

**CARDIOVASCULAR RISK IN HIV-POSITIVE PATIENTS: ASSESSMENT AND
PHARMACOLOGICAL TREATMENT**

by

Kevin Johns

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Abstract

In countries where it is available, highly active antiretroviral therapy (HAART) has transformed HIV infection into a manageable, chronic illness rather than an ultimately fatal condition. As HIV/AIDS-related morbidity and mortality have declined, adverse metabolic effects risen in frequency due to the combined effects of HAART and HIV infection itself. Adverse effects include blood lipid elevations that, in turn, lead to increased cardiovascular risk, potentially resulting in cardiovascular disease (CVD) or death. Treatment and management of these metabolic effects is becoming paramount within the HIV-positive (HIV+) population to extend the lifespan and improve quality of life.

A variety of studies were employed in order to accurately gauge both the risk of CVD posed to the HIV+ population and the efficacy of novel and accepted treatments for metabolic abnormalities in this population. A longitudinal cohort study served to assess the incidence of important metabolic endpoints in HAART-naïve patients initiating therapy. A cross-sectional study was used to assess the prevalence of peripheral arterial disease (PAD), a largely unexplored but clinically relevant cardiovascular endpoint. Two clinical trials investigated the efficacy of treatment in HIV+ patients with elevated cardiovascular risk. One explored the effect of the anti-hyperglycemic agent rosiglitazone on carotid intima media thickness and total plaque area. The second compared the effectiveness of two treatment strategies in patients not reaching lipid targets with rosuvastatin 10 mg: increasing the dose to 20 mg or adding ezetimibe 10 mg to ongoing rosuvastatin.

Findings of the cohort study included a unique and unexpected pattern of treatment-associated lipid abnormalities in HIV+ patients initiating therapy with non-nucleoside reverse transcriptase inhibitors. A low prevalence of PAD was observed in our population of HIV+ subjects most likely secondary to the young age of the participants and factors that confounded the method of assessment. Rosiglitazone did not prove to be an effective agent at reducing surrogate markers for CVD but did have positive effects on endothelial function and inflammatory markers. Finally, the addition of ezetimibe to ongoing rosuvastatin therapy was effective at lowering relevant endpoints, including apolipoprotein B, but did not perform significantly better than a doubled dose of rosuvastatin.

Preface

A version of chapter 1 has been published (Bennett MT, Johns, KW and Bondy GP. [2008] Current and future treatments of HIV-associated dyslipidemia. *Future Lipidology*. 3 (2):175-188). *Future Lipidology*. 3 (2):175-188). I performed the literature search with Bennett MT, wrote the sections on “Lipid-lowering Therapy”, edited the manuscript and updated the paper with recent literature for this thesis submission. Bennett MT wrote the first draft of the manuscript and designed the study with Bondy GP who also served to edit the manuscript. All authors read and approved the final manuscript.

Chapter 2 is based on work conducted with the Canadian Observational Cohort (CANOC) Collaboration. CANOC is an integrated network of all registered HIV/AIDS treatment information from eight cohort databases across British Columbia, Ontario and Quebec. The principal investigator is Hogg RS. Co-investigators are Cooper C, Klein M, Loutfy M, Machouf N, Montaner J, Raboud J, Rourke S and Tsoukas C. I designed the project and was the primary author. The principal investigators serve as part of a steering committee that reviews all work produced by the collaboration.

A version of chapter 3 has been published (Johns K, Saeedi R, Mancini GB, Bondy GP. [2010] Ankle brachial index screening for occult vascular disease is not useful in HIV-positive patients. *AIDS Research and Human Retroviruses*. 26(9):955-9). I submitted the project protocol through the ethics board; performed the Doppler ultrasound assessment; collected, managed and analyzed the data and was the primary author of the manuscript. Saeedi R aided in construction of the manuscript and with data analysis. Mancini GB

aided in study design and provided valuable insight into interpretation of the data. Bondy GP designed the study and recruited patients. All authors read and approved the final manuscript. This study was approved by the University of British Columbia-Providence Health Care Research Ethics Board (Certificate H06-50037).

A version of chapter 4 has been submitted for publication. I collected and managed the trial data, performed the statistical analysis and was the primary author of the manuscript. Dr. S. Chan authored the section of protocol related to flow-mediated dilation. Dr. R. Saeedi performed the inflammatory cytokine assays and aided in drafting the manuscript. Dr. G. Mancini authored ultrasound protocols and supervised interpretations of ultrasound recordings at his Cardiovascular Imaging Research Core Laboratory. Drs. M. Harris, J. Montaner and Dr. G. Bondy were the primary authors of the protocol and recruited patients for the trial. All authors read and approved the final manuscript. This study was approved by the University of British Columbia-Providence Health Care Research Ethics Board (Certificate H02-50086).

A section of chapter 5 has been published (Bennett MT, Johns KW and Bondy GP. [2007] Ezetimibe is effective when added to maximally tolerated lipid-lowering therapy in patients with HIV. *Lipid in Health and Disease*. 6(15): 1-5). I was the primary collector of data by means of chart review, conducted statistical analysis and edited the manuscript. Bennett MT was the principal author of the paper, participated in design of the project and aided in data acquisition. Bondy GP designed the project, aided in data acquisition and was the principal editor of the manuscript. All authors read and approved

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A portion of chapter 5 is based on work performed in the HIV metabolic clinic at the John Ruedy Immunodeficiency Clinic at St. Paul's Hospital. I was responsible for ethical considerations; designing the study protocol; enrolling and following up with patients; collection, management and analysis of data; and I was the principal author of the first draft of the manuscript. Dr. M.T. Bennett conceptualized the study and Dr. G. Bondy aided in design and construction of the study protocol and recruited patients. This study was approved by the University of British Columbia-Providence Health Care Research Ethics Board (Certificate H08-00287).

Table of Contents

| | |
|---|------|
| Abstract..... | ii |
| Table of Contents..... | vii |
| List of Tables..... | xv |
| List of Figures..... | xvi |
| List of Abbreviations..... | xvii |
| Acknowledgements..... | xix |
| | xx |
| Chapter 1: Introduction..... | 1 |
| 1.1 Cardiovascular Risk..... | 1 |
| 1.1.1 Risk of Myocardial Infarction..... | 2 |
| 1.1.2 Peripheral Arterial Disease in HIV..... | 3 |
| 1.1.3 Risk Stratification..... | 3 |
| 1.1.4 Atherosclerotic Process..... | 4 |
| 1.2 Effect of HIV on the Lipid Profile..... | 5 |
| 1.2.1 HIV-mediated HDL Reduction..... | 5 |
| 1.2.2 HIV-mediated Triglyceride Elevation..... | 6 |
| 1.3 Antiretroviral Therapy and its Effect on the Lipid Profile..... | 6 |
| 1.3.1 Protease Inhibitors..... | 7 |
| 1.3.2 Nucleoside Reverse Transcriptase Inhibitors..... | 9 |
| 1.3.3 Non-Nucleoside Reverse Transcriptase Inhibitors..... | 10 |
| 1.3.4 Combination ARV..... | 11 |
| 1.3.5 Fusion Inhibitors..... | 12 |

| | | |
|--|--|----|
| 1.4 | Treatment of Dyslipidemia and Attenuation of Risk..... | 12 |
| 1.4.1 | Diet and Exercise | 12 |
| 1.4.2 | Switch Studies..... | 13 |
| 1.4.3 | Lipid-lowering Therapy | 15 |
| 1.4.3.1 | Statins..... | 15 |
| 1.4.3.2 | Fibrates..... | 18 |
| 1.4.3.3 | Ezetimibe | 19 |
| 1.4.3.4 | Niacin and Fish Oil/Omega-3 Fatty Acid | 20 |
| 1.4.4 | Insulin Sensitizers | 21 |
| 1.4.4.1 | Biguanides..... | 21 |
| 1.4.4.2 | Thiazolidinediones..... | 22 |
| 1.4.4.3 | Pioglitazone..... | 23 |
| 1.5 | Objectives and Hypotheses | 23 |
| Chapter 2: Incidence of Regimen-specific Metabolic Abnormalities in Patients | | |
| | Initiating HAART | 25 |
| 2.1 | Background..... | 25 |
| 2.1.1 | The CANOC Collaboration | 26 |
| 2.1.2 | Comparison of Lipid Abnormalities Between Regimens | 26 |
| 2.1.3 | Atherogenic Index of Plasma..... | 26 |
| 2.2 | Methods..... | 27 |
| 2.2.1 | Participants..... | 27 |
| 2.2.2 | Outcomes | 28 |
| 2.2.3 | Statistical Methods..... | 28 |

| | | |
|--|--|----|
| 2.3 | Results..... | 29 |
| 2.3.1 | Incidence Rate and Risk of Abnormalities by Drug Class | 29 |
| 2.3.1.1 | PI vs. NNRTI | 29 |
| 2.3.2 | Incidence Rate and Risk of Abnormalities by Individual Agent | 30 |
| 2.3.2.1 | Atazanavir vs. Lopinavir..... | 30 |
| 2.3.2.2 | Nevirapine vs. Efavirenz..... | 31 |
| 2.3.3 | NRTI/NtRTI Backbones | 32 |
| 2.3.3.1 | Abacavir vs. Tenofovir | 32 |
| 2.4 | Discussion | 33 |
| 2.4.1 | PI vs. NNRTI | 33 |
| 2.4.1.1 | Efavirenz vs. Nevirapine..... | 34 |
| 2.4.1.2 | Atazanavir vs. Lopinavir..... | 35 |
| 2.4.2 | NRTI/NtRTI Backbones | 35 |
| 2.4.3 | Atherogenic index of plasma | 36 |
| 2.4.4 | Limitations | 37 |
| 2.4.5 | Conclusions..... | 38 |
| Chapter 3: Prevalence of Peripheral Arterial Disease in HIV-positive Patients..... | | 45 |
| 3.1 | Background | 45 |
| 3.2 | Methods..... | 46 |
| 3.2.1 | Participants..... | 46 |
| 3.2.2 | Outcomes | 47 |
| 3.2.2.1 | Ankle-brachial Index | 47 |
| 3.2.2.2 | Cardiovascular Risk..... | 47 |

| | | |
|------------|---|----|
| 3.2.3 | Statistical Analysis..... | 48 |
| 3.3 | Results..... | 48 |
| 3.4 | Discussion..... | 49 |
| 3.4.1 | Risk factors for PAD..... | 49 |
| 3.4.2 | Influence of HAART on PAD..... | 51 |
| 3.4.3 | Limitations..... | 51 |
| 3.4.4 | Conclusions..... | 52 |
| Chapter 4: | Cardiovascular Risk Reduction with Rosiglitazone..... | 55 |
| 4.1 | Methods..... | 56 |
| 4.1.1 | Participants..... | 56 |
| 4.1.2 | Ethics..... | 57 |
| 4.1.3 | Interventions..... | 57 |
| 4.1.4 | Outcomes..... | 58 |
| 4.1.4.1 | IMT and Plaque Burden..... | 58 |
| 4.1.4.2 | Flow-mediated Dilation..... | 58 |
| 4.1.4.3 | Body Composition and Anthropometry..... | 59 |
| 4.1.4.4 | Laboratory Assessments..... | 59 |
| 4.1.5 | Measurement of Biomarkers of Inflammation..... | 59 |
| 4.1.6 | Sample Size Calculation..... | 60 |
| 4.1.7 | Statistical Analysis..... | 60 |
| 4.1.8 | Randomization..... | 60 |
| 4.1.8.1 | Sequence Generation..... | 60 |
| 4.1.8.2 | Allocation Concealment..... | 61 |

| | | |
|---------|--|----|
| 4.1.8.3 | Implementation | 61 |
| 4.1.8.4 | Blinding..... | 61 |
| 4.2 | Results..... | 61 |
| 4.2.1 | Participant Flow | 61 |
| 4.2.2 | Recruitment..... | 62 |
| 4.2.3 | Baseline Data | 62 |
| 4.2.4 | Numbers Analyzed..... | 62 |
| 4.2.5 | Outcomes | 62 |
| 4.2.5.1 | Intima Media Thickness..... | 63 |
| 4.2.5.2 | Plaque Area..... | 63 |
| 4.2.5.3 | Flow-mediated Dilation | 63 |
| 4.2.5.4 | Biomarkers of Inflammation..... | 64 |
| 4.2.5.5 | DXA and Anthropometry | 64 |
| 4.2.5.6 | Safety Parameters..... | 65 |
| 4.2.6 | Adverse Events | 65 |
| 4.3 | Discussion..... | 65 |
| 4.3.1 | Primary Outcomes | 65 |
| 4.3.1.1 | Intima Media Thickness..... | 66 |
| 4.3.1.2 | Plaque Burden..... | 67 |
| 4.3.2 | Secondary Outcomes | 67 |
| 4.3.2.1 | Endothelial Function and FMD | 67 |
| 4.3.2.2 | Inflammatory Markers | 68 |
| 4.3.2.3 | Markers of Insulin Resistance and Lipid Parameters | 69 |

| | | |
|------------|---|----|
| 4.3.2.4 | Fat Distribution and Lipodystrophy..... | 69 |
| 4.3.2.5 | Safety Parameters..... | 70 |
| 4.3.3 | Rosiglitazone and Cardiovascular Risk | 70 |
| 4.3.4 | Conclusions..... | 71 |
| 4.4 | Clinical Trial Registration..... | 72 |
| Chapter 5: | Pharmacological Treatment of HIV-associated Dyslipidemia..... | 80 |
| 5.1.1 | Objectives | 81 |
| 5.1.1.1 | Effectiveness of Ezetimibe | 81 |
| 5.1.1.2 | Effectiveness of Rosuvastatin | 81 |
| 5.1.1.3 | Increased Dose of Rosuvastatin vs. Addition of Ezetimibe..... | 82 |
| 5.2 | Methods..... | 82 |
| 5.2.1 | Effectiveness of Ezetimibe | 82 |
| 5.2.1.1 | Study Design and Setting..... | 82 |
| 5.2.1.2 | Outcomes | 83 |
| 5.2.1.3 | Statistical Analysis..... | 83 |
| 5.2.2 | Effectiveness of Rosuvastatin..... | 84 |
| 5.2.2.1 | Study Design and Setting..... | 84 |
| 5.2.2.2 | Outcomes | 84 |
| 5.2.2.3 | Statistical Analysis and Ethics | 85 |
| 5.2.3 | Increased Dose of Rosuvastatin vs. Addition of Ezetimibe..... | 85 |
| 5.2.3.1 | Study Design..... | 85 |
| 5.2.3.2 | Outcomes | 86 |
| 5.2.3.3 | Randomization | 86 |

| | | |
|---------|--|-----|
| 5.2.3.4 | Eligibility | 87 |
| 5.2.3.5 | Sample Size and Power Calculation | 88 |
| 5.2.3.6 | Statistical Analysis..... | 89 |
| 5.3 | Results..... | 89 |
| 5.3.1 | Effectiveness of Ezetimibe | 89 |
| 5.3.1.1 | Overall Effect of Addition of Ezetimibe..... | 90 |
| 5.3.1.2 | Ezetimibe in Combination with Other Lipid-lowering Therapies | 90 |
| 5.3.1.3 | Patients Reaching Lipid Targets | 91 |
| 5.3.2 | Effectiveness of Rosuvastatin | 91 |
| 5.3.2.1 | Lipids Effects | 92 |
| 5.3.2.2 | Dose-response Analysis..... | 92 |
| 5.3.2.3 | Combination Therapy vs. Monotherapy | 92 |
| 5.3.2.4 | Effect of Protease Inhibitors on Rosuvastatin..... | 93 |
| 5.3.3 | Increased Dose of Rosuvastatin vs. Addition of Ezetimibe..... | 93 |
| 5.3.3.1 | Overall Effects of Treatments..... | 93 |
| 5.3.3.2 | Effect on Apolipoproteins..... | 94 |
| 5.3.3.3 | Patients Reaching Target Endpoints | 95 |
| 5.4 | Discussion | 95 |
| 5.4.1 | Effectiveness of Ezetimibe | 95 |
| 5.4.2 | Effectiveness of Rosuvastatin..... | 97 |
| 5.4.3 | Increased dose of Rosuvastatin vs. Addition of Ezetimibe..... | 100 |
| 5.4.3.1 | Apolipoproteins as CVD Risk Markers in HIV | 102 |
| 5.4.3.2 | Attaining Canadian Cardiovascular Society Targets | 104 |

| | | |
|-------------|---|-----|
| 5.4.4 | Ezetimibe for Attenuation of CVD Risk..... | 106 |
| 5.4.4.1 | Limitations | 107 |
| 5.4.5 | Conclusions..... | 107 |
| Chapter 6: | Future Directions..... | 121 |
| 6.1 | Determination of Time to Metabolic Syndrome | 121 |
| 6.2 | Utility of New CVD Risk Markers | 122 |
| 6.3 | Agents for Attenuation of CVD Risk..... | 123 |
| 6.4 | Biochemical Mechanism of Statin Resistance | 123 |
| 6.5 | Prevention of Dyslipidemia | 124 |
| Chapter 7: | Conclusions | 126 |
| Works Cited | | 128 |

List of Tables

| | |
|---|-----|
| Table 2.1 Prevalence of metabolic abnormalities at pre-ARV baseline | 39 |
| Table 2.2 Demographic characteristics of study participants. | 40 |
| Table 3.1 Demographic and clinical characteristics. | 53 |
| Table 3.2 Cardiovascular risk factor constellations for patients with low ABI..... | 54 |
| Table 4.1 Traditional cardiovascular risk factors of study subjects at baseline..... | 74 |
| Table 4.2 Baseline characteristics of study subjects. | 75 |
| Table 4.3 Ultrasound outcomes at baseline and following 48 weeks of treatment with rosiglitazone or placebo. | 76 |
| Table 4.4 Metabolic parameters at baseline and following 48 weeks of treatment with rosiglitazone or placebo | 77 |
| Table 4.5 Anthropometric and body composition variables at baseline and following 48 weeks of treatment with rosiglitazone or placebo..... | 78 |
| Table 4.6 Safety variables at baseline and following 48 weeks of treatment with rosiglitazone..... | 79 |
| Table 5.1 Baseline demographics of 33 patients initiating ezetimibe. | 109 |
| Table 5.2 Baseline demographics of 130 patients initiating rosuvastatin..... | 110 |
| Table 5.3 Baseline characteristics of the clinical trial group..... | 111 |
| Table 5.4 Effects of adding ezetimibe to rosuvastatin or increasing rosuvastatin dosage on lipid and metabolic outcomes. | 112 |

List of Figures

| | |
|---|-----|
| Figure 2.1 Overall incidence of metabolic abnormalities within CANOC..... | 41 |
| Figure 2.2 Incidence of metabolic abnormalities between patients initiating PIs or NNRTIs..... | 42 |
| Figure 2.3 Incidence of metabolic abnormalities between patients initiating atazanavir/ritonavir or lopinavir/ritonavir..... | 43 |
| Figure 2.4 Incidence of metabolic abnormalities between patients initiating NRTI backbones containing FTC/TDF and 3TC/TDF or 3TC/ABC. | 44 |
| Figure 4.1 Flow chart of patient disposition. | 73 |
| Figure 5.1 Overall effect of addition of ezetimibe to maximally tolerated lipid-lowering therapy..... | 113 |
| Figure 5.2 Effect of ezetimibe on lipids by baseline lipid-lowering therapy..... | 114 |
| Figure 5.3 Percent of patients reaching targets for moderate or high cardiovascular risk following addition of ezetimibe..... | 115 |
| Figure 5.4 Dose response analysis for rosuvastatin..... | 116 |
| Figure 5.5 Change in lipid parameters with either rosuvastatin alone or in combination with fenofibrate..... | 117 |
| Figure 5.6 Change in lipid parameters in statin-naïve patients initiating rosuvastatin on PI-containing or PI-sparing HAART regimens at baseline. | 118 |
| Figure 5.7 Effect on apolipoprotein B for patients with a baseline level >0.80g/L..... | 113 |
| Figure 5.8 Percentage of patients at target for high-risk subjects at baseline and following 12 weeks of treatment with 20 mg rosuvastatin (A) or ezetimibe and 10 mg rosuvastatin (B)..... | 120 |

List of Abbreviations

| Term | Definition |
|-------------------|---|
| 3TC | Lamivudine |
| ABC | Abacavir |
| ABI | Ankle-brachial index |
| AIP | Atherogenic index of plasma |
| ALT | Alanine transaminase |
| ANCOVA | Analysis of covariance |
| AST | Aspartate transaminase |
| ApoA1 | Apolipoprotein A1 |
| ApoB | Apolipoprotein B |
| ApoB:ApoA1 | Apolipoprotein B to Apolipoprotein A1 ratio |
| ARV | Antiretroviral |
| AUC | Area under the curve |
| AZT | Zidovudine |
| CAD | Coronary artery disease |
| CANOC | Canadian Observational Cohort |
| CCS | Canadian Cardiovascular Society |
| CK | Creatine Kinase |
| C _{max} | Maximum concentration |
| CRP | C-reactive protein |
| CTN | CIHR Canadian HIV Trials Network |
| CVD | Cardiovascular disease |
| CYP | Cytochrome P450 |
| d4T | Stavudine |
| DAD | Data collection on adverse events of anti-HIV drugs |
| ddI | Didanosine |
| ddC | Zalcitabine |
| DL | Dyslipidemia |
| DXA | Dual X-ray Absorptiometry |
| FFA | Free fatty acids |
| FMD | Flow-mediated dilation |
| FTC | Emtricitabine |
| HAART | Highly active antiretroviral therapy |
| HbA _{1c} | Hemoglobin A _{1c} |
| HDL | High-density lipoprotein cholesterol |
| HIV+ | HIV positive |
| HOPS | HIV outpatients study |
| HR | Hazard ratio |
| IDC/HIVMC | Immunodeficiency clinic/HIV metabolic clinic |
| IDL | Intermediate-density lipoprotein cholesterol |
| IL-6 | Interleukin-6 |
| IMT | Intima media thickness |

| | |
|----------------|---|
| INF- α | Interferon- α |
| LDL | Low-density lipoprotein cholesterol |
| MCP-1 | Monocyte chemotactic protein-1 |
| MI | Myocardial infarction |
| MS | Metabolic Syndrome |
| NCEP | National Cholesterol Education Program |
| NMD | Nitroglycerin-mediated dilation |
| NNRTI | Non-nucleoside reverse transcriptase inhibitor |
| NRTI | Nucleoside reverse transcriptase inhibitor |
| PAD | Peripheral arterial disease |
| PI | Protease inhibitor |
| PPAR- γ | Peroxisome proliferator-activated receptor-gamma |
| PY | Pack-years |
| PYFU | Patient years follow-up |
| RBP-4 | Retinol-binding protein-4 |
| SD | Standard deviation |
| SREBP-1 | Sterol regulatory element-binding protein-1 |
| Statin | HMG-CoA reductase inhibitor |
| T20 | Enfuvirtide |
| T2DM | Type 2 diabetes mellitus |
| TC | Total cholesterol |
| TC:HDL | Total cholesterol to high-density lipoprotein cholesterol ratio |
| TDF | Tenofovir disoproxil fumarate |
| TG | Triglycerides |
| TNF | Tumour necrosis factor- α |
| TPA | Total plaque area |
| TZD | Thiazolidinedione |
| ULN | Upper limit of normal |
| VAT | Visceral adipose tissue |
| VLDL | Very low-density lipoprotein cholesterol |
| WTOH | Waist-to-hip ratio |

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To my parents Lawrence and Barbara Johns

Chapter 1: Introduction

It is estimated that 33.2 million people are infected with HIV worldwide [1]. In North America the prevalence of HIV continues to rise and it is estimated that 1,000,000 North Americans may be infected with HIV [1]. With the advances in antiretroviral (ARV) therapy the prognosis of patients infected with HIV is improving. The advent of highly active antiretroviral therapy (HAART) has lessened AIDS-related death and rendered HIV into a chronic, manageable disease wherein more than 85% of patients receiving HAART live longer than 10 years after becoming infected [2]. With the reduction of AIDS related deaths there has been a steady increase in mortality from other diseases, including cardiovascular disease (CVD), which has risen to be the second most frequent cause of death in HIV-positive patients [3]. The reason for the rise in CVD in the population is multifactorial, and includes non-modifiable risk factors such as age and gender, but a large proportion of the attributable risk is secondary to the dyslipidemia associated with the HIV virus and to treatment with HAART [3-6].

1.1 Cardiovascular Risk

HAART has reduced morbidity and mortality in the HIV positive population while extending life expectancy [7, 8]. However, the well-documented HAART-associated metabolic abnormalities such as dyslipidemia, insulin resistance and central obesity coupled with an aging HIV-positive (HIV+) population have lead to an increased incidence of cardiovascular events [6, 9-13].

1.1.1 Risk of Myocardial Infarction

The Data Collection on Adverse events of Anti-HIV Drugs (DAD) study group examined the risk factors that were associated with the risk of myocardial infarction in 23, 468 patients with HIV. The risk of myocardial infarction was increased with hypercholesterolemia and hypertriglyceridemia. In this group, the risk of myocardial infarction was increased by 26% per year with the use of ARV therapy. This finding was even seen in the first four to six years [11].

The updated DAD study, which had a total follow-up of 94,469 patient years, showed that the increased risk of myocardial infarction was 16% [14]. Conversely, a decrease in risk for myocardial infarction was reported in the HIV outpatient study (HOPS) [15]. The HOPS authors hypothesize that this is due to the recognition of vascular risk in patients with HIV and the associated use of anti-hypertensive and lipid-lowering medication [16]. Klein et al were also able to show that the risk of myocardial infarction was statistically higher in a group of 4159 HIV-positive men when compared with HIV negative controls. It appears the risk of myocardial infarction is elevated in both patients taking and not taking ARVs [17].

Grover et al. examined the risk of vascular disease in patients with HIV using a Markov model to estimate the effect of dyslipidemia on coronary disease risk and life expectancy. They estimated dyslipidemia increased this risk over 10 years by approximately 50% regardless of gender or the presence of other coronary risk factors. They further proposed that this risk was significant enough to decrease long term survival despite the mortality associated with HIV infection itself [18].

1.1.2 Peripheral Arterial Disease in HIV

Peripheral arterial disease (PAD) of lower extremities is a manifestation of systemic atherosclerosis and a predictor of cardiovascular events [19]. In the general population PAD is associated with traditional cardiovascular risk factors including smoking, type 2 diabetes mellitus (T2DM), hypertension and dyslipidemia [20]. These factors are more prevalent in the HIV positive population [13, 21].

HIV-positive patients are subject to insulin resistance as a result of complications from HAART, the causal mechanism of which has not yet been elucidated, and may experience chronic hyperglycemia similar to diabetes [9, 22]. The resulting hyperglycemia can lead to arterial stiffening due to calcification. The calcified peripheral arteries become incompressible resulting in an elevated ankle brachial index (ABI). As a result the arterial calcification can effectively mask any subclinical cardiovascular disease that is present [23-26].

1.1.3 Risk Stratification

The decision on when and how aggressively to treat patients with dyslipidemia depends on their overall risk for vascular disease. Dyslipidemia is an important risk factor for the development of vascular disease. In the non-HIV population, the Framingham risk score is commonly used to estimate a patient's vascular risk. This combines modifiable risk factors such as total cholesterol (TC) and high-density lipoprotein-cholesterol (HDL) in addition to systolic blood pressure and smoking status as well as the non-modifiable risk factors of age and gender. Together these risk factors estimate the 10-year risk of cardiac death or non-fatal myocardial infarction. Patients are then determined as low, medium or high risk if their 10 year risk is <10%, 10-19% or \geq 20% respectively. Patients with

known vascular disease (coronary artery disease, cerebrovascular disease or peripheral artery disease) and most patients with chronic kidney disease or established T2DM are considered high risk [27].

Guidelines for the treatment of dyslipidemia in HIV have been published by the International AIDS Society, the HIV Medicine Association of the Infectious Disease Society of America and the Adult AIDS Clinical Trials Group [28, 29]. These guidelines were published in 2002 and 2003, respectively. These guidelines have not yet incorporated the aggressive cholesterol targets suggested by most of the major societies for the non-HIV population. The most recent guidelines published by the Canadian Cardiovascular Society (CCS) suggest that the target for high and moderate risk groups be LDL <2.0 mmol/L or apolipoprotein B (ApoB) <0.80 g/L, or a $\geq 50\%$ decrease in LDL be the target for low risk patients warranting therapy [30]. The CCS guidelines currently recommend that HIV patients with chronic HIV infection who are on HAART have their lipid profile and cardiovascular risk factors screened and be treated according to their determined risk [13, 30].

1.1.4 Atherosclerotic Process

Atherogenesis is a complex process that involves oxidative processes, endothelial dysfunction and inflammation resulting in deposition of lipids and other materials within the vascular wall. At the core of atherosclerosis is hypercholesterolemia specifically, elevations in low-density lipoprotein cholesterol (LDL) [31]. LDL that is small and dense is susceptible to oxidation [32]. This oxidized LDL induces endothelial dysfunction more potently than does native LDL [33]. The dysfunctional endothelium allows penetration of the LDL into the subendothelial space. Lipid accumulation triggers the exposure of

inflammatory and adhesive proteins (selectins and cellular adhesion molecules) that promote the recruitment of monocytes into the intima where they transform into macrophages and foam cells by engulfing the accumulated lipids [34]. The toxicity of oxidized lipids induces apoptotic death in the lipid-rich macrophages triggering release of their cytosolic content and generating the necrotic lipid core typical of advanced atherosclerotic lesions [35]. The release of further factors, such as matrix metalloproteinases, facilitates plaque rupture leading to thrombus formation and cardiovascular events [36].

The small, dense LDL particles that play an integral role in atherogenesis are more abundant in patients with insulin-resistance and type 2 diabetes [37]. Furthermore, small-LDL particles and low plasma concentrations of HDL cholesterol can induce endothelial dysfunction independently of LDL plasma concentration [38]. Lipid abnormalities are common complications of HIV infection and its treatment and are at the root of the elevated cardiovascular risk in HIV+ patients.

1.2 Effect of HIV on the Lipid Profile

The dyslipidemia due to the HIV virus is characterized by reductions in serum HDL and TC followed by elevations in triglycerides (TG), very low-density lipoprotein (VLDL) and intermediate density lipoprotein (IDL) [4, 5, 39-42]. The decrease in HDL is associated with immune activation markers, including CD4+ count, associated with HIV infection [43].

1.2.1 HIV-mediated HDL Reduction

A mechanism for the decrease in HDL seen with HIV infection was proposed by Mujawar et al who found that HIV impairs cholesterol efflux from macrophages through

deleterious effects on ATP-binding cassette transporter A1, which mediates efflux of cholesterol to lipid-poor apolipoprotein A1 (ApoA1) to form nascent HDL [44]. Furthermore the inflammatory state caused by HIV infection contributes to reduced plasma HDL by activating endothelial lipase and lipoprotein-associated phospholipase A2, which function to degrade HDL [45]. Finally, as HDL becomes triglyceride-rich with the concomitant hypertriglyceridemia due to HIV it becomes increasingly subject to hepatic lipase-mediated clearance [45].

1.2.2 HIV-mediated Triglyceride Elevation

The proposed mechanisms of hypertriglyceridemia in HIV are related to the inflammatory milieu created by the virus. Patients with advanced HIV disease develop a dyslipidemia similar to that seen in other chronic infections, such as hepatitis, wherein elevated interferon- α (INF- α) interferes with TG clearance [46-48]. Another cytokine, tumour necrosis factor- α (TNF- α), has been shown to interfere with free fatty acid (FFA) metabolism, lipid oxidation and suppression of insulin-mediated lipolysis [49]. These factors together with anomalous, accelerated lipogenesis seen in HIV [5] and the compromised dietary state of HIV-positive patients [39, 40] all likely contribute to the unique pattern of dyslipidemia seen in HIV.

1.3 Antiretroviral Therapy and its Effect on the Lipid Profile

In the current era of HIV treatment most patients who have access to ARV will be treated with them at some point over the course of their disease. The current standard of care is to treat patients with HIV with multiple combination ARV therapy referred to as HAART. Although these treatments are lifesaving and markedly prolong survival, they adversely affect the lipid profile. Each class of ARV (protease inhibitors [PI], nucleoside

reverse transcriptase inhibitors [NRTI], non-nucleoside reverse transcriptase inhibitors [NNRTI] and fusion inhibitors) differs with respect to its effect on the lipid profile. In general, the dyslipidemia associated with HAART includes elevated TG, TC, LDL and decreased HDL [6, 50-53] further proatherogenic complications include increased levels of small, dense LDL [42], lipoprotein (a), ApoB [54, 55], C-III, E and H.

1.3.1 Protease Inhibitors

While the additive effects of the different classes of ARV likely contribute to the constellations of lipid abnormalities seen with HAART the effects of PIs on the lipid profile are well documented and are the most severe by clinical standards. Accordingly, a number of PIs have been shown to induce the characteristic dyslipidemia seen in HAART-treated HIV patients [6, 9, 10, 14, 22, 56-62]. There is a wide variation in the frequency of dyslipidemias induced by PIs. The PI that is associated with the highest frequency of dyslipidemias is ritonavir. Ritonavir is commonly used in PI-based ARV regimens [63]. Ritonavir is a potent inhibitor of cytochrome P450 (CYP) 3A4 and is used to increase or “boost” the circulating blood levels of PIs co-administered with ritonavir. Purnell et al. showed that ritonavir induces a characteristic dyslipidemia in HIV-negative individuals [58]. These effects appear to be dose dependent. Shafran et al. showed that although ritonavir still adversely affected the lipid profile when administered at a low dose these effects were less severe when tested in HIV-negative individuals [64]. Other PIs show varying effects on lipids. Atazanavir does not cause significant changes in lipid levels when administered in monotherapy [65]. When atazanavir is co-administered with ritonavir in boosted regimes it can induce dyslipidemias [66]. Since most PIs are

administered in boosted regimes with ritonavir it can be difficult to assess the effect of an individual PI with regards to the induction of a dyslipidemia.

A limited number of studies have analyzed the effects of specific protease inhibitors against one another. Periard et al. compared the effect of ritonavir, indinavir and nelfinavir on the fasting lipid profile in HIV-infected individuals. Although all three protease inhibitors induced elevations of plasma cholesterol, levels were higher in patients treated with ritonavir than those treated with indinavir or nelfinavir. Furthermore marked hypertriglyceridemia was seen only in the ritonavir group. There was no sequential worsening in the lipid profile when saquinavir was added to ritonavir on nelfinavir [6]. Young et al. also examined the lipid profile alterations of nelfinavir alone compared to boosted PIs lopinavir/ritonavir and indinavir/ritonavir in 1065 ARV-naïve patients. Overall, nelfinavir had minimal effect on the lipid profile at follow up. Although both combinations adversely affected the lipid profile by increasing non-HDL cholesterol and TG, these effects were more pronounced in the group taking indinavir/ritonavir [67]. A comparison of fosamprenavir/ritonavir and lopinavir/ritonavir revealed significant increases in TC, TG, LDL and VLDL as well as a decrease in HDL particle size for both the fosamprenavir/ritonavir (6.6%) and lopinavir/ritonavir groups (10.8%) [68]. Johnson et al compared commonly used PIs atazanavir and lopinavir in ritonavir-boosted regimens in treatment-experienced patients. The lopinavir/ritonavir regime caused significant increases in TC and TG compared with atazanavir/ritonavir [69]. The large, international, multicenter, open-label CASTLE study further confirmed significant elevations in TC, TG and non-HDL cholesterol with lopinavir/RTCV while also reporting that patients on atazanavir/ritonavir reached National Cholesterol

Education Program (NCEP) targets more frequently and fewer required lipid-lowering therapy during the 96-week trial [70]. Darunavir, a new protease inhibitor, like atazanavir, has a moderate effects on lipid parameters [71]. Studies directly comparing darunavir to other PIs are limited but one trial reported significantly elevated TC and TG in lopinavir/ritonavir compared to darunavir/ritonavir. No comparisons have been made between darunavir and atazanavir in HIV-positive patients but one study, in healthy volunteers, reported similar, minor changes to lipid endpoints in the two groups [72].

The specific mechanism by which protease inhibitors induce the metabolic complications of insulin resistance and dyslipidemia remains unclear. One possible mechanism is that PIs inhibit cellular proteases that are involved in the processing and/or regulation of key proteins involved on lipid metabolism. For example, ApoB, the major protein component of VLDL and LDL particles and regulator of their degradation, can be affected by ritonavir. It has been shown that ritonavir can profoundly affect ApoB degradation and this leads to the accumulation of TG and cholesterol-rich lipid particles (such as VLDL) leading to hypertriglyceridemia, low HDL and hypercholesterolemia [73].

Certain PIs have been shown to cause defective activation and nuclear translocation of the transcription factor sterol regulatory element-binding protein 1 (SREBP-1), inhibition of insulin-sensitive glucose transporter-4 and inhibition of the proteasomal pathway involved in degradation of SREBP-1 [74]. These factors alter lipid metabolism and result in the lipid changes seen with protease inhibitor treatment.

1.3.2 Nucleoside Reverse Transcriptase Inhibitors

NRTIs also adversely affect the lipid profile. The predominant effect of this class is an increase the serum TG [22, 75, 76]. The degree of induced dyslipidemia varies widely

within this class of ARV therapy. Zidovudine (AZT), tenofovir (TDF) and emtricitabine (FTC) appear to be associated with the fewest disturbances in lipid endpoints. Conversely, Stavudine (d4T), Didanosine (ddI), zalcitabine (ddC), lamivudine (3TC) and abacavir (ABC) are associated with hypertriglyceridemia and other lipid disturbances [75, 77-79]. A large, longitudinal cohort study investigating the effects of NRTI agents on lipid profile further confirmed the heterogeneity with respect to endpoints in this class. They concluded that initiation of therapy with 3TC/TDF led to the smallest increase in lipid endpoints whereas ddI/3TC therapy was associated with increases in LDL [80]. Effects of individual NRTI agents remain difficult to ascertain as these drugs are often used in combination.

1.3.3 Non-Nucleoside Reverse Transcriptase Inhibitors

Commonly used NNRTIs in HAART, nevirapine and efavirenz, differ in their effects on lipid end points in HIV-positive patients. While both have been linked to increases in the serum concentrations of TC, HDL, LDL and TG [81], initiation of nevirapine has been associated with an anti-atherogenic lipid profile that included increased apolipoprotein AI and HDL particle size in addition to a reduced TC to HDL ratio (TC:HDL) versus indinavir [82]. In addition, nevirapine has been shown to reverse atherogenic lipoprotein profiles caused by PIs [83, 84]. The anti-atherogenic metabolic profile associated with nevirapine was further confirmed in the ARTEN trial, which compared nevirapine to atazanavir/ritonavir. Nevirapine led to greater decreases in TC:HDL and did not raise TG compared to atazanavir/ritonavir [85]. One study directly compared effects of nevirapine or efavirenz in ARV naïve patients beginning treatment with either nevirapine or efavirenz. Efavirenz significantly increased serum TG but not HDL. Although both

agents increased the TC and LDL cholesterol, these increases were similar between the two groups [81]. The adverse effects of efavirenz were further confirmed by the ACTG 5142 trial, which compared combination of lopinavir/ritonavir plus two NRTIs, efavirenz plus two NRTIs and lopinavir/ritonavir/efavirenz. Although TC and HDL levels were significantly elevated in the lopinavir/ritonavir/efavirenz group, there was no difference in the lipids between the efavirenz plus two NRTs group and the lopinavir/ritonavir plus two NRTIs group. Both the lopinavir/ritonavir/efavirenz group and the efavirenz plus two NRTIs group resulted in higher TG than the lopinavir/ritonavir plus NRTIs group [86, 87]. The unfavourable lipid profile associated with efavirenz may be due, in part, to adverse effects on mitochondrial activity, an effect not seen with nevirapine [88].

1.3.4 Combination ARV

Single dose ARV combination therapies are becoming more widespread due to convenience and reduced pill burden for patients. One study investigated the effect of three ARV combinations on lipid profile. Trizivir® (ABC/3TC/AZT), Combivir® (3TC/AZT) plus nelfinavir and d4T/3TC plus nelfinavir were compared in 254 ARV-naïve patients. Following 96 weeks, there were marked increases in the serum TGs in each group. When the groups' effects on the lipid profile were compared, both d4T+3TC+nelfinavir and Combivir®+nelfinavir showed statistically significant elevations in serum concentrations of TC and LDL versus to Trizivir®. None of the three groups had significant effects on HDL. Another commonly used combination treatment is abacavir/lamivudine, trade name Kivexa®, which has been associated with elevated lipids [89, 90]. One trial showed elevations in TC, LDL and TC, but also reported an increase in HDL, versus patients randomized to receive tenofovir/emtricitabine. A second

study also found that abacavir/lamivudine raised TC and LDL levels compared to tenofovir/emtricitabine but found no increases in other markers for CVD [89]. No studies have directly investigated the effects of the commonly used triple combination Atripla® (efavirenz/FTC/TDF) but studies have noted modest improvements in lipid parameters when AZT/3TC is switched to emtricitabine/tenofovir with ongoing efavirenz therapy [91, 92].

1.3.5 Fusion Inhibitors

Enfuvirtide (T20) is the currently the only available member of this class. Data regarding its metabolic effects has only been presented in abstract form. There appear to be no statistically significant adverse lipid effects of this drug [93].

1.4 Treatment of Dyslipidemia and Attenuation of Risk

1.4.1 Diet and Exercise

The first approach to the treatment of dyslipidemia is diet and exercise therapy. The current guidelines published by the NCEP recommend reducing saturated fat (<20% of total fat) and increase in polyunsaturated (25-30% of total fat) and monounsaturated fatty acid intake (~55% of fat energy) while maintaining total dietary fat within the range of 25-35% of total consumed energy [94]. Batterham et al. demonstrated that this diet reduced the total cholesterol by 13% in patients with HIV [95]. Barrios et al. also noted significant reductions in TGs with dietary modification. Unfortunately, good diet compliance was followed by less than half of the 230 HIV+ patients in this study [96].

Gavrila et al. examined the effect of habitual exercise in 120 HIV+ patients in a cross-sectional study. Both the total and aerobic exercise index (number of sessions per week x duration per session x exercise intensity) were significantly and negatively associated

with fasting plasma triglyceride levels. There was no effect of exercise on total cholesterol, HDL or LDL in this group [97]. Yarasheski et al. demonstrated that a 16-week resistance exercise training program could also reduce triglyceride levels in ARV treated patients with hypertriglyceridemia [98].

As HIV is typically a wasting disease the benefits of weight loss have not been thoroughly investigated [99]. However, there has been a recent upwards trend in obesity in the HIV+ population [100] to the point where weight loss is becoming a clinically important strategy. While quality of life has been shown to improve with this strategy, a positive effect on metabolic parameters has yet to be demonstrated [101].

Following attempts at lifestyle modification, the current guidelines suggest two approaches to the treatment of the dyslipidemia associated with HIV: manipulating the ARV regimen and lipid-lowering therapy [28, 29]. Although both approaches appear to be effective in improving the lipid profile, alterations to the ARV regimen should only be considered if there is good viral suppression, low viral resistance, the dyslipidemia appears to be induced by the current ARV regimen, or the current ARV regimen is known to cause dyslipidemia.

1.4.2 Switch Studies

Many studies have examined the effect of manipulating the ARV regimen. These studies can be grouped into studies that replace the protease inhibitor with an NNRTI (efavirenz or nevirapine), studies where the thymidine analogue was changed to another ARV or, more recently, switching the current ARV regimen to one containing atazanavir. Studies switching patients to an NNRTI regimen from a PI-based regimen have shown that switch from a PI-containing regimen to one containing nevirapine is associated with

an improvement in lipid endpoints [83, 84, 102, 103]. Conversely, switching from a PI to efavirenz had little impact on lipid profile [102, 104]. A large, multicentre clinical trial reported improvements in hypertriglyceridemia and lipodystrophy in patients switched to nevirapine whereas those endpoints worsened in those who remained on PIs [103]. Another trial reported significant decreases in TC, LDL and TG during 12 months of follow-up in patients switched to nevirapine compared to patients who continued on their PI regimen [102]. HIV-positive patients with hypercholesterolemia did not experience lasting improvements in any metabolic endpoints when switched from indinavir or nelfinavir to efavirenz [104]. Finally, a trial comparing an efavirenz to nevirapine switch found that those switching to nevirapine showed significantly decreased LDL levels compared to those who remained on efavirenz [105].

Some trials analyzing the effect of replacing the thymidine analogue have shown this approach has minimal effect on lipid levels [106-109]. Other trials that exchanged stavudine for tenofovir found improvements in TC, LDL and TG with this approach [110-115]. Recently, the TOTEM trial reported that switching the NRTI backbone to fixed-dose therapy with tenofovir/emtricitabine improved TG and LDL [116]. No improvements in HDL have been found with NRTI switch studies.

Many switch studies have investigated replacement of the current first-line therapy, usually a ritonavir-boosted PI, with atazanavir, which is associated with fewer metabolic side effects compared to other PIs [65, 117-119]. Trials have found generally favourable outcomes with respect to lipid endpoints when patients were switched to atazanavir from their current ARV regimen whether it be an unboosted [120] or a ritonavir-boosted regime [121-124]. Improvements in TC, LDL, TG and non-HDL cholesterol were seen

with the switch to atazanavir when switched from any boosted/unboosted PI [120, 124], any non-atazanavir-based ARV regimen [121] or if the patients were specifically switched from lopinavir/ritonavir to atazanavir/ritonavir [123, 125]. None of the aforementioned trials report a compromise of virological safety or immunological control with the switch to atazanavir.

Many clinicians fear that altering the ARV regimen will induce viral resistance [106]. Although there appears to be no risk of viral resistance with this approach, patients must be closely monitored following ARV manipulation. There remains a proportion of patients, however, who are unable to alter their ARVs or who remain dyslipidemic despite a switch. In these patients lipid-lowering therapy may be the necessary course of action.

1.4.3 Lipid-lowering Therapy

1.4.3.1 Statins

Statin are competitive inhibitors of the 3-hydroxy-3-methyl-glutaryl CoA reductase, which catalyzes the rate limiting step in cholesterol biosynthesis [126]. Statins are activated and eliminated by a number of different metabolic pathways [127]. For example simvastatin and lovastatin are metabolically activated by CYP3A4 and the active metabolites are inactivated/eliminated from circulation by the same enzyme system. In many ARV regimes, ritonavir is used to inhibit CYP3A4. Because of the potential for drug-drug interactions, patients receiving ritonavir should not be treated with simvastatin or lovastatin. Fichtenbaum et al. found that when simvastatin was combined with ritonavir/saquinavir there was a 30-fold increase in the area under the curve (AUC) of simvastatin. The AUC of atorvastatin was increased by 79% when combined with

ritonavir/saquinavir [128]. The AUC of atorvastatin has been found to decrease by 43% when combined with efavirenz [129]. Lovastatin is also metabolized by CYP3A4. Penzak et al. reported that 4 out of 12 patients treated for dyslipidemia with lovastatin had adverse drug reactions. Most of these patients were receiving PIs [130].

Pravastatin and rosuvastatin are water-soluble statins that have minimal metabolism by the CYP3A4 enzyme system is metabolized by sulfation [127, 131]. This lack of CYP3A4 metabolism makes these statins suitable for use when treating patients with dyslipidemias who are receiving CYP3A4 inhibitors such as ritonavir. Pravastatin, which is inactivated by sulfation [127], has been studied in a limited number of controlled clinical trials involving HIV patients [132-135]. Pravastatin's ability to lower LDL in HIV patients is relatively modest. When combined with ritonavir/saquinavir the area under the curve of pravastatin was decreased by 50% [128]. This lowering of the effective circulating blood level of pravastatin may account for the relative lack of efficacy of pravastatin in HIV patients [136].

An analysis of the effect of darunavir /ritonavir on pravastatin has been published in abstract form. This showed that when pravastatin was combined with darunavir/ritonavir the maximum concentration (C_{max}) of pravastatin increased by 63% and the AUC increased by 81% [137].

Rosuvastatin is a third generation statin and it is the most potent statin currently available [138]. Rosuvastatin is metabolized by CYP2A9 (10%) and eliminated by the fecal route (90%) [139]. Trials investigating the effects of PIs on rosuvastatin found that, despite not being metabolized by CYP3A4, the pharmacokinetics of rosuvastatin were significantly effected [140-143]. Van der Lee et al. found that trough levels of rosuvastatin were 1.5

to 2 times greater when combined with lopinavir/ritonavir [140]. A second study investigating co-administration of lopinavir/ritonavir with rosuvastatin reported a 2.1 fold increase in AUC and a 4.7 fold increase in C_{max}, in addition attenuation of the LDL-lowering effects of rosuvastatin was also reported in this trial [141]. One small trial (n=6) exploring the pharmacokinetic effects of the oft prescribed combination of atazanavir/ritonavir on rosuvastatin found that AUC increase 213% and C_{max} increased 600% whereas no significant pharmacokinetic effects were observed when rosuvastatin was administered with fosamprenavir/ritonavir [142]. Finally, a recent trial investigating the effects of tipranavir/ritonavir, used treatment experienced patients with resistance to more than one PI, reported modest increases of 37% and 123% in AUC and C_{max} respectively [143]. Studies analyzing the efficacy of rosuvastatin report minimal side effects seen with rosuvastatin in HIV+ patients [139, 144].

Fluvastatin is metabolized by CYP 2C9 [145]. It has minimal potential for drug interactions in HIV+ patients. It is, however, a relatively weak statin [136] and because of this, it is not widely used to treat HIV-related dyslipidemias. Pitavastatin is a new potent statin that, like fluvastatin, is metabolized by CYP2C9. No data currently exists regarding its effect on HIV-positive patients but it seems to be as potent as rosuvastatin indicating there is potential for this novel statin to be of use in the HIV-positive population, especially given the low likelihood of drug-drug interactions [146].

The current HIV dyslipidemia guidelines suggest that fluvastatin and pravastatin are safe to use and atorvastatin can be used with caution when combined with protease inhibitors. Simvastatin and lovastatin should not be used in conjunction with PIs and simvastatin should not be used in conjunction with delavirdine [28]. It is anticipated that the updated

guidelines will also support the use of rosuvastatin. Numerous trials have examined the use of statins in HIV+ patients with dyslipidemia and they appear to be largely effective in reducing TC, LDL and TG levels [56, 130, 132-134, 139, 144, 147-151]. A small number of trials have directly compared the effects of statins in HIV+ patients [152-154]. A retrospective study comparing statin use in HIV+ patients concluded rosuvastatin and atorvastatin to be more effective at lowering TC, LDL and non-HDL compared to pravastatin [152]. One randomized clinical trial found that rosuvastatin was significantly more effective at reducing TC (25.2%), and LDL (26.3%) than either atorvastatin (19.8%, 20.3%) or pravastatin (17.6%, 18.1%) [153]. A second randomized of comparing statins trial confirmed rosuvastatin to be more potent in terms of LDL-lowering (37%) vs. pravastatin (19%) while also reporting significantly greater reduction in TG (19% vs. 7%) [154]. Neither trial reported any significant changes with regards to HDL. These results confirm those found in the non-HIV literature with respect to the greater potency of rosuvastatin at lowering lipid endpoints [155].

1.4.3.2 Fibrates

At present, guidelines recommend the use of fibrates as first line therapy for hypertriglyceridemia. This is supported by studies that show fibrates effectively lower triglycerides and, in certain cases, improve TC, LDL and HDL. Furthermore, it appears that the incidence side effects is not higher in patients with HIV than those without HIV [56, 132, 150, 156-163].

Aberg et al. examined whether patients with mixed dyslipidemia, defined as which is characterized by low levels of high-density lipoprotein cholesterol and elevated levels of triglycerides, with or without elevated levels of LDL, were more likely to meet NCEP

targets if they were randomized to fenofibrate or pravastatin. Following 12 weeks of therapy only 1% of the patients in the fenofibrate group and 5% of the pravastatin group met target indicating that neither strategy alone is likely to be successful in the treatment of mixed dyslipidemia. After 12 weeks, clinicians were allowed to combine the two agents in the patients not at target. The combination of fenofibrate and pravastatin resulted in further improvements in lipid endpoints and appeared to have minimal side effects [132].

Two other trials also examined the effect of combination therapy, with statin and a fibrate, on HIV+ patients with dyslipidemia; both demonstrated improvements in lipid endpoints with combined therapy [56, 144]. This combination must be used with caution due to the increased risk of myositis and myopathy [127]. Both studies reported few adverse events and no cases of rhabdomyolysis [56, 144]. This may be due to the small sample size and short-term follow-up of these studies. Despite the clinical evidence indicating the positive effect of fibrates on the lipid profile, a recent meta-analysis raised concerns over the lack of conclusive data for these agents having any cardioprotective effects [164, 165].

1.4.3.3 Ezetimibe

Ezetimibe, the lone member of a new class of drugs for lowering cholesterol, functions by blocking intestinal absorption of dietary and biliary cholesterol. Ezetimibe is effective at optimizing lipid levels in HIV negative patients when added to either statin or fibrate therapy [166, 167]. In contrast, the recent ENHANCE and SEAS trials showed no difference in intima-media thickness or aortic stenosis, respectively, following extended therapy with simvastatin and ezetimibe when compared to simvastatin alone [168, 169].

It should be noted that simvastatin is contraindicated in HIV+ patients due to adverse reactions with ARV medications. However, favourable effects have been demonstrated with ezetimibe in the HIV+ population by reducing LDL as effectively as fluvastatin when used as monotherapy [170]. Few trials have analyzed the effects of therapy with ezetimibe and a statin in HIV but findings have indicated there to be a beneficial effect of this combination on lipid end points in this population. [171-175]. Two studies reported improvements in TC, LDL, HDL, TG when added to statin alone or when added to maximally tolerated lipid-lowering therapy [171, 172]. One observational study noted significant improvements in TC and TG regardless of ARV regimen or ongoing statin therapy [174]. Addition of ezetimibe to unspecified low-dose statin therapy led to significant improvements in LDL (12.4%) and TC (9.1%) with no significant changes to TG in HIV+ patients treated with lopinavir/ritonavir [173]. A second trial (n=44) added ezetimibe to ongoing statin therapy with pravastatin, atorvastatin or fluvastatin and reported significant improvements in TC, LDL, non-HDL and ApoB. To date, no trials have been conducted examining the effect of ezetimibe with rosuvastatin in HIV-positive patients with dyslipidemia. No elevations in ARV concentration have been reported with concomitant use of ezetimibe [171, 173].

1.4.3.4 Niacin and Fish Oil/Omega-3 Fatty Acid

Niacin and fish oil are both recommended as second line therapy for HIV patients with hypertriglyceridemia. Gerber et al. examined the effect of ER-Niacin on lipid levels in HIV+ patients with dyslipidemia. Overall, a reduction in both total cholesterol and triglycerides was observed. There were no significant elevations in transaminase levels or incidence of myopathy and despite cases of niacin-induced insulin resistance, there

were no cases of frank diabetes. Furthermore, none of the patients discontinued the ER-niacin due to flushing when co-administered with 325 mg of aspirin [176].

Fish oil/Omega-3 fatty acid has also been recommended for the treatment of hypertriglyceridemia in patients with HIV. Two studies examining the response to fish oil/omega-3 fatty acid in this population produced conflicting results. One showed a 56.9% reduction in triglycerides with this therapy while the second was unable to demonstrate any statistically significant changes in total cholesterol, LDL, HDL or triglycerides [177, 178].

1.4.4 Insulin Sensitizers

1.4.4.1 Biguanides

Metformin is a biguanide whose mechanism of action is to sensitize peripheral tissues to insulin [179]. This has been found to have favourable effects on the lipid profile in patients with type T2DM [179]. Studies investigating the effect of metformin on total cholesterol, LDL, HDL and triglycerides in the HIV-positive population have produced variable results [180-182, 182-185]. A meta-analysis summarizing the effects of metformin on lipid parameters concluded that it had no significant effect on TC, HDL or LDL but TG was improved [186]. Metformin has been shown to exhibit positive effects on weight loss and weight maintenance [187], giving it the potential to favourably affect cardiovascular risk endpoints such as visceral adipose tissue (VAT), body mass index (BMI) and waist-to-hip ratio (WTOH). Again, results have been mixed with some trials reporting a beneficial effect on VAT [180, 185] while others reported no effect [184, 186, 188]. Results were more consistent for BMI and WTOH as studies tended to support a

favourable effect of metformin on these endpoints compared to controls [180, 183, 185, 186].

1.4.4.2 Thiazolidinediones

Thiazolidinediones (TZDs) are agonists of the peroxisome proliferator-activated receptor-gamma (PPAR- γ) that are true insulin sensitizers affecting insulin action in peripheral tissues such as skeletal muscle and adipose tissue [189]. TZDs have been used to treat insulin resistance in several studies [182, 190-193]. Rosiglitazone also has potential to stimulate adipocyte differentiation making it an appealing agent in HIV as the insulin resistance in HIV is associated with changes in body fat distribution including loss of adipose tissue from the peripheral subcutaneous regions [9, 194, 195]. Improved insulin sensitivity has been associated with an improvement in body fat distribution with rosiglitazone treatment [190-193] although altered body fat has not been reported in all studies [196, 197].

There has been hope that an increase in insulin sensitivity would also lead to improved lipid levels, their effect on the lipid profile and surrogate markers for vascular disease have been mixed with most reporting undesirable increases in lipid endpoints [182, 188, 193, 196-202]. Several randomized, placebo-controlled trials reported increases TC and LDL [193, 201, 202] while others have reported increases in TG [197-199]. Two meta-analyses have confirmed the increased risk of hypercholesterolemia and hypertriglyceridemia with rosiglitazone use [186, 203]. One trial expanded upon the adverse lipid effects of rosiglitazone and found that rosiglitazone led to an increase in small, dense LDL particles, which is associated with susceptibility to oxidation and increased vascular risk [204, 205] and a decrease in large HDL particles [206]. Only one

trial has shown no significant changes with respect to lipid endpoints in patients treated with rosiglitazone as compared to placebo [207].

Further trials have compared the effects of metformin and rosiglitazone on lipids observed contrasting effects within the rosiglitazone treatment group. One reported increases in TC, LDL and HDL with rosiglitazone over a 48 week period while a second trial, with a duration of 26 weeks, observed no significant increases in any cholesterol parameter but did observe a significant increase in TG [182, 208]. A recent study suggested that while rosiglitazone may improve FFA metabolism it also correlated with an increase in cholesterol remnant that correlate with increased risk for CVD [209].

1.4.4.3 Pioglitazone

Like rosiglitazone, pioglitazone has been shown to improve insulin resistance in non-HIV populations [210]. Investigations with pioglitazone have been limited in the HIV+ population compared to rosiglitazone. To date there have been only two trials comparing the effects of pioglitazone to placebo [211, 212]. Neither trial showed an significant impact of pioglitazone on insulin or fasting glucose but, unlike rosiglitazone, favourable effects HDL were reported. The effect on LDL was not consistent between trials as the larger trial (n=127) reported no difference [212] and the smaller trial (n=14) reported a borderline significant increase [211]. No significant effects were observed on VAT or WTOH but a significant increase in BMI was reported [211, 212].

1.5 Objectives and Hypotheses

There are many facets to the cardiovascular risk associated with the HIV+ population and this dissertation will serve to address many of these aspects. First, the rate of metabolic abnormalities that occur among HIV+ patients initiating HAART across Canada will be

determined with investigations into the Canadian Observational Cohort (CANOC) database. Given association between HAART it is expected that there will be a high rate of lipid abnormalities and that patients initiating therapy with PIs will have a higher rate of abnormalities compared to those beginning therapy with NNRTIs.

These metabolic abnormalities put HIV+ at risk for development of CVD. Determination of the prevalence of CVD in HIV+ is an important aspect treating HIV+ patients and this dissertation will seek to determine the prevalence of PAD using the ankle brachial index (ABI) in patients with metabolic abnormalities. The elevated risk for CVD with HAART and HIV disease itself combined along with the high rate of peripheral neuropathy in HIV indicate there would be a high prevalence of PAD.

Treating HIV+ patients with an elevated risk for CVD prior to development of any disease will prove to be an important aspect in preventing high rates CVD in this vulnerable population. Rosiglitazone will be used as an agent to prevent progression of CVD by analyzing its effects on intimal medial thickening and plaque burden. As rosiglitazone has shown to improve insulin resistance and have a favourable effect on adipocyte differentiation we expect significant improvements with rosiglitazone compared to placebo.

Finally, primary treatment of dyslipidemic effect of ARVs is continuing to evolve as the HIV+ population ages. Staying ahead of the curve in treating elevation in cholesterol is important and this dissertation will examine the effect of two lipid-lowering medications, rosuvastatin and ezetimibe, on relevant lipid parameters and will then go one step further by directly comparing the novel combination of these two medications to the standard practice of increasing the dose of ongoing therapy of rosuvastatin.

Chapter 2: Incidence of Regimen-specific Metabolic Abnormalities in Patients Initiating HAART

2.1 Background

Dyslipidemia that includes elevated total cholesterol (TC), LDL-cholesterol (LDL), and triglycerides (TG), and decreased HDL cholesterol (HDL) are common side effects of antiretroviral (ARV) regimens [213]. Dyslipidemia together with hyperinsulinemia and insulin resistance, in addition to other factors e.g. high rates of smoking, put HIV-positive (HIV+) patients at risk for further complications such as development of metabolic syndrome and increased risk of cardiovascular disease (CVD) [9, 13, 14, 214].

Adverse effects on the lipid profile of HIV+ patients with regimens that contain protease inhibitors (PIs) have been well documented in clinical trials assessing the complications of specific ARV agents [215-217]. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) have not been associated with the same frequency of lipid disturbances and long-term CVD risk as PIs; however, trials have linked certain NNRTIs, such as efavirenz but not nevirapine, with metabolic abnormalities that include dyslipidemia [13, 67].

Nucleoside reverse transcriptase inhibitors (NRTIs) have had less of an association with adverse lipid effects than NNRTIs and PIs, with the exception of abacavir and the older agents, didanosine and stavudine [218, 219]. Given the complex nature of HAART, which includes three or more agents given concurrently, it is difficult to rule out effects of a specific ARV class or individual agent.

2.1.1 The CANOC Collaboration

While rigorous clinical trials assessing adverse effects of PIs and NNRTIs in the HIV population are commonplace [9, 79, 215, 220-222], the incidence of dyslipidemia within clinics where these agents are prescribed is less clearly understood. The Canadian Observational Cohort (CANOC) collaboration is Canada's first integrated network of all registered HIV/AIDS treatment information from eight cohort databases across the provinces of British Columbia, Ontario and Quebec.

2.1.2 Comparison of Lipid Abnormalities Between Regimens

This study sought to determine the incidence of metabolic abnormalities in HIV+ patients initiating HAART within CANOC. Furthermore, this study aimed to compare the incidence rate of lipid abnormalities between ARV-naïve patients initiating PI-containing regimens versus those initiating NNRTI-containing regimens, and to compare the incidence of lipid abnormalities between patients initiating therapy with the nucleotide reverse transcriptase inhibitor (NtRTI) tenofovir (TDF)-containing NRTI backbones versus those initiating therapy with abacavir (ABC).

2.1.3 Atherogenic Index of Plasma

The atherogenic index of plasma (AIP), the logarithm of molar ratio of triglyceridemia to high-density lipoprotein cholesterol (TG/HDL), is a novel marker of cardiovascular risk that is predictive of elevated blood pressure [223], small low-density lipoprotein cholesterol (LDL) particle size [224] and vascular events [223] in the general population. AIP has not been thoroughly investigated in the HIV+ population. As AIP is predictive of particle size it is thought to be superior to other ratios, such as the TC to HDL ratio (TC:HDL) as it is a reflection of elevations of atherogenic lipids (TG) relative to anti-

atherogenic HDL particles [225]. This study sought to determine the incidence of treatment-emergent elevations in AIP between patients within CANOC who were prescribed HAART regimens that include PIs and those prescribed regimens that contain NNRTIs.

2.2 Methods

2.2.1 Participants

Patients included in the study were HIV-positive, ARV-naïve adults initiating HAART in any of the eight CANOC cohorts between February 2000 and August 2010. To be included in CANOC, patients must have documented HIV infection, be at least 18 years old, live in Canada, have initiated combination ARV therapy with at least three individual agents naively on or after January 1, 2000, and have baseline (within the six months prior to ARV therapy initiation) CD4 cell count and viral load testing results. Further details of the participating cohorts and the general CANOC structure are available [226].

For this specific study, eligible patients were those who had visited a laboratory prior to initiating any ARV therapy regimen and had a measurement of at least one of the outcome parameters as defined below. Patients who had a measurement of any of the selected parameters within the corresponding cutoff range from pre-ARV baseline and who had a minimum of one follow-up visit were included. Subjects were excluded if they lacked laboratory values or presented with an abnormality at their pre-ARV baseline. Prevalence of abnormalities present at baseline is shown in Table 2.1.

2.2.2 Outcomes

Endpoints were defined as treatment-emergent laboratory abnormalities that included: TC >5.2 mmol/L, HDL <0.9 mmol/L, TC:HDL >5.0, LDL >3.5 mmol/L, TG >2.5 mmol/L, and AIP of >0.11 and >0.21 (indicative of moderate and high cardiovascular risk in the general population) [227]. The HDL and TG endpoints were set outside usual targets due to the high rate of abnormalities of these parameters in HIV+ population. Secondary outcomes included the following safety parameters: creatinine >120 µmol/L as an indicator of kidney function, alanine aminotransferase (ALT) >5 times the upper limit of normal (ULN) and aspartate aminotransferase (AST) >5 times ULN as markers of liver inflammation, and creatine kinase (CK) >10 times ULN as a marker of muscle damage.

2.2.3 Statistical Methods

Incidence rate was calculated as the number of patients who developed a abnormality over length of follow-up time, where follow-up time was calculated as the sum of time from baseline to first recorded abnormality or from baseline to last recorded value for a given parameter. Incidence rate is presented as rate per 100 patient-years follow-up (PYFU). Hazard ratios (HR) were used to compare the hazard of developing an abnormality associated with different ARV classes and with individual ARV agents.

Poisson regression was used to compare incidence rate and Cox proportional hazard model was used to compare HR between ARV classes (PI and NNRTI) and between individual agents. Significance was set at $\alpha=0.05$ and determined by chi-squared tests for comparing incidence rate and HR.

2.3 Results

A total of 3247 patients met the criterion of initiating therapy with a PI- or NNRTI-based HAART regimen. Of those, 1784 patients who lacked baseline and/or follow-up data were excluded. Demographic data for the remaining 1462 patients are shown in Table 2.2. Patients were excluded from analysis of each study endpoint if they had a pre-existing abnormality in that specific laboratory value at baseline (Table 2.1).

Overall incidence of metabolic abnormalities is displayed in Figure 2.1. The most common abnormality was the AIP moderate risk cutoff of ≥ 0.11 (65.75 PYFU), followed by the AIP high-risk cutoff of ≥ 0.21 (58.99 PYFU) and elevated TC (36.45 PYFU). Elevations in liver transaminases (ALT 4.27 PYFU, AST 3.77 PYFU) and creatinine (4.54 PYFU) were not common. By contrast, CK elevations were moderately frequent (17.10 PYFU).

2.3.1 Incidence Rate and Risk of Abnormalities by Drug Class

2.3.1.1 PI vs. NNRTI

The majority of patients initiating ARV therapy within CANOC either began HAART with a PI (59%) or NNRTI (41%). Class comparison of incidence rates for primary outcomes are displayed in Figure 2.2. A significantly increased incidence rate was seen in patients treated with NNRTI as compared to PI for TC (44.25 vs. 32.35, $p=0.021$) and LDL (30.03 vs. 18.46, $p=0.003$). Conversely, increased incidence rate was observed in patients on PI-based regimens for HDL (24.02 vs. 10.34, $p=0.005$) and TG (37.98 vs. 20.17, $p<0.001$). Accordingly, patients on PI-based therapy had significantly greater incidence rate for both the moderate (81.40 vs. 49.60, $p=0.028$) and high-risk (74.42 vs. 43.11, $p=0.007$) AIP cutoffs, while no difference was observed between groups in

incidence rate for TC:HDL. Significantly higher incidence rate was observed in the PI group as compared to the NNRTI group for both creatinine (5.18 vs. 3.48, $p=0.02$) and ALT (4.80 vs. 3.38, $p=0.032$). No significant differences were observed between groups for incidence rate of AST or CK. The pattern of increased rates of lipid elevations observed in patients initiating NNRTI was not expected.

Comparison of time to develop a given abnormality revealed a significantly increased risk for LDL elevations with NNRTI (HR 1.52, 95% confidence interval [CI]: 1.14 – 2.02, $p=0.004$) compared with PI. Hazard of developing an elevated TC did not differ significantly between groups (HR: 1.23, 95% CI: 0.99 – 1.52, $p=0.059$). Patients taking PIs had a significantly greater risk for developing low HDL (HR: 2.36, 95% CI: 1.44 – 2.53, $p<0.001$), elevated TG (HR: 1.91, 95% CI: 1.45 – 2.53, $p<0.001$), moderate AIP (HR: 1.63, 95% CI: 1.19 – 2.24, $p=0.004$) and high AIP (HR: 1.72, 95% CI: 1.28 – 2.29, $p<0.001$) sooner than those taking NNRTIs. The hazard of developing an abnormal creatinine did not differ between PI and NNRTI (HR: 1.17, 95% CI: 0.89 – 1.53). Overall, the hazard ratio for a given abnormality was similar to the pattern observed with risk of development of an abnormality.

2.3.2 Incidence Rate and Risk of Abnormalities by Individual Agent

2.3.2.1 Atazanavir vs. Lopinavir

A total of 858 patients initiated PI-containing HAART regimens within CANOC. Of those patients, the majority began therapy with either ritonavir-boosted atazanavir [atazanavir/ritonavir](56.1%) or the combination drug lopinavir/ritonavir [lopinavir/ritonavir](31.6%), with a small percentage of patients initiating unboosted atazanavir (3.7%) and the remainder beginning a variety of other PIs (Table 1). Due to

the relatively small number (n=32) of patients initiating unboosted atazanavir, these patients were excluded from the analysis and only ritonavir-boosted PIs were considered. Comparison of incidence of abnormalities between atazanavir/ritonavir and lopinavir/ritonavir revealed a higher incidence rate for elevations in TC (47.65 vs. 27.07, p=0.002), TG (71.17 vs. 25.11, p<0.001), TC:HDL (39.81 vs. 23.22, p=0.043), moderate-risk AIP (140.00 vs. 64.94, p=0.012) and high-risk AIP (130.00 vs. 53.00, p<0.001) in patients initiating therapy with lopinavir/ritonavir (Figure 2.3).

There was a significantly greater hazard associated with lopinavir/ritonavir for development of elevated TC (HR: 1.62, 95% CI: 1.21 – 2.16, p=0.001), TG (HR: 1.81, 95% CI: 1.81 – 3.59, p<0.001), TC:HDL (HR: 1.63, 95% CI: 1.10 – 2.41, p=0.015), moderate-risk AIP (HR 1.81 95% CI: 1.17 – 2.82, p=0.008) and high-risk AIP (HR: 2.12, 95% CI: 1.43 – 3.14, p<0.001). There were no significant adverse effects of atazanavir/ritonavir as compared to lopinavir/ritonavir.

2.3.2.2 Nevirapine vs. Efavirenz

Of the 604 patients who began an NNRTI-based HAART regimen, 17.9% began treatment therapy with nevirapine (nevirapine) while the majority (82.1%) began treatment with efavirenz. Unlike patients initiating therapy with PIs, patients who began NNRTI-containing HAART showed few differences with respect to incidence rate of individual metabolic outcomes. The lone parameter that differed between NNRTIs was an increased incidence rate of TC:HDL in patients who began therapy with nevirapine vs. those who started with efavirenz (43.48 vs. 19.81, p=0.025). However, neither elevated TC (52.46 vs. 42.60, p=0.41) nor decreased HDL (10.00 vs. 10.43, p=0.95) was seen more often in the NEV group.

The hazard of developing an elevated TC:HDL was also significantly less in patients beginning nevirapine than those starting efavirenz (HR: 1.94, 95% CI: 1.14 – 3.30). Again, neither time to elevated TC nor to decreased HDL was significantly greater in the nevirapine group than the efavirenz group. Hazard of developing an elevated LDL was slightly shorter in the nevirapine group than the efavirenz group (HR: 1.36, 95% CI: 0.86 – 2.16), but the difference was not significant ($p=0.18$). Increased rates of and increased hazard of elevated TC:HDL in patients initiating nevirapine is a novel finding.

2.3.3 NRTI/NtRTI Backbones

2.3.3.1 Abacavir vs. Tenofovir

A total of 1553 patients began ARV therapy with an NRTI/NtRTI backbone that contained either ABC or TDF. Within CANOC, the most common NRTI backbones consist of either emtricitabine (FTC)/TDF (49.4%), lamivudine (3TC)/ABC (28.3%), or 3TC/TDF (22.2%). For our analyses the two TDF-containing regimens were considered together. IRs of metabolic abnormalities for ABC and TDF were compared but no significant differences were found between groups with respect to any of the primary outcomes (Figure 2.4). Incidence rate of elevated TC approached but did not reach significance in the 3TC/ABC group as compared to TDF-based regimens (44.69 vs. 33.99, $p=0.065$); however, the time to develop elevated TC was shorter with 3TC/ABC-containing regimens as compared to those containing TDF (HR: 1.30, 95% CI: 1.03 – 1.64, $p=0.027$). The only between-group difference was a significantly greater incidence rate of elevated ALT in patients initiating therapy with 3TC/TDF or FTC/TDF vs. those starting 3TC/ABC (4.92 vs. 3.05, $p=0.006$).

When the effect of different NRTI backbones was examined amongst patients on ritonavir-boosted PI- based HAART regimens (n=567), there was an increased incidence rate of elevated TC in patients who began ABC- vs. TDF-containing backbones (45.26 vs. 29.79, p=0.032). Accordingly hazard of developing high TC was also significantly shorter among patients on ABC (HR: 1.47, 95% CI: 1.09 – 1.99, p=0.012). No other outcomes were significantly different between groups in terms of incidence rate. Furthermore, when the effects of NRTI backbones on incidence rate and HR for metabolic outcomes were considered amongst patients initiating HAART with an NNRTI, no differences were observed between the ABC and TDF groups.

2.4 Discussion

Overall, there was a high-rate of treatment-emergent lipid abnormalities in our study cohort as would be expected in ARV-naïve patients initiating HAART. Elevations in TC, TG and LDL accompanied by HDL decreases are well-established complications of ARV therapy. Unique findings included the discovery of the elevated rate of the novel cardiovascular risk marker AIP in HIV+ patients as well as a moderately elevated incidence of increased CK, which has not been noted in the current literature.

2.4.1 PI vs. NNRTI

Despite established adverse effects on lipids seen with ritonavir [6, 50, 228], ritonavir-boosted PIs are recommended in current treatment guidelines, and these are the most common PI regimens in use today. In this study, we found that patients initiating PI-containing HAART followed this well-established pattern, with significant increases in incidence rate for elevated TG and decreased HDL observed in those patients. A unique

finding in this study was the elevated incidence rate of increased TC and LDL in patients beginning HAART with an NNRTI. Usually NNRTIs have a more favourable lipid profile as HDL levels tend to increase and TG levels decrease with increasing exposure to NNRTI-based therapy [67]. However, the effects of the two most commonly prescribed NNRTIs, nevirapine and efavirenz, on the lipid profile have been shown to differ significantly [67, 229]. Furthermore one study comparing ARV-naïve patients initiating therapy with a PI or an NNRTI reported similar LDL levels between groups [230]. Previous studies have assessed the effect of switching from a PI to an NNRTI, and our results seemingly contrast with these trials that found less severe LDL abnormalities associated with NNRTIs [105, 231]. Specifically, a decrease in LDL was seen when patients with PI-associated dyslipidemia were switched to either nevirapine or efavirenz. To our knowledge this is the first study that has compared between-class incidence of lipid abnormalities in patients initiating HAART.

2.4.1.1 Efavirenz vs. Nevirapine

In the CANOC cohort, efavirenz is prescribed more frequently than nevirapine (82.1% vs. 17.9%), which may influence the incidence of TC and LDL elevations; however, when the individual NNRTIs were compared, no significant differences were found between them for any lipid outcomes. Clinical trials directly assessing the effect of efavirenz and nevirapine concluded that efavirenz resulted in higher levels of LDL [81, 102]. Similarly, cohort studies comparing efavirenz and nevirapine found that the two agents affect the lipid profile differently, with efavirenz being associated with more lipid abnormalities. Efavirenz was associated with significantly elevated TC and TG

compared to nevirapine; however, no significant changes in LDL were reported [67, 229, 232].

2.4.1.2 Atazanavir vs. Lopinavir

Comparison of atazanavir/ritonavir and lopinavir/ritonavir showed a pattern of dyslipidemia consistent with previous trials comparing these two regimens. Development of less favourable lipid profiles including elevated TC and TG with lopinavir/ritonavir are well-established [69, 70, 233, 234]. Furthermore, lipid profiles have been shown to improve when patients are switched from lopinavir/ritonavir to atazanavir/ritonavir [123, 125, 235]. The elevations in TC and TG are the likely driving factor in the observed increased risk and incidence for elevated TC:HDL and AIP. In spite of the known risk of lipid abnormalities associated with lopinavir/ritonavir, this regimen still comprised 31.6% of PI regimens initiated in CANOC between February 2000 and August 2010, but lopinavir use is now on the decline. As the lipid profile associated with the lopinavir/ritonavir combination is less favourable than that of atazanavir/ritonavir[70] it is likely driving the elevated frequencies of TC and TG abnormalities observed with PIs as compared to NNRTIs; however, no direct comparisons between agents of different ARV classes were made in this study.

2.4.2 NRTI/NtRTI Backbones

In CANOC, the majority of patients (71.5%) initiated therapy with TDF-containing regimens and, accordingly, our results reflected those seen in previous studies, in that there was neither an increased risk of development of lipid abnormalities nor was there any increased incidence as compared to regimens containing ABC. Our study, like previous studies investigating the effect of ABC on lipids, found an increased risk of

elevated TC with ABC vs. TDF, but incidence and hazard of developing of elevated LDL with ABC was not observed as compared to TDF [89]. Patients on either FTC/TDF or 3TC/TDF regimens showed no increased risk of development of lipid abnormalities in our study. This is in accordance with a study by Crane et al. that found NRTI backbones containing FTC/TDF or 3TC/TDF were associated with a less pro-atherogenic lipid profile compared to other NRTI pairs [80]. Large cohorts, including the multinational DAD study, have shown an increased risk of myocardial infarction with recent use of ABC [215, 236, 237]; however, more recently both a cohort study and a meta-analysis have refuted these findings [238, 239]. Furthermore no other biological cardiovascular risk factors have been associated with ABC therapy[89] casting further doubt on the cardiovascular risk associated with ABC.

2.4.3 Atherogenic index of plasma

The atherogenic index of plasma (AIP) has been recognized by the Adult Treatment Panel III as an important risk factor for coronary heart disease in the general population. It is a promising marker for assessment of cardiovascular disease risk in the HIV positive population as it combines two parameters, HDL and TG, which are adversely affected by both the HIV virus and HAART. In addition to combining these two integral endpoints, AIP has been correlated with other cardiovascular risk factors that include lipoprotein particle size, elevated blood pressure and vascular events [223, 224]. As the incidence of moderate or high cardiovascular risk, defined as an AIP ≥ 0.11 and ≥ 0.21 respectively, is largely unknown in the HIV positive population, this study served as an initial estimate and found this parameter to be the most prevalent of all outcomes assessed. Predictably, elevated AIP, likely driven by elevated TG, was associated with PI use, particularly with

lopinavir/ritonavir use, though the rate of AIP elevation and the risk of developing an elevated AIP were greater than that for TG. This marker requires further validation of its clinical significance before being used as a hard endpoint or clinical target in HIV positive populations.

2.4.4 Limitations

Due to high rates of TG and HDL abnormalities in this population, these endpoints were set outside usual targets, which may have resulted in an underestimation of the incidence and prevalence of these endpoints. CANOC does not yet collect information on lipid-lowering therapy, which may result in an underestimation of the overall incidence of metabolic abnormalities. In addition, CANOC does not record fasting status, the effect of which is likely to be minimal given that fasting-dependent outcomes such as TG and LDL are not collected during non-fasting visits. However, inability to determine fasting status did preclude assessment of elevated glucose in the study. AIP is a relatively new marker for cardiovascular risk and its validity in the HIV positive population requires investigation. Observed effects on ALT should be interpreted cautiously as this study was not designed to specifically assess safety outcomes. There is a possibility of selection bias as the testing patterns of the physicians are not known; some physicians may have sent patients for assessment of lipids regularly from the time of HIV diagnosis, while others may only test their patients' lipids if abnormalities were suspected.

Finally, CANOC includes data from only three provinces, and a clinic-based selection bias may exist, as included data from British Columbia includes the entire population of people on ARV therapy province-wide, while data from Ontario and Quebec come from a selection of clinics.

2.4.5 Conclusions

There is a high incidence of metabolic laboratory abnormalities in patients initiating current ARV therapies in the CANOC cohort. A unique and unexpected pattern of lipid abnormalities was seen in our study cohort with treatment-associated increase in TC driven by LDL elevations in patients initiating NNRTIs. A more detailed analysis is required to confirm any causal effects of the individual agents on incidence of lipid outcomes.

AIP reflects lipid abnormalities common in HIV-positive patients and may prove to be an effective measure of cardiovascular risk associated with ARVs given the pattern of dyslipidemia inherent to this population. Further investigation is required to confirm the correlation of AIP with clinical events in the HIV-positive population.

Tables

Table 2.1 Prevalence of metabolic abnormalities at pre-ARV baseline

| Variable | N* | Prevalence (per 100 PYFU) |
|-------------------------|-----------|----------------------------------|
| TC >5.2 mmol/L | 886 | 10.84 |
| HDL <0.9 mmol/L | 831 | 51.38 |
| TC:HDL ratio \geq 5.0 | 815 | 36.81 |
| LDL >3.5 mmol/L | 667 | 10.04 |
| TG >2.5 mmol/L | 699 | 14.59 |
| AIP \geq 0.11 | 680 | 59.26 |
| AIP \geq 0.21 | 680 | 45.15 |
| Creatinine | 1326 | 1.73 |
| AST >5xULN | 1374 | 2.04 |
| ALT >5xULN | 1398 | 2.43 |
| CK >10xULN | 837 | 7.41 |
| Phosphate | 534 | 3.37 |

*Patients were excluded variable by variable according to selected cutoffs
 PYFU, Patient years follow-up; TC, total cholesterol; HDL, high-density cholesterol; TC:HDL, total cholesterol to high-density cholesterol ratio; LDL, low-density cholesterol; TG, triglycerides; AIP, atherogenic index of plasma; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CK, creatine kinase.

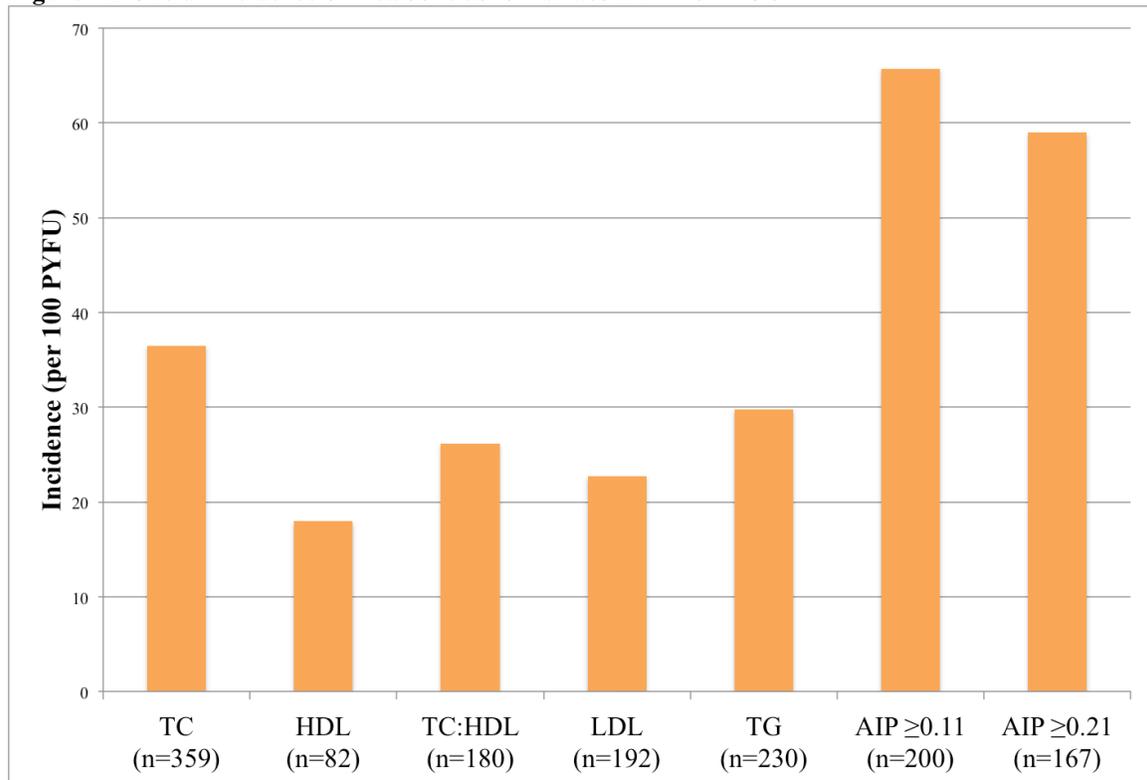
Table 2.2 Demographic characteristics of study participants.

| Variable | |
|-----------------------------------|-----------------------|
| Age, years* | 41 (35 – 47) |
| Male sex | 1298 (89) |
| Follow-up time, years† | 2.1±1.5 |
| CD4+ cell count/mm ³ * | 225 (150 – 300) |
| Viral load copies/mL* | 70800 (19565 – 10010) |
| Hepatitis C co-infection | 266 (18) |
| PI | 858 (58.7) |
| Atazanavir | 513 (59.8) |
| Ritonavir-boosted | 481 (93.8) |
| Unboosted | 32 (6.2) |
| Lopinavir/ritonavir | 271 (31.6) |
| Other PI | 74 (8.6) |
| NNRTI | 604 (41.3) |
| Efavirenz | 496 (82.1) |
| Nevirapine | 108 (17.9) |
| FTC/TDF | 722 (49.4) |
| 3TC/TDF | 324 (22.2) |
| 3TC/ABC | 414 (28.3) |
| Missing NRTI | 2 (0.1) |

Data are frequency (%) unless otherwise noted, *Data are Median (IQR), †Data are mean±SD
 PI, protease inhibitor; FTC, emtricitabine; TDF, Tenofovir; 3TC, lamivudine; ABC, Abacavir; NRTI, nucleoside reverse transcriptase inhibitor.

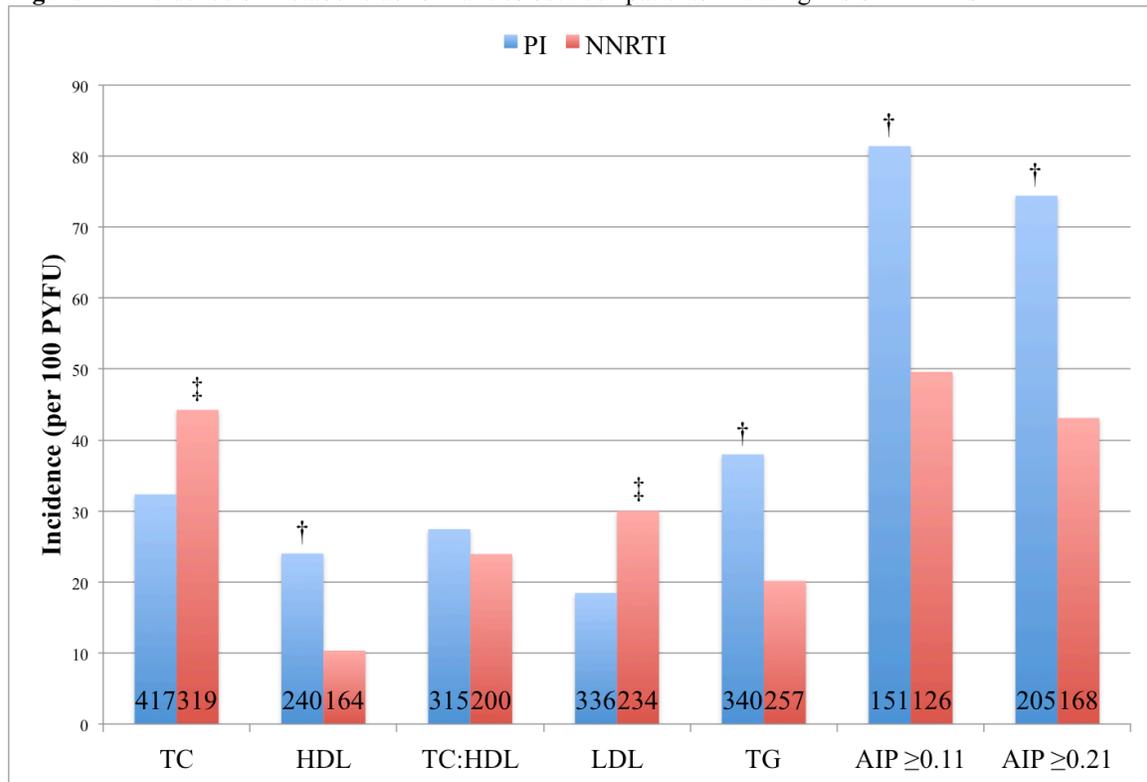
Figures

Figure 2.1 Overall incidence of metabolic abnormalities within CANOC



CANOC, Canadian observational cohort; PYFU, patient years follow-up TC, total cholesterol >5.2 mmol/L; HDL, high-density cholesterol <0.9 mmol/L; TC:HDL, total cholesterol to high-density cholesterol ratio ≥ 5.0 ; LDL, low-density cholesterol >3.5 mmol/L; TG, triglycerides >2.5 mmol/L; AIP, atherogenic index of plasma.

Figure 2.2 Incidence of metabolic abnormalities between patients initiating PIs or NNRTIs

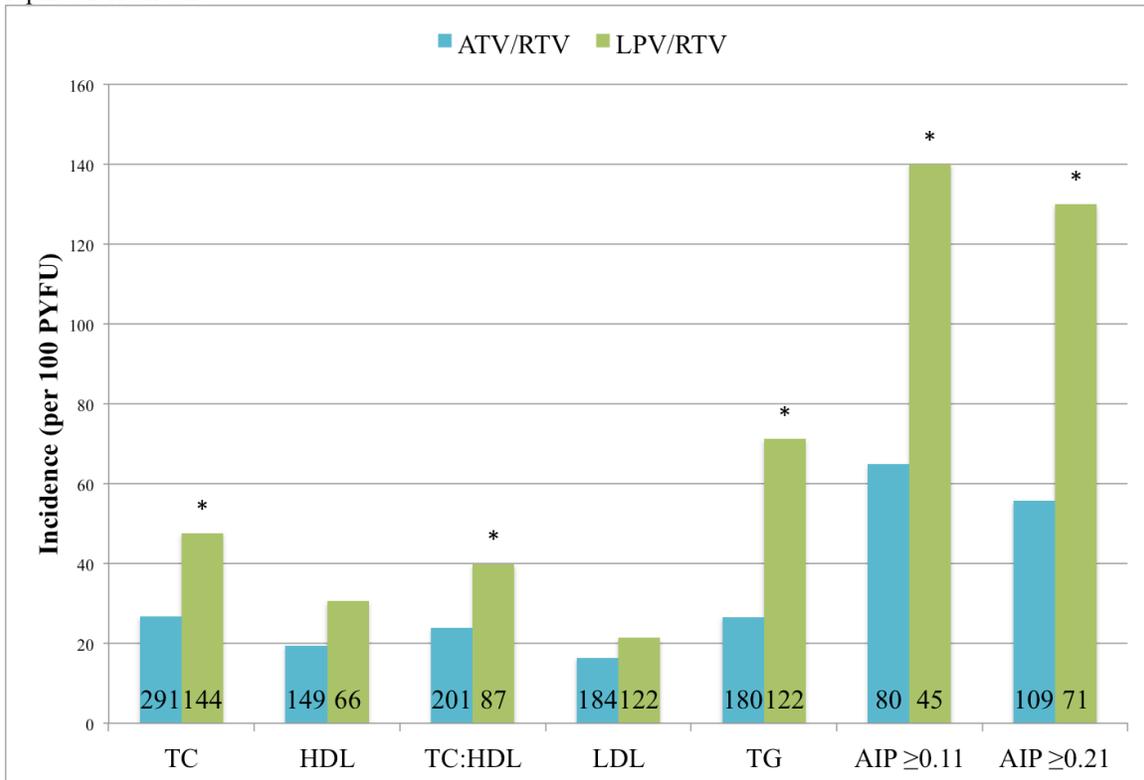


†p<0.05 in favour of PI, ‡ p<0.05 in favour of NNRTI

Numbers inside columns are the number of patients included for each endpoint.

PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PYFU, patient years follow-up; TC, total cholesterol >5.2 mmol/L; HDL, high-density cholesterol <0.9 mmol/L; TC:HDL, total cholesterol to high-density cholesterol ratio ≥5.0; LDL, low-density cholesterol >3.5 mmol/L; TG, triglycerides >2.5 mmol/L; AIP, atherogenic index of plasma.

Figure 2.3 Incidence of metabolic abnormalities between patients initiating atazanavir/ritonavir or lopinavir/ritonavir.

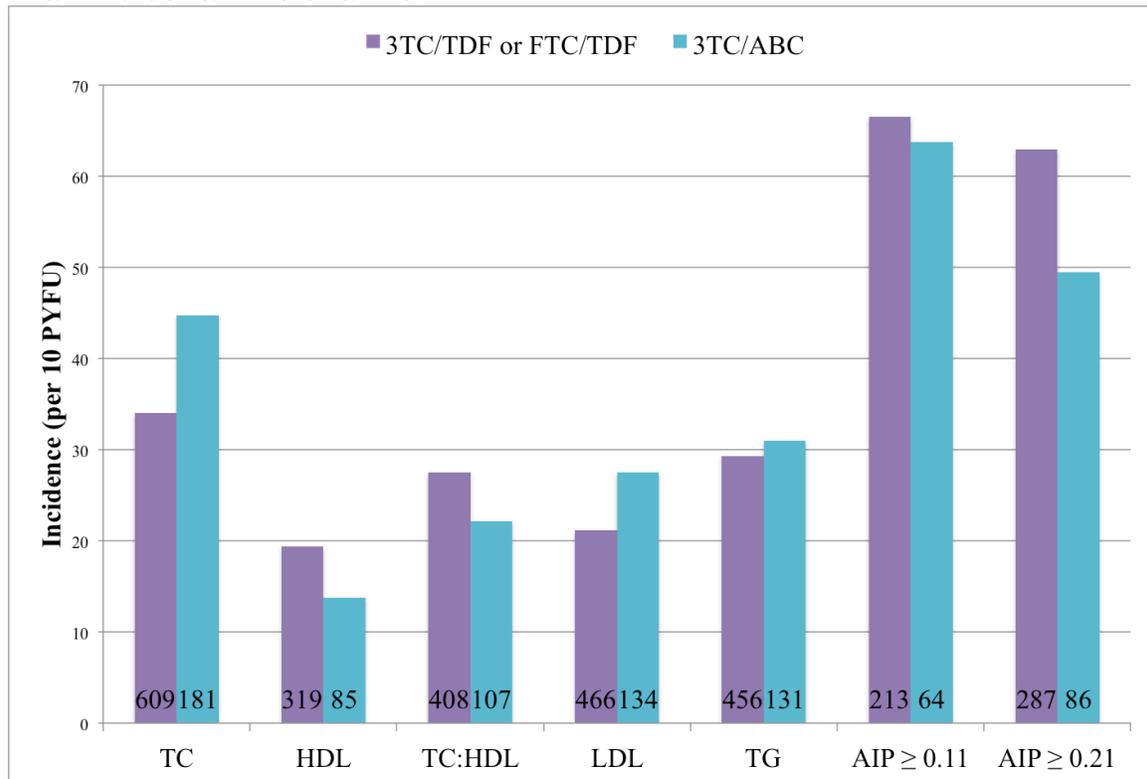


*p<0.05 in favour of lopinavir/ritonavir

Numbers inside columns are the number of patients included for each endpoint.

PYFU, patient years follow-up; TC, total cholesterol >5.2 mmol/L; HDL, high-density cholesterol <0.9 mmol/L; TC:HDL, total cholesterol to high-density cholesterol ratio ≥5.0; LDL, low-density cholesterol >3.5 mmol/L; TG, triglycerides >2.5 mmol/L; AIP, atherogenic index of plasma.

Figure 2.4 Incidence of metabolic abnormalities between patients initiating NRTI backbones containing FTC/TDF and 3TC/TDF or 3TC/ABC.



Numbers inside columns are the number of patients included for each endpoint.

NRTI, nucleoside reverse transcriptase inhibitor; 3TC, lamivudine; TDF, tenofovir; FTC, emtricitaine; ABC, abacavir; PYFU, patient years follow-up; TC, total cholesterol >5.2 mmol/L; HDL, high-density cholesterol <0.9 mmol/L; TC:HDL, total cholesterol to high-density cholesterol ratio ≥5.0; LDL, low-density cholesterol >3.5 mmol/L; TG, triglycerides >2.5 mmol/L; AIP, atherogenic index of plasma.

Chapter 3: Prevalence of Peripheral Arterial Disease in HIV-positive

Patients

3.1 Background

Both HIV infection and highly active antiretroviral therapy (HAART) have been linked to accelerated subclinical atherosclerosis in HIV-positive (HIV+) patients than those without HIV [240]. HIV+ patients are subject to insulin resistance as a result of complications from HAART by mechanisms that have not been fully elucidated and, as a result, may experience chronic hyperglycemia similar to diabetes [9, 22]. The hyperglycemia that stems from this insulin resistance, in combination with elevated insulin levels, can cause calcification that results in arterial stiffness. The calcified peripheral arteries become incompressible resulting in an elevated ABI when in fact subclinical cardiovascular disease is present [23-26]. Vascular complications such as these have yet to be demonstrated in HIV+ patients.

Peripheral arterial disease (PAD) of lower extremities is a manifestation of systemic atherosclerosis and a predictor of cardiovascular events [19]. Early diagnosis of PAD may help identify patients at high risk for CV events and provide important opportunity for intensification of preventative measures. In the general population PAD is associated with traditional cardiovascular risk factors such as smoking, type 2 diabetes mellitus (T2DM), hypertension and dyslipidemia (DL) [20]. These factors are more prevalent in the HIV+ population [13, 21]. PAD is a strongly age-dependent disease [241, 242] and the aging population of HIV+ patients may be at an elevated risk of developing PAD. In

the majority of cases, patients with PAD do not experience the symptoms of intermittent claudication and remain asymptomatic.

Thus, the ankle brachial index (ABI) is an attractive, simple, non-invasive screening method used to detect subclinical PAD [20]. It has been suggested that the ABI is a serviceable tool for assessment of vascular risk both in the non-HIV and HIV populations [243, 244]. The test has low sensitivity in predicting cardiovascular outcomes [19] but its utility as a screening tool in an aging HIV+ population remains to be determined. Our study aimed to assess the prevalence of PAD in an unselected group of HIV+ patients attending an outpatient clinic and to evaluate the utility of the ABI in our population as an adjunct to traditional risk stratification.

3.2 Methods

A cross-sectional study was performed at the HIV Metabolic Clinic in the John Ruedy Immunodeficiency Clinic (IDC) at St. Paul's Hospital in Vancouver, British Columbia.

3.2.1 Participants

The patients seen in the clinic are referred due to metabolic abnormalities in blood lipids or endocrine function. Recruitment took place in the attending physician's (GB) office between June 2006 and April 2007. Exclusion criteria were previous diagnosis of peripheral arterial disease, arterial compliance problems (such as arterial incompressibility, preventing proper calculation of the ABI) and an inability to give informed consent (i.e. mental illness). All eligible patients were asked to participate in the study by the study coordinator (KJ). Patients who agreed to participate in the study

signed an informed consent. This study was approved by the University of British Columbia-Providence Health Care Research Ethics Board.

3.2.2 Outcomes

3.2.2.1 Ankle-brachial Index

ABI was obtained with a handheld, high sensitivity 8 MHz Doppler ultrasound probe (Rheo Dopplex II; Huntleigh Healthcare, Cardiff, UK) and a blood pressure cuff. The patient lied in a supine position for 5-10 minutes prior to blood pressure measurements, according to standard procedures [245]. Briefly, the higher of the two blood pressures measured in the leg (dorsalis pedis or posterior tibial) and the higher of the two brachial pressures were used to calculate the ABI for each lower limb and the lower of the two ABI was recorded as the patient's overall ABI. ABI was defined as low if it was ≤ 0.9 and elevated if it was ≥ 1.3 , according to international guidelines [20].

3.2.2.2 Cardiovascular Risk

Prior to ultrasound assessment, participants were asked a brief series of questions covering smoking status, history of diabetes, hypertension and any prior diagnosis of vascular disease (cerebrovascular disease, ischemic heart disease/coronary artery disease) in order to assess cardiovascular risk. Medical history and clinic records were obtained to identify the following information: age, gender and current ARV regimen, antihypertensive and lipid-lowering medication regimens. Each patient's visit to the IDC coincided with a visit to the hospital laboratory for routine blood work, which was subsequently reviewed to obtain fasting blood glucose and fasting lipid profile. If fasting blood work was not available the most recent non-fasting blood work was used. 10-year Framingham risk score (FRS) was calculated using blood pressure measured upon

recruitment to the study, smoking status and most recent lipids profile. Dyslipidemia was defined according Canadian Diabetes Association treatment clinical practice guidelines [246].

3.2.3 Statistical Analysis

Statistical analysis was performed using a statistical software package (SPSS version 14.0, SPSS Inc., Chicago, IL, USA). Logistic regression was used to determine the influence of clinical characteristics (age, gender), cardiovascular risk factors (smoking, diabetes, hypertension, dyslipidemia) and medication regimens (ARV, antihypertensive, lipid-lowering) on ABI.

3.3 Results

ABI was measured in 172 patients. One patient was excluded due to arterial calcification and one patient withdrew consent following assessment. Demographic and relevant clinical data of the 170 patients included in the study are shown in 3.1. Low ABI (≤ 0.9), indicating presence of PAD, was found in four patients (2.4%), high ABI (≥ 1.30) was taken as suggesting reduced arterial compliance and was found in 11 patients (1.6%).

Patient distribution according to Framingham Risk Score (FRS) into low ($< 10\%$), moderate (10-20%) and high ($> 20\%$) cardiovascular risk categories revealed proportions of 47.3%, 34.7% and 18.0% respectively. No patients with a positive ABI for PAD had a low or moderate FRS. All 4 patients with a low ABI were among the 30 patients with a high FRS. The resulting prevalence of PAD among patients with high cardiovascular risk was 13.3%.

Among the 30 patients with high cardiovascular risk, 18 had previous vascular disease. Three of the four patients with positive tests for PAD had existing vascular disease (stroke or CAD). This leaves one of the remaining 12 (8.3%) high-risk patients with a low ABI indicating previously undiagnosed, asymptomatic peripheral arterial disease. As an internal control, the three patients with previous PAD were screened and all had a positive ABI. Relevant cardiovascular risk factors for each patient with low ABI are displayed in Table 3.2.

3.4 Discussion

This study showed a lower proportion of HIV+ patients with PAD than previously observed [243, 247-249]. The patient cohort was representative of the patient population attending an HIV metabolic clinic specializing in treatment of HIV+ patients suffering metabolic complications, such as dyslipidemia, arising from HAART. As a result, patients attending the clinic have a high prevalence of cardiovascular risk factors, represented by the increased prevalence of risk factors in the study cohort (Table 3.1).

Despite the high proportion of patients with cardiovascular risk factors, including an established history of vascular disease, there was a low prevalence of symptomatic PAD and also a low prevalence of asymptomatic PAD based on ABI. Sample size does not readily explain the low prevalence of PAD observed as the study was large in comparison to prior PAD studies in HIV cohorts [243, 247-249].

3.4.1 Risk factors for PAD

Risk of PAD increases as patients age and, as a result, the prevalence of PAD in the general population has been reported to be anywhere between 1% and 30% depending on

age and presence of cardiovascular risk factors [250-254]. The mean age of our cohort was 52 years and is young compared to non-HIV studies investigating prevalence of PAD [255-257]. Irrespective of the young mean age, prevalence of PAD was lower (4.4% vs. 2.4%) than that found in a study with similar design that screened HIV-infected patients with multiple cardiovascular risk factors for PAD [247]. That study also used ABI as the screening tool and had a cohort with a similar age (50 years vs. 52 years) and similar sex ratio (89.7% male vs. 97.9% male) but had a current smoker prevalence more than double that measured in the current study (72.5% vs. 32.9%)[247]. This may partially explain the low prevalence of PAD. However, this is contradicted by other studies, one reporting a higher proportion of smokers but a similar prevalence of PAD and the other reporting a similar prevalence of PAD but more than double the proportion of smokers [243, 247-249]. In spite of the contradictory literature, smoking is considered a major risk factor for PAD [258-262], illustrated by all patients with positive screening tests for PAD being current or ex-smokers in our cohort (Table 3.2).

Cardiovascular risk assessment of the cohort according to FRS revealed that no patients with a low-moderate FRS were identified as having PAD. Furthermore, the majority of patients who had a positive ABI for PAD had previously been diagnosed with PAD or had a history of established vascular disease such as stroke or coronary artery disease (CAD). Screening patients with pre-existing vascular disease will lead to a high-rate of detection of disease in other vascular beds. However, the most beneficial use of ABI screening is discerning patients with asymptomatic PAD, a prevalent condition among non-HIV patients with a high-risk for cardiovascular disease [255, 256]. As high-risk patients are already targeted for maximally aggressive therapy and given the paucity of

positive tests among the low-moderate risk group the ABI appears to have limited utility in this cohort of HIV+ patients.

3.4.2 Influence of HAART on PAD

The effect of combination ARV therapy on cardiovascular health has been well documented in the literature linking HAART to an increased prevalence of carotid plaques [263], an increased proportion of carotid plaques and intima-media thickening among patients taking PIs[264] as well as decreased flow-mediated vasodilation [265]. The direct effect of ARVs on ankle-brachial index was explored by Olalla et al and an altered ABI, defined as an ABI <0.9 or >1.3, was found to be associated with PI use independent of dyslipidemia [243]. The present study; however, found no link between ARV regimen and low ABI. With the increased risk that is posed to patients on HAART it would be expected that the proportion of PAD amongst HIV+ patients on HAART would increase the prevalence of PAD in this population. In spite of this no study exploring PAD in HIV+ patients has shown a high prevalence of PAD.

3.4.3 Limitations

The low prevalence of PAD observed in our population of HIV+ subjects with varied degrees of cardiovascular risk is most likely related to the relatively young age of the subjects. Also, the low prevalence of PAD shown may be attributed, at least in part, to the method used for screening for the PAD. In the study by Periard et al they measured ABI both at rest and following exercise, and observed a doubling of the proportion of patients with an ABI positive for PAD following exercise [249].

Possible confounders are peripheral neuropathy and chronic hyperglycemia. These conditions are prevalent among HIV+ patients on HAART [266-268]. Increased arterial

stiffness in the peripheral vasculature is another confounder. As a result, the ABI can be affected in a similar fashion as is seen in patients with diabetes [23, 269, 270]. Prevalence of peripheral neuropathy was not ascertained and future research needs to be undertaken to explore the connection, if any, between peripheral neuropathy and ABI in the HIV+ population.

3.4.4 Conclusions

Prevalence of PAD in our cohort of HIV+ patients was not increased in this study, which, to our knowledge, is the first in North America to investigate PAD in such a cohort. The lack of positive diagnoses of PAD among low and moderate risk patients combined with the redundancy of performing an ABI in patients with pre-existing cardiovascular disease or high cardiovascular risk indicates that ABI has limited utility in this cohort. ABI remains a simple, portable diagnostic tool but given the largely negative results obtained here it is not cost effective to perform an ABI with regularity in HIV clinics.

Tables

Table 3.1 Demographic and clinical characteristics.

| Characteristic | Value |
|------------------------------------|------------|
| Age, y | 52.0±8.4 |
| Sex, male, n (%) | 163 (97.6) |
| Medications, n (%) | |
| Current ARV regimen | 163 (97.7) |
| PI | 142 (85.0) |
| Non-PI | 21 (12.6) |
| None | 4 (2.4) |
| Lipid-lowering Therapy | 96 (57.5) |
| Statin | 81 (48.5) |
| Metabolic parameters | |
| Fasting blood glucose, mmol/L | 6.3±2.0 |
| Total Cholesterol, mmol/L | 5.07±1.45 |
| Total Cholesterol:HDL Ratio | 5.1±2.6 |
| Triglycerides, mmol/L | 3.58±3.59 |
| HDL-cholesterol, mmol/L | 1.11±0.42 |
| LDL-cholesterol, mmol/L | 2.52±0.89 |
| Apolipoprotein B, g/L | 1.01±0.30 |
| Cardiovascular risk factors, n (%) | |
| Dyslipidemia | 130 (77.8) |
| Current smoker | 53 (31.7) |
| Ex-smoker* | 23 (13.8) |
| Non-smoker† | 26 (15.6) |
| Never smoker | 65 (38.9) |
| Type 2 diabetes mellitus | 44 (26.3) |
| Hypertension | 68 (40.7) |
| History of cardiovascular disease | 18 (11.4) |
| Framingham score | |
| Low risk (<10%) | 79 (47.3) |
| Medium risk (10-20%) | 58 (34.7) |
| High risk (>20%) | 30 (18.0) |

Data are presented as mean±SD, unless otherwise indicated.

*Quit smoking <5 years prior and/or >20 pack years.

†Quit smoking >5 years prior and <20 pack years.

ARV, antiretroviral; PI, protease inhibitor.

Table 3.2 Cardiovascular risk factor constellations for patients with low ABI

| Patient | Sex | Age | Risk Factors | ABI | FRS* |
|---------|-----|-----|--|------|------|
| 1 | M | 65 | DM, Hypertension, Smoking (10 PY), CAD | 0.78 | N/A |
| 2 | M | 72 | Hypertension, Smoking (55 PY), DL, High CVD Risk | 0.86 | 25 |
| 3 | M | 48 | DM, Hypertension, Smoking (49 PY), DL, Stroke | 0.90 | N/A |
| 4 | M | 58 | Hypertension, Ex-Smoker (20 PY), DL, MI | 0.85 | N/A |

*Patients with previous cardiovascular events had no FRS calculated as by convention they are classified as high-risk.

DM, diabetes mellitus; PY, Pack years; CAD, coronary artery disease; DL, dyslipidemia; MI, myocardial infarction

Chapter 4: Cardiovascular Risk Reduction with Rosiglitazone

The advent and widespread availability of highly-active antiretroviral therapy (HAART) has resulted in a dramatic reduction of HIV-associated morbidity and mortality in the Western world [271, 272]. There is substantial evidence, however, that both antiretroviral (ARV) therapy and HIV infection can be associated with an increased risk of T2DM, atherosclerosis and cardiovascular disease (CVD) progression [9, 10, 273, 274] and myocardial infarction [13, 15].

Carotid intima media thickness (IMT) with high-resolution B-mode ultrasound is a well-accepted, non-invasive method to evaluate cardiovascular risk [275]. Increased IMT positively correlates with progression of atherosclerosis and predicts myocardial infarction and stroke [276, 277]. HIV-positive (HIV+) patients have been shown to have a higher mean carotid IMT and a greater rate of IMT progression compared to age- and gender-matched healthy individuals [278].

Inflammation and endothelial dysfunction have been shown to play a significant role in plaque formation and progression of atherosclerosis in both general population as well as HIV+ individuals [279-281]. Adipose tissue, by producing several adipocytokines, has been recognized to contribute to the inflammatory milieu. High sensitivity C-reactive protein (CRP) levels are correlated with adipose tissue mass and are thought to predict type 2 diabetes mellitus (T2DM) and CVD [282-284]. Alterations in adipocytokines, including elevated retinol-binding protein-4 (RBP-4), interleukin-6 (IL-6), monocyte chemotactic protein-1 (MCP-1) and decreased adiponectin have been implicated in vascular damage and the development of atherosclerosis [285]. Accumulating data indicate that rosiglitazone may exert anti-inflammatory effects within the vessel walls, thereby preventing progression of atherosclerosis [286].

Rosiglitazone, a peroxisome proliferator-activated receptor-gamma (PPAR- γ) agonist belonging to the thiazolidinedione (TZD) drug class, is normally used as an insulin-sensitizing agent in

treating T2DM [287]. In addition to improving glycemic control, TZDs have pleiotropic effects including anti-inflammatory and anti-atherogenic properties [285, 288-290]. While the effects of rosiglitazone on glycemic control were not explored in the present study it is the anti-inflammatory properties of the drug that are credited with its potential to reverse carotid IMT progression in patients with and without diabetes [291, 292]. Rosiglitazone may also stabilize atherosclerotic plaques by reducing the expression of critical biomarkers of inflammation such as CRP [293, 294]. In addition, TZDs stimulate the differentiation of adipocytes and rosiglitazone has improve body composition by decreasing visceral fat and increasing subcutaneous fat [190, 295].

The effect of rosiglitazone on subclinical carotid atherosclerosis has not previously been investigated in the HIV+ population. This prospective pilot study was conducted to investigate the effect of rosiglitazone on carotid IMT and focal plaque progression as well as inflammatory biomarkers, insulin resistance biomarkers and adipocytokines in HIV+ adults with at least one risk factor for cardiovascular disease.

4.1 Methods

4.1.1 Participants

Men and women between 30 and 70 years of age with documented evidence of HIV-1 infection were recruited from the Immunodeficiency Clinic/HIV Metabolic Clinic (IDC/HIVMC) at St. Paul's Hospital in Vancouver, British Columbia, Canada, between January 2004 and January 2006. Inclusion criteria were stable HAART treatment for a minimum of 12 weeks prior to study entry and at least one risk factor for CVD: hyperlipidemia (fasting triglycerides >2.3 mmol/L or total cholesterol >5.2 mmol/L); smoking (\geq 1 pack/day); hypertension (blood pressure >140/90, treated or non-treated); diagnosis of diabetes mellitus (DM); insulin resistance (fasting blood

glucose ≥ 6.1 mmol/L) or metabolic syndrome (3 or more of the following: waist circumference >102 cm for men, 88 cm for women; triglycerides >1.7 mmol/L; HDL cholesterol <1.0 mmol/L; fasting blood glucose ≥ 6.1 mmol/L; blood pressure $>130/90$). Distributions of risk factors at baseline are summarized in Table 1.

Exclusion criteria were: poorly controlled (DM) (Hemoglobin A₁C [HbA₁C]) $> 8\%$; uncontrolled hypertension ($>140/90$) or clinical evidence of heart failure; presence of unstable or severe angina or coronary insufficiency; established CVD; stroke or peripheral vascular disease or an active-AIDS-defining condition; pancreatitis or hepatitis (including hepatitis C co-infection) within the previous 6 months.

4.1.2 Ethics

All patients provided written informed consent for study participation. The study was approved by institutional Research Ethics Board.

4.1.3 Interventions

The study had a parallel design. Eligible participants were randomized to receive either rosiglitazone (8 mg/day) or matching placebo orally for 48 weeks. Rosiglitazone and matching placebo tablets were supplied by GlaxoSmithKline (Mississauga, Ontario, Canada). The duration of each patient's participation in the study was 48 weeks in total, with follow-up evaluations every 8 weeks. At baseline, all participants received standardized dietary advice from a registered dietician, based on the National Cholesterol Education Program [94], with suggestions of physical activity, as part of the regular management of the HIV metabolic syndrome.

4.1.4 Outcomes

4.1.4.1 IMT and Plaque Burden

The primary outcome measures were the difference between rosiglitazone and placebo groups following 48 weeks of treatment in terms of IMT, measured with B mode ultrasonography as described previously [296] and plaque burden as determined by total plaque area (TPA). In brief, the common carotid artery was measured with a 7.5-10MHz linear array transducer. IMT was obtained by measuring over a uniform length of 10 mm in the far wall of the common carotid artery devoid of focal plaques in both the right and left carotid arteries within 2 cm proximal to the carotid bulb. Multiple frames were measured and the region with the thickest IMT was recorded. The measurements from the right and left sides were summed and averaged to yield average IMT. For TPA the common carotid artery and the internal and external carotid arteries were examined by ultrasonography for evidence of focal plaques. Plaques found in any of the carotid segments were identified by consensus of least two observers as wall thickness that is increased focally relative to IMT on either side of the focal area. TPA was calculated by summing the product of plaque length and average lesion thickness for each plaque identified in the carotid tree, giving a result in millimetres squared (mm^2).

4.1.4.2 Flow-mediated Dilation

Endothelial function was determined by means of brachial artery reactivity as measured by flow-mediated dilation (FMD) at weeks 0, 24 and 48. The brachial artery was imaged using the same ultrasound equipment described above. The mid-portion of the forearm was targeted and the basal luminal diameter of the brachial artery was recorded. FMD was induced by occluding the vessel distal to the elbow by inflation of a sphygmomanometer to 300 mmHg for 5 minutes. Imaging began 30 seconds prior to cuff release and continued for the first five minutes of

hyperemia. Peak FMD was recorded. After brachial artery diameter returned to baseline patients were given 0.3 mg of nitroglycerin sublingually. The artery was then imaged for 6 minutes to measure endothelium-independent vasodilation. Peak nitroglycerin-mediated dilation (NMD) was recorded.

4.1.4.3 Body Composition and Anthropometry

Body composition was estimated with whole-body Dual X-ray Absorptiometry (DXA) using a Hologic QDR-4500 Delphi A instrument (Hologic, Waltham, Ma, USA) and Enhanced Whole body software. DXA was performed at 0, 24 and 48 weeks while anthropometric measurements including weight, height, waist and hip circumferences were performed in conjunction with a physical examination every 8 weeks. Weight and height were used to calculate body mass index (BMI).

4.1.4.4 Laboratory Assessments

Participants had laboratory evaluations performed every 8 weeks. Fasting blood samples were analyzed for TC, LDL and HDL, TG, ApoB, CRP, glucose, HbA_{1c}, CD4 cell count, HIV RNA, aspartate transaminase (ALT), alanine transaminase (AST), bilirubin and hemoglobin. A serum sample was collected at each visit and stored frozen for later assessment of biomarkers.

Clinical safety assessments were performed 8 weeks after completion of the study and a non-fasting blood sample was taken for assessment of ALT/AST, bilirubin, hemoglobin, CD4 cell count and HIV RNA.

4.1.5 Measurement of Biomarkers of Inflammation

Serum samples were stored at -80⁰C until analyzed. Serum levels of CRP, IL-6, adiponectin, MCP-1 and RBP-4 were analyzed using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Inc. Minneapolis, MN, USA).

4.1.6 Sample Size Calculation

Sample size calculations were based on a previous publication investigating IMT changes in response to an equivalent dose of a similar drug, pioglitazone, in participants with type 2 diabetes [288]. Sample size was calculated with an estimated mean difference of the progression rate of IMT of 0.1 mm/year between treatment groups, a within group standard deviation of 0.125 mm/year, a power of 0.8 and a two tailed false positive rate of 0.05. This yielded a sample size of 25 participants per group.

4.1.7 Statistical Analysis

Homogeneity of variance and normality assumptions were checked. As the sample size was small data and many variables had a non-normal distribution is presented as medians (IQR). Within group differences were assessed using the Wilcoxon rank-sum test and between-group differences at baseline and at week 48 were tested for using the Mann-Whitney U test. Intention-to-treat analyses were performed. P-values were not adjusted for as this was a small, pilot study and adjustment, while conserving the type-I error rate would have further reduced the power (increasing the type-II error rate) [297]. Statistical analyses were performed using SPSS v.19.0 (SPSS, Inc., Chicago, IL, USA).

4.1.8 Randomization

4.1.8.1 Sequence Generation

The random allocation sequence was generated using a computer programme by a statistician unassociated with the study. There was no stratification and permuted blocks of size of four were used.

4.1.8.2 Allocation Concealment

The allocation file was loaded by a systems administrator with sole access to the codes into a randomization program.

4.1.8.3 Implementation

When a patient was deemed eligible and consenting by the site coordinator, this was reported to a study pharmacist who then called the randomization computer via telephone and logged into the system using a unique authorization code and password. The patient's initials and date of birth were entered into the system by the pharmacist and the computer subsequently issued the allocation to the pharmacist. The transaction, including data and time of the randomization, was logged into a file by the computer.

4.1.8.4 Blinding

The trial was double blinded. Both participants as well as study coordinators doctors were blinded to group assignments. In addition, the readers of the IMT and TPA assessment were blinded as to the study treatment arm of each patient.

4.2 Results

4.2.1 Participant Flow

Fifty-one patients were screened for entry into the study and subsequently randomized. One patient failed screening due to ineligible laboratory criteria and five withdrew consent before randomization. Forty-five eligible began the study and received either placebo (n=23) or rosiglitazone (n=22) for 48 weeks. In the rosiglitazone arm, three patients withdrew due to adverse events, one patient withdrew consent and a fifth was lost to follow-up prior to week 24

leaving 17 who completed the study. The placebo group had one patient lost to follow-up after week 48 leaving 22 who completed the study. Patient disposition is displayed in Figure 4.1.

4.2.2 Recruitment

Patients were recruited between January 2004 and January 2006 with follow-up visits taking place between March 2004 and February 2007.

4.2.3 Baseline Data

At baseline, the rosiglitazone and placebo groups were similar with respect to age, sex and duration of HIV therapy; however, time since first positive HIV test differed significantly between groups (Table 4.2). In terms of ARV medications at baseline, distribution of individual agents was similar between groups with exception of fusion inhibitors as all three were randomized to the rosiglitazone group. There were considerably more patients on lipid-lowering therapy allocated to the rosiglitazone arm (14) compared to the placebo arm (8).

4.2.4 Numbers Analyzed

In the primary analysis investigating IMT and TPA and secondary analyses on FMD and lipid parameters, 17 of the 26 patients randomized to the rosiglitazone group and 22 of the 25 patients randomized to the placebo group completed the trial. For the secondary analyses of inflammatory markers and DXA 15 of the 26 patients randomized to rosiglitazone and 20 of the 25 patients randomized to placebo had baseline and follow-up data for inflammatory markers whereas 17 of 26 randomized to rosiglitazone and 20 of 25 randomized had complete DXA data.

4.2.5 Outcomes

As non-parametric tests cannot account for baseline difference between groups Mann-Whitney U tests were performed to compare each outcome variable at baseline and no significant differences

($p < 0.05$) were found. Complete results are found in Tables 4.3 – 4.6. Data are presented as median (IQR) for all variables.

4.2.5.1 Intima Media Thickness

After 48 weeks of treatment, there were no significant differences in IMT or TPA as determined by carotid ultrasound, in either the placebo or rosiglitazone group. Following 48 weeks of treatment there was a small median difference in IMT between rosiglitazone (0.71 mm, [0.65 – 0.86]) and placebo (0.68 mm, [0.60 – 0.83]), which did not reach significance ($p = 0.54$). There was a small, significant median increase in IMT of 0.02 mm (0.00 – 0.05) in the placebo group ($p = 0.03$) whereas an identical median increase of 0.02 mm (–0.02 – 0.04) in the rosiglitazone group did not reach significance ($p = 0.28$). When considered as a whole the study cohort showed a median increase in IMT of 0.02 mm [IQR –0.01 – 0.04].

4.2.5.2 Plaque Area

Carotid ultrasound revealed plaques for 32 patients at baseline (14 rosiglitazone, 18 placebo), which increased to 33 patients by week 48 (15 rosiglitazone, 18 placebo). Median difference in TPA after 48 weeks of treatment was not significant between groups despite seemingly large difference (11.5 mm² [IQR 4.3 – 27.2] vs. 23.4 mm² [IQR 9.8 – 34.0]). This large disparity can be attributed to the concordant median difference at baseline (9.54 mm² [2.52 – 32.1] vs. 20.8 mm² [7.8 – 32.9]). However, the within placebo group median increase of 0.15mm² (–1.7 – 4.7) approached but did not reach significance ($p = 0.07$). When the groups were combined there was a median increase in TPA of 0.58mm² [IQR –0.33 – 0.58] over the course of the study.

4.2.5.3 Flow-mediated Dilation

No significant between-group difference in FMD was observed ($p = 0.34$). FMD significantly improved within the rosiglitazone group by 0.84% (–0.35 – 3.82, $p = 0.03$) while no significant

improvement was seen in the placebo group. A median increase of 0.32% [IQR -1.33 – 2.48] indicated a trend towards improvement when both groups were considered together.

4.2.5.4 Biomarkers of Inflammation

After 48 weeks of treatment CRP in the rosiglitazone was significantly lower than the placebo group (0.8 mg/L [0.5 – 1.9] vs. 3.6 mg/L [-1.1 – 4.8], $p=0.003$). Accordingly, significant within group improvement in CRP of -0.3 mg/L (-2.3 – -0.5) was observed in the rosiglitazone group. Similar improvements were seen in adiponectin: at 48 weeks, adiponectin was significantly increased in the rosiglitazone group compared to placebo (7.3 mg/mL [3.4 – 17.9] vs. 2.4 mg/mL [0.8 – 5.7], $p=0.01$) and adiponectin significantly increased 4.3 mg/mL (1.6 – 12.1, $p=0.004$) within the rosiglitazone group. In patients treated with rosiglitazone, serum levels of MCP-1 455.4 pg/mL (382.2 – 489.8) vs. 538.3 pg/mL (396.3 – 657.7) and IL-6 1.4 pg/mL (0.9 – 2.2) vs. 3.1 (1.5 – 6.1) were significantly lower ($p=0.04$ for both) in the rosiglitazone group. MCP-1 decreased by -100.8 pg/mL (-157.0 – -11.6, $p=0.004$) vs. baseline and no significant changes were observed in the placebo group. The drop in RBP-4 -18.8 mg/L (-30.8 – -3.2) approached significance $p=0.06$, however, the difference at baseline between the two groups also approached significance ($p=0.06$). An unexpected decrease in insulin was observed in the rosiglitazone group but this did not reach significance ($p=0.81$). No changes were observed within groups or between groups for HbA1c or any lipid markers (**Table 4.4**).

4.2.5.5 DXA and Anthropometry

Treatment with rosiglitazone had no effect on any body fat measurement or anthropometric parameters with the exception of lean body mass, which decreased 0.5 kg (-2.0 - -0.02, $p=0.007$) but the between group difference was not significant ($p=0.11$). A median increase in both trunk fat (0.4 kg [0.02 – 1.6], $p=0.007$) and head fat (0.04 kg [0.01 – 0.10], $p=0.001$) was

observed in the placebo group. Anthropometric and body composition variables are displayed in Table 4.5.

4.2.5.6 Safety Parameters

Hemoglobin differed significantly between groups (146 g/L [137 – 159] for placebo vs. 134 g/L [125 – 147] for rosiglitazone, $p=0.02$) driven by the significant median decrease in hemoglobin of 11 g/L (-15 – -3) in the rosiglitazone group. CD4 cell fraction increased 4% (1-5, $p=0.002$) in the placebo group and in the rosiglitazone group (2-5, $p=0.02$) in the rosiglitazone ($p=0.02$) while absolute CD4 count increased 100 cells/mm³ (20 – 160) ($p<0.001$) in the placebo group. Neither CD4 parameter differed significantly between groups ($p=0.81$ and $p=0.88$). No significant differences were observed between groups for any other safety parameter (Table 4.6).

4.2.6 Adverse Events

Three patients in the rosiglitazone arm withdrew due to adverse events: two discontinued therapy due to abnormal lipids at weeks 8 and 16 respectively, possibly due to the study drug. One further patient withdrew at week 8 because of acute renal failure secondary to tenofovir.

4.3 Discussion

4.3.1 Primary Outcomes

This randomized, placebo-controlled, double-blinded pilot study is the first to investigate the effect of rosiglitazone on cardiovascular disease progression in HIV⁺ patients. In contrast to studies in patients with T2DM or coronary artery disease, treatment with rosiglitazone did not result in a significant difference in IMT as compared to placebo [291, 292, 298]. Rosiglitazone had no significant effect on plaque burden as measured by TPA; however, CRP, IL-6, MCP-1 and adiponectin levels were significantly improved with rosiglitazone as compared to placebo. A

revealing finding was the high proportion of patients that presented with atherosclerotic plaques at baseline (32/45 = 71%), considering the relatively young age of the cohort (median age 49.2 years). This validates the finding that HIV infection is a risk factor for cardiovascular disease [30].

The lack of a demonstrated treatment effect on IMT and TPA could be partially attributed to the small sample size of the study, and therefore lack of statistical significance could be due to limited statistical power. Additionally, it is possible that the study population had too low a risk to properly assess short-term effects on cardiovascular disease progression, as previously described by the ENHANCE trial, which had similar baseline IMT values to the present study [299].

4.3.1.1 Intima Media Thickness

Previous trials have been conducted that examined the effect of rosiglitazone in different patients populations. One trial with patients with T2DM had a similar design and showed a significant change in IMT in the common carotid artery with rosiglitazone [298], although these patients had a higher baseline vascular risk demonstrated by an elevated baseline IMT score than those in the present study. A further study compared the effects of rosiglitazone versus metformin on carotid IMT in T2DM and found a significant regression following 24 weeks of rosiglitazone treatment [291]. Baseline IMT data were not provided, thus severity of baseline vascular risk could not be comparatively assessed. A beneficial effect of rosiglitazone is not limited to patients with T2DM: one trial undertaken in patients with established coronary artery disease without T2DM showed that rosiglitazone significantly reduced common carotid artery IMT progression after 48 weeks of treatment [292]. As expected, this trial had substantially higher baseline IMT

scores than the present study. These latter studies used similar designs to our own but recruited more patients and were thus more adequately powered to detect small changes in IMT.

4.3.1.2 Plaque Burden

Measurement of plaque burden is an emerging aspect of cardiovascular disease diagnosis. In this trial plaque burden was assessed by measuring TPA, the sum of the area of all plaques found within a specific vessel; accordingly, ours is the first study to investigate the effects of rosiglitazone on TPA. Typically, TPA is amalgamated into the measurement of IMT whether the vessel is diffusely thickened or focally thickened by a plaque. Currently, limited data are available on the utility of TPA, measured by B-mode ultrasound, as a surrogate marker for subclinical atherosclerosis. One trial in non-diabetic patients with carotid artery stenosis found that rosiglitazone significantly reduced vascular inflammation leading to increased plaque stability [293]. Large plaques are generally less stable and more prone to rupture and it is plaque rupture that often leads to a cardiovascular event. This demonstrates the importance of determining plaque area in a cohort with elevated risk for cardiovascular disease.

4.3.2 Secondary Outcomes

4.3.2.1 Endothelial Function and FMD

Endothelial function was impaired in both placebo and rosiglitazone groups at baseline, at 5.05% and 4.71% respectively versus 8-11% in the normal population [300]. This is in accordance with the study by Kovacic et al. that recruited a sample size similar to our own and used the same dosage and treatment time [199]. Similarly, we observed a modest yet significant improvement in FMD% in rosiglitazone patients but the between-group difference was <1% and the study was not adequately powered to detect this small difference. It has been suggested that the failure of TZDs to elicit a significant effect on endothelial function in HIV-positive patients, as seen in

T2DM patients, may be due to ritonavir reducing endothelium-dependent vasorelaxation or intrinsic differences between the two conditions [199, 301].

4.3.2.2 Inflammatory Markers

PPAR- γ is expressed in vascular cells and stimulation by agonists, such as TZDs like rosiglitazone, has anti-inflammatory and antihypertensive effects including improved endothelial function and decreased expression of adhesion molecules [302-304]. Rosiglitazone has been shown to reduce CRP and MCP-1, markers of inflammation and cellular adhesion, in both healthy and disease-burdened subjects with varying degrees of cardiovascular disease risk [289, 305, 306], but in the HIV⁺ population, the anti-inflammatory effect of rosiglitazone is less well established.

CRP plays a role in endothelial dysfunction [307] whereas MCP-1 is implicated in atherogenesis as it recruits monocytes into the sub-endothelial space [308] and the inflammatory cytokine, IL-6, has been shown to be elevated in HIV [309]. Elevations in these markers have been linked to CVD and mortality in HIV⁺ patients [310-312]. The improvement in MCP-1 confirms the findings from a similar study by Coll et al that investigated the effects of rosiglitazone and metformin on HIV lipodystrophy [312], however, the modest improvements in CRP and IL-6 with rosiglitazone in HIV⁺ patients is a novel finding. As such, TZDs may prove to be a useful anti-inflammatory agent in attenuation of cardiovascular disease in the HIV⁺ population through lowering of CRP and IL-6. As elevated MCP-1 is related to lipodystrophy [312] a positive effect on body fat distribution may have been expected with rosiglitazone treatment but DXA scan outcomes revealed no such amelioration. The improvements in adiponectin with rosiglitazone are in accordance with other studies [200, 313]. Adiponectin has been negatively correlated with

plaque formation [314, 315], which indicates a potentially positive effect on plaque however no clear effect was observed.

4.3.2.3 Markers of Insulin Resistance and Lipid Parameters

An unusual finding was the lack significant effect of rosiglitazone on markers related to insulin resistance and hyperglycemia despite the observed improvement in adiponectin improvement in insulin resistance generally seen with TZDs [316, 317]. The rosiglitazone group did show a decrease in insulin, which is indicative of a trend toward improvement of insulin resistance given the high median insulin levels at baseline. No effect was observed on RBP-4, a marker for insulin resistance [318] nor was there any appreciable effect on HbA1c, the latter is likely explained by the relatively normal HbA1c at baseline (5.00% in each group). There were no alterations in total cholesterol, HDL, LDL or apolipoprotein B, perhaps due to use of lipid-lowering therapy in both groups, especially the rosiglitazone group.

4.3.2.4 Fat Distribution and Lipodystrophy

TZDs have been shown to redistribute subcutaneous adipose tissue [319, 320] indicating the rosiglitazone may have a positive effect on lipodystrophy. Here, as with previous trials, no improvements in body composition or fat were attained [196, 197, 201, 207] despite higher rosiglitazone dosage and longer treatment time than a recent study [201]. One trial found an improvement of altered body composition with rosiglitazone as trunk fat decreased while limb fat increased [321] and while trunk fat and head fat were shown to increase in placebo no direct benefits of rosiglitazone could be ascertained. Lean body mass decreased in patients on rosiglitazone, in contrast to results from Schindler et al who found that lean body mass increased significantly in both placebo and rosiglitazone groups secondary to lifestyle and/or nutritional changes [201].

4.3.2.5 Safety Parameters

The observed decrease in hemoglobin is a common finding in patients treated with rosiglitazone [193, 197]. The underlying mechanism behind this decrease has not yet been delineated. CD4+ fraction improved in both groups however median CD4+ count trended towards a decrease. It is difficult to draw a direct connection between these changes and rosiglitazone other than one study that found rosiglitazone decreases bioavailability of nevirapine [322].

4.3.3 Rosiglitazone and Cardiovascular Risk

The results of this trial follow large trials and a meta-analysis with conflicting conclusions regarding rosiglitazone treatment and risk of myocardial infarction and death from cardiovascular causes [323-327]. Results from the ADOPT and DREAM trials, and interim results from the RECORD trial, showed no increased risk from taking the drug [323, 324, 327]. In contrast, the meta-analysis conducted by Nissen et al concluded that there was an increased risk for myocardial infarction and a borderline significant increase in the risk of death from cardiovascular causes [325]. This latter study prompted the United States Food and Drug Administration (FDA) to place “black box” restrictions on rosiglitazone [328] and a review of the safety of rosiglitazone by an FDA advisory panel. A recent update of this meta-analysis, including results from the ADOPT, DREAM and RECORD trials, echoed initial findings but no longer showed a significant increase in risk of death due to cardiovascular events. When trials were considered separately, pooled analysis revealed that neither myocardial infarction nor death due to cardiovascular causes reached statistical significance [329]. No cardiovascular events occurred in our study cohort throughout the duration of the study. These results should be interpreted with caution as the study was not powered to detect a safety signal.

4.3.4 Conclusions

In conclusion, the primary outcomes of this pilot study did not reach significance; therefore, we can neither rule out a clinically important effect of rosiglitazone on IMT and TPA, nor can the results provide insight into the increased risk of myocardial infarction observed with rosiglitazone. This study was limited by the small sample size resulting from a high rate of post-randomization drop out, particularly in the rosiglitazone group. The relatively short duration of the study is a further limitation as, in practice, TZD treatment would be ongoing during clinical care. However, the duration of treatment in this study was longer than at least one other positive trial [291] and at least as long as other trials with discordant conclusions [292, 298]. The analysis was limited by the non-normal distribution of many of the variables preventing adjustment for covariates that may have affected the outcomes, such as fibrate use between groups.

Rosiglitazone has been shown to be an effective agent to prevent progression of T2 DM as evidenced by the DREAM and CANOE trials [327, 330]. In our study rosiglitazone was confirmed as having beneficial effects on inflammatory markers in HIV+ patients but these did not translate into improvements in the specific surrogate markers for cardiovascular disease, IMT and TPA, assessed in this trial. While rosiglitazone will not likely be used as an agent to prevent CVD, TZDs should not yet be abandoned. The only other TZD agent on the market, pioglitazone, unlike rosiglitazone, has been shown to reduce the composite of all-cause mortality, non-fatal myocardial infarction, and stroke in patients with type 2 diabetes who have a high risk of macrovascular events [331]. As a result any further trial examining the effects of TZDs on CVD in HIV should study these effects early rather than late in pathogenesis of atherosclerosis.

4.4 Clinical Trial Registration

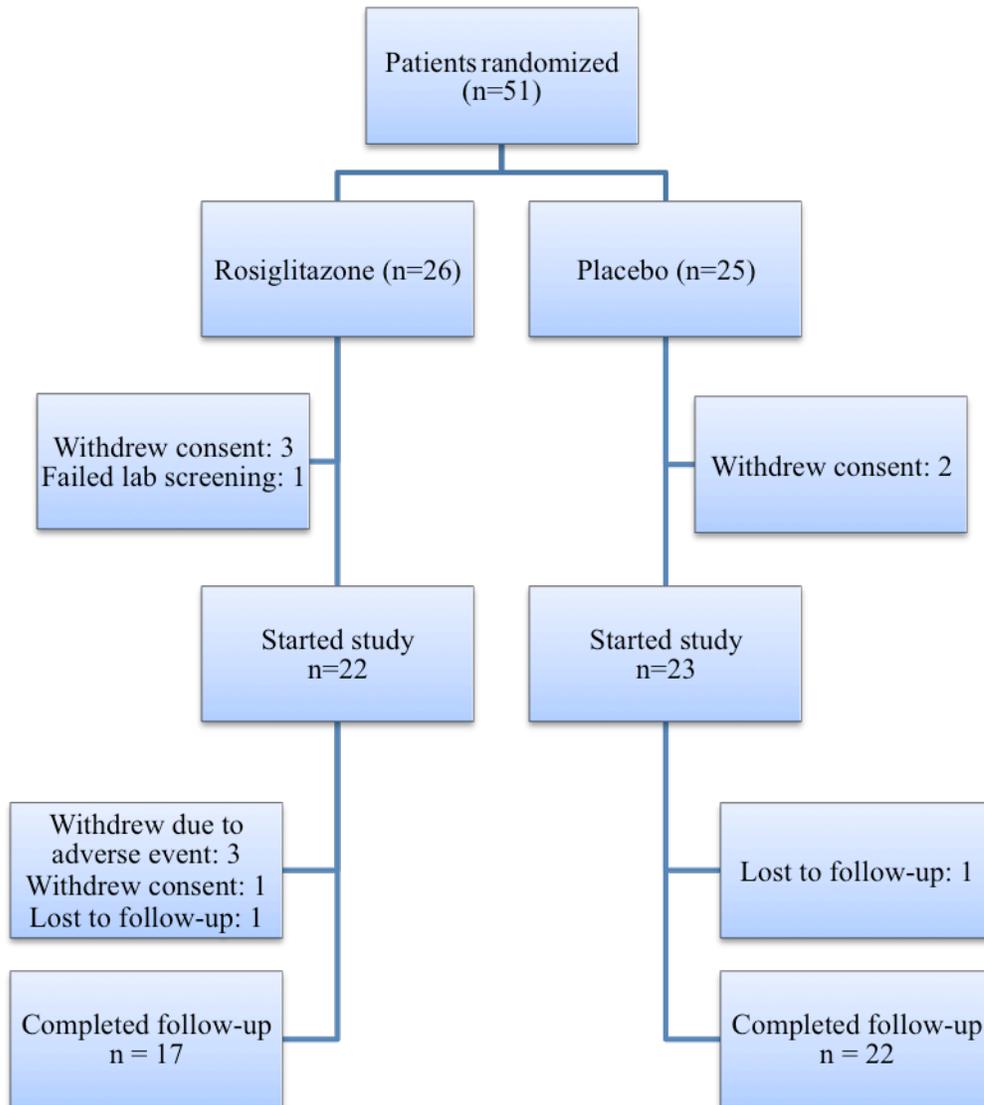
The complete protocol can be found online at ClinicalTrials.gov.

ClinicalTrials.gov Identifier: NCT00143624

URL: <http://clinicaltrials.gov/ct2/show/NCT00143624?term=Rosiglitazone+AND+IMT&rank=2>

Figures

Figure 4.1 Flow chart of patient disposition.



Tables

Table 4.1 Traditional cardiovascular risk factors of study subjects at baseline.

| Risk factor | Both groups (n=45) | Rosiglitazone (n=22) | Placebo (n=23) |
|-------------------------------|-----------------------|-------------------------|-------------------|
| Hyperlipidemia* | 40 (88.9) | 20 (90.9) | 20 (87.0) |
| Smoker | 11 (24.4) | 4 (18.2) | 7 (30.4) |
| Hypertension | 5 (11.1) | 3 (13.6) | 2 (8.7) |
| Diabetes | 3 (6.7) | 1 (4.5) | 2 (8.7) |
| Impaired glucose tolerance† | 6 (13.3) | 2 (9.1) | 4 (17.4) |
| Metabolic syndrome‡ | 15 (33.3) | 7 (31.8) | 8 (34.8) |
| Framingham Risk Score 10-20%§ | 16 (35.6) | 8 (36.4) | 8 (34.8) |

Data are frequency (%)

* total cholesterol >5.2mmol/L or fasting triglycerides >2.3 mmol/L.

† fasting blood glucose \geq 6.1mmol/L .

‡ Any three of the following: waist circumference >102 cm for men, 88 cm for women or Body Mass Index >30; triglycerides >1.7 mmol/L; high-density lipoprotein cholesterol <1.0 mmol/L; fasting blood glucose \geq 6.1 mmol/L; blood pressure >130/90.

§ Intermediate 10-year cardiovascular risk.

Table 4.2 Baseline characteristics of study subjects.

| Variable | Both groups (n=45) | Rosiglitazone (n=22) | Placebo (n=23) |
|--|-------------------------------|---------------------------------|---------------------------|
| Age, years* | 49.2 (43.9 – 52.9) | 49.8 (45.4 – 53.7) | 48.6 (43.5 – 53.0) |
| Male sex | 42 (93.3) | 21 (95.4) | 21 (91.3) |
| Time since first positive HIV test, years* | 12.8 (7.4 – 19.0) | 16.6 (11.5 – 19.7)‡ | 10.1 (7.2 5.8 – 7.2)‡ |
| CD4+ count, cells/mm ³ * | 420 (250 – 564) | 465 (313 – 563) | 300 (240 - 360) |
| Undetectable Viral Load† | 45 (100) | 22 (100) | 23 (100) |
| ARV duration, years* | 1.3 (0.9 – 2.1) | 1.1 (0.7 – 1.6) | 1.4 (0.9 – 2.7) |
| ARV regimen | 45 (100) | 22 (100) | 23 (100) |
| PI | 36 (80.0) | 18 (81.8) | 18 (78.3) |
| NNRTI | 23 (51.1) | 12 (54.5) | 11 (47.8) |
| NRTI | 45 (100) | 22 (100) | 23 (100) |
| Fusion Inhibitors | 3 (6.7) | 3 (13.6) | 0 (0.0) |
| Lipid-lowering therapy | 22 (48.9) | 14 (63.6) | 8 (34.8) |
| Antihypertensive therapy | 5 (12.8) | 3 (13.6) | 2 (9.1) |

Data are frequency (%) unless otherwise indicated

*Data are median (IQR)

†<50 copies per mL.

‡Between group difference <0.05.

ARV, antiretroviral; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor.

Table 4.3 Ultrasound outcomes at baseline and following 48 weeks of treatment with rosiglitazone of placebo.

| Variable | Unit | Placebo | | | Rosiglitazone | | | p* |
|----------|-----------------|--------------------|--------------------|-------------------------|--------------------|--------------------|-----------------------|------|
| | | Baseline | 48 Weeks | Change | Baseline | 48 Weeks | Change | |
| IMT | mm | 0.64 (0.59 – 0.81) | 0.68 (0.60 – 0.83) | 0.02 (-0.0013 – 0.053)† | 0.70 (0.63 – 0.81) | 0.71 (0.65 – 0.86) | 0.02 (-0.020 – 0.038) | 0.36 |
| TPA | mm ² | 9.54 (2.52 – 32.1) | 11.5 (4.3 – 27.2) | 0.15 (-0.23 – 4.82) | 20.8 (7.8 – 32.9) | 23.4 (9.8 – 34.0) | 1.5 (-1.7 – 4.7) | 0.31 |
| FMD | % | 4.13 (2.33 – 7.11) | 5.20 (2.85 – 6.87) | -0.33 (-1.50 – 1.57) | 4.39 (3.73 – 5.30) | 5.45 (3.69 – 9.60) | 0.84 (-0.35 – 3.82)† | 0.35 |
| NMD | % | 17.1 (9.2 – 21.3) | 16.2 (11.5 – 22.3) | -0.61 (-3.61 – 2.33) | 15.5 (12.6 – 21.5) | 19.6 (16.3 – 21.7) | 2.36 (-2.20 – 5.90) | 0.89 |

Data are Median (IQR)

* Between group difference at 48 weeks

† Within group change from baseline p<0.05

IMT, intima media thickness; TPA, total plaque area; FMD, flow-mediated dilation; NMD, nitroglycerin-mediated dilation.

Table 4.4 Metabolic parameters at baseline and following 48 weeks of treatment with rosiglitazone or placebo

| Variable | Unit | Placebo | | | Rosiglitazone | | | p * |
|----------|--------|--------------------------|--------------------------|---------------------------|--------------------------|--------------------------|-----------------------------|--------------|
| | | Baseline | 48 Weeks | Change | Baseline | 48 Weeks | Change | |
| HbA1c | % | 5.00 (4.85 – 5.10) | 5.10 (4.85 – 5.35) | 0.2 (-0.1 – 0.5) | 5.00 (4.85 – 5.20) | 4.90 (4.73 – 5.18) | 0.15 (-0.1 – 0.2) | 0.13 |
| Insulin | pmol/L | 124.5 (79.5 – 227.0) | 105.5 (60.8 – 207.0) | 2.5 (-56.2 – 9.5) | 101.0 (53.5 – 133.0) | 86.0 (47.0 – 124.5) | -10.0 (-29.5 – 33.5) | 0.26 |
| CRP | mg/L | 2.0 (1.0 – 4.9) | 3.6 (0.9 – 7.6) | 0.5 (-1.1 – 4.8) | 1.2 (0.8 – 3.8) | 0.8 (0.5 – 1.9) | -0.3 (-2.3 – -0.5)† | 0.003 |
| Adipo | mg/ml | 2.1 (0.6 – 4.2) | 2.4 (0.8 – 5.7) | 0.5 (-0.3 – 1.5) | 3.9 (1.4 – 5.0) | 7.3 (3.4 – 17.9) | 4.3 (1.6 – 12.1)† | 0.01 |
| RBP-4 | mg/L | 59.7 (39.1 – 63.1) | 58.2 (41.9 – 74.6) | 1.2 (-6.6 – 15.6) | 74.9 (56.7 – 84.0) | 57.0 (45.5 – 70.4) | -18.8 (-30.8 – -3.2) | 0.82 |
| MCP-1 | pg/ml | 618.7 (419.7 – 744.5) | 538.3 (396.3 – 657.7) | -75.1 (-217.6 – 132.0) | 503.4 (428.0 – 736.4) | 455.4 (382.2 – 489.8) | -100.8 (-157.0 – -11.6)† | 0.04 |
| IL-6 | pg/mL | 3.1 (1.6 – 4.2) | 3.1 (1.5 – 6.1) | 1.9 (0.2 – 3.5) | 1.9 (1.0 – 3.1) | 1.4 (0.9 – 2.2) | -0.15 (-0.84 – 0.42) | 0.04 |
| TC | mmol/L | 5.43 (4.43 – 5.92) | 5.49 (4.41 – 5.72) | -0.32 (-0.82 – 0.31) | 5.46 (4.93 – 6.45) | 5.86 (5.01 – 6.21) | 0.70 (-1.23 – 0.98) | 0.15 |
| HDL | mmol/L | 1.02 (0.74 – 1.28) | 1.01 (0.84 – 1.20) | 0.08 (-0.03 – 0.25) | 1.12 (0.84 – 1.25) | 1.03 (0.81 – 1.52) | -0.01 (-0.13 – 0.08) | 0.71 |
| TC:HDL | -- | 5.9 (5.0 – 7.0) | 5.1 (4.1 – 6.8) | -0.8 (-1.2 – 0.2) | 5.1 (4.4 – 6.7) | 5.1 (4.1 – 6.8) | 0.3 (-0.8 – 1.0) | 0.61 |
| LDL | mmol/L | 2.77 (1.96 – 3.95) | 3.03 (2.29 – 3.41) | 0.24 (-0.38 – 0.78) | 2.91 (2.14 – 3.68) | 2.66 (2.15 – 3.59) | 0.22 (-0.72 – 1.15) | 1.00 |
| ApoB | g/L | 1.06 (0.96 – 1.24) | 1.12 (0.89 – 1.28) | -0.03 (-0.14 – 0.14) | 1.09 (0.91 – 1.37) | 1.16 (0.92 – 1.28) | 0.01 (-0.23 – 0.19) | 0.73 |
| TG | mmol/L | 3.38 (2.19 – 5.51) | 2.66 (1.88 – 3.99) | -0.61 (-1.75 – 0.36) | 3.19 (2.89 – 4.03) | 3.92 (1.92 – 4.53) | 0.29 (-1.21 – 1.61) | 0.23 |
| AIP | -- | 0.54 (0.36 – 0.87) | 0.38 (0.20 – 0.65) | -0.14 (-0.29 – 0.05) | 0.48 (0.40 – 0.63) | 0.57 (0.18 – 0.79) | 0.08 (-0.24 – 0.20) | 0.55 |

Data are median (IQR)

*Between group difference at 48 weeks

†Within group change from baseline p<0.05

HbA1c, hemoglobin A1C; CRP, C-reactive protein; RBP-4, retinol-binding protein-4; MCP-1, monocyte chemotactic protein-1; IL-6, interleukin-6; TC, total cholesterol; HDL, high-density lipoprotein cholesterol; TC:HDL total cholesterol to high-density lipoprotein cholesterol ratio; LDL, low-density lipoprotein cholesterol; ApoB, apolipoprotein B; TG, triglycerides; AIP, atherogenic index of plasma.

Table 4.5 Anthropometric and body composition variables at baseline and following 48 weeks of treatment with rosiglitazone or placebo

| Variable | Unit | Placebo | | | Rosiglitazone | | | p * |
|-----------|-------------------|--------------------|--------------------|----------------------|--------------------|--------------------|----------------------|------|
| | | Baseline | 48 Weeks | Change | Baseline | 48 Weeks | Change | |
| Weight | kg | 75.0 (67.0 – 87.4) | 75.6 (69.1 – 87.9) | 2.3 (-1.8 – 6.4)† | 77.0 (67.8 – 86.5) | 79.5 (68.9 – 86.0) | 0.0 (-2.3 – 3.3) | 0.93 |
| BMI | kg/m ² | 25.0 (21.5 – 28.1) | 25.2 (22.4 – 27.9) | 0.8 (-0.7 – 2.6) | 26.5 (23.0 – 29.5) | 27.2 (23.1 – 28.5) | 0.0 (-0.8 – 1.1) | 0.72 |
| Waist/Hip | ratio | 1.03 (1.01 – 1.07) | 1.04 (1.02 – 1.08) | -0.12 (-0.02 – 0.02) | 1.05 (1.00 – 1.09) | 1.02 (1.00 – 1.04) | -0.01 (-0.02 – 0.02) | 0.06 |
| Total fat | kg | 15.5 (11.6 – 22.7) | 17.7 (12.7 – 23.0) | 0.2 (-0.5 – 2.9) | 12.9 (10.6 – 21.0) | 14.6 (12.3 – 22.2) | 1.3 (-0.4 – 2.3) | 0.59 |
| Trunk fat | kg | 9.4 (6.7 – 13.4) | 10.2 (6.5 – 14.7) | 0.4 (0.02 – 1.6)† | 7.5 (6.0 – 13.7) | 7.9 (7.0 – 14.0) | 0.7 (-0.8 – 1.5) | 0.13 |
| Limb fat | kg | 5.6 (3.6 – 6.7) | 5.9 (3.7 – 6.8) | 0.0 (-0.4 – 0.6) | 4.4 (3.1 – 7.5) | 5.5 (3.7 – 7.3) | 0.4 (-1.0 – 0.9) | 0.50 |
| Head fat | kg | 0.83 (0.72 – 0.97) | 0.86 (-0.71 – 1.0) | 0.04 (0.005 – 0.1)† | 0.87 (0.3 – 1.09) | 0.90 (0.82 – 1.0) | 0.0 (-0.1 – 0.06) | 0.23 |
| Body fat | % | 21.3 (16.8 – 26.4) | 21.9 (18.8 – 26.0) | 0.4 (-1.3 – 3.3) | 19.1 (14.0 – 25.3) | 18.8 (15.5 – 25.5) | 1.3 (-0.7 – 2.7) | 0.91 |
| Lean mass | kg | 56.9 (48.4 – 64.6) | 56.3 (49.8 – 63.0) | 0.2 (-2.0 – 2.1) | 56.6 (51.4 – 66.0) | 55.0 (51.0 – 64.8) | -0.5 (-2.0 – -0.02)† | 0.11 |

Data are Median (IQR)

* Between group difference at 48 weeks.

† Within group change from baseline p<0.05.

BMI, body mass index.

Table 4.6 Safety variables at baseline and following 48 weeks of treatment with rosiglitazone.

| Variable | Unit | Placebo | | | Rosiglitazone | | | p* |
|-------------|-----------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-------------|
| | | Baseline | 48 Weeks | Change | Baseline | 48 Weeks | Change | |
| AST | U/L | 26 (15 – 30) | 32 (26 – 38) | 3 (-4 – 11) | 28 (23 – 39) | 32 (27 – 38) | 3 (-4 – 11) | 0.17 |
| ALT | U/L | 44 (29 – 70) | 33 (25 – 43) | -5 (-23 – 2) | 38 (25 – 44) | 36 (25 – 47) | -2 (-8 – 10) | 0.84 |
| Hemoglobin | g/L | 148 (140 – 158) | 134 (125 – 148) | -2 (-5 – 4) | 146 (136 – 157) | 134 (125 – 147) | -11 (-15 – -3)† | 0.02 |
| Bilirubin | µmol/L | 14 (8 – 19) | 16 (7 – 24) | 5 (-6 – 11) | 11 (7 – 14) | 12 (8 – 23) | 4 (-5 – 13) | 0.55 |
| CD4% | % | 20 (17 – 25) | 24 (17 – 30) | 4 (1 – 5)† | 21 (18 – 25) | 24 (22 – 27) | 4 (2 – 5)† | 0.81 |
| CD4 count | cells/mm ³ | 320 (240 – 613) | 500 (298 – 720) | 100 (20 – 160)† | 510 (420 – 620) | 470 (375 – 630) | -20 (-95 – 45) | 0.88 |
| Viral load‡ | number | 22 | 21 | -1 | 17 | 15 | -2 | -- |

Data are median (IQR)

* Between group difference at 48 weeks.

† Within group change from baseline p<0.05.

‡ <50 copies per mL.

AST, asparate transaminase, ALT, alanine transaminase.

Chapter 5: Pharmacological Treatment of HIV-associated

Dyslipidemia

With the advances in antiretroviral (ARV), therapy the prognosis of patients infected with HIV is improving. Accompanied by the increase in survival is an associated increase in the prevalence of cardiovascular disease (CVD) and its complications in the HIV-positive (HIV+) population. Although the reason for this appears to be multifactorial, a large proportion of the attributable risk is likely secondary to the dyslipidemia associated with HIV and with the ARV drugs used to treat it [17, 332, 333].

The dyslipidemia guidelines for the non-HIV population, which were updated in 2009, suggest that the choice of lipid-lowering therapy depends on the degree and type of dyslipidemia [30]. According to the guidelines, dyslipidemia featuring primarily an elevation in cholesterol (total cholesterol [TC] and/or low-density lipoprotein-cholesterol [LDL]) should be treated with an HMG-CoA reductase inhibitor (statin). Pravastatin, atorvastatin and fluvastatin are used for treatment of highly-active antiretroviral therapy (HAART)-related dyslipidemia as they pose a lower risk of pharmacological interaction with ongoing HAART [28, 130]. However, often the lipid goals of HIV+ patients are not achieved by the therapy recommended in the current lipid-lowering guidelines [28].

Rosuvastatin is a highly potent, third generation statin that is not metabolized by the cytochrome P450 (CYP)3A4 enzyme system, which is frequently inhibited by certain ARV drugs [138]. The lack of requirement of CYP3A4 metabolism for rosuvastatin makes it an attractive lipid-lowering drug to treat the dyslipidemia associated with HAART [334]. A limited number of studies have assessed the efficacy of rosuvastatin in patients with HIV [139, 144, 153, 154]. Presently, the pattern of practice in both patients

with and without HIV is to increase the rosuvastatin dose from 10 mg to 20 mg daily if the lipid targets are not met with the initial dose.

A novel treatment option for dyslipidemia in the HIV+ population is ezetimibe, which functions to block the intestinal absorption of dietary cholesterol and bile acid absorption at the intestinal brush border [335]. Ezetimibe has been effective in optimizing lipid levels when added to traditional therapy in non-HIV+ patients [167, 170]. In HIV+ patients, two retrospective studies have assessed the efficacy of ezetimibe with results indicating the positive lipid-lowering potential of ezetimibe both alone [170] and in combination with a statin [171]. Elucidating any reduced response to this potent statin would be of great clinical importance given the potential for wide use of rosuvastatin in this population.

5.1.1 Objectives

The study objectives listed here comprise three studies undertaken to determine the effectiveness of ezetimibe and rosuvastatin in HIV+ patients with dyslipidemia.

5.1.1.1 Effectiveness of Ezetimibe

The ability of ezetimibe to further optimize the serum lipid profile when added to maximally-tolerated lipid-lowering therapy will be examined. The proportion of patients not at target that reach lipid targets following addition of ezetimibe to ongoing therapy will be determined. The safety profile of these combinations will also be described.

5.1.1.2 Effectiveness of Rosuvastatin

The overall lipid-lowering effect and dose response of rosuvastatin will be evaluated as will the lipid-lowering effect of rosuvastatin among patients initiating different ARV therapy regimens.

5.1.1.3 Increased Dose of Rosuvastatin vs. Addition of Ezetimibe

In a prospective study we will seek to determine whether patients not reaching targets while on rosuvastatin 10 mg who are then treated with a combination of rosuvastatin plus ezetimibe show a greater improvement in their fasting lipid profile when compared to those treated with an increased dose of rosuvastatin. To our knowledge, this is a novel investigation within the HIV+ population.

5.2 Methods

5.2.1 Effectiveness of Ezetimibe

5.2.1.1 Study Design and Setting

A retrospective review was undertaken to determine the effectiveness of ezetimibe when added to ongoing lipid-lowering therapy in HIV+ patients. Patients attended the Immunodeficiency Clinic/HIV Metabolic Clinic (IDC/HIVMC) at St. Paul's Hospital in Vancouver, BC, Canada. This is a tertiary referral centre where over 700 patients receive care for HIV-associated metabolic disorders. Patients who were treated with ezetimibe during the time period January 2003 to May 2006 were included. Ezetimibe was initiated only after they were on the maximally-tolerated doses of standard lipid-lowering therapy, defined as the dosage of lipid-lowering therapy(s) that the attending physician deems as the threshold beyond which a potential adverse drug reaction is risked. Maximum therapeutic response for ezetimibe is reached between two to four weeks [336], accordingly patients who took ezetimibe for less than four weeks were excluded. Patients with no clearly ascertainable drug start date or one that was more than three months after

their most recent lipid profile were excluded. In addition, any patients who did not have a follow-up lipid profile within one year from initiating ezetimibe were excluded.

5.2.1.2 Outcomes

The effect of ezetimibe on the serum concentrations of TC, LDL, high-density lipoprotein-cholesterol (HDL), triglycerides (TG) and apolipoprotein B (ApoB) were analyzed. The most recent lipid profile from no more than three months prior to ezetimibe initiation served as baseline with follow-up being the most recent lipid profile more than four weeks but less than 12 months following drug start. In addition, adverse events, as defined by an elevation in aspartate transaminase (AST) or alanine transaminase (ALT) over 5 times upper limit of normal (ULN) or creatine kinase (CK) over 10 times ULN or any symptom requiring discontinuation, were documented. Sub-analysis included determination of the percentage of patients reaching lipid targets according to those set by the guidelines for management of HIV dyslipidemia [28]. The study was approved by the institutional Research Ethics Board.

5.2.1.3 Statistical Analysis

Univariate analysis was conducted to compare the differences between baseline and follow-up laboratory values using the Wilcoxon rank sum test for paired non-parametric samples. SPSS version 14.0 was used to compare primary outcomes using independent sample t-tests or Wilcoxon tests between-group changes and paired sample t-tests or Wilcoxon test for within-group changes.

5.2.2 Effectiveness of Rosuvastatin

5.2.2.1 Study Design and Setting

A retrospective analysis was undertaken to determine the effect of rosuvastatin on the lipid profile of patients with HIV. All patients were seen at IDC/HIVMC. Patients who were treated with rosuvastatin between January 2003 and July 2006 and had both a baseline and follow-up lipid profile were included in the analysis. Maximum therapeutic response for rosuvastatin is reached between four to six weeks [337], accordingly patients who took rosuvastatin for less than four weeks were excluded. Patients with no clearly ascertainable drug start date or one that was more than three months after their most recent lipid profile were excluded. In addition, any patients who did not have a follow-up lipid profile within one year from initiating ezetimibe were excluded. Primary analyses compared doses of rosuvastatin monotherapy (10 or 20 mg), and effectiveness of rosuvastatin alone or in combination with other lipid-lowering medications (fenofibrate, ezetimibe). Due to small numbers, patients taking rosuvastatin 5 mg (n=1) or 40 mg (n=1) were not included in this dose-response analysis.

All relevant concomitant medications were recorded. These included other lipid-lowering therapies, namely cholesterol transport blockers (ezetimibe) and/or fibrates (fenofibrate) as well as each patient's current ARV regimen, simplified to either regimens containing PIs or those not containing PIs (non-PI). Any previous use of statins was also documented.

5.2.2.2 Outcomes

The primary outcomes were the effects of rosuvastatin on serum concentrations of TC, LDL, HDL, TG and ApoB. The most recent lipid profile from no more than three months

prior to rosuvastatin initiation served as baseline with follow-up being the most recent lipid profile more than four weeks but less than 12 months following drug start. Adverse events, as indicated by ALT or AST elevations five times ULN or CK ten times ULN or any other condition requiring discontinuation of the drug, were recorded.

5.2.2.3 Statistical Analysis and Ethics

Student's t-test for related samples was used to compare baseline and follow-up values among all patients initiating rosuvastatin. Differences between subgroups were analyzed using independent sample t-tests. Within group differences were calculated using paired sample t-tests. The study was approved by the institutional Research Ethics Board.

5.2.3 Increased Dose of Rosuvastatin vs. Addition of Ezetimibe

5.2.3.1 Study Design

This was a prospective, randomized, open-label study comparing the effect of the combination of rosuvastatin and ezetimibe to an increased dose of rosuvastatin on the lipid profile of HIV+ patients.

Serum samples were be obtained and tested for TC, TG, LDL, HDL, ApoB, apolipoprotein A1 (ApoA1), C-reactive protein (CRP), AST, ALT, CK, creatinine and fasting blood glucose. Participants were then randomized to one of two groups. One group had their dose of rosuvastatin increased to 20 mg once daily and the other continued to take rosuvastatin 10 mg once daily and had 10 mg ezetimibe once daily added to their medication regimen.

Following three months of treatment, serum samples were again obtained and analyzed for the same apolipoprotein (ApoB, ApoA1), lipid (TC, TG, LDL, HDL), safety (AST, ALT, CK, creatinine), metabolic (fasting blood glucose) and inflammatory (CRP)

parameters as at baseline. This testing regimen is in keeping with standard medical care and does not include any extra phlebotomies. At the conclusion of the study (approximately 12 weeks) participants will be asked a series of questions regarding side effects (including myalgias and gastrointestinal side effects).

5.2.3.2 Outcomes

Apolipoprotein B, the major lipoprotein component of LDL whose measure is not affected by TG and serves as a measurement of LDL particle number was chosen as the primary endpoint of the study. Specifically, the difference in final value of serum apolipoprotein B between participants treated with rosuvastatin versus participants treated with both rosuvastatin and ezetimibe were used as primary endpoints. Secondary endpoints included the percent change in apolipoprotein B, as well as difference in percent and absolute change in serum concentrations of total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, total cholesterol to HDL ratio, non-HDL cholesterol, apolipoprotein A1, apolipoprotein B to apolipoprotein A1 ratio, and CRP between participants treated with rosuvastatin versus participants treated with both rosuvastatin and ezetimibe. Further secondary endpoints included within-group changes in the above endpoints and the assessment of safety parameters, specifically incidence of complications as measured by an increase in AST and/or ALT ≥ 3 -fold ULN and a CK ≥ 10 -fold ULN in participants treated with rosuvastatin versus participants treated with both rosuvastatin and ezetimibe.

5.2.3.3 Randomization

A list of computer-generated treatment allocation codes were supplied by a statistical programmer unassociated with the study. No study personnel had access to the list.

When a participant was deemed eligible and consenting, the site coordinator contacted the study pharmacist. The study pharmacist then provided an authorization code that permitted access to a web page, which issued the study treatment allocation. The randomization employed blocks of random size 4,6 or 8 to minimize the ability of the coordinator to guess the next treatment allocation.

5.2.3.4 Eligibility

Patients who were on a stable HAART regimen with abnormalities in their fasting lipid profile (ApoB >0.90 g/L) and were currently taking 10 mg rosuvastatin were contacted by the research team. They were then screened further to ensure that all of the inclusion criteria are met and that none of exclusion criteria apply. If these criteria were met and the patient consented to be included in the trial they were enrolled in the study.

5.2.3.4.1 Inclusion Criteria

Participants were included in the study if they provided signed, witnessed informed consent prior to study entry and they met all of the following criteria: aged 19 or older; known to have HIV-1 infection; taking 10 mg of rosuvastatin; lipid profile with a serum apolipoprotein B is >0.9 g/L; taking three or more ARV agents at unchanged doses for the previous three months and anticipated that these were to continue at the same dose for the subsequent three months and if taking any of the following medications, were on a stable dosage for one to six months prior to study initiation: testosterone replacement [e.g Depo-Testosterone, Delatestryl, Andriol, AndroGel), rosiglitazone (Avandia) or pioglitazone (Actos) and fish oil (including salmon oil).

5.2.3.4.2 Exclusion Criteria

Participants were excluded from the study if meeting any of the following conditions: any previous adverse reaction to ezetimibe or had taken ezetimibe within 30 days of starting the study; previously had an allergic reaction or muscle problems while taking any statin; had uncontrolled high blood pressure; were currently taking gemfibrozil (Lopid), niacin/nicotinic acid, colestipol (Colestid), cholestyramine (Novo-Cholamine), any agent with a potential drug-drug interaction as listed on pages 27-28 of the ezetimibe (Ezetrol) product monograph or the following post-transplant immune suppressants: cyclosporine (Neoral, Sandimmune), tacrolimus (Protopic, Prograf), lymphocyte immune globulin (Atgam), rho(d) immune globulin, azathioprine sodium (Imuran), muromonab-CD3 (Orthoclone OKT 3); patients of child bearing potential or who are sexually active (male or female), and do not agree to avoid pregnancy (subject or partner); Females who were pregnant, breast-feeding or expecting to conceive or donate eggs during the study; males that were to impregnate a woman or provide sperm donation during the study; use of excessive amounts of alcohol or recreational drugs; previous or current liver disease; serum AST or ALT elevations ≥ 3 -fold ULN and serum CK concentration elevation ≥ 10 -fold ULN.

5.2.3.5 Sample Size and Power Calculation

The planned sample size for this study was 50 participants. The type 1 error was set at 0.05 and type 2 error was 0.1 (to achieve 90% power). A standard deviation of 0.27g/L in apolipoprotein B from similar patient populations and previous studies involving ezetimibe was assumed [338]. Based on the literature, a between-group difference in ApoB of 0.25 g/L is achievable when adding ezetimibe to a lipid-lowering medication

regimen that includes a statin [338] From our previous work, we ascertained there was a negligible difference between 10 mg and 20 mg doses of rosuvastatin [172]. According to these assumptions, this study was adequately powered to detect such a change with a sample size of 50 patients.

5.2.3.6 Statistical Analysis

Data are expressed as mean \pm standard deviation (SD). Assumptions of normality were tested by Shapiro-Wilk tests and review of plots. The primary analysis was to compare treatment effects between groups. For variables with a symmetrical distribution, we used analysis of covariance (ANCOVA) adjusted for baseline values. For variables with skewed distributions, between-treatment changes from baseline were compared with Mann-Whitney U tests. The secondary analysis was to compare changes within each group with paired *t* tests or Wilcoxon tests, as appropriate. Calculations were performed using computer software (SPSS/Mac v. 19.0.0).

Any patients who did not return for the 12-week visit were excluded from the analysis except those subjects who have withdrawn for reason of intolerance or toxicity. These patients were considered not to have changed from baseline if they do not appear for their follow-up visit. The proportion of patients who have experienced complications as measured by an increase in AST and ALT \geq 3-fold ULN or a CK \geq 10-fold ULN will be compared between treatment groups using the Fisher's exact test.

5.3 Results

5.3.1 Effectiveness of Ezetimibe

Forty patients seen in the IDC-HIV Metabolic Clinic had been prescribed ezetimibe 10 mg per day orally between January 2003 and May 2006. Seven of these patients were not

included in the retrospective analysis (two had a statin added within the first four weeks of ezetimibe therapy, three took ezetimibe for less than four weeks [discontinued due to reasons other than adverse events such as financial constraints] and two had insufficient data) leaving 33 patients for analysis. Relevant demographics of these patients are displayed in Table 5.1.

The serum TGs of eight patients were significantly elevated precluding the calculation of serum LDL concentration. Only seven of the patients had ApoB measurements both before and after the initiation of ezetimibe.

5.3.1.1 Overall Effect of Addition of Ezetimibe

Addition of ezetimibe to all maximally-tolerated lipid-lowering therapies resulted in significant improvements in each of the lipid outcomes (Figure 5.1).

5.3.1.2 Ezetimibe in Combination with Other Lipid-lowering Therapies

Patients were compared according to baseline lipid-lowering therapy (figure 5.2). When ezetimibe was added to maximally tolerated doses of a statin and a fibrate, significant improvements were seen in serum concentrations of TC (16%, 95% confidence interval [CI] 18 – 24, p=0.004), TG (21%, 95% CI 5 – 38 , p=0.02), LDL (24%, 95% CI 11 – 37, p=0.008), HDL (21%, 95% CI 0 – 43 p=0.04) and TC:HDL ratio (27%, 95% CI 15 – 29, p=0.01) over a mean follow-up time of 79.6 days (range 37-193). Ezetimibe added to maximally tolerated doses of statin monotherapy yielded significant improvements TC (31%, 95% CI 21 – 41, p=0.001), TG (25%, 95% CI 13 – 63, p=0.03), LDL (42%, 95% CI 27 – 57, p=0.008) and TC:HDL ratio (36%, 95% CI 21 – 46, p=0.002). Ezetimibe in combination with maximally tolerated doses of fibrate monotherapy resulted in a significant decreases in serum LDL (20%, 95% CI 7 – 33, p=0.04) and TC:HDL ratio

(17%, 95% CI 1 – 32, p=0.04). No significant improvements were seen in ApoB, possibly related to small sample size for this parameter (n=8).

5.3.1.3 Patients Reaching Lipid Targets

An important facet of treating HIV patients with lipid-lowering therapy is attaining lipid targets. We determined the percentage of patients who reached lipid targets for moderate and high cardiovascular risk following addition of ezetimibe to maximally-tolerated lipid-lowering therapy (Figure 5.3). The greatest improvements were seen when ezetimibe was added to ongoing statin therapy or statin in combination with a fibrate.

5.3.2 Effectiveness of Rosuvastatin

A total of 161 patients seen in the IDC/HIVMC had been prescribed rosuvastatin in dosages of 5, 10, 20 or 40 mg per day orally between January 2003 and May 2006. Fourteen patients were excluded from the retrospective analysis because the exact start date of rosuvastatin could not be determined and an additional 17 were excluded because they lacked sufficient data for analysis leaving 130 patients for analysis. Five patients discontinued rosuvastatin therapy due to adverse events (three due to elevated liver enzymes and two due to complaints of muscle soreness). For these patients, the most recent lipid parameters measured prior to discontinuation were used in study analyses, as they remained on the medication for longer than 4 weeks prior to being discontinued.

The serum TGs of 33 patients were elevated beyond the limit for accurate calculation of serum LDL concentration.

Relevant demographics of patients included in the analysis are found in Table 5.2.

5.3.2.1 Lipids Effects

Overall, there was a significant, beneficial effect of rosuvastatin on lipid parameters (mean [95%CI]) including: TC (-1.66 [$-1.34 - -1.98$] mmol/L, $p<0.001$), TG (-1.57 [$-0.82 - -2.31$]) mmol/L, $p<0.001$), LDL (-1.00 [$-0.75 - -1.25$] mmol/L, $p<0.001$) and ApoB (-0.27 [$-0.21 - 0.39$] g/L, $p<0.001$). Mean follow-up time on rosuvastatin was 131 days (range 30 – 357). Mean TC:HDL decreased 2.17 ([95% CI $-1.70 - -1.63$], $p<0.001$) driven by the drop in TC, while a non-significant increase in mean HDL of 0.01 ([95% CI $-0.04 - 0.07$) mmol/L was observed ($p=0.67$).

5.3.2.2 Dose-response Analysis

A dose-response analysis was conducted wherein patients on rosuvastatin monotherapy ($n=68$) were subdivided according to initial rosuvastatin dosage to either 10 mg ($n=45$) or 20 mg ($n=23$) groups. Of the patients initiating therapy with 10 mg rosuvastatin, 33 (48.5%) were statin-naïve compared to only one (0.4%) patient that began rosuvastatin therapy with a 20 mg dose.

Both groups saw significant within-group improvements in TC, TG, LDL and TC:HDL ratio. ApoB was significantly decreased in the 10 mg group only. HDL did not improve in either group. The 10 mg subgroup showed greater improvements across all lipid parameters compared to the 20 mg subgroup, with the exception of HDL (Figure 5.4). Only change in mean \pm SD TC:HDL ratio reached borderline significance (-2.14 ± 2.18 for 10 mg vs. -1.10 ± 1.67 for 20 mg, $p=0.05$).

5.3.2.3 Combination Therapy vs. Monotherapy

Secondary analysis examined the effectiveness of a regimen consisting of rosuvastatin as the sole lipid-lowering medication (monotherapy, $n=70$) vs. having rosuvastatin added to

an ongoing fenofibrate regimen (combination therapy, n=42). Of these patients, 34 from the monotherapy group (48.6%) were statin naïve and 36 (51.4%) had switched to rosuvastatin. In the combination group, 16 (38.1%) were statin naïve and 26 (61.9%) had switched to rosuvastatin. None of the patients in the combination group had been on previously been on rosuvastatin monotherapy.

Between-group comparisons revealed a greater improvement in TG and TC in patients treated with combination therapy compared to those on monotherapy (Figure 5.5). Additionally, the monotherapy group showed a mean±SD decrease in HDL of 0.05 ± 0.37 mmol/L compared to a mean±SD) increase of 0.07 ± 0.27 mmol/L in the combination therapy group but only borderline significance was attained (p=0.08). All lipid parameters in both groups showed an improvement from baseline following initiation of rosuvastatin therapy with exception of HDL in the monotherapy group.

5.3.2.4 Effect of Protease Inhibitors on Rosuvastatin

A sub-analysis was conducted to determine any difference in rosuvastatin's effect on lipid outcomes between statin-naïve patients currently receiving PI-based HAART and those not taking PIs. The study did not reveal any significant differences between the groups for any lipid endpoints (Figure 5.6). There was a large discrepancy in size of the non-PI group (n=13) as compared to the PI group (n=63).

5.3.3 Increased Dose of Rosuvastatin vs. Addition of Ezetimibe

5.3.3.1 Overall Effects of Treatments

Due to slow enrollment, the analysis presented here includes 39 patients that were enrolled and completed the 12-week study between May 2010 and September 2011. There were no differences at baseline between groups in terms of age or clinical

characteristics of HIV (Table 5.3). However, despite randomization, there were some observable differences between groups in terms of cardiovascular risk factors. More patients receiving ezetimibe had a history of hypertension and more were current smokers, whereas more patients in the rosuvastatin group self-identified as past smokers (quit within last 12 months). The overall effects of patients receiving either an increased dose of rosuvastatin or ezetimibe in addition to rosuvastatin on lipid and metabolic parameters are displayed in Table 5.4. Both treatments significantly lowered TC, LDL and TC:HDL ratio. Addition of ezetimibe resulted in additional improvements in TG and AIP. No between-group differences were found, with only differences in TC and TG approaching significance ($p=0.06$ and 0.09 , respectively). These differences are potentially clinically important as 0.94 (95% CI $0.56 - 1.33$) mmol/L drop in TC was observed in the ezetimibe group vs. a 0.47 (95% CI $0.15 - 0.80$) mmol/L decrease in the increased dose group. For TG the reduction seen with the addition of ezetimibe more than tripled (-0.6 [95% CI $-0.26 - -0.94$] mmol/L vs. that seen with an increased dose (0.16 [95% CI $-0.48 - 0.17$] mmol/L).

Two patients, both in the increase dose of rosuvastatin group, experienced myalgias one of which suffered moderate to severe cramping. Neither discontinued the study medication due to these events. A statistically significant increase in ALT was observed in the ezetimibe treatment group but this increase was small and not likely to be clinically relevant.

5.3.3.2 Effect on Apolipoproteins

Due to low enrollment and inconsistency between screening and baseline levels of ApoB, patients with a baseline ApoB above target for high-risk subjects (>0.80 g/L) [30] were

included in analysis of the primary outcome. Both treatments significantly improved Apolipoprotein B compared to baseline but no difference was observed between groups (Figure 5.7). Both groups showed a trend toward improvement of the ApoB to ApoA1 (ApoB:ApoA1) ratio but the between-group difference did not reach significance ($p=0.09$). Initially, a significant decrease in apolipoprotein A1 was observed in the ezetimibe group but this effect disappeared when analysis was limited to patients with ApoB >0.80 g/L.

5.3.3.3 Patients Reaching Target Endpoints

Sub-analysis was conducted to determine the proportion of patients reaching primary (LDL <2.0 mmol/L, ApoB <0.80 g/L) and secondary (TC:HDL ratio <4.0 , TG <1.7 mmol/L, ApoB:ApoA1 ratio <0.80 , CRP <2 mg/L) high-risk targets for cardiovascular risk as determined by the current Canadian Cardiovascular Guidelines [30] as well as the high risk limit for AIP (<0.21) [227] (Figure 5.8A and B). Both treatments resulted in an increased proportion of patients reaching primary and secondary, targets relative to baseline, with the exception of CRP, which demonstrated no further improvements in the ezetimibe group.

5.4 Discussion

5.4.1 Effectiveness of Ezetimibe

In our retrospective analysis of 33 HIV+ patients, we observed significant reductions in serum concentrations of TC, TG, LDL and ApoB following the addition of ezetimibe to ongoing lipid-lowering therapy. Serum concentrations of HDL also rose significantly following this intervention. These gains were achieved without any adverse events. Our

findings are concordant with studies that have assessed the efficacy of ezetimibe in non-HIV populations [166, 167].

Only two studies other have described the use of ezetimibe in HIV+ patients. One study compared ezetimibe monotherapy to fluvastatin monotherapy in 20 HIV+ patients and reported a 20% reduction in LDL [170]. As standard practice was to add ezetimibe to lipid-lowering therapy, only patients intolerant of statins were on ezetimibe monotherapy. LDL was lowered by 32% but this was non-significant owing to the small subgroup size. The second study added ezetimibe to ongoing statin therapy in 19 patients not at target [171]. This study succeeded in demonstrating the lipid-lowering effect of adding ezetimibe to statin therapy, with 61.5% [95% CI 36 – 80] of patients achieving LDL target (<3.36 mmol/L) that was not reached with pravastatin alone. Comparatively, there were 14 patients in our study on statin therapy who had a serum LDL concentration >3.36 mmol/L. Following addition of ezetimibe, nine (64.3% [95% CI 29 – 84]) of these patients achieved an LDL < 3.36 mmol/L.

In our centre, in the absence of consensus guidelines for the treatment of dyslipidemia in patients with HIV, we currently treat HIV+ patients to at least moderate risk lipid profile targets (TC \leq 5.0 mmol/L, LDL \leq 3.5 mmol/L and TC:HDL ratio \leq 5.0). Prior to ezetimibe therapy, none of the patients met all targets despite maximally tolerated lipid-lowering therapy. Following the addition of ezetimibe, TC targets were reached in 42% [95% CI 27 – 59] (up from 0%), LDL was reached in 88% [95% CI 70 – 96] of patients (up from 24% [95% CI 11 – 43]) and 61% [95% CI 44 – 75] reached TC:HDL ratio the target (up from 12% [95% CI 5 – 27]). Sub-analysis further categorized patients according to concurrent lipid-lowering therapy (Figure 5.2), which demonstrated that further

improvements in lipid targets were possible when adding ezetimibe to statin therapy and even therapy with a statin and a fibrate.

5.4.2 Effectiveness of Rosuvastatin

While this study found that rosuvastatin is indeed effective at improving potentially atherogenic lipid parameters in HIV+ patients, an emerging theme from our results is an apparent resistance to therapy in our study population relative to previous studies involving non-HIV+ patients. Previous studies have shown statins and fibrates to be less effective in people with HIV compared to healthy subjects indicating possible resistance to lipid-lowering therapy inherent to the virus itself or HAART [160, 339]. As rosuvastatin has previously been shown to be effective in non-HIV populations both with and without the metabolic syndrome, when compared to other statins [340-347], we expected an improvement of a similar magnitude but this was not observed in our study.

One large randomized clinical trial examining the effectiveness of rosuvastatin at improving plasma lipids, in a non-HIV population, in comparison to other statins noted improvements of -33.6% for TC, -46.7% for LDL +9.3% for HDL and -23.4% for TG from baseline in patients with metabolic syndrome taking rosuvastatin (n=240) [160]. By comparison, in our study we saw much more modest effects on lipids with changes from baseline of -25.1% for TC, -31.5% for LDL, -4.1% for HDL and TG -21.3% for TG with rosuvastatin monotherapy (n=70). In addition, observational analysis of dose response (Figure 5.2) revealed little very difference between the 10 and 20 mg doses of rosuvastatin on lipid endpoints. This further supports the possibility of an underlying resistance to statin therapy in HIV+ patients.

At publication time, the only other study examining the effects of rosuvastatin in HIV+ patients also showed improvements that were of lesser magnitude than expected from this highly potent statin. In their small trial (n=16), Calza et al. randomized HIV+ patients to rosuvastatin or placebo for 24 weeks and reported median changes from baseline of -21.7% (TC), -22.4% (LDL) and -30.1% (TG), along with a 28.5% median increase in HDL [139]. These results are similar to ours with the exception of the drastic difference in change in HDL. The lack of effect a clinically important effect (-0.012 [95% CI -0.045 – 0.070] mmol/L) of rosuvastatin on HDL is a novel finding as, to date, no trials have reported significant decreases in HDL with rosuvastatin. Conversely, trials in both HIV and non-HIV populations have observed a positive effect on HDL with rosuvastatin [139, 340]. The lack of effect on HDL cannot be readily explained, but may be related to an underlying dyslipidemia induced by HIV itself. Since publication, two additional trials have assessed effectiveness of rosuvastatin. A second study by Calza et al. compared the effectiveness of three statins (rosuvastatin, atorvastatin and pravastatin) in HIV+ patients taking PIs. While rosuvastatin was shown to be the most effective in improving lipid endpoints, again improvements were less than expected.

Resistance to statin therapy is not unique to rosuvastatin, as evidenced by studies examining the effects of pravastatin in the HIV+ population. These studies observed no better than a 20.4% decrease in LDL in HIV+ patients compared to a 32.7% decrease in the general population [134, 348, 349]. In both studies, the treatment group was randomized to 40 mg of pravastatin.

PI-containing HAART is often as a causal factor in development of HIV-related dyslipidemia and lipodystrophy [6, 17, 79] and metabolism of these agents may play a

role in the resistance to therapy observed here. A sub-analysis was conducted wherein statin-naïve patients were stratified according to their current HAART regimen. A similar pattern to that seen in the whole cohort was observed wherein within-group change in lipid parameters was less pronounced in both the PI and no-PI groups. Improvements in LDL in the PI group (mean [SD] change of -1.34 (1.40) mmol/L) were less pronounced than that seen in study cohort as a whole. However, there were no significant differences between groups for any lipid endpoints, likely owing to the small number of patients on PI-sparing regimens (n=14).

Other possible explanations for the lack of effect rosvastatin shown in our study include interaction with antacids, reduced compliance and inability to afford the medication. These explanations, while valid in certain populations, are unlikely for the present study as all patients at our clinic are advised against taking antacids by the resident pharmacist. Furthermore, the pharmacist reviews each patient's drug compliance every three months and has found that, generally, patients in our clinic are very compliant. Finally, in this centre, the cost of rosvastatin is covered by the provincial drug formulary and lack of financial resources should not play a role in patient compliance.

The most recent guidelines for dyslipidemia in patients on ARV therapy recommend diet and exercise counselling, alteration of ARV regimen or adding lipid-lowering medications [28]. A statin is suggested for LDL elevations and a fibrate is suggested for serum triglycerides elevation. Secondary analysis assessed patients on combination therapy, specifically rosvastatin with fenofibrate (Figure 2). This combination was more successful in improving in both TG (45.3% vs. 23.4%, $p<0.05$) and TC:HDL ratio (33.9% vs. 26.0%, $p<0.05$) as compared to the monotherapy group, but the latter was

driven by a non-significant increase in HDL in the combination. Despite not reaching significance, this increase in HDL in the combination group may warrant further investigation as there have been few studies that have shown increases in HDL in HIV+ patients [154].

In view of the possibility of resistance to rosuvastatin therapy in the HIV+ population, combination therapy should be investigated. The combination of rosuvastatin and fenofibrate is common practice in our clinic, however, and has been moderately successful (as demonstrated here), but improvements have still not matched those of non-HIV studies[350] and more effective combinations should be explored. One such treatment option is the combination of rosuvastatin with ezetimibe. This combination is promising because it will provide two forms of cholesterol reduction, as ezetimibe blocks absorption of cholesterol in addition to the lowering of endogenous cholesterol with a statin. In addition, our preliminary data (Figures 5.2 and 5.3) and one study demonstrating the effectiveness of ezetimibe monotherapy[178] indicate a potential role for this combination in HIV+ patients.

5.4.3 Increased dose of Rosuvastatin vs. Addition of Ezetimibe

This is the first study to investigate the efficacy of rosuvastatin in combination with ezetimibe in HIV+ patients. This is also the first study of any kind to use a recommended dose of rosuvastatin (10 mg) in combination with ezetimibe in comparison with an increased dose of rosuvastatin (20 mg).

Overall, the primary endpoint of ApoB was lowered by 0.12 g/L (11.6%) in the rosuvastatin 20 mg group and by 0.19 g/L (17.5%) in ezetimibe with rosuvastatin group. Both improvements were significant compared to baseline values but the 6% greater

reduction seen with combination therapy was not significantly different from that seen with rosuvastatin alone. A similar trend was observed for secondary lipid endpoints wherein improvement was seen with both treatments. These included TC, which dropped 0.91 mmol/L (17.3%) in the combination group and 0.47 mmol/L (9.4%) in the increased dose group; LDL decreased 0.64 mmol/L (23.7%) vs. 0.45 mmol/L (17.8%) and TC:HDL ratio improved 0.9 (20.1%) and 0.5 (11.4%). In each case, the improvement with the ezetimibe/rosuvastatin combination was greater but not to a significant degree. The combination of ezetimibe and rosuvastatin also ameliorated TG (0.60 mmol/L, 23.0%) and AIP (0.12, 41.8%) from baseline, whereas no such improvement was seen within the increased dose group following 12 weeks of treatment. No improvement in HDL was seen in either group.

Ezetimibe in combination with a statin has been thoroughly studied in the general population, with the bulk of the research investigating the combination of simvastatin/ezetimibe. Generally it has been found that this combination is superior to rosuvastatin alone [351-353]. However, as simvastatin is contraindicated in HIV+ patients due to potential interactions between it and ARV medications, other ezetimibe/statin combinations had to be investigated.

The improvements shown here are greater than those reported from other trials that have investigated the combination of ezetimibe with a statin in HIV+ patients. Chow et al. conducted a 24-week crossover study wherein patients were randomized to receive either ezetimibe or placebo for 12 weeks (then the alternative) in addition to ongoing statin therapy (atorvastatin 10 mg/day or pravastatin 20 mg/day). Improvements were observed in ApoB (12.4%), TC (17.8%), LDL (20.8%) and TG (8.4%)[175]. Berg-Wolf et al.

conducted an 18-week single-arm trial wherein ezetimibe was added to ongoing statin (atorvastatin or pravastatin) and only reported modest improvements in LDL (12.4%) and TC (9.0%) together with a non-significant decrease (5.5%) in HDL, whereas there was no improvement in TG [173]. Finally, Negrodo et al. added ezetimibe to 20 mg pravastatin for 24 weeks. Addition of ezetimibe resulted in a 7% reduction in LDL, 5% reduction in TC, 8% reduction in TG, and 8% increase in HDL at 24 weeks [171]. The facts that less potent statins were used in these other trials, and the improvements we observed were greater in magnitude, both speak to the ability of ezetimibe to lower lipid endpoints in HIV+ patients and provide further evidence for an underlying mechanism of resistance to rosuvastatin in this population.

One study has previously compared the effect of ezetimibe together with rosuvastatin in the non-HIV+ population. In the EXPLORER study, Ballantyne et al. investigated the efficacy and safety of rosuvastatin 40 mg alone or in combination with ezetimibe 10 mg in patients at high risk of coronary heart disease [354]. The improvements seen in this trial were far greater than those reported here, possibly owing to the fact that all lipid-lowering therapy was stopped prior to initiating treatment with either rosuvastatin 40 mg alone or in combination with ezetimibe, preventing any direct comparison. Regardless, the combination therapy group was still proven to be more effective and equally as safe as rosuvastatin alone.

5.4.3.1 Apolipoproteins as CVD Risk Markers in HIV

LDL has traditionally been used as an endpoint for cardiovascular risk; however, the paradigm of cardiovascular risk assessment has recently shifted. Now the number of atherogenic lipoprotein particles is considered to be the most important determinant of

risk [355]. Specifically, ApoB, the major apolipoprotein component of LDL, accurately accounts for the number LDL particles in a given individual. Accordingly, ApoB has proven to be superior to LDL in predicting vascular events in a number of populations [356-361] including patients with type 2 diabetes [362]. Accordingly, ApoB has been suggested as a primary clinical target, and has been incorporated into the CCS as a treatment target for individuals with moderate-high CVD risk [30, 355, 363].

The purpose of this trial was to compare the effectiveness of the standard practice of increasing the dose of rosuvastatin to the addition of ezetimibe to ongoing rosuvastatin therapy in HIV+ patients above ApoB target. Despite screening for elevated ApoB, only 31 of the 39 subjects had an ApoB above target (>0.80 g/L) following baseline assessment. Among these patients a near-identical drop in ApoB was observed in each group. ApoB dropped from 1.12 g/L to 0.91 g/L (18.8%) in the combination group and dropped from 1.16 g/L to 0.96 g/L (17.2%) in the rosuvastatin 20 mg group. These improvements in ApoB are similar to those reported in a large trial in non-HIV patients beginning ezetimibe monotherapy [364]. In terms of patients reaching the CCS goal of ApoB <0.80 g/L, results were similar between groups following 12 weeks of treatment. When ezetimibe was added to rosuvastatin, 45% (from 15.0% at baseline) of patients reached target compared to 50% (from 27.8% at baseline) in patients treated with rosuvastatin.

ApoB presents as a valuable measure of cardiovascular risk in patients where hypertriglyceridemia may preclude accurate measure of LDL, such as in the HIV+ population [9]. However, to date, ApoB has gone largely uninvestigated as a marker for CVD risk in HIV. Studies have confirmed a high prevalence and association of ApoB

with cardiovascular risk factors but a prospective analysis directly assessing the association of long-term CVD risk with elevated ApoB is lacking [365, 366].

Another risk marker that has yet to be thoroughly assessed in the HIV+ population is the ApoB:ApoA1 ratio. In the non-HIV population this ratio has been shown to be significantly more informative and predictive of cardiovascular risk than any of the conventional cholesterol indices: TC:HDL, LDL:HDL or non-HDL:LDL [367]. It follows then that this ratio should be equally as valuable in determining CVD risk in the HIV+ population. To date, this is the first study to use the ApoB:ApoA1 ratio as a marker for effectiveness of lipid-lowering therapy in HIV+ patients. Our small study revealed that, among all study subjects, an elevated ApoB:ApoA1 ratio (>0.80) was not as common (33% of patients) as the more commonly used CCS target of a TC:HDL above 4.0 (62%) [30]. This suggests greater specificity of the ApoB:ApoA1 compared to the TC:HDL ratio. Accordingly, nearly every patient reached the ApoB:ApoA1 target following 12 weeks of treatment: 94.4% (from 65.0% at baseline) of the combination group and 83.3% (from 66.7% at baseline) of the increased dose rosuvastatin group reached this goal. This may suggest that CVD risk is not drastically elevated in the HIV+ population, but as our sample size is small, further investigation into prevalence of elevated ApoB:ApoA1 ratio in this population is warranted.

5.4.3.2 Attaining Canadian Cardiovascular Society Targets

While there is evidence that apolipoprotein endpoints may be able to more accurately predict CVD risk than traditional lipid endpoints, lowering of these parameters (LDL, TC:HDL ratio, TG, CRP) is still of clinical value and may still play an important role in attenuating CVD risk in HIV+ patients.

Attainment of cholesterol goals is an important aspect of clinical care in HIV and targets for patients with moderate or high risk are often sought. Negredo et al. found that 61.5% of HIV+ patients reached the LDL NCEP target of <130 mg/dL (3.36 mmol/L) when ezetimibe was added to ongoing pravastatin therapy [171]. This endpoint was also used by Berg-Wolf et al., but in that study only 35% of patients reached the goal following 18 weeks of therapy with ezetimibe added to ongoing statin therapy [173]. In our study the more aggressive CCS target of LDL <2.0 mmol/L[30] was reached by 50% (from 23.1%) in the combination group and 46.1% (from 18.8%) in the increased dose group.

Secondary CVD risk targets have gone largely unexamined in the HIV+ population, partially because of the lack of updated guidelines with respect to treatment of HIV+ patients. We examined the proportion of patients in each treatment group that reached secondary CCS targets according to recent guidelines for the general population[30]. More patients in the combination group reached a TC:HDL ratio of <4.0 (78.9%) and TG <1.7 mmol/L (53.3%) than in the increased dose group (66.7% and 29.4%). The latter finding is noteworthy, as TG goals can be especially difficult to attain in HIV+ population given the adverse effects of both HIV disease and HAART on this endpoint. However, the difference between groups seen here may be partially explained by a greater number of patients on concomitant fibrate therapy in the increased dose group (33% vs. 19%).

As with other studies, no improvements in CRP were seen with ezetimibe in addition to rosuvastatin as there was not a significant decrease in CRP nor did an increased proportion of patients reach the CCS target of <2.0 mg/L following 12 weeks of therapy

[171, 175]. In the increased dose group, the observed improvement in CRP was not significant but 77.8% of patients (up from 55.6% at baseline) were at target at week 12.

A novel and largely uninvestigated marker of cardiovascular risk in the HIV+ population is the atherogenic index of plasma (AIP), calculated as the $\log_{10}(\text{TG}/\text{HDL})$. AIP has been shown to be predictive of elevated blood pressure[223], small low-density lipoprotein cholesterol (LDL) particle size[224] and vascular events[223] in the general population. As such, AIP may prove to be a useful predictor of risk in the HIV+ population given its predictive utility in non-HIV population and the fact that it combines two parameters that are often adversely affected by HIV and HAART. At baseline, 60% of our study cohort were above the high-risk AIP target of >0.21 [227]. This modestly improved to 65% of patients following 12 weeks of treatment. AIP improvements were more common in the combination therapy group as 60% (from 42.8% at baseline) in that group were below the target compared to 41.2% (from 37.8% at baseline) in the increased dose group.

5.4.4 Ezetimibe for Attenuation of CVD Risk

Ezetimibe is well-tolerated and effective in lowering lipid endpoints but questions have been raised with regard to its long-term benefit. Results from the ENHANCE trial, which compared ezetimibe and high-dose simvastatin vs. simvastatin alone in patients without HIV, found that it did not improve IMT, a surrogate marker for CVD [168]. Conversely, the SANDS trial noted a significant improvement in IMT with ezetimibe in combination with a statin in their study population of HIV uninfected patients with type 2 diabetes [368]. Data are limited with regard to the effect of ezetimibe on clinical endpoints. The SEAS trial assessed simvastatin in combination with ezetimibe on aortic stenosis and showed a small reduction in incidence of ischemic events with simvastatin and ezetimibe,

but no improvements were seen with this combination in the primary outcome of combined aortic-valve ischemic events [169]. In addition, the recent SHARP trial was conducted in non-HIV+ patients with and found that ezetimibe 10 mg with simvastatin 20 mg reduced the incidence of major atherosclerotic in patients with advanced chronic kidney disease.

More such trials that assess the effectiveness of ezetimibe on clinical cardiovascular outcomes are currently underway[369], but further trials in the HIV+ population are still required in order to accurately gauge the potential benefit of this treatment.

5.4.4.1 Limitations

Limitations of this pilot study include that this was a single-centre pilot study with a small sample size. At 12 weeks the study duration may be considered too short to demonstrate the full treatment effect but the maximum therapeutic response of ezetimibe is reached within two to four weeks[336] and trial length is comparable to that of other studies investigating ezetimibe in combination with statins in HIV+ (12-24 weeks) [171, 173, 175]. Lastly, our assumption, based on our previous study[144], of no effect between 10 mg and 20 mg of rosuvastatin may have underestimated the effect of the 20 mg dose as the majority of the patients reviewed had previous statin experience.

5.4.5 Conclusions

Our study was the first to examine the efficacy of adding ezetimibe to maximally tolerated doses of the lipid-lowering therapy including highly potent statins, fibrates and combinations of a statin and a fibrate. Significant improvements in the lipid profile following the addition of ezetimibe were seen. Furthermore, there were no adverse

events. We conclude that if the lipid targets are not met after maximally tolerated doses of lipid-lowering therapy with a statin and/or fibrate, ezetimibe is safe and effective.

Rosuvastatin is well suited for the treatment of HIV dyslipidemia as it is not metabolised by the CYP3A4 pathway. The resistance to therapy exhibited in our study cohort is of importance to clinicians treating HIV dyslipidemia as it demonstrates that simply increasing the dosage of rosuvastatin may not be the most effective way to improve lipid parameters in this population. The results of this study and the literature suggest that rosuvastatin in combination with either a fibrate[334] or ezetimibe[172] is an effective treatment in this population.

The addition of ezetimibe to ongoing therapy with rosuvastatin is effective at improving lipid and reaching lipid targets that were not being reached with rosuvastatin alone. Due to the small sample size of this pilot study, the greater improvements with the combination therapy as compared to an increased dose of rosuvastatin did not reach significance. Despite the lack of difference between groups, the results here have served to raise further questions with regard to resistance to rosuvastatin therapy. In addition, these findings cannot rule out the possibility of a resistance to ezetimibe therapy in HIV+ patients since greater improvements in LDL would have been expected compared to doubling the dose of rosuvastatin [370, 371]. These questions should be investigated with larger trials as well as studies and those aimed at determining a biochemical mechanism of statin or ezetimibe resistance in HIV+ patients.

Tables

Table 5.1 Baseline demographics of 33 patients initiating ezetimibe.

| Variable | |
|--------------------------------------|------------|
| Age, years† | 51.37±8.18 |
| Male sex | 31 (93.9) |
| Duration of ezetimibe therapy, days† | 79.6±31.8 |
| T2DM | 8 (22.9) |
| History of hypertension | 16 (48.5) |
| Previous vascular disease | 9 (27.3) |
| Smoking | |
| Previous | 10 (30.3) |
| Current | 3 (9.1) |
| Statin | 24 (72.7) |
| Rosuvastatin | 15 (45.5) |
| Atorvastatin | 7 (21.2) |
| Pravastatin | 2 (6.1) |
| Fenofibrate | 17 (51.5) |
| Salmon oil | 4 (12.1) |
| Niacin | 2 (6.1) |
| ARV therapy | 30 (90.9) |
| PI | 24 (72.7) |
| NNRTI | 25 (75.8) |
| NRTI | 29 (87.9) |
| Fusion inhibitor | 1 (3.0) |
| Off treatment | 3 (9.1) |

Data are frequency (%) unless otherwise indicated, †Data are mean (±SD).

T2DM, type 2 diabetes mellitus; ARV, antiretroviral; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor.

Table 5.2 Baseline demographics of 130 patients initiating rosuvastatin.

| Variable | |
|---|---------------|
| Age, years† | 52.6 ± 8.29 |
| Male sex | 128 (98.5) |
| Duration of rosuvastatin therapy, days† | 140.8 ± 112.8 |
| T2DM | 20 (15.4) |
| Previous statin | 78 (60.0) |
| Atorvastatin | 64 (49.2) |
| Pravastatin | 10 (7.7) |
| Other | 4 (3.1) |
| ARV therapy | 123 (94.6) |
| PI | 109 (83.8) |
| Non-PI | 14 (10.8) |
| None | 7 (5.4) |

Data are frequency (%) unless otherwise indicated, †Data are mean (±SD). T2DM, type 2 diabetes mellitus; ARV, antiretroviral; PI, protease inhibitor.

Table 5.3 Baseline characteristics of the clinical trial group.

| Characteristic | Ezetimibe (n=21) | Rosuvastatin (n=18) |
|--|-----------------------------|--------------------------------|
| Age, years* | 56.0±7.5 | 56.6±9.9 |
| Male sex | 20 (95) | 15 (83.3) |
| Caucasian | 17 (81.0) | 13 (72.2) |
| ARV duration, months† | 24 (16 – 34) | 25 (14 – 48) |
| CD4+ cell count, cells/mm ³ * | 616±202 | 527±206 |
| Undetectable VL‡ | 19 (90.5) | 17 (94.4) |
| PI | 19 (90.5) | 15 (83.3) |
| NNRTI | 6 (28.6) | 6 (33.3) |
| NRTI | 21 (100) | 17 (94.4) |
| Fibrate | 4 (19.1) | 6 (33.3) |
| Antihypertensive agents | 10 (47.6) | 6 (33.3) |
| Current Smoker | 6 (28.6) | 1 (5.6) |
| Previous Smoker§ | 8 (38.1) | 14 (77.8) |
| T2DM | 8 (38.1) | 7 (38.9) |
| History of hypertension | 10 (47.6) | 6 (33.3) |
| Family History of CVD | 9 (42.9) | 10 (55.6) |
| Body Mass Index, kg/m ² * | 25.8±4.4 | 25.3±5.0 |
| Waist Circumference, cm* | 98±14 | 93±16 |

Data are frequency (%) unless otherwise indicated, *Data are mean (±SD) †Data are median (IQR), ‡<40 copies/mL, §Quit smoking less than one year prior to study recruitment. ARV, antiretroviral; VL, viral load; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; T2DM, type 2 diabetes mellitus; CVD, cardiovascular disease.

Table 5.4 Effects of adding ezetimibe to rosuvastatin or increasing rosuvastatin dosage on lipid and metabolic outcomes.

| Variable | Baseline | | Change after 12 weeks | | <i>P</i> Value (Difference Between Rosuvastatin and Ezetimibe) |
|--------------------|--|--------------------------------------|--|-----------------------------------|---|
| | Ezetimibe add-on group (n=21) | Increased dose group (n=18) | Ezetimibe add-on group (n=21) | Increased dose group (n=18) | |
| ApoB, g/L | 1.06±0.25 | 1.01±0.40 | -0.18±0.18* | -0.14±0.26 | 0.23 |
| ApoA1, g/L | 1.53±0.26 | 1.51±0.33 | -0.07±0.26* | 0.06±0.25 | 0.15 |
| ApoB:ApoA1 | 0.71±0.22 | 0.72±0.41 | -0.10±0.16* | -0.14±0.35 | 0.31 |
| TC, mmol/L | 5.24±1.06 | 5.05±0.52 | -0.95±0.81* | -0.47±0.65* | 0.06 |
| LDL, mmol/L | 2.75±0.85 | 2.51±0.58 | -0.64±0.54* | -0.45±0.57* | 0.55 |
| HDL, mmol/L | 1.21±0.28 | 1.31±0.42 | 0.0±0.3 | 0.02 ± 0.15 | 0.93 |
| TC:HDL ratio | 4.5±1.2 | 4.2±1.2 | -0.9±0.8* | -0.5±0.7* | 0.13 |
| TG, mmol/L | 2.68±1.20 | 3.03±1.51 | -0.60±0.59* | -0.16±0.59 | 0.09 |
| AIP | 0.32±0.29 | 0.34±0.35 | -0.12±0.20* | -0.03±0.12 | 0.15 |
| Glucose, mmol/L | 6.7±1.8 | 6.3±2.4 | 0.2±1.4 | -0.4±1.9 | 0.66 |
| CRP, mg/L | 5.6±10.0 | 2.8±3.6 | -1.2±12.7 | -1.2±3.6 | 0.38 |
| Creatinine, µmol/L | 93±22 | 99±31 | 7±50 | 60±274 | 0.80 |
| ALT, U/L | 33±14 | 33±13 | 11±19* | 4±15 | 0.33 |
| AST, U/L | 28±11 | 30±12 | 6±14 | 0±9 | 0.38 |
| CK, U/L | 143±98 | 170±96 | -23±87 | 14±72 | 0.31 |

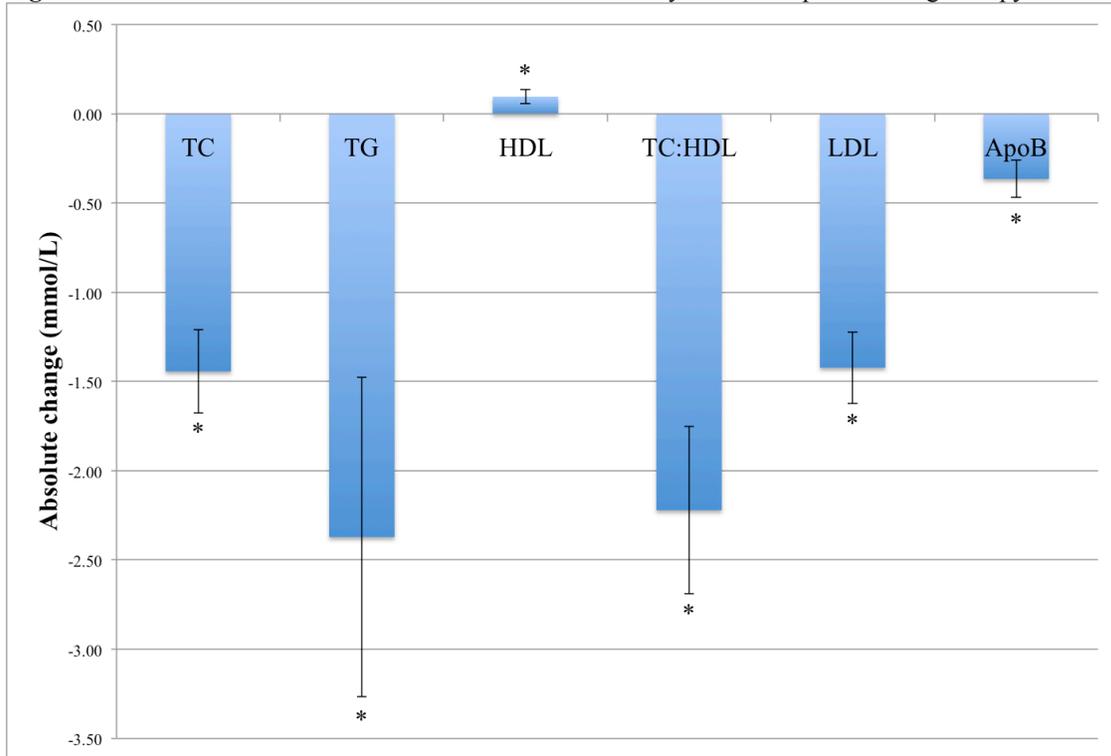
Data are given as mean (±SD)

*Significant change from baseline (p<0.05).

ApoB, apolipoprotein B; ApoA1, apolipoprotein A1; ApoB:ApoA1, apolipoprotein B to apolipoprotein A1 ratio; TC, total cholesterol; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; TC:HDL ratio, total cholesterol to high-density lipoprotein cholesterol ratio; TG, triglycerides; AIP, atherogenic index of plasma; CRP, C-reactive protein; ALT, alanine transaminase; AST, aspartate transaminase; CK, creatine kinase.

Figures

Figure 5.1 Overall effect of addition of ezetimibe to maximally tolerated lipid-lowering therapy.

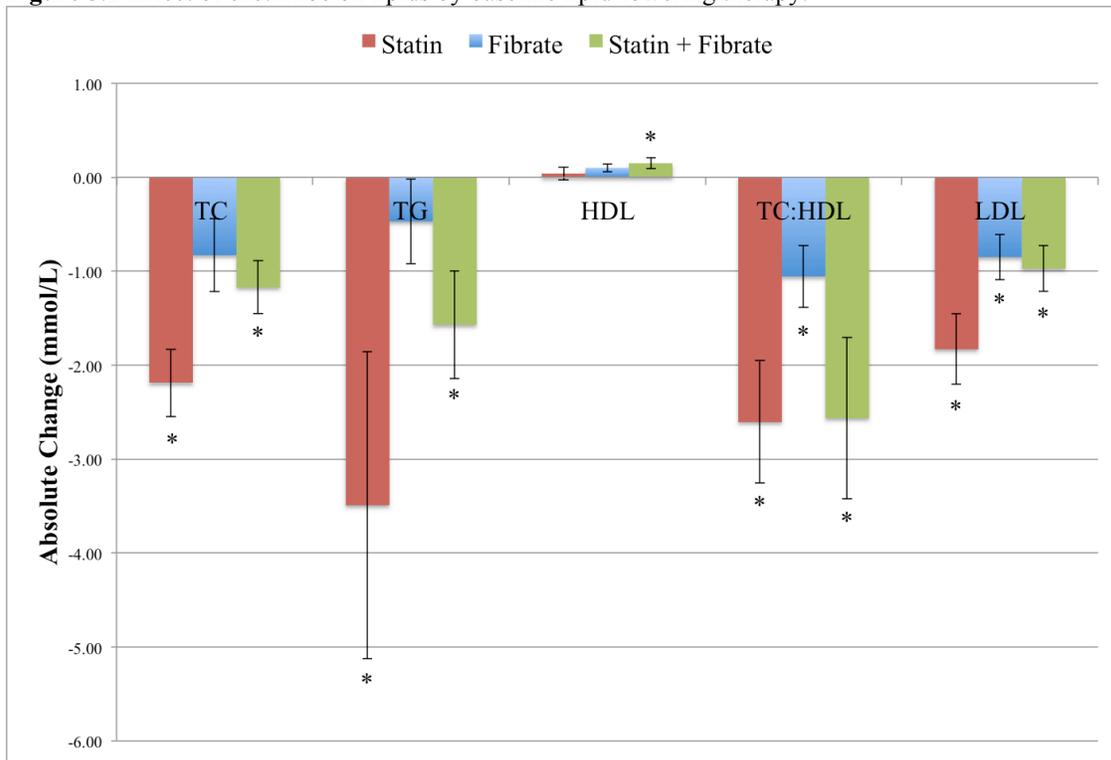


Data are mean (\pm SEM)

* $p < 0.05$.

TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein cholesterol; TC:HDL, total cholesterol to high-density lipoprotein cholesterol ratio; LDL, low-density lipoprotein cholesterol; ApoB, apolipoprotein B.

Figure 5.2 Effect of ezetimibe on lipids by baseline lipid-lowering therapy.

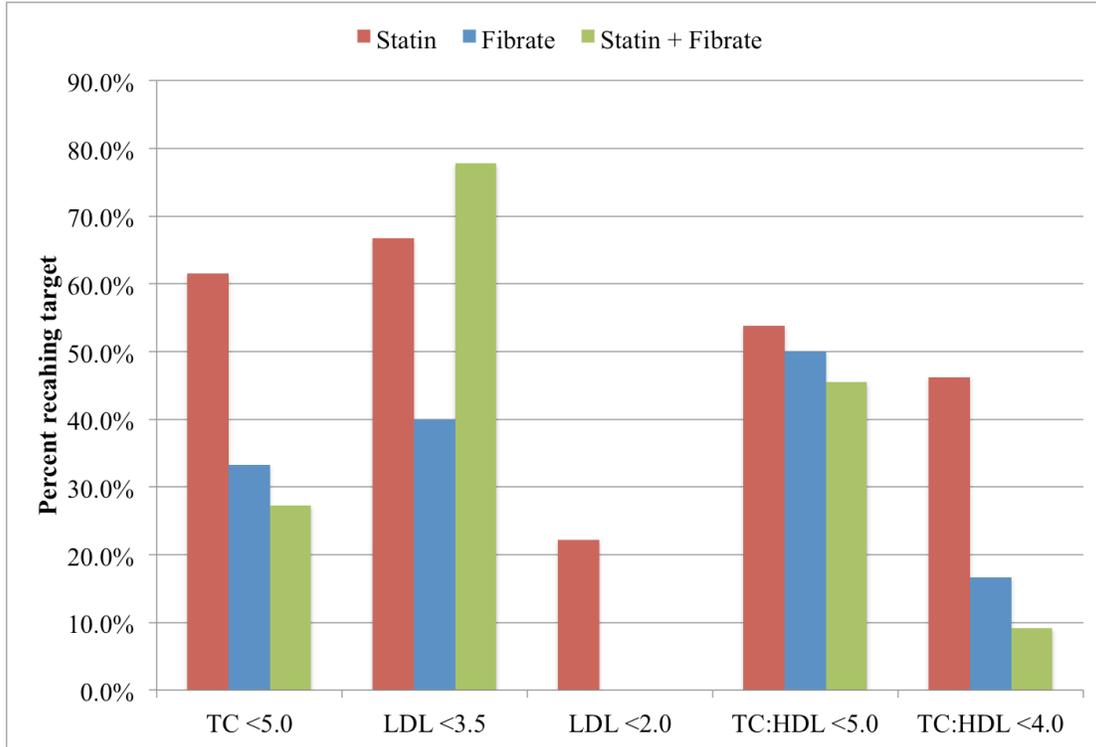


Data are mean (\pm SEM)

* $p < 0.05$.

TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein cholesterol; TC:HDL, total cholesterol to high-density lipoprotein cholesterol ratio; LDL, low-density lipoprotein cholesterol.

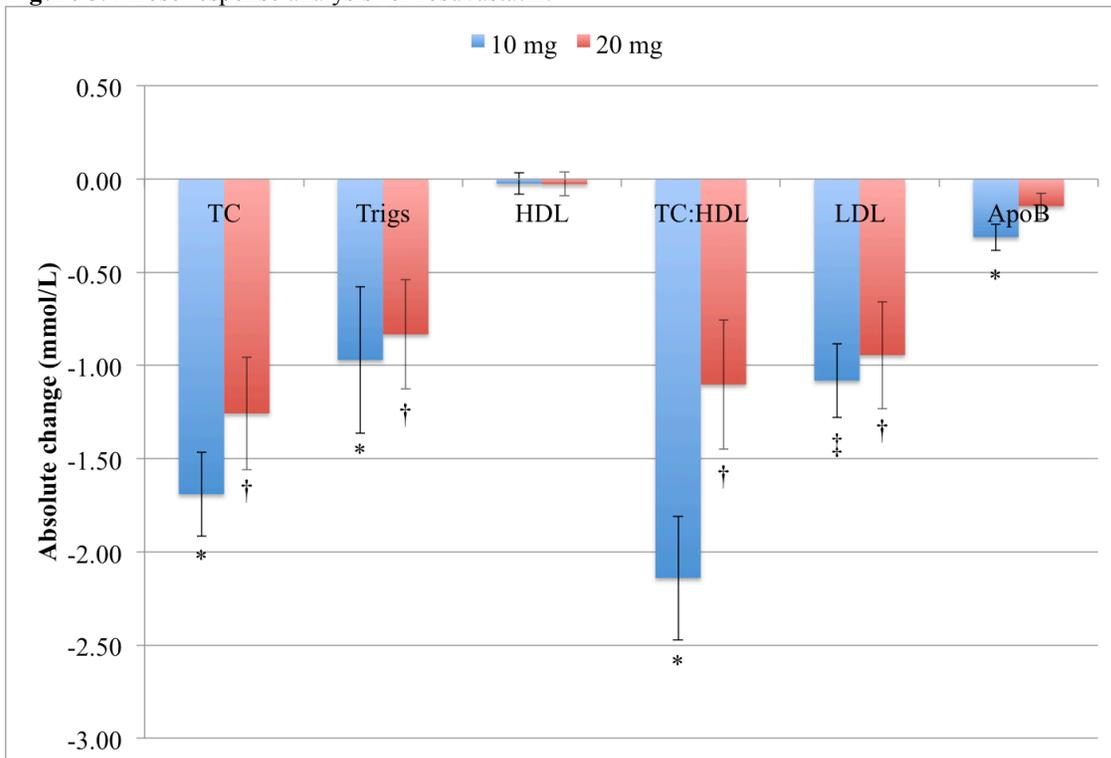
Figure 5.3 Percent of patients reaching targets for moderate or high cardiovascular risk following addition of ezetimibe.



Moderate risk: TC <5.0 mmol/L, LDL <3.5 mmol/L, TC:HDL<5.0; High-risk: LDL <2.0 mmol/L, TC:HDL<4.0.

TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein cholesterol; TC:HDL, total cholesterol to high-density lipoprotein cholesterol ratio; LDL, low-density lipoprotein cholesterol.

Figure 5.4 Dose response analysis for rosuvastatin.



Data are mean (\pm SEM)

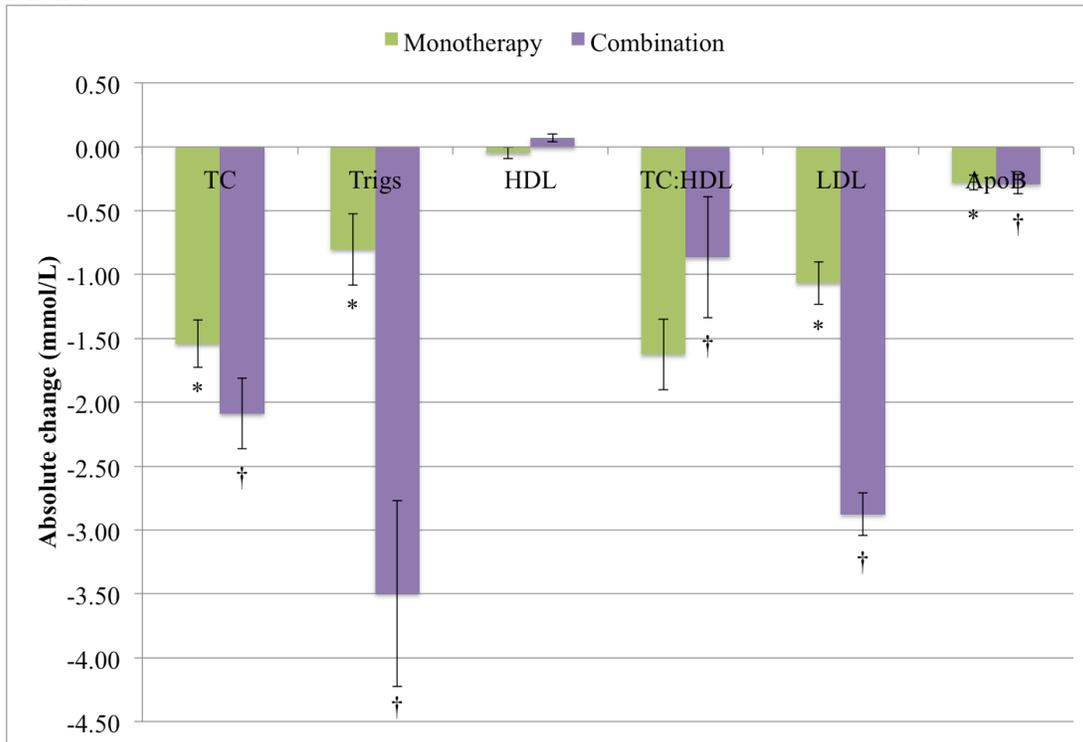
* $p < 0.05$ within 10 mg group.

† $p < 0.05$ within 20 mg group

‡ $p < 0.05$ between groups in favour of 10 mg group.

TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein cholesterol; TC:HDL, total cholesterol to high-density lipoprotein cholesterol ratio; LDL, low-density lipoprotein cholesterol; ApoB, apolipoprotein B.

Figure 5.5 Change in lipid parameters with either rosuvastatin alone or in combination with fenofibrate.



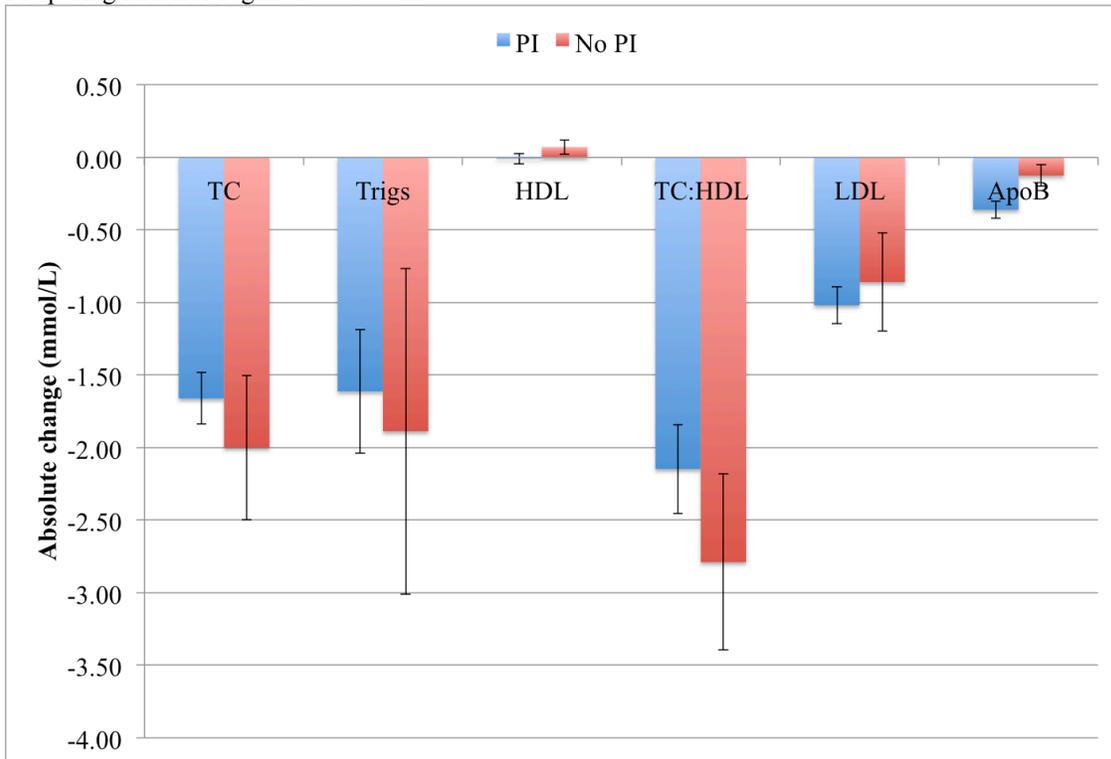
Data are mean (\pm SEM)

* $p < 0.05$ within monotherapy group.

† $p < 0.05$ within combination therapy group.

TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein cholesterol; TC:HDL, total cholesterol to high-density lipoprotein cholesterol ratio; LDL, low-density lipoprotein cholesterol; ApoB, apolipoprotein B.

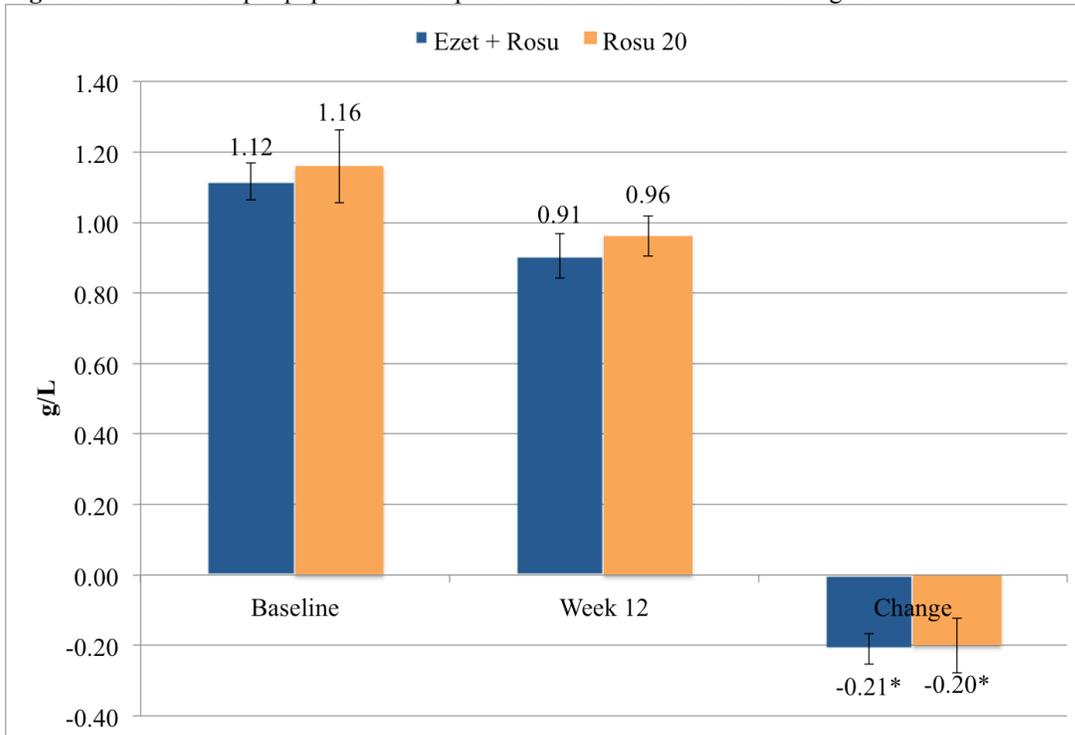
Figure 5.6 Change in lipid parameters in statin-naïve patients initiating rosuvastatin on PI-containing or PI-sparing HAART regimens at baseline.



Data are mean (\pm SEM)

TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein cholesterol; TC:HDL, total cholesterol to high-density lipoprotein cholesterol ratio; LDL, low-density lipoprotein cholesterol; ApoB, apolipoprotein B.

Figure 5.7 Effect on apolipoprotein B for patients with a baseline level >0.80g/L.



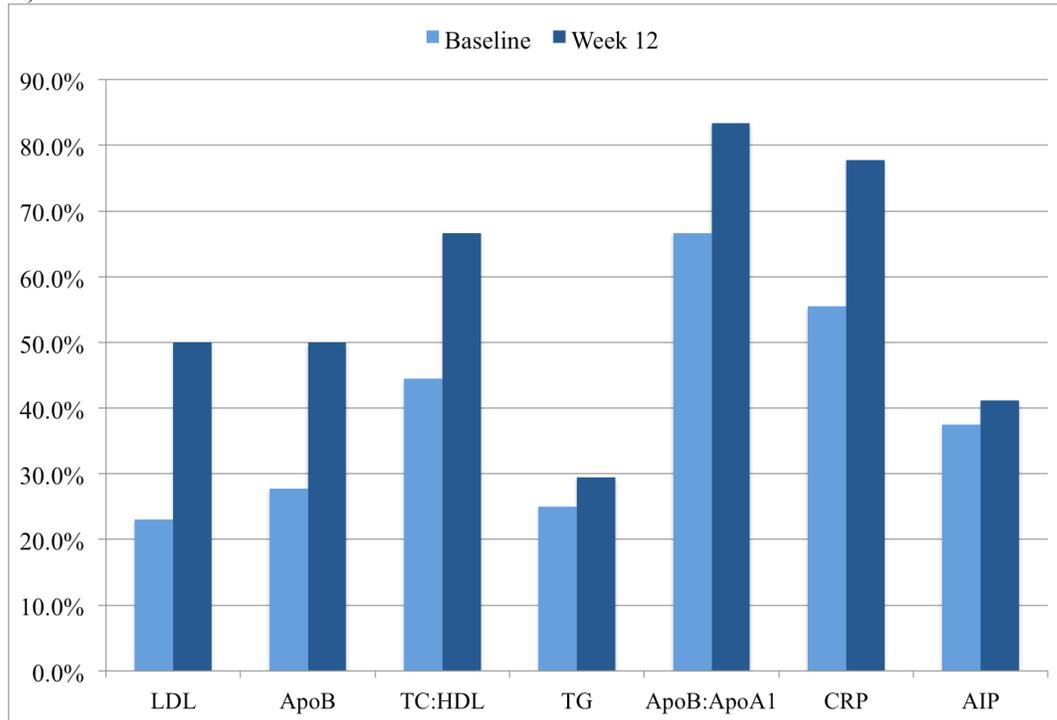
Data are mean (\pm SEM)

* $p < 0.05$.

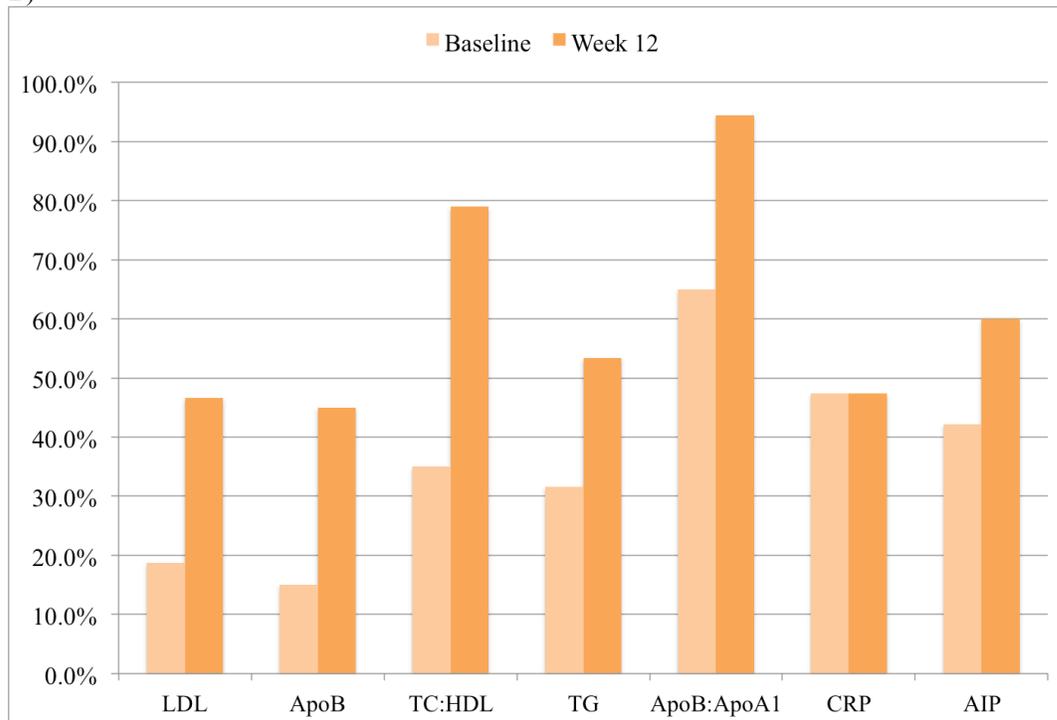
Ezet + Rosu, 10 mg ezetimibe added to 10 mg rosuvastatin; Rosu 20, rosuvastatin increased to 20 mg.

Figure 5.8 Percentage of patients at target for high-risk subjects at baseline and following 12 weeks of treatment with 20 mg rosuvastatin (A) or ezetimibe and 10 mg rosuvastatin (B).

A)



B)



LDL, low-density lipoprotein cholesterol <2.0mmol/L; ApoB, apolipoprotein B <0.80 g/L; TC:HDL, total cholesterol to high-density lipoprotein cholesterol ratio <4.0; TG, triglycerides <1.7mmol/L; ApoB:ApoA1, apolipoprotein B to apolipoprotein A1 ratio <0.80; CRP, C-reactive protein <2.0 mg/L; AIP, atherogenic index of plasma <0.21.

Chapter 6: Future Directions

6.1 Determination of Time to Metabolic Syndrome

Continuing on from the work described in chapter 2 that determined the incidence of abnormal lipid parameters in Canadian HIV-positive (HIV+) patients initiating highly-active antiretroviral therapy (HAART), the next course of action is to measure the time course of developing the constellation of parameters that constitute the metabolic syndrome (MS). The new, worldwide definition of MS was published by the International Diabetes Federation in 2006 and consists of the following parameters: central obesity (defined as waist circumference over ethnicity specific value or body mass index (BMI) $>30 \text{ kg/m}^2$) and any two of the following: elevated triglycerides (TG): $>1.7 \text{ mmol/L}$, or specific treatment for this lipid abnormality; reduced high-density lipoprotein (HDL) cholesterol: $<1.03 \text{ mmol/L}$ in males, $<1.29 \text{ mmol/L}$ in females, or specific treatment for this lipid abnormality; elevated blood pressure (BP): systolic BP > 130 or diastolic BP $>85 \text{ mm Hg}$, or treatment of previously diagnosed hypertension; elevated fasting plasma glucose: $>5.6 \text{ mmol/L}$, or previous diagnosis of type 2 diabetes [372]. As MS comprises a cluster of cardiovascular disease (CVD) risk factors, it provides an important clinical picture of CVD risk. Estimates of the prevalence of MS in HIV+ patients have varied widely owing to the existence of different criteria [373]. The determination of the incidence of MS and its time course following initiation of HAART in a large multi-centre database such as CANOC will aid in specifying the CVD risk posed to HIV+ patients and will provide further insight into treatment of metabolic abnormalities in this population with elevated CVD risk.

6.2 Utility of New CVD Risk Markers

In chapter 5 of this dissertation, we assessed the effects of two lipid-lowering therapy strategies on the primary endpoint of apolipoprotein B (ApoB) as well as the apolipoprotein B to apolipoprotein A1 (ApoA1) ratio in HIV+ patients not reaching target. Measurement of apolipoproteins is not new in the HIV+ population but the predictive value of the simple and accurate endpoints has not been examined in depth. Research in the non-HIV+ population indicates that ApoB, ApoA1 and the ApoB to ApoA1 (ApoB:ApoA1) ratio are more informative than LDL, HDL and associated cholesterol ratios [363, 367, 374]. Our research suggests that the ApoB:ApoA1 ratio is a potentially useful endpoint in the HIV+ population. In addition, apolipoproteins have the potential to be especially useful in HIV+ patients, in view of the fact that the high TG associated with the disease and its treatment that may prevent accurate measurement of LDL. While apolipoproteins appear to be a logical endpoint in HIV+ patients, studies also indicate the potential utility of lipoprotein remnant-like particle cholesterol (RLP-C). RLP-C has also been linked to increased CVD risk in the general population[375] and has been shown by at least one study to be elevated in HIV+ patients receiving HAART[376]; therefore, further investigation into its potential as a marker for CVD in HIV+ patients is warranted. Determination of the specificity of these endpoints requires design and execution of prospective studies that follow HIV+ patients to cardiovascular endpoints.

6.3 Agents for Attenuation of CVD Risk

We investigated the effects of rosiglitazone, a peroxisome proliferator-activated receptor-gamma, on CVD progression. As described in Chapter 4, we confirmed the drug's ability to improve inflammatory markers, though its effect on surrogate endpoints of CVD was not remarkable. From this investigation we did determine a higher than expected prevalence of carotid plaques in HIV+ patients. Attenuating CVD progression in HIV+ patients will continue to be an important clinical goal as the HIV+ population ages thanks to modern ARV regimens lengthening the life span. As such it is important to identify agents that can effectively slow and halt the progression of CVD. Future work should assess the effect of potential agents on the progression of carotid plaques in people with HIV. Due to the restrictions placed on rosiglitazone by Health Canada and the United States Food and Drug Administration due to its purported CVD risk, it is unlikely that further studies will explore its utility. Pioglitazone, the other member of the TZD class on the market, has not been restricted and may prove to be a useful agent in attenuating CVD progression. The search for such agents can be expanded to include rosuvastatin, which has shown promise in preventing cardiovascular events in people with normal cholesterol levels [377, 378] and ezetimibe given its potential to reduce CVD events when combined with a statin [379].

6.4 Biochemical Mechanism of Statin Resistance

In chapter 5 of this dissertation it has been suggested that HIV+ patients treated with HAART may experience resistance to the lipid-lowering effects of statin therapy, including rosuvastatin, the newest and most potent agent in this class. In the same

chapter, we compared the efficacy of the standard treatment (increasing the dose of rosuvastatin) and a new treatment (adding ezetimibe to ongoing rosuvastatin) in order to further explain the reduced effect of statins in HIV; however, a conclusion regarding the superiority of one of these treatment strategies could not be reached. Regardless, clinically important questions still remain with respect to the resistance to lipid-lowering therapy. The organic anion transporter (OAT) has been identified as a delivery vehicle of drugs into the liver and HIV protease inhibitors act to inhibit OAT, providing a possible reason for an attenuated response to statins in patients on HAART [380, 381]. Biochemical mechanistic studies are thus warranted in order to assess the prevalence of this inhibition and identify which ARV agents have the greatest effect on OAT in patients on HAART who begin statin therapy.

6.5 Prevention of Dyslipidemia

Management of the dyslipidemia associated with HIV and HAART now plays a major role in treatment of HIV+ patients, as prevention of the development of CVD becomes paramount in this aging population. The logical course of action is to determine therapies that are effective at preventing the onset of HAART-induced dyslipidemia. There is potential for agents such as rosuvastatin, ezetimibe, or non-traditional agents such as vitamin D for preventing dyslipidemia [168, 377, 378, 382]. The JUPITER study was able to show that prevention of cardiovascular events in HIV-uninfected patients with normal cholesterol levels was possible with rosuvastatin[377, 378]; however, these results have been disputed [383]. Similarly, the ENHANCE trial was not able to conclusively show a benefit of ezetimibe on lessening surrogate markers for CVD again

in an HIV-negative population [168]. This does not preclude the possible effectiveness of either of these agents alone or in combination for prevention of dyslipidemia in HIV+ patients initiating HAART. Randomized placebo-controlled trials that investigate the ability of low-dose rosuvastatin and/or ezetimibe taken in conjunction with the patients' initial HAART may prove informative.

Chapter 7: Conclusions

The work presented in this dissertation has contributed to specific understanding of cardiovascular risk in the HIV+ population. Specifically, we have demonstrated that there is a high incidence of metabolic laboratory abnormalities in HIV+ patients initiating current ARV therapies and that there may exist a unique underlying pattern of lipid abnormalities in patients initiating NNRTIs. Insight was also provided into direct assessment of vascular disease but the current method of assessment of vascular disease in the lower extremities may not be sensitive enough to accurately gauge the prevalence of this potentially serious and clinically important outcome in the HIV+ population. In addition, a number of novel and clinically relevant endpoints were measured in the course of the investigations described here. Assessment of these endpoints, which included atherogenic index of plasma, total plaque area and the apolipoprotein B:apolipoprotein A1 ratio, and their response to current treatments, help to further piece together the picture of CVD risk that is present in HIV+ patients on HAART. In addition, the effectiveness of statins has been questioned due to the reduced response to the rosuvastatin in our cohort. No difference could be found between the standard practice of increasing the dose of rosuvastatin and the newer practice of adding the cholesterol transport blocker, ezetimibe, to ongoing statin therapy in patients not reaching targets but trends do indicate that the latter is a safe and equally (if not more) effective treatment.

In terms of the overarching hypotheses delineated at the outset of this dissertation, it is possible that the overall risk of CVD in the HIV+ population may not be as high as initially thought. Furthermore, the divide between treatment options may not be as great

as hypothesized. However, the small sample sizes and the fact that the majority of the research conducted for this dissertation was performed at a single centre may have limited accurate, generalizable conclusions regarding CVD risk and effectiveness of current therapies treatment in the HIV+ population as a whole. On the other hand, strengths of the work include the wide array of methodologies employed in order to assess the many facets of cardiovascular risk and the treatments used to lessen this risk. The work presented in this dissertation has generated important hypotheses and provided a framework for both construction of clinical guidelines for treatment of HIV+ patients with elevated cardiovascular risk and the development of large, multicentre trials that can accurately assess utility of clinical endpoints and effectiveness of treatments in the HIV+ population.

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