. P. Castelli (1983) Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. *Circulation.* **67**, 968-77.

Hulley, S. B. (1988) Risk factors for coronary heart disease. Selected recent epidemiological advances. *Drugs.* **36 Suppl 3**, 1-4.

Hundal, R. S. and S. E. Inzucchi (2003) Metformin: new understandings, new uses. *Drugs.* **63**, 1879-94.

Inaba, T., N. E. Fischer, D. S. Riddick, D. J. Stewart and T. Hidaka (1997) HIV protease inhibitors, saquinavir, indinavir and ritonavir: inhibition of CYP3A4-mediated metabolism of testosterone and benzoxazinorifamycin, KRM-1648, in human liver microsomes. *Toxicol Lett.* **93**, 215-9.

Jan, V., P. Cervera, M. Maachi, M. Baudrimont, M. Kim, H. Vidal, P. M. Girard, P. Levan, W. Rozenbaum, A. Lombes, J. Capeau and J. P. Bastard (2004) Altered fat differentiation and adipocytokine expression are inter-related and linked to morphological changes and insulin resistance in HIV-1-infected lipodystrophic patients. *Antivir Ther.* **9**, 555-64.

Janneh, O., P. G. Hoggard, J. F. Tjia, S. P. Jones, S. H. Khoo, B. Maher, D. J. Back and M. Pirmohamed (2003) Intracellular disposition and metabolic

effects of zidovudine, stavudine and four protease inhibitors in cultured adipocytes. *Antivir Ther.* **8**, 417-26.

JBS2 (2005) Joint British Societies' guidelines on prevention of cardiovascular disease in clinical practice (JBS 2). *Heart.* **91 Suppl 5**, v1-52.

Jenkins, P. J., S. Khalaf, W. Ogunkolade, K. McCarthy, T. David, R. E. Hands, D. Davies and S. A. Bustin (2005) Differential expression of IGF-binding protein-3 in normal and malignant colon and its influence on apoptosis. *Endocr Relat Cancer.* **12**, 891-901.

Jin, D. K., M. Nowakowski, M. Kramer and D. W. Essex (2000) Hyperviscosity syndrome secondary to a myeloma-associated IgG(1)kappa paraprotein strongly reactive against the HIV-1 p24 gag antigen. *Am J Hematol.* **64**, 210-3.

Johannsson, G., P. Marin, L. Lonn, M. Ottosson, K. Stenlof, P. Bjorntorp, L. Sjostrom and B. A. Bengtsson (1997) Growth hormone treatment of abdominally obese men reduces abdominal fat mass, improves glucose and lipoprotein metabolism, and reduces diastolic blood pressure. *J Clin Endocrinol Metab.* **82**, 727-34.

Jones, R., S. Sawleshwarkar, C. Michailidis, A. Jackson, S. Mandalia, J. Stebbing, M. Bower, M. Nelson, B. G. Gazzard and G. J. Moyle (2005a) Impact of antiretroviral choice on hypercholesterolaemia events: the role of the nucleoside reverse transcriptase inhibitor backbone. *HIV Med.* **6**, 396-402.

Jones, S. P., O. Janneh, D. J. Back and M. Pirmohamed (2005b) Altered adipokine response in murine 3T3-F442A adipocytes treated with

protease inhibitors and nucleoside reverse transcriptase inhibitors. *Antivir Ther.* **10**, 207-13.

Jones, S. P., N. Qazi, J. Morelese, D. Lebrecht, J. Sutinen, H. Yki-Jarvinen, D. J. Back, M. Pirmohamed, B. G. Gazzard, U. A. Walker and G. J. Moyle (2005c) Assessment of adipokine expression and mitochondrial toxicity in HIV patients with lipoatrophy on stavudine- and zidovudine-containing regimens. *J Acquir Immune Defic Syndr.* **40**, 565-72.

Kamin, D., C. Hadigan, M. Lehrke, S. Mazza, M. A. Lazar and S. Grinspoon (2005) Resistin levels in human immunodeficiency virus-infected patients with lipoatrophy decrease in response to rosiglitazone. *J Clin Endocrinol Metab.* **90**, 3423-6.

Kannisto, K., J. Sutinen, E. Korsheninnikova, R. M. Fisher, E. Ehrenborg, K. Gertow, A. Virkamaki, T. Nyman, H. Vidal, A. Hamsten and H. Yki-Jarvinen (2003) Expression of adipogenic transcription factors, peroxisome proliferator-activated receptor gamma co-activator 1, IL-6 and CD45 in subcutaneous adipose tissue in lipodystrophy associated with highly active antiretroviral therapy. *Aids.* **17**, 1753-62.

Kaplan, R. C., L. A. Kingsley, S. J. Gange, L. Benning, L. P. Jacobson, J. Lazar, K. Anastos, P. C. Tien, A. R. Sharrett and H. N. Hodis (2008) Low CD4+ T-cell count as a major atherosclerosis risk factor in HIV-infected women and men. *AIDS.* **22**, 1615-24.

Kappes, A. and G. Loffler (2000) Influences of ionomycin, dibutyryl-cycloAMP and tumour necrosis factor-alpha on intracellular amount and secretion

of apM1 in differentiating primary human preadipocytes. *Horm Metab Res.* **32**, 548-54.

Khoo, S., D. Back and P. Winstanley (2005) The potential for interactions between antimalarial and antiretroviral drugs. *Aids.* **19**, 995-1005.

Kim, R. B., M. F. Fromm, C. Wandel, B. Leake, A. J. Wood, D. M. Roden and G. R. Wilkinson (1998) The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors. *J Clin Invest.* **101**, 289-94.

Kim, R. J., C. G. Wilson, M. Wabitsch, M. A. Lazar and C. M. Steppan (2006) HIV protease inhibitor-specific alterations in human adipocyte differentiation and metabolism. *Obesity (Silver Spring).* **14**, 994-1002.

Kinlaw, W. B. and B. Marsh (2004) Adiponectin and HIV-lipodystrophy: taking HAART. *Endocrinology.* **145**, 484-6.

Kis, O., K. Robillard, G. N. Chan and R. Bendayan (2009) The complexities of antiretroviral drug-drug interactions: role of ABC and SLC transporters. *Trends Pharmacol Sci.* **31**, 22-35.

Kiser, J. J., J. G. Gerber, J. A. Predhomme, P. Wolfe, D. M. Flynn and D. W. Hoody (2008) Drug/Drug interaction between lopinavir/ritonavir and rosuvastatin in healthy volunteers. *J Acquir Immune Defic Syndr.* **47**, 570-8.

Kivisto, K. T. and M. Niemi (2007) Influence of drug transporter polymorphisms on pravastatin pharmacokinetics in humans. *Pharm Res.* **24**, 239-47.

Klein, D., L. B. Hurley, C. P. Quesenberry, Jr. and S. Sidney (2002) Do protease inhibitors increase the risk for coronary heart disease in patients with HIV-1 infection? *J Acquir Immune Defic Syndr.* **30**, 471-7.

Knobel, H., J. Alonso, J. L. Casado, J. Collazos, J. Gonzalez, I. Ruiz, J. M. Kindelan, A. Carmona, J. Juega and A. Ocampo (2002) Validation of a simplified medication adherence questionnaire in a large cohort of HIV-infected patients: the GEEMA Study. *Aids.* **16**, 605-13.

Koenig, W., M. Sund, M. Frohlich, H. G. Fischer, H. Lowel, A. Doring, W. L. Hutchinson and M. B. Pepys (1999) C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. *Circulation.* **99**, 237-42.

Kohli, R. (2008) A randomized placebocontrolled trial of metformin for the treatment of HIV lipodystrophy.

Poster 148. http://www.retroconference.org/2006/Abstracts/27142.HTM

(accessed 19/05/2008).

Koutkia, P., B. Canavan, J. Breu, M. Torriani, J. Kissko and S. Grinspoon (2004a) Growth hormone-releasing hormone in HIV-infected men with lipodystrophy: a randomized controlled trial. *JAMA.* **292**, 210-8.

Koutkia, P., K. Eaton, S. M. You, J. Breu and S. Grinspoon (2006) Growth hormone secretion among HIV infected patients: effects of gender, race and fat distribution. *AIDS.* **20**, 855-62.

Koutkia, P., G. Meininger, B. Canavan, J. Breu and S. Grinspoon (2004b) Metabolic regulation of growth hormone by free fatty acids, somatostatin, and ghrelin in HIV-lipodystrophy. *Am J Physiol Endocrinol Metab*. **286**, E296-303.

Kralisch, S., M. Bluher, R. Paschke, M. Stumvoll and M. Fasshauer (2007) Adipokines and adipocyte targets in the future management of obesity and the metabolic syndrome. *Mini Rev Med Chem*. **7**, 39-45.

Kruger, E., P. M. Kloetzel and C. Enenkel (2001) 20S proteasome biogenesis. *Biochimie*. **83**, 289-93.

Kubota, N., Y. Terauchi, T. Yamauchi, T. Kubota, M. Moroi, J. Matsui, K. Eto, T. Yamashita, J. Kamon, H. Satoh, W. Yano, P. Froguel, R. Nagai, S. Kimura, T. Kadowaki and T. Noda (2002) Disruption of adiponectin causes insulin resistance and neointimal formation. *J Biol Chem*. **277**, 25863-6.

Kuller, L. H., R. Tracy, W. Belloso, S. De Wit, F. Drummond, H. C. Lane, B. Ledergerber, J. Lundgren, J. Neuhaus, D. Nixon, N. I. Paton and J. D. Neaton (2008) Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. *PLoS Med*. **5**, e203.

Lagathu, C., J. P. Bastard, M. Auclair, M. Maachi, M. Kornprobst, J. Capeau and M. Caron (2004) Antiretroviral drugs with adverse effects on adipocyte lipid metabolism and survival alter the expression and secretion of proinflammatory cytokines and adiponectin in vitro. *Antivir Ther*. **9**, 911-20.

Lagathu, C., B. Eustace, M. Prot, D. Frantz, Y. Gu, J. P. Bastard, M. Maachi, S. Azoulay, M. Briggs, M. Caron and J. Capeau (2007) Some HIV antiretrovirals increase oxidative stress and alter chemokine, cytokine or adiponectin production in human adipocytes and macrophages. *Antivir Ther.* **12**, 489-500.

Lagathu, C., M. Kim, M. Maachi, C. Vigouroux, P. Cervera, J. Capeau, M. Caron and J. P. Bastard (2005) HIV antiretroviral treatment alters adipokine expression and insulin sensitivity of adipose tissue in vitro and in vivo. *Biochimie.* **87**, 65-71.

Landovitz, R. J. and J. S. Currier (2009) Clinical practice. Postexposure prophylaxis for HIV infection. *N Engl J Med.* **361**, 1768-75.

Lang, S., M. Mary-Krause, L. Cotte, J. Gilquin, M. Partisani, A. Simon, F. Boccara and D. Costagliola (2010) Impact of individual antiretroviral drugs on the risk of myocardial infarction in human immunodeficiency virus-infected patients: a case-control study nested within the French Hospital Database on HIV ANRS cohort CO4. *Arch Intern Med.* **170**, 1228-38.

Laughlin, G. A., E. Barrett-Connor, S. May and C. Langenberg (2007) Association of adiponectin with coronary heart disease and mortality: the Rancho Bernardo study. *Am J Epidemiol.* **165**, 164-74.

Law, M., N. Friis-Moller, R. Weber, P. Reiss, R. Thiebaut, O. Kirk, A. d'Arminio Monforte, C. Pradier, L. Morfeldt, G. Calvo, W. El-Sadr, S. De Wit, C. A. Sabin and J. D. Lundgren (2003) Modelling the 3-year risk of myocardial

infarction among participants in the Data Collection on Adverse Events of Anti-HIV Drugs (DAD) study. *HIV Med.* **4**, 1-10.

Ledergerber, B., J. D. Lundgren, A. S. Walker, C. Sabin, A. Justice, P. Reiss, C. Mussini, F. Wit, A. d'Arminio Monforte, R. Weber, G. Fusco, S. Staszewski, M. Law, R. Hogg, F. Lampe, M. J. Gill, F. Castelli and A. N. Phillips (2004) Predictors of trend in CD4-positive T-cell count and mortality among HIV-1-infected individuals with virological failure to all three antiretroviral-drug classes. *Lancet.* **364**, 51-62.

Lee, J. H., J. L. Chan, E. Sourlas, V. Raptopoulos and C. S. Mantzoros (2006) Recombinant methionyl human leptin therapy in replacement doses improves insulin resistance and metabolic profile in patients with lipoatrophy and metabolic syndrome induced by the highly active antiretroviral therapy. *J Clin Endocrinol Metab.* **91**, 2605-11.

Lenert, L. A., M. Feddersen, A. Sturley and D. Lee (2002) Adverse effects of medications and trade-offs between length of life and quality of life in human immunodeficiency virus infection. *Am J Med.* **113**, 229-32.

Leszczyszyn-Pynka, M., S. Pynka, A. Boron-Kaczmarska and K. Pilarska (2005) [Serum leptin and adiponectin concentrations in patients infected with human immunodeficiency virus type 1 (HIV-1) on antiretroviral therapy]. *Endokrynol Pol.* **56**, 19-24.

Levy, J. A. (1993) Pathogenesis of human immunodeficiency virus infection. *Microbiol Rev.* **57**, 183-289.

Lewden, C., G. Chene, P. Morlat, F. Raffi, M. Dupon, P. Dellamonica, J. L. Pellegrin, C. Katlama, F. Dabis and C. Leport (2007) HIV-infected adults

with a CD4 cell count greater than 500 cells/mm3 on long-term combination antiretroviral therapy reach same mortality rates as the general population. *J Acquir Immune Defic Syndr.* **46**, 72-7.

Lieb, W., L. M. Sullivan, T. B. Harris, R. Roubenoff, E. J. Benjamin, D. Levy, C. S. Fox, T. J. Wang, P. W. Wilson, W. B. Kannel and R. S. Vasan (2009) Plasma leptin levels and incidence of heart failure, cardiovascular disease, and total mortality in elderly individuals. *Diabetes Care.* **32**, 612-6.

Lihn, A. S., S. B. Pedersen and B. Richelsen (2005) Adiponectin: action, regulation and association to insulin sensitivity. *Obes Rev.* **6**, 13-21.

Lihn, A. S., B. Richelsen, S. B. Pedersen, S. B. Haugaard, G. S. Rathje, S. Madsbad and O. Andersen (2003) Increased expression of TNF-alpha, IL-6, and IL-8 in HALS: implications for reduced adiponectin expression and plasma levels. *Am J Physiol Endocrinol Metab.* **285**, E1072-80.

Lindback, S., C. Brostrom, A. Karlsson and H. Gaines (1994) Does symptomatic primary HIV-1 infection accelerate progression to CDC stage IV disease, CD4 count below 200 x 10(6)/l, AIDS, and death from AIDS? *BMJ.* **309**, 1535-7.

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

Lipscombe, L. L., T. Gomes, L. E. Levesque, J. E. Hux, D. N. Juurlink and D. A. Alter (2007) Thiazolidinediones and cardiovascular outcomes in older patients with diabetes. *JAMA*. **298**, 2634-43.

Liu, D., T. Schuster, M. Baumann, M. Roos, D. Sollinger, J. Lutz, U. Heemann and M. von Eynatten (2009) Comparison of immunoassays for the selective measurement of human high-molecular weight adiponectin. *Clin Chem*. **55**, 568-72.

Lundgren, J. (2009) Risk of Myocardial Infarction with Exposure to Specific ARV from the PI, NNRTI, and NRTI Drug Classes: The D:A:D Study. Abstract 44LB. CROI 2009. http://retroconference.org/2009/Abstracts/36644.htm. Accessed 19/10/2011.

Lundgren, J. D. (2008) Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients. *Aids*. **22**, F17-24.

Lundgren, J. D., A. Babiker, W. El-Sadr, S. Emery, B. Grund, J. D. Neaton, J. Neuhaus and A. N. Phillips (2008a) Inferior clinical outcome of the CD4+ cell count-guided antiretroviral treatment interruption strategy in the SMART study: role of CD4+ Cell counts and HIV RNA levels during follow-up. *J Infect Dis*. **197**, 1145-55.

Lundgren, J. D., M. Battegay, G. Behrens, S. De Wit, G. Guaraldi, C. Katlama, E. Martinez, D. Nair, W. G. Powderly, P. Reiss, J. Sutinen and A. Vigano (2008b) European AIDS Clinical Society (EACS) guidelines on the prevention and management of metabolic diseases in HIV. *HIV Med*. **9**, 72-81.

Luzi, L., E. Meneghini, S. Oggionni, G. Tambussi, L. Piceni-Sereni and A. Lazzarin (2005) GH treatment reduces trunkal adiposity in HIV-infected patients with lipodystrophy: a randomized placebo-controlled study. *Eur J Endocrinol.* **153**, 781-9.

Lyall, E. G., M. Blott, A. de Ruiter, D. Hawkins, D. Mercy, Z. Mitchla, M. L. Newell, S. O'Shea, J. R. Smith, J. Sunderland, R. Webb and G. P. Taylor (2001) Guidelines for the management of HIV infection in pregnant women and the prevention of mother-to-child transmission. *HIV Med.* **2**, 314-34.

Maeda, N., I. Shimomura, K. Kishida, H. Nishizawa, M. Matsuda, H. Nagaretani, N. Furuyama, H. Kondo, M. Takahashi, Y. Arita, R. Komuro, N. Ouchi, S. Kihara, Y. Tochino, K. Okutomi, M. Horie, S. Takeda, T. Aoyama, T. Funahashi and Y. Matsuzawa (2002) Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med.* **8**, 731-7.

Maeda, N., M. Takahashi, T. Funahashi, S. Kihara, H. Nishizawa, K. Kishida, H. Nagaretani, M. Matsuda, R. Komuro, N. Ouchi, H. Kuriyama, K. Hotta, T. Nakamura, I. Shimomura and Y. Matsuzawa (2001) PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. *Diabetes.* **50**, 2094-9.

Magkos, F., A. Brennan, L. Sweeney, E. S. Kang, J. Doweiko, A. W. Karchmer and C. S. Mantzoros (2011) Leptin replacement improves postprandial glycemia and insulin sensitivity in human immunodeficiency virus-infected lipoatrophic men treated with pioglitazone: a pilot study. *Metabolism.* **60**, 1045-9.

Maher, B., A. Alfirevic, F. J. Vilar, E. G. Wilkins, B. K. Park and M. Pirmohamed (2002) TNF-alpha promoter region gene polymorphisms in HIV-positive patients with lipodystrophy. *Aids*. **16**, 2013-8.

Mallon, P. W., J. Miller, D. A. Cooper and A. Carr (2003) Prospective evaluation of the effects of antiretroviral therapy on body composition in HIV-1-infected men starting therapy. *AIDS*. **17**, 971-9.

Mallon, P. W., J. Miller, J. C. Kovacic, J. Kent-Hughes, R. Norris, K. Samaras, M. P. Feneley, D. A. Cooper and A. Carr (2006) Effect of pravastatin on body composition and markers of cardiovascular disease in HIV-infected men--a randomized, placebo-controlled study. *AIDS*. **20**, 1003-10.

Manfredi, R. and F. Chiodo (2001) Disorders of lipid metabolism in patients with HIV disease treated with antiretroviral agents: frequency, relationship with administered drugs, and role of hypolipidaemic therapy with bezafibrate. *J Infect*. **42**, 181-8.

Martin, A., M. Bloch, J. Amin, D. Baker, D. A. Cooper, S. Emery and A. Carr (2009) Simplification of antiretroviral therapy with tenofovir-emtricitabine or abacavir-Lamivudine: a randomized, 96-week trial. *Clin Infect Dis*. **49**, 1591-601.

Martin, A., D. E. Smith, A. Carr, C. Ringland, J. Amin, S. Emery, J. Hoy, C. Workman, N. Doong, J. Freund and D. A. Cooper (2004) Reversibility of lipoatrophy in HIV-infected patients 2 years after switching from a thymidine analogue to abacavir: the MITOX Extension Study. *AIDS*. **18**, 1029-36.

Martin, P. D., M. J. Warwick, A. L. Dane, S. J. Hill, P. B. Giles, P. J. Phillips and E. Lenz (2003) Metabolism, excretion, and pharmacokinetics of rosuvastatin in healthy adult male volunteers. *Clin Ther.* **25**, 2822-35.

Martinez, E., M. Larrousse, D. Podzamczer, I. Perez, F. Gutierrez, M. Lonca, P. Barragan, R. Deulofeu, R. Casamitjana, J. Mallolas, J. Pich and J. M. Gatell (2010) Abacavir-based therapy does not affect biological mechanisms associated with cardiovascular dysfunction. *Aids.* **24**, F1-9.

Martinez, E., M. Tuset, A. Milinkovic, J. M. Miro and J. M. Gatell (2004) Management of dyslipidaemia in HIV-infected patients receiving antiretroviral therapy. *Antivir Ther.* **9**, 649-63.

Mary-Krause, M., L. Cotte, A. Simon, M. Partisani and D. Costagliola (2003) Increased risk of myocardial infarction with duration of protease inhibitor therapy in HIV-infected men. *AIDS.* **17**, 2479-86.

Matthews, D. R., J. P. Hosker, A. S. Rudenski, B. A. Naylor, D. F. Treacher and R. C. Turner (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* **28**, 412-9.

Maziere, J. C., J. C. Landureau, P. Giral, M. Auclair, L. Fall, A. Lachgar, A. Achour and D. Zagury (1994) Lovastatin inhibits HIV-1 expression in H9 human T lymphocytes cultured in cholesterol-poor medium. *Biomed Pharmacother.* **48**, 63-7.

MCC (2009) Manchester City Council-Crumpsall Population Factsheet A26-I. http://www.manchester.gov.uk/.../a26i_crumpsall_ward_population_fact sheet2009. (Accessed 01/09/2009).

McComsey, G. A. (2009) Similar reductions in markers of inflammation and endothelial activation after initiation of abacavir/lamivudine or tenofovir/emtricitabine. http://retroconference.org/2009/Sessions/127.htm. Accessed 18/10/2011.

McGoldrick, C. and C. L. Leen (2007) The management of dyslipidaemias in antiretroviral-treated HIV infection: a systematic review. *HIV Med.* **8**, 325-34.

McTaggart, F. (2003) Comparative pharmacology of rosuvastatin. *Atheroscler Suppl.* **4**, 9-14.

Merck (2010) Zocor (simvastatin). Summary of product characteristics. http://www.Merck.com/product/usa/pi_circulars/z/zocor/zocor_pi.pdf. Accessed 6-09-2010

Merck(a) (2010) Crixivan (IDV). Summary of product characteristics. http://www.Merck.com/product/usa/pi_circulars/c/crixivan_pi.pdf. Accessed 6-09-2010.

Mikhail, N., E. Iskander and D. Cope (2009) Rhabdomyolysis in an HIV-infected patient on anti-retroviral therapy precipitated by high-dose pravastatin. *Curr Drug Saf.* **4**, 121-2.

Miller, C. J., Q. Li, K. Abel, E. Y. Kim, Z. M. Ma, S. Wietgrefe, L. La Franco-Scheuch, L. Compton, L. Duan, M. D. Shore, M. Zupancic, M. Busch, J. Carlis, S. Wolinsky and A. T. Haase (2005) Propagation and dissemination of infection after vaginal transmission of simian immunodeficiency virus. *J Virol.* **79**, 9217-27.

Millipore (2008) CytokineProfilerTM. http://www.millipore.com/drugdiscovery/dd3/milliplex_analytes. (Accessed 25/05/2008).

Montaner, J. S., V. D. Lima, R. Barrios, B. Yip, E. Wood, T. Kerr, K. Shannon, P. R. Harrigan, R. S. Hogg, P. Daly and P. Kendall Association of highly active antiretroviral therapy coverage, population viral load, and yearly new HIV diagnoses in British Columbia, Canada: a population-based study. *Lancet.* **376**, 532-9.

Mora, S., N. Rifai, J. E. Buring and P. M. Ridker (2008) Fasting compared with nonfasting lipids and apolipoproteins for predicting incident cardiovascular events. *Circulation.* **118**, 993-1001.

Morrow, G., L. Vachot, P. Vagenas and M. Robbiani (2007) Current concepts of HIV transmission. *Curr HIV/AIDS Rep.* **4**, 29-35.

Moyle, G. J. (2004) Bridging a gap: surgical management of HIV-associated lipoatrophy. *AIDS Read.* **14**, 472-5.

Moyle, G. J., N. E. Buss and B. G. Gazzard (2002) Pravastatin does not alter protease inhibitor exposure or virologic efficacy during a 24-week period of therapy. *J Acquir Immune Defic Syndr.* **30**, 460-2.

Moyle, G. J., M. Lloyd, B. Reynolds, C. Baldwin, S. Mandalia and B. G. Gazzard (2001) Dietary advice with or without pravastatin for the management of hypercholesterolaemia associated with protease inhibitor therapy. *Aids.* **15**, 1503-8.

Moyle, G. J., L. Lysakova, S. Brown, N. Sibtain, J. Healy, C. Priest, S. Mandalia and S. E. Barton (2004) A randomized open-label study of immediate versus delayed polylactic acid injections for the cosmetic management of facial lipoatrophy in persons with HIV infection. *HIV Med.* **5**, 82-7.

Moyle, G. J., C. A. Sabin, J. Cartledge, M. Johnson, E. Wilkins, D. Churchill, P. Hay, A. Fakoya, M. Murphy, G. Scullard, C. Leen and G. Reilly (2006) A randomized comparative trial of tenofovir DF or abacavir as replacement for a thymidine analogue in persons with lipoatrophy. *Aids.* **20**, 2043-50.

Mulligan, K. (2006) Effects of metformin and rosiglitazone on body composition in HIV-infected patients with hyperinsulinemia and elevated waist/hip ratio: a randomized, placebocontrolled trial. Poster 147. http://www.retroconference.org/2006/Abstracts/27715.HTM. Accessed 19/05/2008.

Mulligan, K. (2007) Improvements in hepatic and adipocyte insulin sensitivity, dyslipidemia, and visceral fat during leptin treatment in HIV-infected men with lipoatrophy and hypoleptinemia. Poster 805. http://www.retroconference.org/2007/Abstracts/29114.htm.

Nachega, J. B., M. P. Trotta, M. Nelson and A. Ammassari (2009) Impact of metabolic complications on antiretroviral treatment adherence: clinical and public health implications. *Curr HIV/AIDS Rep.* **6**, 121-9.

Natali, A. and E. Ferrannini (2006) Effects of metformin and thiazolidinediones on suppression of hepatic glucose production and stimulation of glucose

uptake in type 2 diabetes: a systematic review. *Diabetologia.* **49**, 434-41.

NCEP (2002) Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation.* **106**, 3143-421.

Neaton, J. D. and D. Wentworth (1992) Serum cholesterol, blood pressure, cigarette smoking, and death from coronary heart disease. Overall findings and differences by age for 316,099 white men. Multiple Risk Factor Intervention Trial Research Group. *Arch Intern Med.* **152**, 56-64.

Negredo, E., B. Clotet, J. Puig, N. Perez-Alvarez, L. Ruiz, J. Romeu, J. Molto, C. Rey-Joly and J. Blanco (2006) The effect of atorvastatin treatment on HIV-1-infected patients interrupting antiretroviral therapy. *AIDS.* **20**, 619-21.

Neuvonen, P. J., J. T. Backman and M. Niemi (2008) Pharmacokinetic comparison of the potential over-the-counter statins simvastatin, lovastatin, fluvastatin and pravastatin. *Clin Pharmacokinet.* **47**, 463-74.

NHL-BMI (2008) National Heart Lung metric BMI calculator. www.nhlbisupport.com/bmi/bmi-m.htm. (Accessed 30/05/2008).

NHS (2006) "Statistics on smoking: England, 2006". http://www.ic.nhs.uk/webfiles/publications/smokingeng2006/StatisticsOnSmoking300806_PDF.pdf. (Accessed 25/01/2010).

NICE-TA94 (2009) Statins for the prevention of cardiovascular events. Technology Appraisal 94. www.nice.org.uk/nicemedia/pdf/TA094guidance.pdf.(Accessed 5/01/2011).

Nissen, S. E. and K. Wolski (2007) Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N Engl J Med.* **356**, 2457-71.

Nolan, D., C. Moore, A. Castley, D. Sayer, C. Mamotte, M. John, I. James and S. Mallal (2003) Tumour necrosis factor-alpha gene -238G/A promoter polymorphism associated with a more rapid onset of lipodystrophy. *Aids.* **17**, 121-3.

Noor, M. A., O. P. Flint, J. F. Maa and R. A. Parker (2006) Effects of atazanavir/ritonavir and lopinavir/ritonavir on glucose uptake and insulin sensitivity: demonstrable differences in vitro and clinically. *Aids.* **20**, 1813-21.

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

Obel, N., H. F. Thomsen, G. Kronborg, C. S. Larsen, P. R. Hildebrandt, H. T. Sorensen and J. Gerstoft (2007) Ischemic heart disease in HIV-infected and HIV-uninfected individuals: a population-based cohort study. *Clin Infect Dis.* **44**, 1625-31.

Ofei, F., S. Hurel, J. Newkirk, M. Sopwith and R. Taylor (1996) Effects of an engineered human anti-TNF-alpha antibody (CDP571) on insulin

sensitivity and glycemic control in patients with NIDDM. *Diabetes.* **45**, 881-5.

Ogata, T., M. Yasaka, M. Yamagishi, O. Seguchi, K. Nagatsuka and K. Minematsu (2005) Atherosclerosis found on carotid ultrasonography is associated with atherosclerosis on coronary intravascular ultrasonography. *J Ultrasound Med.* **24**, 469-74.

Opravil, M., B. Ledergerber, H. Furrer, B. Hirschel, A. Imhof, S. Gallant, T. Wagels, E. Bernasconi, F. Meienberg, M. Rickenbach and R. Weber (2002) Clinical efficacy of early initiation of HAART in patients with asymptomatic HIV infection and CD4 cell count > 350 x 10(6) /l. *AIDS.* **16**, 1371-81.

Oral, E. A., V. Simha, E. Ruiz, A. Andewelt, A. Premkumar, P. Snell, A. J. Wagner, A. M. DePaoli, M. L. Reitman, S. I. Taylor, P. Gorden and A. Garg (2002) Leptin-replacement therapy for lipodystrophy. *N Engl J Med.* **346**, 570-8.

Ouchi, N., S. Kihara, T. Funahashi, Y. Matsuzawa and K. Walsh (2003) Obesity, adiponectin and vascular inflammatory disease. *Curr Opin Lipidol.* **14**, 561-6.

Pacenti, M., L. Barzon, F. Favaretto, K. Fincati, S. Romano, G. Milan, R. Vettor and G. Palu (2006) Microarray analysis during adipogenesis identifies new genes altered by antiretroviral drugs. *Aids.* **20**, 1691-705.

Pai, J. K., T. Pischon, J. Ma, J. E. Manson, S. E. Hankinson, K. Joshipura, G. C. Curhan, N. Rifai, C. C. Cannuscio, M. J. Stampfer and E. B. Rimm

(2004) Inflammatory markers and the risk of coronary heart disease in men and women. *N Engl J Med.* **351**, 2599-610.

Pal, D. and A. K. Mitra (2006) MDR- and CYP3A4-mediated drug-drug interactions. *J Neuroimmune Pharmacol.* **1**, 323-39.

Palacios, R., J. Santos, M. Gonzalez, J. Ruiz, P. Valdivielso, M. Marquez and P. Gonzalez-Santos (2002) Efficacy and safety of atorvastatin in the treatment of hypercholesterolemia associated with antiretroviral therapy. *J Acquir Immune Defic Syndr.* **30**, 536-7.

Palella, F. J., Jr., S. J. Gange, L. Benning, L. Jacobson, R. C. Kaplan, A. L. Landay, R. P. Tracy and R. Elion (2010) Inflammatory biomarkers and abacavir use in the Women's Interagency HIV Study and the Multicenter AIDS Cohort Study. *AIDS.* **24**, 1657-65.

Palella, F. J., Jr. and J. P. Phair (2011) Cardiovascular disease in HIV infection. *Curr Opin HIV AIDS.* **6**, 266-71.

Parker, R. A., O. P. Flint, R. Mulvey, C. Elosua, F. Wang, W. Fenderson, S. Wang, W. P. Yang and M. A. Noor (2005) Endoplasmic reticulum stress links dyslipidemia to inhibition of proteasome activity and glucose transport by HIV protease inhibitors. *Mol Pharmacol.* **67**, 1909-19.

Payne, R. (2005) University of Edinburgh Cardiovascular Risk Calculator (BNF).

Pepys, M. B. and G. M. Hirschfield (2003) C-reactive protein: a critical update. *J Clin Invest.* **111**, 1805-12.

Pere, D., S. L. Ignacio, T. Ramon, L. Fernando, T. Alberto, V. Pompeyo, G. Juan, M. J. G, G. Paloma, V. Antonio, C. Jaime, R. Esteban, R. Bernardino, M. L. GA, S. Trinitario, T. Ferran, L. Juan Ramon and G. Myriam (2008) Dyslipidemia and Cardiovascular Disease Risk Factor Management in HIV-1-Infected Subjects Treated with HAART in the Spanish VACH Cohort. *Open AIDS J.* **2**, 26-38.

Periard, D., A. Telenti, P. Sudre, J. J. Cheseaux, P. Halfon, M. J. Reymond, S. M. Marcovina, M. P. Glauser, P. Nicod, R. Darioli and V. Mooser (1999) Atherogenic dyslipidemia in HIV-infected individuals treated with protease inhibitors. The Swiss HIV Cohort Study. *Circulation.* **100**, 700-5.

Pfizer (2009) Lipitor (Atorvastatin). Summary of product characteristics. http://www.pfizer.com/files/products/uspi_lipitor.pdf. (Accessed 6-09-2010).

Phillips, A. N., B. Gazzard, R. Gilson, P. Easterbrook, M. Johnson, J. Walsh, C. Leen, M. Fisher, C. Orkin, J. Anderson, D. Pillay, V. Delpech, C. Sabin, A. Schwenk, D. Dunn, M. Gompels, T. Hill, K. Porter and A. Babiker (2007) Rate of AIDS diseases or death in HIV-infected antiretroviral therapy-naive individuals with high CD4 cell count. *AIDS.* **21**, 1717-21.

Pickup, J. C., M. B. Mattock, G. D. Chusney and D. Burt (1997) NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. *Diabetologia.* **40**, 1286-92.

Piliero, P. J. (2004) Pharmacokinetic properties of nucleoside/nucleotide reverse transcriptase inhibitors. *J Acquir Immune Defic Syndr.* **37 Suppl 1**, S2-S12.

Pirmohamed, M. (2009) Clinical management of HIV-associated lipodystrophy. *Curr Opin Lipidol.* **20**, 309-14.

Plutzky, J. (2001) Inflammatory pathways in atherosclerosis and acute coronary syndromes. *Am J Cardiol.* **88**, 10K-15K.

Pollard, R. B., P. Robinson and K. Dransfield (1998) Safety profile of nevirapine, a nonnucleoside reverse transcriptase inhibitor for the treatment of human immunodeficiency virus infection. *Clin Ther.* **20**, 1071-92.

Pommier, Y., A. A. Johnson and C. Marchand (2005) Integrase inhibitors to treat HIV/AIDS. *Nat Rev Drug Discov.* **4**, 236-48.

Pope, M., M. G. Betjes, N. Romani, H. Hirmand, P. U. Cameron, L. Hoffman, S. Gezelter, G. Schuler and R. M. Steinman (1994) Conjugates of dendritic cells and memory T lymphocytes from skin facilitate productive infection with HIV-1. *Cell.* **78**, 389-98.

Popovic, M., J. M. Shenton, J. Chen, A. Baban, T. Tharmanathan, B. Mannargudi, D. Abdulla and J. P. Uetrecht (2009) Nevirapine hypersensitivity. *Handb Exp Pharmacol*, 437-51.

Pozniak, A. L., J. E. Gallant, E. DeJesus, J. R. Arribas, B. Gazzard, R. E. Campo, S. S. Chen, D. McColl, J. Enejosa, J. J. Toole and A. K. Cheng (2006) Tenofovir disoproxil fumarate, emtricitabine, and efavirenz

versus fixed-dose zidovudine/lamivudine and efavirenz in antiretroviral-naive patients: virologic, immunologic, and morphologic changes--a 96-week analysis. *J Acquir Immune Defic Syndr.* **43**, 535-40.

Pradhan, A. D., J. E. Manson, N. Rifai, J. E. Buring and P. M. Ridker (2001) C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *Jama.* **286**, 327-34.

Q.A. Karim, S. A. Karim, J. A. Frohlic, A. C. Grobler and C. Baxter (2010) Effectiveness and Safety of Tenofovir Gel, an Antiretroviral Microbicide, for the Prevention of HIV Infection in Women. . *Science.* **329**, 1168-1174

Rajala, M. W. and P. E. Scherer (2003) Minireview: The adipocyte--at the crossroads of energy homeostasis, inflammation, and atherosclerosis. *Endocrinology.* **144**, 3765-73.

Ranade, K., W. J. Geese, M. Noor, O. Flint, P. Tebas, K. Mulligan, W. Powderly, S. K. Grinspoon and M. P. Dube (2008) Genetic analysis implicates resistin in HIV lipodystrophy. *Aids.* **22**, 1561-8.

Rathmann, W., B. Haastert, C. Herder, H. Hauner, W. Koenig, C. Meisinger, R. Holle and G. Giani (2007) Differential association of adiponectin with cardiovascular risk markers in men and women? The KORA survey 2000. *Int J Obes (Lond).* **31**, 770-6.

Ray, N. and R. W. Doms (2006) HIV-1 coreceptors and their inhibitors. *Curr Top Microbiol Immunol.* **303**, 97-120.

Reaven, G. M. (1988) Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes.* **37**, 1595-607.

Ribaudo, H. J., C. A. Benson, Y. Zheng, S. L. Koletar, A. C. Collier, J. J. Lok, M. Smurzynski, R. J. Bosch, B. Bastow and J. T. Schouten (2011) No risk of myocardial infarction associated with initial antiretroviral treatment containing abacavir: short and long-term results from ACTG A5001/ALLRT. *Clin Infect Dis.* **52**, 929-40.

Riddler, S. A., X. Li, J. Otvos, W. Post, F. Palella, L. Kingsley, B. Visscher, L. P. Jacobson and A. R. Sharrett (2008) Antiretroviral therapy is associated with an atherogenic lipoprotein phenotype among HIV-1-infected men in the Multicenter AIDS Cohort Study. *J Acquir Immune Defic Syndr.* **48**, 281-8.

Riddler, S. A., E. Smit, S. R. Cole, R. Li, J. S. Chmiel, A. Dobs, F. Palella, B. Visscher, R. Evans and L. A. Kingsley (2003) Impact of HIV infection and HAART on serum lipids in men. *JAMA.* **289**, 2978-82.

Ridker, P. M., N. Rifai, M. Pfeffer, F. Sacks, S. Lepage and E. Braunwald (2000) Elevation of tumor necrosis factor-alpha and increased risk of recurrent coronary events after myocardial infarction. *Circulation.* **101**, 2149-53.

Ridker, P. M., N. Rifai, L. Rose, J. E. Buring and N. R. Cook (2002) Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med.* **347**, 1557-65.

Rietschel, P., C. Hadigan, C. Corcoran, T. Stanley, G. Neubauer, J. Gertner and S. Grinspoon (2001) Assessment of growth hormone dynamics in human immunodeficiency virus-related lipodystrophy. *J Clin Endocrinol Metab.* **86**, 504-10.

Rosenson, R. S. and C. C. Tangney (1998) Antiatherothrombotic properties of statins: implications for cardiovascular event reduction. *JAMA.* **279**, 1643-50.

Rosenson, R. S., C. C. Tangney and L. C. Casey (1999) Inhibition of proinflammatory cytokine production by pravastatin. *Lancet.* **353**, 983-4.

Ross, A. C., N. Rizk, M. A. O'Riordan, V. Dogra, D. El-Bejjani, N. Storer, D. Harrill, M. Tungsiripat, J. Adell and G. A. McComsey (2009) Relationship between inflammatory markers, endothelial activation markers, and carotid intima-media thickness in HIV-infected patients receiving antiretroviral therapy. *Clin Infect Dis.* **49**, 1119-27.

Ross, R. (1999) Atherosclerosis is an inflammatory disease. *Am Heart J.* **138**, S419-20.

Rosso, R., A. Parodi, G. d'Annunzio, F. Ginocchio, L. Nicolini, C. Torrisi, M. P. Sormani, R. Lorini, C. Viscoli and M. Vignolo (2007) Evaluation of insulin resistance in a cohort of HIV-infected youth. *Eur J Endocrinol.* **157**, 655-9.

Ruan, H. and H. F. Lodish (2003) Insulin resistance in adipose tissue: direct and indirect effects of tumor necrosis factor-alpha. *Cytokine Growth Factor Rev.* **14**, 447-55.

Sabin, C. A., S. W. Worm, R. Weber, P. Reiss, W. El-Sadr, F. Dabis, S. De Wit, M. Law, A. D'Arminio Monforte, N. Friis-Moller, O. Kirk, C. Pradier, I. Weller, A. N. Phillips and J. D. Lundgren (2008) Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients enrolled in the D:A:D study: a multi-cohort collaboration. *Lancet.* **371**, 1417-26.

Saint-Marc, T. and J. L. Touraine (1999) Effects of metformin on insulin resistance and central adiposity in patients receiving effective protease inhibitor therapy. *AIDS.* **13**, 1000-2.

Samaras, K., H. Wand, M. Law, S. Emery, D. Cooper and A. Carr (2007) Prevalence of metabolic syndrome in HIV-infected patients receiving highly active antiretroviral therapy using International Diabetes Foundation and Adult Treatment Panel III criteria: associations with insulin resistance, disturbed body fat compartmentalization, elevated C-reactive protein, and [corrected] hypoadiponectinemia. *Diabetes Care.* **30**, 113-9.

Samineni, D., P. B. Desai, L. Sallans and C. J. Fichtenbaum (2011) Steady-State Pharmacokinetic Interactions of Darunavir/Ritonavir With Lipid-Lowering Agent Rosuvastatin. *J Clin Pharmacol.*

Sax, P. E. and P. Kumar (2004) Tolerability and safety of HIV protease inhibitors in adults. *J Acquir Immune Defic Syndr.* **37**, 1111-24.

Schacker, T., A. C. Collier, J. Hughes, T. Shea and L. Corey (1996) Clinical and epidemiologic features of primary HIV infection. *Ann Intern Med.* **125**, 257-64.

Schambelan, M., C. A. Benson, A. Carr, J. S. Currier, M. P. Dube, J. G. Gerber, S. K. Grinspoon, C. Grunfeld, D. P. Kotler, K. Mulligan, W. G. Powderly and M. S. Saag (2002) Management of metabolic complications associated with antiretroviral therapy for HIV-1 infection: recommendations of an International AIDS Society-USA panel. *J Acquir Immune Defic Syndr.* **31**, 257-75.

Schindler, K., D. Haider, M. Wolzt, A. Rieger, B. Gmeinhart, A. Luger, P. Nowotny and B. Ludvik (2006) Impact of antiretroviral therapy on visfatin and retinol-binding protein 4 in HIV-infected subjects. *Eur J Clin Invest.* **36**, 640-6.

Schindler, K., A. Rieger, A. Tura, B. Gmeinhardt, V. Touzeau-Romer, D. Haider, G. Pacini and B. Ludvik (2009) The effect of rosiglitazone on insulin sensitivity, beta cell function, bone mineral density, and body composition in hiv-positive patients on highly-active antiretroviral therapy (HAART). *Horm Metab Res.* **41**, 573-9.

Schmidt, G. A., J. D. Hoehns, J. L. Purcell, R. L. Friedman and Y. Elhawi (2007) Severe rhabdomyolysis and acute renal failure secondary to concomitant use of simvastatin, amiodarone, and atazanavir. *J Am Board Fam Med.* **20**, 411-6.

Sekar, V. J. (2007) Pharmacokinetic drug-drug interaction between the new HIV protease inhibitor darunavir (TMC114) and the lipid-lowering agent pravastatin. 8th International Workshop on Clinical Pharmacology of HIV Therapy. 16-18 April, 2007. Budapest, Hungary. Abstract 54.

Sesmilo, G., B. M. Biller, J. Llevadot, D. Hayden, G. Hanson, N. Rifai and A. Klibanski (2000) Effects of growth hormone administration on inflammatory and other cardiovascular risk markers in men with growth hormone deficiency. A randomized, controlled clinical trial. *Ann Intern Med.* **133**, 111-22.

Shelton, J. D., D. T. Halperin and D. Wilson (2006) Has global HIV incidence peaked? *Lancet.* **367**, 1120-2.

Shitara, Y. and Y. Sugiyama (2006) Pharmacokinetic and pharmacodynamic alterations of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors: drug-drug interactions and interindividual differences in transporter and metabolic enzyme functions. *Pharmacol Ther.* **112**, 71-105.

Shlay, J. C., G. Bartsch, G. Peng, J. Wang, C. Grunfeld, C. L. Gibert, F. Visnegarwala, S. S. Raghavan, Y. Xiang, M. Farrough, H. E. Perry, D. Kotler and W. M. El-Sadr (2007) Long-term body composition and metabolic changes in antiretroviral naive persons randomized to protease inhibitor-, nonnucleoside reverse transcriptase inhibitor-, or protease inhibitor plus nonnucleoside reverse transcriptase inhibitor-based strategy. *J Acquir Immune Defic Syndr.* **44**, 506-17.

Shlay, J. C., S. Sharma, G. Peng, C. L. Gibert and C. Grunfeld (2008) Long-term subcutaneous tissue changes among antiretroviral-naive persons initiating stavudine, zidovudine, or abacavir with lamivudine. *J Acquir Immune Defic Syndr.* **48**, 53-62.

Shlay, J. C., S. Sharma, G. Peng, C. L. Gibert and C. Grunfeld (2009) The effect of individual antiretroviral drugs on body composition in HIV-infected persons initiating highly active antiretroviral therapy. *J Acquir Immune Defic Syndr.* **51**, 298-304.

Shlay, J. C., F. Visnegarwala, G. Bartsch, J. Wang, G. Peng, W. M. El-Sadr, C. Gibert, D. Kotler, C. Grunfeld and S. Raghavan (2005) Body composition and metabolic changes in antiretroviral-naive patients randomized to didanosine and stavudine vs. abacavir and lamivudine. *J Acquir Immune Defic Syndr.* **38**, 147-55.

Sierra, S., R. Kaiser, A. Thielen and T. Lengauer (2007) Genotypic coreceptor analysis. *Eur J Med Res.* **12**, 453-62.

Silverberg, M. J., W. Leyden, L. Hurley, A. S. Go, C. P. Quesenberry, Jr., D. Klein and M. A. Horberg (2009) Response to newly prescribed lipid-lowering therapy in patients with and without HIV infection. *Ann Intern Med.* **150**, 301-13.

Simonson, S. G., A. Raza, P. D. Martin, P. D. Mitchell, J. A. Jarcho, C. D. Brown, A. S. Windass and D. W. Schneck (2004) Rosuvastatin pharmacokinetics in heart transplant recipients administered an antirejection regimen including cyclosporine. *Clin Pharmacol Ther.* **76**, 167-77.

Slama, L., E. Lanoy, M. A. Valantin, J. P. Bastard, A. Chermak, A. Boutekatjirt, D. William-Faltaos, E. Billaud, J. M. Molina, J. Capeau, D. Costagliola and W. Rozenbaum (2008) Effect of pioglitazone on HIV-1-related

lipodystrophy: a randomized double-blind placebo-controlled trial (ANRS 113). *Antivir Ther.* **13**, 67-76.

Sluis-Cremer, N., N. A. Temiz and I. Bahar (2004) Conformational changes in HIV-1 reverse transcriptase induced by nonnucleoside reverse transcriptase inhibitor binding. *Curr HIV Res.* **2**, 323-32.

SMART/INSIGH/D:A:D (2008) Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients. *AIDS.* **22**, F17-24.

Sommeijer, D. W., M. R. MacGillavry, J. C. Meijers, A. P. Van Zanten, P. H. Reitsma and H. Ten Cate (2004) Anti-inflammatory and anticoagulant effects of pravastatin in patients with type 2 diabetes. *Diabetes Care.* **27**, 468-73.

Sonmez, A., Y. Baykal, M. Kilic, M. I. Yilmaz, K. Saglam, F. Bulucu and I. H. Kocar (2003) Fluvastatin improves insulin resistance in nondiabetic dyslipidemic patients. *Endocrine.* **22**, 151-4.

Spranger, J., A. Kroke, M. Mohlig, M. M. Bergmann, M. Ristow, H. Boeing and A. F. Pfeiffer (2003) Adiponectin and protection against type 2 diabetes mellitus. *Lancet.* **361**, 226-8.

Squires, K. E. (2001) An introduction to nucleoside and nucleotide analogues. *Antivir Ther.* **6 Suppl 3**, 1-14.

Stamler, J., D. Wentworth and J. D. Neaton (1986) Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary

screenees of the Multiple Risk Factor Intervention Trial (MRFIT). *Jama.* **256**, 2823-8.

Staszewski, S., V. Miller, C. Sabin, C. Schlecht, P. Gute, S. Stamm, T. Leder, A. Berger, E. Weidemann, A. Hill and A. Phillips (1999) Determinants of sustainable CD4 lymphocyte count increases in response to antiretroviral therapy. *AIDS.* **13**, 951-6.

Stein, J. H., C. E. Korcarz, R. T. Hurst, E. Lonn, C. B. Kendall, E. R. Mohler, S. S. Najjar, C. M. Rembold and W. S. Post (2008) Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. *J Am Soc Echocardiogr.* **21**, 93-111; quiz 189-90.

Sutinen, J., U. A. Walker, K. Sevastianova, H. Klinker, A. M. Hakkinen, M. Ristola and H. Yki-Jarvinen (2007) Uridine supplementation for the treatment of antiretroviral therapy-associated lipoatrophy: a randomized, double-blind, placebo-controlled trial. *Antivir Ther.* **12**, 97-105.

Sweeney, B. P. and J. Bromilow (2006) Liver enzyme induction and inhibition: implications for anaesthesia. *Anaesthesia.* **61**, 159-77.

Sweeney, L. L., A. M. Brennan and C. S. Mantzoros (2007) The role of adipokines in relation to HIV lipodystrophy. *Aids.* **21**, 895-904.

Tai, V. W., M. Schambelan, H. Algren, C. Shayevich and K. Mulligan (2002) Effects of recombinant human growth hormone on fat distribution in

patients with human immunodeficiency virus-associated wasting. *Clin Infect Dis.* **35**, 1258-62.

Thompson, M. A., J. A. Aberg, P. Cahn, J. S. Montaner, G. Rizzardini, A. Telenti, J. M. Gatell, H. F. Gunthard, S. M. Hammer, M. S. Hirsch, D. M. Jacobsen, P. Reiss, D. D. Richman, P. A. Volberding, P. Yeni and R. T. Schooley (2010) Antiretroviral treatment of adult HIV infection: 2010 recommendations of the International AIDS Society-USA panel. *JAMA.* **304**, 321-33.

Tien, P. C., M. F. Schneider, S. R. Cole, A. M. Levine, M. Cohen, J. Dehovitz, M. Young and J. E. Justman (2008) Antiretroviral Therapy Exposure and Insulin Resistance in the Women's Interagency HIV Study. *J Acquir Immune Defic Syndr.*

Tindall, B., S. Barker, B. Donovan, T. Barnes, J. Roberts, C. Kronenberg, J. Gold, R. Penny and D. Cooper (1988) Characterization of the acute clinical illness associated with human immunodeficiency virus infection. *Arch Intern Med.* **148**, 945-9.

Tracy, R. P., R. N. Lemaitre, B. M. Psaty, D. G. Ives, R. W. Evans, M. Cushman, E. N. Meilahn and L. H. Kuller (1997) Relationship of C-reactive protein to risk of cardiovascular disease in the elderly. Results from the Cardiovascular Health Study and the Rural Health Promotion Project. *Arterioscler Thromb Vasc Biol.* **17**, 1121-7.

Travers, K. J., C. K. Patil, L. Wodicka, D. J. Lockhart, J. S. Weissman and P. Walter (2000) Functional and genomic analyses reveal an essential

coordination between the unfolded protein response and ER-associated degradation. *Cell.* **101**, 249-58.

Triant, V. A., H. Lee, C. Hadigan and S. K. Grinspoon (2007) Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. *J Clin Endocrinol Metab.* **92**, 2506-12.

Tsai, C. C., P. Emau, K. E. Follis, T. W. Beck, R. E. Benveniste, N. Bischofberger, J. D. Lifson and W. R. Morton (1998) Effectiveness of postinoculation (R)-9-(2-phosphonylmethoxypropyl) adenine treatment for prevention of persistent simian immunodeficiency virus SIVmne infection depends critically on timing of initiation and duration of treatment. *J Virol.* **72**, 4265-73.

Tsiodras, S. and C. Mantzoros (2006) Leptin and Adiponectin in the HIV Associated Metabolic Syndrome: Physiologic and Therapeutic Implications. *Am J Infect Dis.* **2**, 141-152.

Tsuchida, A., T. Yamauchi and T. Kadowaki (2005) Nuclear receptors as targets for drug development: molecular mechanisms for regulation of obesity and insulin resistance by peroxisome proliferator-activated receptor gamma, CREB-binding protein, and adiponectin. *J Pharmacol Sci.* **97**, 164-70.

Tungsiripat, M., D. E. Bejjani, N. Rizk, A. O'Riordan M, A. C. Ross, C. Hileman, N. Storer, D. Harrill and G. A. McComsey (2010) Rosiglitazone improves lipoatrophy in patients receiving thymidine-sparing regimens. *AIDS.* **24**, 1291-8.

UNAIDS (2007) UNAIDS Global Report 2007. http://www.data.unaids.org/pub/Report/2008/jc1535_annual_report07_en.pdf. (Accessed 03/01/2011).

UNAIDS (2010) UNAIDS Global Report 2010. http://www.unaids.org/documents/20101123_GlobalReport_em.pdf. (Accessed 03/01/2011).

Unknown (1984) Lipid Research Clinics Program. *Jama*. **252**, 2545-8.

van der Lee, M., R. Sankatsing, E. Schippers, M. Vogel, G. Fatkenheuer, A. van der Ven, F. Kroon, J. Rockstroh, C. Wyen, A. Baumer, E. de Groot, P. Koopmans, E. Stroes, P. Reiss and D. Burger (2007) Pharmacokinetics and pharmacodynamics of combined use of lopinavir/ritonavir and rosuvastatin in HIV-infected patients. *Antivir Ther*. **12**, 1127-32.

Van Maele, B. and Z. Debyser (2005) HIV-1 integration: an interplay between HIV-1 integrase, cellular and viral proteins. *AIDS Rev*. **7**, 26-43.

Vandegraaff, N. and A. Engelman (2007) Molecular mechanisms of HIV integration and therapeutic intervention. *Expert Rev Mol Med*. **9**, 1-19.

Vanhems, P., J. Lambert, D. A. Cooper, L. Perrin, A. Carr, B. Hirschel, J. Vizzard, S. Kinloch-de Loes and R. Allard (1998) Severity and prognosis of acute human immunodeficiency virus type 1 illness: a dose-response relationship. *Clin Infect Dis*. **26**, 323-9.

Venhoff, N., B. Setzer, K. Melkaoui and U. A. Walker (2007) Mitochondrial toxicity of tenofovir, emtricitabine and abacavir alone and in combination

with additional nucleoside reverse transcriptase inhibitors. *Antivir Ther.* **12**, 1075-85.

Veny, A., A. Bonjoch, J. Romeu and et al. (1998) Cumulative risk for developing protease inhibitor-associated lipodystrophy (PI-AL) in HIV-infected patients. *38th Interscience Conference on Antimicrobial Agents and Chemotherapy. San Diego.*, Abstract I-92.

Vernochet, C., S. Azoulay, D. Duval, R. Guedj, F. Cottrez, H. Vidal, G. Ailhaud and C. Dani (2005) Human immunodeficiency virus protease inhibitors accumulate into cultured human adipocytes and alter expression of adipocytokines. *J Biol Chem.* **280**, 2238-43.

Vigano, A., G. M. Aldrovandi, V. Giacomet, M. Merlo, L. Martelli, S. Beretta, P. Luraschi, G. Rombola and S. Mora (2005) Improvement in dyslipidaemia after switching stavudine to tenofovir and replacing protease inhibitors with efavirenz in HIV-infected children. *Antivir Ther.* **10**, 917-24.

Vigouroux, C., M. Maachi, T. H. Nguyen, C. Coussieu, S. Gharakhanian, T. Funahashi, Y. Matsuzawa, I. Shimomura, W. Rozenbaum, J. Capeau and J. P. Bastard (2003) Serum adipocytokines are related to lipodystrophy and metabolic disorders in HIV-infected men under antiretroviral therapy. *Aids.* **17**, 1503-11.

Vourvahis, M. and A. D. Kashuba (2007) Mechanisms of pharmacokinetic and pharmacodynamic drug interactions associated with ritonavir-enhanced tipranavir. *Pharmacotherapy.* **27**, 888-909.

Walker, U. A. (2003) Update on mitochondrial toxicity: where are we now? *J HIV Ther.* **8**, 32-5.

Walker, U. A., M. Bickel, S. I. Lutke Volksbeck, U. P. Ketelsen, H. Schofer, B. Setzer, N. Venhoff, V. Rickerts and S. Staszewski (2002) Evidence of nucleoside analogue reverse transcriptase inhibitor--associated genetic and structural defects of mitochondria in adipose tissue of HIV-infected patients. *J Acquir Immune Defic Syndr.* **29**, 117-21.

Wang, E., C. N. Casciano, R. P. Clement and W. W. Johnson (2001) HMG-CoA reductase inhibitors (statins) characterized as direct inhibitors of P-glycoprotein. *Pharm Res.* **18**, 800-6.

Weiss, J., J. Rose, C. H. Storch, N. Ketabi-Kiyanvash, A. Sauer, W. E. Haefeli and T. Efferth (2007) Modulation of human BCRP (ABCG2) activity by anti-HIV drugs. *J Antimicrob Chemother.* **59**, 238-45.

Weyer, C., T. Funahashi, S. Tanaka, K. Hotta, Y. Matsuzawa, R. E. Pratley and P. A. Tataranni (2001) Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab.* **86**, 1930-5.

Whittle, H., J. Morris, J. Todd, T. Corrah, S. Sabally, J. Bangali, P. T. Ngom, M. Rolfe and A. Wilkins (1994) HIV-2-infected patients survive longer than HIV-1-infected patients. *AIDS.* **8**, 1617-20.

WHO (2007) "Cardiovascular Disease, Guidelines for assessment and management of cardiovascular risk". http://www.who.int/cardiovascular_diseases/guidelines/Full%20text.pdf. (Accessed 1/07/2009).

WHO (2009) Use of antiretroviral drugs for treating pregnant women and preventing HIV infection in infants. WHO Rapid Advice, Version 2. http://whqlibdoc.who.int/publications/2009/9789241598934_eng.pdf. Accessed 03/01/2011.

Williams, D. and J. Feely (2002) Pharmacokinetic-pharmacodynamic drug interactions with HMG-CoA reductase inhibitors. *Clin Pharmacokinet.* **41**, 343-70.

Wilson, P. W., R. B. D'Agostino, D. Levy, A. M. Belanger, H. Silbershatz and W. B. Kannel (1998) Prediction of coronary heart disease using risk factor categories. *Circulation.* **97**, 1837-47.

Worm, S. W., C. Sabin, R. Weber, P. Reiss, W. El-Sadr, F. Dabis, S. De Wit, M. Law, A. D. Monforte, N. Friis-Moller, O. Kirk, E. Fontas, I. Weller, A. Phillips and J. Lundgren (2010) Risk of myocardial infarction in patients with HIV infection exposed to specific individual antiretroviral drugs from the 3 major drug classes: the data collection on adverse events of anti-HIV drugs (D:A:D) study. *J Infect Dis.* **201**, 318-30.

Wunder, D., N. A. Bersinger, C. Fux, R. Weber, E. Bernasconi, M. Cavassini, H. C. Bucher, V. Schiffer, P. Schmid and H. Furrer (2005) Plasma leptin levels in men are not related to the development of lipoatrophy during antiretroviral therapy. *Aids.* **19**, 1837-42.

Yee, H. S. and N. T. Fong (1998) Atorvastatin in the treatment of primary hypercholesterolemia and mixed dyslipidemias. *Ann Pharmacother.* **32**, 1030-43.

Yin, M. T. and M. J. Glesby (2005) Recombinant human growth hormone therapy in HIV-associated wasting and visceral adiposity. *Expert Rev Anti Infect Ther.* **3**, 727-38.

Zhang, R., M. L. Brennan, X. Fu, R. J. Aviles, G. L. Pearce, M. S. Penn, E. J. Topol, D. L. Sprecher and S. L. Hazen (2001) Association between myeloperoxidase levels and risk of coronary artery disease. *Jama.* **286**, 2136-42.

Zhang, W., B. N. Yu, Y. J. He, L. Fan, Q. Li, Z. Q. Liu, A. Wang, Y. L. Liu, Z. R. Tan, J. Fen, Y. F. Huang and H. H. Zhou (2006) Role of BCRP 421C>A polymorphism on rosuvastatin pharmacokinetics in healthy Chinese males. *Clin Chim Acta.* **373**, 99-103.

Zou, M. H., S. S. Kirkpatrick, B. J. Davis, J. S. Nelson, W. G. t. Wiles, U. Schlattner, D. Neumann, M. Brownlee, M. B. Freeman and M. H. Goldman (2004) Activation of the AMP-activated protein kinase by the anti-diabetic drug metformin in vivo. Role of mitochondrial reactive nitrogen species. *J Biol Chem.* **279**, 43940-51.

APPENDICES

Appendix 1

LIPODYSTROPHY QUESTIONNAIRE

For each body part described please indicate whether you currently have body shape changes that have occurred since you were diagnosed with HIV and if YES, note the nature of the change as of today and the current severity of any change.

Severity is to be described as:
Mild (noticeable only when specifically looked for)
Moderate (readily obvious to the patient)
Severe (obvious to the other people)

Fat gain refers to a general increase in fat in a specific region. Lipomata are localised fatty lumps under the skin.

1. Is there any fat loss in your face?
   No □ Yes □
   If yes ticked indicate how severe you think it is
   Mild □ Moderate □ Severe □
   Duration

2. Is there any fat gain in your face?
   No □ Yes □
   If yes ticked indicate how severe you think it is
   Mild □ Moderate □ Severe □

3. Is there any fat loss in the front or sides of your neck?
   No □ Yes □
   If yes ticked indicate how severe you think it is
   Mild □ Moderate □ Severe □

4. Is there any fat gain in the front or sides of your neck?
   No □ Yes □
   If yes ticked indicate how severe you think it is
   Mild □ Moderate □ Severe □

5. Is there any fat loss in the back or base of your neck?
   No □ Yes □
   If yes ticked indicate how severe you think it is
   Mild □ Moderate □ Severe □

6. Is there any fat gain in the back or base of your neck?
   No □ Yes □
   If yes ticked indicate how severe you think it is
   Mild □ Moderate □ Severe □

7. Is there any fat loss on your arms?
   No □ Yes □
   If yes ticked indicate how severe you think it is
   Mild □ Moderate □ Severe □

8. Is there any fat gain on your arms?
   No □ Yes □
   If yes ticked indicate how severe you think it is
   Mild □ Moderate □ Severe □

9. Has the size of your breasts increased?
   No □ Yes □
   If yes ticked indicate how severe you think it is
   Mild □ Moderate □ Severe □

10. Has the size of your breasts decreased?
    No □ Yes □
    If yes ticked indicate how severe you think it is
    Mild □ Moderate □ Severe □

11. Has the size of your waist decreased?
    No □ Yes □
    If yes ticked indicate how severe you think it is
    Mild □ Moderate □ Severe □

12. Has the size of your waist increased?
    No □ Yes □
    If yes ticked indicate how severe you think it is
    Mild □ Moderate □ Severe □

13. Has the amount of fat on your buttock decreased?
    No □ Yes □
    If yes ticked indicate how severe you think it is
    Mild □ Moderate □ Severe □

14. Has the amount of fat on your buttock increased?
    No □ Yes □
    If yes ticked indicate how severe you think it is
    Mild □ Moderate □ Severe □

15. Has the amount of fat on your legs decreased?
    No □ Yes □
    If yes ticked indicate how severe you think it is
    Mild □ Moderate □ Severe □

16. Has the amount of fat on your legs increased?
    No □ Yes □
    If yes ticked indicate how severe you think it is
    Mild □ Moderate □ Severe □

17. Since you were diagnosed with HIV have the veins on your arms and/or legs become more prominent?
    No □ Yes □
    If yes ticked indicate how severe you think it is
    Mild □ Moderate □ Severe □

18. Since you were diagnosed with HIV have the veins on your arms and/or legs become less prominent?
    No □ Yes □
    If yes ticked indicate how severe you think it is
    Mild □ Moderate □ Severe □

19. Since you were diagnosed with HIV have you developed any fat lumps (lipomata)?
    No □ Yes □
    Tick the Regions below where the fat lumps have developed:
    Site:
    - Head □
    - Front/sides of Neck □
    - Back/base of neck □
    - Breasts □
    - Trunk □
    - Arms □
    - Legs □
    - Other □

20. Total Duration of symptom: [ ]

Date completed: [ ]

Completed by Patient (initials): [ ]

ADHERENCE QUESTIONNAIRE

Study Title: Effects of switching a thymidine nucleoside reverse transcriptase inhibitor (zidovudine (ZDV) or stavudine (D4T)), or didanosine (DDI), on lipid profiles, adipocytokines and insulin resistance in HIV positive patients.

Patient ID	Patient Initials	Date

Please answer the following questions by ticking the boxes as appropriate. Please answer each question truthfully. The way you are treated will not be affected if you answer that you do not take medication all the time as instructed:

1. Do you ever forget to take your medicine?
   Yes □ No □

2. Are you careless at times about taking your medicine?
   Yes □ No □

3. If at times you feel worse, do you stop taking your tablets?
   Yes □ No □

4. Did you miss any of your medications last weekend?
   Yes □ No □

5. Thinking about last week, how often have you miss taking your medicine? (Please tick one as applicable)
   A: Never □
   B: 1-2 □
   C: 3-5 □
   D: 6-10 □
   E: More than 10 □

6. How many days have you missed taking all your medicines during the past 3 months?

   Days: _____

Appendix 3 (audit proforma)

Audit 1: Dyslipidaemia and Highly Active Antiretroviral drugs in HIV positive patients AuditNorth Manchester General Hospital – Infectious Diseases Research Unit

Date: ___/___/_____ Doctors Initials

Demographics:

Age (years): ☐ Sex: M ☐ F ☐

Ethnicity: White ☐ Black-African ☐ Afro-Caribbean ☐ Asian ☐

Other (specify) _____

HAART:

Current combination:

NRTI Backbone: _____

PI(s): _____

NNRTI: _____

Total Duration of current HAART (months): _____

Previous exposure to: NRTIs ☐ PIs ☐ NNRTI ☐

Duration of All ART (Years): _____

Lipids/Blood Sugar: Lab results previous 1 year

Date					
TC					
LDL					
HDL					
Fasting TGs					
TC:HDL					
Blood sugar					

TC ≥5 at any time point ☐

Cardiovascular Risk Factor:

	Yes	No
Smoking	☐	☐
FHx of IHD	☐	☐
Diabetes	☐	☐
Raised BP	☐	☐
Dyslipidaemia	☐	☐

GTT done? Yes ☐ No ☐

Blood Pressure (when well) ☐ BP not recorded ☐

CV Risk % ☐ CV Risk not calculated ☐

Management aspects:

Life style advice given Yes ☐ No ☐

If yes Specify ☐

Drugs Given Yes ☐ No ☐

If yes Specify: LLA (type eg. statins, fibrates, fish oil, nicotinic acid derivatives)
Antihypertensives
Antidiabetics
Aspirin

☐

Referred for further care (eg. lipid clinic, dietician, GP, smoking cessation services, alcohol services, cardiologist, diabetologist)

Yes ☐ No ☐

If yes Specify ☐

Appendix 4

Audit 2: North West HIV Network Audit CVD Risk Management in HIV Patients on HAART (Nov 14th 2007)

SECTION 1

Date: ___/___/_____ Hospital name/dept: _____

Patient ID: [ ] Audit number: (1-6 per consultant) No.____

Demographics:

Age (years): [ ]

Sex: M ☐ F ☐

Ethnicity: White ☐ Black-African ☐ Afro-Caribbean ☐

Asian ☐ Other (specify) _____

HAART:

Current combination:

NRTI Backbone: _____

PI(s): _____

NNRTI: _____

Total Duration of current HAART (months): _____

Previous exposure to: NRTIs ☐ PIs ☐ NNRTI ☐

Duration of All ART (Years): _____

SECTION 2

	Recorded	Not Recorded
Smoker:	Yes☐ No ☐	☐
Past relevant CVD history (e.g. Hypertension, IHD, CVA and DM, dyslipidaemia etc):	Yes☐ No☐	☐
FHx of CVD 1° relatives (♀<65y, ♂<55y):	Yes☐ No ☐	☐

Total Chol: Highest level past 1 year (mmol/L): _____

AND

HDL-Chol level (any) (mmol/L): _____

OR

Total Chol:HDL Ratio, highest past 1 year: _____

Euglycaemic (glucose < 6.0) ☐ Raised Glucose ☐ Borderline ☐

BP: _____/_____ mmHg No record in notes ☐

SECTION 3:

Do you use the Joint British Societies' Guidelines on the Prevention of Cardiovascular Disease in Clinical Practice (Jan 2006)?

NO ☐ → please fill in the **yellow** coloured **BOX 1** below **ONLY** (page 2), then enter the consultant name in the box at the bottom of page 3. The questionnaire is then complete.

YES☐ → please complete the following subsection on CVD risks, then fill in **EITHER** the **purple BOX 2** (<20% CVD risk) on page 2, **OR** the **green BOX 3** (>20% CVD risk) on page 3. Then enter the consultant name in the box at the bottom of page 3. The questionnaire is then complete.

BOX 1

JBS Guidelines not used

Smoker: Counselled (stop smoking) ☐
Referred smok cessn ☐
Ref to GP/acup/hypno ☐
Started meds ie (patches) ☐

BP: Not Recorded ☐
Normal ☐
High ☐ →
Counselled re salt/exercise Yes☐ NR☐
Ref to GP/BP clinic/medics Yes☐ NR☐
Started meds Yes☐ NR☐
Specify: _____

Lipids: Not Recorded ☐
Normal ☐
Abnormal: ☐ →
dietary advice given Yes☐ NR☐
Ref to GP/lipid clinic/dietician Yes☐ NR☐
HAART changed Yes☐ NR☐
Started meds Yes☐ NR☐
Specify: _____

Weight Not Recorded ☐
Normal ☐
Big/overwt ☐ →
Counselled diet/exercise Yes☐ NR☐
Ref to GP/dietician Yes☐ NR☐

FPG Not Recorded ☐
Normal ☐
>6/Diabetic ☐ →
Counselled diet/exercise Yes☐ NR☐
Ref to GP/DM clinic/dietician Yes☐ NR☐

BOX 2 JBS Guidelines used

Patients at <20% risk of CVD over next 10 years

Life style advice:

- Smoker: Counselled (stop smoking) Yes☐ NR☐
- Diet Yes☐ NR☐
- Exercise Yes☐ NR☐
- Wt Reduction (if over-wt) Yes☐ NR☐
- Alcohol moderation Yes☐ NR☐

Addressed any isolated high risk factors:

- BP(>160 syst or >100 diast) Yes☐ NR☐
- TC/HDL >6 Yes☐ NR☐
- Familial dyslipidaemia Yes☐ NR☐

Targets reached ☐ Targets NOT reached ☐ →Action/Referred to (specify): _____

BOX 3 JBS Guidelines used

Patients at >20% risk of CVD in next 10 years

Life style advice:

- Smoker: Counselled (stop smoking) Yes☐ NR☐
- Diet Yes☐ NR☐
- Exercise Yes☐ NR☐
- Wt Reduction (if over-wt) Yes☐ NR☐
- Alcohol moderation Yes☐ NR☐

BP: Not Recorded ☐
Normal ☐
High ☐ → Counselled re salt/exercise Yes☐ NR☐
Ref to GP/BP clinic/medics Yes☐ NR☐
Started meds Yes☐ NR☐
Specify: _____

Lipids: Not Recorded ☐
Normal ☐
Abnormal: ☐ → Ref to GP/lipid clinic/dietician Yes☐ NR☐
HAART changed Yes☐ NR☐
Started meds Yes☐ NR☐
Specify: _____

RPG Not Recorded ☐
Normal (<6) ☐
Diabetic(>11) Or Impaired Glucose Tolerance (7-11)
☐ → Counselled diet/exercise Yes☐ NR☐
Ref to GP/DM clinic/dietician Yes☐ NR☐

Targets reached ☐ Targets NOT reached ☐ →Action/Referred to (specify):
_____

Consultant's Name: _____ *Thank you!*

Appendix 5

North Manchester general Hospital Infectious Diseases Department
Annual Patient Assessment

Patient Name: _____ Date: ___/___/_____
Unit number: _____
Date of birth: ____/____/____

Consultant: _____

CD4 counts and Viral loads previous 1 year:

	1	2	3	4	5	6
Dates						
CD4 (absolute)						
CD4 %						
Viral Load						

Vaccination Status:

Hep A: Immune Yes ☐
Non-immune ☐ ⇒ Vaccination given ___/___/_____

Hep B: Immune (Anti-Hbs Ab >100) ☐
Non-immune ☐ ⇒ Vaccination/Booster given: ____/____/_____
Previous infection (HBsAg –/HBcAb+) ☐

Hep C: Positive ☐ Negative ☐ ⇒ repeat serology date: ___/___/_____

Influenza vaccination Date: ___/___/_____

Renal Function:

Phosphate Normal ☐ Low ☐
Creatinine Clearance (ml/min): _____
(Cockcroft-Gault formula for CrCl: M = 1.23x(140-age) x Wt(Kg)/creatinine (μmol/l); F = 1.04 x (140-age) x Wt(Kg)/creatinine (μmol/l)

Urine Dipstick: Normal Abnormal (specify): _____

Syphilis serology: Date: ___/___/_____ Pos ☐ Neg☐

Cervical Smear (in Women) Date: ___/___/_____

Sexual screen Date: ___/___/_____

BP: _____/_____ mmHg

Weight: _____ Kg

Cardiovascular Risk Management:

CV Risks: Smoker: Yes ☐ No ☐

↑Chol/TGs Yes ☐ No ☐ TC:HDL ratio: ☐

Blood glucose: Normal ☐
Diabetes ☐
OGTT: Results (if performed) _____

CV Risk %: ☐

(Risk Assessor at http://www.bnf.org/bnf/extra/current/popup/risk.xls OR charts in BNF))

All CV Risk + established vascular disease (DM, IHD, CVA, PVD):

Life-style advice given:

Smoking cessation ☐ Wt ↓ ☐ Diet ☐ Exercise ☐ ↓Alcohol ☐

CV Risk > 20% or established vascular disease (DM, IHD, CVA, PVD)

If Drug treatment started: ↑BP ☐ ↑Lipids ☐ DM ☐ Aspirin ☐

Referrals for un-resolving Risk factors/established vascular disease

↑Lipids ⇒ lipid clinic ☐

↑BP ⇒ hypertension clinic/GP/medics ☐

DM ⇒ DM clinic/GP ☐

IHD (angina) ⇒ cardiologist ☐

Smoking ⇒ smoking cessation clinic/GP ☐

Participation in clinical trials Yes ☐ No ☐

Signature _____ Name _____ Bleep _____

North Manchester General Hospital Infectious Diseases Department
New Patient Assessment

PLEASE COMPLETE ON BOTH SIDES OF THIS FORM

Patient Name: _____ Date: ___/___/_____

Unit number: _____ Consultant: _____

Date of birth: ____/____/____

Demographics:

Age: _____ years

Sex: M ☐ F ☐

Ethnicity: White ☐ Black-African ☐ Afro-Caribbean ☐

Asian ☐ Other (specify) _____

Country of Origin _____

Occupation/Immigration status: _____

Mode of HIV acquisition:
MSM ☐ Heterosexual ☐ IVDU ☐
Other (specify) _____

Social: Smoker Yes ☐ (Cigs/day: ____) No ☐
Alcohol Yes ☐ (units/week: ____) No ☐

CV risk factors: DM ☐ Hypertension ☐ CVA ☐ IHD (angina, MI ☐

FHx of: IHD 1° relatives (♀<65y, ♂<55y) ☐ Dyslipidaemia ☐

BP: _____/_____ mmHg Weight: _____ Kg

HIV diagnosis (Date): ___/___/_____

CD4 Nadir: _____ cell/ml % ____ Date: ___/___/_____

Viral Load: _____ copies/ml Date: ___/___/_____

Serologies:

Hep A: Immune (IgG+) ☐
Non-immune ☐ ⇒ Date vaccinated: 1___/___/_____, 2___/___/_____

Hep B: Positive (sAg+ ☐ ⇒ e antigen + ☐ e antigen- ☐ DNA Viral load ____ copies/ml

Negative (sAg-ve/cAb-ve) ☐
⇒ Dates vaccinated: 1___/___/_____, 2___/___/_____, 3___/___/_____

Previous infection (HBsAg-/HBc+) ☐

Hep C: Positive ☐ ⇒ If CD4 <200: HCV RNA PCR ____ copies/ml ⇒ Genotype:___
Negative ☐

Syphilis: Positive ☐ Negative ☐ Date: ___/___/_____

CMV IgG: Positive ☐ Negative ☐ Date: ___/___/_____

Toxoplasma (IgG): Positive ☐ Negative ☐ Date: ___/___/_____

EBV IgG: Positive ☐ Negative ☐ Date: ___/___/_____

Cryptococcal Antigen (if CD4 <100):
Positive ☐ Negative ☐ Date: ___/___/_____

VZV IgG: Positive ☐ Negative ☐ Date: ___/___/_____

If lived in tropics, Tropical screen (Strongyloides, Schistosomiasis):

Positive ☐ Negative ☐

Haemoglobinopathy Screen (if appropriate) _____

Urine Dipstick: Normal ☐ Abnormal ☐ (specify): _____

CXR: Date ___/___/___ **Comment:** _____

ECG: Date ___/___/___ **Comment:** _____

Last Cervical Smear (in Women) Date: ___/___/_____

Sexual screen Date: ___/___/_____

CV RISK % ☐ **(if >20% follow Joint British Guidelines for CVD prevention)**
(To assess CVD risk: Need age, sex, smoking history, systolic BP, TC/HDL ratio then use graph at back of BNF or risk calculator program to calculate CVD risk)

Signature _____

Name_____

Bleep _____