HIV and Cardiovascular Disease: The Unmeasured Risk

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

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Declaration of authorship

I declare that this work is my own except where indicated by references. Work done in collaboration with, or assistance of others is specified and acknowledged. I conceived the studies and obtained all the necessary institutional, ethical, and regulatory approvals. I carried out data collection (in collaboration with Jean Matthew in chapters 6 and 7). All the statistical analyses within this work were conceived and implemented by myself. In chapter 3 the publication was prepared with the help of Sandra Ortega-Martorell and Ivan Olier who provided statistical oversight and input into the machine learning sensitivity analysis. They also provided statistical oversight for chapter 4 and chapter 5. The image analysis for chapter 4 was performed jointly by Elen Hughes and myself.

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The completion of this thesis marks the conclusion of a long and significant journey. The origins of this work lie in the now defunct Royal Liverpool cardiology office, in which my interests in preventative cardiology and cardiovascular imaging were born. I am grateful for the guidance, humour and academic upbringing of my friend and colleague Dr Scott Murray. I am certain that without his influence this PhD would not be possible. I am also grateful to my primary supervisor Professor Saye Khoo. The opportunities, direction and support were integral to this thesis and has inspired me to make academic work part of my long-term career plans. I would also like to thank clinical staff at Liverpool University Hospital Trust, in particular Dr Jonathan Hasleton and Professor Michael Fisher. Without their steadfast support and guidance, it would have made completion of this work extremely difficult.

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Conflict of interest statement

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6. **Heseltine TD,** Hughes E, Mathew J, Murray S, Khoo S. The effect of changing to Bictegravir on lipids using real world data: A brief report. Journal of Clinical Pharmacy and Therapeutics. 2022;47(12):2182–7.
7. **Heseltine TD**, Hughes E, Mattew J, *et al*. The association of epicardial adipose tissue volume and density with coronary calcium in HIV-positive and HIV-negative patients. J Infect. 2023;S0163-4453(23)00089-0.

Abstract

CVD is an emerging health economic emergency. Following the successful introduction of ART, PLWHIV have a close to normal life expectancy and are succumbing to traditional diseases of ageing. CVD is the leading cause of morbidity and mortality in individuals established on ART and they have roughly double the risk of a CVD event compared to age and risk matched general population individuals.

Historically specific ART drugs were known to induce changes in body morphology, promote dyslipidaemia, insulin resistance and diabetes. However, modern preparations have a more nuanced impact on traditional risk factors but are known to impact on adipocyte function and energy metabolism. HIV and specific ART agents have also been implicated in development of fatty liver with a significantly higher prevalence of "lean NAFLD" seen in PLWHIV. In addition, traditional CVD risk calculators, and HIV-specific risk calculators, are known to be inaccurate in PLWHIV. This work sought to investigate the unmeasured risk seen in HIV. Specifically, this focused on measurement of ectopic fat, including HS and EAT volume, with incident CVD. We also investigated the effect of switching ART on lipid panels.

The data demonstrated that HS was independently associated with incident CVD in PLWHIV but not so in general population controls (OR: 3.13, p=0.005 versus OR: 1.08, p=0.60). These findings were backed up in a sensitivity analysis using Random Forest ML algorithms (age, male sex and HS were the top three variables of importance in the Gini index). In addition, EAT volume was lower in PLWHIV compared to general population people (68mm3 versus 118.3mm3, p<0.005). Again, after adjustment for traditional risk factors EAT volume was significantly associated with coronary calcification in PLWHIV but not in general population individuals (OR 1.14, p<0.005 versus OR 1.04, p=0.104).

When assessing changes in lipid profiles in real world settings TAF/FTC/BIC was found to have no significant effect on TC, TG or HDL (all p>0.05). However, when comparing the highest quartile for TC and TG and the lowest quartile for HDL we found significant

improvements in lipid profiles. Furthermore, in a wide-ranging assessment of switching ART in 329 individuals TC and TG were higher after 6 months ($p<0.005$ and $p=0.031$) whilst there was no significant differences in HDL ($p=0.520$). When investigating associations with these changes D/C/FTC/TAF was associated with the highest quartile of TC (OR2.85, $p<0.005$) whilst RPV/FTC/TDF was negatively associated with this (OR 0.09, $p=0.021$).

A risk calculator model was developed and compared against FRS. Using multiple variable selection techniques including an MFP model. The MFP model contained age, HS, male sex, and LDL. The MFP model demonstrated superior discriminatory ability during internal validation compared to the FRS model (AUC: 0.886 versus 0.712, $p<0.001$ Wilcoxon test).

These finding suggest that HS and EAT are associated with coronary artery disease in PLWHIV but not in general population groups. This hints at different mechanistic drivers of excess atherosclerotic risk seen in HIV disease, of which adipocyte and energy metabolism plays a key role.

Table of Contents

Declaration of authorship **2**

Acknowledgement **2**

Conflict of interest statement **3**

Published work associated with this thesis. **3**

Abstract **5**

List of abbreviations **13**

Chapter 1: Introduction **17**

1.1 AIDS pandemic *17*

1.1.1 Advent of ART 18

1.2 Cardiac disease and HIV *19*

1.2.1 Epidemiology 20

1.2.2 Protease Inhibitors and CVD 21

1.2.3 NRTI 23

1.2.4 Abacavir Therapy 25

1.2.5 Integrase strand inhibitors 29

1.2.6 Risk calculators 31

1.2.7 Current paradigm of CVD and ART 32

1.3 Mechanisms of drug effects on CVD development *32*

1.3.1 Protease Inhibitors 32

1.3.2 NRTI 34

1.3.3 INSTI 35

1.4 HIV – Viral Related effects on adipocyte function *36*

1.5 The origin of insulin resistance: dysfunctional adipose tissue and fatty liver *38*

1.6 The role of Cardiovascular CT as an imaging biomarker *43*

1.6.1 Introduction 43

1.6.2 Coronary Artery Calcium Score 45

1.6.3 CTCA and functional assessment 48

1.6.4 Plaque Quantification and Morphology 50

1.6.5 Cardiovascular CT in HIV 53

1.6.6 Coronary Artery Calcium and HIV 54

1.6.7 CTCA and HIV 55

1.7 The role of ectopic fat in CVD risk *59*

1.7.1 Hepatosteatosis 59

1.7.2 Epicardial Adipose Tissue 61

1.8 The rationale for this thesis *63*

2. Chapter 2: Thesis overview 65

2.1 Aims and objectives *65*

3. Chapter 3: Associations of Hepatosteatosis with cardiovascular disease in HIV-positive and general population Patients: The Liverpool HIV-Heart Project. 66

3.1 Introduction *66*

3.2 Methods *67*

3.2.1 Statistical Analysis 68

3.3 Results *70*

3.3.1 Multivariate analysis 70

3.3.2 Sensitivity analysis 71

3.3 Discussion *72*

3.3.1 Limitations and strengths 74

3.4 Conclusion *76*

3.5 Tables and Figures *77*

3.5.1 Table 1: Summary statistics of the cohort and stratified by HIV serostatus. 77

3.5.2 Table 2: Multivariate analysis in HIV-positive patients for the association of coronary calcification 78

3.5.3 Table 3: Multivariate Analysis in HIV Negative Patients for the association of coronary calcification 79

3.5.4 Figure 1: Comparison of HIV-positive and general population models including and excluding HS. 80

3.5.5 Figure 2: Random Forest analysis of the variables of importance in HIV-positive and general population patients 82

3.5.6 Supplementary Figure 1: Receiver operator characteristic curves demonstrating the predictive ability of regression and random forest models in HIV-positive and general population groups..... 84

3.5.7 Supplementary Figure 2: Box plots to show the performance of models in HIV-positive and general population groups. 86

4. Chapter 4: The Association of epicardial adipose tissue volume and density with coronary calcium in HIV-positive and general population patients..... 87

4.1 Introduction..... 87

4.2 Methods 88

4.2.1 Statistical Analysis 90

4.2.2 Sensitivity Analysis 91

4.3 Results 92

4.3.1 EAT volume and Coronary Calcification 92

4.3.2 EAT volume and clinical covariates 92

4.3.3 EAT Density 93

4.3.4 Sensitivity Analysis 94

4.4 Discussion..... 94

4.4.1 Limitations and strengths..... 97

4.5 Conclusions..... 98

4.6 Tables and Figures 99

4.6.1 Table 1: Patient characteristics stratified by HIV-serostatus. 99
4.6.2 Table 2: Univariate and multivariate predictors of coronary calcification 100
4.6.3 Figure 1: An example of QFAT quantification of epicardial adipose tissue volume and density..... 102
4.6.4 Figure 2: Logit odds of coronary calcification and continuous covariates 104
4.6.5 Figure 3: The association of EAT density with coronary calcification in HIV-positive and General population groups 107
4.6.6 Supplementary Figure 1: Correlation between EAT volume and EAT density in HIV positive and HIV negative groups. 109
4.6.7 Supplementary figure 2: Distribution of Propensity Scores 111
4.6.8 Supplementary figure 3: Balance of Covariate in Propensity Score Matching 112
4.6.9 Supplementary Table 1: Multiple linear regression for the association of clinical covariates and EAT volume 113
4.6.10 Supplementary Table 2: Univariate and multivariate predictors of EAT volume 115
4.6.11 Supplementary Table 3: HIV-specific covariates 116

Chapter 5: Development of a calcium risk prediction tool in people living with HIV: The Liverpool HIV-Heart Project 117

5.1 Introduction 117

5.2 Methods 118

5.2.1 Statistical Analysis 119
5.2.2 Multivariate Modelling 120
5.2.3 Model Selection 120
5.2.4 Internal Validation 120
5.2.5 Sample Size 121

5.3 Results 121

5.3.1 Model Development: Discrimination and Calibration 121
5.3.2 Internal Validation 122

5.4 Discussion 122

5.4.1 Limitations and Strengths 124

5.5 Conclusions *125*

5.6 Tables and Figures *126*

5.6.1 Table 1: Summary statistics of the cohort stratified by coronary artery calcification 126

5.6.2 Table 2: Comparison of accuracy statistics in training dataset 127

5.6.3 Table 3: Intercept, coefficients, and significance of the variables in the MFP model 127

5.6.4 Figure 1: Comparison of the receiver operator characteristics curves for the development models 128

5.6.5 Figure 2: Comparison of the receiver operator characteristic curves for the final model and FRS model 129

5.6.6 Figure 3: Calibration plot of the final model and FRS model 130

Chapter 6: Changes in lipid profiles after switching to TAF/FTC/BIC 131

6.1 Introduction *131*

6.2 Methods *132*

6.2.1 Statistical Analysis 132

6.3 Results *133*

6.4 Discussion *134*

6.5 Conclusion *137*

6.6 Tables and Figures *138*

6.6.1 Table 1: Description of baseline demographics and clinical covariates in patients switched to TAF/FTC/BIC. 138

6.6.2 Table 2: Comparison of mean lipid profiles pre and post antiretroviral therapy change 139

6.6.3 Table 3: Regression coefficients and statistical significance of the multiple regression models assessing associations with change in lipid markers. 139

6.6.4 Figure 1: ANCOVA for quartiles of baseline lipid parameter 140

7 The effect of switching antiretroviral therapy on lipid profiles in a real-world setting. 141

7.1 Introduction *141*

7.2 Methods *142*

7.2.1 Study Outcomes 142

7.2.2 Statistical Analysis 143

7.3 Results *143*

7.4 Discussion *145*

7.5 Conclusions *148*

7.6 Figures and Tables *149*

7.6.1 Table 1: Demographic and clinical covariates for the whole cohort 149

7.6.2 Table 2: Clinical variable stratified by ART regime 150

7.6.3 Table 3: Mean lipid change for the whole cohort 151

7.6.4 Table 4: Mean lipid panel change stratified by ART regime 152

7.6.5 Table 5: Logistic Regression models for the highest quartile of lipid change 153

7.6.6 Figure 1: Lipid change per ART clustered by baseline preparation of tenofovir 154

7.6.7 Figure 2: Variable of importance plots for each constituent of the lipid panel. 157

8. General Discussion 159

9. References 162

List of abbreviations

3TC	Lamivudine
ACS	Acute coronary syndrome
ACTG	AIDS clinical trial group
AHA	American Heart Association
AIC	Akaike information criterion
AIDS	Acquired immunodeficiency syndrome
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ART	Antiretroviral therapy
ARV	Antiretroviral
ASCVD	Atherosclerotic cardiovascular disease
AT	Adipose tissue
ATZ	Atazanavir
AZT	Zidovudine
AUC	Area under the curve
BHIVA	British HIV Association
BIC	Bictegravir
BMI	Body mass index
BSCI	British Society of Cardiovascular Imaging
CAC	Coronary artery calcium
CACS	Coronary artery calcium score
CAD	Coronary artery disease
ChREBP	Carbohydrate response element binding protein
CI	Confidence interval
CKD	Chronic kidney disease
CT	Computed tomography
CTCA	Computed tomography coronary angiography
CVD	Cardiovascular disease
D4T	Stavudine

D:A:D	Data Collection on Adverse Events of Anti-HIV Drugs
DEXA	Dual X-ray absobtiometry
DMII	Diabetes mellitus II
DNA	Deoxyribonucleic acid
DNL	de novo lipogenesis
DRV	Darunavir
DTG	Dolutegravir
EAT	Epicardial adipose tissue
EFV	Efavirenz
ETG	Elvitegravir
FDA	Food and Drug Administration
FFR	Fractional flow reserve
FFRCT	Fractional flow reserve computed tomography
FRS	Framingham risk score
FTC	Emtricitabine
GLP-1	Glucagon-like peptide
GLUT	Glucose transporter
HAART	Highly active antiretroviral therapy
HDL	High density lipoprotein
HFrEF	Heart failure with reduced ejection fraction
HIV	Human immunodeficiency syndrome
HS	Hepatosteatosis
HSCRP	High sensitivity C-reactive protein
HTN	Hypertension
HU	Hounsfield unit
ICD	Implantable cardiac defibrillator
IDL	Intermediate-dense lipoprotein
IDV	Indanavir
IL6	Interleukin-6
INSTI	Integrase strand inhibitor
IR	Insulin resistance

IVUS Intravascular ultrasound

LPV Lopinavir

LXR Liver X-receptor

MACE Major adverse cardiac event

MACS Multicentre AIDS Cohort Study

MAFLD Metabolic associated fatty liver disease

MESA Multi-ethnic study of atherosclerosis

MFP Multivariate fractional polynomial

MI Myocardial infarction

ML Machine learning

MRI Magnetic resonance imaging

NAFLD Non-alcoholic fatty liver disease

NASH Non-alcoholic steatohepatitis

NEF Negative-regulating factor

NEFA Non-esterified fatty acid

NHS National Health Service

NICE National institute for clinical excellence

NNRTI Non-nucleoside reverse transcriptase inhibitor

NPV Negative predictive value

NRI Net reclassification index

NRTI Nucleoside reverse transcriptase inhibitor

OCT Optical coherence tomography

PI Protease inhibitor

PLWHIV People living with HIV

PPAR Peroxisome proliferator-activated receptors

PPV Positive predictive value

PVAT Perivascular adipose tissue

RAL Raltegravir

RLP Rilpivirine

ROC Receiver operator curve

ROI Region of interest

RR Relative risk

RTG Raltegravir

RTV Ritonovir

SAT Subcutaneous adipose tissue

SGLT-2 Sodium-glucose transporter

SIS Segmental involvement score

SIV Simian immunodeficiency virus

SPECT Single photo emission computed tomography

SREBP Sterol receptor binding product

SSS Segmental stenosis score

TAF Tenofovir alafenamide

TAG Triacyclglycerol

TAT Trans-activator of transcription

TC Total cholesterol

TCFA Thin-capped fibroatheroma

TNF Tumour necrosis factor

USA United States of America

VACS Veterans Aging Cohort Study

VAT Visceral adipose tissue

VLDL Very-low density lipoprotein

VPR Viral protein-R

VP Vulnerable plaque

WAT White adipose tissue

Chapter 1: Introduction

The aim of this introduction is to explore the increased risk of CVD seen in HIV-positive individuals. It focuses on the timeline of the AIDS-pandemic with reference to increasing observations of adverse CVD outcomes through pre and early-generation ART. The introduction will analyse the performance of risk prediction tools in HIV-positive individuals before reviewing the paradigm of adipocyte dysfunction, visceral adiposity, and insulin resistance. It will review contemporary ART and its associations with CVD. It also focuses on the use of imaging modalities, particularly cardiovascular CT, assessing imaging biomarkers as surrogates of CVD risk. The overall objective is to appraise the current literature whilst highlighting the gaps in knowledge that this work aims to fill.

1.1 AIDS pandemic

The onset of HIV in human populations is thought to have begun in the 1920's in Kinshasa, Democratic Republic of Congo. The oldest evidence of human involvement in the HIV/AIDS pandemic is from a blood sample obtained from West Africa in 1959 (1). The origins of the virus have been traced back to two different subspecies of Chimpanzee in equatorial West Africa (2). Most HIV infection relates to HIV-1 infection which is genetically distinct from HIV-2. Both viruses can cause AIDS: the HIV-1 virus is responsible for the worldwide infection whilst HIV-2 is limited to West Africa. HIV-2 is gradually being replaced by HIV-1 as the dominant strain.

The overall annual incidence of HIV peaked in 1999 (3.16 million) and has subsequently decreased (1.94 million) but has contributed to an increased prevalence of the disease (36. 8 million). HIV is the underlying cause of death for >1 million people a year with the greatest concentration in Sub-Saharan Africa. The UK had an annualised rate of change for new infections of 1.1% between 2007 and 2017 with reduction of 3.9% in death rate (3). In cohort data collected by Public Health England, CVD and stroke accounted for most of the non-AIDS related death alongside non-HIV associated malignancy (4).

Traditional diseases of aging, including CVD and stroke, are becoming increasingly important sources of morbidity and mortality as access to ART improves. CVD will represent a significant health economic burden as patients with HIV disease age. Finding mechanisms to reduce the impact of this burden are therefore paramount. Strategies to improve prediction, evaluate the mechanisms and potentially offer novel therapeutic targets for CVD are highly desirable.

1.1.1 Advent of ART

Zidovudine was the first antiretroviral developed to treat HIV / AIDS. It is an NRTI and works by competitively inhibiting HIV-1 DNA polymerase, thus reducing HIV replication (5). Zidovudine was approved for treatment of HIV-1 in 1987. The pre-ARV era (1986-1996) had a 10-year survival of 50% and rate of AIDS-defining illness 30.7/100 patient years (6). By the end of the 1990's the risk of death reduced by 64% and the rate of AIDS-defining illness reduced to 2.5/100 patient years (7).

Although the introduction of zidovudine was a significant success, there was an emergence of drug resistance. Much in the same way as cancer treatments, there was an early understanding that multi-drug combinations would be required to treat HIV. Further developments led to new classes of ART including saquinavir (first PI) in 1995 and nevirapine (NNRTI) in 1996. The first combination ART – 3TC/AZT was approved by the FDA in 1997 (5). These early preparations laid the groundwork for sequential generation of ART agents through to the modern era. This has had a transformative effect on HIV life expectancy.

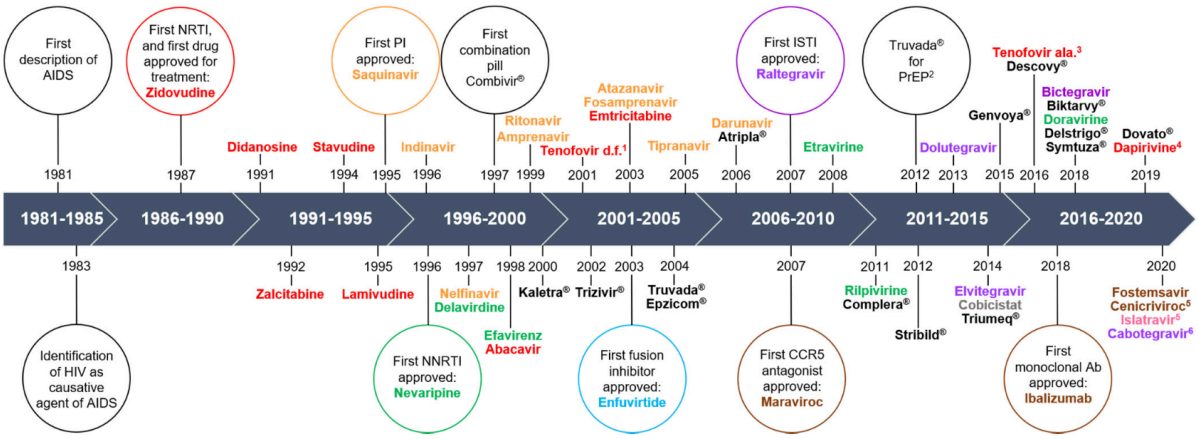


Figure 1: Timeline of milestones and introduction of ART (8)**.**

1.2 Cardiac disease and HIV

In the pre-ART era cardiac involvement in HIV disease was increasingly recognised. Cardiac manifestations of AIDS included myocarditis, dilated cardiomyopathy, pericardial disease, and endocarditis (9). The association of coronary artery disease and AIDS was not established although early autopsy results demonstrated significant atherosclerotic disease in patients with no overt CVD risk factors (10–12). In addition, an autopsy series of six HIV-infected children demonstrated evidence of coronary artery disease (13).

During the early stages of the AIDS-pandemic the interplay between HIV and the endothelium with a resulting pro-thrombotic environment was thought to be causative. It was increasingly recognised that HIV-infected patients were prone to dyslipidaemia. Hypertriglyceridemia with reduced HDL and small-dense LDL was typically recognised in patients with AIDS (14). As early generation ART was introduced concerns emerged regarding cardiovascular risk due to increasingly adverse lipid profiles. In addition, body composition changes including lipodystrophy and peripheral lipoatrophy were increasingly common and was usually associated with the incidence of DMII and adverse lipid profiles. HIV-positive individuals have an increased risk of development of DMII with a pooled incidence rate of 19/1000 patient years in the United States and 8/1000 patient years in Europeans (15).

The effects of HIV and ART on CVD risk have changed over time. The heavy inflammation-driven atherosclerosis seen in pre-ART era's is increasingly rare. Similarly, the overt dyslipidaemia and DMII associated with early generation ART has diminished over time as modern preparations of ART have less of an impact on these traditional CVD risk factors. Despite advances in ART, the modern preparations have challenges related to specific CVD risk factors which will be explored extensively later. Given the success of ART, PLWHIV are now living longer and succumbing to traditional diseases of ageing. CVD is currently a leading cause of morbidity and mortality in PLWHIV and increasing burden on healthcare systems. One model has suggested that the median age of PLWHIV will increase from 43.9 years in 2010 to 56.6 years in 2030 with 78% being diagnosed with CVD (16).

1.2.1 Epidemiology

The overall relative risk of CVD in HIV-positive patients, independent of traditional risk factors, based on data from >790,000 participants in a recent meta-analysis, is 2.6 (95% CI, 1.68-2.77) (17). This has also been confirmed in other cohort studies (18,19). There are also sex-specific and regional differences in the prevalence of CVD. In a registry study from the US, HIV-positive women had a higher relative risk of acute myocardial infarction (compared to general population individuals) than men (RR 2.98, $p<0.0001$ versus 1.40, $p=0.0003$) (20). In a French nested cohort study HIV-positive women had nearly double the standardized morbidity ratio for events than men. The majority of this was seen in women under the age of 45 (21). Using the same cohort women were also found to present with an event at a younger age compared to general populations (-6.2 years, $p<0.01$, men: -2.1 years, $p=0.02$) (22). These findings have been replicated from cohorts in Italy (23). Analysis of the US-based VACS cohort found that HIV-positive women had an increased hazard ratio for a CVD event compared to general population women (HR: 2.8, 95% CI 1.7-4.6, $p<0.001$) (24).

CVD is the leading cause of morbidity and mortality in Western nations however, data from poorer countries including Sub-Saharan Africa is lacking (25). In their large systematic review and meta-analysis, Shah *et al* demonstrated that poorer regions, Sub-Saharan Africa, and

Asia-Pacific, had the highest disability-adjusted life years lost to CVD from HIV. The highest impacted nations were Swaziland, Botswana and Lesotho (17).

1.2.2 Protease Inhibitors and CVD

The introduction of PI therapy had dramatic impacts on life expectancy in patients with HIV disease (26,27). However, PI therapy was increasingly recognised to be associated with increased incidence of dyslipidaemia, insulin resistance and lipodystrophy (28). The clinical syndrome of lipodystrophy relates to peripheral fat wasting with central fat accumulation. This may or may not be accompanied by a change in body weight. In their early cohort study, Carr *et al* initially demonstrated that 83% experienced lipodystrophy, 74% developed dyslipidaemia and 16% developed impaired glucose tolerance in those prescribed PI therapy (29). The United States Food and Drug Agency issued an advisory statement in 1997 relating to emerging diabetes and raised blood sugars the four approved PIs at the time: saquinavir, indinavir, ritonavir and nelfinavir (30).

Modern preparations of PI are thought to not have such a dramatic effect on fat redistribution. A systematic review assessing 27 studies concluded that PIs may have an ameliorating effect on subcutaneous lipoatrophy (31). In addition, there was no difference in central and peripheral fat depots, as assessed by computed tomography, between PI therapy (boosted ATZ and boosted DRV) versus raltegravir (32).

Early concerns regarding these metabolic concerns prompted the development of D:A:D group in 1999. This prospective observational study initially followed >23,000 patients for 36,199 patient years for risk factors and the incidence of MI. In 2003 they published their initial findings demonstrating that cumulative use of ART was independently associated with MI (adjusted relative rate 1.26 per year of exposure, $p<0.001$). In addition, higher TC, TG level and presence of diabetes were associated with increased risk of MI (33).

Further data from the D:A:D group demonstrated odds ratios of 3.26 (p=0.0001) and 5.87 (p=0.0001) for single and double PI use for high TG (>2.3mmol/L) compared to ART-naïve individuals. NNRTIs were also associated with increased TC and LDL-cholesterol compared to ART-naive patients (34). A systematic review, including three observation studies with >20000 participants, demonstrated an association of early generation PI use with myocardial infarction (35).

There were several early trials in the 2000's demonstrating an association of PI and myocardial infarction (36,37). The D:A:D consortium published further data in 2007 showing the unadjusted risk of MI for PI therapy of 6.01 / 1000 patient years compared to 1.53 / 1000 patient years in the non-PI group. This increased risk was somewhat attenuated after adjusting for traditional risk factors (1.16 versus 1.05 events / 1000 patient year) and lipids (1.10 versus 1.00 events / 1000 patient years) (38). The incidence of dyslipidaemia with first generation PIs has been shown to range from 24% to 75% (39–41). Cohort studies performed early after the advent of first generation PIs demonstrated an up to 30% increase in TC and up to 80% increase in TG (42).

Initial investigations demonstrated RTV, both in combination and isolation, was specifically associated with increased rates of total cholesterol and triglyceride (43). This risk with ritonavir was also demonstrated in the 2010 publication of D:A:D data on specific antiretroviral preparation. Cumulative exposure to LPV-RTV and IDV (along with didanosine and ABC) were significantly associated with increased risk of myocardial infarction (1.12 /1000 patient years and 1.09/1000 patient years) (44). There was no increased risk from other preparations studied including tenofovir, d4T, AZT and zalcitabine.

Modern preparations of PI, DRV/ritonavir and ATZ/ritonavir, were analysed in a 2018 assessment of the D:A:D data. DRV/ritonavir was found to be associated with an increased risk of CVD whilst ATZ/ritonavir was not. Furthermore, in those at high CVD risk (>10% over 5 years) DRV/ritonavir increased the risk of acute myocardial infarction by 60% over a 5-year

period. This effect was independent of changes in lipids suggesting alternate causative pathways (45). However, other published registry data has shown no association with DRV/ritonavir or ATZ/ritonavir and myocardial infarction (46). In a further analysis of the D:A:D data using Poisson regression analysis, ATZ was found not to be associated with myocardial infarction (47). In the SCOLTA study switching away from boosted PI therapy resulted in improvements in lipid profile (48).

In summary, modern PI therapy has a minimal effect on weight alteration and insulin resistance. Although modern preparations have a beneficial effect on lipid profiles compared to older generations swapping away from boosted PIs improves lipid profiles. Despite this there seems to be a trend towards CVD risk in some modern preparations of PI which is independent of lipid profiles. However, this data is discrepant. Further work is needed to understand possible mechanistic drivers of any potential increased CVD risk seen in PI based regimes.

1.2.3 NRTI

NRTI therapy is considered backbone therapy in many ART regimes and therefore teasing out specific associations with risk of MI is difficult. Despite this, ABC was initially shown to be associated with MI by D:A:D investigators and data relating to ABC is considered separately (49). Despite this difficulty, different preparations of NRTI have been demonstrated to have significant impacts on CVD risk factors in PLWHIV.

AZT was the first class of NRTI and, as described in chapter 1.2.1, had profound impacts on outcomes for PLWHIV. TDF was introduced in and approved by the FDA in 2001 and remains a significant contributor to ART backbone therapy. A further preparation of tenofovir, TAF, was later introduced in 2015 with improved activity against HIV (50,51). Furthermore, there are significant differences in side effect profiles of both drugs.

In a 15-year follow up of the D:A:D study it was demonstrated that TDF/FTC and TDF/3TC had a favourable effect on TG compared to ABC/3TC and 3TC/d4T. In general there was a longitudinal improvement in lipid profiles in those taking ART (52). TAF is lipid neutral (53) whilst TDF has lipid suppressive effects (54). In a study investigating body changes in those switching from TDF to TAF it was shown that TAF was associated with increased weight gain and CVD risk scores (55,56). TAF was also associated with increased rates of DMII compared to TDF in the EMERALD study (57). Despite the slight worsening of lipid parameters when switch to TAF-coformulations, compared to TDF-coformulations, there was no significant increase in CVD risk (58). In the OPERA cohort, patients switching to TAF from TDF demonstrated significant increases in LDL and TG over the first 9-16 months. These changes could not be attributed to changes in third agents (59).

TAF has been demonstrated to be associated with weight gain compared to ABC and TDF (60,61). In the same pooled analysis DTG and BIC were associated with more weight gain than EVG/cobicistat and RPV was associated with more weight gain than efavirenz. Despite the weight gain there were no clinically significant changes in markers of metabolic syndrome although there was limited follow up for this (60). In a national Spanish cohort study investigating changes in weight, lipids, and clinical events in those switching from TDF to TAF, it was demonstrated that TAF was associated with modest weight gain and increased total cholesterol. There were no significant differences in diabetes, hypertension, NAFLD or lipid lowering drug prescriptions (62).

In the COCOMO study, visceral adipose tissue volumes and subcutaneous adipose tissue volumes were assessed using abdominal CT. Previous exposure to the thymidine analogues were associated with increased visceral adipose tissue, hypertension, hypercholesterolaemia and raised LDL-cholesterol. This effect continued even years after termination of treatment (63). Increased insulin resistance has also been reported in those with prior exposure to thymidine analogues (64). These findings hint at a sustained impact of these agents on normal adipose tissue regulation and increased atherosclerotic risk. In their follow up study Gelpi *et al* also demonstrated low adipose tissue density and impaired

adiponectin production was again associated with thymidine analogous in 848 patients from the COCOMO study (65).

Although abacavir has been linked with myocardial infarction there has been no evidence linking other NRTI agents to increased CVD risk (49). This is despite the body of evidence suggesting differing effects on weight gain and lipid profiles between agents.

1.2.4 Abacavir Therapy

In 2008 the D:A:D study group updated published data on their increasing cohort and patient years (33,347 patients and 157,912 person-years). The purpose of this study was to analyse the effect of individual drugs in the era of increasing availability of different ART preparations. This revealed the unexpected finding that ABC therapy was independently associated with increased risk of MI (RR 1.9, 95% CI 1.47-2.45 [p=0.001]). This effect was reversed after patients had not taken ABC for 6 months. There was no association with MI with d4T or zidovudine (49).

This association was unexpected as ABC was not known at the time to have any significant metabolic effect and had not previously been implicated as a contributor to CVD. More so, it was exposure within the preceding 6 months rather than cumulative exposure that had a significant association with myocardial infarction.

This initial finding sparked a wave of publications from cohorts across the world. Within a few months of the initial D:A:D finding the SMART/INSIGHT group published an investigation from their ongoing study. The primary endpoint of the SMART study was to investigate the rate of death / development of opportunistic disease between a drug conservation strategy versus a viral suppression strategy (66). They found that current use of ABC was associated with a HR for myocardial infarction of 4.3 (95% CI: 1.4-13) (67). Further insights from these studies demonstrated CD4 guided treatment interruption resulting in an increase in MI and single IL-6 or HS-CRP measurement was a far better predictor of cardiac events than

traditional risk scores (68,69). This underscored definitively the inflammatory basis for cardiac disease in HIV.

Initial criticism of these two trials centred around the fact that the unexpected result may, in part, be due to channelling or prescription bias. The patients taking ABC tended to have worse CVD risk profiles, previous adherence problems and in patients with chronic kidney disease. These factors may favour the trend towards clinically relevant CVD in patients taking ABC. In addition, there was no clear biological explanation for this effect.

In 2009 Brothers *et al* published data from GlaxoSmithKline-sponsored clinical trials involving ABC including 9502 patients who had received ABC (70). They found no association between ABC and the risk of MI (RR 0.81, CI: 0.38 to 1.75). Some of the potential reasons why the results from this study contradict the D:A:D and SMART findings are that the follow up in these randomised control trials was short. The follow up period tended to be 24 to 48 weeks. In addition, the characteristic of a patient recruited to these studies tends to have less CVD risk as they are clinically stable with fewer co-morbidities.

The Brothers *et al* pooled analysis was the first in a series of investigations that seemed to contradict the initial findings in both the D:A:D and SMART analyses (70). Obel *et al* published results from a Danish cohort in the HAART era (1995-2005). After adjusting for confounders they found the incidence rate ratio of MI, with ABC therapy, to be 2 (95% CI: 1.07-3.76) (71). In contrast to the D:A:D data this analysis showed that the increased risk of MI persisted beyond 6 months. The authors conclude that this effect may represent remaining confounding within their analysis. However, after initiating propensity scoring, they found no single factor associated with increased risk of MI. Also, the risk of MI was also increased in patients who initiated ABC within 2 years of commencing HAART.

Lang *et al* performed a case-control study nested within a French registry. Their initial analysis revealed recent ABC initiation was associated with increased risk of MI (OR 2.01;

95% CI: 1.11-3.64), however, this association disappeared when adjusted for cocaine and intravenous drug abuse (OR 1.27, p>0.05). They conclude that the differences seen in their study could be attributed to the fact that D:A:D and SMART did not adjust for cocaine and intravenous drug use, their study included lower numbers of patients who had received previous ART (24%) thus reducing selection bias and their study did not include recurrent MI unlike D:A:D and SMART (72). Similar results were obtained from a registry study by Triant *et al*. They demonstrated that CD4 count <200/mm^3 was predictive of MI (OR 1.74; 95% CI: 1.07-2.81 p=0.02). In addition, hypertension and being non-white were predictive of MI whilst no individual ART reached significance (73). Similarly, Ribaudo *et al* showed ABC had no significant association with MI but rather traditional CVD risk factors were the biggest contributor (74).

In contrast to these publications in 2011 Durand *et al* performed a nested case-control study analysing the associations of different components of ART. MI was identified in 125 cases and were matched to 1084 HIV-positive controls without MI. Any exposure to ABC was significantly associated with MI (OR 1.79; 95% CI: 1.16 to 2.76, p=0.02) along with EFV (OR 1.83; 95% CI: 1.21to 2.76, p=0.004), LPV (OR 1.98; 95% CI:1.24 to 3.16, p=0.004) and RTV (OR 2.29; 95% CI: 1.48 to 3.54, p=<0.001) (75). This study addressed concerns regarding channelling bias of ABC in patients with higher CVD due to renal failure as TDF was largely unavailable in Quebec (study location) at the time.

Between 2011 and 2016 there were several cohort studies that demonstrated a significant association with recent or cumulative exposure to ABC with myocardial infarction (75–81). During the same period there were also cohort studies showing no association (74,82). Interestingly, three of these analyses were done on the same cohort with differing results. Choi *et al* (76) and Desai *et al* (78) both found positive associations with recent ABC exposure whilst Bedimo *et al* found no association with cumulative or current ABC use with MI. There are several important differences in how these studies were conducted. Firstly, the statistical model used differed between the studies. Both Bedimo *et al* and Choi *et al* used extended cox models whilst Desai *et al* incorporated both extended cox models and

marginal structural models. The rationale of using marginal structural models over extended cox models was to reduce selection bias. Secondly, the comparison group differed amongst the studies. Bedimo *et al* reported 1-year ABC exposure relative to no ART exposure whilst Choi *et al* reported recent ABC relative to no ABC or TDF. Desasi *et al* reported on the combination of ART and their risk of MI as well as individual components.

In 2016 the follow up to the original D:A:D was published (83). Current ABC use was associated with MI with a RR of 1.98 (95% CI: 1.72 to 2.29). There was no difference in the pre, and post March 2008 risk associated with ABC. This analysis was included to address concerns around channelling bias regarding the renal associated CVD risk. There was a reduction in ABC prescriptions following the publication of the original D:A:D data in 2008 which fell from around 20% of the total cohort pre 2008 to 18-19%. There was a greater reduction in ABC prescriptions in higher CVD risk from approximately 29% pre D:A:D 2008 to just above 20% in 2012. This reduction in ABC prescriptions was also confirmed in a separate analysis (84). The authors conclude that this demonstrates that the excess risk seen in ABC is not a consequence of channelling bias of ABC towards patients with a higher CVD risk (83). Similarly, in an analysis of the NA-ACCORD registry in the US and Canada the inclusion of FRS to the marginal structural model did not modify the risk associated with ABC (p=0.14) (85). After adjustment for FRS in the marginal structural model the HR for type 1 MI and type 2 MI and recent ABC use remained at 1.88 (95% CI: 1.20-2.95) (85).

There have been several meta analyses since 2010 which both agree with MI risk from ABC (86,87) and disagree (74,88,89). The latest meta-analysis by Dorjee *et al* shows that the summary RR for MI and recent ABC is significant with a summary RR of 1.61 (95% CI: 1.48-1.75). This increases to a summary RR of 1.91 (95% CI: 1.48-2.46) in ART naïve patients although far few patients were included in this analysis.

The weight of evidence suggests that ABC is associated with CVD risk and work is ongoing to understand possible mechanisms. This is also reflected in modern guidelines which advise avoidance of ABC in high CVD risk groups (90).

1.2.5 Integrase strand inhibitors

INSTI are modern preparation of ART that have proven efficacy in HIV disease (91). There have been increasing concerns regarding metabolic dysfunction and weight gain in those taking INSTI-containing regimes. INSTI are often combined with TAF back-bone therapy and have been shown to cause exaggerated weight gain (92–96). The ADVANCE trial investigating the effect TAF/DTG versus TDF/EFV on weight gain demonstrated mean increases of 6.4kg and 1.7kg respectively. There was also an increase of 3.2kg in the TDF/DTG group (97). There is also emerging evidence regarding increased CVD risk from metabolic syndrome (98).

There have been sex-specific differences in weight gain reported with INSTI although this is not ubiquitous. In the ADVANCE study, women had greater weight gain in the TAF/DTG group compared to the TDF/DTG group, a difference which was not seen in men (97). There were also differences in the NAMSAL study where women had greater weight gain with dolutegravir based regime compared to efavirenz. Again, an effect which was not seen in men (99).

In the ADVANCE study, TAF/DTG had significantly higher increases in QRISK scores and development of DMII compared with TDF/DTG and TDF/EFV (98). There was also greater development of metabolic syndrome in the TAF/DTG group. INTSI have also been shown to be associated with increased HBA1c (100). A recent meta-analysis and systematic review found that DTG-based and TAF-based backbone therapies led to higher weight gains than other ART (101). Again, the NAMSAL study reported a significantly increase in glucose levels in those who reported >10% weight gain (102).

The effect of INSTI on lipids was assessed in a publication of the NA-ACCORD cohort study. They demonstrated that dyslipidaemia was less common with INTSI compared to boosted PIs. Amongst the INSTI group, dyslipidaemia was more common with ETG and RAL compared to DTG (102). Further studies have shown the INSTI has a mostly neutral effect on lipid (103–105). Switching to dolutegravir combined with ABC/3TC has been shown to improve lipids when switching from boosted-PI or NNRTI therapy (106). Specifically, there was a mean reduction in total cholesterol and triglyceride to HDL ratios. A recent study exploring the effect of INSTI on lipid profiles, in both treatment naïve and switching, found that INSTI to have favourable lipid profiles compared to PI and EFV. In treatment naïve individuals RTG and DTG were favourable compared to PI and NNRTI whilst ETG was similar to boosted ATZ In the switching studies (away from boosted PI or EFV) RTG, DTG and BIC were all superior whilst ETG was similar (107). Similar results were found in the RESPOND prospective cohort (>4500 participants). However, the RESPOND paper reports that compared with dolutegravir both ETG and RTG were both associated with higher rates of dyslipidaemia (102).

In a retrospective analysis switching analysis, there were significant increases in TC in the ETG/TAF and RLP/TAF group compared to DTG/ABC groups (108). In further switching studies RTG and ETG based regimes have been shown to have superior lipid profiles compared to boosted PIs (109–111).

In the NA-ACCORD cohort an initial therapy with INSTI or PI versus NNRTI resulted in greater risk of development of diabetes which is mediated through weight gain (112). Further to this, in a study analysing >42,000 cases, INSTI therapy was associated with new onset diabetes in the first 6 months after starting treatment. INSTI therapy was associated with a 31% increased risk compared to non-INSTI regimes with the highest risk attributable to ETG and lowest risk RTG. However, in a retrospective French registry including >19,000 patients INSTI (and other third line agents) was not associated with DMII development. In the multivariate analysis the age >46 and BMI>30 were the only variables associated with development of type II diabetes (113). Reasons for the variation in the main findings of

these studies is not clear. Both are retrospective in design and include large numbers of patients. There are differences in the make-up of the cohorts and time duration for the incidence of DMII. Both studies had <5% use of BIC and similar compositions of the other drugs in class. In both these studies there is an underrepresentation of women. Neither study was able to assess for weight gain and its impact on breakthrough type II diabetes. In a further retrospective study involving 1118 women INSTI was found to be associated with greater increases in HBA1c and systolic blood pressure compared to non-INSTI groups. In addition, the greatest impacts on HBA1c were seen in those with >5% weight gain (114).

The RESPOND cohort, including >29,000 patients (median follow up 6.16 years) demonstrated an increased risk of MI in those taking INSTI. The increased risk was similar across all risk groups (low, intermediate, and high 5-year risks). There was a rapid increase in incidence of outcomes over a two year period which diminished thereafter (115). These findings remained after adjustment for traditional cardiovascular risk factors. This is the first study to report on cardiovascular outcomes in INSTI.

INSTI has seen rapid deployment as a first line ART, largely due to its efficacy against HIV and preferable safety profile. As demonstrated, there are emerging concerns regarding weight gain, particularly in women. Its effects on CVD risk remain unknown.

1.2.6 Risk calculators

Traditional risk prediction algorithms have been shown to underperform in PLWHIV (116). The D:A:D calculator was developed as a HIV-specific calculator and was shown to have superior discriminatory ability compared to FRS (117). A recent comparison of traditional risk calculators, including D:A:D, demonstrated reasonable discriminatory ability for CVD endpoints but statistically did not fit the data (118). Traditional risk calculators have also been used to predict the presence of subclinical CVD (119). Current UK guidelines recommend QRISK2 CVD risk assessment in patients aged >40 years (90). QRISK2 is a previous iteration of a widely adopted traditional risk algorithm for general populations in

the UK. A recent systematic review and meta-analysis assessed 10 different risk prediction models and concluded that in general risk calculators underpredict true risk. However, the Framingham and Pooled Cohort Equation seemed to be better calibrated (120).

FRS, PCE and D:A:D have also been demonstrated to be ineffective at predicting incident coronary artery calcium and plaque progression on cardiovascular CT (discussed later in the chapter) in HIV-positive groups (121)

1.2.7 Current paradigm of CVD and ART

The current BHIVA guidelines (updated 2022) recommend avoidance LPV/ritonavir containing regimes and avoidance of abacavir in those with high CVD risk or established CVD. In addition, if PI therapy is desired ATZ-based regimes may have advantages over DRV-based regimes (90). This is also mirrored in European Guidelines (122).

1.3 Mechanisms of drug effects on CVD development

1.3.1 Protease Inhibitors

First generation PI therapy caused significant metabolic disturbances with impacts on rates of dyslipidaemia, lipodystrophy, inflammation and development of diabetes (35,43,123). Lipodystrophy has three recognised phenotypes: central fat accumulation (lipohypertrophy), peripheral fat loss (lipoatrophy) and a combination. There is no unifying mechanism that drives fat redistribution and is likely a combination of several factors including baseline fat volume, diet / caloric intake, and host genetic response. The underpinning pathological response to ART/HIV pressures involves reduced SAT energy storage with increased VAT expansion.

PI therapy has been demonstrated to have an independent effect on the development of metabolic syndrome (124). The mechanism by which PI therapy exert its effects on this metabolic disturbance are thought to be multifactorial and include the following:

- Activation of SREBP1-c in the nucleus of preadipocytes causing reduction of PPAR-γ and reduction in differentiation of preadipocytes.
- Reduced clearance and increased circulating time of LDL-cholesterol.
- GLUT-4 inhibition reducing peripheral uptake of glucose, increasing insulin resistance, and pushing toward diabetic physiology.
- SREBP induced unfolded protein response causing endoplasm reticulum stress.

These findings have been demonstrated *in vitro* and *in vivo*. The first study to demonstrate reduced glucose uptake analysed the effect of indinavir on cultured adipocytes. Murata *et al* showed that indinavir potently reduced glucose uptake but did not affect GLUT-4 translocation or insulin signalling. They concluded this to be the major source of PI associated insulin resistance (125). This has been reproduced in multiple studies across first generation PI therapy (126,127). Further studies demonstrated similarities in the structure of PIs with the binding site of glucose with GLUT-4 (128). However, whilst this is true of older generation PIs, ATZ has been demonstrated in healthy volunteers to have no effect on insulin sensitivity (127).

PIs have been shown to stimulate hepatocyte SREBP activation in the cell nucleus and inhibits its breakdown via proteasome inhibition in both hepatocytes and adipocytes (129). The consequence of this is increased intracellular cholesterol synthesis in endoplasmic reticulum stress, causing degradation of unfolded proteins and, ultimately, apoptosis. However, there are fundamental differences in the effects of SREBP activation between hepatocytes and adipose tissue. Hepatic SREBP activation promotes increased TG synthesis and promotion of lipogenic / gluconeogenic gene expression. In contrast, adipose tissue demonstrates reduced lipogenic gene expression including PPAR-γ and SREBP-1c (130). The changes in adipocytes favour cell senescence with blunted abilities to store lipid which favours insulin resistance.

Indinavir has been demonstrated to reduce expression of PPAR-γ and SREBP-1c with reduction in differentiation of preadipocytes (131). These effects are mirrored clinically with the common findings of PI induced lipodystrophy and fatty liver (132). ATZ has been shown

to have less of an effect on both hepatocytes and adipocytes compared to older generation PIs (130). Furthermore, it has been shown to have less of an effect on preadipocyte differentiation compared to ritonavir (133). The effect of PI on adipocytes differs by location with SAT being more effected compared to VAT (134). This effect may not be surprising given the fundamental differences between these two fat depots. VAT contains larger numbers of hypertrophied adipocytes which have been demonstrated to have higher insulin resistance and reduced storage ability. Furthermore, VAT has increased infiltration of macrophages with the ability to secrete proinflammatory cytokines that can potentiate insulin resistance and cell damage. There are also major differences in the secretion of adipokines (135).

In summary, PI therapy induces SREBP expression inducing endoplasmic reticulum stress (the unfolded protein response) causing increased hepatic TG production with reduced storage ability of adipocytes (through a combination of reduced lipogenic gene expression and pre-adipocyte differentiation). Taken together, these effects explain the increased trend towards metabolic syndrome and body fat redistribution that is associated with PI therapy. Whilst modern preparations, including ATZ and DRV, have less of an impact on insulin sensitivity than early generation preparations (136), they still may interfere with normal adipocyte homeostasis, specifically in cells undergoing differentiation (137).

1.3.2 NRTI

NRTI agents have been implicated in playing a role in the development of metabolic syndrome and fat redistribution. This is principally through the effect of mitochondrial toxicity inhibiting mitochondrial DNA transcription via inhibition of DNA-polymerase-γ (138). This, in turn, causes impaired energy substrate generation with increased intracellular lipid accumulation which contributes to increasing liver fat accumulation. In addition, production of reactive oxidative species can contribute to proinflammatory signalling driving insulin resistance. First generation NRTI's have been demonstrated to be associated with VAT accumulation which is irreversible (63). They have also been linked with a reduction in SAT mitochondrial density and lipoatrophy (139).

Modern preparations of NRTI may have such a pronounced impact on mitochondrial dysfunction. Tenofovir, 3TC and ABC have been demonstrated to have a minimal effect on mitochondrial DNA polymerase-γ (140–142). In addition, cumulative use of dideoxynucleoside analogues was associated with non-alcoholic fatty liver (as measured by hepatic attenuation on CT) but cumulative NRTI was not associated (143). However, in a study analysing 4 contemporary ART regimes, both TDF/FTC and ABC/3TC caused reduction in mitochondrial density and surrogates of mitochondrial respiratory chain function. In the same study ATZ and EFV were compared and both caused a similar reduction in mitochondrial density and function on fat biopsy through 96 weeks (144).

1.3.3 INSTI

Mechanisms for INSTI induced weight gain are still not fully elucidated. As previously discussed, other ART preparations interfere with normal adipose tissue function through several pathways. Data around mechanistic drivers of weight gain and metabolic dysfunction with INSTI are currently lacking. One study analysed the effect of RTG and DTG on adipose tissue in human and simian models in vivo. Both drugs promoted oxidative stress, mitochondrial dysfunction, and insulin resistance. There was also a shift towards adipocyte hypertrophy and adipocyte inflammation (145). ETG has been shown to impair adipogenesis (reduced expression of PPAR-γ and adiponectin) and promote expression of proinflammatory cytokine in a similar fashion to efavirenz (146).

DTG and BIC have also been demonstrated to promote hypertrophic, insulin resistant adipose cells with greater levels of fibrosis in SIV infected macaques. There was also inhibition of beiging of WAT, a process which promotes fatty acid oxidation and reduces adipocyte size. There was suppression of lipolysis and fatty acid flux with promotion of lipogenic genes (147).

DTG has been associated with reduced expression of PPAR-γ, increased secretion of IL-6 and reduced expression and excretion of adiponectin and leptin when compared to BIC and RTG in differentiating adipocytes (148). Downregulation of adiponectin with DTG treatment has been described before (149). Dysregulation of adiponectin and leptin may induce insulin resistance and a proinflammatory phenotype (150). Some data also suggested that DTG increases appetite through interference with melanocortin-4 receptors (151), although not all data is concordant (152).

In summary, INTSI have been shown to interfere with normal adipocyte function through inhibition of normal preadipocyte differentiation through its effects on PPAR-γ. There are also signs in animal models and cultured cells of a trend towards lipogenic gene expression and reduced lipolysis. In human studies, reduction of adipokines has been shown with potential effects on insulin resistance and a pro-inflammatory phenotype. Studies are currently lacking which offer definitive mechanistic insight into the profound weight gain seen with these agents and the effect on CVD risk.

1.4 HIV – Viral Related effects on adipocyte function

HIV infection per se is thought to contribute to adipocyte dysfunction and promote increasing visceral adiposity, reducing SAT volume and ectopic fat storage. The phenotypical changes seen in HIV positivity also include increasing subcutaneous fibrosis (with and without lipodystrophy) and altered adipokine signalling (153,154). As discussed later in this chapter these changes can cause significant metabolic dysfunction including adverse cardiometabolic profiles.

Latent HIV is present in adipocyte stores in reservoir T cells and macrophages and has not been shown to directly infect adipocytes (155–157). There is local release of accessory HIV proteins including VPR, NEF and TAT. The presence of accessory proteins is detected even in the absence of an undetectable viral load (158). In untreated HIV-positive patients there is reduced gene expression of proadipogenic genes including CEPBA and PPARG – encoding for

C/EBPα and PPAR-γ respectively (159). In addition, there is reduced expression of lipid storage genes and expression of adipokines (leptin and adiponectin) with increased expression of proinflammatory cytokines such as TNF-α in subcutaneous adipose (154,160,161). PPAR-γ is also required for the normal function of mature adipocytes including regulation of GLUT4 expression, expressions of adipokines and tumour necrosis factor-α (TNF-α) (162). Adipocyte hypertrophy is associated with reduction in adipocyte differentiation markers including PPAR-γ (163).

Specifically, VpR has been found to increase hepatic *de novo* lipogenesis, reduce β-oxidation and reduce VLDL-TG export in wild type mice injected with synthetic VpR (164). VpR has also been found, in vitro, to block preadipocyte differentiation and increase lipolysis in mature adipocytes through inhibiting PPARγ and its promotors. These changes induced accelerated whole body lipolysis, hyperglycaemia and hypertriglyceridemia (158). In addition, VpR may contribute to development of hepatosteatosis by reducing hepatic fatty acid ß-oxidation via interactions with the liver X-receptor (164). Tat and Nef have also been shown to induce adipose tissue ageing and induce oxidative stress and mitochondrial dysfunction in human and macaque subcutaneous and visceral adipose tissue (165). Both have also been shown in-vitro to induce a profibrotic phenotype in adipose stem cells which may contribute to increasing adipose tissue fibrosis seen in HIV-positive individuals (166). Nef has also been associated with reduced glucose uptake in adipocytes via inhibition of insulin-stimulated GLUT4 expression (167).

HIV also has a deleterious effect on adipocyte mitochondrial DNA. In one study, involving both treated and untreated HIV positive patients, there was reduced mitochondrial DNA content and reduced expression of mitochondrial encoded proteins. This may cause mitochondrial dysfunction from inadequate respiratory chain function causing promotion of reactive oxygen species which impairs normal lipid storage (168).

In biopsy studies of subcutaneous fat with lipoatrophy there was reduced PPAR-y expression and increased apoptosis (169,170). In their study comparing fat biopsies in PLWHIV with elderly "healthy" individuals, Domingo *et al* found reduced markers of adipocyte differentiation and mitochondrial dysfunction in PLWHIV. In the elderly group, there were markers of senescence and reduced telomere length demonstrating that premature ageing of adipose tissue is only partially responsible for adipocyte dysfunction in PLWHIV (171).

1.5 The origin of insulin resistance: dysfunctional adipose tissue and fatty liver

Insulin resistance refers to the reducing effects of insulin on body tissues. In the context of caloric overconsumption and DMII this stems from chronic over exposure to nutrient excess and insulin. This causes alterations to the proximal insulin signalling cascade including the insulin receptor, insulin receptor substrate and AKT. There is also reduction of translocation of GLUT4 to the cell membrane causing reduced cell entry of glucose (172). To maintain glucose homeostasis there is a compensatory increase in insulin from pancreatic beta cells. In the development of DMII beta cell production of insulin becomes gradually impaired leading to increasing blood sugars.

Insulin has differing effects depending on cell type. Hepatocytes are exposed to up to three times the insulin concentration of peripheral tissues as insulin is secreted directly into the portal circulation (173). Insulin binds to insulin receptor-1 and insulin receptor-2 on the surface of hepatocytes causing phosphorylation and 'cascade' intracellular signalling. There is rapid reduction in hepatic gluconeogenesis and increased glycogen production through direct and indirect pathways. There are also significant effects on lipid metabolism. There is promotion of de-novo lipogenesis through insulin-stimulated lipogenic proteins, the principal one being SREBP1c (174). With increasing de-novo lipogenesis in hepatocytes, there is a reduction in NEFA flux to peripheral tissues.

WAT is the principal energy depot of the body. There are two main depots for WAT: subcutaneous WAT and visceral WAT. WAT regulates energy homeostasis by balancing

glucose absorption with fatty acid lipolysis. Insulin promotes rapid reduction in circulating NEFA by inhibition of adipocyte lipolysis. It causes GLUT4 translocation to the cell membrane increasing glucose uptake. Glucose is used as substrate for DNL and energy storage with reduced lipid delivery to hepatocytes and myocytes. There is also glucose mediated stimulation of ChBREP which promotes lipogenic gene activity. In the context of low insulin, such as the fasted state, lipolysis increases with increasing NEFA flux. The predominant source of lipogenesis in this state is circulating NEFA rather than DNL (175). NEFA delivery to hepatocytes induces increased beta oxidation, decreased glycogenesis, and increased gluconeogenesis.

Skeletal muscle is responsible for most circulating glucose consumption. Insulin receptor-1 is predominant. Insulin binding causes translocation of GLUT4 to the cell membrane which facilitates glucose uptake by the myocyte. Glucose is then utilised for glycogenesis or energy consumption.

The pathological consequences of insulin resistance are complex and not fully described. HS is commonly associated with insulin resistance and describes accumulation of lipid within hepatocytes. There are differing models as to how HS develops in the context of insulin resistance but the concept of selective insulin resistance is thought to play a key role (176). The lipogenic pathway, insulin mediated SREBP1-c upregulation, may remain unaltered or paradoxically may become upregulated which further enhances DNL. The source of substrate for DNL occurs from four main sources: 1) NEFA released from WAT lipolysis, 2) post prandial chylomicrons, 3) hepatic lipolysis from plasma lipoproteins delivered to the liver 4) fatty acids synthesised de novo from non-lipid precursors. DNL is thought to be a major contributor to overall hepatic triglyceride content (174).

One hallmark of insulin resistance is the failure of suppression of hepatic gluconeogenesis. This is thought to be driven through both direct and indirect pathways, but WAT plays a key role. In Insulin resistant WAT there is a relative failure to suppress lipolysis with continued

NEFA delivery to peripheral tissues and hepatocyte. Given the relative increased NEFA substrate there is continued hepatic gluconeogenesis. Hepatic gluconeogenesis is exquisitely sensitive to NEFA and hence WAT insulin resistance may be the first step in development of insulin resistance and DMII.

The principal function of WAT is to modulate energy storage and deliver substrate to peripheral tissues. Maintaining flexibility to deal with states of fasting and over-nutrient supply is an essential function of WAT. In the context of chronic nutrient oversupply there is stimulation of preadipocyte differentiation within WAT to expand the reservoir of storage for fatty acid. This is governed by PPAR-γ which is highly expressed in adipose tissue. There are two key mechanisms of WAT expansion: hyperplasia and hypertrophy. Expanding, hyperplastic WAT has been shown to demonstrate normal cellular function with low levels of insulin resistance / type II diabetes, normal adipokine signalling and low macrophage recruitment and inflammation. In contrast, increasing adipocyte size (adipocyte hypertrophy) is associated with inflammation, reduced levels of adiponectin / leptin and insulin resistance (177).

In general populations, adipocyte expansion is governed by genetic factors, inflammation, and cell senescence. With ongoing energy oversupply there is 'spill-over' of fatty acid causing increased circulating fatty acid promoting visceral and ectopic fat storage. Loss of the "safe storage" of fatty acid promotes inflammation and cellular dysfunction at the site of ectopic fat which further promotes insulin resistance (178).

The prevalence of NAFLD has dramatically increased in recent years. The current global prevalence is estimated at 25% (179). NAFLD is closely associated with a host of deleterious metabolic pathologies including hypertension, DMII and insulin resistance which is commonly characterised as the metabolic syndrome. The most sensitive clinical predictor of NAFLD is DMII with a high global prevalence of NAFLD in patients with DMII of 55% (180).

The overall prevalence of NAFLD in the United States is predicted to increase to 33.5% of the adult population by 2030 (181).

The histopathological consequence of NAFLD, which encompasses a range of progressive inflammatory hepatic syndromes, is underpinned by HS. The defining hallmark of hepatosteatosis is when >5% of hepatocytes accumulates triglyceride. This is driven through TAG deposition in hepatocytes. Progressive hepatic ectopic fat deposition results in inflammation giving rise to the unique clinical entity of NASH. Continued inflammation induces fibrosis causing cirrhotic liver disease which is strongly associated with liver related outcomes and a threefold increase in mortality (182). Hepatocellular carcinoma occurs most frequently in those with established cirrhosis (183) but is also increased in NALFD without cirrhosis compared with the general population (184).

The main sources of hepatic TAG arise from dietary sources (15%), NEFA from expanded WAT (60%) and hepatic DNL (25% depending on fed state) (185). Beyond dietary intake of lipid, fructose consumption has been highlighted as a key player in the development of fatty liver. Fructose is taken up by hepatocytes via unregulated GLUT2 facilitated absorption. It then undergoes metabolism to form glucose, lactate, and fatty acids. This unregulated entry contributes to triglyceride production. In addition, fructose is potent promoter of DNL via upregulation of SREBP-1 and ChREBP (186).

Fatty liver is thought of as the hepatic manifestation of the metabolic syndrome due to its close association. Metabolic syndrome refers to dyslipidaemia, insulin resistance and hypertension and is usually associated with obesity. The huge increase in prevalence is due to increasing rates of inactivity and high calorie food consumption. Although many patients with hepatosteatosis are overweight there has been recent interest in MAFLD in normal weight individuals. There are significant regional variations in rates of lean-MALFD with the highest risk being in Latinx individuals (187). The significant genetic contribution to this process is still being fully characterised (188).

The dyslipidaemia associated with metabolic syndrome is traditionally characterised by high LDL low HDL and high TG. This profile has consistently been shown to be associated with CVD and is thought to be causative. The composition of LDL is important in atherogenesis, and two phenotypes have been described. Type A is characterised by large 'buoyant' LDL particles and type B by small-dense LDL. Hepatic TG availability plays a key role in determining the subclass phenotype an individual produces. In the context of low triglyceride availability hepatocytes produce TG rich VLDL and IDL which is triglyceride poor. Following the effects of lipoprotein lipase and hepatic lipase larger, type A, LDL particles are produced. In triglyceride rich environment hepatocytes are pushed to produce two classes of VLDL, one which is triglyceride rich, VLDL-1 and one which is triglyceride poor, VLDL-2 (189). The end product of this pathway is small dense-LDL which has a longer circulating time and enhanced rates of oxidation which drives atherosclerotic disease.

The inflammatory pressures from HS and WAT are key drivers in the atherosclerotic process. In the liver, this process is demonstrated by the histopathological process of progressive NASH and fibrosis. Increasing NEFA delivery to hepatocytes causes mitochondrial stress from increased reactive oxygen species. There is production of TNF-α and IL-6 which activate Kupffer cells and further heightens insulin resistance through effects on the insulin receptor signalling (190). WAT contains significant amounts of immunomodulatory cells. Macrophages account for 10% of the mass in lean individuals but can reach 50% in obese people (191). As obesity and insulin resistance proceeds there is increased expression of M1, proinflammatory macrophages which is mediated through TNF-α, IL-6 and lipopolysaccharide. In contrast, pressures to express M2-anti-nflammatory macrophages are driven by adiponectin and PPAR-γ through weight loss (192). WAT increased rates of macrophage recruitment have been demonstrated with increased adipose size (193).

Alongside the liver parenchyma EAT is a principal depot for ectopic fat. EAT lies between the myocardium and visceral pericardium. Embryologically it is distinct from pericardial adipose tissue, and they have different vascular supplies. EAT lies directly over the myocardium and

adventitia of the coronary vessels. They share a common microcirculation and EAT supplies the adventitia of the vasculature via the vasa vasorum. Physiologically, EAT acts as an energy source for the underlying myocardium. It can rapidly synthesise fatty acids which acts as the energy substrate of approximately 70% of myocardial energy expenditure. Furthermore, it rapidly absorbs free fatty acids and thus acts in a cardioprotective role from the effects of lipotoxicity.

Beyond its regulation of myocardial energy, EAT has several other cardioprotective functions. It provides structural support to the myocardium and coronary arteries. EAT also displays brown adipose tissue functions and protect the heart from hypothermia via non-shivering thermogenesis. EAT also exerts significant local metabolic regulation by production of anti and proinflammatory cytokines and adipokines. The close relationship via a common vasculature allows EAT to exert a paracrine and vasocrine effect on myocardium and coronary vessels.

The impact of HS, EAT and ectopic fat deposition augmented by HIV-specific factors is of keen interest. HIV and ART clearly interfere with normal adipocyte function and may promote changes which shift energy metabolism towards visceral and ectopic storage with all the deleterious metabolic dysfunction that ensues. This may be a key driver for the excess risk CVD seen in PLWHIV, which as of yet, is largely undetected by traditional risk scoring algorithms.

1.6 The role of Cardiovascular CT as an imaging biomarker

1.6.1 Introduction

The impact of cardiovascular CT within diagnostic cardiology has been far reaching and profound. The clinical utility of this modality is underpinned by excellent sensitivity (99%) and negative predicative value (97%) for detecting significant coronary artery disease (194). Over the last decade, technological advances in form of increased gantry spin times and fast single heartbeat scanning have driven improved temporal resolution together with lower

radiation exposure. These advances have been realised through prospective “gating” of the CT study. Coronary arteries are mobile throughout the cardiac cycle and imaging during the phases where they are least mobile is paramount to image quality. This occurs typically at 50-80% of the R-R interval. The X-ray tube is only active for a specific duration of the cardiac cycle, for example 50-80% of the R-R interval, thus reducing radiation exposure (195).

Increased gantry spin times and multi-detector technology have allowed development of specific algorithms that allow the whole heart scanning in a single heartbeat. For example, the time required for one rotation of an X-ray tube is reduced by increasing the gantry spin times and effectively halved by adding a second X-ray tube. This allows acquisition of the whole heart volume in a single beat at a specific point of the R-R interval. The patient is then moved rapidly through the Z axis to cover the whole heart in a single acquisition.

In addition, vendors offer models with increased number of detector rows (256 and 320 slice) which allows coverage of the entire heart in a single acquisition. The advantages of these techniques are that the X-ray tube is only on for a small amount of time, and it alleviates stitching artefact as the whole image is acquired in one sequence. All the major CT vendors have recently introduced systems incorporating these advances. Rigorous heart rate control also essential for keeping the effective radiation dose down. The effective dose given to an average patient doubles when a heart rate increases from less than 55 to 60. It doubles again with an increase to 65 (196).

The culmination of recent advances led to a change in NICE guideline for assessment of chest pain of recent onset. CTCA is now recommended as the first line investigation in patients with typical or atypical chest pain. This represents a dramatic change from previous versions of the guidance with a move away from calculations of pre-test probability and the previous emphasis on functional imaging (197). The radiological infrastructure within the UK is significantly behind other European health systems and the change in NICE guidance will

contribute significantly to increased demand. The BSCI produced a report on the provision of cardiac CT which estimated a UK wide shortfall of 43% (198).

The importance of CTCA as the principal non-invasive imaging modality in stable chest pain patients is highlighted in the 5-year outcomes from the SCOT-HEART study. When added to standard care, CTCA was associated with fewer CVD deaths or non-fatal MIs (HR: 0.59; CI 0.41-0.84. p=0.004) (199).

1.6.2 Coronary Artery Calcium Score

The pathological evolution of atherosclerosis is a dynamic process involving varying inflammatory insults to the arterial intima which ultimately results in the development of coronary plaque (200). The evolution of atherosclerosis is highly variable and there is a spectrum of atherosclerotic disease with distinct plaque characteristics. One common final pathway for plaque progression is calcification (201). Coronary artery calcification (is associated with total atherosclerotic burden and advancing disease phenotypes (202). There are several methods used to quantify the burden of calcification including the Agatston score, calcium volume and more recently calcium density. The Agatston score is the most established of these methods due to its good reproducibility and high accuracy. The Agatston calcium score was initially developed in the 1990s as a tool for quantifying the degree of calcium within the coronary arteries. Calcification within the coronary artery is defined as 130 HU and >1mm^2 in size. The method for calculating the Agatston score relies on calcium density and total area of calcification (described in table 1). Early work was performed using single beam electron beam computed tomography and later expanded to multi-detector computed tomography (203).

Agatston Calcium Score
Density in Hounsfield Units (HU): • 1: 130-199HU • 2: 200-299HU • 3: 300-399HU • 4: >400HU This weighted score is then multiplied by the area mm^2.
Table 1: The calculation of an Agatston calcium score (8).

Several large cohort studies have established CACS as a valuable tool to assess future CVD risk for general populations (204–206). The outcome of CACS is traditionally categorised into different strata reflecting the differing CVD risk. Unsurprisingly, the lowest risk of any coronary event is in the CACS 1-100 strata (HR: 3.61) and highest in those greater than 300 (HR: 9.67) (9). Although the CACS technique has been in utilization for nearly two decades, there have been recent advances incorporating its use regarding risk stratifying individuals. It provides incremental benefit above traditional CVD risk prediction models and is underpinned by the ability to potentially reclassify patients assessed by traditional CVD risk scores (207–211).

Recently CACS has been added to traditional risk factors to make a risk prediction tool. The ASTROCHARM tool measures 10 year atherosclerotic CVD risk and was developed using three large cohorts and validated against a fourth (212). The ASTROCHARM calculator again demonstrated incremental value of added CACS above FRS (improvement in C-statistic of 0.03 and NRI of 0.12). A previous CVD risk calculator developed using MESA data was validated in an older cohort (mean age 65 in MESA and mean age 51 in ASTROCHARM) (213). Statins are indicated in patients over 50 with a CVD risk profile >7.5%-10% respectively (214,215). Given that age is a significant independent CVD risk factor the ASTROCHARM tool has potentially greater clinical utility (216).

The development of integrated risk prediction tools enables enhanced decision making around primary prevention medications and risk factor modification. The current European guidelines recommend use of CACS in intermediate risk patients (evidence level: IIa) (217). In a review of the current evidence Greenland *et al* have made specific recommendations regarding the CVD risk in context of CACS. Essentially, if the 10 year risk of a CVD event is 7.5 to 20% but the CACS is zero, then statin therapy may not be required due to the lack of impact on events (218). In addition, a recent Danish follow up study demonstrated that CVD events in CACS of zero (and no non-calcified plaque) were still low through 4.2 years in the highest LDL strata (219).

The scope for CACS +/- CTCA to be utilised in high-risk patient groups in which FRS may underestimate true CVD risk is increasing. For example, CVD risk calculators in HIV (and other chronic inflammatory diseases) are known to underestimate true CVD risk (116). Patients with HIV have been shown to have greater CACS than matched non-HIV patients, illustrating the greater burden of subclinical coronary atherosclerosis (220). There are currently no validated risk predication tools which incorporate CACS in these higher risk populations.

Recently data from the PROMISE cohort has been reported around the prognostic benefit of a CACS of zero in patients (n=8811) symptomatic of chest pain. Evaluating the performance of CACS to functional imaging the investigators found that, in symptomatic patients with CACS of 0, the annualised event rate was <1%. Out of 133 events there were 21 patients who had a CACS of 0 (15.8%) and of those patients only two had a severe non-calcified (>70%) stenosis. The risk of having an event with a severe non-calcified stenosis (>70%) with a CACS=0 was 1.4%. Of these 21 patients roughly half had normal coronary arteries (52%) thus the “event” may have been secondary to a type II MI, embolism, or coronary spasm. There were no data on plaque morphology. Having a positive CACS (one or above) was able to predict 83% of future CVD events, whilst a positive functional test only predicted 33% (221). These data add to historical evidence that CACS of zero is associated with a very low risk of CVD outcomes (222).

Coronary artery calcification can be detected on non-dedicated CT scans of the thorax with excellent diagnostic accuracy (223,224). It is also possible to quantify the severity of coronary calcification using a simple ordinal scoring system (mild to severe). This is recommended by the BSCI (225). Incidental findings of coronary calcification should prompt review of modifiable CVD risk factors including preventative pharmacotherapy. The extent by which this reduces CVD events is yet to be determined.

1.6.3 CTCA and functional assessment

Fractional flow reserve computed tomography (CT derived FFR) utilizes computational fluid dynamics to predict the functional significance of coronary artery lesions. In the current, and only commercially available model, data from standard CTCA datasets is transferred to the vendor's server with a report emailed back within a few hours. The report demonstrates the coronary tree with CT derived FFR results reported in all coronary segments. In the similar fashion to invasive FFR, <0.8 is considered functionally significant (226). The ability to combine both an anatomical and functional test is extremely appealing and this technology is well validated in terms of accuracy and safety (227–230). Invasive FFR is a well-established technique for quantifying lesion specific ischaemia and is comparable to functional imaging (231). To date, no trials with large numbers of patients have presented a comparison of myocardial functional imaging versus CT derived FFR.

Modality	Lesion	Sensitivity	Specificity	PPV	NPV
CT derived FFR(232)	>50%	86 (77-92)	79 (72-84)	65 (56-74)	93 (87-96)
CT derived FFR(229)	Per patient	90	54	67	84
Table 2: Comparison of accuracy of CT derived FFR					

The technique has several constraints. Firstly, good image quality is paramount. A recent series of CT derived FFR reported the rate of unsuitable studies as high as 13% (232). In clinical practice, the rate of unsuitable datasets may be higher secondary to inappropriate heart rates (including atrial fibrillation), patients body habitus, poor contrast evolution and problems with artefact (such as breathing and movement artefact). Patient preparation and heart rate control must be rigorous to obtain quality images to utilise CT derived FFR. The

advent of modern CT scanners will also enhance quality. Secondly, concerns remain around the diagnostic accuracy in "FFR grey-zone" – CT derived FFR 0.7-0.8. This was reported as 46.1% in a recent systematic review assessing 536 patients from five studies (233). Thirdly, there is no data in patients having already undergone revascularisation.

Currently this service is limited to a few dedicated centres in the UK from a single vendor, HeartFlow. NICE have recently updated their guidance to suggest how CT derived FFR may be considered in patients with recent onset or stable chest pain (234).

As this technology continues to mature the initial results from the ADVANCE registry unsurprisingly show the strongest predictor of positive CT derived FFR is stenosis >70% (235). Invasive studies of FFR have proven there is a disconnect between anatomical assessment of coronary stenosis and the physiological impact of those lesions (236). The ADVANCE registry again demonstrates this disconnect as despite >70% stenosis being the greatest predictor of CT derived FFR <0.8, there was nearly a third of severe lesions (28.4%) that were functionally insignificant. Similarly, in patients with non-obstructive coronary anatomy (stenosis grading 30-49%) there was positive CT derived FFR rate of 20.8%.

Very recently the 1-year data from the ADVANCE registry was published demonstrating low rates of MI in CT derived FFR >0.8 (0.19% of total FFR>0.8 group). In addition, 92.9% of individuals in which medical therapy was recommended remained free from revascularisation or MACE at 1 year (237).

As the CT derived FFR technology is refined over the coming years, it is expected to become increasingly available across the NHS. The economic impact of this has been considered by NICE when compared to invasive angiography. Conservative estimates show this test could be used in approximately 40,000 patients per year with savings per year of £9.1 million from reduced referral for functional investigation or invasive investigation (234). CT derived FFR is certain to have an increasing presence in the diagnostic armoury of the cardiologist over the

near future, particularly as it offers results on anatomy and physiology in a single test/single visit.

As with all non-invasive cardiac imaging – patient selection will be paramount in delivering this service. In addition, centres will require robust systems for optimizing CTCA dataset acquisition to ensure quality images for CT derived FFR analysis.

1.6.4 Plaque Quantification and Morphology

The analysis of plaque morphology is becoming increasingly important. The ability of CTCA to visualise the entire vessel has several advantages in terms of assessing plaque. There has been extensive recent work on how plaque morphology may impact primary prevention, predictors of ischaemia and prognosis.

The seminal work by Motoyama *et al* established the CT based concept of vulnerable plaque (VP) identification on CT, which confers a significantly heightened risk of acute coronary syndrome. They assessed 3158 patients for three high risk features which are readily identifiable on routine CTCA: low-attenuation plaque (<30 Hounsfield units), positive remodelling of the coronary vessel (remodelling index of >1.1) and spotty calcification. The event rate was 16% over a median following up of 3.9 years (238). High risk plaque found on CTCA, specifically the plaque composition (necrotic core / fibrous plaque ratio), correlates with TCFA which is seen on IVUS (239). Positive remodelling and low-attenuation plaque have also been demonstrated to be associated with TCFA with macrophage infiltration on OCT (240).

Further work around vulnerable plaque in type II diabetics has recently been published which challenges the high-risk nature of vulnerable plaque morphology. A series by Halon *et al,* which included 630 patients, showed that vulnerable plaque caused acute coronary syndrome in 3.5% of instances over median follow up of 9.2 years compared with 0.6% of other plaques (241). Put in other words, 96.5% of vulnerable plaque never cause an event

and the risk attributable to vulnerable plaque was the same as stenosis >50%. The rate of statin therapy was similar at baseline and follow up between ACS cases and non-ACS cases (75-80%). Whilst there are important differences in the demographics between these two studies, some of the difference in event rate attributable to VP could be due to baseline statin use. In the Motoyama *et al* (41) cohort, the statin rate after initial CTCA was 38.9% compared to 80% in Halon *et al* cohort (44).

Despite some disagreement on the exact magnitude of the risk conferred by vulnerable plaque, the detection of vulnerable plaque on CTCA is frequently regarded as an indication for aggressive primary prevention strategies. Whilst statins are considered to help reduce the size of plaque and increase calcification there does not seem to be major evidence that treating those patients with a CACS of zero confers any benefit in terms of hard cardiovascular endpoints. In a cohort of 13644 patients statin therapy was shown to reduce the CVD endpoints in patients with evidence of any coronary calcification (adjusted sub-hazard ratio 0.76) whilst in CACS=0 group there was no risk reduction (sub-hazard ratio =1) (242). We do not yet know the impact of treating only non-calcified plaque with aspirin and statin therapies, however this seems the obvious thing to do.

Beyond vulnerable plaque there are several markers obtained on routine CTCA datasets that are used to attribute risk. There have been recent calls to standardise the reporting of CTCA and move away from a qualitative approach that is mostly employed clinically. This is outlined in the CAD-RADS document and may involve utilizing semi-automated scoring systems alongside traditional luminal stenosis (243). Diameter stenosis, specifically obstructive coronary artery disease, is still the most clinically relevant tool that prompts referral for invasive assessment and is the marker associated with adverse outcome (244). Other markers such as SSS and SIS are used to quantify the burden of disease and both show prognostic value (245,246).

The addition of individual plaque characteristics to diameter stenosis can improve prediction of functionally significant lesions. Sophisticated semi-automated systems are used to quantify plaque characteristics such as AutoPlaque©. These systems are limited to research tools and currently have limited scope in the clinical domain but may well enter routine clinical practice in the foreseeable future.

This disconnect between anatomical assessment and functional importance of a lesion is likely multifactorial. Firstly, the grading of a stenosis may be inaccurate. Secondly, the location of the lesion and the amount of myocardium subtended is likely to have a role. Thirdly, the plaque morphology at the site of stenosis is likely to portray underlying endothelial dysfunction. The metabolically active plaque confers more endothelial dysfunction which may contribute to ischaemia secondary to reduced nitric oxide bioavailability (247). To this end, Doris *et al* recently published an analysis of CT derived FFR in non-severe lesions on CTCA. They demonstrated that the best predicator of total vessel ischaemia was total plaque volume (OR 2.09) compared with calcified plaque volume, non-calcified plaque volume and low density non-calcified plaque volume (OR 1.36, 1.95 and 1,95 respectively) (248).

Total plaque volume has also been shown to be discriminatory of ischaemia when added to stenosis severity. Øverhus *et al* recently published data demonstrating the superiority of total vessel plaque versus proximal plaque in predicating ischaemia. This sub-study of the NXT trial demonstrated improvement of the area under the curve of 0.83 versus 0.81 when whole vessel low density non-calcified plaque volume was added to diameter stenosis versus proximal plaque. Stenosis severity alone had a AUC of 0.78.(249) A whole vessel approach is more predicative of overall plaque burden as it takes into account distal disease.

Total plaque volume across the entire coronary tree is also associated with cardiac related death. Hell *et al* retrospectively assessed 2748 patients and found that total plaque volume >179mm^3 was associated with a HR of 2.3 (CI 1.09-4.58; p=0.022) of cardiac death over a

mean follow up of 5.2 years (250). This is unsurprising as higher plaque volume portrays more advanced disease phenotypes.

Changes in plaque morphologies over serial CTCAs have the potential to show benefit from primary preventative strategies; the hypothesis being that one would be able to demonstrate to patients reduced plaque volume / vulnerable plaque / stenosis severity in response to specific interventions. Statin therapy is the single most significant pharmacological primary prevention medication and is well documented to reduce events in secondary prevention cohorts and moderate to high risk primary prevention cohorts (251). It's influence on coronary plaque has been described in IVUS-VH studies previously which show reduction in total plaque volumes and increases in dense calcium plaque volumes, although there was no effect on lipid rich cores (252). Also, the statin effect on coronary plaque is not uniform across agents or dosages (253).

On serial CTCA, total plaque volume has consistently been shown to be reduced and calcified plaque volume increased if a significant LDL-lowering target is achieved (254). This progressive calcification in the setting of lipid lowering therapy is yet to be fully elucidated but the effect likely represents plaque stabilization. This should not be confused with the fact that increasing CAC score confers increasing risk as discussed. Calcium on CACS is a surrogate for total plaque volume and the extent of potential disease. Increasing calcium arcs within a particular plaque is a sign of a more stable plaque (255,256). These facts call into question the utility of performing serial CACS on any patients taking statin medication without considering density. There are also newer models being developed to try and improve on the basic Agatston score (257).

1.6.5 Cardiovascular CT in HIV

Cardiovascular CT has been used to investigate subclinical CVD in HIV-positive populations. Its ability to inform on multiple metrics relating to atherosclerotic burden as led to its increasing use as a surrogate for CVD risk. Outcome studies relating to CVD in HIV-positive

populations would require several thousand participants due to the low number of events per person-years. This has led to the emergence of CTCA as a research tool to investigate the prevalence and pattern of coronary atherosclerosis, as well as informing on mechanistic insights.

1.6.6 Coronary Artery Calcium and HIV

CACS was first utilised for assessing CVD in PLWHIV nearly two decades ago. Talwani *et al* utilised electron beam CT to assess differences in coronary artery calcium in 60 patients whom 41 were being treated with PI (258). Further work in 2005 demonstrated that HIV patients had a higher amount of CACS above the 90th centile compared to controls (259). The initial coronary calcium report from the Multicentre AIDS Cohort Study suggests that, after adjustment for common clinical covariates, both HIV infection and increasing age had significant odds ratios for the presence of coronary calcium. However, this was tempered by data showing that patients taking ART for greater than 8 years had significantly lower CACS (260). Sequential studies demonstrated an increased vascular age (261) and increased CACS with HIV-associated metabolic syndrome (262). However, despite these positive associations, a meta-analysis of observational studies by Hulten *et al* concluded that HIV positivity was associated with a small increase in carotid intima media thickening but not the presence of coronary calcification (263).

Interpretation of these early studies looking at the association of CACS and HIV covariates is difficult due to the cross-sectional methodology. Furthermore, the study size was limited in most trials. With the advent of CTCA, the focus on CACS as a surrogate for CVD risk shifted to interpretations of plaque morphologies and stenosis quantification.

The application of CACS in risk prediction in PLWHIV is limited thus far. Raggi *et al* demonstrated that CACS >100 was an independent predictor of CVD events in PLWHIV (264). Pereira *et al* assessed the performance of traditional CVD risk prediction tools and found that the application of CACS reclassified 43.1% of intermediate risk patients (265).

However, CACS is known to underestimate risk in PLWHIV as they have been shown to have higher proportions of non-calcified plaque compared to general population groups (266). The ongoing REPRIEVE study investigating the effect of statin therapy using CACS and CTCA will further help delineate the benefit of statin therapy in PLWHIV (267).

1.6.7 CTCA and HIV

CTCA has several clinical applications. There are several large multicentred CT registries that have furthered our understanding of the influence of plaque burden on CVD outcomes. The number of obstructed vessels and number of segments with plaque have been shown to be associated with adverse outcomes (268). CTCA has been shown to demonstrate incremental prognostic information above traditional risk factors and CACS (269).

The application of CTCA in PLWHIV has been increasingly used to delineate plaque morphology, plaque burden and CVD risk. The initial studies investigating the relationship of HIV characteristics and CVD centred on CACS, as previously described. An initial meta-analysis, published in 2015, summarised early CTCA studies showing that HIV infection was associated with >3-fold prevalence of non-calcified plaque but no difference with rates of coronary stenosis or prevalence of calcified plaques. Rates of non-calcified plaque were also associated with lower CD4 cell counts (270). Further to non-calcified plaque, HIV positive patients have also been demonstrated to have higher prevalence of vulnerable plaque (271).

Increased rates of non-calcified plaque seen in patients with more advanced disease likely point towards an inflammatory and immune-mediated cause. Increased prevalence of non-calcified plaque is associated with markers of inflammation even in those with low or undetectable viraemia (272). A higher burden of non-calcified plaque is also demonstrated in elite controllers, HIV-positive patients who maintain suppressed viral load without ARVs, compared to general population controls (273). Given the association of HIV-positivity and non-calcified plaque, unsurprisingly, work involving the MACS cohort have demonstrated an increased prevalence of CACS of zero with non-calcified plaque (266). This may have an

impact on the utility of CACS for risk quantification. Very recently, Pereira *et al* demonstrated how use of CACS changed patients risk group (265). However, given the above studies the extent to which CACS alone can be used to accurately delineate CVD remains to be seen.

Two large cohorts have utilised CTCA in PLWHIV: the MACS cohort and the Swiss HIV Cohort. Both cohorts compare HIV-positive patients to general population patients. Recent publications from both groups suggest different burdens and morphologies of coronary plaque. The Swiss HIV cohort found no significant difference between rates of non-calcified/mixed plaque between HIV-positive and general population patients, with lower calcified plaque in the HIV group (274). Advanced immunosuppression was associated with non-calcified and mixed plaque and viral load >100,000 was associated with CAC. The MACS cohort found a significant association with all types of plaque type but not with significant stenosis (275). There are obvious and important differences between these two cohorts which may explain the differing results. The Swiss cohort may represent a better treated group with lower rates of smoking and lower rates of HIV-related risks. There were also significant sex differences (MACS included only men who had sex with men) and racial differences and differences in BMI. A further cohort from Baltimore, USA investigated the presence and extent of coronary plaque in African Americans. They found that coronary plaque was significantly associated with CVD risk calculation and that HIV infection was not significantly associated with the presence of subclinical CVD. The authors conclude that differences may be due to methodological differences in measurement of non-calcified plaque, older age and worse risk profiles in the MACS cohort compared to the Baltimore cohort (276).

The impact of ARV regimes on atherosclerotic plaque has also been assessed although the data is discrepant. Data from the MACS cohort was published in 2016 showing no consistent significant association between ARV regimes and presence and extent of coronary plaque in well treated patients (277). Data from the large Swiss HIV Cohort suggested noncalcified and mixed plaque was significantly associated in patients with exposure to abacavir. Negative associations were found for emtricitabine with noncalcified/mixed plaque, TDF with any

plaque and efavirenz with calcified plaque (278). Both papers utilised the statistical technique of inverse probability of treatment weighting to adjust for channelling bias for certain ARV regimes. Important differences between the MACS cohort and Swiss HIV cohort exist including racial, sex, cardiac risks and use of lipid lowering agents. These differences make it difficult to directly compare these two cohorts.

The influence of statin therapy on CVD risk in PLWHIV is of great interest. Traditional CVD risk calculators recommend statin therapy in intermediate and high-risk patients which has an emphasis on levels of LDL-c (279–281). Whilst LDL-c lowering is the principle aim of lipid lowering therapy statins are known to attenuate CVD risk via their immunomodulatory and anti-inflammatory effects (282–284). In 2019 Whelton *et al* investigated the relationship between lipids and coronary plaque in the MACS cohort. They concluded that the relationship between atherogenic lipid markers was weaker for HIV-positive patients compared to negative patients. TC/HDL has the strongest association for both HIV-positive and negative patients (285). The triglyceride to HDL ratio was not reported on despite triglyceride levels demonstrating a stronger relationship across plaque phenotypes and different multivariate models. TG/HDL ratio is also strongly associated with metabolic syndrome and CVD in non-HIV populations (286,287). Given that the relationship between lipid indices and coronary plaque seems to differ in PLWHIV it suggests differences in the pathogenesis of coronary plaque. Therefore, traditional risk calculators (which rely on these indices), and preventative pharmacotherapy are likely to have different applications in PLWHIV.

The impact of statin therapy and the degree to which it attenuates risk is the subject of the REPRIVE trial which is currently recruiting (267). Work by Lo *et al* in 2015 reported on a randomised control trial investigating the effect of atorvastatin on aortic inflammation and coronary plaque in PLWHIV using serial CTCA. No significant changes were demonstrated in aortic inflammation but there were significant reductions in non-calcified plaque volume and high risk plaque features compared to placebo (288). Further work by the same group demonstrated that statin therapy has also been demonstrated to reduce HS (289). A further report by Foldyna *et al* assessed changes in coronary plaque on a lesion-by-lesion basis. They

found that statin therapy reduced plaque progression by reducing the fatty and fibrotic plaque components (290). Similar findings have been found in non-HIV populations, principally from the PARADIGM registry trial (291).

The impact of sex and race on the development of CVD in PLWHIV is under investigated. The MACS cohort is exclusively male while the Swiss HIV Cohort is 85% male. Foldyna *et al* compared 48 HIV-infected women to 97 HIV-infected men demonstrating lower prevalence of plaque and lower VP in women (292). This may suggest there a sex-specific reduction in cardiovascular risk in PLWHIV although the mechanistic process for this is unknown. In a general population black people are known to exhibit lower calcified plaque compared to other races (293). There was no significant difference between sexes in the Swiss HIV cohort (274). In a sub-study of the MACS cohort, Miller *et al* demonstrated lower incidence of calcified plaque and stenosis >50% in HIV-positive black men compared to non-black men. This suggests a similar pattern of atherosclerosis development seen in non-HIV populations.

Serial CTCA has also been utilised to assess plaque progression. Increasing burdens, usually quantified as volumes of non-calcified and calcified plaque, have been used as a surrogate for disease and risk progression. The Swiss HIV-cohort study published longitudinal data comparing HIV-positive and general population individuals undergoing sequential CTCA. Using CACS, SIS and SSS, they demonstrated no significant differences between rates of progression between HIV-positive and general population patients. High FRS in both groups was associated with increased rates of progression (294). In the MACS cohort they assessed progression through increasing luminal stenosis, new stenosis of >50% and SIS increase. They also found that there was no significant difference in rates of progression between HIV-positive and general population patients. However, in those with evidence of ongoing viraemia (31% of the HIV-positive group), there was significant progression compared to well controlled (no viraemia) HIV-positive patients and general population patients (RR 2.3, 95% CI: 1.32-4, p=0.003) (295).

In a further analysis of the MACS cohort Shaikh *et al* measured total plaque volume and non-calcified plaque volume in 495 cases (284 HIV-positive, 211 general population) who had sequential CTCA's. They assessed the impact of CVD risk scores on plaque progression. They demonstrated that FRS and PCE better predicted progression in the general population group compared to the HIV-positive group. In the HIV-positive group those identified as high-risk by the D:A:D calculator had roughly double the risk of progression compared to the moderate and low risk groups (adjusted OR:2.08, 95% CI: 1.12-3.88m $p<0.05$). Furthermore, ROC curves compared the ability of each risk score to predict plaque progression in both groups. The PCE performed slightly better than FRS in predicting progression using total plaque volume in the HIV-positive group (AUC 0.60 versus 0.52, $p<0.05$) (121). These plaque progression studies broadly demonstrate no significant differences in atherosclerotic plaque progression depending on serostatus. It also highlights the deficiencies in risk prediction tools in HIV-positive patients.

1.7 The role of ectopic fat in CVD risk

HIV-positive individuals have been shown to develop NAFLD at lower body weights compared to general population individuals (296,297). As previously discussed, this may be because of viral and ART related changes on adipocyte and hepatocyte metabolism with pressures towards 'central' fatty acid deposition. The principal sites for this are visceral adipose tissue, hepatic parenchyma and epicardial adipose tissue.

1.7.1 Hepatosteatosis

The prevalence of NAFLD has dramatically increased in recent years. The current global prevalence is estimated at 25% (179). NAFLD is closely associated with a host of deleterious metabolic pathologies including hypertension, DMII and insulin resistance which is commonly characterised as the metabolic syndrome. The most sensitive clinical predictor of NAFLD is DMII with a high global prevalence of NAFLD in patients with DMII of 55% (180). The overall prevalence of NAFLD in the United States is predicted to increase to 33.5% of the adult population by 2030 (181).

The histopathological consequence of NAFLD, which encompasses a range of progressive inflammatory hepatic syndromes, is underpinned by HS. Progressive hepatic ectopic fat deposition results in inflammation, giving rise to the unique clinical entity of non-alcoholic steatohepatitis. Continued inflammation induces fibrosis causing cirrhotic liver disease which is strongly associated with liver related outcomes and a threefold increase in mortality (182). Hepatocellular carcinoma occurs most frequently in those with established cirrhosis (183) but is also increased in NALFD without cirrhosis compared with the general population (184).

HS has been linked with increased subclinical CVD (298), increased risk of CVD events (299) and CAC progression (300) in general populations. A comprehensive systematic review has demonstrated a positive association with HS and multiple surrogates for CVD including carotid intima media thickness, CAC, endothelial dysfunction and arterial stiffness (301). HS has also been demonstrated to be a risk factor for CVD on invasive coronary angiography (302). In contrast several studies have shown no significant association with NAFLD and CAC (303–305). The degree to which HS influences CVD risk in PLWHIV is yet to be fully described.

In PLWHIV the prevalence of HS is between 13 to 65% (306–308) and is the most common cause of liver disease (307). The traditional metabolic risk factors for development of HS in non-HIV populations are common in PLWHIV. Dyslipidaemia and diabetes are a common association with HS (309). Whilst the relationship of obesity and HS is well defined in general populations it remains more complex in PLWHIV. Lower BMI has been shown to be associated with visceral adiposity and reductions in peripheral fat (296,310,311). In a study comparing PLWHIV with and without NAFLD Kaplan *et al* demonstrated that patients with NAFLD had significantly higher rates of hypertension, BMI, DMII and lower CD4 counts (310). In their study analysing gene expression and liver attenuation Gabriel *et al* demonstrated significant associations with increasing liver fat and expression of genes associated with metabolic syndrome (312).

Quantification of HS via CT correlates well with biopsy results (the gold standard). Using non-contrast acquisition, the mean attenuation of the hepatic parenchyma is quantified by drawing two region of interest (ROI) circles (10mm^2) in the opposing lobes of the liver. A further ROI is drawn on the spleen and a liver spleen ratio of <1 indicates HS (example in figure 3). A mean attenuation of <40HU on the liver parenchyma also indicates moderate to severe HS (>30% steatosis) (313,314). Quantification of hepatic fat content using the liver/spleen ratio is also possible using contrast enhanced studies, but is less reliable due to differences in scan timing and contrast administration (313,315).

Given that HS shares common CVD risk factors, the degree to which it can be considered independently associated remains to be fully elucidated. Many studies investigating this link do not publish collinearity statistics of their multivariate models which may limit the applicability of regression models commonly utilised in such studies. It may be intuitive to think that HS represents an advanced metabolic phenotype or is the hepatic manifestation of metabolic syndrome. The causal role of HS in CVD development, with reference to PLWHIV, is yet to be established beyond the description of known associations of clinical risk factors. Investigation into the role of NAFLD using non-invasive imaging strategies has been recently recommended as *a priori*ty by an expert panel review (316).

1.7.2 Epicardial Adipose Tissue

Increased EAT volume has been shown to be independently associated with coronary stenosis, ischaemia and major adverse cardiac events (317). EAT volume has been associated with increased CACS, non-calcified plaque, increased CACS progression and adverse CVD events in both symptomatic and asymptomatic populations (318–322). EAT volume and density has also been demonstrated to be associated with thin-cap fibroatheromas on intravascular ultrasound (320). EAT volume has also been demonstrated to be a risk factor independent of VAT and BMI (323). EAT has also been shown to enhance risk predication models in studies utilising ML techniques (324). Furthermore, increasing obesity has been linked to EAT adipocyte hypertrophy, hypoxia, inflammation and oxidative stress with reduction of adiponectin (325). The mechanism by which EAT drives the atherosclerotic

process is complex and not fully understood. One proposed mechanism is that proinflammatory cytokines, released from dysfunctional EAT, exerts its influence on vascular biology from the intimate relationship with EAT and the epicardial coronary arteries. There is a shared common microcirculation, the vasa vasorum, that may allow proinflammatory cytokines to cause endothelial dysfunction and subsequent vascular injury (326,327).

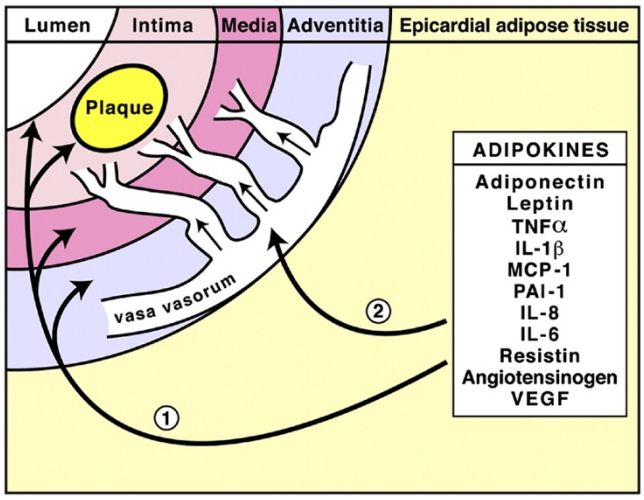


Figure 1: Potential mechanism driving atherogenesis from EAT (326).

The role of EAT in PLWHIV is less well defined. Guaraldi *et al* demonstrated in a large cross sectional study the association between EAT and CACS >100 (328). Increasing EAT volume (along with increasing age, male sex, and type II diabetes) was independently associated with presence of CACS >100. EAT also had a trend towards greater predictive value for the presence of CACS >100 than waist circumference, BMI and visceral adipose tissue although this difference didn't reach statistical significance (328). In 2014 Brener *et al* published findings from the MACS cohort comparing both HIV positive men and general population men. HIV infection was associated with significantly higher EAT volume after adjustment for CVD risk factors. Further significant HIV associated variable were duration of ART use, and treatment with ATZ. Assessment of plaque morphologies demonstrated that both non-calcified plaque and presence of any plaque were significantly associated with EAT after adjustment for risk factors (329).

The association of EAT with CVD in PLWHIV may differ between sex. Srinivasa *et al* assessed 55 HIV-positive and 27 general population women for the impact on EAT on CVD (330). Whilst both groups exhibited a similar EAT volume in the general population group the EAT volume was significantly associated with CVD risk scores (p=0.02). This association was not present in the HIV-positive group (p=0.99). Furthermore, the HIV-positive women had higher non-calcified plaque with the highest percentage in patients with excess EAT. Markers of calcified plaque were highest in the general population women (330).

Quantification of EAT is readily achievable using CT. Software from multiple vendors allows semi-quantitative assessment of EAT volume, distribution, and density. Machine learning systems have also been demonstrated to be effective in quantifying EAT. A ROI is drawn around the epicardium. This typically begins at the bifurcation of the pulmonary artery and continues to the apex. Threshold values for detection of fat vary slightly but typically are set to a lower limit of -190HU and an upper limit of -30HU. Quantification of EAT is also possible with contrast enhanced datasets with adjustment of the upper limit to 0HU. Good correlation is also shown between gated and non-gated studies.

1.8 The rationale for this thesis

PLWHIV have an increased risk of developing CVD compared to risk matched general population patients (17). CVD is a leading cause of morbidity and mortality in PLWHIV and increasing burden on healthcare systems. One model has suggested that the median age of PLWHIV will increase from 43.9 years in 2010 to 56.6 years in 2030 with 78% being diagnosed with CVD (16). Traditional risk predication tools have been demonstrated to perform poorly in this patient cohort (120). The excess risk seen in PLWHIV is therefore inadequately described by traditional CVD risk factors. Given the impending health economic emergency and poor predictive tools, urgent work to elucidate this excess risk is urgently required.

The data around dysfunctional adipocyte induced ectopic fat deposition is of increasing interest. The relationship between viral related factors and ART is complex and not fully described. Increasing utilisation of non-invasive imaging tools to measure fat volume fat quality may unlock the missing link in the excess risk seen in PLWHIV. The expected dramatic increase in CVD from this ageing patient cohort means that work is urgently needed to reduce healthcare burdens and improve prevention strategies. We have therefore designed a series of works investigating the link between ectopic fat storage and CVD with a focus on risk assessment.

2. Chapter 2: Thesis overview

This study aimed to investigate the missing links between the excess CVD risk seen in HIV-positive individuals. This study builds on the accumulating evidence analysing the links between HIV and CVD. This is of increasing importance as the predicted morbidity and mortality from CVD in this aging cohort is predicted to significantly increase in the near future.

2.1 Aims and objectives

The main objectives from this study were to firstly explore the HIV-specific associations with CVD from the existing literature. It also sought to analyse differences in traditional and novel risk factors between HIV-positive groups and general population groups. The specific aims of each study are discussed in detail in each chapter but a broadly defined by the following:

- To appraise the current evidence base for HIV-specific risk factors with CVD.
- To compare the association of HS with CVD in HIV-positive and general population individuals.
- Develop a CVD risk prediction tool using conventional risk factors and imaging biomarkers.
- To compare the association of EAT with CVD in HIV-positive and general population patients.
- To assess the impact on lipid profiles in those switching to modern preparations of ART.

3. Chapter 3: Associations of Hepatosteatosis with cardiovascular disease in HIV-positive and general population Patients: The Liverpool HIV-Heart Project.

3.1 Introduction

HS is the most common cause of liver disease in PLWHIV, affecting between 13 to 65% (306–308) individuals. HS describes hepatic ectopic fat accumulation and is present when it affects >5% of the liver by weight. HS encompasses a spectrum of clinically entities including NAFLD. The prevalence of HS is under reported.

Histologically, progressive hepatic fat accumulation is associated with lipotoxicity and chronic inflammation, progressing in many cases to cirrhotic liver disease, and a threefold increase in mortality (182). The relationship of obesity, insulin resistance, DMII and HS is well defined in non-HIV populations (180). The estimated prevalence of NAFLD in the United States is predicted to reach 33% of the adult population by 2030 (181).

PLWHIV have unique risk factors for the development of HS compared to non-HIV populations. They have been shown to develop lean NAFLD, defined as NAFLD in BMI< 25Kg/m^2, at increased rates compared to non-HIV populations (296). The complex interplay of viral related factors, ARV medications and chronic inflammation may cause PLWHIV to be more susceptible to the development of HS. Liver disease represents a huge source of morbidity and mortality in PLWHIV with up to 13% of deaths in the D:A:D cohort attributable to liver disease (331). In both HIV-positive and general population populations dyslipidaemia, insulin resistance and overt type II diabetes are strongly associated with the presence of HS.

HS has been shown to be associated with CVD in General population populations (298,300,332) although this is not universal (303–305). Given the increasing burden of HS in

HIV-positive populations we sought to examine if HS was independently associated with CVD in HIV-positive compared to general population populations.

3.2 Methods

We conducted a real-world retrospective analysis to compare the associations of HS and coronary calcification in both HIV positive and HIV negative patients. Data was collected from the Royal Liverpool University Hospital HIV clinical database and CTCA clinical database. Both the HIV and CTCA databases exist for both clinical use and quality improvement purposes and are approved by the host institution's audit committee. All demographic and clinical variables present on the databases were cross checked by the research team using the Trust's electronic patient record (clinical notes). The Royal Liverpool University Hospital audit committee approved this study. The HIV database includes all patients under follow up with service. It contains clinical co-morbidities, current and previous medications, anthropometric measurements, blood chemistry and CVD risk.

The HIV database was cross checked for patients that had a received a CT thorax within the last 10 years. The images were inspected by an independent imaging cardiologist for the presence of coronary calcification which was recorded in a binary fashion (yes or no). HS was assessed using non-contrast CT scans or venous phase CT scans in those with liver parenchyma visible on the images. Both imaging techniques are established methods for quantifying liver fat content. Two regions of interest circles covering 100mm^2 were drawn on the right lobe of the liver and one on the left lobe. The mean attenuation (Hounsfield unit [HU]) was recorded for liver parenchyma. Regions with non-uniform attenuation and hepatic vessels were avoided. A further 100mm^2 region of interest was drawn on the spleen and the HU recorded. HS was confirmed if the liver to spleen ratio (L/S ratio) was less than 1 and/or the mean hepatic measurement was <40HU in non-contrast scans (333–336). Liver attenuation of 20HU less than spleen attenuation was used for the venous phase imaging (315). Hepatosteatosis was also confirmed in patients that had a prior imaging (ultrasound) or biopsy confirmed diagnosis of fatty liver. The HIV-positive group were taken exclusively from this database.

The CTCA database contains demographic and clinical variables for all patients referred for cardiac CT (either CAC scoring or CTCA) between January 2014 and November 2016. The majority of patients were referred for the investigation of atypical angina and were considered low to medium risk in line with guidelines from the National Institute for Clinical Excellence that were established at the time (337). The presence of HS had previously been calculated by two independent radiologists and added to the clinical database. This was done using gated, non-contrast studies from the CAC protocol which is an established mechanism for detecting HS (333). The methodology and criteria used to establish HS was the same as outlined with the HIV-positive cohort. Patients were labelled as having coronary calcification if the calcium score was >0.

Variables were collected by reviewing patients' case notes, including consultation letters, for prior and current diagnoses. Patients with prior diagnoses of CVD (including clinical diagnosis, imaging diagnosis of coronary plaque or coronary event or intervention) were excluded. Patients were also excluded if quantification of coronary calcifications or quantification of HS was not possible.

3.2.1 Statistical Analysis

Summary statistics were calculated to compare the difference in clinical and demographic covariates between HIV-positive and general population groups. The prevalence of categorical variables was presented in absolute prevalence and percentages. All data were inspected using graphical representation for normality (histograms and Q-Q plots) and Shapiro-Wilk test. The proportions between categorical variables were compared using Chi-Squared test. Continuous variables were presented as means and standard deviations where normally distributed. The means were compared using an independent t-test. P-values were considered statistically significant if <0.05. The data used was complete. Any case with missing values was excluded from the analysis.

A multiple logistic regression (LR) model was developed to ascertain the association between coronary calcifications and CVD risk co-variates in HIV-positive and general population patient groups, using odd ratios (OR) as a measure of association. Additionally, we performed a sensitivity analysis using random forest (RF), to assess variables of importance in both HIV-positive and general population patients. Variables of importance were determined by Gini index. The Gini index is an established measure of accumulated nodal impurity for each variable in random forest algorithms. A high mean decrease in Gini indicates a high variable importance (338).

For the development of the models, we performed bootstrapping, with 1000 repetitions (including replacements), each with the same size as the original datasets. This was done to calculate the standard errors and confidence intervals. For the sensitivity analysis we split the datasets into 70% training and 30% test in both group of patients. The area under the receiver operator curve (AUC) for the test sets were calculated, and 95% confidence intervals (95% CI) were estimated from the standard error.

The statistical analysis and development of the machine learning models were performed using RStudio, version 1.3.1056.

3.3 Results

The HIV database contains data on 1294 patients who are under follow up. After removing those with prior documented CVD and those where assessment of coronary calcification or HS was not possible there were 209 cases available for analysis. There were 1744 patients within the CTCA database. Again, after removing those with prior CVD, there were 1097 cases available for assessment. This left an overall total of 1306 cases (mean age 52.32; 46.6% male) to be included in the analysis.

The clinical and demographic co-variates for the whole cohort and stratified by HIV status are displayed in table 1. The groups differed in terms of the proportion of clinical and demographic covariates. Mean age was lower in the HIV-positive group (49.9 versus 52.3, p=0.002) and the proportion of males was higher (76.6% versus 40.9%, p<0.001). The proportion of patients with hypertension, type II diabetes and dyslipidaemia were significantly higher in the general population group (p<0.005 in all). The proportion of current smokers was higher in the HIV-positive group (28.7% versus 18.8%, p=0.002).

The overall prevalence of HS was 44.6% and was significantly higher in the general population group (46% versus 36.8%, p=0.018). The rate of coronary calcification was similar between the 2 groups (37.4% in general population versus 32.5% in HIV-positive, p=0.128). Within the HIV-positive group 91.9% had HS quantified by CT.

3.3.1 Multivariate analysis

In the multivariate model, including all clinically relevant demographic and clinical risk factors, HS was significantly associated with CVD in HIV-positive patients (OR: 3.13, 95% CI: 1.51-6.63 p=0.005). The only other significant co-variates associated with CVD were increasing age (OR: 1.15, 95% CI: 1.10-1.20, p<0.005) and male sex (OR: 3.77, 95% CI:1.37-11.69 p=0.014 (table 2). The associations of clinical covariates with CVD differed in the general population group. Current smoking (OR:1.96, 95% CI: 1.37-2.81p<0.005), dyslipidaemia (OR:1.66, 95% CI: 1.24-2.22 p <0.005) along with increasing age and male sex

were significantly associated with CVD. HS was not significantly associated with CVD in this group (OR:1.08, 95%CI: 0.81-1.44 p=0.60) (table 3). The AUC for the logistic regression was 0.841 (95% CI: 0.785– 0.897) for the HIV-positive model and 0.796 (95% CI: 0.770 – 0.822) for the general population model. Upon removing HS from the model, the AUC dropped to 0.819 (95% CI: 0.758-0.881) for the HIV-positive model and 0.796 (95% CI: 0.770-0.822) (figure 1). There was no statistically significant difference between either AUC (HIV positive: 0.619, general population: 0.993, Wilcoxon test).

3.3.2 Sensitivity analysis

For the HIV-positive model age, HS and male sex were top three variables of importance using, according to the mean decrease in Gini index. In the general population group the top three variables of importance were age, male sex, and hypertension (figure 2). The mean AUC for the HIV-positive and HIV negative models were 0.877 (95% CI: 0.755-0.959) and 0.828 (95% CI: 0.780-0.873), respectively. The logistic regression models and the random forest models were significantly different for both cohorts ($p<0.001$, Wilcoxon test) (supplementary material).

3.3 Discussion

In this retrospective analysis we sought to assess the differences in the association of HS with CVD between HIV-positive and general population patients. The principal finding from this study is HS is independently associated with CVD in HIV-positive patients whilst there was no significant association in general population patients. To our knowledge, our study is the first to directly compare the effect of HS on CVD in both HIV-positive and general population patients. The finding that HS was independently associated with CVD in HIV-positive patients, but not general population patients was confirmed in our sensitivity analysis.

Despite a higher proportion of HS in the general population group (p=0.018) and similar rates of coronary calcification, (p=0.128), we found HS to be independently associated with CVD in the HIV-positive group (table 2) but not in general population group (table 3) after adjusting for CVD risk factors. We also observed a significant association of the traditional risk factors of age and male sex with subclinical CVD). The association of HS and CVD in HIV-positive patients has been assessed in previous analyses. However, there have been no study comparing HS and CVD in HIV-positive and general population groups. Kaplan *et al* (310) compared 232 HIV-positive patients with and without NAFLD and found that NAFLD, as assessed by ICD-9 codes, was independently associated with CVD (OR: 3.08, 95%CI: 1.37-6.94). Our HIV-positive patients were similar in age and proportions of male sex. However, the definition of CVD differed from this study as they used a broad composite which included coronary artery disease, heart failure, peripheral vascular disease, stroke, transient ischaemic attack, myocardial infarction, and revascularisation. In our analysis we assessed the association with subclinical CVD found on CT rather than hard clinical outcomes.

In a separate analysis, using a mainly male HIV-positive cohort, Crum-Cianflone *et al* found that HS diagnosed on non-contrast CT was significantly associated with coronary calcification (OR: 3.8, p<0.01) (339). In a further analysis, Guaraldi *et al* assessed HIV-positive patients and found no significant association with NAFLD and coronary calcium (340).

Studies assessing the impact of HS as an independent CVD risk factor in general population groups demonstrate conflicting results. In their meta-analysis Kapuria *et al* concluded that NAFLD was associated with increased prevalence of subclinical atherosclerosis (based largely on CAC score) (304). Vanwagner *et al* analysed the association of NAFLD from CT scans, defined as liver attenuation <40HU, and found no significant association with coronary calcium after adjustment for obesity (303). In this current study we found that HS was not significantly associated with CVD in our multivariate analysis (OR 1.08, 95%CI: 0.81-1.44).

Current smoking, hypertension, type II diabetes and dyslipidaemia are established risk factors for CVD in both HIV-positive and general population groups. The relatively low incidence of these risk factors within this study makes it difficult to interpret the magnitude of effect. In the case of dyslipidaemia only 7.2% (n=15) of the HIV-positive had this risk factor documented. This is reflected in the wide confidence interval produced by the multivariate analysis (95% CI: 0.85-10.58). Although these results did not show an association between traditional risk factors and CVD, the study was underpowered to demonstrate any association. The purpose of this retrospective analysis was to compare the associations of HS with CVD in HIV-positive and general population groups. It was not designed to determine the impact of traditional risk factors.

In the sensitivity analysis we utilised a random forest algorithm to investigate the variables of importance in HIV-positive and general population groups (figure 2). The benefit of using this technique in a sensitivity analysis is that it is not constrained by the same assumptions that logistic regression and offers an alternative methodology to examine the results. In both groups age was the most important variable. In the HIV-positive group HS was the second most important variable followed by dyslipidaemia. In the general population group HS was the fifth most important variable. This further illustrates the difference in the impact of HS as an independent risk factor between HIV-positive and general population groups.

We utilised random forest as an alternative statistical technique as it does not require the same assumptions from the data as logistic regression. The random forest models demonstrated good discriminatory ability and was statistically significantly superior to logistic regression performed on the same testing data. The performance of each model is summarised in the box plots. Although the random forest models demonstrated superior discriminatory ability compared to logistic regression models, they demonstrated similar results. By utilising this contemporary alternative analysis, it increases the robustness of the finding that HS is significantly associated with CVD in HIV-positive groups and not associated in the general population group.

The association of HS as an independent risk factor may be attenuated by adjustment for other metabolic disease related conditions such as diabetes, obesity, dyslipidaemia, and hypertension. Our finding, showing that HS is an independent predictor of CVD in HIV-positive but not general populations, suggest potential differences in drivers of CVD between these two cohorts. HS that manifests in HIV-positive populations may represent a more severe adverse metabolic phenotype compared to non-HIV populations.

HS drives cardiovascular risk through increased atherogenic lipid profiles, inflammation, and insulin resistance. HIV-positive patients have been shown to have increased rates of lean adiposity compared to general population groups (296). Ectopic fat deposition has also been shown to be associated with prior CVD events (341). An individual's susceptibility to this process may also be derived from genetic factors (342). The findings from this study further demonstrate the unique pathophysiological process underpinning the increased CVD risk seen in PLWHIV.

3.3.1 Limitations and strengths

There were several limitations to our study. First, the way in which patients had coronary calcifications assessed in the HIV-positive group was based on the presence of a non-dedicated CT scan. These scans had taken place historically for different indications. Whilst

it is recognised that assessment of coronary calcification using non-dedicated CT thorax (343) the assessment between groups was not homogenous. Second, by opportunistically selecting patients who had received CT scans of the thorax for alternative indications we may have introduced selection bias into the study. We attempted to reduce any differences between groups by undertaking the sensitivity analysis. Third, the general population group were a pre-defined group of patients with low to intermediate risk chest pain. This may affect the generalisability of this result. We did not adjust for HIV-related co-variates including ARV medications. However, this study was designed to assess the differences between two different cohort rather than determinants of CVD risk specific to HIV-positive patients. In addition, prior studies did not demonstrate any significant HIV-related associations with HS and CVD (310).

Finally, we were not able to adjust for alcohol consumption or other secondary causes of hepatosteatosis (such as hepatitis C) in either group due to the retrospective design of the study. Although these influences are of significant interest it is outside the scope of this retrospective study to assess their impact on the presence of hepatosteatosis. Crum Cianflone *et al* performed a sensitivity analysis in which participants with significant alcohol consumption were excluded. This did not significantly alter their result (339). Further prospective work is required to assess their impact on the presence of HS and CVD.

Despite these limitations there were multiple strengths to our analysis. Both cohorts used in this comparison were extremely well characterised and data used within this analysis was 100% complete. This study is also unique in the fact that it is the first to compare the impact of HS on CVD between HIV-positive and general population cohorts. We were able to confirm the validity of the result by utilising a random forest algorithm in our sensitivity analysis. The broad agreement between logistic regression models and random forest models confirms the robustness of the result. This study was designed to be hypothesis generating rather than show definitive causality. Future studies quantifying CVD risk, plaque burden and homogenous assessment of HS and coronary calcium scores are required to

further delineate this emerging field. These findings may have important clinical implications for the way in which CVD risk quantified in HIV-positive patients.

3.4 Conclusion

In these well characterised cohorts, we have demonstrated a significant difference in the impact of HS as an independent CVD predictor between HIV-positive and general population groups. This may represent a unique metabolic process that drives the excess CVD risk seen in PLWHIV.

3.5 Tables and Figures

3.5.1 Table 1: Summary statistics of the cohort and stratified by HIV serostatus.

	Overall	**HIV negative**	**HIV positive**	**P Value**
n	1306	1097	209	
Age (mean (SD))	52.32 (±12.00)	52.77 (±12.26)	49.92 (±10.22)	<0.005
Male Sex (%)	609 (46.6)	449 (40.9)	160 (76.6)	<0.005
Current smoker (%)	266 (20.4)	206 (18.8)	60 (28.7)	<0.005
HTN (%)	375 (28.7)	342 (31.2)	33 (15.8)	<0.005
DMII (%)	124 (9.5)	113 (10.3)	11 (5.3)	0.032
Dyslipidaemia (%)	389 (29.8)	374 (34.1)	15 (7.2)	<0.005
Statin (%)	343 (26.3)	304 (27.7)	39 (18.7)	0.008
Coronary calcium (%)	489 (37.4)	421 (38.4)	68 (32.5)	0.128
HS (%)	582 (44.6)	505 (46.0)	77 (36.8)	0.018
Obesity (%)	135 (10.3)	72 (6.6)	66 (31.2)	<0.005

SD, standard deviation; HTN, hypertension; DMII, type II diabetes; HS, hepatosteatosis

3.5.2 Table 2: Multivariate analysis in HIV-positive patients for the association of coronary calcification

	Odds Ratio (95% CI)	**P Value**
Age	1.15 (1.10-1.20)	<0.005*
Male Sex	3.77 (1.37-11.69)	0.014*
Current smoker	2.14 (0.93-5.06)	0.077
HTN	0.58(0.19-1.67)	0.317
DMII	0.75 (0.14-3.45)	0.718
Dyslipidaemia	2.89 (0.84-10.73)	0.097
HS	3.13 (1.51-6.63)	0.005*
Obesity	1.58 (0.70-3.56)	0.269

HTN, hypertension; DMII, type II diabetes; HS, hepatosteatosis

* Denotes significant association

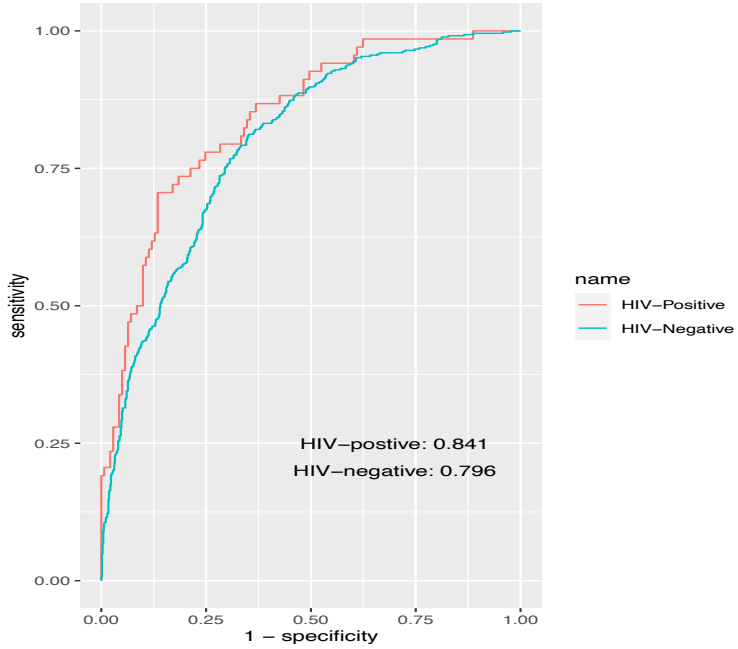
3.5.3 Table 3: Multivariate Analysis in HIV Negative Patients for the association of coronary calcification

	Odds Ratio (95% CI)	P value
Age	1.11 (1.09-1.13)	<0.005*
Male Sex	2.97 (2.19-4.05)	<0.005*
Current Smoker	1.96 (1.37-2.81)	<0.005*
HTN	1.39 (1.02-1.90)	0.04*
DMII	1.14 (0.72-1.82)	0.58
Dyslipidaemia	1.66 (1.24-2.22)	<0.005*
HS	1.08 (0.81-1.44)	0.60
Obesity	0.95 (0.54-1.65)	0.87

HTN, hypertension; DMII, type II diabetes; HS, hepatosteatosis

* Denotes significant association

3.5.4 Figure 1: Comparison of HIV-positive and general population models including and excluding HS



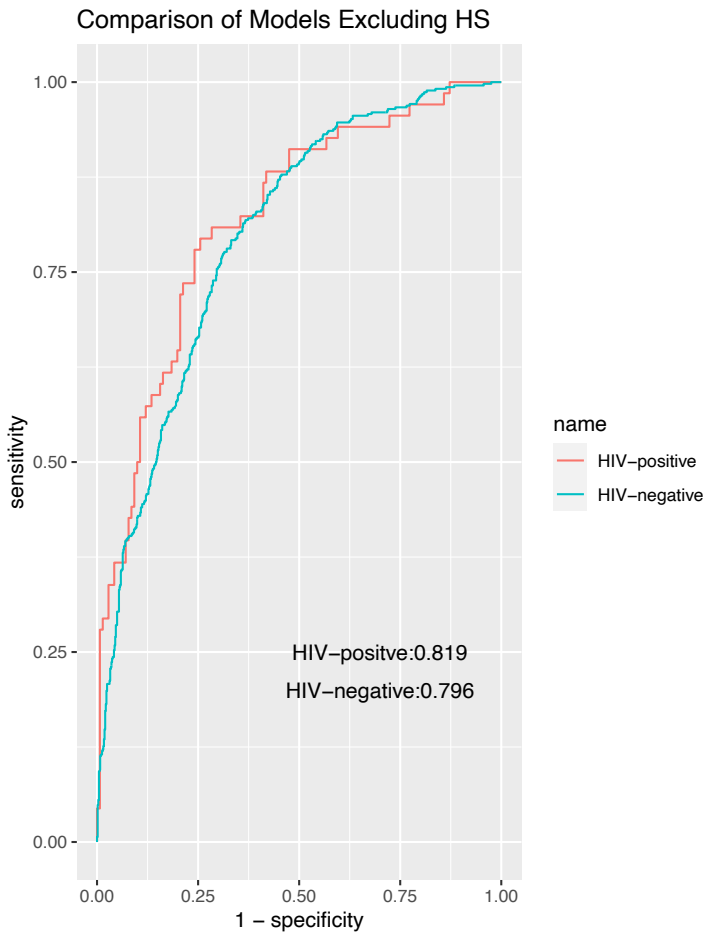


Figure Legend: Comparison of receiver operator characteristic curves for the logistic regression models. The first plot demonstrates the predictive ability between HIV-positive and HIV negative groups. The second plot demonstrates the predictive ability of the models not including HS.

3.5.5 Figure 2: Random Forest analysis of the variables of importance in HIV-positive and general population patients

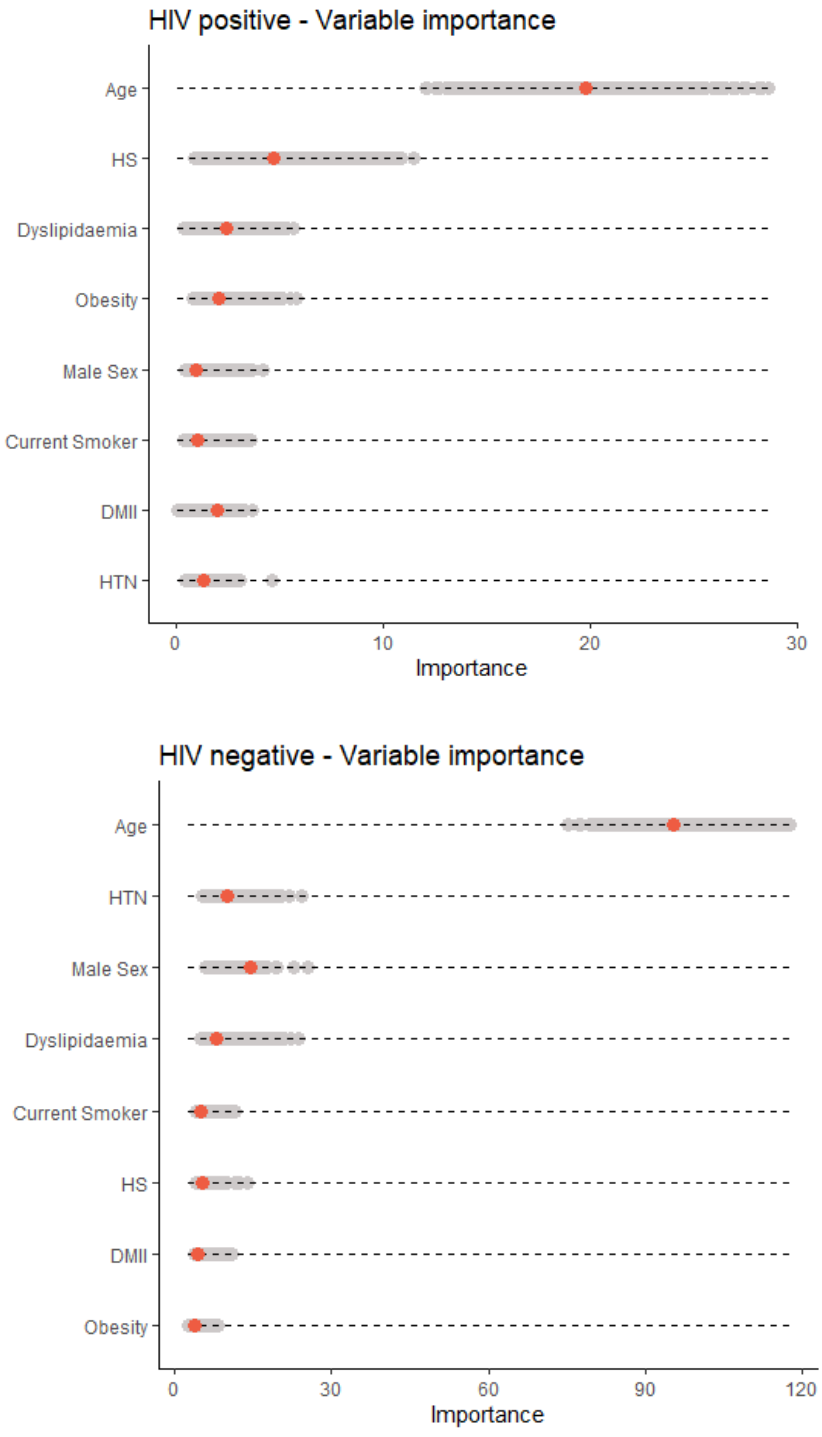
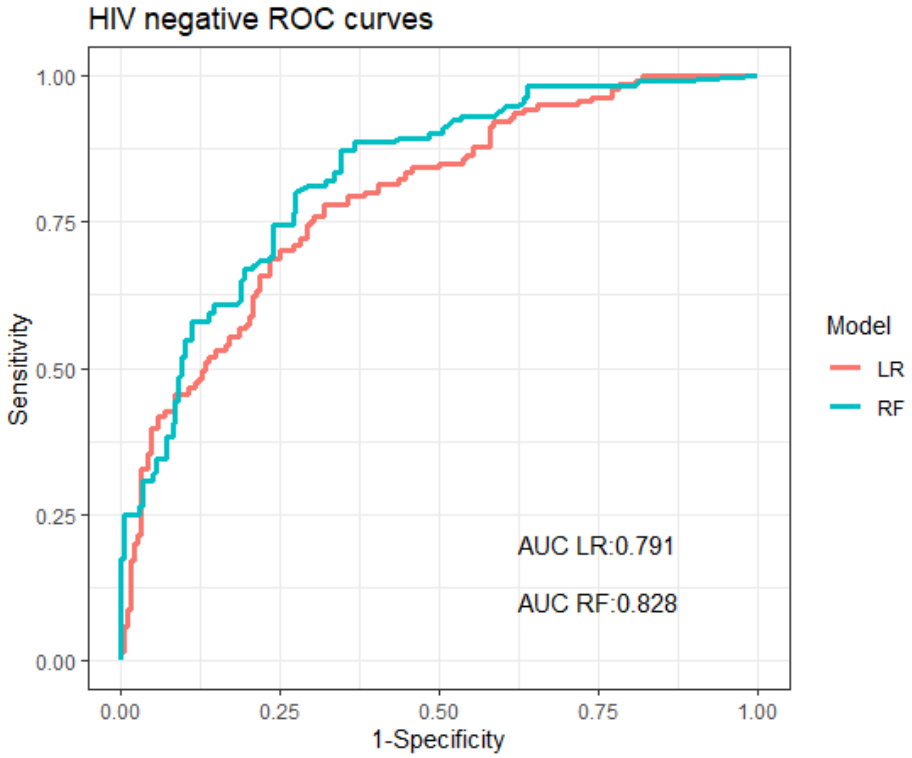


Figure legend:

Variables of importance given by mean decrease in Gini index. The shaded area (grey points) is the variable importance given by all models. The variables of importance of the model with the best AUC is highlighted in red. Variable abbreviations are the same as table 1.

3.5.6 Supplementary Figure 1: Receiver operator characteristic curves demonstrating the predictive ability of regression and random forest models in HIV-positive and general population groups.



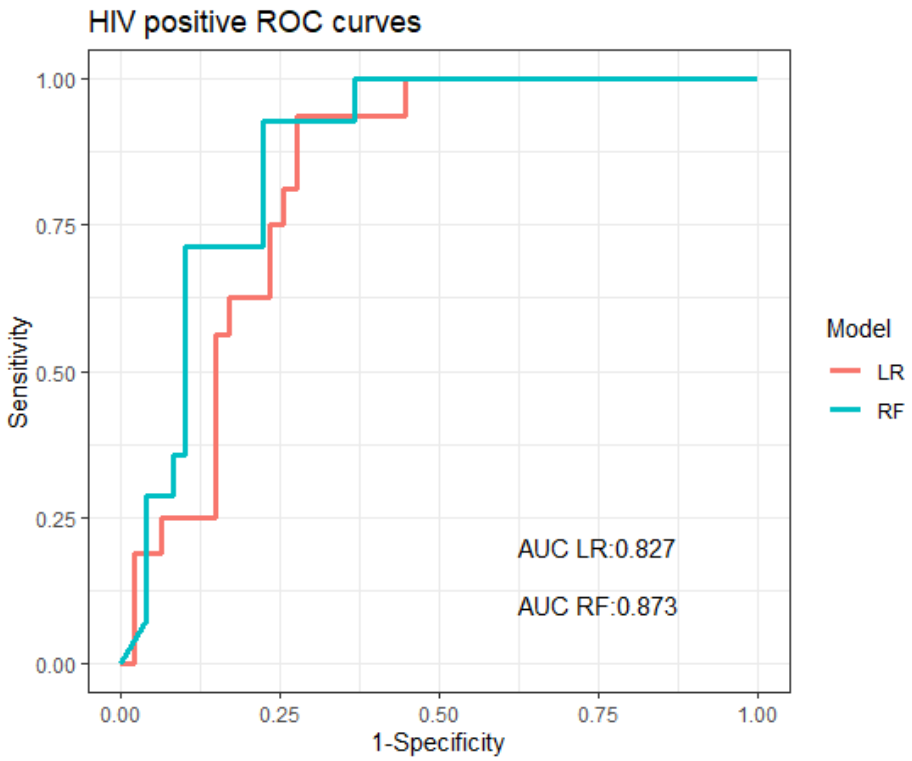


Figure Legend: Comparison of receiver operator characteristic curves and corresponding area under the curves for LR models and RF models. In the HIV negative plot, the mean AUC for LR was 0.791 (95% CI: 0.741-0.842) and for RF a mean AUC of 0.828 (95% CI: 0.781-0.872). The difference between these models was also statistically significant ($p<0.001$, Wilcoxon test). The HIV positive plot shows a mean AUC for LR of 0.827 (95% CI: 0.705-0.933) and for RF a mean AUC of 0.873 (95% CI: 0.768-0.959. The difference between the models was statistically significant ($p<0.001$, Wilcoxon test).

Abbreviations; AUC: area under the curve; LR: logistic regression; RF: random forest

3.5.7 Supplementary Figure 2: Box plots to show the performance of models in HIV-positive and general population groups.

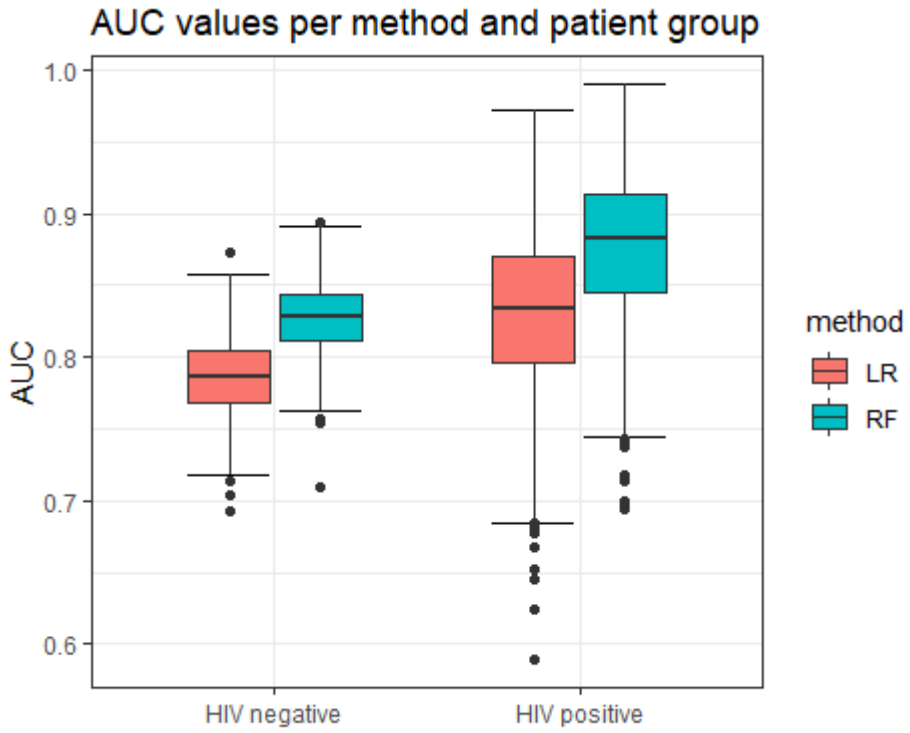


Figure Legend: Box plots to summarise the performance of RF and LR models in HIV-positive and general population groups.

4. Chapter 4: The Association of epicardial adipose tissue volume and density with coronary calcium in HIV-positive and general population patients.

4.1 Introduction

PLWHIV have an increased risk of developing CVD compared to risk matched general population patients (17). This increased risk may be driven by several factors. Viral related mechanisms, ART, and increased prevalence of traditional risk factors (smoking, poor diet, sedentary lifestyle) act in a synergistic fashion to enhance the cardiovascular risk seen in HIV-positive patients.

EAT is increasingly recognised as a potential risk factor for CVD. EAT functions as an energy depot for normal cardiac function and has an intimate relationship with the epicardial coronary arteries, with both EAT volume and density strongly associated with CVD (317,318,344). EAT shares a common microcirculation with the epicardial coronary arteries and exerts influence on vascular function through adipokine secretion, buffering of fatty acids, and secretion of inflammatory cytokines.

Increasing energy storage requirements causes adipocytes to increase in size and number to accommodate extra lipid. Hypertrophic adipocytes have been shown to be dysfunctional due to lipotoxicity and ischaemia. This induces a proinflammatory phenotype with secretion of pro-inflammatory cytokines and adipokines. The close anatomical relationship between EAT and the epicardial coronary arteries may indicate a novel substrate for atherogenesis in the context of EAT dysfunction (345).

EAT is readily quantifiable using non-invasive imaging techniques. CT allows accurate quantification of EAT volume and density (346). Increased EAT volumes have been shown to be associated with markers of subclinical CVD and cardiac events in HIV-positive populations (264,347). This association has also been demonstrated in general populations (348).

Lower EAT density is associated with adipocyte hypertrophy and hyperplasia due to increased lipid content (349). Lower EAT density has been shown to be associated with markers of metabolic syndrome, inflammation and coronary artery disease in general populations (350,351). However, the converse has been demonstrated in patients presenting with acute myocardial infarction (352). EAT density has not been substantially studied in HIV-positive populations.

In this study, we aimed to assess the association of EAT volume and density with the presence of coronary artery disease using a large dataset of HIV-positive and general population individuals.

4.2 Methods

We conducted a real-world retrospective analysis to compare the associations of EAT volume and CVD in both HIV positive and HIV negative patients. Data were collected from the Royal Liverpool University Hospital HIV clinical database and CT Coronary Angiography (CTCA) clinical database. Both databases are clinically approved by the Liverpool University Hospitals Foundation Trust's audit committee. All demographic and clinical variables present on the databases were cross checked by the research team using electronic patient records (clinical notes). The primary objective of this study was to assess the association between EAT volume and coronary calcium in HIV-positive and general population groups. The secondary objective was to assess the association between EAT density and coronary calcium in both groups.

The HIV database includes all patients under follow up with the service. It contains clinical co-morbidities, current and previous medications, anthropometric measurements, blood chemistry, and CVD risk. The HIV database was cross checked for patients that had received a CT thorax within the last 5 years. The images were inspected by an independent imaging

cardiologist for the presence of coronary calcification which was recorded in a binary fashion (yes or no). This method has been described previously (353).

The CTCA database contains demographic and clinical variables for all patients referred for cardiac CT (either CAC scoring or CTCA). We included all sequential patients referred for cardiovascular CT from 1st July to 31st December 2019. Most patients were referred for the investigation of atypical angina and were considered low to medium risk. The presence of hepatosteatosis (HS) had previously been calculated by two independent radiologists and added to the clinical database. Patients were labelled as having coronary calcification if the calcium score was >0.

Clinical variables were collected and checked by reviewing patient case notes, including consultation letters, for prior and current diagnoses. The inclusion criteria were age >18 and CAC score or CTCA between July 1st and December 31st 2019. Patients with prior diagnoses of CVD (including clinical diagnosis, imaging diagnosis of coronary plaque or coronary event, or intervention) were excluded. Patients were also excluded if quantification of coronary calcifications was not possible.

EAT volumes and density were calculated using automated, deep learning software specifically designed to quantify EAT. We used QFAT 2.0 software developed by Cedars-Sinai Medical Center. Contours are generated along the visceral pericardium from the main pulmonary artery bifurcation to apex of the heart on sequential axial slices (figure 1). Operators, specifically trained in EAT volume measurement, were able to adjust the contours as required. Epicardial fat was defined tissue between the visceral pericardium and myocardium meeting the predefined attenuation thresholds for adipose tissue. The overall mean density was quantified using the QFAT software and displayed in mean Hounsfield Units (HU). Hepatosteatosis (HS) was confirmed if the liver to spleen ratio (L/S ratio) was less than 1 and/or the mean hepatic measurement was <40HU in non-contrast scans. This method has previously been described (353). Liver/spleen ratio of <0.8 was used for the

venous phase imaging. HS was also confirmed in patients that had a prior imaging (ultrasound) or biopsy confirmed diagnosis of fatty liver.

In the HIV-positive group CT datasets, including contrast and non-contrast studies, were analysed. For non-contrast enhanced studies, attenuation thresholds were set between -190 and -30 HU. For contrast enhanced studies the upper threshold limit was adjusted to 0HU as described in previous work (346). In the general population group EAT volume was assessed from non-contrast enhanced studies using the attenuation thresholds.

4.2.1 Statistical Analysis

Summary statistics were calculated to compare the difference in clinical and demographic covariates between HIV-positive and general population groups. The prevalence of categorical variables was presented in absolute prevalence and percentages. All data were inspected using graphical representation for normality (histograms and Q-Q plots) and Shapiro-Wilk test. The proportions between categorical variables were compared using the Chi-Squared test. Continuous variables were presented as means and standard deviations (normal distribution) or median and interquartile ranges (non-normal distribution). The means were compared using an independent t test where normally distributed and Mann-Whitney-U test where non-normally distributed. P-values were considered statistically significant if <0.05. Missing data (<5%) was imputed using a random forest multiple imputation technique.

Linear regression analysis was performed on continuous data and the logit of the probabilities of coronary calcification. Variables were inspected for linear associations. Outliers and influential datapoints were assessed using graphical representation of Cook's distance and plotting the standardized residuals. Multicollinearity was assessed by measuring the variance inflation factors of the covariates.

Multiple logistic regression (LR) models were developed to ascertain the association between coronary calcifications (binary) and EAT volumes after adjustment for clinical covariates in the HIV-positive and HIV negative groups. Clinical covariates were added to the model *a priori*. Further multiple logistic models were developed to assess the influence of lipid subfractions and finally HIV-serostatus as independent predictors of coronary calcifications. Odd ratios (OR) and 95% confidence intervals (95% CI) were used to demonstrate the degree of association.

Multiple linear regression models were developed to assess the association of predictor variables and EAT volume for HIV-positive and general population groups. In the subgroup analysis, models were developed to assess the association with EAT density and predictor variables.

4.2.2 Sensitivity Analysis

We performed a propensity score matching analysis using the "matchit" package on the imputed dataset. This technique uses a 'nearest-neighbour' with propensity score estimated using logistic regression models using HIV status as the dependent variable with baseline clinical covariates as independent variables. We then inspected the model for balance of covariates from the resulting model outputs (see supplementary data). The propensity score matched cohort were then inspected for the association of EAT volume and coronary calcification The statistical analysis and development of the regression models were performed using RStudio, version 1.3.1056.

4.3 Results

A total of 700 patients were included in the analysis. Of these, there were 195 HIV positive and 505 general population subjects. The demographic and clinical covariates for the cohort, stratified by HIV-serostatus, are summarised in table 1. Mean age was lower in the HIV-positive group (49.2 versus 57.8, p<0.005) whilst the HIV-positive group had a higher proportion of male sex (75.9% versus 48.1%, p<0.005). The presence of traditional CVD risk factors (hypertension, diabetes, hypercholesterolaemia) was significantly lower in the HIV-positive group compared to the general population group (all p<0.005). Current smoking was more prevalent in the HIV positive group whilst ex-smoking status was more prevalent in the general population group (both p<0.005).

EAT volume was significantly lower in the HIV positive group (68mm^3 versus 118.3mm^3, p<0.005) whilst the prevalence of HS was significantly higher (31.3% versus 19.6%, p<0.005). Coronary calcifications were higher in the general population group (58.2% versus 29.2%, p<0.005). BMI was significantly lower in the HIV-positive group (27.3 versus 29.6, p<0.005). There were no significant differences in parameters of the lipid profile (all >0.05).

4.3.1 EAT volume and Coronary Calcification

Univariate and multivariate predictors of coronary calcification are presented in table 2. In the HIV-positive group, after adjustment for CVD risk factors, age, sex, statin use and BMI both EAT volume (OR: 1.14 per 10mls, p<0.005) and HS (OR: 3.17, p<0.005) remained significantly associated with coronary calcification. In the general population group the only significant association in the final model was TC (OR: 0.75, p=0.012).

4.3.2 EAT volume and clinical covariates

Multiple linear regression demonstrated that HS was significantly associated with EAT volume in the HIV-positive group but not in the general population group after adjustment for sex, age, and BMI (p<0.005 versus 0.066). There were no significant associations with constituents of the lipid panel and EAT volume in the HIV-positive group. In the general

population group, TG was the only marker significantly associated with increasing EAT volume (p=0.009). There was no significant association with hypertension, diabetes or hypercholesterolaemia in either the HIV-positive or the general population group. HIV positivity was negatively associated with EAT volume after adjusting for age, sex, BMI, and HS (p<0.005). The logit odds of each clinical covariate were calculated and compared between HIV-positive and general population groups (figure 2).

4.3.3 EAT Density

In the subgroup analysis the association of EAT density with clinical covariates was assessed in 79 HIV-positive patients (40.5%) in whom non-contrast CT datasets were available. Associations with EAT density were assessed using linear regression models. Significant univariate predictors of lower EAT density for the HIV-positive group were ex-smoking (p=0.039), statin prescription (p=0.030), EAT volume (p<0.005), and BMI (p=0.030). For the general population group, age (p<0.005), male sex (p=0.043), statin use (p=0.024), EAT volume (p<0.005), triglyceride (p=0.025), and BMI (p<0.005) were significantly associated with lower EAT density.

In the HIV-positive group only EAT volume (p<0.005) was significantly associated with lower EAT density on multivariate analysis. In the general population group male sex (p<0.005) and EAT volume (p<0.005) were significantly associated with EAT density. The correlation between EAT volume and EAT density was significantly negatively correlated in both HIV-positive and general population groups.

The association of EAT density and coronary calcification was assessed using multiple logistic regression. In both HIV-positive and general population group, EAT density was a significant predictor of coronary calcification. After adjusting for age, sex (model 2) and age, sex, HS, and EAT volume (model 3) the association became non-significant (supplementary figure 1).

4.3.4 Sensitivity Analysis

There were 300 matched participants following completion of the matching algorithm (195 HIV-positive, 195 general population). Multivariate logistic regression demonstrated the significant association of EAT volume ($p<0.005$) and HS ($p<0.005$) with coronary calcium when adjusted for age, sex, BMI, hypertension, diabetes, hypercholesterolaemia and BMI. In the general population matched group there was no significant association with both EAT volume and HS and coronary calcification ($p>0.05$ for both).

4.4 Discussion

In this retrospective analysis, involving 700 participants, we sought to investigate the association of EAT volume and EAT density with coronary calcification in HIV-positive and general population groups. The principal findings from our study were that increasing EAT volume was strongly and independently associated with coronary calcium in HIV-positive individuals. In the general population group there was no significant association. Secondly, lower EAT density was significantly associated with coronary calcification in both groups, however, this association became non-significant when adjusted for traditional CVD risk factors.

HIV-positive populations are known to have approximately double the risk of developing CVD events than risk matched general population patients (17). The aetiology of this excess risk is multifactorial and includes increased burdens of traditional risk factors and metabolic pressures derived from HIV-positivity, inflammation, and ART (354). The impact of ectopic fat deposition and its role in driving adverse cardiometabolic profiles in HIV-positive populations is of great interest. The principal sites of ectopic fat deposition are the hepatic parenchyma, EAT, and skeletal muscle. Ectopic fat and visceral adipose tissue (VAT) are used interchangeably to describe fat deposition outside specialist tissue. VAT has been demonstrated to be an important risk factor and HIV-positive populations have high rates of visceral adipose tissue at lower body weights than general populations (296). Our own data, and others, have demonstrated the role of HS as an independent predictor of CVD (353). Specific pressures relating to HIV and ART may cause dysregulation of adipose tissue and

drive ectopic fat deposition (355). The finding of increasing EAT volume being associated with coronary calcification is therefore unsurprising given these pressures towards ectopic fat deposition. The intimate relationship of EAT with epicardial coronary arteries may explain how dysfunctional proinflammatory phenotypes of fat contribute to increasing atherosclerosis.

The significant association of EAT volume with coronary calcification in the HIV-positive group was demonstrated as a univariate predictor and through sequential multivariate models (table 2). In addition, HS remained an independent predicator of coronary calcification in the fully adjusted model. In contrast, both EAT volume and HS became non-significant after adjustment for age, sex, and BMI in the general population group. We calculated the logit odds of EAT volume (along with other continuous covariates) and demonstrated a strong linear relationship with the odds of coronary calcification (figure 2).

The association of EAT volume and coronary artery disease in HIV-positive patients has been assessed before. Sandouni *et al* demonstrated a significant association with EAT volume and non-calcified plaque on CTCA. The association with calcified plaque was not significant (356). Brener *et al*. found a significant association with calcified plaque and EAT volume in their minimally adjusted model, however, this association became non-significant in the fully adjusted models (347). The association of non-calcified plaque and any plaque was significant through all adjusted models. Other studies have demonstrated significant associations of coronary calcium and EAT volume (357,358).

We assessed the association of clinical covariates with EAT density in a subgroup of the HIV-positive group and all the general population group. Both groups had similar covariates that were associated with EAT density including EAT volume and BMI. We also assessed the association of EAT density on coronary calcification. Lower EAT density was associated with coronary calcification in both HIV-positive and general population groups (figure 2). However, after adjustment this association became non-significant. Data on EAT density in

HIV-positive groups is lacking. The association of EAT density, CVD risk factors, and coronary calcium has been assessed in general population populations. EAT density has been previously shown to be independently associated with coronary calcium, in low to medium CVD risk groups (359). However, higher EAT attenuation has been shown to be independently associated with those suffering myocardial infarction versus stable coronary artery disease (352). The SMART study analysed 140 patients at high risk of CVD. They found the strongest associations of EAT density and clinical covariates were male sex, BMI, and lower adipose tissue attenuation (360). Our data is broadly in keeping with previous work, demonstrating similar associations of clinical covariates and EAT density. Both HIV-positive and general population groups had similar predictors of EAT density. Both groups also demonstrated no significant association with coronary calcification after adjustment. The role of EAT density in development of CVD risk in HIV-positive groups requires further study. Our data is the first to our knowledge which assesses the association of EAT density in HIV-positive patients.

There are differences in the data of studies assessing the association of EAT volume and CVD in HIV-positive groups. The EAT volumes vary significantly across studies. The mean EAT volume in our study was 68mm^3. Srinivasa *et al* reported a mean EAT volume of 65mm^3 in HIV infected women (330). Other studies have reported mean volumes >100mm^3 despite a similar BMI to our study (347,356,358). The reason for these differences is not clear but may represent differences in patient demographics and ethnicity. The method for quantifying EAT volume also differs across studies with different attenuation thresholds used.

We found a significant association with EAT volume and coronary calcium. Whilst previous studies have demonstrated EAT volume to be associated with coronary artery disease this has principally been non-calcified plaque (330,347,356). HIV-positive patients have previously been demonstrated to have higher burdens of non-calcified plaque compared to matched general population patients (361). This is thought to be caused by the persistent immune activation and inflammation seen in HIV. Coronary calcification represents the natural evolution of atherosclerosis, and quantification allows assessment of overall

atherosclerotic disease burden. We demonstrated a modest incidence of coronary calcification in the HIV-positive group (29.2%). Other groups reported coronary calcium incidences of 53-65.8% with similar rates between HIV-positive and general population groups (347,356). Our data show that the incidence of coronary calcium was double in the general population group. This is likely due to this population having a higher mean age and prevalence of CVD risk factors. There were also higher rates of statin prescription which may contribute to coronary calcification.

4.4.1 Limitations and strengths

We acknowledge several limitations to our study. First, assessment of coronary calcium and EAT volumes was performed in some instances on non-dedicated CT datasets of the thorax. These non-dedicated scans had taken place historically for different indications. Although it is recognised that assessment of EAT volumes and coronary calcification can be performed in a robust manor which is highly correlated to dedicated datasets, our datasets were not homogenous. By using non-contrast datasets, we were unable to assess different plaque morphologies. Second, by opportunistically selecting patients who had received CT scans of the thorax for alternative indications we may have introduced selection bias into the study. Thirdly, the general population cohort were a pre-defined group of patients with symptomatic low to medium risk chest pain and this may affect the generalisability of the result. We did not adjust for HIV-specific covariates or ART. However, this study was designed to assess the differences in associations of EAT volume and attenuation between HIV-positive and general population cohorts. In addition, due to the design of this study it was not possible to investigate for hard clinical outcomes such as cardiovascular events and death. This is due to the relatively low number of events per patient years. Statistical significance would require many thousands of patients to show any true differences on this outcome. Instead, we measured against a well-established surrogate for cardiovascular risk (coronary artery calcium).

Despite these limitations our study had several strengths. Our data was extremely well characterised with <5% missing values. This study is unique in the way that HS, an important

visceral adiposity depot and marker of CVD risk, was assessed with EAT metrics to assess associations with coronary calcification. This is also the first study comparing EAT attenuation between HIV-positive and general population groups. We were also able to perform a sensitivity analysis which confirmed the associations detected in the principal analysis. Finally, this study is designed to be hypothesis generating and no causality can be inferred from the retrospective nature.

4.5 Conclusions

The principal implications of this study are that increasing EAT volume may be an independent predictor of CVD risk in HIV-positive populations. This suggests a specific role for EAT in development of coronary atherosclerosis and hints at an altered underlying pathological mechanism compared to general population populations. Despite EAT volumes being lower, EAT was still associated with coronary calcification in the HIV-positive group. This suggests altered function of this fat depot irrespective of the volume of EAT. The impact of EAT density requires further evaluation and mechanistic analyses are required to elucidate the impact of adipose tissue function on CVD risk.

In conclusion, in this retrospective analysis we demonstrated a strong and significant independent association of EAT volume with coronary calcification in the HIV-positive cohort, but not in the general population group. Drivers of EAT volume differed in each group, suggesting different mechanistic drivers of coronary atherosclerosis depending on HIV-serostatus.

4.6 Tables and Figures

4.6.1 Table 1: Patient characteristics stratified by HIV-serostatus.

	General population	HIV-positive	P Value
Number	505	195	<0.005
Age (Mean (SD))	57.84 (12.2)	49.24 (10.6)	<0.005
Male Sex (%)	243 (48.1)	148 (75.9)	<0.005
Smoker (%)	81 (16.0)	57 (29.2)	<0.005
Ex Smoker (%)	105 (20.8)	7 (3.6)	<0.005
Hypertension (%)	218 (43.2)	28 (14.4)	<0.005
Diabetes (%)	81 (16)	5 (2.6)	<0.005
Hypercholesterolaemia (%)	184 (36.4)	14 (7.2)	<0.005
Statin use (%)	190 (37.6)	34 (17.4)	<0.005
Hepatosteatosis (%)	99 (19.6)	61 (31.3)	<0.005
EAT Volume (mm^3) (mean (SD))	118.35 (26.7)	68.03 (49.94)	<0.005
TC (mean (SD)) mmol/L	4.64 (1.03)	4.76 (1.11)	0.188
TG (mean (SD)) mmol/L	1.74 (0.80)	1.89 (1.45)	0.096
LDL (mean (SD)) mmol/L	2.40 (0.93)	2.51 (1.08)	0.160
HDL (mean (SD)) mmol/L	1.36 (0.36)	1.34 (0.48)	0.555
BMI (mean (SD)) mmol/L	29.62 (4.68)	27.33 (6.38)	<0.005
Coronary calcium (%)	294 (58.2)	57 (29.2)	<0.005

HS, Hepatosteatosis; TC, total cholesterol; TG, triglyceride; LDL, low-density lipoprotein; HDL, high density lipoprotein; BMI; body mass index.

4.6.2 Table 2: Univariate and multivariate predictors of coronary calcification

	HIV-positive						General population					
	Univariate OR	p value	Model 1	p value	Model 2	p value	Univariate OR	p value	Model 1	p value	Model 2	p value
Age	1.10	<0.005					1.11 (1.09-1.14	<0.005				
Sex	2.91 (1.28-7.53)	0.016					1.90 (1.33-2.74)	<0.005				
Smoker	1.48 (0.75-2.85)	0.249	2.78 (1.23-6.48)	0.015			1.27 (0.78-2.09)	0.345	1.87 (1.06-3.35)	0.031		
Ex-smoker	1.86 (0.36-8.71)	0.426	0.92 (0.13-5.77)	0.924			1.57 (1.01-2.49)	0.050	1.71 (1.03-2.89)	0.042		
Hypertension	1.42 (0.59-3.25)	0.417	0.85 (0.36-2.16)	0.738			1.84 (1.28-2.66)	<0.005	1.08 (0.70-1.67)	0.733		
Diabetes	1.64 (0.21-10.13)	0.595	1.38 (0.15-9.96)	0.754			2.16 (1.29-3.71)	<0.005	1.18 (0.65-2.19)	0.580		
Hypercholesterolaemia	3.59 (1.19-11.41)	0.024	2.15 (0.67-7.16)	0.197			1.90 (1.31-2.80)	<0.005	1.46 (0.94-2.28)	0.092		
Statin	3.52 (1.64-7.63)	<0.005	2.19 (0.91-5.27)	0.079			3.17 (2.15-4.74)	<0.005	1.77 (1.12-2.81)	0.014		
EAT Volume (10mls)	1.22 (1.14–1.32)	<0.005	1.14 (1.05-1.25)	<0.005	1.14 (1.05-1.26)	<0.005	1.12 (1.08-1.17)	<0.005	1.05 (0.99-1.10)	0.089	1.04 (0.99-1.10)	0.104
HS	4.29 (2.23-8.39)	<0.005	3.41 1.62-7.28)	<0.005	3.17 (1.46-7.02)	<0.005	1.07 (0.69-1.69)	0.757	0.85 (0.49-§.46)	0.549	0.81 (0.47-1.45)	0.444
TC	1.16 (0.87-1.53)	0.306	1.01 (0.73-1.39)	0.953	1.05 (0.74-1.48)	0.778	0.60 (0.50-0.73)	<0.005	0.76 (0.61-0.93)	0.010	0.75 (0.60-0.93)	0.012
TG	1.32 (1.07-1.68)	0.014	1.26 (1.00-1.61)	0.052	1.23 (0.94-1.64)	0.142	0.91 (0.73-1.14)	0.421	1.10 (0.84-1.44)	0.493	1.06 (0.79-1.37)	0.699

LDL	1.12 (0.84-1.50)	0.422	1.03 (0.75-1.43)	0.839	1.09 (0.76-1.57)	0.642	0.67 (0.55-0.82)	<0.005	0.81 (0.64-1.02)	0.070	0.83 (0.65-1.05)	0.115
HDL	0.45 (0.20-0.92)	0.037	0.35 (0.14-0.79)	0.016	0.43 (0.16-1.01)	0.066	0.83 (0.51-1.36)	0.464	0.53 (0.29-0.99)	0.050	0.55 (0.28-0.98)	0.045
BMI	1.05 (1.00-1.11)	0.034					1.05 (1.01-1.09)	0.022				

HS, Hepatosteatosis; TC, total cholesterol; TG, triglyceride; LDL, low-density lipoprotein; HDL, high density lipoprotein; BMI; body mass index.

(Odds ratio)

Model 1: Age, Sex, BMI

Model 2: Age, Sex, BMI, Smoker, HTN, DM, Chol, Statin

4.6.3 Figure 1: An example of QFAT quantification of epicardial adipose tissue volume and density

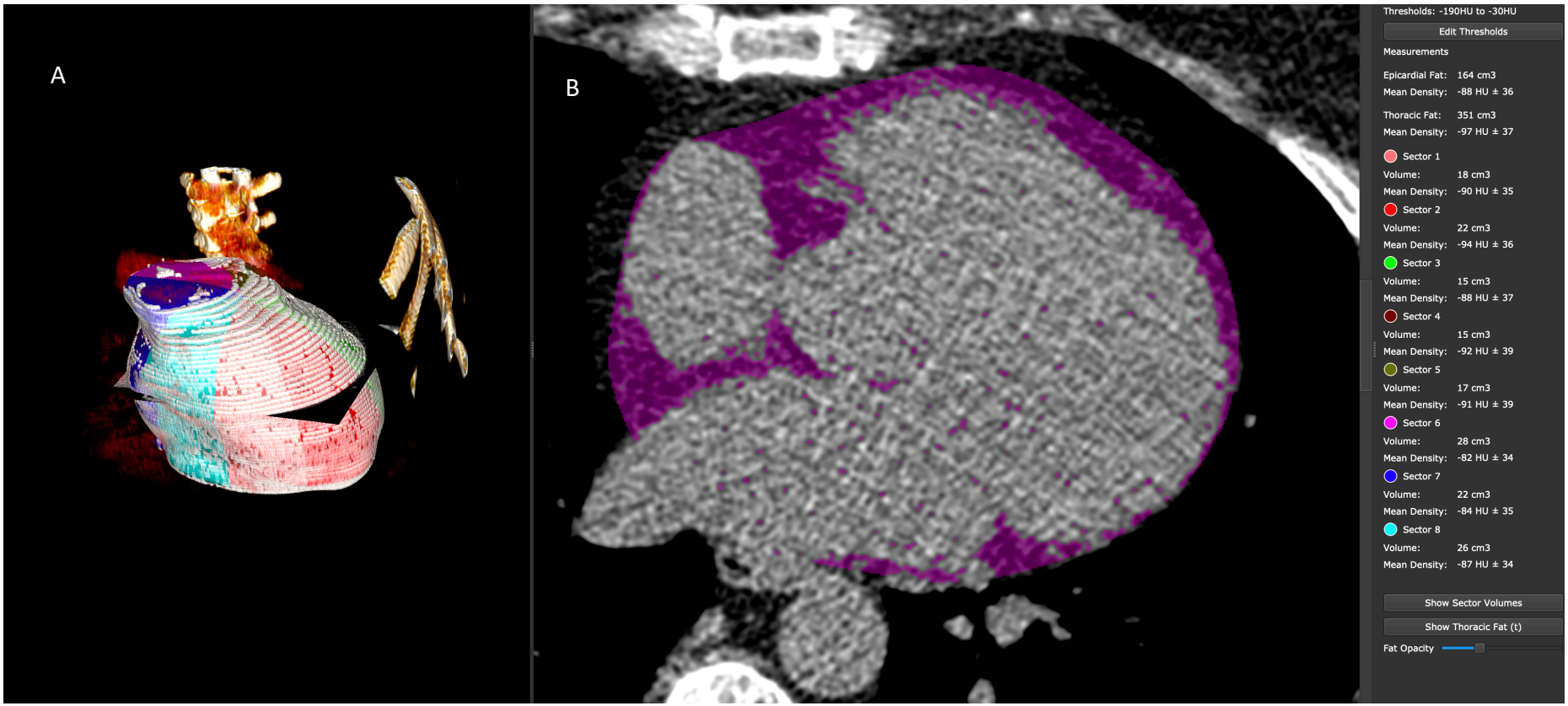


Figure 1 legend: Panel A demonstrates a 3D reconstruction of the epicardial fat. The sub mask colours demonstrates the programmes ability to regionalise epicardial fat volumes and densities. Panel B demonstrates the machine learning depiction of the epicardial contours. Within the contours any voxel within the pre-specified Hounsfield unit range is shaded. The right hand side of the panel demonstrates the quantification of epicardial fat volume and mean density.

4.6.4 Figure 2: Logit odds of coronary calcification and continuous covariates

(a) Associations of continuous covariates with logit odds of coronary calcification in the General population group.

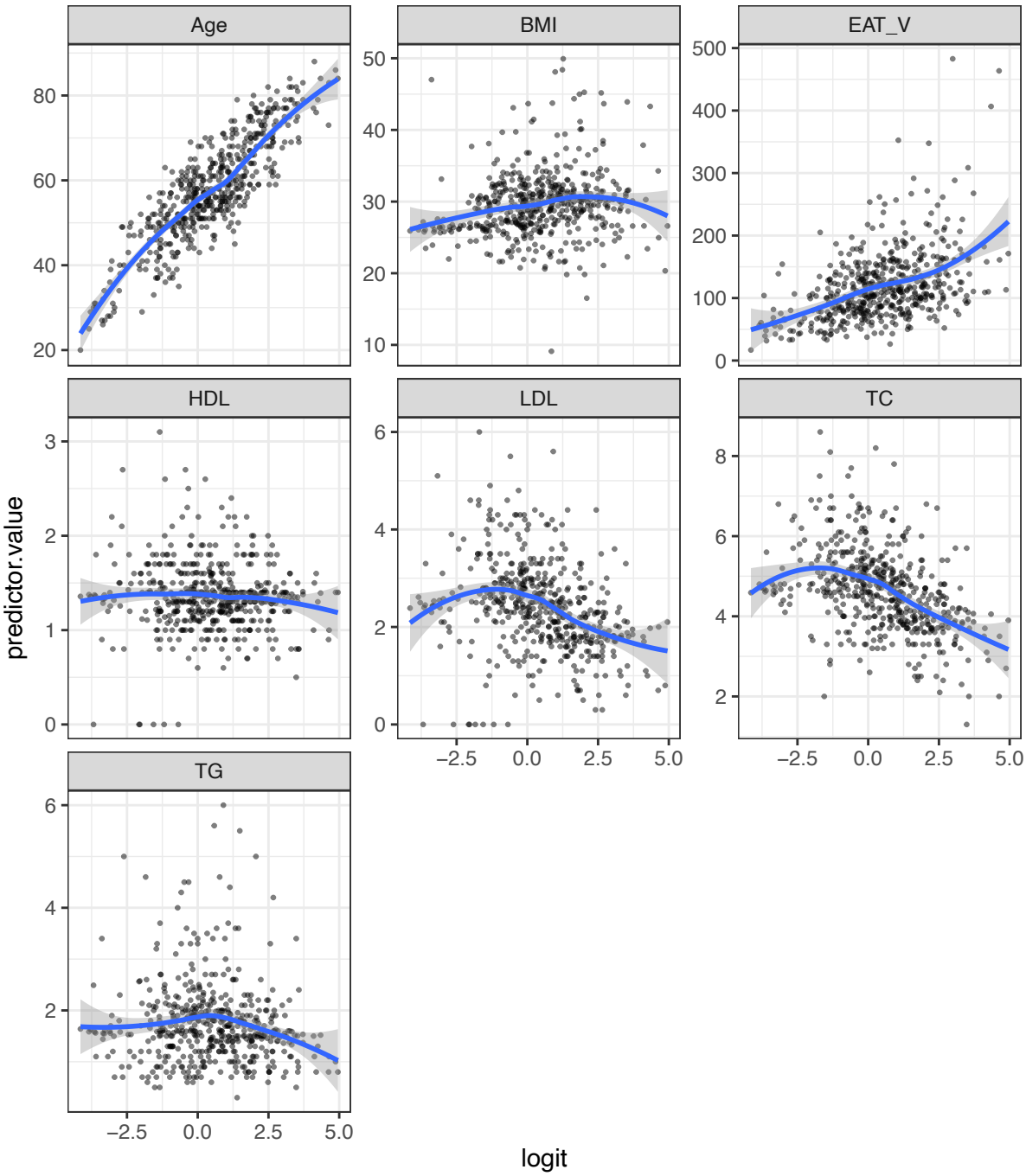


Figure 2 (continued)

(b) Association of continuous covariates with logit odds of coronary calcification in the HIV-positive group.

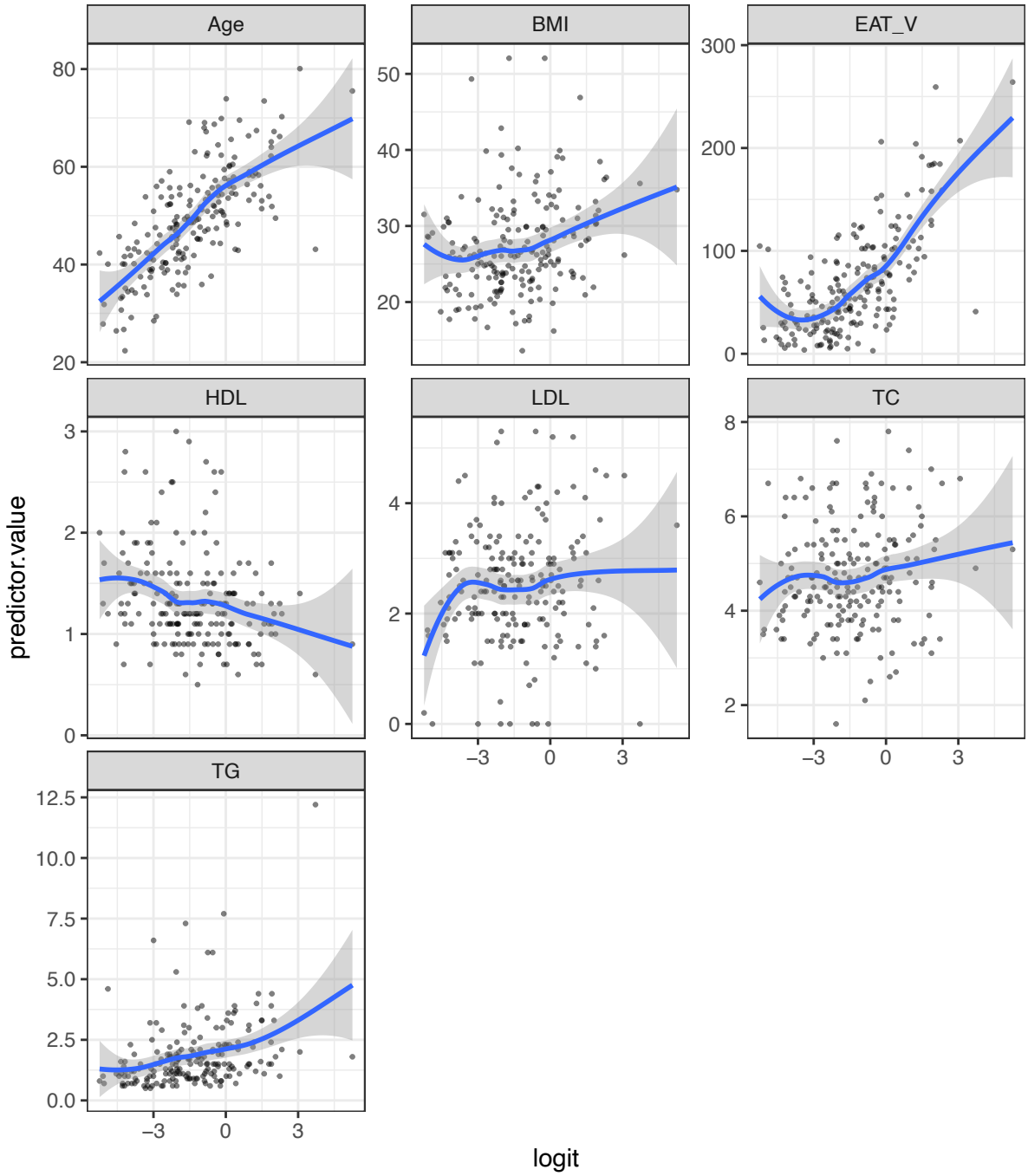
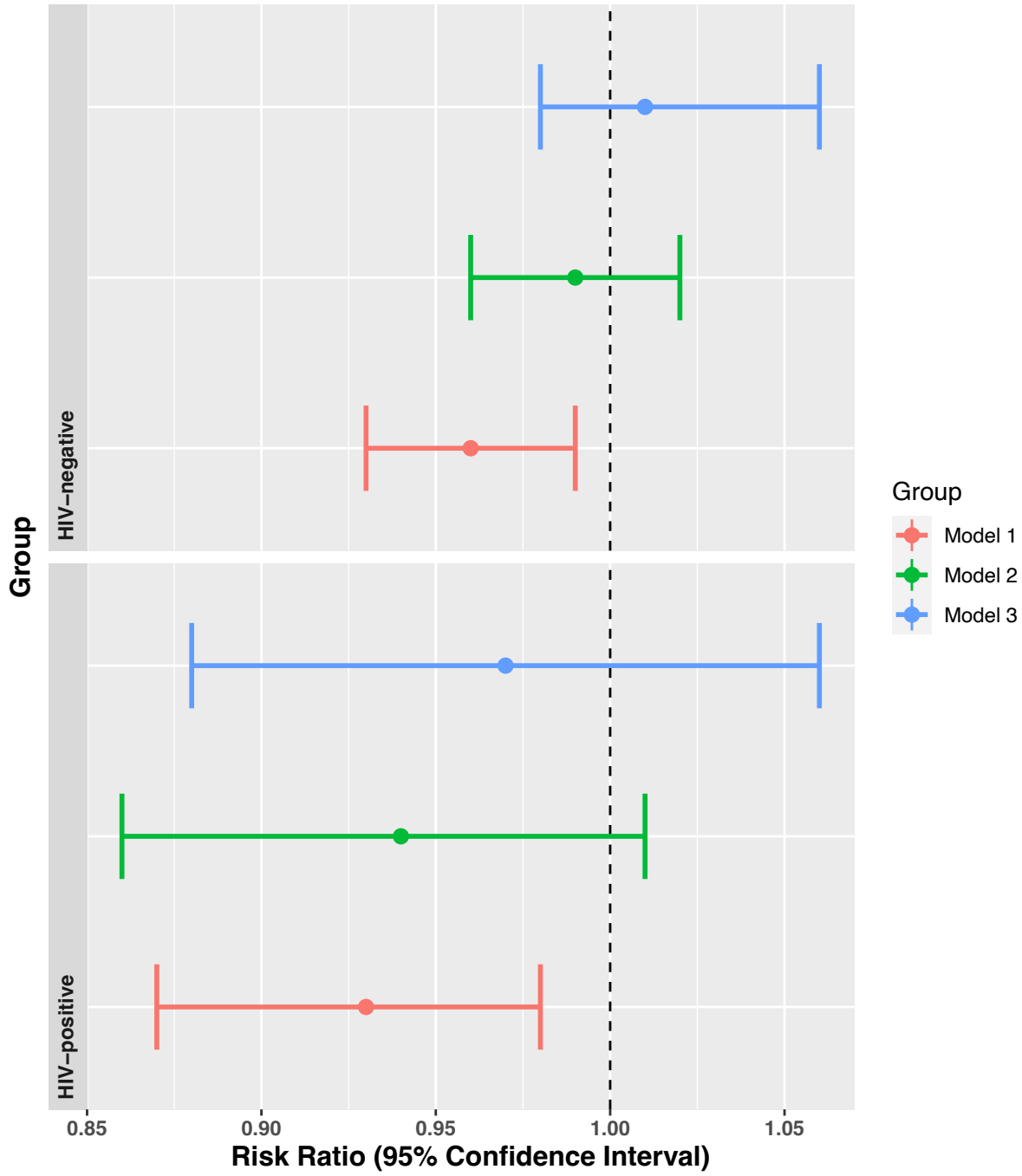


Figure 2 legend: the logtit odds for clinical covariates and their association with coronary calcification in HIV-positive and general population groups. In both groups age has a strong, positive linear relationsip with increasing odds of coronary calcification. EAT volume also

had a linear relationship in both groups. The other clinical covariates do not appear to have a linear relationship with coronary calcification.

BMI, body-mass index; EAT_V, epicardial adipose tissue volume; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglycerid

4.6.5 Figure 3: The association of EAT density with coronary calcification in HIV-positive and General population groups



Model 1: Univariate

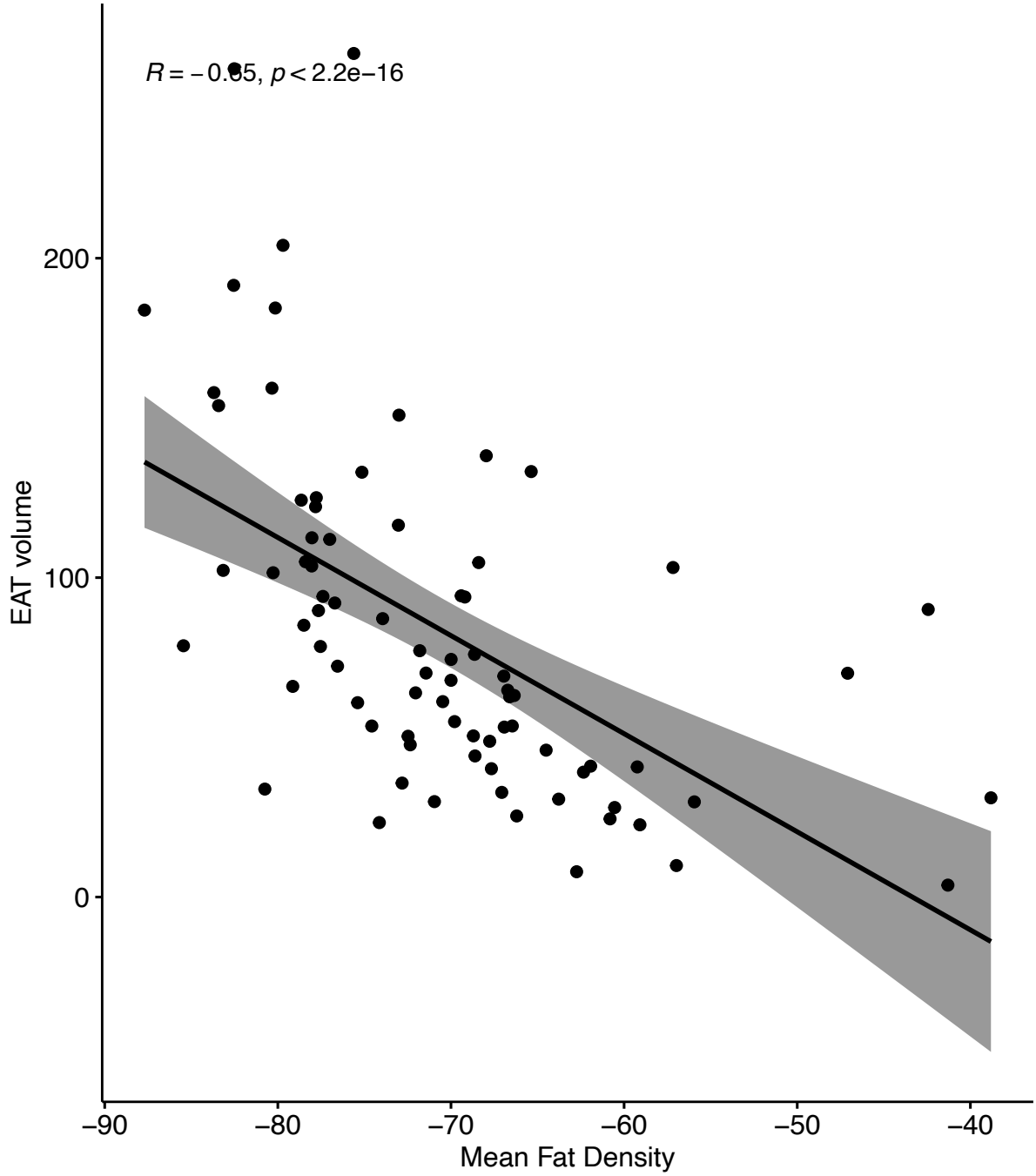
Model 2: Adjusted for age and sex

Model 3: Adjusted for age, sex, HS, and BMI

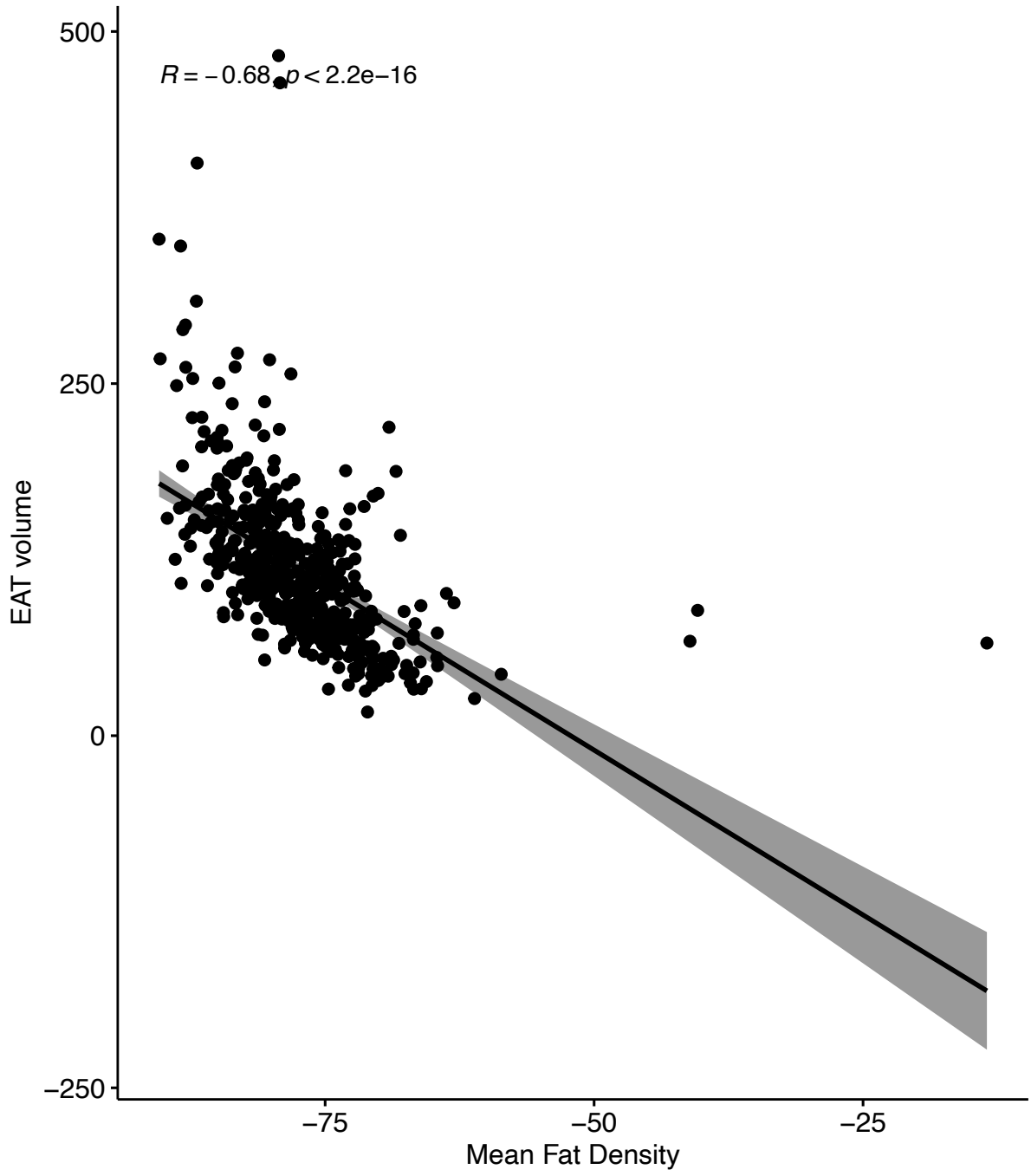
Figure 3 Legend: Illustration of risk ratios with confidence intervals for EAT density and coronary calcification in HIV-positive and general population groups. Model 1(red) models EAT density as a univariate predictor with significant associations in both groups. Model 2 (green) shows a partially adjusted model (non-significant in both groups). Model 3 (blue) shows the fully adjusted model which shows no significant association.

4.6.6 Supplementary Figure 1: Correlation between EAT volume and EAT density in HIV positive and HIV negative groups.

(a) HIV-Positive

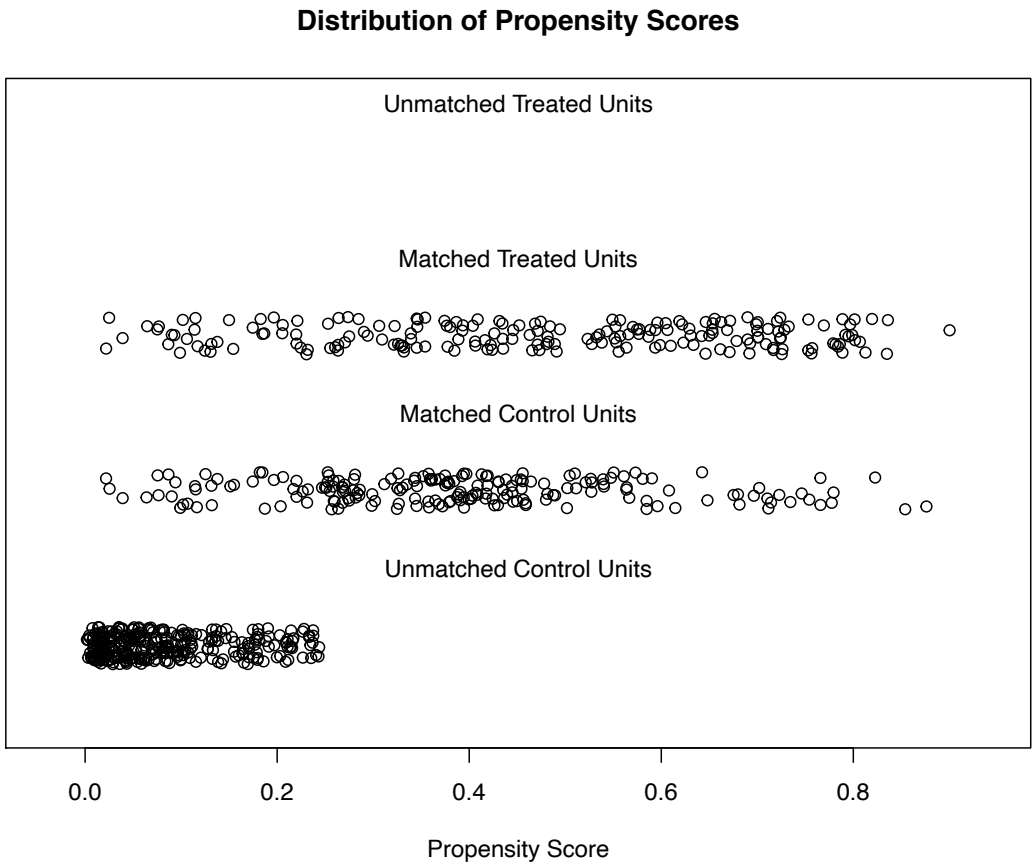


(b) General population



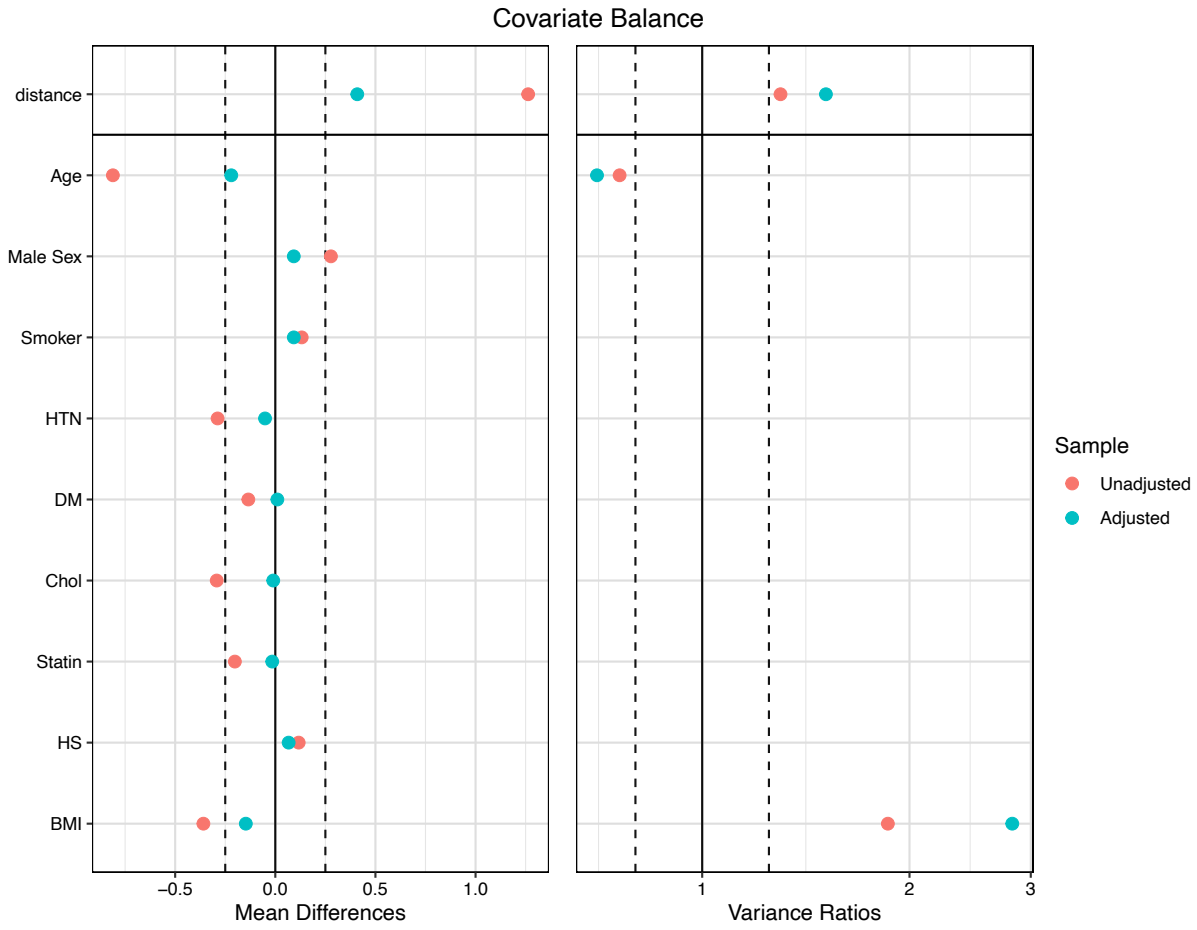
Supplementary figure 1 legend: correlation of mean fat density and EAT volume with corresponding R value. Figure 2 a) shows the HIV-positive group and figure 2 shows the general population group

4.6.7 Supplementary figure 2: Distribution of Propensity Scores



Supplementary figure 2 legend: Graphical representation of matching algorithm performance on HIV-positive and general population individuals

4.6.8 Supplementary figure 3: Balance of Covariate in Propensity Score Matching



Supplementary figure 3 legend: Graphical representation of the adjusted and unadjusted means and variances of covariates in the propensity score matching algorithm

4.6.9 Supplementary Table 1: Multiple linear regression for the association of clinical covariates and EAT volume

	General population				HIV-positive			
	Coefficient	Std Error	P value	Significance	Coefficient	Std Error	P value	Significance
Intercept	-5.61	1.36	<0.005	*	-8.20	2.05	<0.005	*
Age	0.11	0.01	<0.005	*	0.09	0.02	<0.005	*
Male Sex	0.75	0.25	<0.005	*	0.71	0.62	0.249	
Smoker	0.79	0.31	0.011	*	0.80	0.46	0.085	
Ex Smoker	0.81	0.29	<0.005	*	0.01	1.01	0.993	
Hypertension	-0.01	0.23	0.957		-0.73	0.67	0.278	
DM	-0.05	0.34	0.898		-0.47	1.11	0.669	
Dyslipidaemia	0.24	0.27	0.371		1.06	0.75	0.158	
Statin	0.39	0.28	0.160		0.10	0.64	0.882	
Hepatosteatosis	-0.31	0.30	0.289		0.88	0.43	0.040	*
TC	-0.61	0.28	0.030	*	-1.07	1.00	0.281	
TG	0.18	0.20	0.374		0.75	0.53	0.153	
LDL	0.36	0.41	0.317		1.10	0.97	0.256	
HDL	0.02	0.03	0.349		0.67	1.16	0.564	
BMI	0.03	0.03	0.349		0.02	0.04	0.593	

HS, Hepatosteatosis; TC, total cholesterol; TG, triglyceride; LDL, low-density lipoprotein; HDL, high density lipoprotein; BMI; body mass index.

4.6.10 Supplementary Table 2: Univariate and multivariate predictors of EAT volume

	HIV-positive			General population		
	Univariate	Model 1	Model 2	Univariate	Model 1	Model 2
Age	<0.005*	<0.005*	-	<0.005*	<0.005*	-
Male Sex	<0.005*	<0.005*	-	<0.005*	<0.005*	-
Smoker	0.443	0.800	0.753	0.858	0.484	0.193
Ex-smoker	0.016*	0.034*	0.104	0.356	0.187	0.187
Hypertension	0.115	0.212	0.747	<0.005*	0.030*	0.593
Diabetes	0.262	0.322	0.401	<0.005*	0.005*	0.183
Hypercholesterolaemia	0.173	0.204	0.772	0.319	0.643	0.187
Statin	<0.005*	<0.005*	0.048*	<0.005*	<0.005*	0.45
TC	0.109	0.175	0.449	<0.005 *	<0.005*	0.576
TG	0.154	0.345	0.931	0.006*	0.104	<0.005*
LDL	0.254	0.265	0.385	<0.005*	<0.005*	0.111
HDL	0.116	0.151	0.276	0.132	0.546	0.5
HS	<0.005*	<0.005*	<0.005*	<0.005*	0.032*	0.067*
BMI	<0.005*	-	-	<0.005	-	-

HS, Hepatosteatosis; TC, total cholesterol; TG, triglyceride; LDL, low-density lipoprotein; HDL, high density lipoprotein; BMI; body mass index.

Model 1: BMI

Model 2: Age, sex, BMI

4.6.11 Supplementary Table 3: HIV-specific covariates

	N=195
Current ABC	31 (15.9%)
Current PI	26 (13.3%)
Current TDF	37 (19%)
Current TAF	114 (58.5%)
Length of diagnosis	11.2 years
Prior AIDS	5 (2.6%)
FRS	9%

ABC, Abacavir; PI, Protease Inhibitor; TDF, Tenofovir Disoproxil; TAF, Tenofovir Alafenamide; FRS, Framingham Risk Score

Chapter 5: Development of a calcium risk prediction tool in people living with HIV: The Liverpool HIV-Heart Project

5.1 Introduction

PLWHIV have an increased risk of developing CVD events compared to general population patients (362). This is due to a complex interaction of viral related factors, antiretroviral related factors, and traditional risk factors. Ectopic fat deposition is becoming increasingly recognised as an important consequence of these factors. This is likely driven by a reduced capacity of adipocytes to accommodate fatty acid storage (363). Hepatosteatosis, epicardial adipose tissue and skeletal muscle are the main recipient of ectopic fat deposition (341). Previous work, including our own data, has suggested that hepatosteatosis is an independent risk factor for CVD in PLWHIV (310,364).

Traditional risk prediction algorithms have been shown to underperform in PLWHIV (116). The D:A:D calculator was developed as a HIV-specific calculator and was shown to have superior discriminatory ability compared to FRS (117). A recent comparison of traditional risk calculators, including D:A:D, demonstrated reasonable discriminatory ability for CVD endpoints but statistically did not fit the data (118).

Treatment decisions, particularly implementation of lipid lowering medications, are based on the perceived CVD risk from these risk prediction tools. The predictive ability of these tools is modest and therefore may lead to inaccurate treatment decisions. Coronary artery calcium (CAC) scoring is a well-established method for predicting CVD risk and gives incremental diagnostic performance over and above algorithm driven risk prediction tools (213,365). Utilisation of CAC scoring to accurately delineate risk is recommended in intermediate risk asymptomatic patients (366).

The clinical utility of CAC scoring has been assessed in PLWHIV. Raggi *et al* demonstrated that CAC score >100 was an independent predictor of myocardial infarction (264). CAC has recently been demonstrated to have the ability to net-reclassify 42% of cases from high risk to non-high risk and vice-versa in PLWHIV (265). Based on this shifting paradigm we sought to develop a clinical risk calculator to accurately identify patients who have subclinical coronary calcification, and will therefore derive clinical benefit from preventative pharmacotherapies and lifestyle interventions. We compared developed models to traditional risk prediction algorithms to assess discriminatory ability and calibration.

5.2 Methods

We conducted a contemporary, retrospective analysis of the association of clinical comorbidities with coronary calcifications in PLWHIV. Data was collected from the Royal Liverpool University Hospital HIV clinical database and CTCA clinical database (Liverpool HIV-Heart Project). Both the HIV and CTCA databases exist for both clinical use and quality improvement purposes and are approved by the host institution's audit committee. All demographic and clinical variables present on the databases were cross checked by the research team using the Trust's electronic patient record.

The CTCA database contains demographic and clinical variables for all patients referred for cardiac CT (either CAC scoring or CTCA) between January 2014 and November 2016. The majority of patients were referred for the investigation of atypical angina and were considered low to medium risk in line with guidelines from the National Institute for Clinical Excellence that were established at the time (337). The presence of hepatosteatosis (HS) had previously been calculated by two independent radiologists and added to the clinical database. Patients were labelled as having coronary calcification if the calcium score was >0.

The HIV database was cross checked for patients that had a received a CT thorax within the last 10 years. The images were inspected by an independent imaging cardiologist for the presence of coronary calcification which was recorded in a binary fashion (yes or no). HS

was assessed using non-contrast CT scans or venous phase CT scans in those with liver parenchyma visible on the images. Both imaging techniques are established methods for quantifying liver fat content (333–336). Two regions of interest circles covering 100mm^2 were drawn on the right lobe of the liver and one on the left lobe. The mean attenuation (Hounsfield unit [HU]) was recorded for liver parenchyma. Regions with non-uniform attenuation and hepatic vessels were avoided. A further 100mm^2 region of interest was drawn on the spleen and the HU recorded. HS was confirmed if the liver to spleen ratio (L/S ratio) was less than 1 and/or the mean hepatic measurement was <40HU in non-contrast scans. Liver/spleen ratio of <0.8 was used for the venous phase imaging.

Hepatosteatosis was also confirmed in patients that had a prior imaging (ultrasound) or biopsy confirmed diagnosis of fatty liver. Patients with prior diagnoses of CVD (including clinical diagnosis, imaging diagnosis of coronary plaque or coronary event or intervention) were excluded. Patients were also excluded if no quantification of coronary calcifications or quantification of HS was not possible.

5.2.1 Statistical Analysis

Summary statistics were calculated to compare the distribution of demographic and clinical covariates in the cohort. All data were inspected using graphical representation for normality (histograms and Q-Q plots) and Shapiro-Wilk test. The proportions between categorical variables were compared using Chi Squared test. Continuous variables were presented as means and standard deviations where normally distributed. The means were compared using an independent t test. P-values were considered statistically significant if <0.05. Missing data occurred in <5% of cells. A random forest generated multiple imputation technique was used to impute the missing values. The data was then split into training (70%) and test (30%) subsets.

The statistical analysis and development of the models were performed using RStudio, version 1.3.1056.

5.2.2 Multivariate Modelling

Multiple logistic regression models were developed to ascertain the association of clinical covariates and coronary calcification. Each covariate was selected *a priori*. We then undertook a variable selection process using multiple techniques including backwards stepwise regression techniques using AIC assess for the best fit. We modelled the impact of continuous data using a multivariate fractional polynomial (MFP) procedure. Continuous data are assessed for linearity with the dependent variable. A closed test backwards elimination technique assessing best fit and modelling transformations of non-linear data was used (367). The full model is entered into the programme and fractional polynomials are examined for the best fit (closed test procedure). The fractional polynomials and best fitting variables are produced in the output (368).

5.2.3 Model Selection

Models generated using the above variable selection methods were compared. The accuracy of the models was compared on the training data set. The discriminatory ability of the models was calculated using the area under receiver operator curve (AUC) and the calibration compared by inspection of the calibration plot and Brier Score. The goodness of fit for each model was compared using a Hosemer-Lemeshow (HL) statistic. The final model was selected according to best discriminatory performance and calibration.

5.2.4 Internal Validation

The final model was then assessed on the test dataset and compared to the FRS model on the test dataset. Receiver operator characteristic (ROC) curves were constructed for each model and the discriminatory ability of the models were compared using area under the curve (AUC). Confidence intervals for the AUC were calculated using a bootstrap technique with 1000 repetitions. Calibration plots were constructed using predicted probabilities and true outcomes. Calibration was also assessed using the HL test and Brier score.

5.2.5 Sample Size

The sample size for predictive logistic regression models is challenging. One accepted technique for the minimum suggested cases is based work by Peduzzi *et al* (369). The minimum number of cases is based on the following equation: N=10k/p. For this cohort k=7, p=0.41 (using forward regression model):

N= (10*7) / 0.41 = 170.7

Therefore, our sample size of 195 should give our model statistical power.

5.3 Results

The overall numbers of participants included was 355. The baseline characteristics for this cohort, stratified for presence of coronary calcium, are displayed in table 1. Mean age (48.06 versus 56.9, p<0.001) and proportion of male sex (69.4% versus 89.2%, p<0.001) were significantly higher in the coronary calcium group. Dyslipidaemia was significantly higher in the coronary calcium group (5% versus 13.5%, p=0.019). There was no other statistically significant difference in other comorbidities.

Within the lipid covariates levels mean triglyceride (1.79 versus 2.18, p=0.021) and mean HDL (1.39 versus 1.25, p=0.023) were the only significantly different variables. Hepatosteatosis (21% versus 56.8%, p<0.001) and FRS (8% versus 14%, p<0.001) were also significantly higher in the coronary calcium group.

5.3.1 Model Development: Discrimination and Calibration

Variables were considered for the model *a priori* and included in the full model. All the covariates were entered into the variable selection techniques. The step-AIC model selected variables according to the best possible fit. We compared the step-AIC model, MFP model and full model with FRS derived prediction of coronary calcification. ROC curves were constructed for the models. The discriminatory ability was compared using AUC (figure 1). The step-AIC model had excellent discriminatory ability to detect coronary calcification (AUC: 0.825, 95% CI: 0.767-0.884). The MFP model also had excellent discriminatory ability

(AUC:0.821 95% CI: 0.761-0.880). Both models had a statistically significant higher discriminatory ability compared to the FRS model (p<0.001 for both, Wilcoxon test).

To assess goodness of fit, we utilised HL test and Brier score. The step-AIC model (χ^2=5.388, p=0.716), MFP model (χ^2=4.117, p=0.846) and FRS model (χ2=9.642, p=0.291) all fit the data well as assessed by HL statistic and subsequent p value. There was also no significant difference in Brier Score's across the models (table 2). Given the similarities in accuracy and data fit between the step-AIC model and the MFP model, we selected the simplest model. The MFP had fewer variables included within the model and hence was the preferred model (table 3).

5.3.2 Internal Validation

Following assessment of our models on the training dataset we selected the MFP model as having a combination of superior discriminatory ability and calibration scores. This final model was assessed on the test set and compared to FRS. The MFP model demonstrated a significantly greater AUC compared to the FRS model (0.886 95% CI: 0.799-0.974 versus AUC 0.712 95% CI:0.604-0.821, p<0.001). The ROC curves are compared in figure 3. The MFP model was significantly higher AUC compared to the FRS model (p<0.001, Wilcoxon test). The MFP model also showed a good overall fit to the testing data (HL statistic: χ^2 = 10.36, p=0.24).

5.4 Discussion

The purpose of this study was to develop a HIV-specific risk calculator to predict the presence of coronary calcification. Using multiple statistical modelling techniques, we were able to demonstrate that our final model was superior to FRS at predicting the presence of coronary calcification (figure 2). This may improve the accuracy of those who would benefit from preventative pharmacotherapy.

Predictive models of CVD outcomes in PLHIV have been shown to both underestimate and overestimate CVD risk. Van Zoest *et al* compared the performance of the D:A:D risk calculator, SCORE-NL, FRS and PCE risk calculators in over 16,000 patients in the ATHENA cohort. They found that D:A:D, SCORE-NL and PCE underestimated risk whilst D:A:D overestimated true CVD risk. Furthermore, CVD risk was underestimated in lower CVD risk groups. All of the risk prediction models produced a significant lack of fit to the data (370).

The enhanced risk prediction demonstrated in our final model may be due to the inclusion of HS as a clinical covariate. Our own data, and data from other groups, support HS as an independent risk factor for the presence of CVD. HS is a marker of visceral adiposity which is significantly increased in PLWHIV (296). This 'fat redistribution' has been associated with increased oxidative stress, inflammation, and dysregulation of adipokines (355). Such inflammation is driven through secretion of proinflammatory cytokines from increasingly lipotoxic, hypertrophied adipocytes (371). This may underpin the reasons why HS is an independent risk factor for CVD. The presence of hepatosteatosis in PLWHIV is an emerging health concern and may be a contributor to the excess CVD morbidity and mortality seen in this patient group (310,364).

The use of CACS to estimate CVD risk is well established in general populations. In the Heinz Nixdorf recall study the addition of CACS reclassified patients risk profile in up to 30.6% (365). Calcium scores have been incorporated into risk prediction models to provide enhanced 10-year CVD risk prediction (212). This enhanced risk prediction is the basis to recommend preventative pharmacotherapy in those with a 10-year CVD risk >7.5%. The AHA guidelines now recommend CACS in asymptomatic patients with intermediate CVD risk to aid treatment decisions (366). In HIV populations Pereira *et al* recently published a cross sectional analysis comparing CACS with FRS, QRISK2 and D:A:D scores. In those with potential eligible for preventative pharmacotherapy (FRS>10%), 23% had no calcium detectable. In those who were low risk (FRS <10%), 19.6% had evidence of detectable calcium (265). This results demonstrate the poor performance of FRS to predict the

presence of coronary calcification and, by extension, those who would benefit from preventative pharmacotherapy.

The emergence of HS as a potential CVD risk factor may represent a potential imaging biomarker to enhance CVD risk prediction. HS has been shown in both HIV-positive and general population groups to be an independent predictor of CVD. The variable selection models produced using the techniques in this study all included HS due to its predictive value on coronary calcification. These results suggest our MFP model may provide a useful clinical risk prediction tool for detecting the presence of coronary calcification. This risk calculator may help delineate PLWHIV who would benefit from enhanced risk prediction in the form of CACS.

5.4.1 Limitations and Strengths

There are several limitations to this study. Firstly, this initial study utilises internal validation of the models. This methodology has inherent biases and may lead to over confidence in the final model as the testing data was generated from the same cohort as the training data. These models require external validation in large diverse cohorts prior to clinical implementation. Secondly, coronary calcifications were assessed retrospectively on CT scans of the thorax. Often these were not dedicated sequences to detect coronary calcification and had been performed for alternative reasons. However, it is recognised that quantification of coronary calcifications on non-dedicated CT is valid (225). Thirdly, in order to undertake internal validation we split our data set (70% training and 30% test) which may reduce the power of the final models. As with all internal validation studies we expect this model to be overfitted to our data and rigorous external validation studies are required.

Despite these limitations there are multiple strengths to our study. The cohorts used to generate these models were extremely well characterised and had very few missing data (<5%). This study is unique in the fact that this is the first attempt to generate a clinical risk

predictor in HIV-positive patients to detect the presence of coronary calcification. This may lead to accurate decisions of whom may be eligible for CACS. We also undertook a robust assessment of the models generated from various variable selection techniques. The models generated demonstrated excellent performance. We also report measures of calibration in different subgroups. The models generated from this initial study requires external validation in a separate cohort.

5.5 Conclusions

We have demonstrated a robust technique for building clinical risk prediction tools for the detection of coronary calcification in PLWHIV. This simple clinical risk calculator demonstrated excellent discriminatory ability and was significantly superior to FRS in determining presence of coronary calcification in internal validation. The presence of HS as a CVD risk factor may improve our ability to determine those at risk of CVD. The development of this risk calculator may enable appropriate decisions regarding whom will benefit from CACS leading to accurate prescriptions of preventative pharmacotherapies. Further external validation studies are required to ascertain the clinical utility of this risk prediction tool.

5.6 Tables and Figures

5.6.1 Table 1: Summary statistics of the cohort stratified by coronary artery calcification

	No Coronary Calcium	Coronary Calcium	p
n	281	74	
Age (mean (SD))	48.06 (±9.73)	56.9 (±9.77)	<0.001*
Male Sex (%)	195 (69.4%)	66 (89.2%)	0.001*
Current smoker (%)	75 (26.7%)	22 (29.7%)	0.707
HTN (%)	52 (18.5%)	15 (20.3%)	0.859
DMII (%)	15 (5.3%)	4 (5.4%)	1
Dyslipidaemia (%)	14 (5%)	10 (13.5%)	0.019*
CKD (%)	30 (10.7%)	13 (17.6%)	0.157
TC (mean (SD))	4.93 (±3.16	4.83 (±1.32)	0.791
TG (mean (SD))	1.79 (±1.2)	2.18 (±1.64)	0.021*
HDL (mean (SD))	1.39 (±0.48)	1.25 (±0.43)	0.023*
LDL (mean (SD))	2.51 (±0.99)	2.6 (±1.24)	0.487
Non-HDL (mean (SD))	3.37 (±1.05)	3.59 (±1.26)	0.118
FRS (mean (SD))	0.08 (±0.06)	0.14 (±0.07)	<0.001*
Length Diagnosis (mean (SD))	11.88 (±7.1)	12.66 (±8.13)	0.414
HS (%)	59 (21%)	42 (56.8%)	<0.001*
BMI (mean (SD))	26.29 (±9.87)	27.45 (±8.58)	0.355

HTN, Hypertension; DMII, Type II diabetes; CKD, Chronic kidney disease; TC, Total cholesterol; TG, Triglyceride; HDL, High-density lipoprotein; LDL, Low-density lipoprotein; FRS, Framingham risk score; HS, Hepatosteatosis; BMI, Body-mass index

*Denotes significant association

5.6.2 Table 2: Comparison of accuracy statistics in training dataset

	Step-AIC	FRS	Polynomial
AUC	0.825	0.760	0.821
HL	5.388	9.642	4.117
HL p value	0.716	0.291	0.846
Brier Score	0.12	0.15	0.13

AUC, Area Under the Curve; HL. Hosmer-Lemeshow test

5.6.3 Table 3: Intercept, coefficients, and significance of the variables in the MFP model

	Estimate	**Std. Error**	**z value**	**P Value**
(Intercept)	-7.91057	1.39607	-5.666	<0.001
Age	0.09894	0.02126	4.654	<0.001
HS	1.24741	0.37284	3.346	<0.001
Male Sex	1.72045	0.69393	2.479	0.013
I((LDL + 0.1)^2)	-0.35699	0.14392	-2.48	0.013
I((LDL + 0.1)^3)	0.08227	0.02864	2.872	0.004

HS, Hepatosteatosis; LDL. Low density lipoprotein

5.6.4 Figure 1: Comparison of the receiver operator characteristics curves for the development models

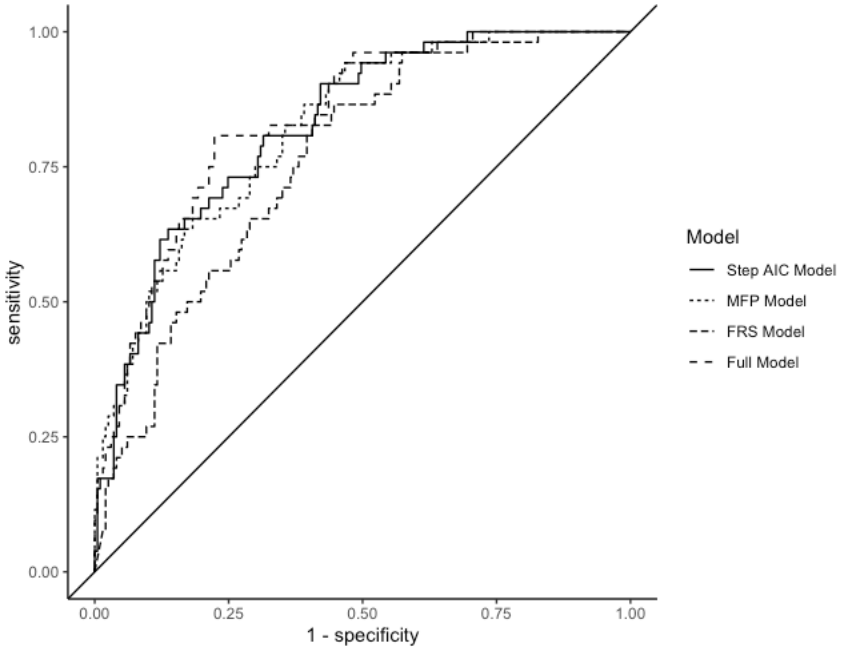


Figure Legend: Comparison of receiver operator characteristic curves for the differing models.

5.6.5 Figure 2: Comparison of the receiver operator characteristic curves for the final model and FRS model

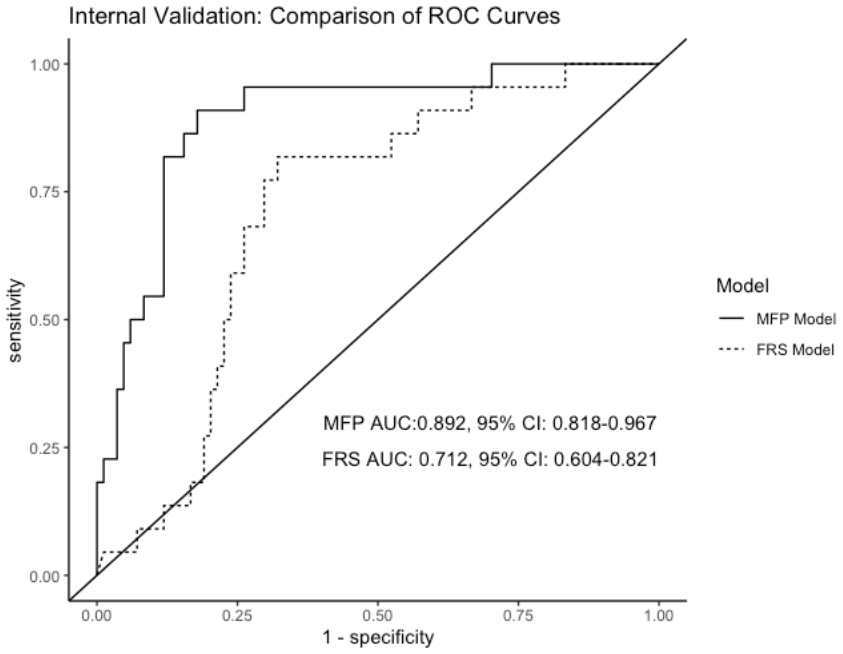


Figure legend: Comparison of receiver operator characteristic curves for the 'final' MFP model compared with the FRS model.

5.6.6 Figure 3: Calibration plot of the final model and FRS model

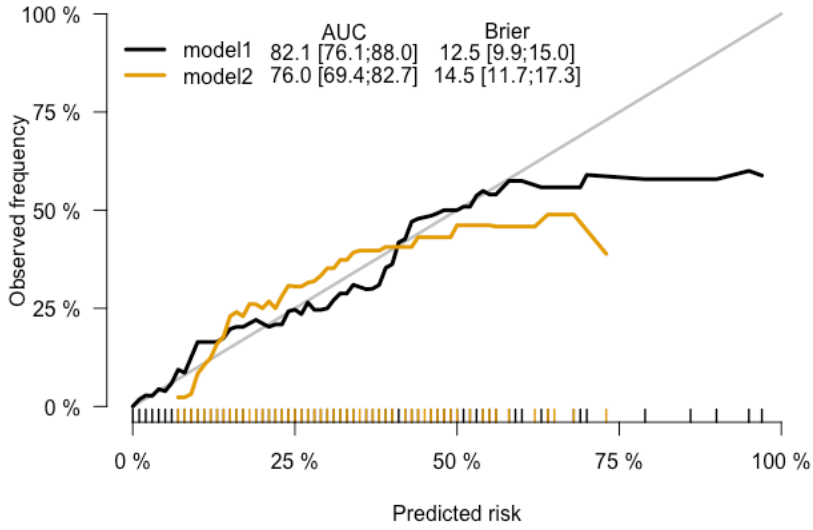


Figure legend: Calibration plot of training

Chapter 6: Changes in lipid profiles after switching to TAF/FTC/BIC

6.1 Introduction

CVD is an increasing health concern amongst PLWHIV. Modern ART has significantly extended the life expectancy of this patient group (372). As a consequence, patients are succumbing to traditional diseases of ageing rather than AIDS related illness (4). PLWHIV have excess CVD risk compared to risk matched general population groups (362). The mechanisms behind this increased risk have not yet been fully elucidated but likely centre on chronic inflammation, immune activation, and dysfunction of lipid storage. Up to 78% of PLWHIV are expected to develop CVD by 2030 (16).

Primary prevention of CVD is therefore of increasing importance. Traditional CVD risk scores are known to underpredict the risk of CVD in PLWHIV. HIV specific risk calculators have been developed but remain inaccurate (373). The cornerstone of risk prediction with these tools is the lipid profile. Data from the D:A:D study suggests that for every 1mmol/L increase in TC the risk of myocardial infarction increases 26% (38). Treatment decisions regarding preventative pharmacotherapy and ART switching continue to be made in response to adverse lipid profiles and a perceived heightened CVD risk.

Prior work has suggested that TAF/FTC/BIC has a neutral effect on the lipid profile and is therefore seen as a CVD safe preparation (374). Compared to dolutegravir based regimes, TAF/FTC/BIC has been shown to have no significant effect on the lipid profile in treatment naïve individuals (375). Recently, switching to TAF/FTC/BIC has been shown to result in significant increases in TC, LDL and TC:HDL in those switching from a ABC/3TC/DTG regime (374). In treatment experienced patients TAF/FTC/BIC has been shown to have no significant effect on lipids compared with those taking boosted PI therapy and TDF. In the same study TAF/FTC/BIC demonstrated favourable lipid changes compared to those continuing boosted PI with ABC/3TC backbone therapy (376).

Most of the safety data related to lipids in those switching to TAF/FTC/BIC comes from clinical trials. These often compare the study drug to the current standard of treatment or in treatment naïve individuals. Given that in real world settings individuals often take a multitude of different ART preparations, we sought to investigate the change in lipid profile after switching to TAF/FTC/BIC in a real-world setting.

6.2 Methods

We conducted a real-world retrospective analysis to analyse the effect of switching ART on lipid profiles. Data was collected from the Royal Liverpool University Hospital HIV clinical database. The HIV database exists for both clinical use and quality improvement purposes and is approved by the host institution's audit committee. All demographic and clinical variables present on the database were cross checked by the research team using the Trust's electronic patient record. The Royal Liverpool University Hospital audit committee approved this study. Lipid data was collected from the Trust electronic reporting system.

Participants were included if they were HIV-positive and had been switched to TAF/FTC/BIC. Participants were excluded if there was no/insufficient lipid data or if there was no follow-up. The lipid results from the point of switching ART up to 50 weeks prior to ART switch were included. We included the nearest to the ART switch. Lipid data post switch was taken from 12 to 100 weeks post ART switch.

6.2.1 Statistical Analysis

Summary statistics were calculated to demonstrate the demographics and clinical covariates of the cohort at baseline. The mean and standard deviation are displayed where normally distributed for continuous data. For non-normal data the median and interquartile ranges are displayed. Categorical data is displayed in absolute terms and the proportion as percentages. Changes in lipid profiles were compared using a paired T-test. Statistical significance is inferred with $p<0.05$.

We performed a sensitivity analysis using multiple regression analysis using the lipid change as the dependent variable for total cholesterol (TC), triglyceride (TG) and high-density lipoprotein cholesterol (HDL). Clinical and demographic covariates were added to the model *a priori*. A power calculation was not utilised as we felt this inappropriate due to the cross-sectional nature of this study.

One way analysis of covariance (ANCOVA) was calculated for each lipid parameter after adjusting for age. Post hoc analysis with the Bonferroni adjustment was performed if statistical significance was detected. A two-way ANCOVA was also calculated for each lipid parameter to investigate the effect of statin prescription on lipid change. We opted to use quartiles of lipid parameter *a priori*. The statistical analysis was performed using RStudio, version 1.3.1056.

6.3 Results

There were 135 patients included in the analysis. The baseline demographics and clinical covariates are outlined in table 1. The mean age was 47 with a proportion of 80% male sex (n=108). The proportion of statin therapy at baseline was 17% (n=23). All patients taking baseline statin continued until the end of the study. Baseline lipids were assessed at a mean of 23 weeks prior to ART switch. Follow up lipids were assessed at a mean of 42 weeks post ART switch. There was no significant difference in mean lipid change for any lipid parameter (table 2).

The multiple regression models demonstrated significant associations for increasing baseline lipid quartile (compared to the first quartile) for TC (all $p<0.001$). The significant associations for TG were the highest baseline quartile only ($p<0.001$). The HDL model did not demonstrate any significant association with quartiles of HDL (table 3).

There were statistically significant differences between quartiles for each lipid parameter as outlined by the ANOVA test. In the post hoc analysis of ANOVA, the highest quartile of TC

had a statistically significantly significant mean change compared to each of the other quartiles ($p<0.001$) (supplementary data). The highest quartile of TG also had a statistically significant mean change from baseline compared to the other quartiles ($p<0.001$). The highest quartile of HDL had significant change compared to the first and third quartiles ($p<0.001$) (figure 1). In the two-sided ANCOVA test to assess for the variance in statin therapy, there was no statistical significance in any group.

In the subgroup analysis, there were 71 participants taking TAF based ART at baseline (52.6%) and 27 taking TDF based ART (20%) at baseline. Details of previous ART regime is provided in the supplementary data. In those switching from TDF there was a statistically significant increases in TC compared to those switching from TAF (mean increase: 0.34mmol/L for TDF versus -0.23mmol/L for TAF, $p<0.001$). In addition, there were significant differences in TG changes between those switching from TDF compared to TAF (mean increase: 0.61mmol/L for TDF versus a mean decrease of -0.06mmol/L, $p<0.001$ for TAF). There was no significant change between groups with HDL.

6.4 Discussion

The main finding from this real-world study investigating lipid change in patients switching to TAF/FTC/BIC for the whole cohort there was no significant change in lipid profile from baseline to follow up. However, our data shows that those with the most adverse lipid profile at baseline (highest quartile of TC, TG, and lowest quartile of HDL) have a significant improvement on follow up after switching. In our subgroup analysis we demonstrate that those switching from TDF based ART regimens have significantly worse lipid profiles than those switching from TAF based ART. These findings were confirmed in our sensitivity analysis.

TAF/FTC/BIC is considered to have a neutral effect on lipids and is recommended as first line treatment for HIV-1. It is also considered to be safe in those with high risk of CVD. Our data suggests that in those with the most adverse lipid profile may gain the most improvement in

lipid profile through switching to TAF/FTC/BIC. This result has not been replicated in other real-world data.

Prior work comparing the efficacy and safety of TAF/FTC/BIC and dolutegravir in treatment-naïve individuals demonstrated that TAF/FTC/BIC-treated patients had significant increases in TC and LDL after 96 weeks compared to DTG/ABC/3TC (53). Stellbrink *et al* also demonstrated no significant difference in lipid change between treatment naïve individuals in those randomised to TAF/FTC/BIC or TAF/FTC/DTG (377). In a study of treatment experienced, virologically suppressed patients, Sax *et al* demonstrated no significant difference in lipids in those switching to TAF/FTC/BIC or TAF/FTC/DTG (378). Overall, our data did not show any significant change in lipid markers after switching to TAF/FTC/BIC. However, unlike these clinical trials our cohort was heterogenous in terms of clinical comorbidities and baseline ART.

The effect on lipid markers in those switching to TDF is well established. TDF is known to have favourable effects on the lipid profile and changing away from TDF to TAF causes worsening of these markers (379,380). TDF has also been shown to reduce lipid markers in healthy volunteers (381). The results of our sub study demonstrate that the adverse effect of changing from TDF to TAF remains consistent for those taking TAF/FTC/BIC.

The impact of switching to TAF/FTC/BIC in real world settings has been studied recently. Rolle *et al* investigated the safety implications of switching to TAF/FTC/BIC in patients >50 years. They demonstrated statistically significant decreases in all lipid markers through 48 weeks (382). The reasons why our results differ may lie in the proportions of clinical covariates and demographics. Our mean age was younger (mean 47 years compared with median 57 years) and we had less concomitant prescription of statins at baseline. In addition, the baseline ART was significantly different in both our studies. There were similar rates of TDF at baseline in both studies (20% in our current study versus 25%). However, in the study by Rolle *et al* there were 55% of patients taking dual NRTI plus INSTI at the point

of switching. There was also higher rates of PI therapy in our study (23% versus 13%). There was also significantly lower rates of lipid lowering therapy at baseline in our study (17% versus 51%).

In our sensitivity analysis we utilised multiple regression models to assess any association of demographic and clinical covariates with change in each lipid marker. The highest quartile of TC and TG were significantly and independently associated with change in TC and TG. These results were independent of prior TDF therapy. Furthermore, statin therapy was independently associated with a reduction in TC (p=0.047). Switching away from PI was also associated with a reduction in TG (p=0.031). The sensitivity analysis also confirmed the results from the tenofovir sub study. Baseline TDF use significantly associated with increased TC and TG (p=0.031 and p=0.009 respectively).

The results from the regression models suggest that switching from PI is significantly associated with improvement in TG (table 3). PI therapy, particularly older generation PIs, has been demonstrated to be associated with adverse lipid profiles and adverse cardiometabolic risk. This risk seems to be most pronounced ritonavir-boosted preparations although modern preparation of PI have been shown to have less of an effect (45).

There were several limitations to our study. Firstly, We did not utilise the LDL measurements as part of this study as this was measured in an indirect way (Friedewald equation). Consequently, LDL data was missing in those that had TG >4.5mmol/L. In addition, although our multiple regression models demonstrated the significance of baseline lipid quartile with overall lipid change the overall fit of the models was poor. This may suggest alternate explanations that predict lipid change that were not measured within this study.

In addition, we were not able to control for change in lifestyle measures such as diet and exercise. The impact of these changes may influence changes in lipid measures to a certain

degree. We also did not quantify the reasons for swapping ARV. This may have introduced bias into the study as there are often broad reasons for switching ARV. For example, patients may have been switched away from PI-based therapy due top perceived CVD risk.

Despite these limitations our study had several strengths. Firstly, our cohort was extremely well characterised with no missing data. We were able to perform multiple assessments on the impact of clinical covariates on lipid change after an ART switch to TAF/FTC/BIC. This study was designed to be hypothesis generating rather than show definitive causality. The main results may have significant clinical relevance in the fact that the patients that stand to benefit the most in CVD risk reduction may be those with the most adverse lipid profiles. This seems to be independent of lipid lowering therapy. Furthermore, we were able to confirm our findings using a regression modelling in our sensitivity analysis.

6.5 Conclusion

We assessed the effect of changing ART to TAF/FTC/BIC on lipid panels in a real-world clinical setting. The results of this study largely confirm data from clinical trials regarding changes in lipid panels. However, we were able to demonstrate that those with the most adverse lipid panels (the highest quartile of TC, TG, and lowest quartile of HDL) had significant improvements in lipid markers after switching. The implications of these results are that clinicians may expect significant improvements in switching to TAF/FTC/BIC in those with the most adverse lipid profiles.

6.6 Tables and Figures

6.6.1 Table 1: Description of baseline demographics and clinical covariates in patients switched to TAF/FTC/BIC.

Baseline demographics and clinical covariates	
Age (mean ±SD)	47.1 ± 11.6 years
Male Sex (%)	108 (80%)
Previous PI therapy (%)	31 (23%)
Previous tenofovir therapy (%)	91 (67.4%)
Current smoker (%)	38 (28.1%)
Hypertension (%)	22 (16.3%)
DMII (%)	8 (5.9%)
Prior CVD (%)	1 (0.7%)
eGFR (mean ±SD)	75.7 ± 13.6
Length of HIV diagnosis (mean ±SD)	9.3 ±7.9 years
Statin therapy (%)	23 (17%)

PI; Protease inhibitor, DMII; type II diabetes, CVD; cardiovascular disease, eGFR; estimated glomerular filtration rate

6.6.2 Table 2: Comparison of mean lipid profiles pre and post antiretroviral therapy change

	Mean Pre-ART change	Mean Post-ART change	P value
TC (±SD)mmol/L	4.76 (±1.15)	4.72 (±1.04)	0.64
TG (±SD)mmol/L	2.00 (±1.44)	2.10 (±1.40)	0.64
HDL (±SD)mmol/L	1.32 (±0.45)	1.27 (±0.42)	0.08

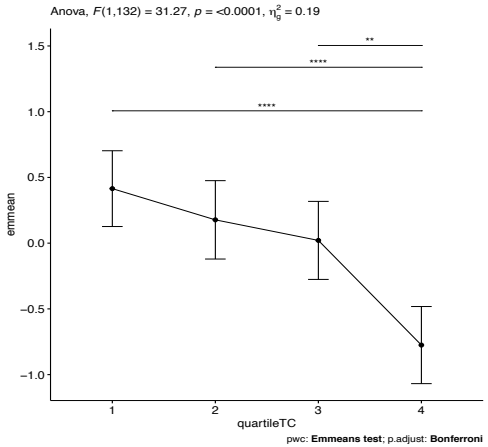
6.6.3 Table 3: Regression coefficients and statistical significance of the multiple regression models assessing associations with change in lipid markers.

	TC	TG	HDL
Intercept	0.16 (p=0.687)	-0.21 (p=0.825)	0.03 (p=0.846)
Baseline quartile 2	-0.32 (p=0.145)	0.14 (p=0.683)	-0.10 (p=0.211)
Baseline quartile 3	-0.55 (p=0.015)*	0.04 (p=0.894)	-0.07 (p=0.353)
Baseline quartile 4	-1.21 (p<0.005)*	-1.38 (p<0.005)*	-0.04 (p=0.594)
Age	0.01 (p=0.277)	0.00 (p=0.932)	0.00 (p=0.897)
Male Sex	-0.01 (p=0.991)	0.47 (p=0.111)	-0.03 (p=0.631)
statin therapy	-0.53 (p=0.047)*	0.04 (p=0.922)	0.04 (p=0.659)
Hypertension	0.20 (p=0.380)	-0.07 (p=0.825)	-0.09 (p=0.246)
DMII	-0.17 (p=0.622)	0.50 (p=0.346)	-0.14 (p=0.244)
Previous PI	-0.16 (p=0.365)*	-0.60 (p=0.031)*	0.04 (p=0.489)
Previous TDF	0.45 (p=0.031)*	0.76 (p=0.009)*	0.04 (p=0.489)

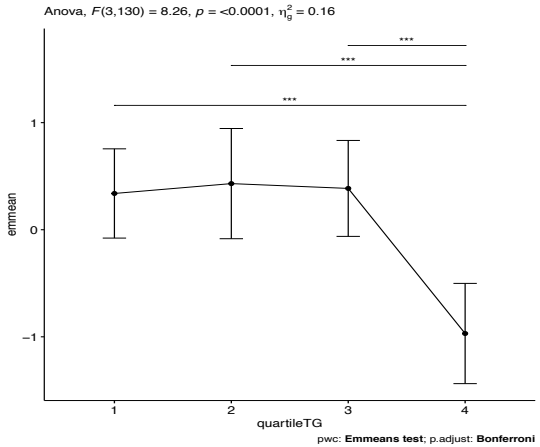
DMII; type II diabetes, PI; protease inhibitor, * denotes statistical significance.

Quartile 2, 3 and 4 compared to the first quartile in the model.

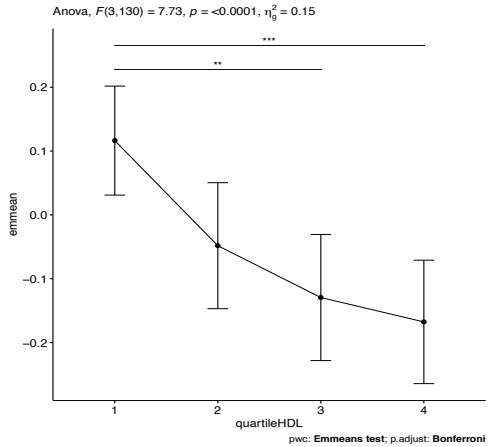
6.6.4 Figure 1: ANCOVA for quartiles of baseline lipid parameter



Panel A: Analysis of variance between quartiles of TC after adjusting for age.



Panel B: Analysis of variance between quartiles of TG after adjusting for age.



Panel C: Analysis of variance between quartiles of HDL after adjusting for age.

Figure 1: Analysis of variance for each individual lipid parameter. Each quartile of lipid parameter was compared to assess the mean lipid change and adjusted for age. The Y-axis represents mean change, the X-axis represents quartiles of lipid profile. The vertical lines in each quartile represent 95 % confidence intervals. The ANOVA result is above each graph. Significance is denoted by ***.

TC; total cholesterol, TG; triglyceride, HDL; high-density lipoprotein cholesterol

7 The effect of switching antiretroviral therapy on lipid profiles in a real-world setting.

7.1 Introduction

The advent of ART has dramatically reduced the prevalence of AIDS-defining illness and increased the life expectancy in patients diagnosed with HIV (372). HIV-positive patients are now succumbing to traditional diseases of ageing. CVD remains one of the leading causes of death in this patient group, with HIV-positive patients having up to double the risk of matched general population patients (383). One of the major contributors to this risk is dyslipidaemia. The D:A:D study found that for every 1mmol/L increase in (TC) the risk of myocardial infarction increased by 26%. The risk dropped by 28% for each 1mmol/L increase in HDL cholesterol (38).

Specific ART regimes are known to contribute to dyslipidaemia. Early generation PIs caused lipodystrophy, insulin resistance and dyslipidaemia due to their effect on insulin signalling and triglyceride storage (384,385). Modern preparations may have less of a cardiometabolic impact which may translate into reduced CVD risk. However, the effect of PI induced CVD risk is only partially explained by dyslipidaemia (45). NNRTI therapy, such as efavirenz, is also associated with increased TC. NNRTI induced dyslipidaemia does not appear to translate to increased cardiac events (38).

INSTI are a modern class of ART that is prescribed in combination with NRTI backbone therapy. They are known to have several metabolic effects including pronounced weight gain, blood pressure changes and unfavourable changes in HBA1c (114,386). Rates of dyslipidaemia with INSTI have been shown to be better than boosted PI therapy (102). Long term data on the translation to CVD risk is awaited.

Data on lipid changes from switching ART is often reported in clinical trials analysing a specific regimes against the previous standard of care. The multiple agents used in ART can

make it difficult to tease out the specific agent that may be contributing to changing lipid panels. Dyslipidaemia remains a significant contributor to CVD risk and in the context of increasing CVD burdens, a full understanding of the implications of ART switching is required. We sought to analyse our real-world data to establish the impact of changing ART on lipid panels.

7.2 Methods

We conducted a retrospective analysis to compare changes in lipid profiles in patients undergoing ART switch. Data were collected from the Royal Liverpool University Hospital HIV clinical database. The database is approved by the host institution audit committee and functions as an electronic patient record. Data, including demographic, clinical covariates, and ART details, were collected from patient visits between January 2018 and December 2020. Laboratory data, including lipid panels, were crossed checked with the Trust clinical systems and recorded in the database.

Participants were eligible if they were >18 years and had switched to ART within the study period. Participants were required to have lipid panels available <12 months before ART switch and <12 months following ART switch. We restricted the analysis to those switching to the six most common ART regimes which gave a sample size of 329. A power calculation was not utilised as we felt this inappropriate due to the cross-sectional nature of this study.

7.2.1 Study Outcomes

The primary outcome was the mean lipid change for each ART regime. We summarised and compared the baseline demographics, clinical covariates, and lipid panels across the whole group. Secondary outcomes included calculation of odds ratios for each ART regime for the highest quartile of TC, TG and HDL change.

7.2.2 Statistical Analysis

Continuous variables are summarised as means (± standard deviations) where normally distributed. Non-normally distributed continuous variables are presented as medians (± interquartile ranges). Categorical variables are summarised as absolute figures with percentages. The mean lipid change for each constituent of the lipid profile are compared using a paired T-test. We compared the demographic and clinical covariates between ART regimes using one-way ANCOVA for continuous variables and chi-squared test for categorical variables. Statistical significance was assumed with $p<0.05$.

The change for each lipid parameter was calculated and placed in quartiles. We performed binary logistic regression to calculate odds ratios for each ART class. The highest quartile as lipid change was used as dependent variable. Covariates were added to the sequential models *a priori*. Odd ratios (OR) and 95% confidence intervals (95% CI) were used to demonstrate the degree of association.

For the sensitivity analysis, we constructed a random forest algorithm to assess the variables of importance in predicting the highest quartile of each lipid constituent. Each clinical and demographic variable used in the logistic regression model was used in the random forest model. The results are displayed using a variable of importance chart using mean decrease in Gini index. The statistical analysis and development of the models were performed using RStudio, version 1.3.1056.

7.3 Results

There were 329 participants included in the analysis. The majority were male (66.9%) and had a mean age of 46.8 (±10.9) years. Baseline therapy was characterised by preparation of tenofovir and presence of PI. The majority (66.6%) of patients were taking a preparation of tenofovir with the majority of those taking TDF (51.4%). There were 26.1% of patients taking PI at baseline (table 1).

There were significant differences between clinical covariates stratified by ART regime switch (table 2). Those switching to RPV/FTC/TAF had a significantly higher proportion of statin prescription compared to other groups ($p<0.05$ for interactions). There were also greater proportions of hypertension, type II diabetes and chronic kidney disease (CKD) in the RPV/FTC/TAF group compared to other groups ($p<0.05$ for interaction terms). The D/C/FTC/TAF group and E/C/FTC/TAF group had a higher proportion of previous tenofovir compared to other groups. Prior PI prescription was also significantly higher in the D/C/FTC/TAF group compared to other groups ($p<0.005$).

For the whole group the mean TC was significantly higher post ART switch ($p<0.005$). The mean TG was also significantly higher post switch ($p=0.031$). There was no significant difference in HDL ($p=0.520$) (table 3). TC increased significantly from baseline in those switching to EFV/FTC/TDF, E/C/FTC/TAF, RPV/FTC/TAF and D/C/FTC/TAF (all $p<0.005$). TC decreased significantly in those switched to RPV/FTC/TDF and DOL/ABC/3TC ($p<0.005$ for both). TG increased significantly from baseline in those switched to EFV/FTC/TDF ($p=0.023$), E/C/FTC/TAF ($p=0.039$), RPV/FTC/TAF ($p=0.032$) and D/C/FTC/TAF ($p=0.033$). TG dropped significantly for those switched to RPV/FTC/TDF ($p=0.010$) and DOL/ABC/3TC ($p=0.033$). There was no significant change in HDL for any of the ART regimes (Table 4).

In the multivariate analysis both D/C/FTC/TAF and RPV/FTC/TDF were associated with the highest quartile of TC in the adjusted model (OR: 2.85, $p<0.005$ and OR:0.09, $p=0.021$ respectively). None of the ART regimes were significantly associated with the highest quartile of TG or HDL in either the univariate or adjusted models (table 5).

In the sensitivity analysis we performed random forest to assess the variables of importance for the highest quartile of each lipid constituent. For the TC model we found that age, followed by ART regime, were the most important by mean decrease in Gini index. This was also true in the TG and HDL models (figure 2).

7.4 Discussion

We sought to analyse our real-world data to establish which specific ART regimes contribute to changing lipid profiles. We assessed data from the top six most prescribed ART regimes within our local service. The main findings from this study are that switching ART resulted in significant alterations in lipid profiles. Switching to TAF (TAF) containing preparations, D/C/FTC/TAF, E/C/FTC/TAF and RPV/FTC/TAF, resulted in significantly increased TC and TG (Table 4). Switching to TDF containing preparations had mixed results with RPV/FTC/TDF demonstrating a significant reduction in TC and TG whilst, in contrast, EFV/FTC/TDF produced significant increases in TC and TG. None of the preparations demonstrated any significant alterations in HDL.

Within the whole group there was a significant increase in TC and TG with no significant change in HDL. Within group analysis demonstrated that TC increases were significantly associated with switching to EFV/FTC/TDF, E/C/FTC/TAF, RPV/FTC/TAF and D/C/FTC/TAF (all $p<0.005$). There was a significant decrease in TC in those switching to RPV/FTC/TDF and DOL/ABC/3TC. Switching to TAF containing preparations from TDF has previously been shown to increase constituents of the lipid panel (387). In the recently published report from the OPERA cohort, it was demonstrated that switching from TDF to TAF resulted in 5-10% increase in TC, 21-27% increase in TG and 30-35% reduction in HDL (387). In our cohort, the three TAF containing ART regimes were associated with significantly increased TC and TG with no significant impact on HDL. In those switching to TAF regimes, just under half had previously been taking TDF based regimes for those switched to D/C/FTC/TAF and E/C/FTC/TAF (49.3% and 48.6% respectively). In those switching to RPV/FTC/TAF, the majority had previously taken a TDF based regime (62.2%). Figure 1 compares changes in lipid based on tenofovir preparation at baseline.

The proportion of clinical covariates, prior ART and statin therapy varied between groups. To assess the differences between different ART regimes on lipid change we constructed a multivariate model using the highest quartile of change in each lipid marker as the dependent variable. D/C/FTC/TAF was independently associated with increasing TC whilst

other TAF containing preparations were not. Reasons for this may lie in the third agents used in these preparations. RPV/FTC/TAF contains RPV which has been shown to have a neutral effect on lipids (48,388) whilst D/C/FTC/TAF contains boosted DRV. The effect of cobicistat boosted DRV on lipid profiles is unclear. Previously, it has been shown that cobicistat-boosted DRV has a beneficial effect in those switching from ritonavir-boosted DRV. However, these results may be confounded by TDF and potential for cobicistat boosting of TDF level (389). Our data suggest that boosted DRV may contribute to worsening lipid profiles, independent of previous ART regime.

E/C/FTC/TAF contains ETG which is an INSTI. ETG has been demonstrated to have a deleterious effect on lipid profile compared to other INSTI in treatment naïve individuals (390). This does not appear to be a class effect, however, switching to INSTI has been associated with significant weight gain (391,392). E/C/FTC/TAF has been shown to be associated with increased rates of dyslipidaemia and weight gain in a Taiwanese population (391). Most patients in this study were taking TDF prior to changing to E/C/FTC/TAF. TDF has been demonstrated to reduce TC and LDL in healthy volunteers, although no significant change in TG was noted (381). In our data less than half of the patients switched to E/C/FTC/TAF were taking TDF at baseline. Although there was a significant increase in TC and TG in those switching to E/C/FTC/TAF there was no independent association with the highest quartile of TC and TG change after adjusting for baseline ART. This may suggest that adverse lipid changes related to switching to E/C/FTC/TAF are related to prior TDF therapy.

RPV/FTC/TDF demonstrated a significant reduction in TC and TG. It was also significantly and independently associated with negative changes in the highest quartile of TC but not TG or HDL. EFV/FTC/TDF, the other TDF containing regime in this cohort, demonstrated a significant increase in both TC and TG but no significant association in the multivariate model. These results are in keeping with previous findings regarding TDF preparations. The third agent in EFV/FTC/TDF, efavirenz, has been shown to have an adverse lipid profile compared to RPV. Significant improvements in lipid profiles has been reported in those switching from efavirenz to RPV (393–395). However, efavirenz has been associated with

increased HDL (394) although these results are not universal (395). Our data suggest that efavirenz containing preparations are associated with worsening lipid profiles compared to RPV containing ART.

In patients switching to DOL/ABC/3TC there was a significant reduction in mean TC and mean TG from baseline ($p<0.05$ and $p=0.033$ respectively). DOL/ABC/3TC has previously been shown to have a neutral effect on lipids (396,397). In our multivariate analysis there was no significant association in those switching to DOL/ABC/3TC with the highest quartile of TC, TG or HDL. The mean reduction in TC and TG in those switching to DOL/ABC/3TC may be because of the high proportion of patients taking prior PI as part of their baseline ART (30% PI at baseline).

The sensitivity analysis was performed using the machine learning technique of random forest. This ignores the assumptions required for traditional regression models and provides an alternative robust assessment into variables that have an impact on the dependent variable. The model demonstrates that age and the ART regime were the most important variables in predicted the highest quartile of lipid change.

The overall results from this study are broadly in keeping with previously documented changes in lipids in those undergoing ART change. The impact of changing ART on lipid change has been well documented but most data is from clinical trials. The magnitude of change on each marker is small and clinical impact of these changes is uncertain. There has been numerous studies investigating the impact on CVD risk scores in patients undergoing ART switch (387). Whilst these scores can offer a guide, they remain an imprecise tool for investigating excess CVD risk, especially in HIV-populations (370). Furthermore, any change in lipid profile because of ART switch has not been demonstrated to increase hard cardiovascular events. The difficulty in studying hard endpoints for CVD in this population is related to the low numbers of events and high numbers to adequately power any study.

However, translational work such as the effect on coronary plaque morphology using CTCA, could be utilised to gain insights into cardiovascular risk in those switching ART.

There were limitations to our study. Firstly, this study was retrospective in design and as such no causal conclusions can be drawn from this study. Secondly, we did not include markers of LDL. Due to the real world nature of this study there were high proportions of laboratory data for LDL that were missing. This was most commonly due to errors in the Friedewald formula to estimate LDL from TC, HDL, and TG. The remainder of the data is extremely well characterised with no missing data. We did not wish to compromise the result with estimations of LDL.

Despite these limitations there several strengths. We sought to investigate the effect of changing ART in a real-world setting. Most data on lipid change comes from clinical trial data. Our study gives insight into the implications of ART switches in a real-world setting. The dataset was extremely well characterised and complete giving a robust analysis. We utilised machine learning techniques to perform a sensitivity analysis which confirmed the main findings from this study.

7.5 Conclusions

The principal implications of this study are that ART choice influences lipid change in patients undergoing ART switch. The significant changes in TC and TG in those switching to TAF containing ART is likely explained by prior TDF use. Similarly, in those switching to TDF based regimes we demonstrated a significant reduction except in those ART containing efavirenz. These results largely confirm data from clinical trials regarding lipid increases with TAF based preparations. The degree to which these changes have any clinical implications, regarding CVD risk, is yet to be fully elucidated.

7.6 Figures and Tables

7.6.1 Table 1: Demographic and clinical covariates for the whole cohort

Clinical variable	n=329
Age (mean(SD))	46.8 (±10.9)
Male Sex (%)	220 (66.9%)
Hypertension (%)	47 (14.3%)
Type II diabetes(%)	21 (6.4%)
Ischaemic heart disease (%)	1 (0.3%)
CKD III or above (%)	26 (7.9%)
Current smoker (%)	76 (23.1%)
Ex-smoker (%)	10 (3%)
Total cholesterol (mean(SD))	4.71 (±1.1)
HDL cholesterol (mean(SD))	1.36 (±0.43)
Triglyceride (mean(SD))	1.62 (±1.09)
Prior TDF (%)	169 (51.4%)
Prior TAF (%)	50 (15.2%)
Prior PI (%)	86 (26.1%)
Statin therapy (%)	32 (9.7%)

CKD; chronic kidney disease, TDF; tenofovir disoproxil fumarate, TAF; tenofovir alafenamide, PI; protease inhibitor

7.6.2 Table 2: Clinical variable stratified by ART regime

		D/C/FTC/TAF	E/C/FTC/TAF	EFV/FTC/TDF	RPV/FTC/TDF	RPV/FTC/TAF	DOL/ABC/3TC	p value
Clinical variable	n=329	71	72	30	26	90	40	
Age (mean(SD))	46.8 (±10.9)	45.6 (±10.4)	47.3 (±10.1)	44.2 (±10.2)	50.3 (±10.1)	51.3 (±10.8)	44.9 (±10.8)	<0.005
Male Sex (%)	220 (66.9%)	48 (67.6%)	49 (68.0%)	22 (73.3%)	12 (46.1%)	61 (67.8%)	28 (70%)	0.319
Hypertension (%)	47 (14.3%)	6 (8.5%)	12 (16.7%)	7 (23.3%)	0 (0%)	19 (21.1%)	3 (7.5%)	0.019
Type II diabetes(%)	21 (6.4%)	1 (1.4%)	3 (4.2%)	3 (10%)	0 (0%)	14 (15.6%)	0 (0%)	<0.005
Ischaemic heart disease (%)	1 (0.3%)	0 (0%)	0 (0%)	1 (3.3%)	0 (0%)	0 (0%)	0 (0%)	0.075
CKD III or above (%)	26 (7.9%)	3 (4.2%)	5 (6.9%)	0 (0%)	0 (0%)	12 (13.3%)	6 (15%)	0.027
Current smoker (%)	76 (23.1%)	23 (32.4%)	19 (26.4%)	7 (23.3%)	0 (0%)	12 (13.3%)	10 (25%)	0.108
Ex-smoker (%)	10 (3%)	1 (1.4%)	2 (2.8%)	2 (6.7%)	0 (0%)	3 (3.3%)	2 (5%)	0.645
Total cholesterol (mean(SD)) (mmol/L)	4.71 (±1.1)	4.60 (±1.09)	4.70 (±1.08)	4.83 (±1.10)	4.84 (±1.03)	4.72 (±1.10)	4.71(±1.10)	0.922
HDL cholesterol (mean(SD)) (mmol/L)	1.36 (±0.43)	1.30 (±0.41)	1.35 (±0.41)	1.46 (±0.41)	1.45 (±0.41)	1.36 (±0.43)	1.36 (±0.42)	0.509
Triglyceride (mean(SD)) (mmol/L)	1.62 (±1.09)	1.68 (±1.11)	1.59 (±1.12)	1.42 (±1.12)	1.38 (±1.07)	1.77 (±0.92)	1.57 (±1.10)	0.517
Prior TDF (%)	169 (51.4%)	35 (49.2%)	35 (48.6%)	21 (70%)	10 (38.4%)	56 (62.2%)	12 (30%)	<0.005
Prior TAF (%)	50 (15.2%)	21 (29.6%)	13 (18.1%)	0 (0%)	3 (11.5%)	7 (7.8%)	6 (15%)	<0.005
Prior PI (%)	86 (26.1%)	54 (76.1%)	13 (18.1%)	0 (0%)	2 (7.7%)	5 (5.6%)	12 (30%)	<0.005

Statin therapy (%)	32 (9.7%)	2 (2.8%)	3 (4.2%)	4 (13.3%)	1 (3.8%)	20 (22.2%)	2 (5%)	<0.005

7.6.3 Table 3: Mean lipid change for the whole cohort

	Mean baseline	Mean (Post ART change)	p value
Total cholesterol (±SD) (mmol/L)	4.71 (±1.1)	4.91 (±1.08)	<0.005
Triglyceride (±SD) (mmol/L)	1.62 (±1.09)	1.75 (±1.18)	0.031
High-density lipoprotein (±SD) (mmol/L)	1.36 (±0.43)	1.37 (±0.40)	0.520

7.6.4 Table 4: Mean lipid panel change stratified by ART regime

ART	Mean baseline TC (mmol/L)	Mean TC post switch (mmol/L)	p value	Mean baseline TG (mmol/L)	Mean TG post switch (mmol/L)	p value	Mean baseline HDL (mmol/L)	Mean HDL post switch (mmol/L)	p value
EFV/FTC/TDF (n=30)	4.83	4.91	<0.005	1.42	1.55	0.023	1.46	1.43	0.322
RPV/FTC/TDF (n=26)	4.89	4.41	<0.005	1.38	1.25	0.010	1.45	1.25	0.330
E/C/FTC/TAF (n= 72)	4.70	5.11	<0.005	1.59	1.80	0.039	1.35	1.42	0.357
RPV/FTC/TAF (n=90)	4.72	4.74	<0.005	1.77	1.80	0.032	1.36	1.35	0.498
DOL/ABC/3TC (n=40)	4.71	4.63	<0.005	1.57	1.45	0.033	1.36	1.39	0.481
D/C/FTC/TAF (n=71)	4.60	5.28	<0.005	1.68	1.99	0.033	1.30	1.34	0.276

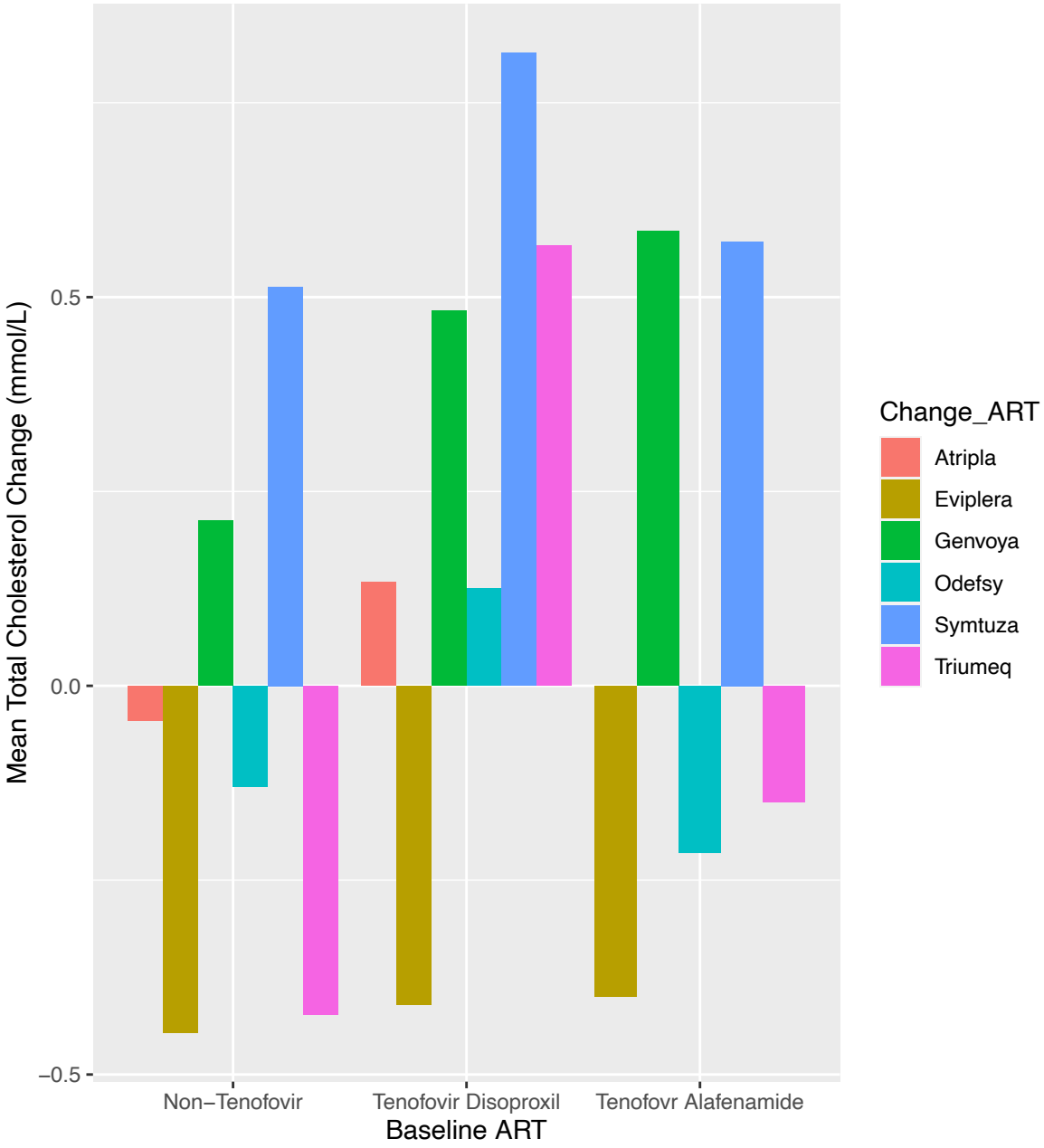
7.6.5 Table 5: Logistic Regression models for the highest quartile of lipid change

	Total Cholesterol				Triglyceride				High Density Lipoprotein Cholesterol			
Predictor	Univariate (95% CI)	p value	Adjusted Model (95% CI)	p value	Univariate (95% CI)	p value	Adjusted (95% CI)	p value	Univariate (95% CI)	p value	Adjusted Model (95% CI)	p value
RPV/FTC/ TDF	0.11 (0.01-0.53	0.031	0.09 (0.01-0.46)	0.021	0.52 (0.15-1.42)	0.248	0.51 (0.14-1.49)	0.256	0.25 (0.04-0.88)	0.066	0.24 (0.04-0.87)	0.062
E/C/FTC/TAF	1.44 (0.80-2.56)	0.213	1.60 (0.86-2.92)	0.132	1.72 (0.96-3.02)	0.064	1.62 (0.88-2.95)	0.114	2.10 (1.17-3.70)	0.011	2.26 (1.24-4.10)	0.007
RPV/FTC/ TAF	0.89 (0.49-1.55)	0.682	1.26 (0.65-2.40)	0.493	0.75 (0.41-1.32)	0.328	0.82 (0.42-1.58)	0.565	0.91 (0.80-11.54)	0.756	0.91 (0.47-1.74)	0.786
D/C/FTC/ TAF	2.67 (1.52-4.68)	<0.005	2.85 (1.33-6.21)	0.007	1.24(0.68-2.21)	0.476	1.54(0.69-3.54)	0.288	0.76 (0.38-1.42)	0.408	0.57 (0.24-1.31)	0.198
DOL/ABC/ 3TC	0.49 (0.18-1.15	0.128	0.47 (0.17-1.13)	0.114	1.00 (0.45-2.09)	0.991	0.99 (0.42-2.18)	0.977	1.69 (0.80-3.41)	0.151	1.93 (0.88-4.08)	0.090
EFV/FTC/ TDF	0.31 (0.07-0.91)	0.060	0.30 (0.07-0.92	0.060	0.58 (0.19-1.44)	0.278	0.42 (0.13-0.96)	0.109	0.38 (0.08-1.00)	0.082	0.27 (0.06-0.83)	0.042

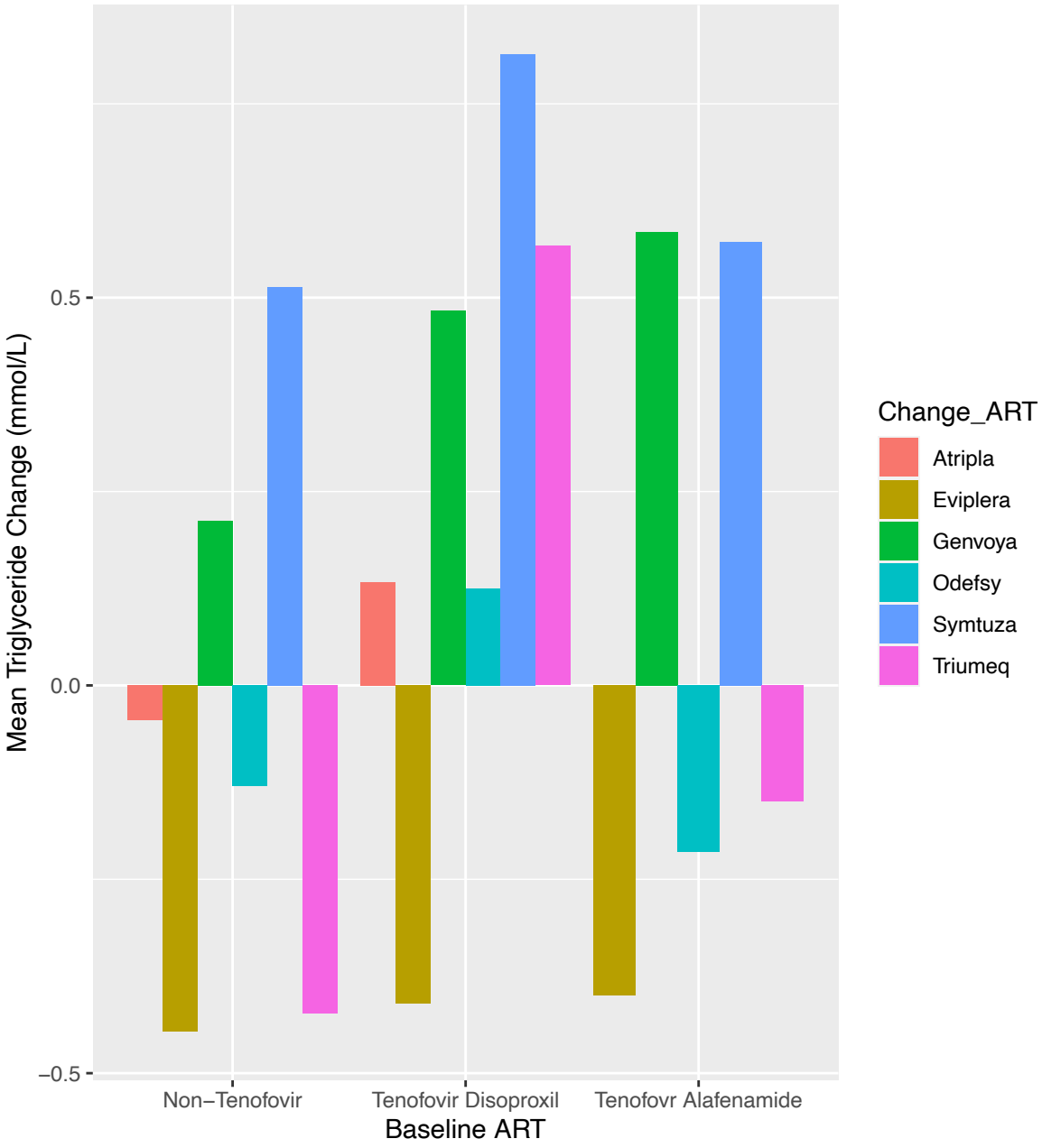
Adjusted for statin therapy, age, sex, diabetes, hypertension, current smoking and previous ART

7.6.6 Figure 1: Lipid change per ART clustered by baseline preparation of tenofovir

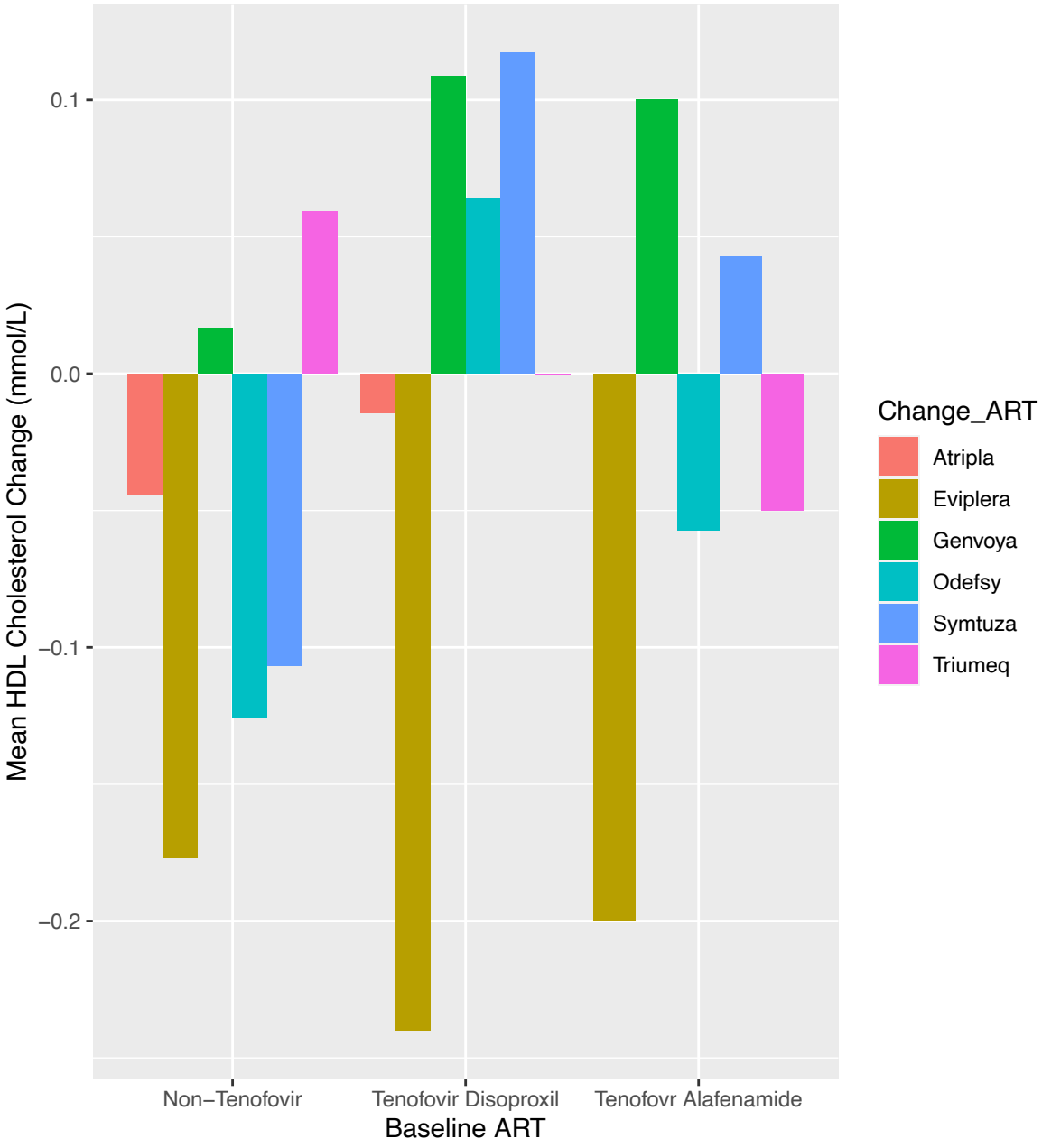
Panel A: Change in total cholesterol



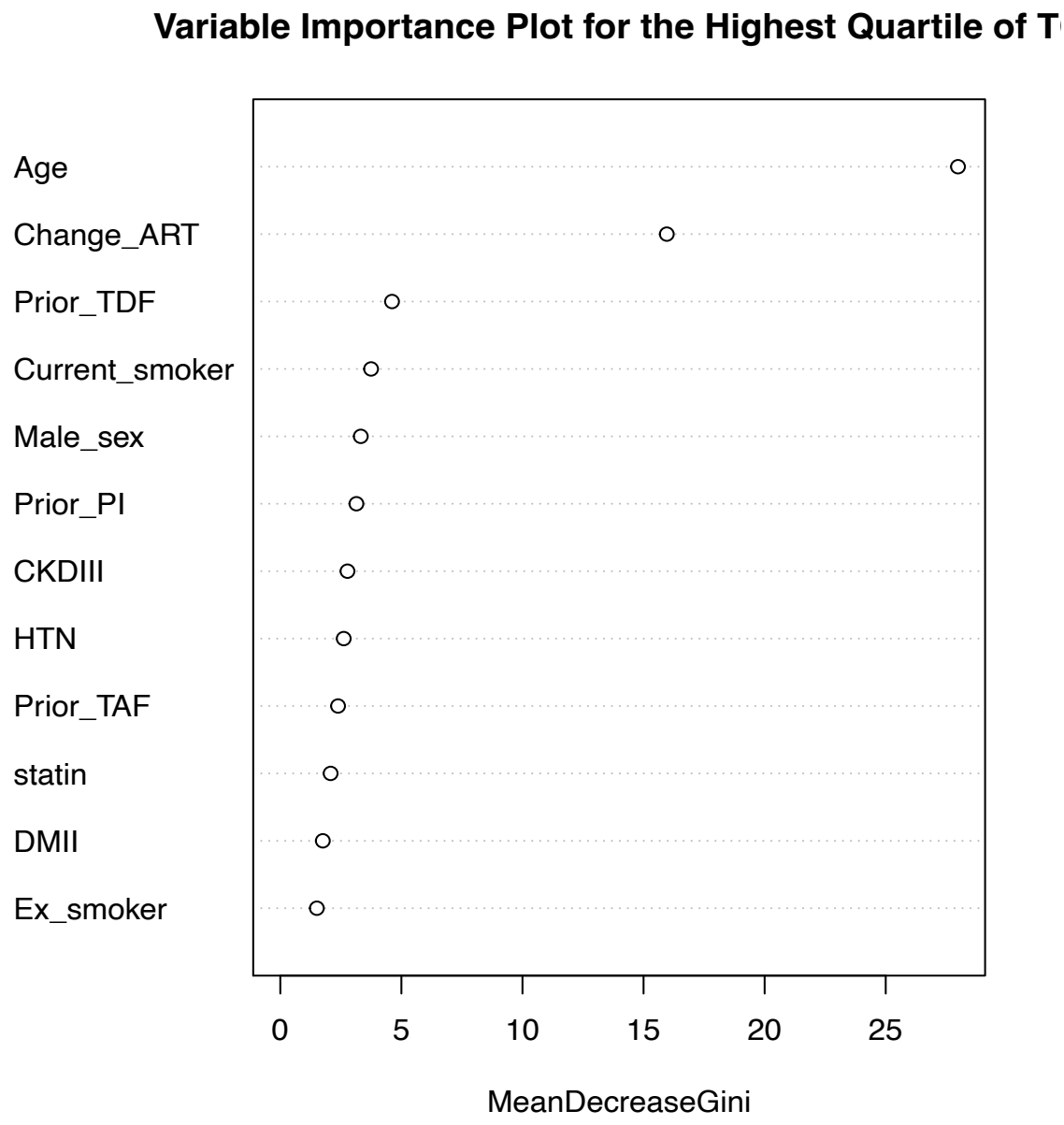
Panel B: Change in triglyceride

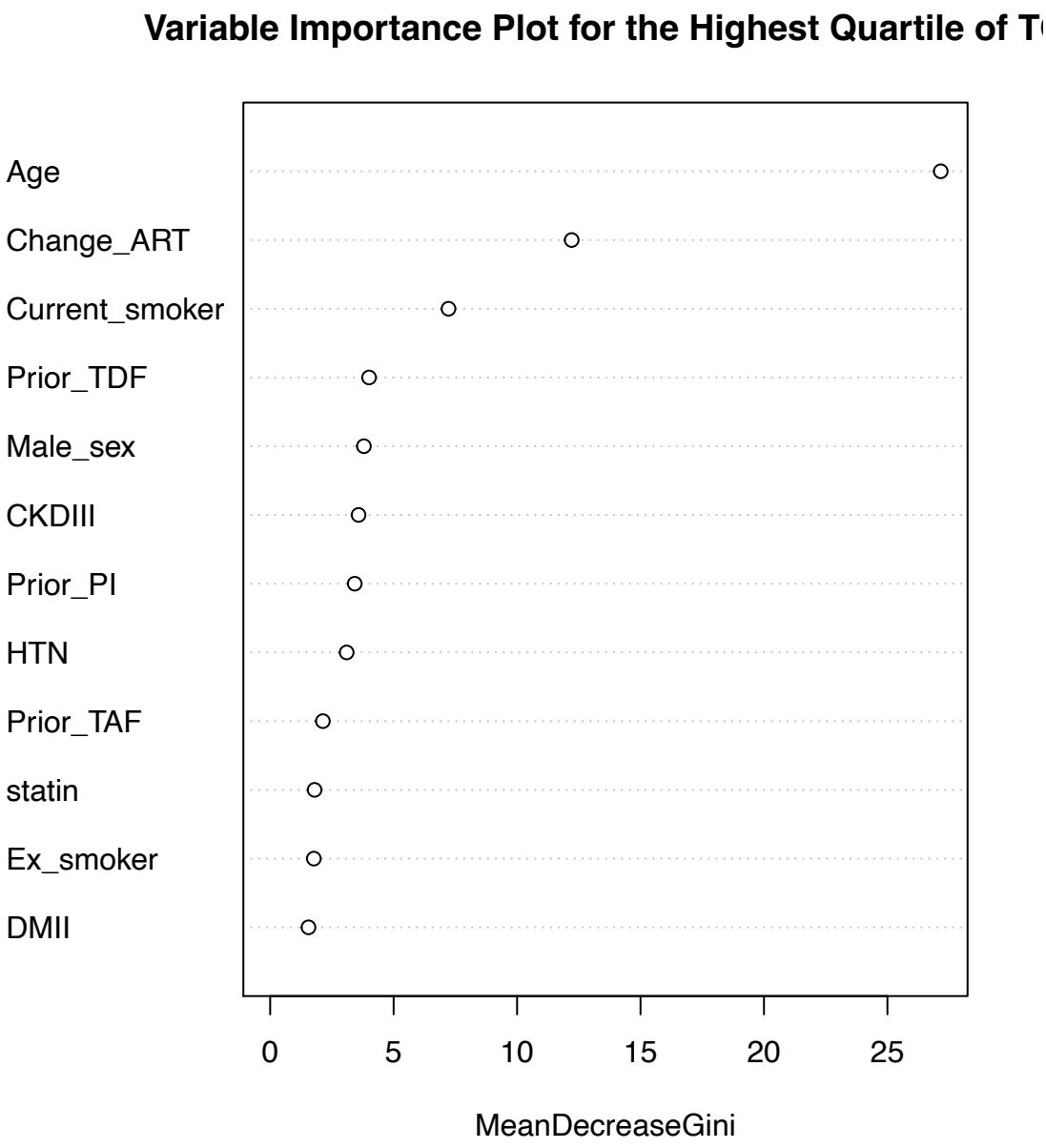


Panel C: Change in high-density lipoprotein cholesterol.



7.6.7 Figure 2: Variable of importance plots for each constituent of the lipid panel.




Variable Importance Plot for the Highest Quartile of T
Age
Change_ART
Current_smoker
Prior_TDF
Male_sex
CKDIII
Prior_PI
HTN
Prior_TAF
statin
Ex_smoker
DMII
0
5
10
15
20
25
MeanDecreaseGini

8. General Discussion

We have demonstrated significant associations between markers of ectopic fat deposition, HS and EAT volume, and incident CVD in PLWHIV. In these two imaging studies this association was not significant in the general population groups. These findings hint at HIV-specific pathways linking ectopic fat with CVD and is consistent with knowledge of HIV and ART induced changes in adipocyte homeostasis and pressures towards 'central' fat accumulation.

This work opens several exciting directions of travel using imaging biomarkers. Firstly, mechanistic studies describing the relationship between multiple markers of ectopic fat (EAT volume, HS, VAT volume) and adipocyte structure and function should be forthcoming. Initial work by Gabriel *et al* have first described some of the relationships between HS and gene expression in SAT (312). This should be further expanded to encompass the effect of specific ART's and the relationship with EAT volume and density. As described, INSTI are a first line ART but is increasingly recognised as causing profound weight gain and metabolic syndrome. In one recent study INTSI have been linked with increased rates if CVD (115). The study investigating use of TAF/FTC/BIC in a real-world setting (chapter 6) does indicate that lipid panels improve, but significantly in those with the worst levels. How this translates to CVD risk needs further work.

CTCA has been used frequently to assess surrogates for CVD risk in PLWHIV. The biggest driver of CVD risk is atherosclerotic plaque burden. The use of plaque volumes and plaque morphology is relatively underutilised in mechanistic studies. Barriers to this may include the time-consuming nature of measuring non-calcified plaque in CTCA post-processing techniques. In an ancillary paper associated with this work, we found significant associations with low-attenuation plaque and HS (398). Studies utilising plaque volumes and plaque morphology with ectopic fat measurements would further add to our understanding of risk. Furthermore, longitudinal studies investigating the natural progression of HS and EAT are lacking in this patient cohort.

There have been several recent developments using CTCA datasets and risk prediction. Perivascular adipose tissue has recently been demonstrated to be significant in enhancing risk predication. The attenuation of the immediate fat surrounding epicardial coronary arteries (within one radial length of the lumen) has been shown to be correlated with adipocyte size, dysfunction and inflammation (399). It is not clear if this reflects inflammation generated at the coronary artery endothelium or inflammation generated at the level of the adipocyte. This has been amalgamated into AI algorithms and found to be additive to risk prediction when using traditional metrics (400). There have been no published studies investigating the role of perivascular adipose tissue attenuation in PLWHIV. Given the associations of ectopic fat and HIV disease demonstrated in this work and the great need to enhance risk prediction, investigating the use of PVAT in PLWHIV is of great interest.

Recent developments in heart failure medical therapy have led to SGLT2-I becoming first line therapy for patients with HFrEF. The mechanism of action involves competitive antagonism of the SGLT2 receptor in the proximal convoluted tubule, reducing glucose resorption and causing glucosuria (401). Beyond its ability to improve insulin sensitivity it has been demonstrated to improve outcomes in patients with heart failure (402). Likely mechanism involves improvement of myocyte energy handling by promotion of switching to beta oxidation (403). It has also been shown to reduce EAT volume and pro-inflammatory cytokines (404–406). Given the HIV-specific risk associated with ectopic fat, as demonstrated here, using ectopic fat as a therapeutic target for risk reduction is an exciting future research focus. Further, therapeutics including GLP-1 agonists, may have the capacity to modulate ectopic fat deposition and provide incremental risk reduction.

The work in this thesis (chapters 6 and 7) has documented the effect of switching ART on lipid panels and thus perceived CVD risk. Switching away from TDF based preparations has been extensively demonstrated to result in increased metrics of the lipid profile. The extent to which this translates into true CVD risk has not been demonstrated. TAF/FTC/BIC is a modern preparation of ART and considered first line therapy. Chapter 6 demonstrates the

beneficial effect on lipid profiles in those in the 'worst' quartile of lipid panels, which may indicate those with the highest CVD risk stand to gain the most from switching to TAF/FTC/BIC. Aside from changes in lipid panels switching to INSTI-based ART has been associated with significant weight gain and metabolic syndrome. Worryingly, INSTI has also been implicated in excess CVD outcomes (115). Translational research utilising CTCA-based radiomics could be deployed to help elucidate the effect of INSTI on coronary plaque, EAT and HS. Furthermore, longitudinal studies using sequential CTCA could be used to understand the effect of ART switches and novel therapeutics on markers of plaque and ectopic fat.

There have been multiple attempts to augment risk calculation tools to be specific for HIV disease. These have incorporated HIV-specific features but still significantly underperform. We attempted to generate a coronary calcium risk prediction tool and compare it against FRS. Whilst this has obvious limitations, (doesn't include non-calcified plaque or EAT volume), it is the only risk prediction tool to include ectopic fat (HS). Future work should focus on development and integration of novel risks and radiomics for inclusion in risk prediction algorithms.

The impending health and economic implications of excess CVD in PLWHIV needs urgent attention. It is hoped that this work will set the foundations for mechanistic studies and ultimately translational research into the aetiology of CVD in PLWHIV. Precise risk prediction in PLWHIV is also a necessity due to the risk of drug-drug interactions (particularly with statins). The use of novel therapeutics targeting ectopic fat is of great interest and likely to drive future investment in randomised control trials in PLWHIV.

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