THE ASSOCIATION OF TRADITIONAL, NON-TRADITIONAL, HIV, AND HIGHLY ACTIVE ANTIRETROVIRAL THERAPY- RELATED RISK FACTORS AND DYSLIPIDEMIA AMONG PEOPLE WHO ARE LIVING WITH HIV IN NOVA SCOTIA: A LONGITUDINAL COHORT STUDY

by

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Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
October 2010

DALHOUSIE UNIVERSITY

DEPARTMENT OF COMMUNITY HEALTH AND EPIDEMIOLOGY

The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled " THE ASSOCIATION OF TRADITIONAL, NON-TRADITIONAL, HIV, AND HIGHLY ACTIVE ANTIRETROVIRAL THERAPY- RELATED RISK FACTORS AND DYSLIPIDEMIA AMONG PEOPLE WHO ARE LIVING WITH HIV IN NOVA SCOTIA: A LONGITUDINAL COHORT STUDY"

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Dated: October 27, 2010

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DALHOUSIE UNIVERSITY

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DEPARTMENT OR SCHOOL: Department of Community Health and Epidemiology

DEGREE: MSc CONVOCATION: May YEAR: 2011

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Abstract

The present study investigated the longitudinal relationships between traditional, non-traditional, HIV and HAART-related risk factors and dyslipidemia in people who are living with HIV living in Nova Scotia. A total of 303 men and 39 women who were patients of the Halifax HIV clinic with at least two measurements of: total cholesterol, low density lipoprotein- cholesterol (LDL-C), high density lipoprotein- cholesterol (HDL-C) or triglyceride concentration, taken between 1997 and 2009 were included in this study. Univariate repeated measures linear mixed effects regression models were developed for men and women separately and multivariate models were developed for men. BMI, produced a significant independent effect on total cholesterol to HDL-C ratio in men living with HIV. Hepatitis C co-infection, a history of injection drug use, and viral load (copies HIV RNA/ ml blood), all found to produce significant independent effects on HDL-C concentration among men living with HIV.

List of Abbreviations and Symbols Used

AIDS	Acquired immune deficiency syndrome
AR	Autoregressive
ART	Antiretroviral therapy
BMI	Body mass index
CDHA	Capital District Health Authority
Cells/mm^3	Cells per cubic milliliter
CHD	Coronary heart disease
CI	Confidence interval
CRABP-1	Cytoplasmic retinoic-acid binding protein-1
CVD	Cardiovascular disease
DAD	Data Collection on Adverse Events of Anti-HIV Drugs
EFV	Efavirenz
HAART	Highly active antiretroviral therapy
HCV	Hepatitis C virus
HDL-C	High density lipoprotein cholesterol
HIV	Human immunodeficiency virus
INF-α	Interferon-alpha
IQR	Interquartile range
kg/m^2	Kilogram per meter squared
LDL-C	Low density lipoprotein cholesterol
LRP	Low-density lipoprotein receptor-related protein
mmol/l	Millimole per liter
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
NSHS95	1995 Nova Scotia health survey
NtRTI	Nucleotide reverse transcriptase inhibitor
NVP	Nevirapine
OR	Odds ratio
PI	Protease inhibitor

SRF	Standard risk factors
TC	Total serum cholesterol
TC:HDL-C	Total cholesterol to high density lipoprotein cholesterol ratio
TG	Triglyceride
TNF-α	Tumor necrosis factor alpha
VLDL	Very low density lipoprotein cholesterol
WHO	World Health Organization

Acknowledgements

I would like to thank my supervisor, Dr. Susan Kirkland, whose guidance, patience, and encouragement were integral to the completion of this thesis. I would also like to thank the members of my thesis committee, Drs. Gordon Flowerdew, and Lynn Johnston, for their helpful comments and suggestions in conducting this study. I am grateful to the Halifax HIV Clinic for the provision of the data. Thanks for financial support are due to the Faculty of Graduate Studies, Dalhousie University, and the Nova Scotia Health Research Foundation. Finally, I would like to thank the department of Community Health and Epidemiology and my family and friends for their support.

Chapter One: Introduction

1.1 Background

Over the past decade there have been significant advances in the treatment of patients with human immunodeficiency virus infection/ acquired immune deficiency syndrome (HIV/AIDS). This has led to improved survival for HIV-infected individuals living in developed countries. Highly active antiretroviral therapy (HAART) is given credit for infected individuals living longer and this therapy continues to represent an important aspect of the treatment for HIV/AIDS. As HIV/AIDS shifts from being a rapidly fatal disease to a potentially treatable, albeit serious chronic disease, one of the most pressing issues for researchers in the HAART era has to do with the presence of co-morbidities, particularly those that have been related to aging. At the present time, it is unclear how HIV infection impacts HIV-related and non- HIV-related co-morbidities.

Several non-HIV-related diseases are being diagnosed earlier and more frequently in the HIV-infected population than would be expected in the non-infected population. (1-3) In particular, both an earlier appearance and increased incidence of cardiovascular disease (CVD) has been noted in HIV-infected individuals.(1,4-6)

One of the major mechanisms suspected to be causing cardiovascular complications in the HIV-infected population is dyslipidemia, or abnormal levels of lipids in the blood. (6-8) It is likely that a number of factors determine blood lipid levels in the HIV-infected population. Figure 1 depicts the possible contributions of traditional (diabetes, smoking, obesity etc), and non-traditional (cocaine use, Hepatitis C infection, etc) risk factors, HIV-infection itself (1,8) and HAART (1,4-6,9) to the risk of dyslipidemia. However, the relative contribution of each of these factors is currently unknown as they have rarely all been studied in the same individuals. It is probable that many of these factors do not independently predict dyslipidemia, but rather, they may may be partially dependent or redundant markers of risk for dyslipidemia.

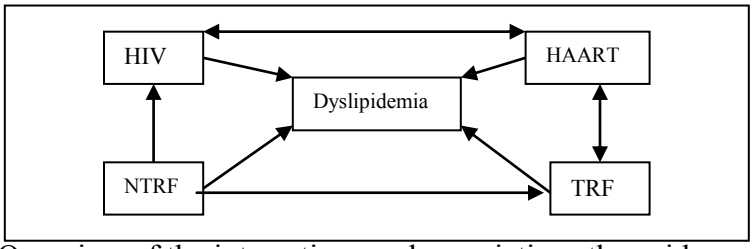


Figure 1. Overview of the interactions and associations the evidence suggests exists between exposures: traditional risk factors (TRF), non-traditional risk factors (NTRF), highly active anti-retroviral therapy (HAART), and HIV-infection, which may be affecting their relative contribution to dyslipidemia.

In order to create models for assessing dyslipidemia in HIV-infected individuals the traditional, non-traditional, HIV-infection and HAART-associated risk factors would all need to be considered. This study assessed prospectively collected patient level information on important covariates that other studies have not been able to capture. Hospital clinic databases often include this information, and using the Halifax HIV clinic database provided a unique opportunity to develop models for assessing the risk factors associated with dyslipidemia in an HIV-infected population.

1.2 Primary Objectives

1. To determine the factors that predict dyslipidemia in people living with HIV as measured by:

(a) total serum cholesterol (TC) concentration;

(b) high-density cholesterol (HDL-C) concentration;

(c) TC to HDL-C ratio;

(d) low-density cholesterol (LDL-C) concentration; and

(e) triglyceride (TG) concentration.

1.3 Secondary Objectives

1. To determine if traditional risk factors independently predict (a) through (e) in people living with HIV.

2. To determine if non-traditional risk factors independently predict (a) through (e) in people living with HIV.

3. To determine if markers of HIV infection independently predict (a) through (e) in people living with HIV.
4. To determine if HAART use independently predicts (a) through (e) in people living with HIV.

Chapter Two: Literature Review

2.1 HIV

HIV was first identified as the cause of the recognized pattern of opportunistic infections now known as AIDS in 1983. (10) HIV is a virus that attacks the immune system, in particular the CD4+ T lymphocytes that are an integral part of cell-mediated immunity. HIV causes a high level of CD4+ T lymphocyte activation, resulting in the rapid proliferation and death of these cells. (11) As CD4+ T lymphocyte levels decrease in advanced HIV infection an individual becomes more susceptible to opportunistic infections including tuberculosis, pneumonia, diarrhea, meningitis and tumors such as Kaposi's Sarcoma.

There are two main strains of HIV: HIV-1, which is the most common and HIV-2, which is predominant in West Africa. The HIV virus is transmitted through direct contact of the blood stream or mucous membrane with infected blood or body fluids such as vaginal fluid and semen. There are several possible modes of transmission including: sexual contact with someone who is infected; injection drug use (through sharing a contaminated needle); transfusion with infected blood; and mother to child transmission (before childbirth, during childbirth, or through breastfeeding). (12)

In 2008 the United Nations program on HIV/AIDS (UNAIDS) and the World Health Organization (WHO) estimated that 33.4 million people are living with HIV worldwide. (13) While HIV is pandemic, the epidemiology of the HIV epidemic varies by geographical region. For example, in Sub-Saharan Africa (where 22.4 million people are living with HIV) the primary mode of transmission is heterosexual intercourse and the disease affects all social and economic groups. Conversely, in Canada the epidemic is concentrated among specific populations, particularly men who have sex with men, those who inject drugs, and immigrants from countries where HIV is endemic. The prevalence of HIV in Canada is also much lower than in Sub-Saharan Africa (0.3 v.s 5.2 %). (13) However, due to improvements in treatment and the fact that new infections continue to occur, the prevalence of HIV in Canada increased by 14% from 2005 to 2008. In 2008 approximately 65000 Canadians were living with HIV. (14)

2.2 HIV in the HAART Era

Even with the introduction of the first treatment strategies in 1987, the prognosis for people living with HIV remained poor. Until the mid-1990s an infected individual was expected to survive for only two years after the appearance of the first AIDS defining-illness. In 1995 however, a new treatment regimen, HAART, was introduced and has proven to increase the life expectancy of people living with HIV to a length similar to the life expectancy of an individual with a chronic disease. (10)

HAART is the combination of at least three antiretroviral therapy (ART) drugs, the backbone of which is usually two nucleoside analogue reverse transcriptase inhibitors (NRTIs) or a nucleotide reverse transcriptase inhibitor (NtRTI) and one protease inhibitor (PI) or nonnucleoside analogue reverse transcriptase inhibitor (NNRTI). (15) Taking these drugs in combination has proven to increase the effectiveness of treatment over single drug therapy because each drug class acts on the virus at a different point in the life cycle, and therefore more effectively decreases viral replication. (16)

The improved survival resulting from the introduction of HAART has meant that research in the area of HIV/AIDS is currently focusing more on the management of the disease as a chronic condition. Research has now begun to address people living with HIV are afflicted by conditions such as diabetes mellitus (DM) and coronary heart disease (CHD), common chronic conditions also prevalent in the non-infected aging population. Several studies have found an increase in the rate of CHD among the HIV-infected population compared to the non-infected population. (1,6,8) Current research findings have led researchers to speculate that the metabolic changes that have been noted in HIV-infected patients, primarily dyslipidemia characterized by decreased HDL-C concentrations and elevated total cholesterol (TC) and triglyceride (TG) concentrations, are partially responsible for this cardiac risk. This is because atherosclerosis, the underlying cause of most types of vascular disease including coronary heart disease, is essentially the build up of cholesterol in the presence of an unfavorable lipid profile. (17,18)

2.3 Etiology and Pathogenesis of Dyslipidemia

Abnormal levels of serum cholesterol and triglycerides are mainly due to abnormalities in the lipoproteins responsible for carrying and clearing cholesterol and triglycerides from the blood stream. Dyslipidemia is the disorder of lipoprotein metabolism that causes serum and triglyceride abnormalities, and it can result in both an overproduction and deficiency of lipoproteins.

When lipoproteins are classified by density there are five classes: chylomicrons, high- density lipoproteins (HDL), intermediate density lipoproteins (IDL), low- density lipoproteins (LDL) and very low- density lipoproteins (VLDL). As the density of these particles decreases the lipid/cholesterol content increases. Each class of lipoprotein performs a different role in the transport of lipids in the blood. LDL carries cholesterol from the liver to other cells of the body and therefore LDL-C is sometimes referred to as "bad cholesterol". HDL-C, on the other hand is often referred to as "good cholesterol" because this lipoprotein transports cholesterol from body cells back to the liver where it can be metabolized and excreted from the body in the form of bile salts. Dietary triglycerides are found in the blood stream as a component of chylomicrons and triglycerides synthesized by the liver are transported as a component of VLDL. (19)

The National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) has determined the TC, LDL-C, HDL-C and triglyceride levels and associated cutpoints that place an individual at risk for cardiovascular disease. These levels are: TC concentrations $\geq$ 6.2 mmol/L, LDL-C concentrations $\geq$4.14mmol/L, HDL-C concentrations $\leq$1.03mmol/L, triglyceride concentrations $\geq$1.7mmol/L. (20) The literature often refers to these high concentrations of TC and triglycerides as hypercholesterolemia and hypertriglyceridemia respectively.

The risk factors that contribute to cholesterol levels consistent with cardiovascular risk and hypertriglyceridemia can be classified into two groups: modifiable and non-modifiable. The modifiable risk factors can be altered by an individual through medications or changes to lifestyle. For unhealthy cholesterol levels and hypertriglyceridemia these include a diet rich in saturated fat and

cholesterol, excess weight and obesity, physical inactivity, type-2 diabetes and cigarette smoking. Additional modifiable risk factors that are specific to low HDL-C and hypertriglyceridemia include, very high carbohydrate intake and certain drugs (beta-blockers, anabolic steroids and progestational agents). High serum triglyceride levels are also a risk factor for low HDL-C levels. Excess alcohol intake is also a risk factor for hypertriglyceridemia. Non-modifiable risk factors that cannot be changed include older age, gender (at younger ages men tend to have higher total cholesterol levels, but after menopause women's LDL-levels rise), and family history of dyslipidemia. Diseases such as chronic renal failure, nephritic syndrome and hypothyroidism are also non-modifiable risk factors for hypertriglyceridemia. (20)

2.4 Dyslipidemia in the HIV-Infected Population

Two patterns of dyslipidemia have been described in the HIV-infected population. The first form occurs in HIV-infected patients who are either treatment naïve or who are currently untreated. The second form of dyslipidemia found in the HIV-infected population occurs in patients currently being treated for HIV.

Studies examining lipid levels in untreated HIV-infected individuals have found dyslipidemia characterized by an increased risk for hypertriglyceridemia and low levels of HDL-C compared to non-infected controls. (21-24) In addition to low levels of HDL-C, the research suggests that untreated HIV-infected patients who are progressing from asymptomatic to late stages of infection also have TC and LDL-C levels below those of uninfected controls. Disease progression has the opposite affect on triglyceride levels, whereby HIV patients with an AIDS defining illness have been shown to have higher rates of hypertriglyceridemia compared to the general population, by almost two-fold. (25)

Most studies examining lipid levels in the HIV-infected population have examined patients who have been treated for the disease. These studies have found that HIV-treated patients have a greater risk of high levels LDL-C, hypercholesterolemia and hypertriglyceridemia than both un-infected individuals and untreated HIV controls. HDL-C levels in patients treated for HIV have been

shown to increase compared to untreated patients, but do not reach pre-infection levels. (26-30) Riddler et al. reported that within 2-3 years of receiving HAART, 50% of HIV-infected men with no history of hypercholesterolemia prior to initiation of treatment had developed the condition. (29) New onset of hypertriglyceridemia has been reported in up to 38.2% of treatment naïve patients who have received HIV treatment for 12 months. (31)

The exact mechanisms underlying the dyslipidemias found among the HIV-infected population are currently unresolved. Three main features of the HIV-infected population separate them from the non-infected population in terms of the risk for dyslipidemia. First, there is an elevated prevalence of traditional and non-traditional risk factors among the HIV positive population due to their lifestyle and demographic profile. Secondly, the HIV infection itself may lead to low HDL-C concentrations and hypertriglyceridemia. Studies of immunodeficiency and inflammation caused by HIV infection have demonstrated associations between this form of dyslipidemia, increased viral load and pro-inflammatory cytokines. (22) Finally, HAART has been shown to unfavorably alter lipid metabolism through a variety of pathways. (32)

2.4.1 Demographics and Lifestyle and the Risk of Dyslipidemia

The populations in which HIV infections are concentrated are recognized as having higher rates of risk factors for dyslipidemia than the general population. This means that due to their demographic and lifestyle factors these populations would have higher rates of dyslipidemia independent of their HIV and treatment. For example, HIV-infected individuals in North America are more likely to be men (32) and Nova Scotia is no exception to this trend, with 85.8% of reported positive HIV tests in men. (33) Furthermore, the most prevalent mode of HIV transmission in Nova Scotia is men who have sex with men. (34) A recent study of Swiss homosexual men found that after adjusting for sociodemographic characteristics and health behaviour (eg smoking and alcohol consumption) homosexual men had a greater risk of high cholesterol compared to men from the general population (OR 1.96 $P<0.05$). (35)

Studies have reported rates of smoking in the HIV-infected population ranging from 35-59% (30,36-38) compared to 18.1 % in the total population of Nova Scotia. (39) Kaplan (2008) reported that out of 1741 people living with HIV participating in their study, 35.5% of men and 43.3% of women were current smokers. This compares to 18.7% of men and 17.5% of women living in Nova Scotia who report smoking daily. (39). A meta-analysis conducted by Craig et al. found that when compared with non-smokers, current smokers had significantly higher levels of TC, triglycerides, and VLDL (levels of LDL-C were also higher but not significantly) and significantly lower levels of HDL-C. (40) There is also a higher rate of excess alcohol consumption among the HIV-infected population compared to the non-infected population. (41) Excessive alcohol consumption has been shown to lead to hypertriglyceridemia (42).

Another emerging non-traditional factor in dyslipidemia is hepatitis C (HCV), which is present at higher rates in the HIV-infected population due to shared methods of transmission and decreased immune function. The Public Health Agency of Canada estimates that 50-90% of HIV-positive people with a history of injection drug use are co-infected with HCV. (43) Lower rates of hypercholesterolemia and hypertriglyceridemia have been demonstrated in HIV and HCV co-infected patients as compared to HIV mono-infected individuals. (44-46)

2.4.2 HIV Infection and risk of Dyslipidemia

The lipid profile of treatment naive HIV-infected individuals is complex and appears to be influenced by several factors including level of immunosuppression, level of viral replication, and the presence of concomitant infection. A prospective study conducted by Riddler et al. found that HIV is likely responsible for decreasing levels of TC, LDL-C and HDL-C since these changes were found in subjects shortly after HIV-infection, but before treatment initiation. (47)

The findings of the prospective cohort study of antiretroviral naïve HIV-infected subjects conducted by El-Sadr et al. provides further evidence for the relationship between HIV-infection and dyslipidemia. This study found associations between HIV disease progression (decreasing number of CD4+ T cells in a cubic ml of blood, increasing number of copies of HIV RNA in a ml of blood, and/or a history

of an AIDS defining event) and decreased concentrations of HDL-C and LDL-C and increased concentrations of TC, VLDL, and triglycerides. Specifically, a low HDL-C concentration was associated with a lower CD4+Tcell count ($p<0.01$) and a higher viral load ($p<0.005$). A high viral load was also associated with a low concentration of LDL-C ($p<0.05$) and high concentrations of VLDL and triglycerides ($p<0.01$). A history of an AIDS defining event was associated with higher TC ($p<0.05$), VLDL ($p<0.01$) and triglyceride ($p<0.01$) concentrations. (48)

These findings strongly suggest that HIV infection directly affects the concentration of HDL-C, LDL-C, VLDL and triglycerides. However, whether or not HIV infection has a direct affect on TC levels is less clear. While El-Sadr et al. did not find an association between TC and CD4+ T cell count or viral load, the Data Collection on Adverse Events of Anti-HIV Drugs (DAD) study, a large international prospective cohort study that followed more than 20 000 people who are living with HIV from 20 countries in Europe, USA, and Australia, did find a negative association between TC and viral load in treatment naïve patients. The DAD study did not find an association between TC levels and CD4+ T cell count in treatment naïve patients (30)

The final pieces of evidence strengthening the argument that HIV itself causes dyslipidemia are the findings of studies assessing the impact of HAART treatment interruption on lipid levels. The study conducted by Tebas et al., found that by week eight after treatment interruption there had been moderate decreases in triglyceride, TC, LDL-C and HDL-C concentrations, which the authors attributed to discontinuing treatment. However, at 48 weeks after treatment interruption HDL-C concentrations had continued to decrease and the number of individuals with HDL-C concentrations consistent with cardiac risk (1.04mmol/L) increased from 53% prior to discontinuation to 76%. Further contributing to cardiac risk among untreated patients, at week 48 triglyceride concentrations were increasing. The authors believed that HIV itself mediated the dyslipidemia seen at week 48 and that it is not representative of any residual effects of HAART. (49)

The findings of the Tebas et al. study confirm the results of an earlier study conducted by Rose et al. This retrospective cohort study found that compared to

uninfected controls and HIV-infected patients receiving HAART, HDL-C levels among men 24 weeks after treatment interruption were 22% and 11% lower respectively. Rose et al. also found a positive correlation between CD4+T cell count and HDL-C ($r=0.247$ $p<0.01$) and a negative correlation between viral load and HDL-C level ($r=-0.21$ $p<0.03$) among currently untreated HIV positive patients. (24)

The pattern of dyslipidemia seen in treatment naïve and long-term untreated HIV patients is similar to that found in individuals with chronic bacterial, parasitic, and other viral infections. This has led researchers to investigate whether inflammation caused by HIV itself and HIV-related opportunistic infections are responsible for causing the observed dyslipidemia. (50,51) During HIV-infection there is increased CD4+ T cell activation, proliferation and death in response to the presence of the HIV virion. Activated CD4+T cells generate pro-inflammatory cytokines, which partially regulate lipid metabolism. In particular, circulating Interferon-alpha (INF-α) has been positively correlated to levels of serum triglycerides, specifically VLDL associated triglycerides. (52)

In terms of opportunistic infections leading to dyslipidemia, high levels of the pro-inflammatory cytokine Tumor Necrosis Factor- alpha (TNF-α) are found in HIV patients with concomitant infections. TNF-α has been linked to the depression of TC, LDL-C, and HDL-C levels. (31) The inflammation hypothesis is consistent with the finding that HDL-C concentration decreases are associated with low CD4+ T cell levels.

In addition to the inflammation hypothesis it is speculated that HIV may secrete a protein that prevents HDL-C mobilization from liver cells. (24) Another possibility is that HIV protein Nef impairs cholesterol efflux from infected macrophages, causing depletion in HDL-C levels. (53) This theory is consistent with the finding that higher viral load is associated with lower HDL-C concentrations.

2.4.3 Highly Active Antiretroviral Therapy and Dyslipidemia

The final factor complicating our understanding of the relationship between dyslipidemia and HIV is HAART, the combination therapy that includes a NRTI or NtRTI backbone and either PIs, NNRTIs or both. Much of the recent research

examining the association between HIV and dyslipidemia has focused on the role of HAART. Researchers have explored the hypothesis that HAART is associated with an excess risk of dyslipidemia through an examination of both the biological and the epidemiological evidence.

The mechanisms by which PIs (protease inhibitors) could cause dyslipidemia have been described. It is hypothesized that PIs bind to cytoplasmic retinoic-acid binding protein-1 (CRABP-1) and low-density lipoprotein receptor-related protein (LRP). The effect of PI binding to CRABP-1 is an increased rate of apoptosis of adipocytes and a reduced rate of differentiation of pre-adipocytes into adipocytes. This results in a reduction in triglyceride storage and an increase in lipid release, increasing the amount of circulating triglycerides. The effect of PIs binding to LRP is impaired endothelial triglyceride clearance and hepatic uptake of chylomicrons, which would also cause an increase in the amount of circulating triglycerides. (54) PIs are also hypothesized to mediate proteasome inhibition and the accumulation of the active portion of the sterol regulatory element binding protein (SREBP)-1c in liver cells and adipocytes, which would ultimately result in an increase in the amount of remnant lipoprotein returning to the liver. (55)

The dyslipidemia found in NNRTI (non-nucleoside reverse transcription inhibitors) treated individuals is speculated to be caused by an NNRTI mediated increase in hepatic synthesis of apolipoprotien A1 (a major component of HDL) and the resultant increased capacity for lipoprotein secretion from body cells. (21) The mechanism by which NRTIs (nucleoside reverse transcriptase inhibitors) are believed to cause dyslipidemia is more indirect than the other two drug classes. It is hypothesized that individuals treated with NRTIs experience some mitochondrial toxicity caused by the drug's interaction with the enzyme required for mitochondrial production, and this toxicity may cause adipocyte damage. (10,55,56)

To date several large epidemiological studies have been conducted in order to assess the relationship between HAART and dyslipidemia. Patients treated with HAART have been shown to have significantly higher levels of TC and triglycerides compared to HIV negative controls (45) and higher levels of TC, LDL-C, and triglycerides compared to non-HAART treated HIV-infected controls. (27,29,30,57)

The DAD study found that 25.3% of patients on some form of HAART had hypercholesterolemia, compared to only 7% of HIV-infected treatment naïve individuals. Similarly, compared to 15.2% of treatment naïve individuals, 37% of patients on some form of HAART had hypertriglyceridemia. (30)

In addition to producing findings similar to those of the DAD study, the research conducted by Kopple et al. showed that LDL-C levels are also higher in patients receiving HAART as compared to treatment naïve patients ($p<0.0001$). HAART treated patients in this study were also more likely to have LDL-C levels consistent with cardiac risk a compared to naïve patients ($p<0.0001$). (27)

HDL-C levels also appear to be moderately affected by HAART use. Riddler (2007) found that HDL-C levels increased by 0.11mmol/L [95%CI 0.06-0.17] 0.5yrs after HAART initiation, but report no further changes after that time. (29) According to Rose et al. patients treated for HIV had a higher risk of HDL-C levels consistent with cardiac risk as compared to uninfected controls, but not as high of a risk as patients untreated for HIV. This indicates that although HDL-C levels among patients treated with HAART may be raised compared to naïve patients they may not be raised above the threshold level that no longer represents a cardiac risk.

The effect that duration of exposure to HAART has on cholesterol levels is unclear. Riddler (2007) found that the odds of elevated total cholesterol increased significantly in the two years after HAART initiation (OR 1.81; 95%CI 1.0-2.32), but remained stable beyond two years of HAART exposure. (29) In contrast, a study conducted by Boulassel et al. found that the proportion of study subjects with hypercholesterolemia increased steadily over the eight year study period with no sign of reaching a plateau. (26) The stable period found by Riddler et al. may be partially explained by an increased use of lipid lowering therapy over time found in this study group.

There have been no studies reporting the effect of duration of exposure to HAART on triglyceride levels. However, studies have examined the effect of the duration of exposure to individual HAART classes on triglyceride levels. The results of these studies will be discussed in the section below.

2.4.3.1 Individual Class of ART and the Risk of Developing Dyslipidemia

While combination HAART has been examined for its role in the development of dyslipidemia, current epidemiological research has moved towards elucidating the role of individual classes of antiretroviral drugs in this association. Due to the biological plausibility that any drug class could cause dyslipidemia, there have been studies examining the individual effects of NRTIs, NNRTIs, and PIs. However, most studies have focused on PIs.

The findings of studies conducted to assess the association between dyslipidemia and PI therapy are all in agreement that PI use is associated with an increased risk of hypercholesterolemia. (24,26,30,31,58) A cohort study with a one year follow up found incident hypecholesterolemia in 25% of study subjects who started a PI containing regimen. (31) This finding is supported by the DAD prospective cohort study, which found hypercholesterolemia to be present among 27% of subjects taking PIs. (30) A cross- sectional study conducted by Pere et al. found that the adjusted odds ratio of hypercholesterolemia for patients on PIs was 4.04 (95%CI 2.12-7.74). (59)While the DAD study also reported an association between hypercholesterolemia and PI use, the effect was smaller than that found by Pere et al. (OR 2.35; 95%CI 1.92-2.87). (30)

An elevation in non-HDL-C levels has been shown to account for the increase in plasma TC associated with PI exposure. HDL-C levels have been found to be unchanged or remain low during PI therapy, while LDL-C levels significantly increase ($p=0.05$) during PI therapy. (23,24,58) These findings are consistent with studies of HAART that have found only minimal changes in HDL-C levels associated with treatment.

Associations have also been found between hypertriglyceridemia and PIs. (30,31,58,60) A five-year cohort study found that after initiation of a PI containing regimen the incidence of hypertriglyceridemia among the study population increased 6 fold. (60) The study conducted by Calza et al. found incident hypertriglyceridemia in 38.2% of patients treated with a PI. (31) Again, these results were consistent with

the findings of the DAD study, which found that 40% of its study population receiving PIs had hypertriglyceridemia.

The effect of duration of exposure to PIs on the development of hypercholesterolemia and hypertriglyceridemia has not been well studied. A prospective cohort study conducted by Montes et al. found that TC and triglyceride levels increased significantly within the first two months of initiating PI therapy. Triglyceride levels showed no significant increase after 2 months and TC levels continued to increase slightly until stabilizing at 6 months. (46) In contrast, both the DAD study group and Tsiodras et al. found a positive association between cholesterol levels and duration of PI exposure. (30,60) The DAD study reported an odds ratio for hypercholesterolemia of 1.42 ($p<10^{-4}$) per year of PI exposure. (30)

Studies examining the associations between hypercholesterolemia and NNRTI therapy have been limited and have produced conflicting results. A prospective cohort study conducted by Boulassel et al. found no significant association between the development of hypercholesterolemia and the use of NNRTIs. (26) In contrast, the DAD study reports the adjusted odds ratio of hypercholesterolemia for patients on NNRTIs to be 1.79 (95%CI 1.45-2.22). (30)

The DAD study also examined the association between hypertriglyceridemia and NNRTI use. In this study the use of NNRTIs was associated with a two-fold increase in the odds of having hypertriglyceridemia. (30)
This finding is consistent with the findings of a retrospective study conducted in Brazil, which found no significant differences between PI and NNRTI containing treatment regimens for the development of hypertriglyceridemia.(61)

The answer to the NNRTI controversy came in 2004 when the study conducted by van Leth et al. found that individual NNRTI drugs produced different lipid profiles. This study examining the two most commonly used NNRTI's, Nevirapine (NVP) and Efavirenz (EFV), showed that EFV treated patients had significantly higher increases in non-HDL-C ($p=0.007$) and triglycerides ($p<0.001$) compared to NVP treated patients. Therefore the conflicting results produced by previous studies may reflect differences in the prevalence of each NNRTI drug. Additionally van Leth et al. found that patients treated with NVP had increases in

HDL-C in the same order of magnitude as those seen with the use of the investigational HDL-C increasing drugs, suggesting that NVP may be cardio protective in comparison to EFV. (62)

Finally, studies have also examined the contribution of NRTIs, the backbone of HAART, to dyslipidemia. A prospective cohort study of individuals exposed to NRTIs with no current or previous exposure to any other class of ART found incident hypercholesterolemia and hypertriglyceridemia in 10.5 and 22.7% of subjects after a median of 2 years of NRTI exposure respectively. (56) Several studies have also noted that the risk of elevated cholesterol increases with the duration of exposure to NRTIs. (60,63) Tsiodras et al. reported that cholesterol levels of previously ART naïve patients increased by an average of 0.29% per month of exposure to NRTI therapy. (60) Specifically Stavudine (d4T) has been associated with an earlier time to and higher risk of hypercholesterolemia as well as a greater risk of developing hypertriglyceridemia compared to patients on alternative NRTI backbones ($p=0.04$). (28,56) In recent years patients taking NRTIs and suffering from dysplipidemia have had Tenofovir, a NtRTI, substituted for the NRTI. Tenofovir has been shown to improve the lipid profile of HIV-infected patients by decreasing non-HDL-C, LDL-C, TC and triglycerides. (64)

2.5 Interactions between Dyslipidemia Risk Factors in the HIV-Infected Population

An HIV-positive patient's risk of developing dyslipidemia may be multi-factorial with traditional, non-traditional, HIV and HAART related risk factors all contributing. Furthermore, many of the significant risk factors for dyslipidemia may not independently predict dyslipidemia. The literature describes well-defined relationships between some of the traditional risk factors for dyslipidemia (e.g. type-2 diabetes) and non-traditional, HIV, and HAART related risk factors. These relationships may make these covariates partially dependent or redundant markers of risk for dyslipidemia.

There are higher rates of diabetes among the HIV-infected population compared to the general population. (6,7,38,51,54,65,66) This may in part be

attributed to increased rates of smoking (67), increased prevalence of hepatitis C infection (68,69), increased prevalence of depression (70), the use of HAART (particularly PIs) (71) and HIV infection itself (50).

The literature on dylipidemias in the HIV-infected population has also recently begun to demonstrate the subtle interactions that may exist between the non-traditional, HIV, and HAART related risk factors. Immunodeficiency is a sign of advanced HIV disease, and it has been examined for its role as risk factor for dyslipidemia. There are factors beyond the viral infection affecting a HIV-infected patient's immune system and thereby increasing the risk of low HDL-C concentrations and hypertriglyceridemia. For instance, the severity of immunodeficiency could be exacerbated by cocaine use. Cocaine has been shown to decrease CD4+ cell count in women living with HIV (72,73) and increase viral load in both men and women living with HIV. (72,74)

Immunodeficiency may also influence the association between HAART use and risk of dyslipidemia, because physicians use CD4+T cell count and viral load as indicators for informing HAART initiation. The associations between cholesterol and triglyceride levels and CD4+ T cell count and viral load have been examined in both treated and untreated HIV positive patients. The DAD study found a positive association between CD4+T cell count and elevated levels of TC in patients treated for HIV, but not in treatment naïve patients, and a negative association between viral load and TC levels in both treated and treatment naïve patients. (4). This suggests that while the association between HAART and TC may not be confounded by CD4+ T cell count, it may be confounded by viral load. Triglyceride levels also appear to be positively associated with CD4+T cell count in treated, but not in treatment naïve patients, again suggesting that the association between HAART and triglycerides is not confounded by CD4+ T cell count. Triglyceride levels are also positively associated with viral load in both treated and treatment naïve patients. (30,48) This suggests that both HAART use and HIV infection contribute to the appearance of hypertriglyceridemia in people living with HIV.

2.6 Summary

To date, the limited literature that has been published in the area of HIV and risk of dyslipidemia suggests that there is an increased risk of abnormal cholesterol levels and hypertriglyceridemia among people living with HIV compared to the general population. Researchers have focused on these outcomes among people living with HIV for several reasons. First, it is well established that some traditional risk factors (male gender, smoking, and diabetes, depression and stress) and some non-traditional risk factors (cocaine use, hepatitis C co-infection) are more prevalent among people living with HIV. It is also well established that these traditional and non-traditional risk factors are associated with dyslipidemias in the general population. It is therefore reasonable to speculate that higher rates of these factors among people living with HIV may contribute to an increased risk of dyslipidemia.

Another reason that research in the field of HIV has focused on dyslipidemia is the biological plausibility behind the hypothesis that HIV itself could be causing an increased risk of HDL-C levels and hypertriglyceridemia consistent with an elevated cardiac risk, as shown in Figure 2. The mechanisms by which immunodeficiency and inflammation caused by HIV infection could be altering lipid metabolism have been described.

The final reason dyslipidemia among people living with HIV is being examined is the biological plausibility and epidemiological evidence behind the hypothesis that HAART (particularly the PI class of ART) could be increasing TC, LDL-C and triglyceride concentration to unhealthy levels in this population, as shown in Figure 2. The mechanisms by which the individual classes of ART are speculated to cause this dyslipidemia have been described. Moreover, several studies have found that HAART, PI, and NRTI exposure is associated with an increased risk of hypercholesterolemia and hypertriglyceridemia when compared to untreated people living with HIV serving as controls.

There are still several issues that remain unresolved in the current literature about dyslipidemia among people living with HIV. The impact of confounders such as smoking, cocaine use, co-infection with hepatitis C, and socio-demographic status on the risk of the dyslipidemias found among people living with HIV is unknown and will be

addressed in this study. Furthermore, this study will examine the relative contribution of traditional, non-traditional, HAART and HIV- related risk factors to changes in cholesterol and triglyceride levels.

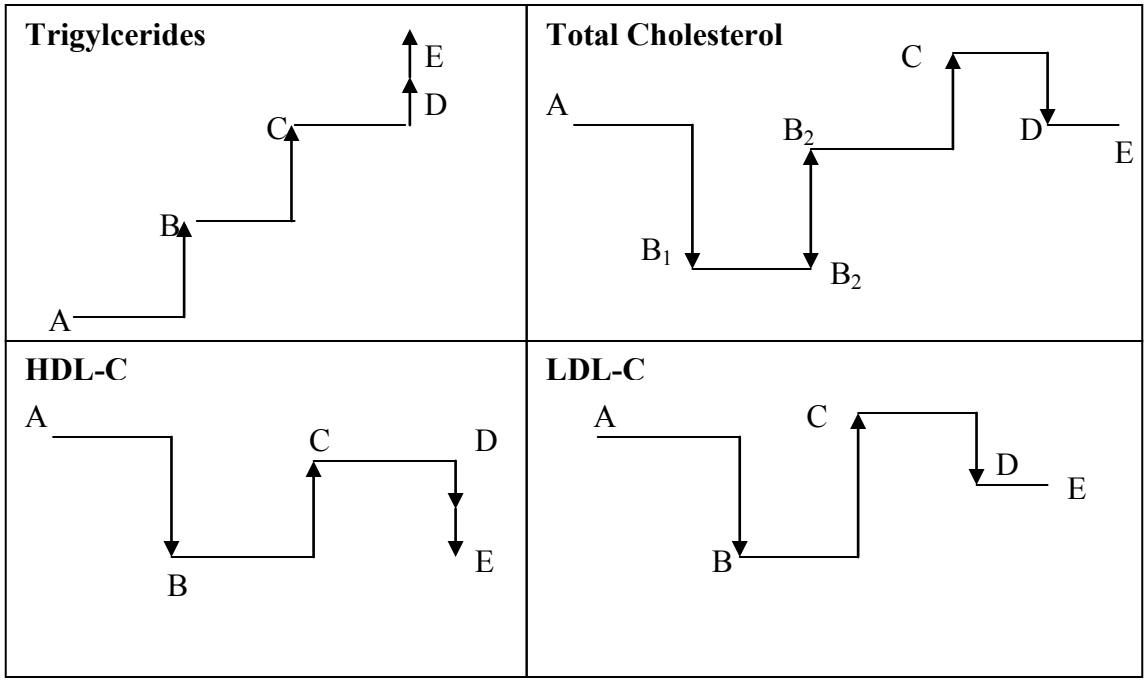


Figure 2. An overview of the lipid concentration changes that occur with HIV infection, HAART use and HAART interruption.
A) Pre-infection B) After infection with HIV C) After initiation of HAART D) 8 weeks after HAART interruption. E) 48 weeks after treatment interruption
*Total Cholesterol: B_1) Immediately after HIV infection total cholesterol concentration decreases. B_2) There is conflicting evidence on the effect of disease progression on total cholesterol concentration. Total cholesterol has been shown to increase with a history of an AIDS defining event and to decrease with an increasing viral load.

Chapter Three: Methods

3.1 Study Design

This research used a retrospective cohort design with repeated measurements to examine the risk factors for dyslipidemia in people living with HIV in Nova Scotia from 1997 to 2009. The Halifax HIV Clinic database was used to identify the study cohort and obtain demographic and descriptive information and blood lipid levels. Prior to the analysis of any data for this project, ethical approval was obtained from the Capital District Health Authority (CDHA) Research Ethics Board.

3.2 Study Participants

The participants of this study were identified using the Halifax HIV Clinic database, a database that contains records for all the people living with HIV in Nova Scotia who have been seen at the clinic since its inception in the early 1980s. The Halifax HIV Clinic is the major point of specialized care for people living with HIV in Nova Scotia. The database is organized according to clinic visits, and thus multiple records exist for individual patients, each patient identifiable by a unique medical record number. At the time of data collection, the database contained 3229 records with blood lipid measurements, entered into the Halifax HIV Clinic database between December 1997 and September 2009. Blood lipid levels were not routinely measured on HIV patients before 1997; for this reason the study period selected for observation was December 1997 to September 2009.

The participants for this study included patients of the Halifax HIV Clinic who had at least two measurements of blood lipid levels within 60 days of a clinic visit, and these two measurements could not be within 60 days of the same clinic visit. If there was more than one blood lipid measurement taken within sixty days of a clinic visit, then the measurement that occurred closest to a visit was used. After this criterion was applied 2456 records remained. All participant entries in the database were sorted line by line and any discrepancies or irregularities (dates, duplications, etc) were checked on a case-by-case basis. Every time a new clinic visit is entered into the database it is automatically assigned an assessment ID number, which is one unit larger than the assessment ID number assigned to the previous clinic visit entered into the database. For every patient,

the lower the assessment ID number is, the more remote in time the clinic visit. There were 28 records of a patient having multiple clinic visits with the same date and five instances of two clinic visits being associated with the same blood lipid measurement. When these two situations occurred the visit with the lower assessment ID was included. By selecting the visit with the lowest assessment ID among the visits with the same dates, we were able to choose the first clinic visit entered into the database with that date, which is also the clinic visit most likely to have occurred on the recorded date. There were five instances of blood lipid measurements being the same number of days before or after a clinic visit. When this occurred the blood lipid measurement with the lowest blood lipid ID was included. Similar to the assessment ID, the blood lipid ID is a number automatically assigned to a blood lipid measurement when it is entered into the database.

Finally, the initial criterion of each patient having at least two blood lipid measurements within 60 days of a clinic visit was re-applied to the remaining records because the previous data cleaning steps may have left some patients with only one blood lipid measurement. When this was done 92 records were excluded. As a result of the study inclusion criteria and data cleaning, the study database used for this research contained 2326 visit records for 343 patients, a total of 32 patients were excluded in this process.

3.3 Database

The Halifax HIV Clinic, located at the Victoria General site of the Queen Elizabeth II Health Sciences Centre, sees approximately 90% of people living with HIV in mainland Nova Scotia who are aware of their status. People living with HIV in Cape Breton attend a clinic in Sydney. Patients of the Halifax HIV Clinic are scheduled to visit an infectious diseases physician annually; during these visits the physician conducts a physical examination, a review of blood tests (CD4 count, viral load, lipids, etc), and an interview. All the demographic, descriptive and outcome information used in this study was drawn from the Halifax HIV Clinic database. The database contained a record of the initial clinic visit and at least one follow- up visit for most patients, including patients considered inactive or expired. The only visits that were relevant to this study were the initial visit, because it included the patient's social history, and the follow-up visits that

were associated with a blood lipid measurement. The data obtained from this database included: patient demographics (age, gender, sexual preference, level of education), traditional risk factors (BMI, type-2 diabetes, smoking, alcohol), non-traditional CHD risk factors (history of recreational drug use, hepatitis C co-infection), markers of disease progression (CD4+ cell count and viral load), history of ART use, and blood lipid levels (triglycerides, total cholesterol, HDL-C and LDL-C).

3.4 Inclusion Criteria

Residents of Nova Scotia living with HIV who were at least 18 years of age and patients of the Halifax HIV Clinic who had at least two serum lipid measurements recorded within sixty days of a clinic visit.

3.5 Outcome Variables

The outcome variables used in this study were repeated measurements of fasting lipids: total cholesterol, triglyceride, HDL-C and LDL-C concentrations in millimoles per liter (mmol/l) and total cholesterol to HDL ratio (TC:HDL-C). All lipid measures were examined for any errors that may have occurred during data entry and any measures that were not clinically possible were set as missing values; this included four triglyceride measurements and one total cholesterol measurement.

3.6 Selected Demographic, Traditional, Non-Traditional, HAART and HIV Risk Factor Variables

The variables under investigation in this study were divided into five categories: demographics (gender, sexual preference, level of education), traditional risk factors (diabetes, smoking, alcohol consumption, BMI) non-traditional risk factors (cocaine use, IV drug use, hepatitis C co-infection), HIV infection related factors (HIV viral load, CD4+Tcell count, duration of HIV infection), and HAART related risk factors (class of ART use). All of the information on these variables was obtained from the HIV Clinic database.

Detailed information regarding the sociodemographic variables (level of education and sexual preference) traditional risk factor variables (smoking and alcohol

consumption) and non-traditional risk factor variables (cocaine use and IV drug use) was obtained from patients during their initial visit to the HIV clinic during the years 1986-2009.

Within the database, alcohol use is not categorized, rather the field contains the physician's notes about the patient's alcohol consumption (i.e. social drinker, weekend drinker, number drinks/day, alcoholic, etc) therefore categories of alcohol consumption were created for the purposes of this study. Patients were categorized as being non-drinkers, light-moderate drinkers, or heavy drinkers. Men who had more than two drinks a day and women who had more than one drink a day were classified as heavy drinkers; any patients who were noted as alcoholics at the time of their initial visit were also classified as heavy drinkers. Patients who consumed alcohol but who were not heavy drinkers were classified as light-moderate drinkers.

Information on the remaining variables was time dependent and obtained at follow-up visits. At each clinic visit, the patient's weight in kilograms was measured. Body mass index (BMI), calculated as weight in kilograms divided by the square of height in metres (kg/m^2), was calculated from the height measured at the time of the initial visit and the weight measured at each clinic visit. In order to deal with the large number of patients missing either height or weight measurements BMI values were imputed by carrying values forward or backward if the BMI value was missing at the time of the first or last observation or by taking the average of the BMI values before and after the missing value. BMI values were then categorized according to the World Health Organization's international classification standards of obesity, overweight, normal weight and underweight.

At each clinic visit, the patient's current medications were recorded. A patient's diabetic status was determined by examining the medications they were taking at each clinic visit. If a patient was found to be on any of the medications listed in Table 1 he/she was considered to be diabetic from that visit onwards, even if there was no diabetes medication listed at their next follow-up visit. Although the use of lipid lowering drugs was not included as a variable in the analysis, it was measured in order to describe the study population. Patients were considered to be on a lipid lowering drug if they were taking any of the drugs listed in Table 2 at the time of any of their clinic visits. The lists

of medications in Tables 1, 2, and 3 were compiled by consulting the therapeutic guide and the brand and generic name index in the Compendium of Pharmaceuticals and Specialties (CPS) 2009 edition and an infectious diseases specialist, Dr. Lynn Johnston. (75)

Table 1. Medications used to identify patients with diabetes

Class	Generic Name(s)	Brand Name(s)
Biguanide	metformin	CO Metformin Coated, Glucophage, Glumetza, Mylan-Metformin, Novo-Metformin, Nu-Metformin, PMS-Metformin, RAN-Metformin, ratio-Metformin, Sandoz Metformin FC
Meglitinide	repaglinide	GlucoNorm
	nateglinide	Starlix
Sulfonylurea	chlorpropamide	
	glimepiride	Amaryl, ratio-Glimepiride, Sandoz Glimepiride
	gliclazide	Diamicron MR, Diamicron, Mylan-Gliclazide, PMS-Gliclazide
	glyburide	Diabeta, Mylan-Glybe Novo-Glyburide, Nu-Glyburide PMS-Glyburide, ratio-Glyburide, Sandoz Glyburide,
	tolbutamide	
Thiazolidinedione (Glitazone)	pioglitazone	Actos, CO Pioglitazone, Mylan-Pioglitazone, PMS-Pioglitazone, ratio-Pioglitazone, Sandoz Pioglitazone
	rosiglitazone	Avandia
Alpha Glucosidase Inhibitors	acarbose	Glucobay
Dipeptidyl Peptidase Inhibitors	sitagliptin	Januvia®
Combination Oral Diabetes Medications		Avandaryl
		Avandamet
		Janumet®
Insulin	insulin lispro	Humalog, Humalog Mix25, Humalog Mix50
	insulin determir	Levemir
	insulin aspart	NovoRapid, NovoMix 30
	insulin glargine	Lantus
	insulin gluisine	Apidra
	insulin NPH	Humulin-N, Novolinge NPH

Table 2. Medications used to identify patients receiving lipid lowering drugs

Class	Generic Name(s)	Brand Name(s)
Statin	atorvastatin calcium	Lipitor
	fluvastatin sodium	Lescol, Lescol XL
	lovastatin	CO Lovastatin, Mevacor, Mylan-Lovastatin, Nu-Lovastatin, PMS-Lovastatin, RAN-Lovastatin, ratio-Lovastatin, Sandoz Lovstatin,
	pravastatin sodium	CO- Pravastatin, Mylan Pravastatin, Novo-Pravastatin, Nu-Pravastatin, PMS Pravastatin, Pravachol, RAN- Pravastatin, ratio-Pravastatin, Sandoz Pravastatin
	rosuvastatin calcium	Crestor
	simvastatin	CO Simvastatin, Mylan-Simvastatin, Novo-Simvastatin, PMS-Simvastatin, Ratio-Simvastatin, Sandoz Simvastatin, Zocor
Selective cholesterol absorption inhibitors	ezetimibe	Ezetrol
Resins	cholestyramine	Cholestyramine, PMS-Cholestyramine
	colestipol	Colestid
Fibrates	gemfibrozil	Lopid, PMS-Gemfibrozil
	fenofibrate	Apo-Fenofibrate, Apo-Fen-Micro, Sandoz Fenofibrate S , Lipidil Supra, Mylan-Fenofibrate Micro, PMS- Fenofibrate Micro, Lipidil EZ, ratio Fenofibrate MC
	bezafibrate	
Niacin		
Combination Medications		Advicor Caduet

In addition to the list of concurrent medications recorded at each clinic visit, a list of the antiretroviral therapy (ART) the patient is receiving is also entered into the HIV clinic database. The database contains the drug names of the ART each patient is on at each visit. These drugs were categorized according to Table 3. The fusion inhibitors, integrase inhibitors, and CCR5 receptor antagonists, are relatively new therapies and therefore the number of records of patients receiving each of these therapies over the entire study period (4, 45, and 4 records respectively), was insufficient to include these drug classes as individual variables in the analysis. These classes of ART were combined to create the 'Other' category. Patients receiving combination therapy were considered to be receiving all classes of drugs contained in the combination treatment. Patients who did not have a prescription for ART at the time of a clinic visit were considered untreated at that visit.

Table 3. Classification of ART drugs

ART Class	Generic Name	Trade Name
NRTI	Zidovudine (AZT, ZDV, azidothymidine)	Retrovir
	Didanosine (ddI)	Videx , Videx EC
	Zalcitabine (ddC, dideoxycytidine)	Hivid
	Stavudine (d4T)	Zerit, Zerit XR
	Lamivudine (3TC)	Epivir
	Abacavir (ABC,1592)	Ziagen,
	Emtricitabine (FTC)	Emtriva, Coviracil
	Entecavir (INN)	Baraclude
	Apricitabine (ATC)	
NNRTI	Efavirenz	Sustiva, Stocrin
	Nevirapine	Viramune
	Delavirdine	Rescriptor
	Etravirine	Intelence
	MKC 442	Emivirine
PI	Saquinavir	Fortovase, Invirase
	Ritonavir	Norvir
	Indinavir	Crixivan
	Nelfinavir	Viracept
	Amprenavir (141W94)	Agenerase
	Lopinavir	Kaletra
	Atazanavir	Reyataz
	Fosamprenavir	Lexiva, Telzir
	Tipranavir	Aptivus
	Darunavir	Prezista
Fusion Inhibitor	Enfuvirtide (T20)	Fuzeon
Integrase Inhibitor	Raltegravir (MK0518)	Isentress
CCR5 Receptor Antagonist	Maraviroc	Selzentry, Celsentri
NtRTI	Tenofovir	
Combined Formulas	Zidovudine + Lamivudine	Combivir
	Abacavir+ Zidovudine +Lamivudine	Trizivir
	Lopinavir + Ritonavir	Kaletra (ABT-378/r)
	Abacavir + Lamivudine	Kivexia
	Emtricitabine + Tenofovir	Truvada
	Efavirenz + Emtricitabine + Tenofovir	Atripla

Near the time of each clinic visit a patients' viral load (number of copies of HIV RNA per milliliter of blood) and CD4+ T cell count (number of CD4+Tcells per cubic milliliter of blood) were measured. Patients often had their blood work done before or after a clinic visit and therefore viral load and CD4+ T cell count measurements that were recorded within 60 days of a clinic visit were associated with that clinic visit. If a patient had more than one record of blood work being done within this time period the record that was closest to the clinic visit was selected. Viral load had a very wide range of values and so this variable was transformed to a Log_{10} scale measurement.

3.7 Statistical Analysis

The statistical analyses included two parts: the descriptive statistics and analytic statistics. All analyses were conducted using Statistical Analysis System (SAS) version 9.2.

3.7.1 Descriptive Statistics

The descriptive analysis provides a basic characterization of the cohort. Descriptive statistics were performed on each variable of interest at baseline. Means, standard deviations, ranges and medians were calculated to understand the distribution of continuous variables. All independent continuous variables were normally distributed with the exception of BMI and viral load. With respect to the outcome variables, LDL-C was normally distributed, whereas HDL-C, total cholesterol, triglycerides and the TC:HDL-C ratio were not. The correlation between each of the outcomes variables was also assessed.

Additionally, means, standard deviations, ranges and medians were calculated for the calendar year of patients' first blood lipid measurements, the number of patients entered into the study during each year of the study period, the number of blood lipid measurements per patient, the number of months between clinic visits, and the number of months between patients' first positive test for HIV and their first blood lipid measurements, to understand their distributions. The number of participants who had high total cholesterol ($\geq$6.2mmol/L), and triglycerides ($\geq$1.7mmol/L), at the time of first serum lipid measurement was also calculated.

3.7.2 Analytic Statistics

Repeated measures linear mixed effects models (PROC Mixed in SAS) were constructed to assess the longitudinal association between the dependent and independent variables. The linear mixed effects method is suitable for the analysis of the longitudinal relationship between a continuous outcome variable and several time-dependent and time-independent covariates. This method is also appropriate for an unbalanced dataset that results from variation among individuals in the number and timing of observations. An advantage to repeated measures linear mixed effects models is that they allow the researcher to simultaneously estimate the fixed effects across patients and the random effects of individual patients. The repeated measures linear mixed effects model used in the present study can be written first as two parts representing the level-1 (within person) model and the level-2 (between-person) model (76):

Level-1: $Y_{ij=}\pi_{0j}+\pi_{1j}\,(TIME)_{ij}+r_{ij}$, where $r_{ij}\sim N(0,\Sigma)$

and

Level-2: $\pi_{0j=}\beta_{00}+\beta_{01}COVAR_j+\mu_{0j}$,

$$\pi_{1j}=\beta_{10}+\beta_{11}COVAR_j+\mu_{1j,} \quad \text{where} \quad \begin{pmatrix}\mu_{0j}\\ \mu_{1j}\end{pmatrix}\sim N\left[\begin{pmatrix}0\\0\end{pmatrix},\begin{pmatrix}\tau_{00} & \tau_{01}\\ \tau_{10} & \tau_{11}\end{pmatrix}\right]$$

i= 1…n where Y_i denotes the outcome (e.g. HDL-C concentration) for patient i at time j and n is the number of patients. β_{00} represents the average intercept and β_{10} represents the average slope. The time variable was the time elapsed since the first blood lipid measurement, therefore at first blood lipid measurement time=0. β_{10} represents the coefficient for the fixed effect covariate COVAR, which could be any of the covariates being assessed in the present study: this coefficient captures the relationship between the covariate and initial status. β_{11} represents the coefficient for the interaction between the covariate and time elapsed since the time of the first blood lipid measurement. The final two terms in this model, μ_{0j}, and r_{ij} represent the random effects (intercept, and the within person residual) error associated with the i^{th} patient at time j is given by r_{ij}. A time polynomial (the square of time since the patients first blood lipid measurement) was added to the model shown above in order to account for the fact that the relationship between the outcome variables and time is not linear.

The optimal covariance structure for the level-1, within-person, error variance-covariance matrix Σ was the spatial power structure. This structure was chosen over the simpler, more commonly used autoregressive (AR(1)) structure because of its ability to account for both the fact that lipid level values within individuals are correlated over time and the unequal timing of observations between patients and within a patient. The Kenward-Roger degrees of freedom calculation was used because the model contained both a random and repeated statement and the data were unbalanced. (77)

Separate analyses were conducted to assess the relationship between the independent variables and each dependent variable: total cholesterol, triglycerides, LDL-C and HDL-C concentration and TC:HDL-C ratio. The distributions of triglyceride and HDL-C concentrations and the TC:HDL-C ratio were not normal and therefore these dependent variables were transformed prior to the univariate and multivariate analyses. HDL-C concentrations and the TC:HDL-C ratio were winsorized by a factor of two standard deviations and triglyceride concentrations were measured on a Log_{10} scale. These transformations were necessary because a normal distribution is an assumption of the PROC Mixed procedure.

In a univariate analysis individual independent variables were assessed for their association with each of the dependent variables. All the univariate analyses were stratified by gender. Any independent variables found to be significantly associated with the dependent variable at a significance level of $p \leq 0.2$ in the univariate analysis were assessed in the multivariate model. The multivariate analysis was only conducted on men, as the number of women was insufficient to produce reliable results. The continuous variables age, BMI and duration of illness, used in the multivariate analysis were centered around the population mean to avoid problems with multicollinearity.

The multivariate analysis applied a block approach to repeated measures linear mixed effects regression model building. The analysis was done in two steps. In the first step (Figure 3) separate regressions were conducted for each of the five categories of risk factors (demographic, traditional, non-traditional, HIV, HAART) in order to identify the covariates within each category that were associated with the dependent variable ($p=0.05$). All regression analyses included a set of standard risk factors (SRF): age, time elapsed since first blood lipid measurement and the square of the time elapsed since the

first blood lipid measurement. Interactions between covariates were also assessed during step one by adding interaction terms to the model. Backwards selection based on a significance level of $p \leq 0.05$ was used to select the most parsimonious model.

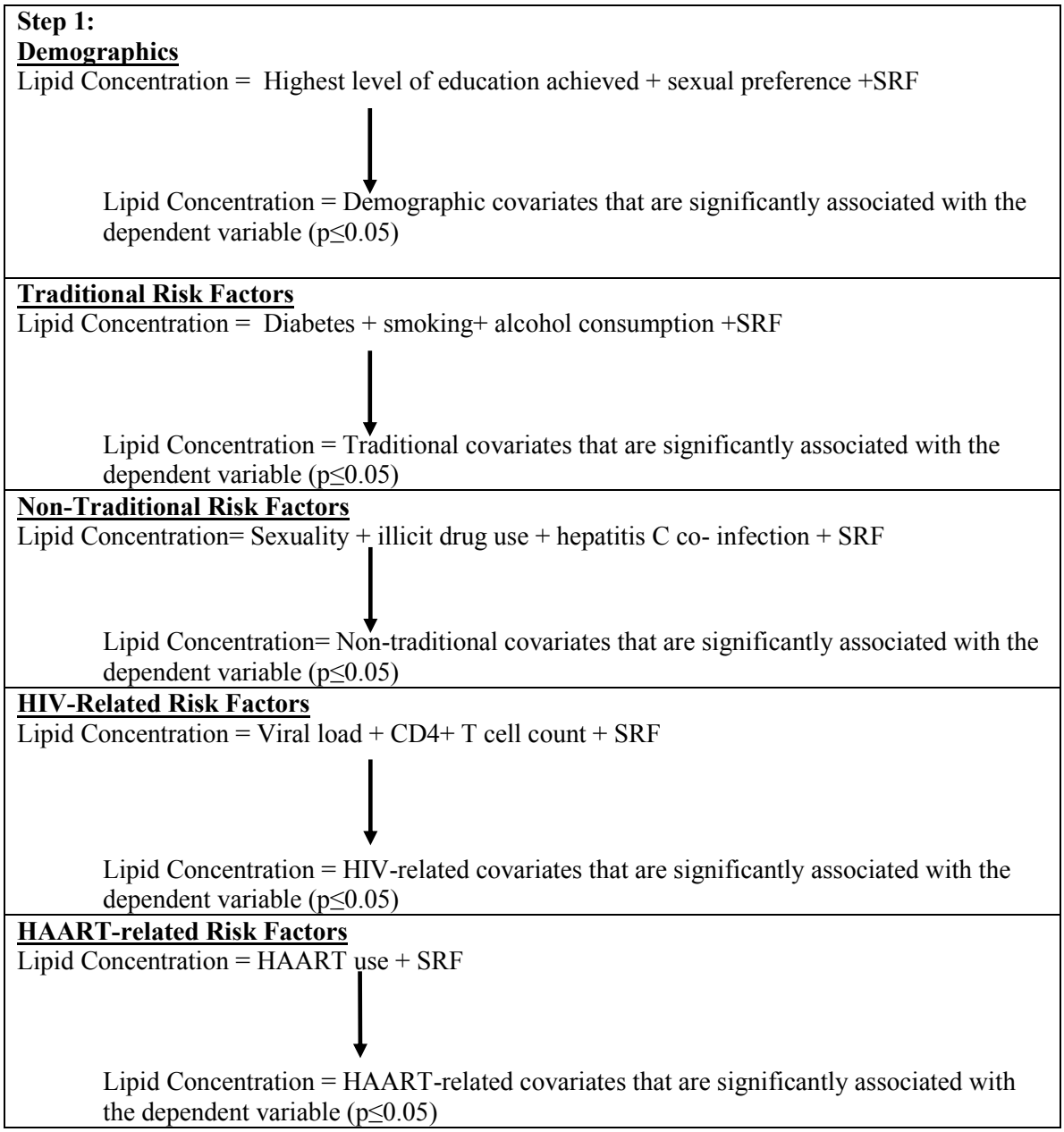


Figure 3. Block Regression Approach Used in the Multivariate Analysis. The first step of the multivariate analysis determined the predictors of lipid concentration included 5 separate regressions, one for each category of risk factor: demographic, traditional, non-traditional, HIV and HAART. Each regression was adjusted for standard risk factors (SRF): age, time since the patients first blood lipid measurement and the square of the time since the patients first blood lipid measurement.

Next, the covariates and any interactions identified as being significantly associated with the dependent variable in step one were added to a repeated measures linear mixed effects model and reassessed for their association with the dependent variables. Backwards selection using a significance level of $p \leq 0.05$ was applied in order to develop the most parsimonious model.

Chapter Four: Results

4.1 Descriptive Statistics

4.1.1 Clinic Visits

The study includes 343 patients who had a total of 2326 records of blood lipid measurements that could be associated with a unique clinic visit between 1997 and 2009. The mean calendar year for the first visit associated with a blood lipid measurement, signifying study entry, was 2002; the distribution of the number of patients entered into the study each year of the study period is presented in Figure 4. The mean and median numbers of records per patient were 6.7 and 5.0 respectively, and ranged from 2 to 30. Those patients with twenty or more records generally had two or three lipid measurements per year over a period of at least ten years. Approximately one quarter of patients (22.5%) had two records (Figure 5). The mean and median numbers of months between records were 11.9 and 7.6 respectively; there were eight records that occurred one month or less before the next record, and there were twenty-one records that occurred 60 months or more before the next record. Seventy six percent of the patients with 60 or more months between blood lipid measurements were followed at the clinic during the intervening years; they simply did not have any lipid measurements taken during that time. Only two of these patients did not have any clinic visits during the intervening years. There were fifty patients who had blood lipid measurements associated with their initial clinic visit; for the remaining 293 patients the median number of months between the initial clinic visit and the first blood lipid measurement was 34.8 and the mean was 54 months.

Time elapsed since a patient's first positive HIV test was determined at the time of the first blood lipid measurement. Three patients were missing data for time since first positive HIV test. The median number of months between a patient's first positive HIV test and their first blood lipid measurement was 54 and the mean was 73.2.

4.1.2 Baseline Cohort Demographics

Baseline characteristics of the study population are presented in Tables 4 and 5. Age was determined by calculating the age at the time of the first blood lipid measurement. Of the 343 patients in the cohort, one patient was missing data for gender, and one was missing data for age. More men (n= 303 ranging in age from 21 to 82) than

women (n=39 ranging in age from 23-70) were seen at the Halifax HIV clinic. At the time of the patient's first blood lipid test most women (51%) were in the age group 31-40, conversely nearly equal numbers (36 and 32%) of men were in the 31-40 and 41-50 age groups. The median age for men was 41, and the mean was 42. The median and mean age for women was 36 and 38 respectively.

During the interview portion of a patient's initial clinic visit sexual preference and highest level of education attained were elicited. There were six patients missing data for sexual preference and twenty-nine missing data for education. Most patients (58%) self-identified as homosexual (n=195). The highest level of education attained by most patients (40.2%) was university (n=126) (Table 4.)

4.1.3 Baseline Indicators of Dyslipidemia

The number of patients taking lipid-lowering drugs at the time of the first blood lipid measurement was determined by searching for any patients taking the medications listed in Table 3. There were fourteen subjects (4.1%) taking lipid-lowering drugs. The prevalence of clinically high cholesterol and triglycerides: total cholesterol $\geq$ 6.2mmol/L, triglycerides $\geq$1.7mmol/L as determined by NCEP ATP III, was also determined for the time of the first blood lipid measurement. (20) There were two patients who were missing data for triglycerides and two patients who were missing data for cholesterol. A total of 180 (52.8%) patients had hypertriglyceridemia and 61 (17.8%) had hypercholesterolemia at the time of their first blood lipid test. Among patients receiving treatment for their disease at the time of the first blood lipid measurement the prevalence of hypercholesterolemia and hypertriglyceridemia was 58.3 % and 22.1% respectively. Among patients not receiving treatment for their disease at the time of the first blood lipid test the prevalence of hypertriglyceridemia and hypercholesterolemia was 40.7% and 8.3% respectively.

4.1.4 Baseline Blood Lipid Levels

Table 6 presents the median total cholesterol, triglyceride, LDL, and HDL concentrations for men and women at baseline. One man and one woman were missing data for triglyceride concentration, two men were missing data for cholesterol concentration, sixty men and two women were missing data for HDL concentration and sixty-six men and four women were missing data for LDL concentration at baseline. The ratio of total

cholesterol to HDL-cholesterol (TC:HDL-C) was also assessed for men and women at baseline. There were sixty-one men and two women missing data for TC:HDL-C ratio. The mean and median TC:HDL-C ratio were 5.3 and 5.2 respectively for men and 4.0 and 3.7 respectively for women.

Table 7 presents a comparison of the mean total cholesterol, triglyceride, LDL and HDL concentrations for people living with HIV at baseline and participants of the 1995 Nova Scotia Health Survey (NSHS95) (a population based survery) stratified by gender. The results of the NSHS95 used in this comparison reflect the entire study group, which was representative of the Nova Scotian population by age and sex.

4.1.5 Distribution of Lipid Levels

Over the study period there were both between subject and within subject changes in lipid concentrations, as shown for triglycerides in Figure 6. The distributions of the mean concentration of triglycerides, total cholesterol, HDL-C, LDL-C, and the total cholesterol to HDL ratio appear to be linear over the entire study period, as shown in Figures 7-11. The results of a correlation analysis of the outcomes are presented in Table 8. All five of the outcome measures are significantly correlated with each other ($p<0.0001$). Due to the fact that TC and LDL-C were so highly correlated ($r^2= 0.759$) only LDL-C was used as an outcome.

4.2Analytic Results

Individual repeated measures linear mixed effects models were developed to assess the association between each of the variables listed in Tables 4 and 5 and each of the outcomes of HDL-C concentration, LDL-C concentration, TC: HDL-C ratio and triglyceride concentration for men and women separately. The results of these univariate analyses are presented in Tables 9 -35 and will be expanded upon in the following sections. Any variable that had a type 3 test of fixed effects significant at the $p \leq 0.2$ level in the univariate analysis was included the first step of the multivariate analysis (multivariate models assessing each of the outcomes were developed for each category of risk factor) and reassessed for significance at the $p \leq 0.05$ level. The results of the first step of the multivariate analysis are also presented in Tables 9-35. Tables 9-14, 16-20, 22-27, and 29-35 present the results of the univariate and the first step of the multivariate

analyses examining HDL-C, LDL-C, TC:HDL-C ratio and triglycerides respectively as an outcome. In order to facilitate the interpretation of these tables, Table 9, which presents the results of the univariate analysis of the association between demographic, traditional and non-traditional risk factors and HDL-C concentration for women living with HIV, has been partially interpreted (demographic risk factors only) in the following section.

The independent variables included in the univariate models included: the risk factor of interest, time since their first blood lipid measurement, the interaction between that risk factor and time since their first blood lipid test (these last two terms are standard to repeated measures mixed effects models), and the square of time since their first blood lipid test (accounts for non-linearity in the association between the risk factor variable and the outcome over time). The interpretation of the demographic risk factor results presented in Table 9 are as follows: for women living with HIV with each year increase in age, HDL-C concentration decreases by 0.0001mmol/l (Beta), however this association is not significant because the 95% confidence interval (CI) spans 1 and the type 3 p value is 0.98 ($p \leq 0.05$ is considered significant). With respect to sexual preference none of the women living with HIV included in this study self-identified as homosexual, therefore the results can be interpreted as follows: compared to women identifying as heterosexual, women identifying as bisexual have HDL-C concentrations that are 0.42 mmol/l lower, however this association is also not significant since the 95%CI spans 1 and the type 3 p value is 0.22. Finally, the association between highest level of education and HDL-C concentration among women living with HIV can be interpreted as follows: Compared to the referent of completing high school those who completed university had an HDL-C that was 0.18 mmol/l higher, and those who completed either trade school or grade school had an HDL-C that was 0.21 mmol/l higher. None of these associations are significant, as indicated by the 95%CI. The type 3 p value in this case is interpreted differently than in the examples above because this categorical variable has more than two categories and therefore the type 3 p value is not simply indicating if there is a significant difference compared to the referent category. The type 3 p assesses all possible combinations of the categories (i.e. university vs trade school, grade school vs university, trade school vs grade school etc) to determine if there is an overall significant difference. The type 3 p

value is not significant for the level of education in Table 9 and therefore overall there is not a significant difference in the HDL-C concentrations between of levels of education.

In the second step of the multivariate analysis any of the variables the had a type 3 test of fixed effect significant at the $p \leq 0.05$ level in step one were assessed in a single multivariate model. The results of the second step of the multivariate analyses of HDL-C, LDL-C, TC:HDL-C and triglycerides are presented in Tables: 15, 21, 28, and 36 respectively. Multivariate models were constructed for each of the outcomes for men only. Multivariate models were not constructed for women due to small numbers and the lack of significant associations found in the univariate analysis.

4.2.1 High Density Lipoprotein- Cholesterol Concentration

As shown in Table 15 a history of injection drug use (IDU), hepatitis C co-infection (HCV), viral load and the interaction between NNRTI and NtRTI use all remained significantly associated with HDL-C concentration in the final step of the multivariate analyses. Compared to the mean HDL-C concentration of patients not having a history of IDU, patients having a history of IDU had an HDL-C concentration that was 0.11mmol/l higher. HCV co-infection was initially associated with a 0.17mmol/l decrease in HDL-C concentration compared to the mean HDL-C concentration of mono-infected patients. However, over time HCV was associated with an increase in HDL-C, compared to mono-infected patients. Viral load was negatively associated with HDL-C, for every unit increase in HIV RNA the mean HDL-C concentration decreased by 0.036mmol/l. Taking an NNRTI but not an NtRTI was associated with an 0.081 mmol/l increase in HDL-C concentration compared to the mean HDL-C concentration of patients receiving neither an NNRTI, nor and NtRTI. Conversely, NNRTI use in conjunction with NtRTI use was associated with a 0.13mmol/l decrease in HDL-C concentration compared to the mean HDL-C concentration of patients receiving neither an NNRTI nor an NtRTI.

4.2.2 Low Density Lipoprotein –Cholesterol Concentration

As shown in Table 21 the only variables found to be significantly associated with LDL-C concentration in the final step of the multivariate modeling analyses were viral load and the interaction between viral load and CD4+ T cell count. The results presented in this table can be interpreted as follows: an individual with the study population mean CD4+T cell count was found to have a LDL-C concentration that decreased by 0.099

mmol/l with each unit increase in viral load. An individual with the study population mean viral load was found to have an LDL-C concentration that increased by 0.00013mmol/l with each unit increase in CD4+ T cell count.

4.2.3 Total Cholesterol to High Density Lipoprotein – Cholesterol Ratio

As shown in Table 28. The variables found to be significantly associated with TC:HDL-C ratio in the final step of the multivariate analyses were, BMI, the interaction between diabetes and time since the first blood test, the interaction between sexual preference and diabetes, the interaction between viral load and CD4+ T cell count and the interaction between NRTI and NNRTI use. BMI was the only independent predictor of TC:HDL-C ratio, compared to individuals with a BMI in the normal range individual with BMIs in the overweight and obese ranges had TC:HDL-C ratios that were 0.50mmol/l and 0.82mmol/l higher respectively. Also, patients whose BMIs were missing had significantly higher TC:HDL-C ratios than patients whose BMIs were in the normal range.

4.2.4 Triglyceride Concentration

As shown in Table 36, none of the risk factor variables assessed in this study were significantly independently associated with triglyceride concentration in the final step of the multivariate analysis. Interactions were found between viral load and time since first blood lipid test, CD4+ T cell count and time since first blood lipid measurement, viral load and CD4+ T cell count, CD4+ T cell count and duration of infection, viral load and protease inhibitor use and viral load and being untreated.

4.2.5 Demographics

As shown in Tables 24 and 31 men living with HIV and self-identifying as bisexual had a higher TC:HDL-C ratio but lower triglyceride concentrations compared to men self-identifying as heterosexual in the univariate (unadjusted) analysis. The positive association found between bisexuality and the TC:HDL-C ratio remained after adjusting for age, however the association between bisexuality and triglyceride concentration did not remain significant after adjusting for age. For women, neither sexual preference nor level of education was significantly associated with any of the outcomes in the univariate models.

4.2.6 Traditional Risk Factors

As shown in Tables 12 and 19, men living with HIV who were current smokers had higher HDL-C and LDL-C concentrations than non-smokers in the univariate analyses. However, these associations did not remain significant after adjusting for alcohol consumption (HDL-C) and BMI (HDL-C and LDL-C). Heavy alcohol consumption was associated with a higher HDL-C concentration and a lower TC: HDL-C ratio in the univariate analyses only. The association between HDL-C and alcohol consumption was no longer significant after adjusting for smoking status and BMI. The association between TC:HDL-C ratio and alcohol consumption was no longer significant after adjusting for diabetes status and BMI.

In the univariate models for men, having a BMI in the overweight range was found to be associated with a lower HDL-C concentration and a higher LDL-C concentration, and TC:HDL-C ratio compared to having a BMI that is in the normal weight range. After adjusting for other traditional risk factors, the associations between having a BMI in the overweight range and LDL-C concentration and TC:HDL-C ratio remained significant. However, only the association between having a BMI in the overweight range and TC:HDL-C ratio remained significant in the second step of the multivariate analyses, after adjusting for demographic, non-traditional, HIV and HAART-related risk factors. Having a BMI that is in the obese range was associated with a higher TC:HDL-C ratio and triglyceride concentration in the univariate analyses. Similar to having a BMI in the overweight range, having a BMI in the obese range remained significantly associated with an increase in TC:HDL-C both the model only adjusted for other traditional risk factors and in the fully adjusted model.

In the univariate analyses of traditional risk factors among women none of the traditional risk factors were significantly associated with any of the outcomes.

4.2.7 Non-Traditional Risk Factors

As shown in Tables 13 and 26, for men a history of injection drug use (IDU) was found to be associated with a lower TC:HDL-C ratio and a higher HDL-C concentration in the univariate models. While the association between a history of IDU and TC:HDL-C ratio did not remain significant in the fully adjusted model, a history of IDU did remain significantly associated with an increase in HDL-C concentration after adjusting for age,

cocaine use, hepatitis C coinfection, viral load, NNRTI use, NtRTI use and the time since the first blood lipid measurement. The mean HDL-C concentration in men with a history of IDU was 0.11mmol/l higher than the mean HDL-C concentration of patients without such a history after adjustment (Table 15).

In the univariate analyses of non-traditional risk factors among women none of the non-traditional risk factors were significantly associated with any of the outcomes.

4.2.8 HIV- Related Risk Factors

Among men as viral load increased all four of the outcomes assessed in the univariate analyses decreased. Viral load remained significantly associated with each of the outcomes after adjusting for demographic, traditional, non-traditional and HAART-related risk factors. In contrast, the univariate analyses of men found that as CD4+ T cell count increases so do HDL-C, LDL-C and triglyceride concentrations. These associations did not remained in the fully adjusted models. A longer duration of HIV infection was found to be associated with an increase in triglyceride concentration in the univariate and HIV-related risk factor adjusted models, however this association was not significant in the fully adjusted model.

Among women, viral load had a significant positive association with HDL-C concentration, the interaction between viral load and time since first blood lipid measurement was also significantly associated with HDL-C in the time-adjusted model.

4.2.9 HAART –Related Risk Factors

Among men NRTI and NNRTI use were both found to be associated with higher HDL-C, LDL-C and triglyceride concentrations in the univariate analyses, however only the association between NNRTIs and HDL-C remained significant in the fully adjusted model. NRTI use was not found to be significantly associated with TC:HDL-C ratio in the univariate analyses, however it was found to be significantly associated with a higher TC:HDL-C in the fully adjusted model.

PI use was found to be associated with higher LDL-C and triglyceride concentrations and TC: HDL-C ratio in the univariate analyses. Only the association between PI use and triglyceride concentration remained significant in the fully adjusted model.

NtRTI use was negatively associated with HDL-C concentration in the univariate analyses, however this association did not remain significant in the fully adjusted model. Being untreated was negatively associated with HDL-C, LDL-C and triglyceride concentrations and TC: HDL-C ratio in the univariate analyses. Only the association between being untreated and triglyceride concentration remained significant after adjusting for demographic, traditional, non-traditional and HIV-related risk factors.

The univariate analyses of HAART related risk factors among women revealed that women receiving PIs had higher triglyceride concentrations than those who were not receiving PIs.

4.2.10 Time Dependent Risk Factors

Several of the associations between the covariates and outcomes under investigation in this study varied over time. For men, the TC:HDL-C ratio of diabetics decreased by 0.15 compared to the mean TC:HDL-C ratio of non-diabetics with each year of observation after adjusting for age, diabetes, BMI, viral load, CD4+ T cell count, NRTI use, NNRTI use, time since the first blood lipid test and the square of the time since the first blood lipid test.

A time dependent association was also found between diabetes and HDL-C concentration. Men with hepatitis C co-infection were found to have a significantly lower initial HDL-C concentration compared to individuals without hepatitis C co-infection. However the HDL-C concentration of co-infected patients was found to increase more than mono-infected patients over the study period.

Finally, the associations between viral load and HDL-C and triglyceride concentrations and the TC:HDL-C ratio were found to be time dependent in the univariate analyses. Only the positive association between viral load and triglyceride concentration was found to be time-dependent after adjusting for demographic, traditional, non-traditional, HIV and HAART related risk factors. A significant positive time- dependent association was also found between triglyceride concentration and the CD4+ T cell count in the fully adjusted model.

4.2.11 Interactions

In the multivariate modeling stage of the analysis several interactions were identified. A significant interaction was found between NNRTI and NRTI use in the fully

adjusted multivariate model that assessed HDL-C concentration as the dependent variable. When LDL-C was assessed as the dependent variable an interaction between viral load and CD4+ T cell count was identified, this interaction was described in the LDL-C section of the results. Several interactions were found to be significantly associated with the TC:HDL-C ratio; interactions were found between sexual preference and diabetes, viral load and CD4+ T cell count and NRTI and NNRTI use. When triglyceride concentration was assessed as the dependent variable significant interactions were found between viral load and CD4+T cell count, CD4+ T cell count and duration of HIV infection, viral load and PI use, and viral load and being untreated.

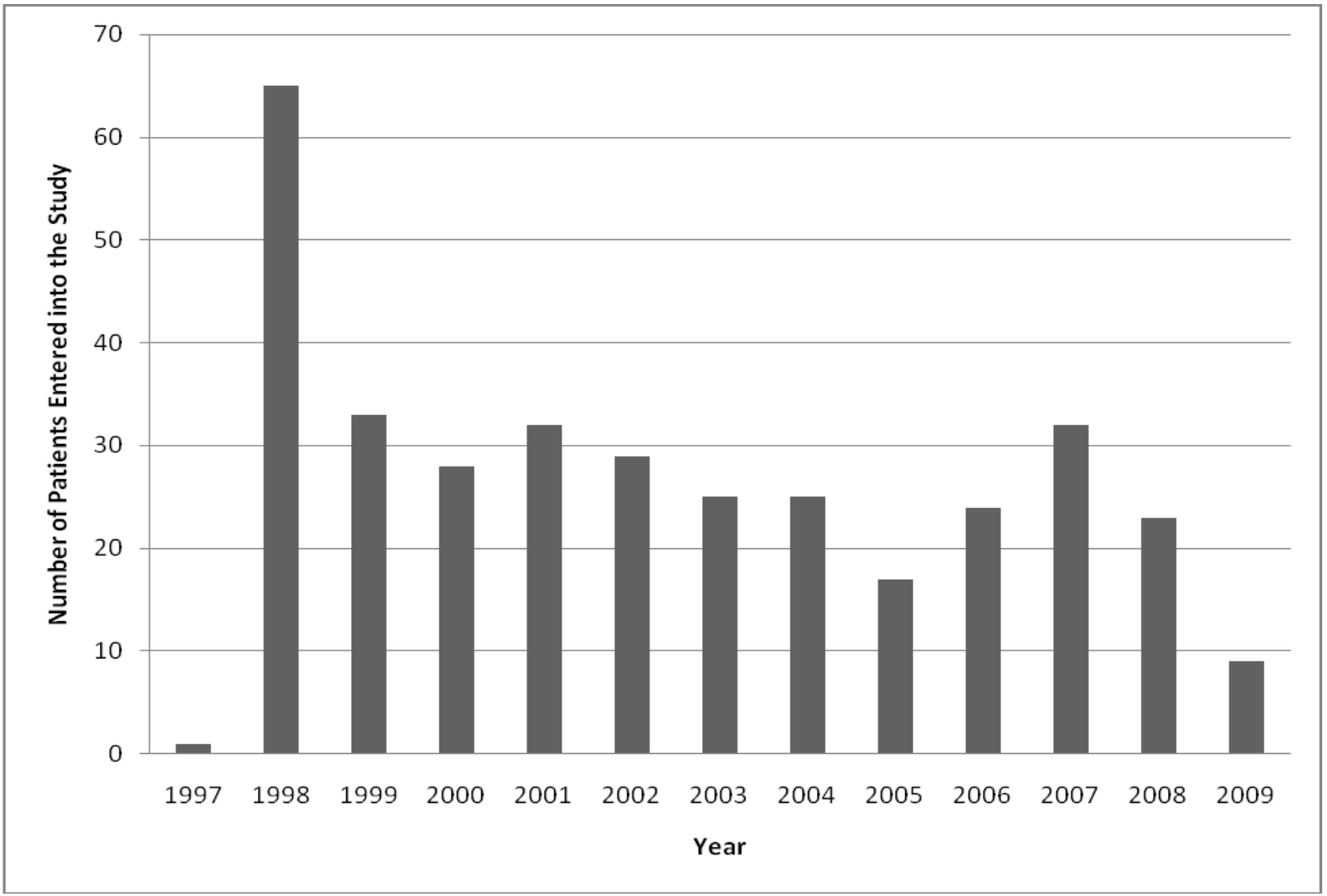


Figure 4. Distribution of the number of patients entered into the study each year of the study period.

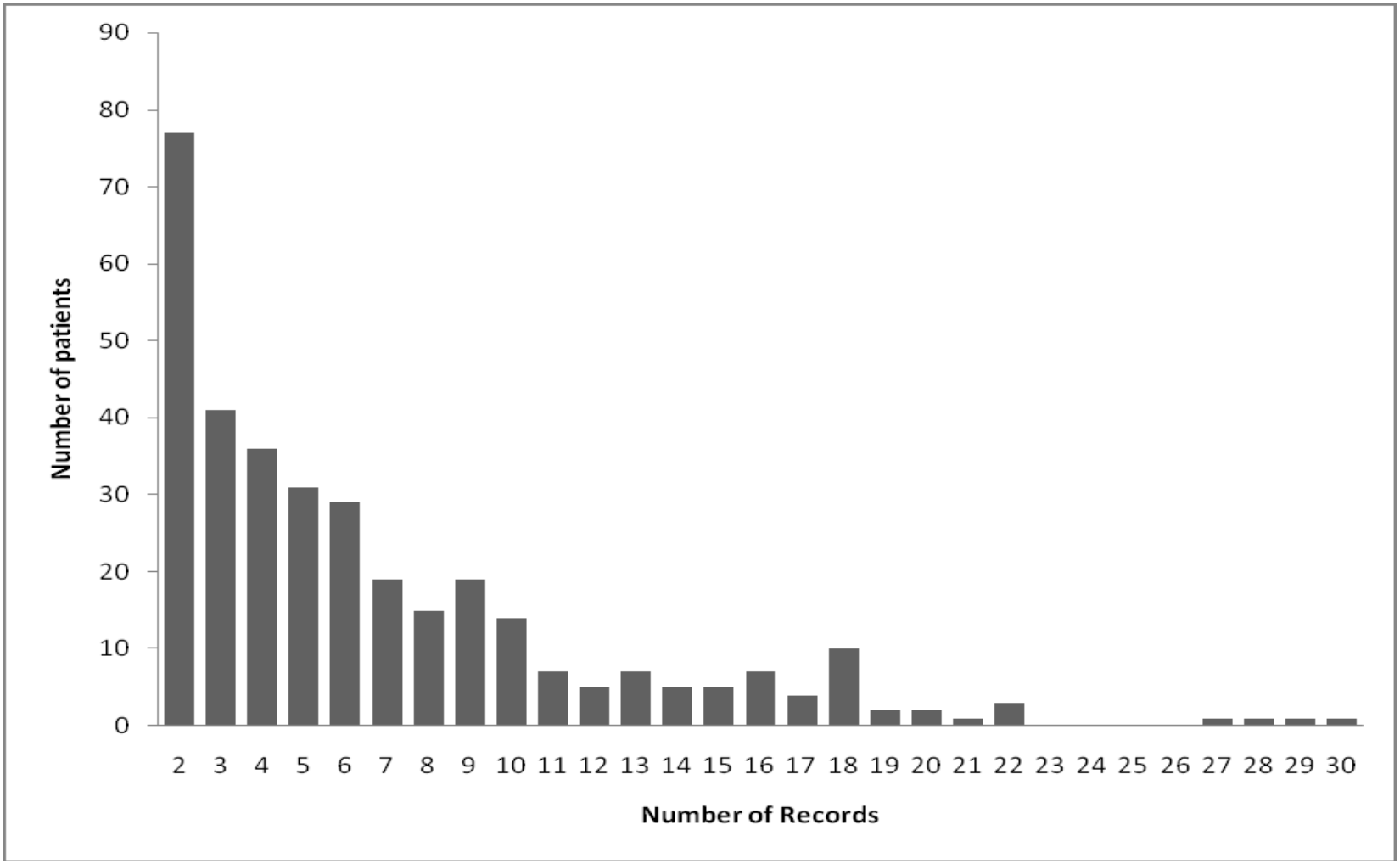


Figure 5. Distribution of the number of blood lipid measurements at distinct clinic visits per patient

Table 4. Baseline Demographic, Traditional and Non-Traditional Risk Factor Characteristics of Study Participants (HIV Clinic, Halifax, NS, 1997-2009)

	Men	Women
	Freq(%)	Freq (%)
Number of subjects n_{total}= 342	**303(100%)**	**39(100%)**
Demographics		
Age (n= 342)		
Median years (IQR)	41 (14)	36 (9)
Sexual Preference (n=337)		
Heterosexual	65 (21.8%)	37 (97.4%)
Homosexual	198 (66.4%)	0(0.0%)
Bisexual	35(11.8%)	1(2.6%)
Highest Level of Education(n=314)		
Grade school	38 (13.7%)	5(13.9%)
High school	83(30.0%)	17(47.2%)
Trade school	28 (10.1%)	4(11.1%)
University	128 (46.2%)	10(27.8%)
Traditional Risk Factors		
Diabetes (n=343)	9 (3.0%)	0(0.0%)
Smoking (n=336)		
Never smokers	65 (21.9%)	10(26.3%)
Previous smokers	72 (24.2%)	7(18.4%)
Current smokers	160 (53.9%)	21(55.3%)
Alcohol consumption (n=326)		
Never drinkers	64 (22.2%)	15(40.5%)
Light to moderate drinkers	187 (64.9%)	21(56.8%)
Heavy drinkers	37 (12.9%)	1(2.7%)
BMI (n=216)		
Underweight (<18.5)	5 (2.6%)	1 (4.0%)
Normal weight (≥18.5-≤24.9)	98 (51.3%)	13 (52.0%)
Overweight (≥25.0-≤29.9)	66 (34.6%)	5(20.0%)
Obese (≥30.0)	22 (11.5%)	6(24.0%)
Non-Traditional Risk Factors		
History of Injection DrugUse(n=334)	26 (8.8%)	11(28.9%)
History of Cocaine use (n=334)	15 (5.1%)	2(5.4%)
Hepatitis C co-infection (n=343)	17 (5.6%)	6(15.4%)

IQR: Interquartile Range

Table 5. Baseline HIV and HAART- Related Risk Factor Characteristics of Study Participants (HIV Clinic, Halifax, NS, 1997-2009)

	Men	Women
	Freq (%)	Freq (%)
Number of Subjects n_{total}=342	303 (100%)	39 (100%)
HIV- Related Risk Factors		
Viral load (n=298)		
Median log10 copies/ml (IQR)	2.7 (1.7)	3.2(2.1)
CD4+ cell count (n=315)		
Median cells/mm^3 (IQR)	366 (306)	367(342)
HAART- Related Risk Factors		
Class of Antiretroviral Therapy Exposure * (n=343)		
Currently untreated	90 (29.7%)	14(36.0%)
NRTIs	207 (68.3%)	25(64.1%)
NNRTIs	66(21.8%)	7 (17.9%)
Nevirapine	16 (5.3%)	2(5.1%)
PI	128 (42.2.%)	8 (20.5%)
NtRTI	12(4.0%)	1(2.6%)
Integrase, Fusion or CCR5 inihibitors	0 (0.0%)	0(0.0%)
HAART regimen (n=235)		
NRTIs only	28 (13.4%)	8(32.0%)
PIs only	3 (1.4%)	0(0%)
NRTIs and PIs	96(46.0%)	8(32%)
NRTIs and NNRTIs	37 (17.4%)	6(24%)
NRTIs and Nevirapine	9 (4.3%)	2(8.0%)
NRTIs and NtRTIs	1 (0.5%)	0(0%)
NRTIs, PIs and NNRTIs	18 (8.5%)	0(0%)
NRTIs, PIs and Nevirapine	7 (3.3%)	0(0%)
NRTIs, PIs and NtRTIs	3 (1.4%)	0(0%)
NRTIs, NNRTIs and NtRTIs	8(3.8%)	1(4.0%)
NNRTIs and PIs	1 (0.5%)	0(0%)
NNRTIs and unknown	2 (0.9%)	0(0%)

*The percentages do not add up to 100% due to the fact that patients are exposed to more than one type of ART

**NNRTIs refers to all drugs within this class of ART with the exception of nevirapine, which was included as a separate variable

IQR: Interquartile Range

Table 6. Mean and median lipid concentrations at time of first lipid measurement according to gender

Lipid	Men					Women				
	N	Mean (mmol/l)	SD	Median (mmol/l)	IQR	N	Mean (mmol/l)	SD	Median (mmol/l)	IQR
Triglycerides	302	2.77	3.22	1.93	1.84	38	1.69	1.26	1.34	0.89
Cholesterol	301	5.05	1.63	4.8	1.86	39	5.01	1.00	4.87	1.48
HDL-C	243	1.02	0.48	0.95	0.39	37	1.39	0.62	1.3	0.5
LDL-C	237	2.95	1.17	2.78	1.43	35	2.89	0.84	3.0	1.12

IQR: Interquartile Range

Table 7. Mean lipid concentrations at time of first lipid measurement and as reported by the 1995 Nova Scotia Health Survey (NSHS95) according to gender

	HIV Patients			NSHS95		
Men						
	N	Mean	SD	N	Mean	SD
Triglycerides (mmol/l)	302	2.77	3.22	1234	1.91	1.42
Total Cholesterol (mmol/l)	301	5.05	1.63	1234	5.29	1.08
HDL-cholesterol (mmol/l)	243	1.02	0.48	1214	1.14	0.28
LDL-cholesterol (mmol/l)	237	2.95	2.78	1193	3.31	0.89
Women						
	N	Mean	SD	N	Mean	SD
Triglycerides (mmol/l)	38	1.69	1.26	1243	1.60	1.02
Total Cholesterol (mmol/l)	39	5.01	1.00	1243	5.2	1.12
HDL-cholesterol (mmol/l)	37	1.39	0.62	1242	1.38	0.35
LDL-cholesterol (mmol/l)	35	2.89	0.84	1219	3.13	0.96

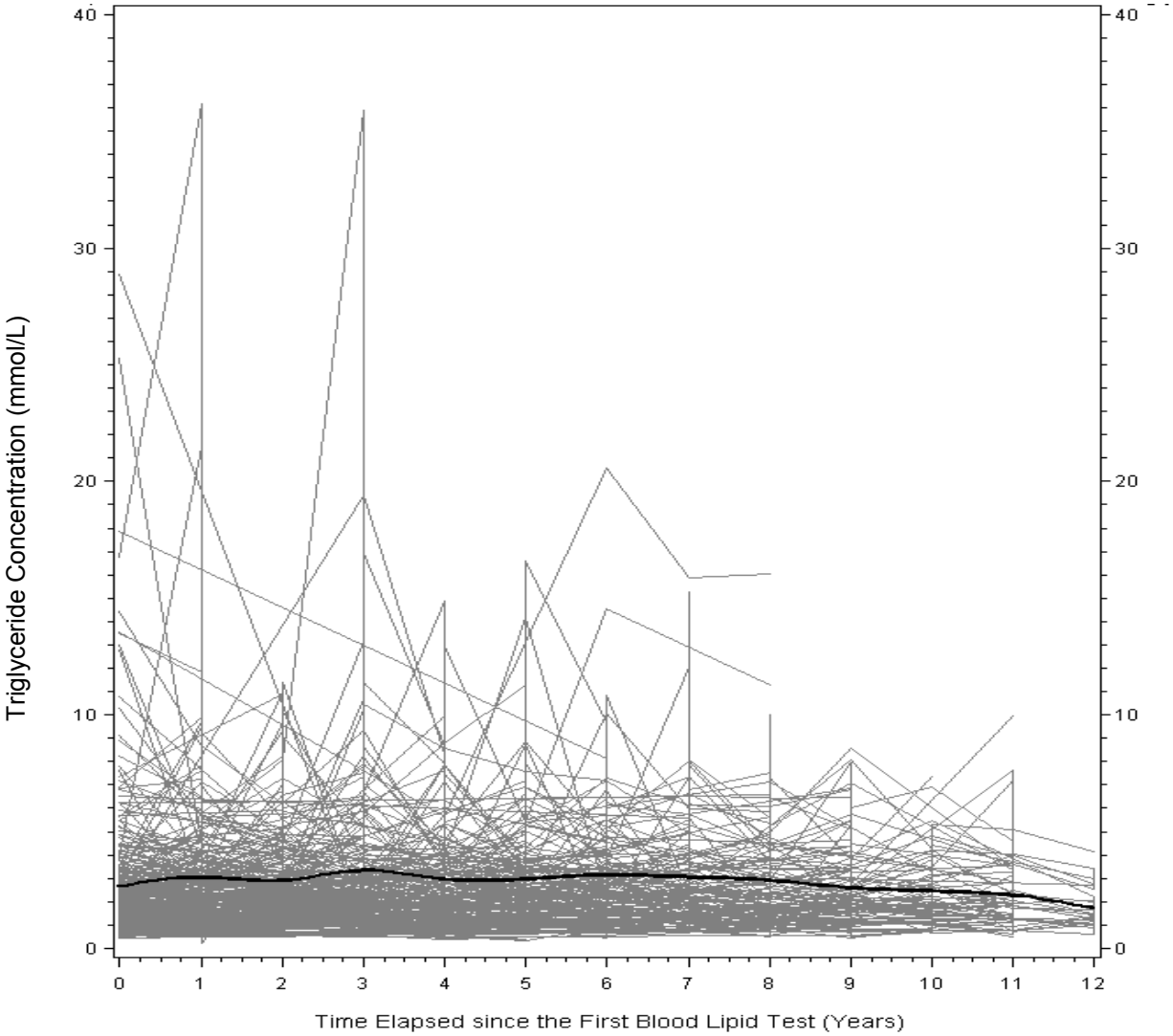
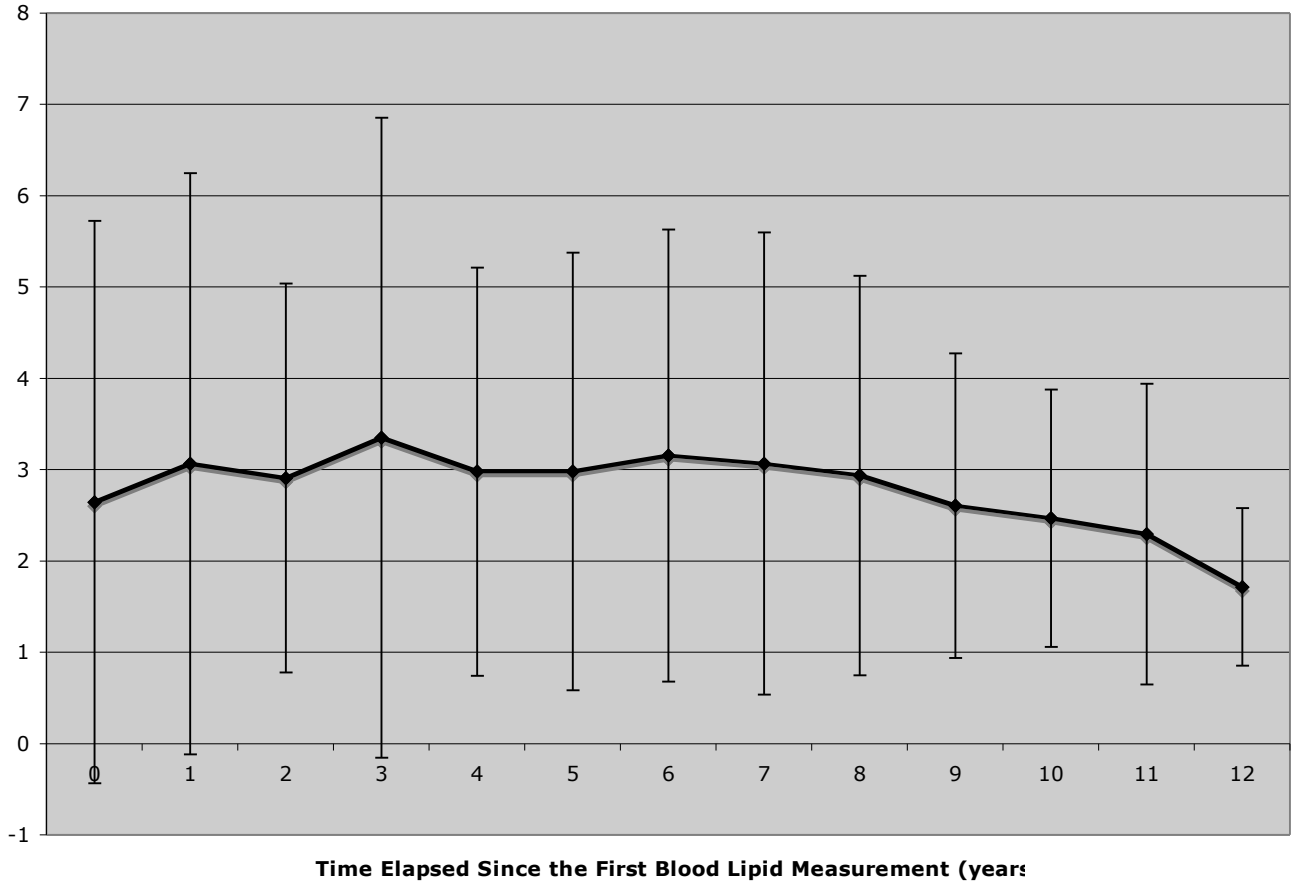
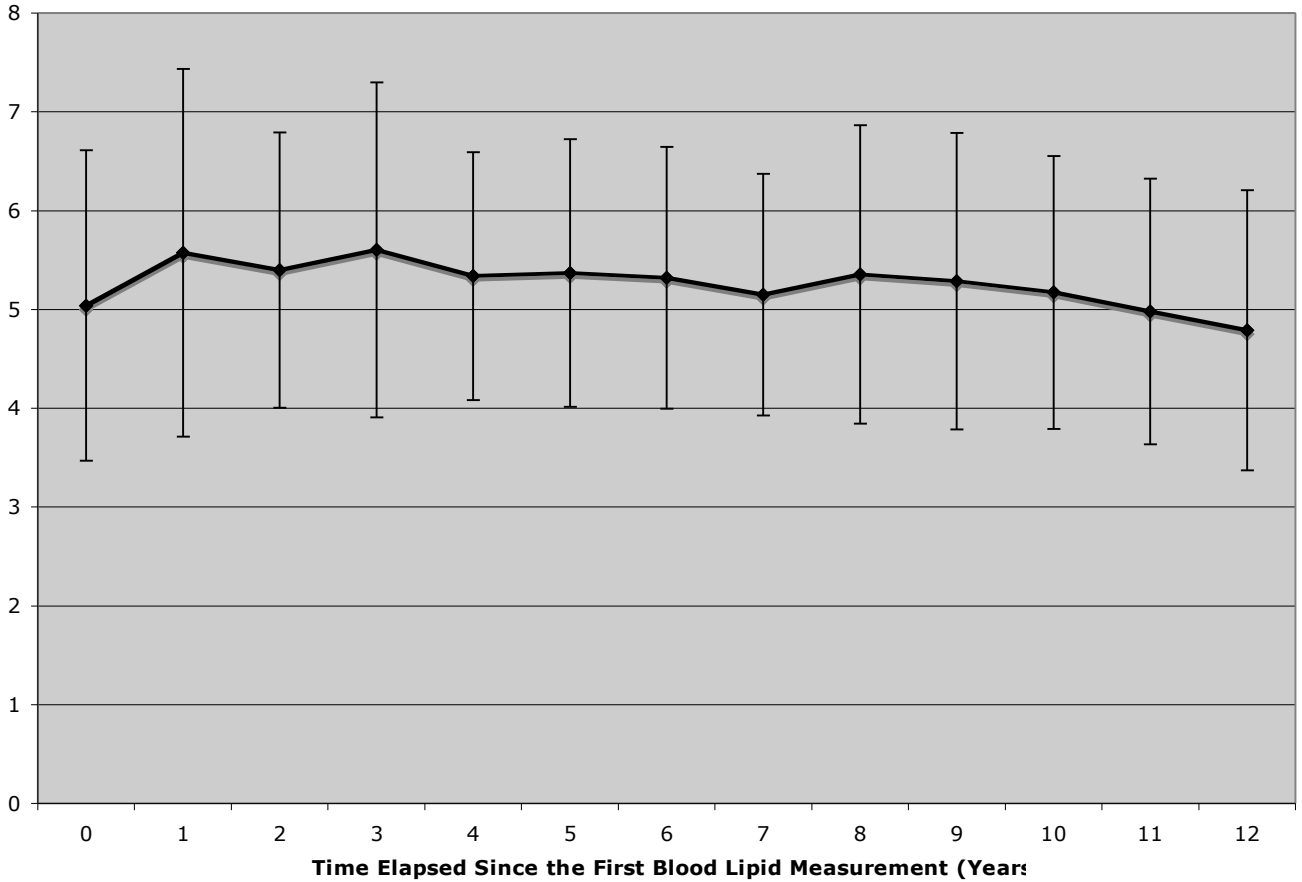


Figure 6. Individual triglyceride profiles with mean trend line



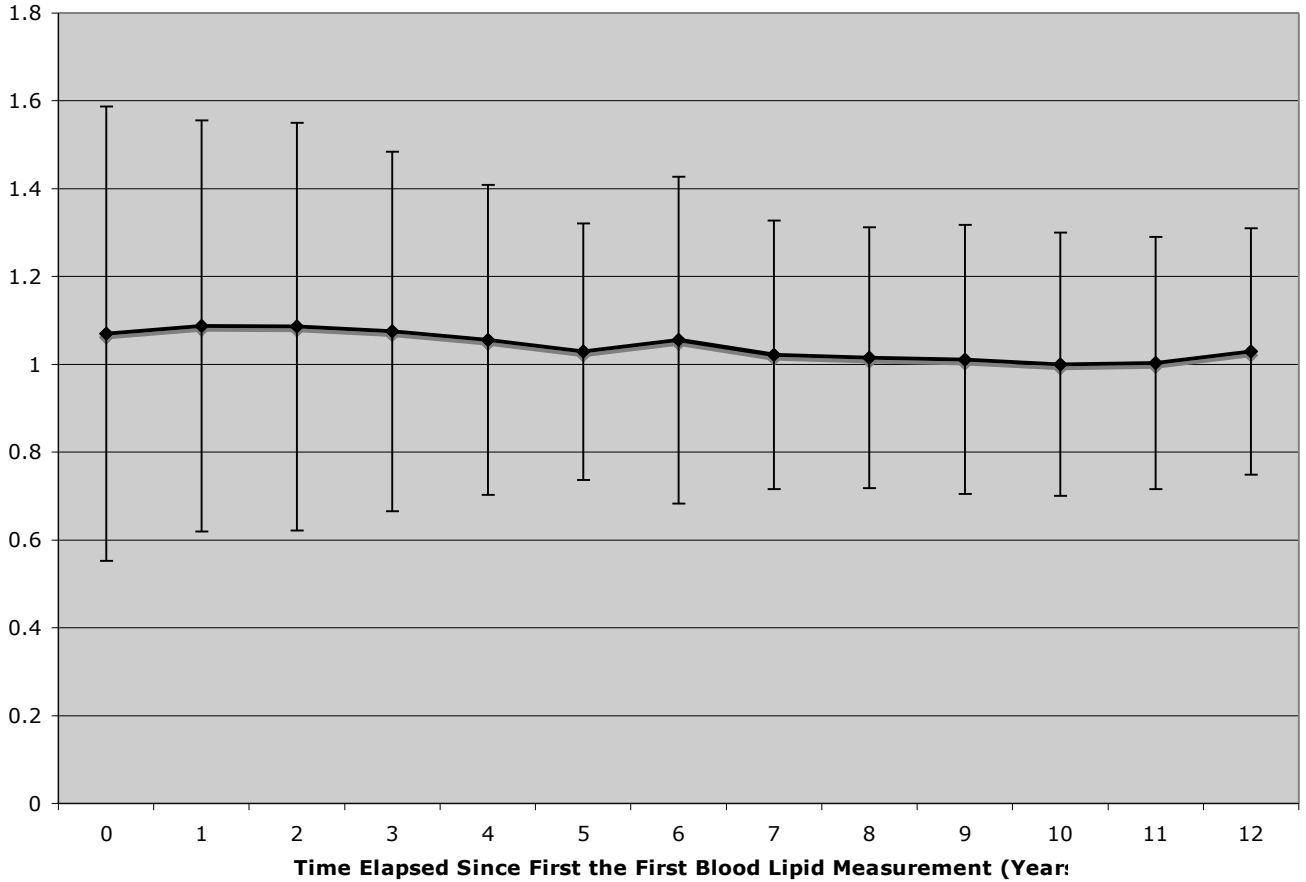
Number of measurements	**341**	**250**	**271**	**247**	**217**	**202**	**180**	**146**	**142**	**132**	**69**	**71**	**31**
Number of patients	**341**	**182**	**192**	**167**	**152**	**143**	**127**	**108**	**98**	**94**	**55**	**55**	**25**

Figure 7. Distribution of mean triglyceride concentration over the study period. Error bars indicate standard deviations.



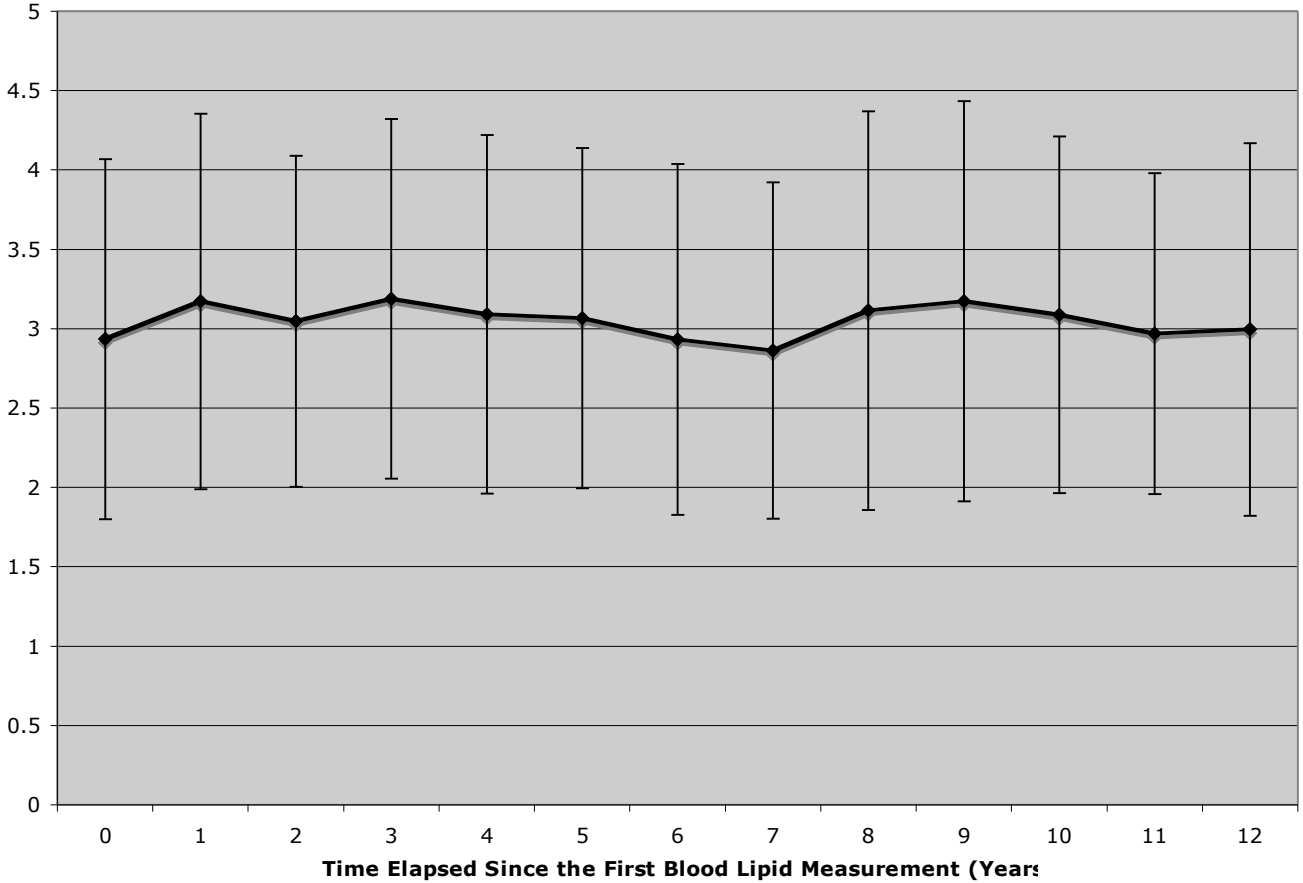
Number of measurements	**341**	**256**	**271**	**249**	**217**	**202**	**181**	**146**	**145**	**132**	**70**	**71**	**32**
Number of Patients	**341**	**164**	**193**	**169**	**153**	**142**	**126**	**108**	**99**	**94**	**56**	**55**	**26**

Figure 8. Distribution of mean total cholesterol concentration over the study period. Error bars indicate standard deviations



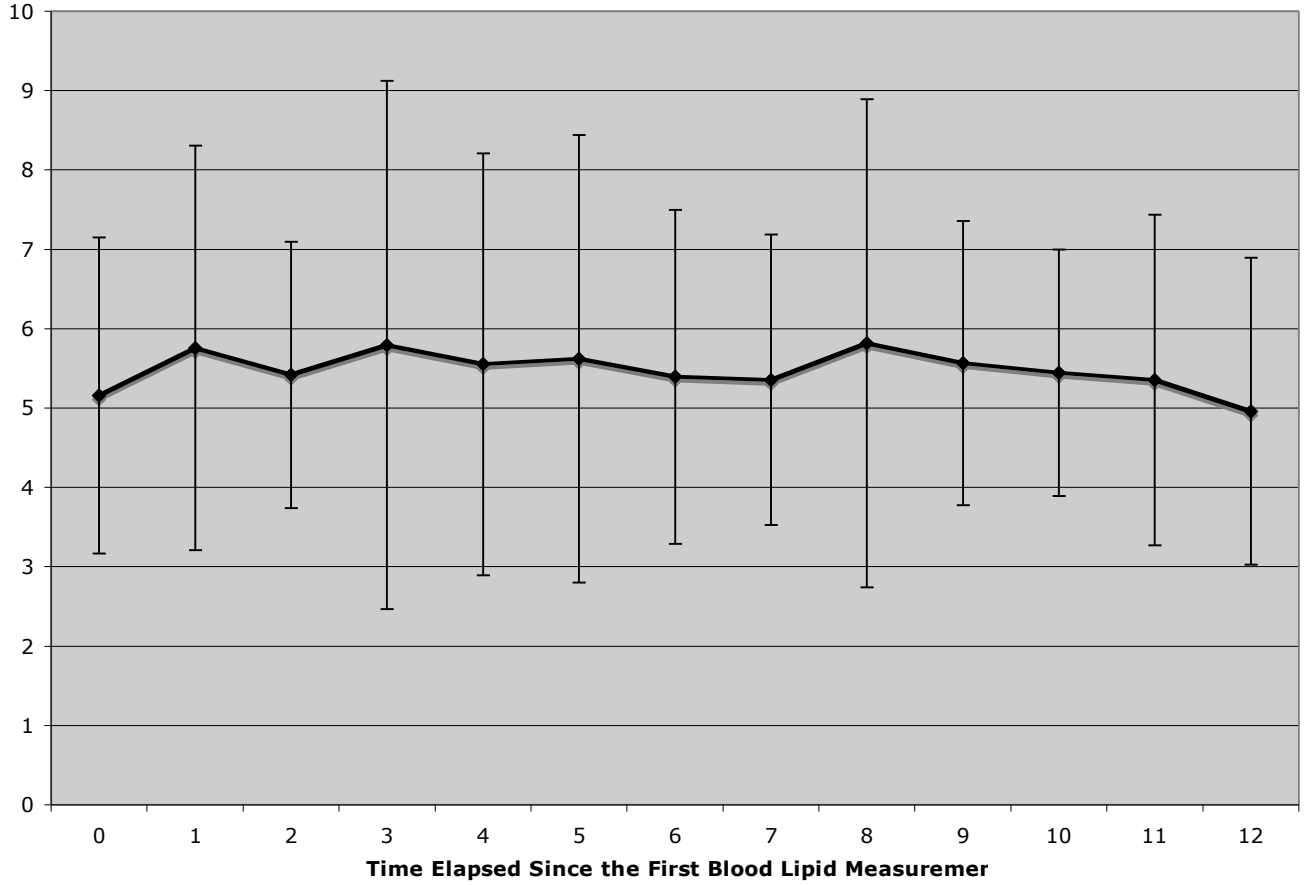
Number of measurements	**281**	**219**	**253**	**239**	**208**	**196**	**178**	**142**	**140**	**132**	**70**	**71**	**32**
Number of patients	**281**	**168**	**182**	**162**	**149**	**138**	**124**	**106**	**97**	**94**	**56**	**55**	**26**

Figure 9. Distribution of HDL-C concentration over the study period. Error bars indicate standard deviation.



Number of measurements	**273**	**204**	**241**	**232**	**203**	**195**	**174**	**139**	**137**	**131**	**69**	**70**	**31**
Number of patients	**273**	**164**	**177**	**160**	**146**	**138**	**122**	**104**	**94**	**93**	**55**	**54**	**25**

Figure 10. Distribution of LDL-C concentration over the study period. Error bars indicate standard deviation.



Number of measurements	**280**	**219**	**252**	**239**	**207**	**196**	**177**	**142**	**140**	**131**	**70**	**71**	**32**
Number of patients	**280**	**168**	**182**	**162**	**149**	**138**	**123**	**106**	**97**	**93**	**56**	**55**	**26**

Figure 11. Distribution of total cholesterol to HDL-C ratio over the study period. Error bars indicate standard deviation.1.5

Table 8. Correlation of outcomes at baseline. Correlation coefficients are Pearson correlation coefficients.

	Triglycerides	Total Cholesterol	HDL-C	LDL-C	TC:HDL-C
Triglycerides	**1**	0.47349	-0.18955	-0.10801	0.54206
Total Cholesterol		**1**	0.18435	0.75972	0.52496
HDL-C			**1**	0.09227	-0.50084
LDL-C				**1**	0.32778
TC:HDL-C ratio					**1**

*****All correlations are significant at $p<0.0001$

Table 9. Results of repeated measures linear mixed effects univariate models regarding the relationship of demographic, traditional and non-traditional risk factors and HDL-C concentration in women.

	Beta	Std Error	95% CI	Type 3 p
Demographics				
Age (years)	-0.0001	0.004	(-0.009,0.009)	0.98
Sexual Preference				
Homosexual	-	-	-	
Bisexual	-0.42	0.34	(-1.10,0.26)	0.22
Heterosexual	0	.	.	
Highest Level of Education				
University	0.18	0.18	(-0.19,0.54)	0.66
Trade school	0.21	0.22	(-0.25, 0.66)	
Grade school	0.21	0.17	(-0.13, 0.56)	
High school	0	.	.	
Traditional Risk Factors				
Diabetes	-0.053	0.25	(-0.55, 0.44)	0.83
Smoking				
Current	-0.17	0.12	(-0.42, 0.076)	0.37
Previous	-0.16	0.16	(-0.47, 0.16)	
Never	0	.	.	
Alcohol Consumption				
Heavy	0.49	0.33	(-0.16, 1.15)	0.11
Light-moderate	-0.13	0.10	(-0.33, 0.078)	
Non- drinker	0	.	.	
BMI (kg/m^2)				
Underweight	0.21	0.34	(-0.48, 0.90)	0.70
Overweight	-0.10	0.11	(-0.33, 0.12)	
Obese	-0.12	0.15	(-0.43, 0.18)	
Missing	-0.11	0.12	(-0.35, 0.12)	
Normal	0	.	.	
Non-Traditional Risk Factors				
History of Injection Drug Use	-0.049	0.12	(-0.28, 0.18)	0.67
History of Cocaine use	-0.039	0.25	(-0.54, 0.46)	0.88
Hepatitis C Co-infection	0.15	0.14	(-0.14, 0.44)	0.30

- : There were no women in this category.

¶ : Univariate model: HDL-C= Risk Factor+ Interaction between risk factor and time+ time+ $time^2$.

Table 10. Results of repeated measures linear mixed effects univariate models regarding the relationship of HIV and HAART- related risk factors and HDL-C concentration in women.

	Beta	Std Error	95% CI	Type 3 p
HIV-Related Risk Factors				
Viral Load (Log_{10} RNA Copies/µl) †	-0.066	0.028	(-0.12, -0.011)	0.019
CD4 +T cell count (cells/mm^3)	0.000028	0.00011	(-0.00018,0.00024)	0.79
Duration of HIV Infection	-0.013	0.010	(-0.033, 0.0079)	0.22
HAART- Related Risk Factors				
Class of ART exposure				
NRTI	-0.0070	0.070	(-0.14, 0.13)	0.92
NNRTI§	0.095	0.072	(-0.047, 0.24)	0.19
Nevirapine	0.054	0.13	(-0.20, 0.30)	0.67
PI	-0.10	0.070	(-0.24, 0.035)	0.14
NtRTI	0.090	0.14	(-0.18, 0.36)	0.65
Other (Integrase, Fusion or CCR5 inihibitors)	-	-	-	-
Untreated	0.000011	0.078	(-0.15, 0.15)	0.99

- : There were no women in this category.

†: Interaction between covariate and time since the first blood lipid measurement is significant ($p \leq 0.05$) in univariate model.

¶: Univariate model: HDL-C= Risk Factor+ Interaction between risk factor and time+ time+ $time^2$.

§ NNRTI refers to all drugs within this class of ART with the exception of Nevirapine, which was included as a separate variable

Table 11. Results of repeated measures linear mixed effects univariate and multivariate models regarding the relationship of demographic risk factors and HDL-C concentration in men.

	Univariate Models				Multivariate Model			
	Beta	Std Error	95%CI	Type 3 p	Beta	Std Error	95%CI	Type 3 p
Age (years)	0.001	0.002	(-0.002, 0.005)	0.42	NS	NS	NS	NS
Sexual Preference								
Homosexual	-0.03	0.04	(-0.12, 0.04)	0.18	NS	NS	NS	NS
Bisexual	-0.11	0.06	(-0.23, 0.007)					
Heterosexual	0	.	.					
Highest Level of Education								
University	-0.02	0.05	(-0.13, 0.08)	0.23	NS	NS	NS	NS
Trade school	0.10	0.07	(-0.05, 0.24)					
Grade school	-0.03	0.06	(-0.14, 0.09)					
High school	0	.	.					

NS: covariate non-significant at the $p \leq 0.05$ level in multivariate model.

¶: Univariate model: HDL-C= Risk Factor+ Interaction between risk factor and time+ time+ time^2.

Table 12. Results of repeated measures linear mixed effects univariate and multivariate models regarding the relationship of traditional risk factors and HDL-C concentration in men.

	Univariate Models				Multivariate Model			
	Beta	Std Error	95%CI	Type 3 p	Beta	Std Error	95% CI	Type 3 p
Traditional Risk Factors								
Diabetes	0.046	0.052	(-0.056, 0.15)	0.38	-	-	-	-
Smoking								
Current	0.093	0.042	(0.011, 0.14)	0.075	NS	NS	NS	NS
Previous	0.048	0.048	(-0.047, 0.14)					
Never	0	.	.					
Alcohol Consumption								
Heavy	0.16	0.058	(0.043, 0.27)	0.024	0.17	0.076	(0.016, 0.31)	0.11
Light-moderate	0.041	0.041	(-0.038, 0.12)		0.14	0.055	(0.028, 0.24)	
Non- drinker	0	.	.		0	.	.	
BMI (kg/m^2)								
Underweight	0.13	0.082	(-0.032,0.29)	0.093	0.48	0.16	(0.18, 0.79)	0.27
Overweight	-0.063	0.032	(-0.13, -0.00066)		-0.0022	0.052	(-0.11,0.10)	
Obese	-0.047	0.047	(-0.14, 0.045)		-0.015	0.072	(-0.16,0.13)	
Missing	-0.060	0.037	(-0.13, 0.012)		0.080	0.067	(-0.052, 0.21)	
Normal	0	.	.		0	.		

- : covariate non-significant at $p \leq 0.2$ in univariate analysis, therefore not assessed in multivariate analysis.

NS: covariate non-significant at the $p \leq 0.05$ level in multivariate model.

¶: Univariate model: HDL-C= Risk Factor+ Interaction between risk factor and time+ time+ time^2.

Multivariate model: HDL-C= Alcohol consumption+ BMI+ interaction between alcohol consumption and BMI+ age+ time+ time^2 (the interaction in this multivariate model is significant ($p<0.05$).

Table 13. Results of repeated measures linear mixed effects univariate and multivariate models regarding the relationship of non-traditional risk factors and HDL-C concentration in men.

	Univariate Models				Multivariate Model			
	Beta	Std Error	95% CI	Type 3 p	Beta	Std Error	95%CI	Type 3 p
Non-Traditional Risk Factors								
History of Injection Drug Use	0.15	0.061	(0.034, 0.27)	0.012	0.21	0.064	(0.081, 1.04)	0.0013
History of Cocaine use†	-0.050	0.075	(-0.20, 0.098)	0.51	-0.078	0.073	(-0.22, 0.067)	0.28
Hepatitis C Co-infection	-0.081	0.061	(-0.20, 0.038)	0.18	-0.20	0.066	(-0.33,-0.070)	0.66

†: Interaction between covariate and the time since the first blood lipid measurement is significant ($p \leq 0.05$) in univariate model

¶: Univariate model: HDL-C= Risk Factor+ Interaction between risk factor and time+ time+ time^2.

Multivariate model: HDL-C= History of IV drug use+ history of cocaine use+ hepatitis C co-infection+ interaction between history of IV drug use and time+ interaction between hepatitis C co-infection and time+ interaction between cocaine use and hepatitis C co-infection+ age+ time+ time^2. (All the interactions present in the multivariate model are significant ($p<0.05$)).

Table 14. Results of repeated measures linear mixed effects univariate and multivariate models regarding the relationship of HIV and HAART- related risk factors and HDL-C concentration in men.

	Univariate Models				Multivariate Model			
	Beta	Std Error	95%CI	Type 3 p	Beta	Std Error	95%CI	Type 3 p
HIV Related Risk Factors								
Viral Load (Log_{10} RNA Copies/µl)	-0.041	0.0085	(-0.058,-0.024)	<.0001	-0.041	0.0055	(0.98, 1.1)	<.0001
CD4 +T cell count (cells/mm^3)	0.00014	0.000048	(0.000046,0.00024)	0.0037	NS	NS	NS	NS
Duration of HIV Infection	0.0041	0.0029	(-0.0015, 0.0097)	0.15	NS	NS	NS	NS
HAART Related Risk Factors								
Class of ART exposure								
NRTI†	0.10	0.021	(0.061, 0.14)	<.0001	NS	NS	NS	NS
NNRTI§	0.13	0.022	(0.083, 0.17)	<.0001	0.095	0.017	(0.061,0.13)	0.40
Nevirapine	0.039	0.038	(-0.036,0.11)	0.31	-	-	-	-
PI	0.027	0.021	(-0.015,0.069)	0.21	-	-	-	-
NtRTI	-0.066	0.037	(-0.14, 0.0062)	0.073	-0.033	0.022	(-0.076, 0.0094)	<.0001
Other (Integrase, Fusion or CCR5 inihibitors)	0.011	0.078	(-0.14, 0.16)	0.89	-	-	-	-
Untreated	-0.11	0.022	(-0.15, 0.064)	<.0001	-0.067	0.017	(-0.10,-0.033)	<.0001

- : covariate non-significant at $p \leq 0.2$ in univariate analysis, therefore not assessed in multivariate analysis.

NS: covariate non-significant at the $p \leq 0.05$ level in multivariate model.

¶: Univariate model: HDL-C= Risk Factor+ Interaction between risk factor and time+ time+ $time^2$.

HIV related risk factor multivariate model: HDL-C= Viral load + age+ time+ $time^2$.

HAART related risk factor multivariate model: HDL-C= NNRTI use+ NtRTI use + untreated+ interaction between NNRTI use and NtRTI use + age+ time+ $time^2$ (the interaction between NNRTI use and NtRTI use were significant ($p<0.05$).

§: NNRTI refers to all drugs within this class of ART with the exception of Nevirapine, which was included as a separate variable.

Table 15. Repeated measures linear mixed effects multivariate model for predicting HDL-C concentration in men living with HIV.

Covariate/ interaction	Beta	Std Error	p
History of IV Drug Use	0.11	0.055	0.048
Hepatitis C Co-infection	-0.17	0.063	0.0075
Hepatitis C Co-infection x Time since the first blood lipid measurement	0.025	0.020	0.019
Viral Load (Log_{10} copies RNA/μL)	-0.036	0.0057	<.0001
NNRTI[§]*NtRTI			
NNRTI=yes, NtRTI=no	0.081	0.017	<.0001
NNRTI=no, NtRTI=yes	-0.037	0.022	0.10
NNRTI=yes, NtRTI=yes	-0.13	0.043	0.0034

* All β estimates have been adjusted for all the variables listed in this table age, time and time^2.

§NNRTI refers to all drugs within this class of ART with the exception of Nevirapine, which was included as a separate variable.

Table 16. Repeated measures linear mixed effects univariate models regarding the relationship between demographic, traditional and non-traditional risk factors and LDL-C concentration in women.

	Beta	Std Error	95% CI	Type 3 p
Demographics				
Age (years) † *	0.032	0.012	(0.0081, 0.057)	0.0098
Sexual Preference				
Homosexual	-	-	-	
Bisexual	0.56	0.94	(-1.32, 2.44)	0.55
Heterosexual	0	.	.	
Highest Level of Education				
University	0.55	0.48	(-0.42, 1.53)	0.71
Trade school	0.28	0.60	(-0.92, 1.49)	
Grade school	0.33	0.46	(-0.59, 1.25)	
High school	0	.	.	
Traditional Risk Factors				
Diabetes	0.12	0.83	(-1.51, 1.76)	0.88
Smoking				
Current	-0.40	0.33	(-1.07, 0.27)	0.49
Previous	-0.30	0.43	(-1.16, 0.56)	
Never	0	.	.	
Alcohol Consumption				
Heavy	-0.90	0.95	(-2.79, 0.98)	0.63
Light-moderate	-0.0011	0.30	(-0.60, 0.59)	
Non- drinker	0	.	.	
BMI (kg/m^2)				
Underweight	-0.63	0.95	(-2.52, 1.25)	
Overweight	-0.056	0.37	(-0.79, 0.68)	0.74
Obese	-0.24	0.43	(-1.10, 0.62)	
Missing	0.25	0.33	(-0.40, 0.91)	
Normal	0	.	.	
Non-Traditional Risk Factors				
History of Injection Drug Use	-0.084	0.32	(-0.73, 0.56)	0.80
History of Cocaine use	1.27	0.67	(-0.052, 2.60)	0.060
Hepatitis C Co-infection	-0.25	0.42	(-1.086, 0.59)	0.55

†: Interaction between covariate and the time since the first blood lipid measurement is significant ($p \leq 0.05$) in univariate model.

*The time elapsed since the first blood lipid measurement is significant ($p \leq 0.05$).

¶: Univariate model: LDL-C= Risk Factor+ Interaction between risk factor and time+ time+ $time^2$.

Table 17. Results of repeated measures univariate models regarding the relationship of HIV and HAART- related risk factors and LDL-C concentration in women.

	Beta	Std Error	95% CI	Type 3 p
HIV Related Risk Factors				
Viral Load (Log_{10} RNA Copies/µl)	0.041	0.11	(-0.17, -0.25)	0.70
CD4 +T cell count (cells/mm^3)	-0.00051	0.00033	(-0.0012, 0.00014)	0.12
Duration of HIV Infection	0.0014	0.028	(-0.055, 0.057)	0.96
HAART Related Risk Factors				
Class of ART exposure				
NRTI	0.056	0.23	(-0.39, 0.50)	0.81
NNRTI[§]	0.43	0.24	(-0.038, 0.90)	0.071
Nevirapine	-0.10	0.39	(-0.86, 0.66)	0.80
PI	-0.096	0.25	(-0.58, 0.39)	0.70
NtRTI	0.13	0.45	(-0.76, 1.017)	0.78
Other(Integrase, Fusion or CCR inihibitors)	-	-	-	-
Untreated	0.07125	0.2567	(-0.44, 0.58)	0.7817

- : There were no women in this category.

¶: Univariate model: LDL-C= Risk Factor+ Interaction between risk factor and time+ time+ $time^2$.

§: NNRTI refers to all drugs within this class of ART with the exception of nevirapine, which was included as a separate variable.

Table 18. Results of repeated measures linear mixed effects univariate and multivariate models regarding the relationship of demographic risk factors and LDL-C concentration in men.

	Univariate Models				Multivariate Model			
	Beta	Std Error	95% CI	Type 3 p	Beta	Std Error	95%CI	Type 3 P
Age (years) †‡ *	0.013	0.0063	(0.00093, 0.026)	0.0353	NS	NS	NS	NS
Sexual Preference								
Homosexual	0.064	0.16	(-0.25, 0.38)	0.89	NS	NS	NS	NS
Bisexual	0.10	0.23	(-0.35, 0.55)					
Heterosexual	0	.	.					
Highest Level of Education								
University	0.26	0.20	(-0.14, 0.67)	0.21	NS	NS	NS	NS
Trade school	0.58	0.27	(0.046, 1.12)					
Grade school	0.24	0.22	(-0.19, 0.66)					
High school	0	.	.					

NS: covariate non-significant at the $p \leq 0.05$ level in multivariate model.

¶: Univariate model: LDL-C= Risk Factor+ Interaction between risk factor and time+ time+ time^2.

Table 19. Results of repeated measures linear mixed effects univariate and multivariate models regarding the relationship of traditional and non-traditional risk factors and LDL-C concentration in men.

	Univariate Models				Multivariate Model			
	Beta	Std Error	95% CI	Type 3 p	Beta	Std Error	95%CI	Type 3 p
Traditional Risk Factors								
Diabetes	-0.12	0.21	(-0.53, 0.29)	0.57	-	-	-	-
Smoking								
Current	0.33	0.16	(0.014, 0.64)	0.084	NS	NS	NS	NS
Previous	0.36	0.18	(-0.0028, 0.72)					
Never	0	.	.					
Alcohol Consumption								
Heavy	-0.23	0.22	(-0.67, 0.20)	0.51	-	-	-	-
Light-moderate	-0.15	0.16	(-0.46, 0.15)					
Non- drinker	0	.	.					
BMI (kg/m^2) †								
Underweight	-0.089	0.33	(-0.73,0.55)	0.16	-0.10	0.33	(-0.74, 0.54)	0.20
Overweight	0.29	0.13	(0.036, 0.54)		0.27	0.13	(0.014, 0.52)	
Obese	0.32	0.19	(-0.050,0.70)		0.34	0.20	(-0.041, 0.73)	
Missing	0.054	0.14	(-0.014, 0.076)		0.037	0.14	(-0.24, 0.32)	
Normal	0	.	.		0	.	.	
Non-Traditional Risk Factors								
History of Injection Drug Use	0.019	0.23	(-0.42, 0.46)	0.93	NS	NS	NS	NS
History of Cocaine use	-0.53	0.28	(-1.08, 0.028)	0.063	NS	NS	NS	NS
Hepatitis C Co-infection	-0.32	0.23	(-0.78, 0.13)	0.16	NS	NS	NS	NS

- : covariate non-significant at $p \leq 0.2$ in univariate analysis, therefore not assessed in multivariate analysis.
NS: covariate non-significant at the $p \leq 0.05$ level in multivariate model.
†: Interaction between covariate and the time since the first blood lipid measurement is significant ($p \leq 0.05$) in univariate model.
¶: Univariate models: LDL-C= Risk Factor+ Interaction between risk factor and time+ time+ $time^2$
Multivariate traditional risk factor model: LDL-C= BMI+ interaction between BMI and time+ age+ time+ $time^2$ (the interaction in this multivariate model is significant ($p<0.05$).

Table 20. Results of repeated measures linear mixed effects univariate and multivariate models regarding the relationship of HIV and HAART- related risk factors and LDL-C concentration in men.

	Univariate Models				Multivariate Model			
	Beta	Std Error	95% CI	Type 3 p	Beta	Std Error	95% CI	Type 3 p
HIV Related Risk Factors								
Viral Load (Log_{10} RNA Copies/µl)	-0.18	0.036	(-0.25,-0.12)	<.0001	-0.99	0.025	(-0.15,-0.049)	<.0001
CD4 +T cell count (cells/mm^3)	0.00046	0.00019	(0.000081, 0.00084)	0.018	0.00013	0.00013	(-0.00013, 0.00040)	0.33
Duration of HIV Infection	-0.00800	0.011	(-0.029, 0.013)	0.46	-	-	-	-
HAART Related Risk Factors								
Class of ART exposure								
NRTI†	0.32	0.090	(0.15, 0.50)	<.0001	0.23	0.064	(0.11, 0.36)	0.66
NNRTI§	0.21	0.091	(0.031, 0.39)	0.021	0.41	0.17	(0.082, 0.74)	0.020
Nevirapine	0.037	0.16	(-0.27, 0.35)	0.81	-	-	-	-
PI	0.23	0.087	(0.062, 0.40)	0.0075	NS	NS	NS	NS
NtRTI	-0.059	0.15	(-0.35, 0.23)	0.69	-	-	-	-
Other (Integrase,Fusion or CCR5 inihibitors)	-0.14	0.33	(-0.52, 0.79)	0.68	-	-	-	-
Untreated	-0.34	0.090	(-0.51, -0.16)	0.0002	NS	NS	NS	NS

- : covariate non-significant at $p \leq 0.2$ in univariate analysis, therefore not assessed in multivariate analysis.

NS: covariate non-significant at the $p \leq 0.05$ level in multivariate model.

†: Interaction between predictor variable and the time since the first blood lipid measurement is significant ($p \leq 0.05$) in univariate model.

¶: Univariate model: LDL-C= Risk Factor+ Interaction between risk factor and time+ time+ $time^2$.

HIV related risk factor multivariate model: LDL-C= Viral load + CD4 T cell count+ interaction between viral load and CD4 T cell count +age+ time+ $time^2$ (the interaction between viral load and CD4 T cell count was significant ($p<0.05$)).

HAART related risk factor multivariate model: LDL-C= NRTI use+ NNRTI use + interaction between NRTI use and NNRTI use + age+ time+ $time^2$ (the interaction between NRTI use and NNRTI use was significant ($p<0.05$)).

§: NNRTI refers to all drugs within this class of ART with the exception of Nevirapine, which was included as a separate variable.

Table 21. Repeated measures linear mixed effects multivariate model for predicting LDL-C concentration in men living with HIV.

Covariate/ Interaction	Beta*	Std Error	p
Viral Load (Log_{10} Copies RNA/ μL)	-0.099	0.025	<.0001
CD4+ T Cell Count (Cells/ mm^3)	0.00013	0.00014	0.33
Viral Load* CD4+ T Cell Count	0.00023	0.000087	0.0087

* All β estimates have been adjusted for all the variables listed in this table age, time and $time^2$

Table 22. Results of repeated measures linear mixed effects univariate models regarding the relationship between demographic, traditional and non-traditional risk factors and total cholesterol to HDL ratio in women.

	Beta	Std Error	95% CI	Type 3 P
Demographics				
Age (years) †	0.029	0.018	(-0.0082, 0.065)	0.13
Sexual Preference				
Homosexual	-	-	-	
Bisexual	1.46	1.40	(-1.33, 4.25)	0.30
Heterosexual	0	.	.	
Highest Level of Education				
University	0.53	0.72	(-0.93, 1.99)	0.54
Trade school	-0.19	0.80	(-1.99, 1.62)	
Grade school	-0.24	0.68	(-1.61, 1.14)	
High school	0	.	.	
Traditional Risk Factors				
Diabetes	0.62	1.12	(-1.60, 2.84)	0.58
Smoking				
Current	-0.057	0.49	(-1.051, 0.94)	0.74
Previous	0.37	0.63	(-0.90, 1.64)	
Never	0	.	.	
Alcohol Consumption				
Heavy	-1.45	1.38	(-4.21, 1.31)	0.13
Light-moderate	0.66	0.42	(-0.18, 1.50)	
Non- drinker	0	.	.	
BMI (kg/m^2)				
Underweight	-1.20	1.38	(-3.96, 1.55)	0.39
Overweight	0.59	0.50	(-0.41, 1.58)	
Obese	0.063	0.62	(-1.18, 1.30)	
Missing	0.65	0.46	(-0.27, 1.57)	
Normal	0	.	.	
Non-Traditional Risk Factors				
History of Injection Drug Use	0.041	0.46	(-0.88, 0.96)	0.93
History of Cocaine use	-0.50	0.79	(-1.35, 2.71)	0.52
Hepatitis C Co-infection	-0.53	0.58	(-1.69, 0.63)	0.37

†: Interaction between predictor variable and the time since the first blood lipid measurement is significant ($p \leq 0.05$).

¶: Univariate model: TC: HDL-C= Risk Factor+ Interaction between risk factor and time+ time+ $time^2$.

Table 23. Results of repeated measures linear mixed effects univariate models regarding the relationship of HIV and HAART- related risk factors and total cholesterol to HDL-C ratio in women.

	Beta	Std Error	95%CI	Type 3 p
HIV Related Risk Factors				
Viral Load (Log_{10} RNA Copies/µl)	0.025	0.14	(-0.25, - 0.30)	0.86
CD4 +T cell count (cells/mm^3)	-0.00050	-0.00050	(-0.0015, 0.00044)	0.30
Duration of HIV Infection	0.070	0.040	(-0.011, 0.15)	0.089
HAART Related Risk Factors				
Class of ART exposure				
NRTI	-0.067	0.059	(-0.099, 1.14)	0.26
NNRTI[§]	-0.065	0.065	(-0.15, 1.17)	0.32
Nevirapine	0.056	0.55	(-1.03, 1.14)	0.92
PI	-0.025	0.067	(0.25, 1.49)	0.71
NtRTI	0.0058	0.64	(-1.26, 1.27)	0.99
Other (Integrase, Fusion or CCR5 inihibitors or NtRTIs)	-	-	-	-
Untreated	-0.41	0.35	(-1.094, 0.27)	0.24

- : There were no women in this category.

¶: Univariate model: TC: HDL-C= Risk Factor+ Interaction between risk factor and time+ time+ $time^2$.

§: NNRTI refers to all drugs within this class of ART with the exception of Nevirapine, which was included as a separate variable

Table 24. Results of repeated measures linear mixed effects univariate and multivariate models regarding the relationship between demographic risk factors and total cholesterol to HDL-C ratio in men.

	Univariate Models				Multivariate Models			
	Beta	Std Error	95%CI	Type 3 p	Beta	Std Error	95%CI	Type 3 p
Age (years) †	0.016	0.0093	(-0.0021, 0.034)	0.083	0.0072	0.038	(0.0090, 0.023)	0.37
Sexual Preference								
Homosexual	0.41	0.23	(-0.030, 0.86)	0.0055	0.42	0.23	(-0.28, 0.86)	0.007
Bisexual	1.063	0.33	(0.42, 1.71)		1.04	0.33	(0.39, 1.7)	
Heterosexual	0	.	.		0	.	.	
Highest Level of Education								
University	0.15	0.30	(-0.43, 0.74)	0.80	-	-	-	-
Trade school	-0.15	0.40	(-0.93, 0.62)					
Grade school	0.048	0.31	(-0.57, 0.67)					
High school	0	.	.					

- : covariate non-significant at $p \leq 0.2$ in univariate analysis, therefore not assessed in multivariate analysis.

†: Interaction between covariate and the time since the first blood lipid measurement is significant ($p \leq 0.05$) in the univariate model.

¶ : Univariate model: TC: HDL-C= Risk Factor+ Interaction between risk factor and time+ time+ time^2.
Multivariate model: TC:HDL-C= Sexual preference+ +age+ time+ time^2 .

Table 25. Results of repeated measures linear mixed effects univariate and multivariate models regarding the relationship of traditional risk factors and total cholesterol to HDL-C ratio in men.

	Univariate Models				Multivariate Models			
	Beta	Std Error	95% CI	Type 3 p	Beta	Std Error	95% CI	Type 3 p
Diabetes†	0.19	0.30	(-0.40, 0.79)	0.53	0.17	0.39	(-0.42, 0.75)	0.58
Smoking								
Current	0.032	0.23	(-0.42, 0.49)	0.65	-	-	-	-
Previous	0.22	0.27	(-0.31, 0.74)					
Never	0	.	.					
Alcohol Consumption								
Heavy	-0.73	0.32	(-1.36,-0.10)	0.069	NS	NS	NS	NS
Light-moderate	-0.34	0.22	(-0.78, 0.092)					
Non- drinker	0	.	.					
BMI (kg/m^2)								
Underweight	-0.66	0.48	(-1.61,0.29)	<.0001	-0.63	0.35	(-1.30,0.050)	<.0001
Overweight	0.74	0.19	(0.38, 1.11)		0.53	0.14	(0.27,0.80)	
Obese	1.12	0.27	(0.59, 1.65)		0.85	0.22	(0.43, 1.28)	
Missing	0.40	0.20	(0.0045, 0.80)		0.43	0.18	(0.085,0.78)	
Normal	0	.	.					

NS: covariate non-significant at the $p \leq 0.05$ level in multivariate model.

- : covariate non-significant at $p \leq 0.2$ in univariate analysis, therefore not assessed in multivariate analysis.

†: Interaction between covariate and the time since the first blood lipid measurement is significant ($p \leq 0.05$) in the univariate model.

¶ : Univariate model: TC: HDL-C= Risk Factor+ Interaction between risk factor and time+ time+ $time^2$.

Traditional risk factor multivariate model: TC:HDL-C= Diabetes+ BMI+ interaction between diabetes and time +age+ time+ $time^2$ (the interaction in this model is significant ($p<0.05$).

Table 26. Results of repeated measures linear mixed effects univariate and multivariate models regarding the relationship of non- traditional risk factors and total cholesterol to HDL-C ratio in men.

	Univariate Models				Multivariate Model			
	Beta	Std Error	95% CI	Type 3 p	Beta	Std Error	95% CI	Type 3 p
History of Injection Drug Use	-0.87	0.34	(-1.54, -0.21)	0.011	-0.77	0.28	(-1.33, -0.21)	0.0068
History of Cocaine use	-0.60	0.43	(-1.43, 0.24)	0.16	NS	NS	NS	NS
Hepatitis C Co-infection	-0.036	0.35	(-0.72, 0.65)	0.92	-	-	-	-

NS: covariate non-significant at the $p \leq 0.05$ level in multivariate model.

- : covariate non-significant at $p \leq 0.2$ in univariate analysis, therefore not assessed in multivariate analysis.

¶ : Univariate model: TC: HDL-C= Risk Factor+ Interaction between risk factor and time+ time+ $time^2$.

Non-traditional risk factor multivariate model: TC:HDL-C= History of injection drug use+ age+ time+$time^2$.

Table 27. Results of repeated measures linear mixed effects univariate and multivariate models regarding the relationship of HIV and HAART- related risk factors and total cholesterol to HDL-C ratio in men.

	Univariate				Multivariate			
	Beta	Std Error	95%CI	Type 3 p	Beta	Std Error	95% CI	Type 3 p
HIV Related Risk Factors								
Viral Load (Log_{10} RNA Copies/µl)†	-0.18	0.052	(-0.29,-0.078)	0.0006	-0.12	0.056	(-0.23, -0.012)	0.03
CD4+ T cell count (cells/mm^3)	0.00046	0.00028	(-0.00009, 0.0010)	0.10	0.00028	0.00020	(-0.00012, 0.00067)	0.17
Duration of HIV Infection	-0.0033	0.016	(-0.035, 0.028)	0.83	-	-	-	-
HAART Related Risk Factors								
Class of ART exposure								
NRTI	0.21	0.13	(-0.046, 0.46)	0.11	0.42	0.096	(0.23, 0.61)	0.67
NNRTI§	-0.18	0.13	(-0.45, 0.07)	0.16	0.31	0.25	(-0.18, 0.61)	0.68
Nevirapine	-0.16	0.23	(-0.61, 0.29)	0.4782	-	-	-	-
PI	0.49	0.13	(0.24, 0.74)	0.0001	NS	NS	NS	NS
NtRTI	0.14	0.22	(-0.30, 0.0058)	0.53	-	-	-	-
Other (Integrase, Fusion or CCR5 inihibitors or NtRTIs)	0.31	0.48	(-0.62, 1.26)	0.51	-	-	-	-
Untreated	-0.31	0.14	(-0.57, -0.040)	0.024	NS	NS	NS	NS

NS: covariate non-significant at the $p \leq 0.05$ level in multivariate model.

- : covariate non-significant at $p \leq 0.2$ in univariate analysis, therefore not assessed in multivariate analysis.

†: Interaction between covariate and the time since the first blood lipid measurement is significant ($p \leq 0.05$) in the univariate model.

¶ : Univariate model: TC: HDL-C= Risk Factor+ interaction between risk factor and time+ time+ $time^2$.

HIV related risk factor multivariate model: TC:HDL-C= Viral load+ CD4 T cell count + interaction between viral load and CD4 T cell count + Interaction between viral load and time+ age+ time+$time^2$ (the interactions in this model are significant ($p<0.05$))

HAART related risk factor multivariate model: TC:HDL-C= NRTI+ NNRTI + interaction between NRTI and NNRTI+ age+ time + $time^2$ (the interaction in this model is significant ($p<0.05$)).

§: NNRTI refers to all drugs within this class of ART with the exception of Nevirapine, which was included as a separate variable.

Table 28. Repeated measures linear mixed effects multivariate model for predicting total cholesterol to HDL ratio in men people living with HIV.

Covariate/ Interaction	Beta	Std Error	p
Diabetes interaction with time since first blood test	-0.15	0.0039	0.0017
Sexual Preference interaction with Diabetes			
Sexual Preference= Homosexual Diabetes=yes	2.45	1.29	0.059
Sexual Preference= Bisexual Diabetes=yes	-2.52	1.29	0.052
Sexual Preference= Heterosexual Diabetes=yes	-1.40	0.29	0.29
Sexual Preference=Homosexual Diabetes=no	0.39	0.21	0.059
Sexual Preference=Bisexual Diabetes=no	0.60	0.30	0.049
Sexual Preference= Heterosexual Diabetes=no	0	.	.
BMI			
Underweight	-0.50	0.36	0.17
Overweight	0.50	0.14	0.0005
Obese	0.82	0.23	0.0005
Missing	0.42	0.18	0.022
Normal Weight	0	.	.
Viral Load (Log_{10} Copies RNA/ μL)	-0.0105	0.042	0.80
CD4+ T Cell Count (Cells/mm^3)	0.00015	0.00020	0.46
Viral Load interaction with CD4+ T Cell Count	0.00034	0.00013	0.0095
NRTI interaction with NNRTI §			
NRTI= Yes, NNRTI= No	0.30	0.11	0.0083
NRTI= No, NNRTI= Yes	0.25	0.26	0.34
NRTI= Yes, NNRTI= Yes	-0.56	0.27	0.034

*All β estimates have been adjusted for all the variables listed in this table age, time and $time^2$

§: NNRTI refers to all drugs within this class of ART with the exception of Nevirapine, which was included as a separate variable

Table 29. Results of repeated measures linear mixed effects univariate models regarding the relationship between demographic, traditional and non-traditional risk factors and Log_{10} triglyceride concentration in women.

	Beta	Std Error	95% CI	Type 3 P
Demographics				
Age (years)	0.0030	0.0037	(-0.0044, 0.010)	0.42
Sexual Preference				
Homosexual	-	-	-	
Bisexual	-0.048	0.29	(-0.62, 0.52)	0.87
Heterosexual	0	.	.	
Highest Level of Education				
University	0.042	0.10	(-0.16, 0.25)	0.87
Trade school	-0.078	0.14	(-0.36, 0.21)	
Grade school	-0.025	0.13	(-0.29, 0.24)	
High school	0	.	.	
Traditional Risk Factors				
Diabetes	-0.19	0.23	(-0.65, 0.27)	0.42
Smoking				
Current	0.14	0.099	(-0.062, 0.33)	0.20
Previous	0.22	0.13	(-0.034, 0.47)	
Never	0	.	.	
Alcohol Consumption				
Heavy	-0.11	0.28	(-0.67, 0.44)	0.24
Light-Moderate	-0.13	0.087	(-0.039, 0.31)	
Non- drinker	0	.	.	
BMI (kg/m^2)				
Underweight	-0.21	0.29	(-0.019, 0.26)	0.85
Overweight	0.037	0.11	(-0.79, 0.36)	
Obese	0.10	0.13	(-0.17, 0.25)	
Missing	0.037	0.099	(-0.16, 0.25)	
Normal	0	.	.	
Non-Traditional Risk Factors				
History of Injection Drug Use	0.066	0.096	(-0.13, 0.26)	0.50
History of Cocaine use	0.0091	0.20	(-0.40, 0.42)	0.96
Hepatitis C Co-infection	0.024	0.12	(-0.22, 0.26)	0.84

- : There were no women in this category.

¶: Univariate model: Log_{10} Triglycerides= Risk Factor+ Interaction between risk factor and time+ time+ $time^2$.

Table 30. Results of repeated measures linear mixed effects univariate models regarding the relationship between HIV and HAART- related risk factors and Log_{10} triglyceride concentration in women.

	Beta	Std Error	95% CI	Type 3 p
HIV Related Risk Factors				
Viral Load (Log_{10} RNA Copies/µl)	-0.031	0.027	(-0.085, 0.022)	0.25
CD4 +T cell count (cells/mm^3)	0.000094	0.00011	(-0.00011, 0.00030)	0.37
Duration of HIV Infection	0.0041	0.0085	(-0.013, 0.021)	0.63
HAART Related Risk Factors				
Class of ART Exposure				
NRTI	0.11	0.064	(-0.020, 0.23)	0.099
NNRTI	0.063	0.066	(-0.069, 0.19)	0.35
Nevirapine	-0.023	0.11	(-0.23, 0.18)	0.83
PI	0.18	0.078	(0.024, 0.33)	0.023
NtRTI	-0.067	0.13	(-0.33, 0.20)	0.61
Other (Integrase, Fusion or CCR5 inihibitors)	-	-	-	-
Untreated	-0.086	0.072	(-0.23, 0.055)	0.23

- : There were no women in this category.

¶: Univariate model: Log_{10} triglycerides= Risk Factor+ Interaction between risk factor and time+ time+ $time^2$.

§: NNRTI refers to all drugs within this class of ART with the exception of nevirapine, which was included as a separate variable.

Table 31. Results of repeated measures linear mixed effects univariate and multivariate models regarding the relationship between demographic risk factors and Log_{10} triglyceride concentration in men.

	Univariate Models				Multivariate Model			
	Beta	Std Error	95%CI	Type 3 p	Beta	Std Error	95%CI	Type 3 p
Age (years) †	0.0033	0.0016	(-0.000077, 0.0066)	0.045	0.0033	0.0016	(-0.000077, 0.0066)	0.045
Sexual Preference*								
Homosexual	-0.031	0.035	(-0.047, 0.11)	0.057	NS	NS	NS	NS
Bisexual	-0.14	0.058	(-0.022, 0.25)					
Heterosexual	0	.	.					
Highest Level of Education*								
University	0.03	0.052	(-0.086, 0.12)	0.75	-	-	-	-
Trade school	0.028	0.070	(-0.14, 0.14)					
Grade school	0.027	0.055	(-0.14, 0.081)					
High school	0	.	.					

NS: covariate non-significant at the $p \leq 0.05$ level in multivariate model.

- : covariate non-significant at $p \leq 0.2$ in univariate analysis, therefore not assessed in multivariate analysis.

***:** The time elapsed since the first blood lipid measurement is significant ($p \leq 0.05$) in the univariate model.

†: Interaction between covariate and the time since the first blood lipid measurement is significant ($p \leq 0.05$) in the univariate model.

¶ : Univariate model: Log_{10} Triglycerides = Risk Factor+ interaction between risk factor and time+ time+ $time^2$.
Multivariate model: Log_{10} Triglycerides = Age+ time+ $time^2$.

Table 32. Results of repeated measures linear mixed effects univariate and multivariate models regarding the relationship between traditional risk factors and Log_{10} triglyceride concentration in men.

	Univariate Models				Multivariate Models			
	Beta	Std Error	95%CI	Type 3 p	Beta	Std Error	95% CI	Type 3 p
Traditional Risk Factors								
Diabetes	0.093	0.052	(-0.0091, 0.19)	0.074	NS	NS	NS	NS
Smoking*								
Current	-0.030	0.041	(-0.11, 0.050)	0.58	-	-	-	-
Previous	-0.049	0.047	(-0.14, 0.044)					
Never	0	.	.					
Alcohol Consumption†								
Heavy	-0.023	0.058	(-0.14, 0.092)	0.27	-0.0055	0.057	(-0.12, 0.11)	0.31
Light-moderate	-0.063	0.041	(-0.14, 0.017)		-0.054	0.040	(-0.13,0.024)	
Non- drinker	0	.	.		0	.	.	
BMI (kg/m^2)*								
Underweight	-0.19	0.082	(-0.35,0.030)	<.0001	-0.15	0.059	(-0.26, -0.032)	<.0001
Overweight	0.083	0.030	(-0.024, 0.14)		0.050	0.023	(0.0049,0.094)	
Obese	0.21	0.046	(-0.12,0.30)		0.18	0.038	(0.11,0.25)	
Missing	0.081	0.035	(0.012, 0.15)		0.053	0.033	(-0.011, 0.12)	
Normal	0	.	.		0	.	.	

NS: covariate non-significant at the $p \leq 0.05$ in multivariate model.

- : covariate non-significant at $p \leq 0.2$ in univariate analysis, therefore not assessed in multivariate analysis.

***:** The time elapsed since the first blood lipid measurement is significant ($p \leq 0.05$) in the univariate model.

†: Interaction between covariate and the time since the first blood lipid measurement is significant ($p \leq 0.05$) in the univariate model.

¶ : Univariate model: Log_{10} Triglycerides = Risk Factor+ interaction between risk factor and time+ time+ $time^2$.

Multivariate model: Log_{10} Triglycerides =Alcohol consumption+ BMI+ interaction between alcohol consumption and time+ age+ time+ $time^2$ (the interaction in this model is significant ($p<0.05$))

Table 33. Results of repeated measures linear mixed effects univariate and multivariate models regarding the relationship between non-traditional risk factors and Log_{10} triglyceride concentration in men.

	Univariate Models				Multivariate Model			
	Beta	Std Error	95% CI	Type 3 p	Beta	Std Error	95% CI	Type 3 p
History of Injection Drug Use	-0.059	0.058	(-0.17, 0.055)	0.31	-	-	-	-
History of Cocaine use	-0.11	0.075	(-0.26, 0.040)	0.15	NS	NS	NS	NS
Hepatitis C Co-infection	0.0069	0.057	(-0.11, 0.12)	0.90	-	-	-	-

NS: covariate non-significant at the $p \leq 0.05$ level in multivariate model.

- : covariate non-significant at $p \leq 0.2$ in univariate analysis, therefore not assessed in multivariate analysis.

¶ : Univariate model: Log_{10} Triglycerides = Risk Factor+ interaction between risk factor and time+ time+ $time^2$.

Table 34. Results of repeated measures linear mixed effects univariate and multivariate models regarding the relationship between HIV related risk factors and Log_{10} triglyceride concentration in men.

	Univariate Models				Multivariate Model			
	Beta	Std Error	95% CI	Type 3 p	Beta	Std Error	95% CI	Type 3 p
Viral Load (Log_{10} RNA Copies/µl) †	-0.054	0.0084	(-0.071,-0.038)	<.0001	-0.046	0.0090	(-0.063,-0.028)	<.0001
CD4 +T cell count (cells/mm^3)	0.00015	0.000045	(0.000063,0.00024)	0.0008	0.000026	0.000050	(-0.00007, 0.00012)	0.61
Duration of HIV Infection	0.0070	0.0028	(0.0015, 0.013)	0.013	0.0055	0.0027	(0.00012, 0.011)	0.045

†: Interaction between covariate and the time since the first blood lipid measurement is significant ($p \leq 0.05$) in the univariate model.

¶ : Univariate model: Log_{10} Triglycerides = Risk Factor+ interaction between risk factor and time+ time+ $time^2$.

Multivariate model: Log_{10} Triglycerides =Viral load+ CD4 T cell count+ Duration of infection+ interaction between viral load and time+ interaction between CD4 T cell count and time + interaction between viral load and CD4 T cell count+ interaction between CD4 T cell count and duration of infection+ age+ time+ $time^2$ (the interactions in this model are significant ($p<0.05$))

Table 35. Results of repeated measures linear mixed effects univariate and multivariate models regarding the relationship between HAART related risk factors and Log_{10} triglyceride concentration in men.

	Univariate Models				Multivariate Models			
	Beta	Std Error	95% CI	Type 3 p	Beta	Std Error	95% CI	Type 3 p
Class of ART Exposure								
NRTI*†	0.10	0.021	(0.061, 0.14)	<.0001	NS	NS	NS	NS
NNRTI	0.058	0.022	(0.016, 0.10)	0.0073	NS	NS	NS	NS
Nevirapine*	-0.022	0.037	(-0.095, 0.052)	0.57	-	-	-	-
PI*	0.11	0.020	(0.071, 0.15)	<.0001	0.057	0.015	(0.028,0.087)	0.0001
NtRTI	-0.038	0.037	(-0.11, 0.035)	0.31	-	-	-	-
Other (Integrase, Fusion or CCR5 inihibitors)	0.036	0.08	(-0.12, 0.19)	0.65	-	-	-	-
Untreated	-0.13	0.022	(-0.17, -0.086)	<.0001	-0.075	0.017	(-0.11, -0.040)	<.0001

NS: covariate non-significant in multivariate model.

- : covariate non-significant at $p \leq 0.2$ in univariate analysis, therefore not assessed in multivariate analysis.

***:** The time elapsed since the first blood lipid measurement is significant ($p \leq 0.05$) in the univariate model.

†: Interaction between covariate and the time since the first blood lipid measurement is significant ($p \leq 0.05$) in the univariate model.

¶ : Univariate model: Log_{10} Triglycerides = Risk Factor+ interaction between risk factor and time+ time+ $time^2$.
Multivariate model: Log_{10} Triglycerides = PI use+ untreated+ age+ time+ $time^2$

Table 36. Repeated measures linear mixed effects multivariate model for predicting Log_{10} triglyceride concentration in men living with HIV.

Covariates/ Interaction	Beta	Std Error	p
Viral Load (Log_{10} Copies RNA/ μL)	-0.0070	0.013	0.59
CD4+ T Cell Count (Cells/mm^3)	0.000017	0.000050	0.72
Duration of HIV Infection	0.0045	0.0027	0.089
Viral Load x Time Elapsed Since the First Blood Lipid Measurement	0.0056	0.0019	0.0032
CD4+ T Cell Count x Time Elapsed Since the First Blood Lipid Measurement	0.000025	0.000010	0.014
Viral Load x CD4+ T Cell Count	0.000044	0.000022	0.044
CD4+ T Cell Count x Duration of HIV Infection	-0.00001	0.0000059	0.030
Protease Inhibitor	0.051	0.016	0.0012
Untreated	-0.017	0.028	0.54
Viral Load x Protease Inhibitor Use	-0.046	0.013	0.0004
Viral Load x Untreated	-0.035	0.017	0.047

* All β estimates have been adjusted for all the variables listed in the table age, time and $time^2$

Chapter Five: Discussion

The literature on co-morbidities found among people living with recognizes the presence of dyslipidemia. Current research speculates that it is likely that a number of factors (demographic, traditional, non-traditional, HIV and HAART-related) determine blood lipid levels in people living with HIV, however, the exact risk factors and their relative contribution is currently unknown. This study provided an opportunity to examine the presence of dyslipidemia among people living with HIV in Nova Scotia, and it also provided the rare opportunity to study all these categories of risk factors in the same individuals, thus, allowing us to begin to elucidate the mechanisms underlying the dyslipidemia found among people living with HIV.

In terms of the describing the presence of dyslipidemia among people living with HIV in Nova Scotia the present study compared lipid levels in people living with HIV to the general population of Nova Scotia, and persons treated for HIV to persons not treated for HIV. The results of the 1995 Nova Scotia Health Survey (NSHS95) (a population based survey) were used to compare people living with HIV to the general population. This comparison revealed that men living with HIV in the present study had a higher mean triglyceride level, but lower mean HDL-C, LDL-C and total cholesterol levels at baseline compared to men in the NSHS95. While lower LDL-C is favorable, low HDL-C is not, and HDL-C combined with high triglyceride levels represents an atherosclerotic risk that could lead to cardiovascular disease. Women in the present study, however, appear to have a somewhat healthier lipid profile compared to uninfected women living in Nova Scotia. In contrast to women who participated in the NSHS95, women living with HIV have slightly higher triglycerides and HDL-C, but lower total cholesterol and LDL-C levels. The gender differences in lipid levels found in this study are not unusual since male gender is a risk factor for a poor lipid profile. (20)

The comparison of people living with HIV who were treated to those who were not showed that there is a higher prevalence of hypertriglyceridemia and hypercholesterolemia among individuals who were HIV treated (58.3% and 22.1 % respectively) than in those who were not (40.7% and 8.3% respectively).

The findings of both of these comparisons are in agreement with the pattern of dyslipidemia that the current literature describes in people living with HIV. People living with HIV, both treated and untreated, have higher triglyceride and lower HDL-C levels compared to uninfected controls. On the other hand, HIV treated and untreated patients differ from uninfected controls with treated individuals having higher LDL-C and total cholesterol, while untreated individuals have lower LDL-C and total cholesterol. (21-30)

After establishing the existence of dyslipidemia, the present study determined the risk factors that significantly contribute to dyslipidemia among men living with HIV. Table 37 presents a summary of the associations found between the risk factors and the four outcomes assessed in this study

Table 37. Summary of results from repeat measures mixed effects multivariate models for all four outcomes assessed.

Risk Factor	Outcome			
	HDL-C	LDL-C	TC:HDL-C	Triglycerides
Sexual Preference			✖	
Highest Level of Education				
Diabetes			✖	
Smoking				
Alcohol				
BMI			✓	
History of IDU	✓			
History of Cocaine use				
Hepatitis C Co-infection	✓			
Viral load	✓	✖	✖	✖
CD4+ T cell count		✖	✖	✖
Duration of Infection				✖
Untreated				✖
NRTI			✖	
NNRTI	✖		✖	
Nevirapine				
PI				✖
NtRTI	✖			
Other				

✓: Indicates where a significant independent association was found.
✖: Indicates risk factors that were part of a significant interaction.

Of the four lipid outcomes assessed in our regression analyses only one, TC:HDL-C ratio, was significantly associated with any of the demographic and

traditional risk factors included in this study. Diabetes and sexual preference had a significant joint effect and BMI had a significant independent effect on TC:HDL-C ratio. While the literature supports the positive association found between diabetes and TC:HDL-C ratio (diabetes is known to increase triglycerides and decrease HDL-C), how this association may be influenced by sexual preference is unclear. (78) It is unlikely that diabetes status influences sexual preference or vice versa, however, it is possible that sexual preference is serving as a marker for some other risk factor(s) for dyslipidemia, which is actually modifying or being modified by diabetes status. For example, higher rates of depression, smoking and alcohol consumption have all been noted among homosexuals compared to the heterosexuals. (35) The positive association between TC:HDL-C ratio and BMI, being overweight or obese, was expected since overweight and obesity are well established risk factors for high total cholesterol. (20)

Few studies have examined the associated between non-traditional risk factors such as hepatitis C (HCV) co-infection and injection drug use (IDU) and dyslipidemia in men living with HIV. The only outcome that this study found to be significantly associated with a non-traditional risk factor is HDL-C concentration. Our analyses revealed that both HCV co-infection and a history of IDU were independently associated with HDL-C concentration. While HCV co-infection was initially associated with having a lower HDL-C concentration, the HDL-C concentration of co-infected patients increased more than that of mono-infected patients over the study period. A history of IDU was also independently associated with an increase in HDL-C concentration. Both of these findings are different from those seen in the current literature, where neither HCV co-infection, nor IDU have been associated with changes in HDL-C concentration in men people living with HIV. (26,44,45,79,80)

This study found that HIV-related risk factors are important contributors to dyslipidemia. Viral load was found to be significantly associated with all four of the outcomes analyzed in this study. However, only the association between viral load and HDL-C was independent, as all the models for the other outcomes included significant interaction terms between viral load and CD4+ T cell count. An increase in viral load was independently associated with a significant decrease in HDL-C. This confirms the findings of an earlier study conducted by El-Sadr et al, and is consistent with the

dyslipidemia that has been described in treatment naïve HIV-infected individuals, characterized by a decrease in HDL-C concentration. (21-24,48). This finding is also consistent with the hypotheses that an HIV protein inhibits HDL-C mobilization from the liver and macrophages. (24,53)

The interaction found between viral load and CD4+T cell count is of interest because although these two measures of HIV disease progression are physiologically related, they have not previously been shown in to modify each other's effect on dylipidemia in the context of an epidemiological study. A biological mechanism for this interaction, however, has previously been proposed. The inflammation hypothesis proposes that the activation of CD4+ T cells and secretion of pro-inflammation cytokines caused by HIV infection or the opportunistic infections that arise as a result of HIV infection results in dyslipidemia. (31,52) Further research is needed to better understand the mechanism of effect of viral load and CD4+ T cell count and their interaction on HDL-C concentration.

HAART-related risk factors also appear to be important contributors to dyslipidemia among men living with HIV since ART was significantly associated with nearly all the outcomes assessed in this study. LDL-C was the only outcome that was not significantly associated with ART. The remaining three outcomes were each associated with at least two classes of ART drugs, however none of the classes of ART examined in this study had an independent effect on lipid or triglyceride levels. NtRTIs and NNRTIs were found to jointly affect HDL-C concentration. Compared to patients taking neither class of ART drugs, patients taking an NNRTI but not an NtRTI had significantly higher HDL-C levels and patients taking both an NNRTI and an NtRTI had significantly lower HDL-C levels. The finding that NNRTIs are associated with an increase in HDL-C is in keeping with the findings of Benal et al., who demonstrated that although nevirapine was associated with the highest HDL-C levels, efavirenz was also associated with higher HDL-C levels than any other ART drug class. (81) Nevirapine was treated as a separate category of ART in this study's analyses. No other study has reported an interaction between these two drug classes and the few studies that have assessed the effect of NtRTIs on HDL-C compare them to NRTI use, and have found no difference in HDL-C concentration. (23,82)

The present study also found that NRTIs and NNRTIs jointly affect the TC:HDL-C ratio. Compared to patients not taking drugs from either class of ART, patients taking NRTIs but not NNRTIs were found to have a significantly higher TC:HDL-C ratio and patients taking both NRTIs and NNRTIs were found to have a significantly lower TC:HDL-C ratio. This suggests that NRTIs increase the TC:HDL-C ratio, creating an unfavorable lipid profile and is supported by several studies that have reported high levels of cholesterol among NRTI treated patients. (56,60,63) The use of an NNRTI with an NRTI appears to negate the effect of NRTI on the TC:HDL-C ratio, by increasing HDL-C levels NNRTI use in conjunction with NRTI use decreases the TC:HDL-C ratio.

Although the literature reports that PI use is associated with increases to both total cholesterol and triglyceride concentrations, this study only found a positive association between PI use and triglyceride concentration. (24,26,30,31,58,60) This association was at least in part mediated by viral load, this study found an interaction between viral load and PI use. The existence of this interaction suggests that the effect of PI on triglyceride concentration depends on the number of copies of HIV RNA per ml of blood. Further research is needed to better understand the mechanism of effect of PIs, and viral load and their interaction on triglyceride concentration.

The fact that HIV and HAART-related risk factors were associated with multiple outcomes in multivariate models and that demographic, traditional and non-traditional risk factors were not suggests that HIV and treatment-related risk factors have a greater influence over cholesterol and triglyceride concentrations in this population of men living with HIV. This may have clinical implications for the treatment of dyslipidemia in men living with HIV since the results of this study suggest that targeting modifiable traditional risk factors may be of limited benefit. The only modifiable traditional risk factor that the results of this study indicate may be worth targeting to reduce dyslipidemia is BMI, which was shown to be independently associated with a high TC:HDL-C ratio . However, these results should be interpreted with caution, as this study was unable to capture potentially important information related to diet and level of physical activity.

Strengths and Limitations

Understanding dyslipidemia in people living with HIV is complex. There are many factors that may be contributing to the abnormal lipid profiles that have been noted among people living with HIV, and as this study demonstrated many of these factors interact with or modify each other. This study is a comprehensive evaluation of cholesterol and triglyceride levels of the HIV-infected population living in Nova Scotia. The Halifax HIV Clinic database provided access to information on a large number of risk factors, some of which had never been assessed for their association with dyslipidemia in the same study. The database also provided a reliable means of capturing the four different lipid measures that were used as outcomes in this study. Having multiple types of lipid measurements as outcomes meant that this study presents a more complete picture of the lipid changes that occur in HIV-infected patients and the risk factors associated with each of them.

Another important aspect of this study is that it is a longitudinal evaluation of the risk factors associated with dyslipidemia in people living with HIV. Compared to cross-sectional studies, longitudinal studies are a more reliable means of observing changes in both the lipid levels and risk factors over time. Longitudinal studies follow the same group of patients for the entire study period, therefore the focus is on the changes that occur within an individual and any inferences that are made to the general population are less biased by between subject variation.

A final strength of this study is the fact that the information in the Halifax HIV Clinic database is mainly collected for clinical purposes. Unlike in a clinical trial, the timing of visits and anthropometric measurements in this study reflect the pattern of visits that occur in the real world.

While the strengths of this research are related to the use of the Halifax HIV clinic database, several of this study's limitations are also a result of using a database created for clinical purposes. One of the limitations of this study is that since the data was collected for the purposes of clinical care there is some variability in the timing and completeness of clinical assessment. Missing data was particularly prevalent for BMI. To deal with this missing information BMI values were interpolated and then categorized, and a category was created for missing data in order to avoid a large number of patients being excluded from the analyses. The amount of missing data on BMI may have

introduced bias into the results of this study; however, this bias is minimized by the fact that after reviewing the raw data BMI values appear to be missing at random.

Another limitation of this study was that there were certain important risk factors that we were unable to assess because the information was not contained in the database. In particular, the traditional risk factors reflecting level of exercise and diet, could not be included in the analysis. If the Halifax HIV Clinic database is going to be used as a source of data for further research in the area of dyslipidemia in people living with HIV it would be prudent to start collecting information related to these two important risk factors. Other possible confounders that were not included in this study included: certain drugs (beta-blockers, anabolic steroids and progestational agents), a family history of dyslipidemia and diseases such as chronic renal failure, nephritic syndrome and hypothyroidism.

This study also relied on measures of alcohol consumption and smoking status that were recorded only at the time of a patient's initial clinic visit. Thus, this study was unable to capture any changes in these measures that may have occurred over the study period. However, studies of patterns of smoking in people living with HIV have shown that smoking status within this population is relatively stable over time. There are several barriers to smoking cessation for people living with HIV, including co-morbid drug use, pain, and emotional distress, which have all been shown to impact smoking status and readiness to quit among this population. (83,84) For those patients who do manage to quit smoking, studies have found that most relapse. (85) Few studies have examined longitudinal patterns of alcohol consumption in people living with HIV. A recent study conducted by Bertholet et al. found that patients who either abstained or consumed moderate amounts of alcohol were consistent in their patterns of consumption. Patients who were heavy consumers of alcohol were less consistent in their pattern of consumption, varying between consuming moderate to heavy amounts of alcohol. (86) Future research on this topic should thus attempt to collect longitudinal data on alcohol consumption, particularly for heavy drinkers.

Finally, the multivariate analysis in this study could only be carried out on men given the small number of women living with HIV included in the Halifax HIV Clinic database and therefore our results should not be generalized to women, particularly given

the gender differences in baseline characteristics. However, the results of this study can be generalized to the men living with HIV outside of Nova Scotia. The distribution of baseline characteristics (age, number of smokers, number of patients exposed to HAART, median viral load and CD4+ T cell count) among the men included in this study are similar to those reported by several other large population based cohort studies of dyslipidemia among men living with HIV. (23,30,87) Additionally, the rates of homosexuality, injection drug use and hepatitis C co-infection among men in this study were similar to those reported by the Public Health Agency of Canada's national HIV surveillance program. (88)

Future directions for research in the area of dyslipidemia among people living with HIV should include further evaluations of the effect of individual ART drugs and their combinations into HAART regimens on lipid levels. Current research being conducted on dyslipidemia among people living with HIV examines individual ART drugs and these studies are finding that there are differences in the dyslipidemic effect of individual drugs within classes of ART. For example the previously discussed HDL-C concentration differences associated with nevirapine and efavirenz, members of the NNRTI class of ART. (81) This means that the lipid levels being associated with classes of ART may be inappropriate and that they are simply a reflection of the composition of the individual drugs with in a study group. Another future direction for research is an investigation into the interaction this study found between viral load and CD4 + T cell count. This study found an independent association between hepatitis C co-infection and HDL-C concentration, however although several studies have examined dylipidemia in HCV mono-infected patients, few studies have examined dyslipidemia in HIV/ HCV co-infected patients, and a consensus has yet to be reached. Research should be conducted to further investigate the association of hepatitis C co-infection and dyslipidemia. In the future, as people living with HIV continue to age, the contribution of aging on lipid levels in this population should also be examined.

Conclusion

Improved management of HIV/AIDS in developed countries has meant there is emerging concern over the presence of co-morbid conditions, in particular dyslipidemia and its subsequent impact on cardiovascular disease, among this population. This study

provides evidence indicating that on average people living with HIV in Nova Scotia have triglyceride levels that are higher than the general population in the NSHS95, which is consistent with the findings of other published studies. However, in contrast to the literature, this study did not find that patients in this study had higher cholesterol levels than those reported in the general population. HDL-C concentrations among men living with HIV were lower than those found among the men of the general population, a result that is supported by the literature. Since low HDL-C concentration is a risk factor for atherosclerosis, this lipid should be considered an important outcome in the discussion of dyslipidemia in men living with HIV.

This study demonstrated that the risk of a men living with HIV developing dyslipidemia is multifactoral; demographic, traditional, non-traditional, HIV and HAART-related risk factors were all associated with at least one outcome (HDL-C, LDL-C, TC:HDL-C, triglycerides). However, our analyses revealed that HIV and HAART-related risk factors were the only categories of risk factors to be consistently associated with multiple lipid outcomes. This study also found that very few risk factors have an independent effect on lipid levels when controlled for other factors. Given that the literature describes well-defined relationships between many of the risk factors interactions were expected. Interactions were found between NtRTI use and NNRTI use, sexual preference and diabetes, viral load and CD4+ T cell count, CD4+ T cell count and duration of HIV infection, viral load and PI use, and viral load and not being treated for HIV. Further research is needed to better understand the mechanism of effect of each of the covariates and their interaction on lipid levels.

The risk factors found to produce a consistent independent effect on lipids were BMI, injection drug use, hepatitis C co-infection, and viral load. The associations found between BMI and increased TC:HDL-C ratio and viral load and decreased HDL-C are well established in the literature. However, the associations between both injection drug use and hepatitis C co-infection and increased HDL-C among people living with HIV have not yet been sufficiently explored. The findings of this study suggest that further research into the effects of these two risk factors is warranted.

References

(1) Currier JS, Taylor A, Boyd F, Dezii CM, Kawabata H, Burtcel B, et al. Coronary heart disease in HIV-infected individuals. J.Acquir.Immune Defic.Syndr. 2003 Aug 1;33(4):506-512.

(2) Goals of HIV Control in Aging Populations. New Frontiers- Fifth International AIDS Society ; July 19-22, 209; Quebec: Medical Education Network.

(3) Guaraldi G, Zona S, Alexopoulos N, Orlando G, Carli F, Ligabue G, et al. Coronary Aging in HIV-Infected Patients. Clin.Infect.Dis. 2009 Nov 3.

(4) Friis-Moller N, Sabin CA, Weber R, d'Arminio Monforte A, El-Sadr WM, Reiss P, et al. Combination antiretroviral therapy and the risk of myocardial infarction. N.Engl.J.Med. 2003 Nov 20;349(21):1993-2003.

(5) Obel N, Thomsen HF, Kronborg G, Larsen CS, Hildebrandt PR, Sorensen HT, et al. Ischemic heart disease in HIV-infected and HIV-uninfected individuals: a population-based cohort study. Clin.Infect.Dis. 2007 Jun 15;44(12):1625-1631.

(6) Triant VA, Lee H, Hadigan C, Grinspoon SK. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. J.Clin.Endocrinol.Metab. 2007 Jul;92(7):2506-2512.

(7) DAD Study Group, Friis-Moller N, Reiss P, Sabin CA, Weber R, Monforte A, et al. Class of antiretroviral drugs and the risk of myocardial infarction. N.Engl.J.Med. 2007 Apr 26;356(17):1723-1735.

(8) Klein D, Hurley LB, Quesenberry CP,Jr, Sidney S. Do protease inhibitors increase the risk for coronary heart disease in patients with HIV-1 infection? J.Acquir.Immune Defic.Syndr. 2002 Aug 15;30(5):471-477.

(9) Mary-Krause M, Cotte L, Simon A, Partisani M, Costagliola D, Clinical Epidemiology Group from the French Hospital Database. Increased risk of myocardial infarction with duration of protease inhibitor therapy in HIV-infected men. AIDS 2003 Nov 21;17(17):2479-2486.

(10) Potthoff AV, Brockmeyer NH. Current therapy of HIV. J.Dtsch.Dermatol.Ges. 2009 Jul 23.

(11) Hunt PW, Martin JN, Sinclair E, Bredt B, Hagos E, Lampiris H, et al. T cell activation is associated with lower CD4+ T cell gains in human immunodeficiency virus-infected patients with sustained viral suppression during antiretroviral therapy. J.Infect.Dis. 2003 May 15;187(10):1534-1543.

(12) Marcovitch H editor. Black's Medical Dictionary 41st Edition. ; 2005.

(13) Joint United Nations Program on HIV/AIDS and World Health Organization. AIDS Epidemic Update December 2009. 2009.

(14) Public Health Agency of Canada, Centre for Communicable Diseases and Infection Control, Surveillance and Risk Assessment Division. Summary: Estimate of HIV Prevalence and Incidence in Canada, 2008. 2008.

(15) Piacenti FJ. An update and review of antiretroviral therapy. Pharmacotherapy 2006 Aug;26(8):1111-1133.

(16) Lucas GM, Chaisson RE, Moore RD. Comparison of initial combination antiretroviral therapy with a single protease inhibitor, ritonavir and saquinavir, or efavirenz. AIDS 2001 Sep 7;15(13):1679-1686.

(17) Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W,Jr, et al. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation 1995 Sep 1;92(5):1355-1374.

(18) Crowther MA. Pathogenesis of atherosclerosis. Hematology Am.Soc.Hematol.Educ.Program. 2005:436-441.

(19) Grundy SM. Cholesterol metabolism in man. West.J.Med. 1978 Jan;128(1):13-25.

(20) National Cholesterol Education Program Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults. Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) Final Report. 2002;III.

(21) Kotler DP. HIV and antiretroviral therapy: lipid abnormalities and associated cardiovascular risk in HIV-infected patients. J.Acquir.Immune Defic.Syndr. 2008 Sep 1;49 Suppl 2:S79-85.

(22) Stein JH. Dyslipidemia in the era of HIV protease inhibitors. Prog.Cardiovasc.Dis. 2003 Jan-Feb;45(4):293-304.
(23) Anastos K, Lu D, Shi Q, Tien PC, Kaplan RC, Hessol NA, et al. Association of serum lipid levels with HIV serostatus, specific antiretroviral agents, and treatment regimens. J.Acquir.Immune Defic.Syndr. 2007 May 1;45(1):34-42.

(24) Rose H, Woolley I, Hoy J, Dart A, Bryant B, Mijch A, et al. HIV infection and high-density lipoprotein: the effect of the disease vs the effect of treatment. Metabolism 2006 Jan;55(1):90-95.

(25) Grunfeld C, Pang M, Doerrler W, Shigenaga JK, Jensen P, Feingold KR. Lipids, lipoproteins, triglyceride clearance, and cytokines in human immunodeficiency virus infection and the acquired immunodeficiency syndrome. J.Clin.Endocrinol.Metab. 1992 May;74(5):1045-1052.

(26) Boulassel MR, Morales R, Murphy T, Lalonde RG, Klein MB. Gender and long-term metabolic toxicities from antiretroviral therapy in HIV-1 infected persons. J.Med.Virol. 2006 Sep;78(9):1158-1163.

(27) Koppel K, Bratt G, Eriksson M, Sandstrom E. Serum lipid levels associated with increased risk for cardiovascular disease is associated with highly active antiretroviral therapy (HAART) in HIV-1 infection. Int.J.STD AIDS 2000 Jul;11(7):451-455.

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

(29) Riddler SA, Li X, Chu H, Kingsley LA, Dobs A, Evans R, et al. Longitudinal changes in serum lipids among HIV-infected men on highly active antiretroviral therapy. HIV.Med. 2007 Jul;8(5):280-287.

(30) Friis-Moller N, Weber R, Reiss P, Thiebaut R, Kirk O, d'Arminio Monforte A, et al. Cardiovascular disease risk factors in HIV patients--association with antiretroviral therapy. Results from the DAD study. AIDS 2003 May 23;17(8):1179-1193.

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

(32) Carr A. Pathogenesis of cardiovascular disease in HIV infection. Curr.Opin.HIV.AIDS. 2008 May;3(3):234-239.

(33) Nova Scotia Health Promotion and Protection. Notifiable diseases in Nova Scotia: Surveillance Report 2007. 2008.

(34) Nova Scotia Department of Health. HIV/ AIDS Surveillance Report 2000. 2001.

(35) Wang J, Hausermann M, Vounatsou P, Aggleton P, Weiss MG. Health status, behavior, and care utilization in the Geneva Gay Men's Health Survey. Prev.Med. 2007 Jan;44(1):70-75.

(36) Saves M, Chene G, Ducimetiere P, Leport C, Le Moal G, Amouyel P, et al. Risk factors for coronary heart disease in patients treated for human immunodeficiency virus infection compared with the general population. Clin.Infect.Dis. 2003 Jul 15;37(2):292-298.

(37) Kaplan RC, Kingsley LA, Gange SJ, Benning L, Jacobson LP, Lazar J, et al. Low CD4+ T-cell count as a major atherosclerosis risk factor in HIV-infected women and men. AIDS 2008 Aug 20;22(13):1615-1624.

(38) Pere D, Ignacio SL, Ramon T, Fernando L, Alberto T, Pompeyo V, et al. Dyslipidemia and Cardiovascular Disease Risk Factor Management in HIV-1-Infected Subjects Treated with HAART in the Spanish VACH Cohort. Open AIDS.J. 2008;2:26-38.

(39) Statistics Canada. CANSIM, table 105-0427 and Catalogue no. 82-221-X. Last modified: 2009-07-06.

(40) Craig WY, Palomaki GE, Haddow JE. Cigarette smoking and serum lipid and lipoprotein concentrations: an analysis of published data. BMJ 1989 Mar 25;298(6676):784-788.

(41) Galvan FH, Bing EG, Fleishman JA, London AS, Caetano R, Burnam MA, et al. The prevalence of alcohol consumption and heavy drinking among people with HIV in the United States: results from the HIV Cost and Services Utilization Study. J.Stud.Alcohol 2002 Mar;63(2):179-186.

(42) Pejic RN, Lee DT. Hypertriglyceridemia. J.Am.Board Fam.Med. 2006 May-Jun;19(3):310-316.

(43) Public Health Agency of Canada. HIV/AIDS Epi Updates, November 2007. 2007.

(44) Bedimo R, Ghurani R, Nsuami M, Turner D, Kvanli MB, Brown G, et al. Lipid abnormalities in HIV/hepatitis C virus-coinfected patients. HIV.Med. 2006 Nov;7(8):530-536.

(45) Patroni A, Torti C, Tomasoni L, Roldan EQ, Bertelli D, Puoti M, et al. Effect of highly active antiretroviral therapy (HAART) and hepatitis C Co-infection on hyperlipidemia in HIV-infected patients: a retrospective longitudinal study. HIV.Clin.Trials 2002 Nov-Dec;3(6):451-461.

(46) Montes ML, Pulido F, Barros C, Condes E, Rubio R, Cepeda C, et al. Lipid disorders in antiretroviral-naive patients treated with lopinavir/ritonavir-based HAART: frequency, characterization and risk factors. J.Antimicrob.Chemother. 2005 May;55(5):800-804.

(47) Riddler SA, Smit E, Cole SR, Li R, Chmiel JS, Dobs A, et al. Impact of HIV infection and HAART on serum lipids in men. JAMA 2003 Jun 11;289(22):2978-2982.

(48) El-Sadr WM, Mullin CM, Carr A, Gibert C, Rappoport C, Visnegarwala F, et al. Effects of HIV disease on lipid, glucose and insulin levels: results from a large antiretroviral-naive cohort. HIV.Med. 2005 Mar;6(2):114-121.

(49) Tebas P, Henry WK, Matining R, Weng-Cherng D, Schmitz J, Valdez H, et al. Metabolic and immune activation effects of treatment interruption in chronic HIV-1 infection: implications for cardiovascular risk. PLoS One 2008 Apr 23;3(4):e2021.

(50) Grinspoon S, Carr A. Cardiovascular risk and body-fat abnormalities in HIV-infected adults. N.Engl.J.Med. 2005 Jan 6;352(1):48-62.

(51) Farrugia PM, Lucariello R, Coppola JT. Human immunodeficiency virus and atherosclerosis. Cardiol.Rev. 2009 Sep-Oct;17(5):211-215.

(52) Christeff N, Melchior JC, de Truchis P, Perronne C, Gougeon ML. Increased serum interferon alpha in HIV-1 associated lipodystrophy syndrome. Eur.J.Clin.Invest. 2002 Jan;32(1):43-50.

(53) Mujawar Z, Rose H, Morrow MP, Pushkarsky T, Dubrovsky L, Mukhamedova N, et al. Human immunodeficiency virus impairs reverse cholesterol transport from macrophages. PLoS Biol. 2006 Oct;4(11):e365.

(54) Carr A, Samaras K, Chisholm DJ, Cooper DA. Pathogenesis of HIV-1-protease inhibitor-associated peripheral lipodystrophy, hyperlipidaemia, and insulin resistance. Lancet 1998 Jun 20;351(9119):1881-1883.

(55) Barbaro G. Cardiovascular manifestations of HIV infection. Circulation 2002 Sep 10;106(11):1420-1425.

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

(57) Rimland D, Guest JL, Hernandez-Ramos I, Del Rio C, Le NA, Brown WV. Antiretroviral therapy in HIV-positive women is associated with increased apolipoproteins and total cholesterol. J.Acquir.Immune Defic.Syndr. 2006 Jul;42(3):307-313.

(58) Periard D, Telenti A, Sudre P, Cheseaux JJ, Halfon P, Reymond MJ, et al. Atherogenic dyslipidemia in HIV-infected individuals treated with protease inhibitors. The Swiss HIV Cohort Study. Circulation 1999 Aug 17;100(7):700-705.

(59) Pere D, Ignacio SL, Ramon T, Fernando L, Alberto T, Pompeyo V, et al. Dyslipidemia and cardiovascular disease risk factor management in HIV-1-infected subjects treated with HAART in the Spanish VACH cohort. Open AIDS.J. 2008;2:26-38.

(60) Tsiodras S, Mantzoros C, Hammer S, Samore M. Effects of protease inhibitors on hyperglycemia, hyperlipidemia, and lipodystrophy: a 5-year cohort study. Arch.Intern.Med. 2000 Jul 10;160(13):2050-2056.

(61) Almeida SE, Borges M, Fiegenbaum M, Nunes CC, Rossetti ML. Metabolic changes associated with antiretroviral therapy in HIV-positive patients. Rev.Saude Publica 2009 Apr;43(2):283-290.

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

(63) Saves M, Raffi F, Capeau J, Rozenbaum W, Ragnaud JM, Perronne C, et al. Factors related to lipodystrophy and metabolic alterations in patients with human immunodeficiency virus infection receiving highly active antiretroviral therapy. Clin.Infect.Dis. 2002 May 15;34(10):1396-1405.

(64) Llibre JM, Domingo P, Palacios R, Santos J, Perez-Elias MJ, Sanchez-de la Rosa R, et al. Sustained improvement of dyslipidaemia in HAART-treated patients replacing stavudine with tenofovir. AIDS 2006 Jun 26;20(10):1407-1414.

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

(66) Lo J, Grinspoon S. Cardiovascular disease in HIV-infected patients: does HIV infection in and of itself increase cardiovascular risk? Curr.Opin.HIV.AIDS. 2008 May;3(3):207-213.

(67) Fagard RH, Nilsson PM. Smoking and diabetes-The double health hazard! Prim.Care.Diabetes 2009 Oct 27.

(68) Lonardo A, Carulli N, Loria P. HCV and diabetes. A two-question-based reappraisal. Dig.Liver Dis. 2007 Aug;39(8):753-761.

(69) Mehta SH, Brancati FL, Sulkowski MS, Strathdee SA, Szklo M, Thomas DL. Prevalence of type 2 diabetes mellitus among persons with hepatitis C virus infection in the United States. Ann.Intern.Med. 2000 Oct 17;133(8):592-599.

(70) Anderson RJ, Freedland KE, Clouse RE, Lustman PJ. The prevalence of comorbid depression in adults with diabetes: a meta-analysis. Diabetes Care 2001 Jun;24(6):1069-1078.

(71) Noor MA. The role of protease inhibitors in the pathogenesis of HIV-associated insulin resistance: cellular mechanisms and clinical implications. Curr.HIV/AIDS Rep. 2007 Aug;4(3):126-134.

(72) Cook JA, Burke-Miller JK, Cohen MH, Cook RL, Vlahov D, Wilson TE, et al. Crack cocaine, disease progression, and mortality in a multicenter cohort of HIV-1 positive women. AIDS 2008 Jul 11;22(11):1355-1363.

(73) Chao C, Jacobson LP, Tashkin D, Martinez-Maza O, Roth MD, Margolick JB, et al. Recreational drug use and T lymphocyte subpopulations in HIV-uninfected and HIV-infected men. Drug Alcohol Depend. 2008 Apr 1;94(1-3):165-171.

(74) Carrico AW, Johnson MO, Morin SF, Remien RH, Riley ED, Hecht FM, et al. Stimulant use is associated with immune activation and depleted tryptophan among HIV-

positive persons on anti-retroviral therapy. Brain Behav.Immun. 2008 Nov;22(8):1257-1262.

(75) Canadian Pharmacists Association editor. Compendium of Pharmaceuticals and Specialties 2009. 44th ed.: Canadian Pharmacists Association; 2009.

(76) Singer JD. Using SAS PROC MIXED to Fit Multilevel Models, Hierarchical Models, and Individual Growth Models. Journal of Educational and Behavioral Statistics Winter 1998;24(4):323-355.

(77) Spilke J, Piepho HP, Hu X. Analysis of Unbalanced Data by Mixed Linear Models Using the mixed Procedure of the SAS System. Journal of Agronomy and Crop Science 2005;191(1):47-54.

(78) Verges B. New insight into the pathophysiology of lipid abnormalities in type 2 diabetes. Diabetes Metab. 2005 Nov;31(5):429-439.

(79) Floris-Moore M, Howard AA, Lo Y, Schoenbaum EE, Arnsten JH, Klein RS. Hepatitis C infection is associated with lower lipids and high-sensitivity C-reactive protein in HIV-infected men. AIDS Patient Care STDS 2007 Jul;21(7):479-491.

(80) Polgreen PM, Fultz SL, Justice AC, Wagner JH, Diekema DJ, Rabeneck L, et al. Association of hypocholesterolaemia with hepatitis C virus infection in HIV-infected people. HIV.Med. 2004 May;5(3):144-150.

(81) Bernal E, Masia M, Padilla S, Gutierrez F. High-density lipoprotein cholesterol in HIV-infected patients: evidence for an association with HIV-1 viral load, antiretroviral therapy status, and regimen composition. AIDS Patient Care STDS 2008 Jul;22(7):569-575.

(82) Ribera E, Paradineiro JC, Curran A, Sauleda S, Garcia-Arumi E, Castella E, et al. Improvements in subcutaneous fat, lipid profile, and parameters of mitochondrial toxicity in patients with peripheral lipoatrophy when stavudine is switched to tenofovir (LIPOTEST study). HIV.Clin.Trials 2008 Nov-Dec;9(6):407-417.

(83) Burkhalter JE, Springer CM, Chhabra R, Ostroff JS, Rapkin BD. Tobacco use and readiness to quit smoking in low-income HIV-infected persons. Nicotine Tob.Res. 2005 Aug;7(4):511-522.

(84) Gritz ER, Vidrine DJ, Lazev AB, Amick BC,3rd, Arduino RC. Smoking behavior in a low-income multiethnic HIV/AIDS population. Nicotine Tob.Res. 2004 Feb;6(1):71-77.

(85) Ingersoll KS, Cropsey KL, Heckman CJ. A test of motivational plus nicotine replacement interventions for HIV positive smokers. AIDS.Behav. 2009 Jun;13(3):545-554.

(86) Bertholet N, Cheng DM, Samet JH, Quinn E, Saitz R. Alcohol consumption patterns in HIV-infected adults with alcohol problems. Drug Alcohol Depend. 2010 Jun 23.

(87) Domingos H, Cunha RV, Paniago AM, Martins DM, Elkhoury EB, Souza AS. Metabolic effects associated to the highly active antiretroviral therapy (HAART) in AIDS patients. Braz.J.Infect.Dis. 2009 Apr;13(2):130-136.

(88) Public Health Agency of Canada. HIV and AIDS in Canada. Surveillance Report to December 31, 2007. 2008.